

## Abstracts of the 15th International Symposium on Bioluminescence and Chemiluminescence

Abstracts are in alphabetical sequence of first author

### The measurement of cytosolic ATP during apoptosis: bioluminescence imaging at the single cell level

R. Akiyoshi and H. Suzuki

Research and Development Division, Olympus Co., Tokyo, Japan. E-mail: ryutaro\_akiyoshi@ot.olympus.co.jp

ATP is the major source for metabolic energy in the cell and affects numerous cellular processes. Generally ATP is measured by luminescence assay using firefly luciferin–luciferase reaction as total ATP content in a mass of cells. We developed a luminescence microscope to measure ATP at the single cell level and applied it to monitor cytosolic ATP level throughout the apoptotic process in a HeLa cell transfected luciferase gene. As a result, apoptotic stimuli by four medicines (staurosporine, carbonyl cyanide *p*-trifluoro-methoxyphenylhydrazide, cycloheximide and actinomycin D) induced elevation of the ATP level after 1–2 h. This result confirmed previous data reported by luminometric assay. However, we observed a phenomenon just before cell death that some cells emit flash luminescence after 4–8 h stimulus. It is uncertain whether or not the flash luminescence is due to increased cytosolic ATP; such a result can not be obtained by conventional luminometric assay. The bioluminescence imaging assay at the cellular level would make a new research field, not only in ATP measurement but also in intracellular signal transduction, regulation of gene expression and other areas.

### Comparison of effects of uranium and americium on bioluminescent bacteria

M. A. Alexandrova,<sup>1</sup> L. G. Bondareva<sup>2</sup> and Y. A. Bolsunovsky<sup>2</sup>

<sup>1</sup>Siberian Federal University, Krasnoyarsk 660041, Russia.

E-mail: maka-alexandrova@rambler.ru

<sup>2</sup>Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia

The effect of  $\text{UO}_2(\text{NO}_3)_2$  on the bioluminescent bacterium *P. phosphoreum* was studied. It was compared with the effect of solutions of the more active radionuclide,  $^{241}\text{Am}(\text{NO}_3)_3$ , studied earlier (Rozhko *et al.*, 2007). Bioluminescence inhibition was observed under uranyl concentrations exceeding  $10^{-5}$  mol/L (30 Bq/L); and bioluminescence activation was not observed under all radionuclide concentrations and exposure times in the experiment. The effect of uranyl was attributed to the chemical component of its impact, not the radiation component. It was shown that solutions of americium were detoxified by humic substances (0.25 mg/mL) but that solutions of uranyl were not.

### Fluorescence of firefly luciferin in DMSO and aqueous solutions with changing excitation wavelength

Y. Ando and H. Akiyama

Institute for Solid State Physics, University of Tokyo and CREST JST, 5-1-5, Kashiwanoha, Kashiwa, Chiba 277-8581, Japan.

E-mail: yori@issp.u-tokyo.ac.jp

We systematically investigated the fluorescence of firefly luciferin in DMSO and aqueous solutions (pH 2, 4, 6 and 8) by continuously changing the excitation wavelength between 350 nm and 600 nm. In DMSO, fluorescence was switched from blue emission (ex 350–400 nm) to yellow-green emission (ex 430–460 nm) and then to red emission (ex 570–600 nm). In aqueous solution, excitation wavelength change caused fluorescence colour change from yellow-green to red at every pH, though excitation wavelength to switch the fluorescence colour was pH dependent. Existence of the red emitting luciferin state in DMSO and in all aqueous solutions with any pH was found, which may be an important clue in investigating firefly bioluminescence colour change.

### Flow-injection chemiluminescence determination of hydroxyl amine hydrochloride

M. R. Baezzat and M. Izadpanah

Department of Chemistry, Fars Bioconversion and Environmental Research Institute, Shiraz, Iran.

E-mail: mrbaezzat@yahoo.com

A new sensitive and selective flow-injection chemiluminescence method for the determination of hydroquinone over the range  $1 \times 10^{-7}$ – $5 \times 10^{-6}$  mol/L is described. The method is based on chemiluminescence emission during the oxidation of hydroquinone by potassium persulphate in alkaline medium. Method development includes optimization of reagent concentrations and flow conditions; the detection limit is  $4.5 \times 10^{-8}$  mol/L. The method also has good selectivity and is simple, fast, selective and precise.

### The effects of sulphur dioxide on activities of fatty acid synthase in livers and lungs

J. Bai and Z. Meng\*

Research Centre of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China.

E-mail: baijuli@sxu.edu.cn

Sulphur dioxide ( $\text{SO}_2$ ) is a ubiquitous air pollutant. In the present study, male Wistar rats were housed in exposure chambers and treated with  $14.00 \pm 1.01$ ,  $28.00 \pm 1.77$  and  $56.00 \pm 3.44$  mg/m<sup>3</sup>  $\text{SO}_2$  for 6 h/day for 7 days, while control rats were exposed to

filtered air in the same condition. The activities of fatty acid synthase (Fasn) in livers and lungs were analysed using UV-Vis absorption at 340 nm, based on the mechanism of NADPH being oxidated to NADH. The results showed that SO<sub>2</sub> inhalation at all concentrations tested caused increase in Fasn activities (for liver: 1.15-fold at 14 mg/m<sup>3</sup>, 1.95-fold at 28 mg/m<sup>3</sup>, 3.57-fold at 56 mg/m<sup>3</sup>; for lungs: 1.37-fold at 28 mg/m<sup>3</sup>, 2.41-fold at 56 mg/m<sup>3</sup>) but at low concentration (14 mg/m<sup>3</sup>) the increase in Fasn activities in lungs was not statistically significant. These results led to the conclusion that SO<sub>2</sub> might affect the metabolism of fatty acid in livers and lungs and suggested that SO<sub>2</sub> might have relations with some metabolic syndrome.

### Bioluminescence of coelenterates: spectral components

N. V. Belogurova,<sup>1,2</sup> N. S. Kudryasheva,<sup>1,2</sup> R. R. Alieva<sup>2</sup> and A. G. Sizykh<sup>2</sup>

<sup>1</sup>Institute of Biophysics SB RAS, Akademgorodok No 50, Krasnoyarsk 660036, Russia. E-mail: nbelogurova@mail.ru, n\_belogurova@yahoo.com

<sup>2</sup>Siberian Federal University, Svobodny No 79, Krasnoyarsk 660041, Russia

Ca<sup>2+</sup>-regulated photoproteins are responsible for the bioluminescence of marine coelenterates. The addition of calcium ions to Ca<sup>2+</sup>-regulated photoproteins results in light emission. This peculiar feature of the photoproteins provides the basis for their analytical application, mainly in monitoring intracellular calcium concentration. During the study, the complex bioluminescence spectra of photoproteins from marine coelenterates, e.g. the jellyfish *Aequorea victoria* and the hydroid *Obelia longissima*, and photoluminescence spectra of products of the bioluminescent reactions (Ca<sup>2+</sup>-discharged photoproteins) were resolved into spectral components. Resolution of the spectra was performed in a combined way, involving methods of: (a) function increment based on Gaussian distribution; (b) secondary derivatives; and (c) optimization of spectral components' parameters and minimization of divergence. The spectral components were attributed to four forms of coelenteramide – unionized and three ionized forms. The bioluminescence spectra were found to include all the four forms of coelenteramide, but the photoluminescence spectra included only two: the Ca<sup>2+</sup>-discharged obelin produces fluorescence of two ionized forms, and the discharged aequorin both unionized and ionized forms. The differences in bioluminescence and photoluminescence spectra of the discharged photoproteins are explained by the different protonic environments of coelenteramide in these proteins.

### *Metridia* luciferase and *Renilla* Ca<sup>2+</sup>-dependent coelenterazine-binding protein as reporters for *in vitro* assay

V. V. Borisova, L. A. Frank, S. I. Korneeva and E. S. Vysotski  
Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia.  
E-mail: vasilisa.borisova@yahoo.com

*Metridia longa* is a marine luminous copepod whose bioluminescence is conditioned by secreted coelenterazine-dependent luciferases. The recombinant *Metridia* luciferase expressed in *E. coli* cells as inclusion bodies was purified and refolded. Luciferase bioluminescence can be initiated by both

free coelenterazine and the recombinant Ca<sup>2+</sup>-dependent coelenterazine-binding protein (CBP) of *Renilla*. Despite the fact that CBP belongs to the *Renilla* bioluminescence system, it is an effective substrate of *Metridia* luciferase, producing more light than free coelenterazine. The background with CBP is significantly lower as compared to free coelenterazine. The luciferase can be detected down to theamol linear range, extending over five orders of magnitude. At biotinylation, luciferase preserved 40% of its initial activity. Tandem bioluminescent solid-phase microassay based on Ca<sup>2+</sup>-regulated photoprotein obelin, *Metridia* luciferase and CBP was developed. The light signals were measured sequentially or simultaneously, for these proteins have different bioluminescence kinetics.

### Bioluminescence imaging of bacteria–host interplay: interaction of *E. coli* with epithelial cells

L. Y. Brovko,<sup>1</sup> H. Wang,<sup>1</sup> J. Elliott,<sup>1</sup> R. Dadarwal,<sup>1</sup> O. Minikh<sup>1,2</sup> and M. W. Griffiths<sup>1</sup>

<sup>1</sup>Canadian Research Institute for Food Safety, University of Guelph, Guelph, ON N1G 2W1, Canada.

E-mail: lbrovko@uoguelph.ca

<sup>2</sup>Department of Chemical Enzymology, Lomonosov Moscow State University, Moscow 117899, Russia

Bioluminescence methods provide a unique opportunity to monitor cell trafficking and intracellular metabolic changes non-invasively and in real-time format. We employed bioluminescence techniques to investigate the interaction of multiple *Escherichia coli* strains with HeLa epithelial cells to provide new insights into bacterial pathogenicity. Fifteen auto-bioluminescent strains were constructed, using both non-pathogenic and pathogenic *E. coli* of different serotypes. Attachment of bacteria to a monolayer of epithelial cells was investigated by monitoring the time-course of bioluminescence during cell attachment and further colonization. A significant difference was observed in lag periods for bioluminescence for both pathogenic and non-pathogenic *E. coli*; indicating a difference in bacterial attachment between strains. However, there was no significant difference in growth rate between pathogenic and non-pathogenic *E. coli*; suggesting that major differences in the behaviour of pathogenic and non-pathogenic *E. coli* with epithelial cells occurred during the early stages of interaction. It is known that host cells play an active role in pathogenicity events. To assess metabolic changes in epithelial cells during pathogen attachment and colonization, HeLa cells carrying genes for the Ca<sup>2+</sup>-dependent photoprotein obelin and ATP-dependent firefly luciferase were used. Time-courses of both Ca<sup>2+</sup>- and ATP-related bioluminescence were recorded. The obtained data were used to assess the possibility of developing a cell-based biosensor for pathogenicity.

### Testing of crucial amino acids for light inactivation of Ca<sup>2+</sup>-regulated photoprotein berovin from the ctenophore *Beroë abyssicola*, using site-directed mutagenesis

L. P. Burakova, S. V. Markova, G. A. Stepanyuk and E. S. Vysotski  
Photobiology Laboratory, Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia. E-mail: burakoval@mail.ru

The Ca<sup>2+</sup>-regulated photoprotein berovin from the ctenophore *Beroë abyssicola* displays blue-green bioluminescence ( $\lambda_{\text{max}}$  =

491 nm). In contrast to photoproteins such as obelin, aequorin or others, berovin is inactivated by light. At present the reasons for photoinactivation of the ctenophore photoproteins are unknown. The substitution of amino acids, which reside in a substrate-binding pocket of berovin and might take part in coelenterazine coordination, or amino acids, which absorb UV light, might influence light-dependent inactivation of this photoprotein. Here we report some properties and sensitivity to light of berovin mutants, with replacement of some tryptophans, tyrosines and other amino acid residues (W61F, W103F, W103Y and W192F; Y133F, Y133H, Y204F, Y204E and Y204K; L38H, R41M, K90M, K90E, N107W, M153Y, M154Y, M154Q, H186Y, H186D, F188H and R189H). Supported by Grant 06-04-89502 of the Russian Foundation for Basic Research and Taiwan National Science Council.

### G-rich sequence-functionalized polystyrene microsphere-based instantaneous derivatization for the chemiluminescence-amplified detection of DNA

Z. Cao,<sup>1</sup> C. Lau,<sup>1</sup> M. Kai<sup>2</sup> and J. Lu<sup>1</sup>

<sup>1</sup>School of Pharmacy, Fudan University, Shanghai 200032, People's Republic of China. E-mail: jzlu@shmu.edu.cn

<sup>2</sup>Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852-8521, Japan

We describe the development of a sensitive chemiluminescence (CL) approach for the determination of sequence-specific DNA, taking advantage of magnetic beads as preconcentration carriers and polystyrene microspheres as an amplification platform. Briefly, a 'sandwich-type' detection strategy is employed in our design, which involves capture probe DNA immobilized on the surface of carboxyl-terminated magnetic beads and multiple biotinylated reporter DNA self-assembled on the surface of streptavidin-modified polystyrene microspheres. The reporter DNA includes a guanine nucleobase-rich sequence domain for the generation of light and an additional tethered nucleic acid domain complementary to the target DNA. The CL signal is obtained via a novel instantaneous derivatization reaction between a specific CL reagent and guanine nucleobases rich in the target and reporter DNA. As a result, we demonstrate that this DNA assay is reproducible, stable, easy to use, and can sensitively detect femtomolar target DNA with excellent differentiation ability for single-base mismatched sequences. The study presented here validates the design and concept of sequence-specific DNA detection, and hence offers great promise for DNA analysis in the diagnosis and treatment of genetic diseases, the detection of infectious agents and forensic analysis, etc.

### A novel gaseous acetone sensor utilizing cataluminescence on nanosized ZrO<sub>2</sub>/Er<sub>2</sub>O<sub>3</sub>

X. Cao, W. Wu, Z. Wen, Z. Chen, Y. Huang, C. Huang, J. Wu, Y. Chen and C. Wu

Environmental Science and Engineering Institute, Guangzhou University, 510006 Guangzhou, People's Republic of China. E-mail: caoxiaoan2003@yahoo.com.cn

The rapid determination of acetone vapour is useful either in environmental monitoring or in the clinical diagnosis of

diabetes. In previous reports, the sensors for detecting acetone vapour were not sufficiently selective, especially for ethanol, which does coexist with acetone in some environments and often produces a similar signal to acetone. In the present study, a new cataluminescence-based gaseous sensor for acetone was developed, using nanosized ZrO<sub>2</sub> doped with Er<sub>2</sub>O<sub>3</sub> as catalyst. The result showed that ZrO<sub>2</sub> doping Er<sub>2</sub>O<sub>3</sub> improves selectivity significantly at an optimal temperature of 247°C, a wavelength of 460 nm and a flow rate of 280 mL/min. The common coexistence of other gases, such as ethanol, chloroform, formaldehyde and benzene, did not interfere with the determination. The linear range of CTL intensity vs. concentrations of acetone vapour was 1.5–2000 ppm, with detection limits (3σ) of 0.4 ppm. The authors gratefully acknowledge financial support by the National Natural Science Foundation of China (20677013) and the Natural Science Foundation of Guangdong Province, China (5001879).

### Quenching effect of malathion on chemiluminescence for the luminol–hydrogen peroxide–haemin system and the fluorescence of fluorescein

M. J. Chaichi,<sup>1</sup> M. R. Kasaai,<sup>2</sup> S. Mohammadkhani<sup>1</sup> and K. Kamel<sup>1</sup>

<sup>1</sup>Department of Chemistry, Mazandaran University, Babolsar, Mazandaran, Iran. E-mail: jchaichi@yahoo.com

<sup>2</sup>Faculty of Agriculture, Mazandaran University, Khazar Abad Road km 9, PO Box 578, Sari, Mazandaran, Iran

The influence of malathion on the luminol–hydrogen peroxide system in alkaline medium, catalysed by haemin using a chemiluminescence method, was investigated. The effect of malathion on fluorescein using a fluorescence method was also studied. Several variables on chemiluminescence response were examined for the determination of optimum conditions, using a central composite design (experimental design) method. The influence of malathion was evaluated through the calculation of fluorescence quantum yield ( $\Phi_f$ ), irradiative rate constant ( $k_f$ ), non-irradiative rate constant ( $k_n$ ) and fluorescence life time ( $\tau_f$ ), with and without its presence. The Stern–Volmer equation was employed to calculate quenching rate constants for the chemiluminescence and fluorescence processes. The results of this study reveal that malathion has a quenching effect on the systems studied. The Stern–Volmer plots were linear for both chemiluminescence and fluorescence processes over the malathion concentration ranges of  $1 \times 10^{-5}$ – $3 \times 10^{-2}$  mol/L and  $1 \times 10^{-5}$ – $1 \times 10^{-3}$  mol/L, respectively.

### Conjugate of (acridinium)<sub>x</sub>–BSA–anti-HCV core to enhance the detection of HCV core antigen

C. Chang, Y. Cheng, L. Jiang, V. Salbilla and D. Shah

Abbott Laboratories, 100 Abbott Park Road, D09GN, AP20, Abbott Park, IL 60064-6016, USA

The detection of HCV core antigen (Ag) in the pre-seroconversion period has been shown to achieve early detection of patients with HCV infections. We investigated conjugates using BSA carrying multiple copies of acridinium (Acr) labels to anti-HCV core antibody for the detection of HCV core antigen. The HCV core assay was a sandwich assay run on

an automated chemiluminescence analyser, the Abbott PRISM®, in which microparticles coated with monoclonal antibodies (MAbs) were utilized to capture the Ag and conjugate of acridinium-labelled monoclonal anti-HCV core to give chemiluminescence for detection. The sensitivity of the assay was evaluated by testing samples from the pre-seroconversion period, i.e. HCV RNA-positive but anti-HCV-negative. The (Acr)<sub>x</sub>-BSA-anti-HCV core showed four- to six-fold signal enhancement compared with the control conjugate of the anti-HCV core IgG directly labelled with Acr. The former conjugate would be useful in a chemiluminescent immunoassay to achieve earlier detection of HCV infections.

### Dye-sensitized silica-coated lanthanide complex nanoparticles for bioassays

Y. Chen

State Key Laboratory of Electronics, Department of Bioscience and Medical Engineering, South-East University, Nanjing 210096, People's Republic of China. E-mail: yc@seu.edu.cn

The preparation and detection of highly luminescent aqueous lanthanide nanoparticle labels for time-resolved fluorometric assays has been demonstrated. The luminescent complexes included in the nanoparticle were composed of a dye sensitizer and a polyaminocarboxylate-based chelator with excellent water-solubility and a high binding constant for lanthanides. The luminescence of lanthanide is greatly enhanced by the sensitization of the dye. The structural design of two functional entities in a single molecule caused the lanthanide complex to have both strong luminescence and good aqueous solubility. SiO<sub>2</sub> nanoparticles containing lanthanide complexes were prepared by the reverse microemulsion method. These nanoparticles were conjugated with biomolecule probes by a surface chemical reaction and used in the DNA time-resolved fluorometric assay. This luminescent nanoparticle label, based on dye sensitization, can be applied in ultrasensitive time-resolved fluorometric assays and imaging.

### Delayed fluorescence and LSCM study on the Cd<sup>2+</sup>-induced progressive senescence of *Arabidopsis*

W.-L. Chen,<sup>1,2</sup> Y. H. Bi,<sup>1,2</sup> Z.-Q. Chen,<sup>1,2</sup> Q. Zhou<sup>3</sup> and D. Xing<sup>2</sup>

<sup>1</sup>College of Life Science, Guangdong Key Laboratory of Biotechnology for Plant Development, South China Normal University, People's Republic of China. E-mail: chenwl@scnu.edu.cn

<sup>2</sup>MOE Key Laboratory of Laser Life Science and Institute of Lasers, South China Normal University, Guangzhou 510631, People's Republic of China

<sup>3</sup>Medical Imaging Centre, First Affiliated Hospital of Jinan University, Guangzhou 510632, People's Republic of China

Delayed fluorescence, hydrogen peroxide generation and morphological change of mitochondria were investigated during the cadmium ion (Cd<sup>2+</sup>)-induced progressive senescence of *Arabidopsis*. Protoplasts of *Arabidopsis* were picked up and their fluorescence images were observed using a laser scanning confocal microscope (LSCM). After incubation of the protoplasts with different concentrations of Cd<sup>2+</sup>, intracellular hydrogen

peroxide (H<sub>2</sub>O<sub>2</sub>) production and distribution, autofluorescence of the chloroplasts and fluorescence of the MitoTracker-stained mitochondria were visualized under LSCM. The mitochondria were evenly distributed around the chloroplasts. After Cd<sup>2+</sup> treatment, the protoplasts showed a concentration-dependent decrease of intracellular H<sub>2</sub>O<sub>2</sub> in comparison with the control cells. Fluorescence images of H<sub>2</sub>O<sub>2</sub> and mitochondria showed that the mitochondria irregularly clumped or clustered surrounding the chloroplasts, or aggregated in other places within the cytoplasm. These results indicate that H<sub>2</sub>O<sub>2</sub> is produced early and is mainly derived from chloroplasts and mitochondria. Cd<sup>2+</sup>-induced H<sub>2</sub>O<sub>2</sub> may act as signalling molecules in Cd<sup>2+</sup>-induced progressive senescence of *Arabidopsis*.

### New techniques for electrochemiluminescence

G. Chen, Y. Chi, Z. Lin, Y. Chen and H. Dai

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and Department of Chemistry, Fuzhou University, Fuzhou 350002, People's Republic of China. E-mail: gnchen@fzu.edu.cn

Electrochemiluminescence (ECL) usually occurs at the surface of electrode or in the solution adjacent to the electrode, therefore adjusting the position of the electrode or changing the material of the electrode can always optimize the intensity and characteristics of ECL. However, no attention has been paid to the effect on ECL of the temperature of the electrode surface. In fact, the ECL occurring at the surface of electrode is related not only to the diffusion of the luminescent compound at the electrode, but also to the convection of luminescent compounds near the electrode surface, whereas convection is greatly affected by temperature. ECL investigations at elevated temperatures can be performed on directly headed electrodes while leaving the bulk solution temperature unchanged. This approach is particularly advantageous for studying the ECL behaviour of volatile substances and dissolved gases at increased temperature under standard pressure. The solubility of these substances in the bulk solution will not be affected by an increase in temperature at the interface. Moreover, there are many electrochemical reactions which are inert at room temperature and take place at appreciable rates if the temperature is raised. Therefore, investigation of the effect of temperature at the electrode surface is very significant in improving the sensitivity, selectivity, stability and reproducibility of ECL. The main problem in investigating the effect of temperature on the ECL reaction at the electrode surface is to design and establish an electrode system; using this electrode system, when the temperature of the surface of electrode is increased, the bulk solution remains unaffected by the increasing electrode temperature. A heating-controlled micro-electrode is probably a useful tool to solve this problem. In this paper, new ECL detection system using an electric heat-controlled microelectrode as the working electrode was established, and it has been used for investigation of the behaviour of some ECL systems. This project was financially supported by the National Nature Sciences Funding of China (20735002, 20575011), the Science and Technology Development Funding of Fuzhou University (826249) and the science and technology project of education department of Fujian province (JB07168).



**Study of the binding mechanism of *meso*-tetra-(3,5-dibromo-4-hydroxyphenyl) porphyrin with protein by a fluorescence method**

X. Chen, Y.-Y. Han, N. Zhang, H.-M. Ma, J. Wang and Q. Wei  
School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, People's Republic of China. E-mail: sdjndxwq@263.net

The interaction mechanism between *meso*-tetra-(3,5-dibromo-4-hydroxyphenyl) porphyrin [T(DBHP)P] and bovine serum albumin (BSA) was investigated using a fluorescence method. Based on the mechanism of fluorescence quenching of BSA by T(DBHP)P, the binding constants between T(DBHP)P and BSA were measured at different temperatures. T(DBHP)P and BSA were shown to have strong interactions. The binding constants of the reactions at 27°C and 48°C decreased with increasing temperature. The type of quenching between T(DBHP)P and BSA was determined as static quenching. By the theory of Förster non-radiation energy transfer, the binding distance and the energy transfer efficiency at 27°C between T(DBHP)P (acceptor of energy) and BSA (donor of energy) were obtained. The binding distance was <7 nm, therefore the interaction was similar to non-radiation energy transfer and the static quenching was further proved. According to the thermodynamic parameters, the main sorts of binding force between T(DBHP)P and BSA could be judged as electrostatic force when  $\Delta G < 0$ ,  $\Delta H < 0$ ,  $\Delta S > 0$ . Using the synchronous fluorescence spectra, the effect of T(DBHP)P on the conformation of BSA was studied. The results indicated that the hydrophobic properties of the environment of residues in BSA decreased.

**A new electrochemiluminescent detection system equipped with an electrically heated indium–tin oxide electrode**

Y. T. Chen,<sup>1,2</sup> Y. Y. Jiang,<sup>1</sup> Z. Y. Lin,<sup>1</sup> J. J. Sun,<sup>1</sup> L. Zhang<sup>1</sup> and G. N. Chen<sup>1</sup>

<sup>1</sup>Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350002, People's Republic of China

<sup>2</sup>Department of Chemistry and Chemical Engineering, Minjiang University, Fuzhou, Fujian 350108, People's Republic of China. E-mail: gnchen@fzu.edu.cn

An electrochemiluminescence (ECL) detection system equipped with an electrically heated indium–tin oxide (ITO) electrode, coupling the advantages of a heated electrode with the optical transparency property of ITO glass, was developed and introduced. The novel heated ITO electrode (HITOE) is integrated into the ECL cell, which could save the expensive luminescence reagent. The temperature of the electrode could be accurately controlled by electrical heating.  $\text{H}_2\text{O}_2/\text{MCLA}$  and  $\text{TPrA}/\text{Ru}(\text{bpy})_3^{2+}$  at different temperatures of the electrode ( $T_e$ ) were performed for evaluating the feasibility and reliability of this detection system. The results revealed several advantages. First, higher sensitivity and smaller RSD could be obtained for ECL by moderately heating the electrode; second, the optical transparent of HITOE, which made the design and operation of the instrument easier; third, the volume of this ECL cell equipped with HITOE was small, which was propitious for saving

the expensive luminescence reagent. The preliminary results indicate that the present ECL detection system, equipped with a heated ITO electrode, is a promising approach with strong potential applications, due to its high sensitivity, excellent reproducibility and less consumption for ECL determination.

**Spin-labelled quantum dots as a novel probe for antioxidants**

W. B. Chen, X. J. Tu, D. J. Pei, Y. Zhao and X. Q. Guo  
Department of Chemistry, MOE Key Laboratory of Analytical Science and Key Laboratory for Chemical Biology of Fujian Province, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, People's Republic of China. E-mail: xqguo@xmu.edu.cn

As an excellent complement to traditional organic fluorophores, quantum dots (QDs) have received intense study in biological systems because of their unique optical properties. In this paper we report a rapid and facile ligand exchange method to engineer QDs with a designed ligand which had a dithiol moiety and a paramagnetic nitroxide radical tail. The results showed that the intrinsic fluorescence of the QDs could be effectively quenched through the electron transfer between QDs and the nitroxide radical. Interestingly, when the paramagnetic properties of the nitroxide moiety were eliminated, the quenched fluorescence of QDs could be readily restored. This mechanism made the spin-labelled QDs a sensitive sensor for biological antioxidant.

**Interaction of  $\text{Tb}^{3+}$ –protocatechuic acid complex with nucleic acids and its use in the determination of nucleic acids based on fluorescence quenching**

Y. Chen,<sup>1,2</sup> Y. Yang<sup>1</sup> and J. Yang<sup>2</sup>

<sup>1</sup>School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, People's Republic of China. E-mail: chm\_chenyj@ujn.edu.cn

<sup>2</sup>School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, People's Republic of China

A study on the interaction of  $\text{Tb}^{3+}$ –protocatechuic acid (PCA) complex with nucleic acid was carried out. Binding of  $\text{Tb}^{3+}$  with PCA leads to a marked enhancement in the fluorescence emission intensity of  $\text{Tb}^{3+}$ , but with the addition of nucleic acids, the fluorescence emission intensity of the  $\text{Tb}^{3+}$ –PCA complex can be greatly quenched. Based on this, a new fluorescence method for the determination of nucleic acids was established. Under optimum conditions, the quenched fluorescence intensity was proportional to the concentration of nucleic acid and the detection limits were 23 ng/mL for ctDNA and 9.9 ng/mL for yRNA. The interaction mechanism was studied using techniques including absorption, light scattering, circular dichroism and viscosity determination. It was considered that the major force between the probe and nucleic acid is electrostatic binding. The competition of the oxygen atom of phosphate in nucleic acid framework with PCA for the  $\text{Tb}^{3+}$  induces the decrease of fluorescence intensity. The fact that the association constant of RNA with  $\text{Tb}^{3+}$ –PCA is much larger than that of DNA also confirms the conclusion. The interaction between  $\text{Tb}^{3+}$ –PCA and DNA causes the double helix

of DNA to partly undo, the viscosity of this system to decrease and the configuration of DNA to change.

### Study on optimal conditions for USPIO and SPIO labelling of SPC-A-1 and HUVEC cells using LSCM

W.-L. Chen,<sup>1,2</sup> M.-X. Yu,<sup>1,2</sup> Q. Zhou<sup>3</sup> and D. Xing<sup>2</sup>

<sup>1</sup>College of Life Science, Guangdong Key Laboratory of Biotechnology for Plant Development, South China Normal University, People's Republic of China.  
E-mail: chenwl@scnu.edu.cn

<sup>2</sup>MOE Key Laboratory of Laser Life Science and Institute of Laser, South China Normal University, Guangzhou 510631, People's Republic of China

<sup>3</sup>Medical Imaging Centre, First Affiliated Hospital of Jinan University, Guangzhou 510632, People's Republic of China

A study was made of the optimal concentration and time of incubation of human lung adenocarcinoma cells (SPC-A-1) and human umbilical artery endothelial cells (HUVEC) labelled with superparamagnetic iron oxide (SPIO) and ultrasmall superparamagnetic iron oxide (USPIO) particles *in vitro*. SPC-A-1 and HUVEC cells were cultured with different concentrations of SPIO and USPIO, respectively, for different incubation times. Iron phagocytoses of the cells were observed by laser scanning confocal microscopy (LSCM) to determine particle uptake and their distribution in the cells. SPC-A-1 cells took up a large amount of iron particles within the first 3 h, while HUVEC cells took up a large amount of iron particles after 5 h. In this study, the concentration of iron with 25 µg/mL SPIO and time of incubation 30 min is the optimal condition for labelling the SPC-A-1 with SPIO; the concentration of iron with 100 µg/mL SPIO and time of incubation 15 min is the optimal condition for labelling the HUVEC cells with USPIO.

### Distribution of fluorescent dissolved organic matter in the South China Sea

J. N. Chen and Y. Zhang

State Key Laboratory of Marine Environmental Science (Xiamen University), Environmental Science Research Centre, Xiamen University, Xiamen 361005, People's Republic of China.  
E-mail: jnchen@xmu.edu.cn

Fluorescent dissolved organic matter (FDOM), including protein-like and humic-like substances, is playing an important role in biological and chemical processes in the natural water column. The vertical distribution of FDOM was determined, both on board and in the laboratory, during two navigations of the South China Sea. The excitation–emission matrix (EEM) technique had been used for identifying different sources of fluorescent signals. At every station, the fluorescence of humic-like (350/450 nm) substances was lowest near the sea surface, due to photodegradation; a great increase occurred in the upper 100 m layer, with a maximum around 100 m and then a slight decreased. The signal increased again slightly and reached its highest value at about 1000 m, then was almost constant. In contrast to humic-like substances, the fluorescence of protein-like substances (220/340 nm) was highest near the

sea surface and showed a minimum below 1000 m depth. The relationship with temperature, salinity, oxygen and nutrients was discussed.

### Bioluminescence of sharks, a case study: *Etmopterus spinax*

J. M. Claes and J. Mallefet

Laboratory of Marine Biology, Catholic University of Louvain, 3 Place Croix du Sud, Kellner Building, B-1348 Louvain-la-Neuve, Belgium.  
E-mail: julien.m.claes@uclouvain.be

Bioluminescence arose independently in a wide range of species, from bacteria to fishes, which are the only luminous vertebrates. Consequently, luminescent species demonstrate a great diversity in the structure, control and function of their photogenic systems. Among luminous organisms, sharks are probably the least investigated group and extremely scanty information is available concerning their bioluminescence, even though more than 50 shark species, i.e. about 13% of current species, are endowed with the ability to emit light. In this paper we present a synthesis of shark luminescence as well as the first results of a pluri-disciplinary approach of the velvet belly lanternshark, *Etmopterus spinax*. Light and epifluorescent microscopy, luminometry and digital imaging analysis were used to follow the development of luminous structures during the ontogeny of this species, from embryos to old individuals. The potential functions of bioluminescence were discussed, based on the light production of the shark and theoretical optical models. In addition, we provide the first pharmacological analysis of luminescence control in this animal.

### Metal nanoparticle-initiated chemiluminescence and electrochemiluminescence

H. Cui, C.-F. Duan, W. Wang, N. Li and H. Zhang

Department of Chemistry, University of Science and Technology of China (USTC), Hefei, Anhui 230026, People's Republic of China. E-mail: hcui@ustc.edu.cn

In recent years, nanoparticles have been widely studied for their excellent properties, and their potential applications in microelectronics, in optical, electronic and magnetic devices, and as catalysts. Light emission accompanied by a chemical reaction, known as chemiluminescence (CL), has been intensively investigated for many years. Recently, the CL study has been extended to nanoparticle systems from traditional molecular systems. We found that metal nanoparticles could initiate CL reactions as reductants, catalysts and luminophors. We report the current progress in our research group. For liquid-phase CL, some novel metal nanoparticle-involved CL reactions were observed, including luminol–H<sub>2</sub>O<sub>2</sub>–metal nanoparticle, gold nanoparticle–KMnO<sub>4</sub>, gold nanoparticle–KIO<sub>4</sub>–NaOH–Na<sub>2</sub>CO<sub>3</sub>, bis-(2,4,6-trichlorophenyl) oxalate–H<sub>2</sub>O<sub>2</sub>–gold nanoparticle, lucigenin–KI–noble metal nanoparticle. For electrochemiluminescence (ECL), noble metal nanoparticles such as Au, Ag, Pt modified on an electrode or dispersed in a solution were found to enhance the ECL of the luminol and lucigenin systems and/or initiate new ECL peaks of these systems. Furthermore, some CL and ECL systems involving metal nanoparticles were used for

the determination of analytes, such as compounds containing –SH and –NH<sub>2</sub>.

### **A high-performance glucose ECL biosensor based on immobilization of glucose oxidase on polyelectrolyte–chitosan–modified glassy carbon electrode**

H. Dai, Y. Wang, X. Wu, G. Chen and H. Chen

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and Department of Chemistry, Fuzhou University, Fuzhou 350002, People's Republic of China. E-mail: gnchen@fzu.edu.cn

There is a great demand for determination of the glucose concentration in several fields, including food, microorganism and medicine, particularly for the control and treatment of diabetes. In this paper, a high-performance ECL glucose biosensor was developed by cross-linking glucose oxidase (GOD) on a sensitive membrane modified glassy carbon electrode, which was prepared by doping poly(diallyldimethylammonium chloride) (PDDA) with chitosan (Chit). This biosensor provided good biocompatibility and a stabilizing microenvironment for the enzyme. In order to obtain optimal performance of the ECL biosensor, the composition of modified membrane and a series of measurement conditions were investigated. Under optimal conditions, this ECL glucose biosensor was able to detect glucose in the luminol system in the range  $5 \times 10^{-10}$ – $4.0 \times 10^{-5}$  mol/L, with a detection limit (s:n = 3) of 0.1 nmol/L. This ECL biosensor exhibited the effectively improved stability of the electron transfer mediator. It showed excellent properties for ultrasensitive determination for glucose, good reproducibility and stability. It has also been used to determine glucose concentrations in real serum samples, with satisfactory results. Thus, this immobilization approach is promising for the construction of analogous biosensors and bioelectronic devices.

### **Electrochemiluminescence determination of metamphetamine hydrochloride with multiwall carbon nanotube–ionic liquid paste electrodes**

H. Dai, Y. Wang, X. Wu, G. Chen, Y. Chen and L. Xu

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and Department of Chemistry, Fuzhou University, Fuzhou 350002, People's Republic of China. E-mail: gnchen@fzu.edu.cn

There is a steady trend to use novel materials in electrochemical sensing systems, with their success largely due to the continuous design and development that meets the needs of modern electrochemical biosensor technology. In our research, room temperature ionic liquids (RTILs) have been proposed to be very interesting and efficient pasting binders in place of non-conductive organic binders for the preparation of multiwall carbon nanotube (MWCNT) ionic liquid [1-butyl-3-methylimidazolium hexafluorophosphate (BMIM·FP<sub>6</sub>)] paste electrodes. We used this paste electrode to immobilize Ru(bpy)<sub>3</sub>Cl<sub>2</sub>. This ECL sensor showed excellent electrochemiluminescent characteristics. With the Ru(bpy)<sub>3</sub><sup>2+</sup>–MWCNT–BMIM·FP<sub>6</sub> paste ECL sensor, we found that metamphetamine hydrochloride (MA·HCl) would enhance its electrochemiluminescence. On this basis, enhanced

electrochemiluminescence detection has been developed for the determination of metamphetamine hydrochloride. The method exhibited good reproducibility, wide-range linearity and high sensitivity and stability, with a detection limit (signal:noise ratio = 3) of  $8.0 \times 10^{-9}$  mol/L and a dynamic concentration range of  $1.0 \times 10^{-8}$ – $8.0 \times 10^{-5}$  mol/L. The relative standard deviation was 3.1% for  $1 \times 10^{-5}$  mol/L metamphetamine hydrochloride (*n* = 10).

### **Fluorescence enhancement of KI for the morin–fsDNA system and its analytical applications**

H. Ding, X. Wu, J. Yang and F. Wang

Key Laboratory of Colloid and Interface Chemistry (Shandong University), Ministry of Education School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, Shandong, People's Republic of China. E-mail: wux@sdu.edu.cn; yjh@sdu.edu.cn

Potassium iodide (KI) is commonly used as a fluorescent quenching agent because of the heavy atom effect. Therefore, it has wide applications in study of the interaction mechanism between fluorescence probes and biomacromolecules. It was found that KI at lower concentrations ( $<1.0 \times 10^{-3}$  mol/L) could enhance the fluorescence intensity of the morin–fsDNA system. Based on this phenomenon, a new and selective method for the determination of fsDNA was developed, and its detection limit was  $10^{-10}$  g/mL. The interaction mechanism of the morin–fsDNA–KI system was studied by absorption, circular dichroism, fluorescence polarization and lifetime, etc. The results indicated that the interactive mode between morin and DNA was mainly groove binding in the presence of KI. Meanwhile, the role of KI in enhancing the fluorescence intensity of the morin–fsDNA system was also considered. The enhancement of KI was attributed to the formation of the more favourable structure for the luminescence, whereas the heavy atom effect of halogen anion within a certain range of concentration was not dominant in the KI–morin–fsDNA system.

### **Ultrasensitive chemiluminescent immunochemical localization of protein components in painting cross-sections**

L. S. Dolci,<sup>1</sup> G. Sciuotto,<sup>2</sup> M. Rizzoli,<sup>1</sup> M. Guardigli,<sup>1</sup> R. Mazzeo,<sup>2</sup> S. Prati<sup>2</sup> and A. Roda<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, University of Bologna, Bologna 40126, Italy

<sup>2</sup>Microchemistry and Microscopy Art Diagnostic Laboratory, University of Bologna, Ravenna 48100, Italy

The characterization of the complex multilayer and multimaterial structure of a painting is fundamental for authentication purposes and for the study of painting techniques. Here we describe an ultrasensitive immunochemical procedure for the detection of ovalbumin (chicken egg white albumin, employed as binding medium or varnish) in cross-sections of tempera paintings. The combination of chemiluminescence (CL) imaging detection with optical microscopy allowed the target protein to be localized with good spatial resolution (i.e. within single painting layers). Furthermore, differently from fluorescence techniques (which are affected by the autofluorescence of

pigments and organic binding media), no interference from other painting components was observed. The method was first applied to standard samples containing different common pigments, then used for the localization of ovalbumin in a Renaissance period wood painting. In perspective, a detailed characterization of organic components (drying oils, resins, etc.) in a painting cross-section could be obtained, either by performing sequential immunoenzymatic assays or employing a mixture of antibodies, each of them labelled with a different CL enzyme.

### Chemiluminescence determination of rutin using a micelle-sensitizing N-bromosuccinimide-H<sub>2</sub>O<sub>2</sub> reaction

J. Du,<sup>1</sup> L. Hao<sup>1,2</sup> and J. Lu<sup>1</sup>

<sup>1</sup>Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China. E-mail: jxdu@snnu.edu.cn

<sup>2</sup>Department of Chemistry, Shaanxi University of Technology, Hanzhong 723001, People's Republic of China

It is found that weak chemiluminescence (CL) can be generated from the reaction of rutin with N-bromosuccinimide and hydrogen peroxide in alkaline condition. The CL signal is enhanced significantly in the presence of cetyltrimethylammonium bromide surfactant micelles. This system was used to develop a simple FI-CL method for the determination of rutin. Under the optimum experimental conditions, the CL signal is proportional to the concentration of rutin in the range  $1.0 \times 10^{-7}$ – $1.0 \times 10^{-5}$  g/mL. The detection limit is  $7 \times 10^{-8}$  g/mL rutin and the sample throughput is 120 injections/h. The relative standard deviation for  $1.0 \times 10^{-6}$  g/mL rutin solution is 2.2% in 11 replicate measurements. The proposed method was applied to the determination of rutin in four Chinese medicinal herbs, with recoveries in the range 91.7–108.1%. The possible CL reaction mechanism was also discussed by examining the CL spectra, UV visible spectra and fluorescence spectra.

### Determination of bisphenol A by using fluorescence immunoassay

L. Du, C. Wang, J. Qian and S. Wang

Institute of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, People's Republic of China. E-mail: shuhaowang@sohu.com

Bisphenol (BPA), a chemical intermediate in the synthesis of polycarbonate and epoxy resins, unsaturated polyester-styrene resins and flame-retardants, is often a contaminant in polluted waters. It is well known as a strong oestrogenic endocrine-disrupting substance. Recently many chemical methods have been developed for detecting bisphenols, in which chromatographic methods were mostly used. Compared with chemical methods, immunoassay is specific, fast and sensitive and is appropriate for monitoring biological samples. A competitive fluorescence immunoassay was developed for detecting BPA in water samples. 4,4-Bis(4-hydroxyphenyl)-valeric acid (BHPVA) was used, and the antigen was obtained by coupling BSA to BHPVA through the carboxyl group. The antibody was

developed from immune rabbits. In the competitive immunoassay, FITC-labelled goat anti-rabbit antibody was applied. The linear range for the determination of BPA was 4.0–1400 ng/mL and the detection limit was 0.8 ng/mL. Some water samples were analysed, with satisfactory results.

### Polymer-stabilized gold nanocluster-enhanced luminol chemiluminescence in neutral solutions

C.-F. Duan, H. Cui, S.-M. Xia, B. Liu and J. Jiang

Department of Chemistry, University of Science and Technology of China, Hefei, Anhui, 230026, People's Republic of China. E-mail: cfduan@mail.ustc.edu.cn

Luminol chemiluminescence (CL) usually proceeds in basic aqueous solutions. It is difficult to use it for the detection of biologically important compounds occurring at physiological pH. In this study we found that in neutral pH conditions luminol could react with hydrogen peroxide in the presence of small gold nanoclusters (~2.3 nm) stabilized by poly(N-vinyl-2-pyrrolidone) (PVP) to produce strong CL by use of a flow-injection system. The effect of 20 amino acids and two thiol-containing compounds, glutathione (GSH) and homocysteine (Hcy), on the luminol-hydrogen peroxide-gold nanocluster CL system were investigated. It was found that the sulphur-containing compounds, including methionine (Met), cysteine (Cys), GSH and Hcy, could inhibit the CL intensity of this system, while other amino acids had no obvious responses. Based on the inhibited CL, a new flow-injection CL method was developed for the selective detection of four sulphur-containing bioactive compounds. The limits of detection for Met, Cys, Hcy and GSH were 5.7, 2.1, 9.6, 2.6 nmol/L, respectively. The CL method proposed in this study provides a wider linear range and higher sensitivity than other reported spectrometric methods based on gold nanoparticles.

### The kinetics of coelenterazine binding with apo-obelin and apo-aequorin

E. V. Fremeieva,<sup>1,3</sup> S. V. Markova,<sup>1</sup> L. A. Frank,<sup>1</sup> J. Lee,<sup>2</sup>

W. J. H. van Berkel,<sup>3</sup> A. J. W. G. Visser,<sup>3</sup> and E. S. Vysotski<sup>1,2</sup>

<sup>1</sup>Photobiology Laboratory, Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia

<sup>2</sup>Department of Molecular Biology and Biochemistry, University of Georgia, Athens, GA 30602, USA

<sup>3</sup>Laboratory of Biochemistry, Wageningen University, 6703 HA Wageningen, The Netherlands

The Ca<sup>2+</sup>-regulated photoproteins obelin and aequorin are bioluminescent proteins emitting light at calcium binding. The recombinant apo-obelin and apo-aequorin can be converted into active photoproteins by incubation with synthetic coelenterazine in the presence of reducing agents under aerobic conditions. The kinetics of coelenterazine binding for both apo-photoproteins at different protein concentrations and various conditions (pH and temperature) was investigated using various techniques. A mathematical model describing the process of coelenterazine binding is suggested. Using this model, the apparent dissociation constants for coelenterazine for obelin and aequorin were estimated. The low values of the apparent



dissociation constants are in agreement with the observed stability of the photoprotein complexes. Supported by Grant 05-04-48271 of the Russian Foundation for Basic Research and Wageningen University Sandwich PhD-Fellowship programme.

### Enzyme-based bioluminescence biosensors: biochemical design and applications

E. N. Esimbekova<sup>1,2</sup> and V. A. Kratasyuk<sup>1,2</sup>

<sup>1</sup>Institute of Biophysics, Akademgorodok, 660036 Krasnoyarsk, Russia. E-mail: Esimbekova@yandex.ru

<sup>2</sup>Siberian Federal University, Pr. Svobodnyi 79, 660041 Krasnoyarsk, Russia

Cost-effective biosensors for use in environmental control, medicine and safety monitoring have been developed. Co-immobilization of bacterial luciferase, oxidoreductase and their substrates into a starch gel is referred to as a 'multifunctional immobilized biosensor'. Two types of biosensors were presented. First, alarm signal assays using coupled and triple reactions attract attention to an extraordinary situation. Biosensors control the effect of the sum of toxicants on luciferase. The biosensors' characteristics are rapidity, simplicity, high sensitivity and accuracy. Second, specific analysis is conducted to identify the analytes or toxicants with enzymatic bioluminescent reactions. The novel biosensors may be designed by searching for bioluminescent reactions with high sensitivity to the analyte, varying the mechanisms of interaction between enzymes in multienzymic reaction, the substrates' and enzymes' concentrations. They are used for bioluminescent analysis, e.g. toxicity bioassays or analyses of NADH, FMN and aldehydes. This work was supported by Grant N 07-04-01340-a from the Russian Foundation for Basic Research.

### Bioluminescent assay of key metabolites in plants

E. N. Esimbekova<sup>1,2</sup>, N. N. Remmel<sup>3</sup> and V. A. Kratasyuk<sup>1,2</sup>

<sup>1</sup>Institute of Biophysics, Akademgorodok, 660036 Krasnoyarsk, Russia. E-mail: Esimbekova@yandex.ru

<sup>2</sup>Siberian Federal University, Pr. Svobodnyi 79, 660041 Krasnoyarsk, Russia

<sup>3</sup>Applied Biosystems RU, Box 8684, Akademgorodok, 660036 Krasnoyarsk, Russia

Pyridine nucleotides are key redox carriers of all living cells and play crucial roles in oxidant metabolism and non-redox mechanisms, particularly in plant stress responses. A bioluminescent assay for monitoring the key metabolites that are indicators of stress in plants has been developed. The levels of NAD(P)H and NAD(P)<sup>+</sup> in radish root extracts from controls and stress-inducing conditions were compared using bacterial coupled enzymatic systems: NAD(P)H:FMN-oxidoreductase-luciferase and triple enzymatic system NAD(P)H:FMN-oxidoreductase-luciferase-alcohol dehydrogenase. The sensitivity of bioluminescent assay for NAD(P)H and NAD(P)<sup>+</sup> was about 1 nmol and 0.1 nmol, respectively. To induce various environmental stresses (low pressure, humidity and oxygen concentration), plants were grown in closed environmental chambers at the University of Florida. Concentrations of NAD(P)<sup>+</sup> and NAD(P)H in plants vary greatly under stress conditions, e.g. decrease of

pressure from 100 kPa to 30 kPa and 20 kPa varies the ratio NAD(P)<sup>+</sup>:NAD(P)H from 0.1 to four and six times, respectively. Therefore, the NAD(P)<sup>+</sup>/NAD(P)H pair can be used as an universal indicator of the redox state and bioenergetic status of tissue. The developed bioluminescent assay for the quantification of pyridine nucleotides in plant tissues is rapid, low cost and easily performed.

### High-throughput screening and live cell imaging using luciferase biosensors

E. Fan, B. Binkowski, P. Stecha, B. Butler and K. Wood

Research and Development, Promega Corporation, 2800, Woods Hollow Road, Madison, WI 53711, USA. E-mail: frank.fan@promega.com

High-throughput screening is the central paradigm for modern drug discovery. Cell-based assays are becoming the preferred format in screening programmes. In recent years, we have developed many bioluminescent assays for drug discovery applications. These bioluminescent assays are uniquely suited for high-throughput screening, due to their inherent high sensitivity, wide dynamic range and low susceptibility to compound interference. In this presentation, we report on using genetically encoded, circularly permuted forms of firefly luciferases as biosensors to monitor intracellular cAMP dynamics in real time. This technology is also ideal for high-throughput screening application for GPCR modulators, using high-density microtitre plates at and beyond the 1536-well format. In addition, we show live cell imaging of cAMP using a luminescent microscope. These bioluminescent assays enable new applications for cell biology research and drug discovery.

### Using a luciferase-based screening assay to identify traditional Chinese medicine targeting nuclear hormone receptors

F. Fang, Z. Huang, W. Liu and C. Wong

Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou International Business Incubator, Guangzhou Science City 510663, People's Republic of China. E-mail: wong\_chiwei@gibh.ac.cn

Traditional Chinese medicine (TCM) has been used for centuries in China for the treatment of an assortment of ailments. Nuclear hormone receptors (NHRs) are transcription factors that bind to small molecule ligands through their ligand-binding domains. Since NHRs can specifically bind to structurally distinct chemicals, and TCM extracts contain chemicals that are thought to be responsible for their therapeutic actions, we aimed to establish a screening assay to investigate whether selective TCM extracts display agonistic or antagonistic activities on selective NHRs. We identified a number of TCM extracts that contained NHR selective agonist and antagonist activities, using a luciferase-based Gal4DBD-NHR-LBD reporter system. By scaling up the extraction, we were able to separate chemicals with these TCM extracts into distinctive fractions using a HPLC method. Individual fractions were then used in the reporter system and we identified several fractions that contain NHR-modulating activities.

### Chemiluminescence reactions with acidic potassium permanganate: a re-evaluation of the emitting species and new analytical applications

P. S. Francis, J. L. Adcock, T. A. Smith and N. W. Barnett  
School of Life and Environmental Sciences, Deakin University,  
Geelong, Victoria 3217, Australia. E-mail: psf@deakin.edu.au

A broad red band of light is emitted when potassium permanganate reacts with a wide range of compounds in acidic solution. This reagent is particularly responsive to certain phenolic compounds, which we have exploited to develop new methods for the determination of antioxidants, neurotransmitter metabolites, adrenergic amines and other biomolecules of interest. The characteristic red luminescence from these reactions has been attributed to several different emitting species, but supporting evidence has not been conclusive. We have directly compared the laser-induced photoluminescence of manganese(II) chloride with the chemiluminescence from reactions with acidic potassium permanganate to confirm that the characteristic red emission from this widely-used reagent emanates from an electronically excited manganese(II) species. However, in many previous cases where permanganate was used in the presence of fluorescent compounds, e.g. enhancers or reaction products, energy transfer to the efficient fluorophore was proposed. We have also examined many reactions of this type and found that in most cases the same characteristic red emission was produced, but there were several notable exceptions.

### Coelenterazine-dependent bioluminescent proteins as the effective labels in immunoassay

L. A. Frank, V. V. Borisova and S. I. Korneeva  
Photobiology Laboratory, Institute of Biophysics, SB RAS,  
Krasnoyarsk, 660036, Russia. E-mail: lfrank@yandex.ru

Coelenterazine and its derivatives are the most frequently encountered luciferins that have been identified in marine organisms. Their bioluminescent reaction involves enzymatic catalysed coelenterazine oxidation by molecular oxygen. The oxidized product in the exciting state, coelenteramide, exists in several forms, differing energetically. Relaxation into the ground state is accompanied by light emission with a high quantum yield. There are two types of coelenterazine-dependent bioluminescent system:  $O_2$ -dependent (luciferase-based) and  $O_2$ -independent (photoproteins). The availability of chemically synthesized coelenterazine and the recombinant luciferases and apophotoproteins made possible the development of bioluminescent analytical technologies. *Metridia longa* luciferases,  $Ca^{2+}$ -dependent photoproteins and coelenterazine-binding protein from *Renilla muelleri* were successfully used as labels in immunoassay. Supported by RFBR Grant 06-04-08076.

### Bioluminescent assay of antibiotic susceptibility of clinical samples

V. G. Frundzhyan and N. N. Ugarova  
Chemistry Faculty, Lomonosov Moscow State University,  
Moscow 119992, Russia. E-mail: vgf@enz.chem.msu.ru

Rapid assay of antibiotic susceptibility of clinical sample is crucial for successful chemotherapy of infectious diseases and

postsurgical complications. The standard microbiology assay used at present in hospitals comprises isolation of the pathogen from a clinical sample, followed by application of a disc or turbidimetric method. The total duration of the assay exceeds 48 h. To simplify and accelerate the assay, we applied bioluminescent ATP-metry. According to the protocol proposed, the clinical sample to be analysed is diluted 10-fold with nutritive media and aliquots (1 mL) supplemented with antibiotics examined (probes) and free of antibiotic (control) are incubated in a 48-cell plate (5 h, 37°C), followed by determination of ATP concentration. Highly sensitive ATP-reagent based on recombinant *L. mingrelica* luciferase and portable luminometer LUM-1 (photon counter) developed in our laboratory were applied. The comparison of the ATP concentrations measured in probes and control allows selection of the most effective antibiotic for chemotherapy within 5–6 h. Our research was performed in several Moscow hospitals. Good correlation between the proposed bioluminescent assay and the standard disk method was observed.

### The fine structure of the larval light organ in the aquatic firefly *Luciola lei*

X. Fu  
Department of Plant Science and Technology, Huazhong  
Agricultural University, Wuhan 430070, People's Republic of  
China. E-mail: fireflyfxh@mail.hzau.edu.cn

The larval light organ was observed in detail from newly-hatched larvae to the final instar stage. Morphological study revealed that the larval light organ of *L. lei* was covered with an acellular layer which was orbicular, with a diameter of 0.38 mm, and was submerged directly in blood. The larval light organ did not consist of a reflecting layer and a photogenic layer; it contained large empty and transparent structures at its edge and consisted of numerous photocytes which contained many typical photocyte granules. The tertiary tracheae entered the light organ and there were two to four muscle chains crossing the larval light organ. The muscles chains can fix the light organ. The larval light organ of *L. lei* contained numerous tracheae and tracheoles. There were large nerves that connected with the larval light organ. This research suggested that the aquatic firefly larval light organ was primitive and had low efficiency of light emission, due to lack of a reflective layer.

### BART applications in medical and food diagnostics

O. A. Gandelman, G. Kiddle, C. J. McElgunn, M. Rizzoli,  
C. R. Pereira, R. L. Harrall and L. C. Tisi  
Lumora Ltd, Denmark House, Cambridgeshire Business Park,  
Angel Drove, Ely CB7 4ET, UK.  
E-mail: o.gandelman@lumora.co.uk

This report details recent improvements and applications of our bioluminescent assay in real-time. BART is a robust detection system, used successfully to monitor the synthesis of inorganic pyrophosphate (PP<sub>i</sub>) liberated during various isothermal nucleic acid amplification tests (iNAAT). PP<sub>i</sub> released during an iNAAT is rapidly converted into ATP, which is consequently consumed by thermostable firefly luciferase to produce detectable levels of light. BART can be coupled to many iNAATs and is suitable for

quantifying target nucleic acids, at temperatures up to 63°C. We demonstrate BART working in diagnostic tests that utilize loop-mediated isothermal amplification (LAMP) and a technique requiring DNA:RNA chimeric primers (RDC). The tests target a wide range of prokaryotic and eukaryotic matrices: *Chlamydia trachomatis* in urine specimens, classical swine fever virus extracted from blood, pathogenic (*Salmonella enterica* and *Listeria monocytogenes*) and genetic (GM) contamination of food. All targets are rapidly (<60 min) detected and quantified using BART, with high sensitivity and specificity comparable to that of conventional quantitative real-time polymerase chain reaction (qPCR). Unlike qPCR, iNAATs and BART only require simple analytical platforms. We have prototyped bespoke hardware designed to suit the needs of our high-throughput and point-of-care customer. Together, these technologies provide an attractive alternative to existing molecular *in vitro* diagnostic tests.

### **BART: smart biochemistry, bright bioluminescence, low-cost hardware**

O. A. Gandelman, G. Kiddle, M. Rizzoli and L. C. Tisi  
Lumora Ltd, Denmark House, Cambridgeshire Business  
Park, Angel Drove, Ely CB7 4ET, UK.  
E-mail: o.gandelman@lumora.co.uk

Isothermal nucleic acid amplification technologies (iNAATs) are increasingly being applied to molecular diagnostics. We developed the bioluminescent assay in real time (BART) technology to act as a real-time reporter system for iNAATs. BART utilizes the firefly luciferase-dependent detection of inorganic pyrophosphate, which is produced in large quantities when 'target' sequences are detected using an iNAAT. As such, molecular diagnostics can be achieved with BART simply by measuring the light emitted from closed tubes in a homogeneous phase assay. BART is proven to work with several different iNAATs operating at 50–63°C. Recently, improvements in the BART reagent have greatly increased the light output from assays. As a consequence, less costly light detection systems can be used in the diagnostic process. This has allowed us to design and prototype two types of low-cost, highly robust hardware platforms for molecular diagnostics: (a) a CCD-based machine capable of high- or ultra-high-throughput applications; and (b) a remarkably simple diode array-based device for point-of-use applications. The availability of low-cost affordable equipment suitable for BART applications removes the major limitation currently imposed on molecular diagnostic testing by, for example, quantitative real-time PCR, and makes BART a much more attractive option for use in all fields.

### **Identification of methane-consuming bacteria by intrinsic fluorescence spectra**

D. Gao,<sup>1</sup> G. Zhang,<sup>1</sup> J. Bai,<sup>2</sup> C. Zhao,<sup>2</sup> S. Shuang<sup>1</sup> and C. Dong<sup>2</sup>  
<sup>1</sup>School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, People's Republic of China  
<sup>2</sup>Research Centre of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China.  
E-mail: dc@sxu.edu.cn

Live bacteria possess numerous intracellular biological molecules. These main intrinsic fluorophores include aromatic amino acids

(AAA), nucleic acids (NA) and co-enzymes (NADPH and FAD). They have been investigated to reliably discriminate bacteria at family, genus, species and subspecies levels. Three different methane-consuming bacteria (*Methylobacterium*, *Pseudomonas* and *Klebsiella*) were studied by intrinsic fluorescence spectra in our work. When the OD<sub>600</sub> of bacteria reached  $0.1 \pm 0.05$ , fluorescence spectral differences of three bacteria were collected in a matrix (Ex 260–480 nm, Em 350–550 nm). With the culture days increasing, the NADPH fluorescence intensity of *Pseudomonas* and *Klebsiella* increased markedly but that of *Methylobacterium* changed only a little. Meanwhile, compared to *Klebsiella*, *Pseudomonas* produced other unknown metabolites. The results were to some extent consistent with previous reports. Moreover, the influence of different carbon resources (CHCl<sub>2</sub>, CHCl<sub>3</sub>) as nutritional material for these bacteria was researched. The results showed that methane may be their only carbon resource.

### **Determination of BSA with 3-(4'-methyl phenyl)-5-(2'-sulphophenylazo)rhodanine by resonance Rayleigh scattering method**

S. Ge, P. Dai, J. Yu, B. Li and Y. Tan  
School of Chemistry and Chemical Engineering, University  
of Jinan, Jinan 250022, People's Republic of China.  
E-mail: chm\_gesg@ujn.edu.cn

The resonance Rayleigh light scattering (RRS) spectrum of 3-(4'-methyl phenyl)-5-(2'-sulphophenylazo) rhodanine (4MRASP) and bovine serum albumin (BSA) in Britton–Robinson (BR) buffer solution at pH 2.8 was studied with the presence of SDS microemulsion. It was found that the RRS intensity of 4MRASP and of BSA is weak in each case, but is sharply enhanced when the two are compounded. The RRS intensity was strong over the wavelength range 380–400 nm and the maximum scattering intensity was obtained at 391 nm. The concentration of BSA is linear with 4MRASP in the range 0–1.4 µg/mL, with a detection limit of  $1.78 \times 10^{-7}$  g/mL. The method was applied to the determination of BSA in real samples, with satisfactory results. The mechanism of enhancement of RRS intensity of the 4MRASP-BSA compound was given.

### **Computational studies of firefly luminescence**

J. D. Goddard,<sup>1</sup> A.-M. Ren,<sup>2</sup> Z.-W. Li<sup>2</sup> and T. Yang<sup>1</sup>  
<sup>1</sup>Department of Chemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada. E-mail: jgoddard@uoguelph.ca  
<sup>2</sup>State Key Laboratory of Theoretical and Computational Chemistry, Institute of Theoretical Chemistry, Jilin University, Changchun 130023, People's Republic of China.  
E-mail: aimin.ren@gmail.com

Modern computational chemistry has been employed to study the ongoing intriguing question of the precise mechanism of the multicolour bioluminescence of the firefly (the *Photinus pyralis* luciferase). The structures and spectral characteristics of the ground and first excited singlet electronic states of a number of forms of oxyluciferin (keto, enol, several different net charges) have been examined. Theoretical approaches include configuration interaction singles (CIS), complete active space self-consistent field (CASSCF) and time-dependent density

functional theory (TDDFT). Most importantly, in establishing a more realistic model, solvent effects on the electronic spectra have been included with a polarized continuum model (PCM). In addition, explicit interactions of the excited state oxyluciferins with small molecules and ions have been included in the model. The present theoretical predictions are discussed in the context of the twisted emitter, variation of the microenvironment and changes in resonance forms models used in the past to explain the colour variations in the luminescence. The results are also considered in the light of the most recent experimental research on firefly bioluminescence and colour change by pH-sensitive green emission.

### New luminescent multi-enzyme assays for genomics and drug discovery applications

V. Golovlev<sup>1</sup> and A. Berthold<sup>2</sup>

<sup>1</sup>Sci-Tec Inc., 10425 Cogdill Road, #300, Knoxville, TN 37932, USA

<sup>2</sup>Berthold Detection Systems GmbH, Bleichstrasse 56–68, 75173 Pforzheim, Germany. E-mail: berthold@berthold-ds.com

Recent advances in luminometer design and progress in the understanding of complex enzymatic systems open new opportunities for expanding luminescent techniques into new research fields. Multi-enzyme luminescent assays have been recently introduced for ultra-sensitive detection of DNA and RNA replication and for DNA/RNA quantitation. Emerging applications of the multi-enzyme assays are expected to include microbial genotyping, microRNA analysis and antiviral drug discovery. A key for success of multi-enzyme assays is the availability of high-performance luminometers capable of measuring luminescence from miniaturized samples. Two photon-counting luminometers from Berthold Detection Systems have been studied and found to be fully suitable for these demanding applications. In this report we review the application of Orion and Sirius luminometers for luminescence detection from the reduced volume samples (<10 µL) and present examples of the implementation of multi-enzyme assays, which utilize the capabilities of the above instruments for DNA/RNA quantitation, monitoring RNA transcription in real time, and high-content screening of HIV-1 inhibitors.

### A comparison between ECLIA and RIA for detection of human type III procollagen in sera

X.-R. Guo

Centre for Clinical Diagnosis, Nantong First People's Hospital, Jiangsu 226001, People's Republic of China

Type III procollagen is commonly regarded as a one of the early and sensitive indicators for liver fibration, the fundamental pathogenesis of hepatocirrhosis caused by chronic hepatitis. In this study, the concentrations of type III procollagen in human sera from 108 clinical cases of chronic hepatitis were detected by enhanced chemiluminescence immunoassay (ECLIA) and radioactive immunoassay (RIA) and their performances, such as repeatability, linear scope and recovery, were compared. The data represented satisfactory results for both ECLIA and RIA, while ECLIA was better in repeatability, linear scope (<900 vs. <600 µg/mL) and recovery (>95%). Meanwhile, the correlation

coefficient between ECLIA and RIA was found to be 0.928, using the equation  $Y = 1.107X - 3.312$ . Based on tests on 60 healthy individuals, the reference interval for ECLIA was estimated as 4.5–126.5 µg/mL. In view of its advantages in sensitivity, stability and flexibility, it is proposed that ECLIA may act as a substitution for RIA to detect type III procollagen in sera at clinical diagnosis.

### Change of expression efficiency of natural and cloned *lux* operon in conditions of famine

A. A. Gusev

Institute of Biophysics SB RAS, Akademgorodok 50/50, 660036 Krasnoyarsk, Russia, E-mail: snowmind@mail.ru

The maintenance of a high level of expression of cloned *lux* genes is an important strategic problem which justifies the cloning of alien genes in new host cells. Decrease of expression of *lux* operons in environmental conditions will occur, due to the reorganization of metabolic processes. The coefficients of effectiveness of bioluminescence expression at the molecular, cell and population levels are offered, to estimate the opportunity for natural and transgenic strains to adapt in water ecosystems, with preservation of *lux* operons with high expression. It was shown that at the population level all transgenic variants of *Escherichia coli* Z905-2/pPHL7 superseded natural variants of *Photobacterium leiognathi*, irrespective of the type of *lux* operon regulation. At the cell level, bright and dim transgenic variants exceeded the effectiveness of bioluminescence expression of natural strains by several orders of magnitude. At the level of one *lux* operon, the effectiveness of expression of the transgenic bright variant was rather higher than in the natural bright variant; in dim variants they were similar and the effectiveness of bioluminescence expression of the dark variant of *E. coli* Z905-2/pPHL7 was lower by two orders than in the dark variant of *P. leiognathi*. Under famine conditions, the transgenic variants, in contrast to natural strains, considerably reduced the efficiency of bioluminescence expression at the population level.

### Luminol-dependent chemiluminescence increases with formation of phenothiazine cation radicals by horseradish peroxidase

V. A. Hadjimitova,<sup>1</sup> T. Traykov<sup>1</sup> and R. Bakalova<sup>2</sup>

<sup>1</sup>Department of Physics and Biophysics, Medical University-Sofia 1421, Bulgaria. E-mail: verah@mail.bg

<sup>2</sup>Department of Biophysics, Molecular Imaging Centre, NIRS, Chiba, Japan

The introduction of phenothiazines into a HRP-H<sub>2</sub>O<sub>2</sub>-luminol system causes a strong increase in chemiluminescence. This investigation includes chlorpromazine, levomepromazine, trifluoperazine, thioridazine and promethazine, in concentrations of 5.10<sup>-8</sup>–5.10<sup>-5</sup> mol/L. The results show that all these substances increase chemiluminescence up to eight times above the control. As a result of the processes occurring in the samples, cation radicals of phenothiazines are generated. Their presence is demonstrated through their characteristic absorption in the visual range. The radicals' concentration was proportional to the chemiluminescence increase. Chlorprothixene's structure is very similar to that of chlorpromazine but does not allow the



formation of a radical, as in the case of chlorpromazine. Putting chlorprothixene instead of chlorpromazine into the system does not cause a chemiluminescence increase. We conclude that the chemiluminescence increase is related to the formation of phenothiazine radicals, as there is a relationship between the increase in chemiluminescence and the type of cation radicals, and a small difference in structure inhibits the process.

### Variety of chemiluminescent methods for antioxidant activity investigation of *Crataegus oxicantha* extract

V. A. Hadjimitova,<sup>1</sup> T. Traykov<sup>1</sup> and R. Bakalova<sup>2</sup>

<sup>1</sup>Department of Physics and Biophysics, Medical University-Sofia 1421, Bulgaria. E-mail: verah@mail.bg

<sup>2</sup>Department of Biophysics, Molecular Imaging Centre, NIRS, Chiba, Japan

In order to investigate the antioxidant activity (AOA) of ethanol extract from hawthorn (*Crataegus oxicantha*) leaves and flowers, we used three methods based on luminol-dependent chemiluminescence (CL). We used CL detection of hydroxyl radicals ( $\cdot\text{OH}$ ), generated by a  $\text{H}_2\text{O}_2$ -( $\text{Fe}^{2+}$ -EDTA) system, hypochlorite generated by  $\text{NaOCl}$ , and decomposition of  $\text{H}_2\text{O}_2$  by a HRP-luminol system. The combination of these three methods, based on a common amplifier of CL, allows for a comparison to be made of the AOA of hawthorn exhibited with regard to the three most common *in vivo* reactive oxygen species. We found that the extract from *C. oxicantha* showed very strong antioxidant properties. Its strongest AOA was exhibited in HRP- $\text{H}_2\text{O}_2$ , followed by scavenger activity with regard to  $\cdot\text{OH}$  and hypochlorite. C-50 values obtained by the three systems were, respectively, <10 mg/L, 90 mg/L and 120 mg/L. By using an alternative photometric method for  $\cdot\text{OH}$  detection, we found that the results observed were not due to a quenching effect.

### Visualization of sequential response in intracellular signal transduction cascade by fluorescence and luminescence imaging in the same living cell

Y. Hatta-Ohashi, T. Takahashi and H. Suzuki

Research and Development Division, Olympus Corporation, 2-3 Kuboyama-cho, Hachioji, Tokyo 192-8512, Japan.  
E-mail: yoko1\_ohashi@ot.olympus.co.jp

Cells recognize changes in their environment through cell surface receptors, resulting in initiation of intracellular signal transduction, followed by gene expression of the downstream transcription factors. We usually observe protein-protein interaction in cell signalling by fluorescence imaging, using green fluorescent protein (GFP) and we detect gene expression through intracellular signalling, using the reporter assay with luminometer. However, it has been impossible to observe both of these processes sequentially in the same living cell. To observe the two processes sequentially, we developed a luminescence imaging system that can be also applied to fluorescence imaging. We successfully monitored the

translocation of protein kinase C (PKC) labelled with GFP from cytoplasm to cell membrane, caused by phorbol myristate acetate (PMA), and the following gene expression of the downstream transcription factor, NF $\kappa$ B, using luciferase in HeLa cells. Thus, sequential response in signal transduction cascade could be visualized in the same cell by using fluorescence and luminescence imaging. Furthermore, the results of Ras signalling pathway analysis are reported.

### Determination of matrine in medicine and bio-fluids based on its inhibited luminol-myoglobin with flow-injection chemiluminescence

X. He, X. Xie and Z. Song

Department of Chemistry, Northwest University, Xi'an 710069, People's Republic of China. E-mail: hexili@mail.xjtu.edu.cn

A novel green method using flow-injection chemiluminescence has been investigated for the rapid and sensitive determination of matrine. It was found that matrine could greatly inhibit the chemiluminescence generated by the reaction between luminol and myoglobin. The decreased chemiluminescence signal was proportional to the logarithm of the matrine concentration over the range 10 ng/mL to 3  $\mu\text{g/mL}$  ( $r^2 = 0.9978$ ), offering a detection limit as low as 3.5 ng/mL ( $3\sigma$ ). At a flow rate of 2.0 mL/min, one analysis cycle, including sampling and washing, could be accomplished in 20 s, with a relative standard deviation (RSD) of <5%. The sensitive flow-injection method was applied successfully to the determination of matrine in pharmaceutical injections, human urine and serum without any pretreatment procedure, with recoveries of 90.0–110.0% and RSD <5.0%.

### Chemiexcitation mechanism for *Cypridina* (Vargula) and *Aequorea* bioluminescence

T. Hirano,<sup>1</sup> H. Ohba,<sup>1</sup> Y. Takahashi,<sup>1</sup> S. Maki,<sup>1</sup> S. Kojima,<sup>1</sup> H. Ikeda<sup>2</sup> and H. Niwa<sup>1</sup>

<sup>1</sup>Department of Applied Physics and Chemistry, University of Electro-Communications, Chofu, Tokyo 182-8585, Japan.  
E-mail: hirano@pc.uec.ac.jp

<sup>2</sup>Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan

The imidazo[1,2- $\alpha$ ]pyrazin-3(7H)-one (imidazopyrazinone) ring is the important core structure for the bioluminescent substrates, *Cypridina* luciferin and coelenterazine, of the ostracod *Cypridina* (Vargula) and the jellyfish *Aequorea*, respectively. A remarkable characteristic of the bioluminescence systems is a high quantum yield of light production ( $\Phi_{\text{BL}} \approx 0.3$ ). This indicates that the chemiexcitation process in the bioluminescence reactions produces an excited molecule with a high efficiency ( $\Phi_s$ ). To establish the chemiexcitation mechanism for the imidazopyrazinone-bioluminescence system, we investigated the chemiluminescence of a series of 6-arylimidazopyrazinone derivatives as a bioluminescence model. We also studied the fluorescent properties of the light-emitter compounds and performed quantum chemical calculations of the molecules

related to the chemiexcitation process. From the results, we discuss the mechanism of the highly efficient chemiexcitation in *Cypridina* and *Aequorea* bioluminescence: thermal decomposition of a neutral dioxetanone intermediate gives a singlet-excited light-emitter molecule ( $S_1$ ) with intramolecular charge transfer (ICT) character via an ICT transition state (TS). The similarity of the ICT character of  $S_1$  and TS leads the chemiexcitation process to be efficient with the charge transfer-induced luminescence (CTIL) mechanism.

### Development of BRET-based auto-illuminated fluorescent-protein (BAF) and application to live-cell imaging

H. Hoshino

Research Institute for Cell Engineering, Advanced Industrial Science and Technology, Osaka 563-8557, Japan.

E-mail: ah-hoshino@aist.go.jp

Bioluminescence resonance energy transfer (BRET) is an intrinsic process that can be observed in sea creatures such as the jellyfish *Aequorea aequorea* and the sea pansy *Renilla reniformis*. Utilizing the BRET phenomenon, we developed a bioluminescent probe composed of EYFP and *Renilla* luciferase (Rluc) [BRET-based auto-illuminated fluorescent-protein on EYFP (BAF-Y)] for the real-time single-cell imaging technique. We show that BAF-Y enhances Rluc luminescence intensity via resonance energy transfer, and exhibits appropriate subcellular distribution with high spatial and temporal resolution when it is fused to targeting signal-peptides or histone H2AX. Our BAF-Y probes would expand further possibilities in GFP use.

### Site-directed mutagenesis of *Lampyrus turkestanicus* luciferase: the effect of conserved residue(s) in bioluminescence emission spectra among firefly luciferases

S. Hosseinkhani,<sup>1</sup> N. Kh.Tafreshi,<sup>2</sup> M. Sadeghizadeh,<sup>2</sup>

R. Emamzadeh,<sup>1</sup> B. Ranjbar<sup>1</sup> and H. Naderi-Manesh<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Basic Sciences, Tarbiat Modares University, Tehran 14115-175, Iran.

E-mail: saman\_h@modares.ac.ir

<sup>2</sup>Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran 14115-175, Iran

The bioluminescence colours of firefly luciferases are determined by assay condition and luciferase structure. Due to the lower energy of red light than green light and less absorption in biological tissues, red-emitter luciferases have been considered as useful reporters in imaging technology. A set of red-emitter mutants of *Lampyrus turkestanicus* luciferase has been made by site-directed mutagenesis. Amongst different beetle luciferases, those from the railroad worm *Phrixothrix* emit either green or red bioluminescence colours naturally. Sequence alignment analysis shows that red-emitter luciferase from *P. hirtus* has an additional Arg residue at 353 which is absent in firefly luciferases. By insertion of an additional residue (Arg 356) using site-specific insertion mutagenesis in a green-emitter luciferase (*L. turkestanicus*), the colour of emitted light was

changed to red and the optimum temperature of activity was also increased. Moreover, substitution of three specific amino acids using site-specific mutagenesis in *L. turkestanicus* luciferase changed the colour of emitted light to red, concomitant with decreasing decay rate. Different specific mutations (H245N, S284T and H431Y) led to changes in the bioluminescence colour. Change in the light colour indicates the critical role of these conserved residues in bioluminescence colour determination among firefly luciferases.

### Fluorescent resonance energy transfer based on quantum dots in antibody-antigen system

S. Hu,<sup>1</sup> H. Yang,<sup>1,2</sup> R. Cai,<sup>1,3</sup> Q. Zhang<sup>1</sup> and X. Yang<sup>1</sup>

<sup>1</sup>College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, People's Republic of China. E-mail: yangxl@mail.hust.edu.cn

<sup>2</sup>Environmental Science Research Institute, Huazhong University of Science and Technology, Wuhan, Hubei, People's Republic of China

<sup>3</sup>College of Chemistry and Molecular Sciences, Wuhan University, Wuhan, Hubei, People's Republic of China

We demonstrate the probability of protein concentration detection by the method of fluorescence resonance energy transfer (FRET) assays. In this assay, quantum dots (QDs) as energy donors conjugated with rabbit anti-mouse IgG F(ab')<sub>2</sub>, and rhodamine B isothiocyanate (RBITC) conjugated with mouse IgG served as energy acceptors. The mutual affinity of antibody [anti-IgG F(ab')<sub>2</sub>] and antibody (IgG) brought QDs and RBITC close enough to result in FRET. F(ab')<sub>2</sub> was used in order to make the conjugation easier and shorten the distance between QDs and RBITC. Competition immunoassays with FRET were demonstrated for the detection of mouse IgG. Immunoassay based on FRET in antibody-antigen system will be a new direction for quantitative analysis.

### A novel method for the determination of L-cysteine, based on the electrochemiluminescence of CdTe quantum dots

L.-J. Hua, H.-Y. Han and D.-L. Lu

College of Science, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China.

E-mail: hyhan@mail.hzau.edu.cn

Electrochemiluminescence (ECL) analytical techniques coupled with quantum dots (QDs) have been rapidly developed in recent years, which undoubtedly extend to both fundamental study and analytical application. In this paper, the direct ECL of prepared thiol-capped CdTe QDs was carried out in air-saturated solution. The results demonstrated that relatively high ECL intensity was observed without adding additional oxidant, and the ECL behaviour of CdTe QDs was sensitive to the sizes of CdTe QDs variation. The mechanisms of these size-dependent ECL properties of CdTe QDs were also discussed. Based on the quenching of ECL by L-cysteine, a novel method for the determination of L-cysteine was developed. It was found that

the ECL signals of CdTe QDs had a linear relationship with the concentration of L-cysteine in the range  $1.3 \times 10^{-6}$ – $3.5 \times 10^{-5}$  mol/L ( $R = 0.996$ ) with a detection limit of  $8.6 \times 10^{-7}$  mol/L. In comparison with previous ECL sensors, the proposed method does not need a complicated modifying process, which will be helpful for the further study of the properties of QDs and to expand their potential applications.

### Quantum dots–ssDNA–fluorescent dye conjugates as bioprobes for specific and sensitive detection of DNase

S. Huang and Z. He

College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, People's Republic of China.  
E-mail: zhkhe@whu.edu.cn

Here we report an easy and simple method for specific and sensitive detection of DNase with QDs–ssDNA–rhodamine conjugates. The conjugates were prepared based on the strong interactions between streptavidin and biotin which result in fluorescence resonance energy transfer (FRET) between the streptavidin-conjugated QDs and the rhodamine labelled on the 5'-end of ssDNA whose 3'-end was functionalized with biotin. However, when ssDNA was cleaved by micrococcal nuclease into small fragments, the fluorescence intensity of the rhodamine (acceptor) decreased rapidly, while that of QDs showed a strong increase. The detection limit of micrococcal nuclease can be as low as 0.4 U/mL in homogeneous solution. This work was supported by the National Science Foundation of China (20575046, 90717111) and the National Key Scientific Program (2006CB933100).

### Construction of recombinant luminescence bacteria vector to evaluate the genetic toxicity of environment pollutant

X.-X. Huang, M. He, H.-C. Shi and Q. Cai

Division of Water Environment, Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, People's Republic of China.  
E-mail: xxhuang@mail.tsinghua.edu.cn

Recombinant luminescence bacteria have an important role in evaluating water toxicity. Two recombinant luminescence bacteria vectors, PUCD-*uvrA* and PUCD-*alkA*, were constructed to investigate the impaired mechanism of pollutant genetic toxicity. The genes *uvrA* and *alkA* were amplified by PCR from *Escherichia coli* W3110 and 237 and 326 bp fragments were acquired. The homology of sequencing results reached 99% when blasted in GenBank, confirming that the amplified results were correct. The PCR products and PUCD615 vector were all digested with *Bam*HI and *Eco*RI, then linked and imported into JM109 with electroporation. Several clones were selected and identified by PCR and sequencing. The results confirmed that *uvrA* and *alkA* had been inserted into the multiple clone site correctly; the insert direction and reading frame were also correct. PUCD615 containing the full *luxCDABE* gene box had the advantage over that containing only *luxAB*, for the latter showed bioluminescence only in the presence of substrate (fatty aldehyde).

Optimizing the condition of ligation and transformation, the large fragment of PUCD615 and the short inserted sequence can be ligated successfully.

### Research on surface-coding technique of NaYF<sub>4</sub>:Yb,Er with polyethylene glycol

M. Huang, F. Meng, X. Yuan, Y. Li and R. Liu

Chemistry Department, Jiaying University, Meizhou City, Guangdong 514015, People's Republic of China.  
E-mail: mhmw@jyu.edu.cn

Hexagonal plates of NaYF<sub>4</sub>:Yb,Er infrared upconversion material were prepared and the technique of NaYF<sub>4</sub>:Yb,Er coated with polyethylene glycol was investigated. The sample was prepared at 40°C, ammonia pH 9, stirring in a three-necked bottle for 1 h. Before coating, the absorbing peaks were 3441.82 cm<sup>-1</sup>, 1383.21 cm<sup>-1</sup> and 1081.08 cm<sup>-1</sup> on the infrared spectrum, but they were 2883.64 cm<sup>-1</sup>, 1470.27 cm<sup>-1</sup>, 1342.25 cm<sup>-1</sup>, 1111.81 cm<sup>-1</sup>, 958.18 cm<sup>-1</sup> and 835.28 cm<sup>-1</sup> after coating. The peaks are the typical polyethylene glycol character. Under near-infrared excitation the spectrum did not show a change of NaYF<sub>4</sub>:Yb,Er upconversion luminescence performance.

### Research on the influence of luminescence with different intensities of Yb<sup>3+</sup>, Lu<sup>3+</sup> in NaYF<sub>4</sub> codoped upconversion material

M. Huang, F. Meng, X. Yuan, Y. Li and R. Liu

Chemistry Department Jiaying University Meizhou City, Guangdong 514015, People's Republic of China.  
E-mail: mhmw@jyu.edu.cn

The influence of upconversion luminescence with different intensities of Yb<sup>3+</sup>, Lu<sup>3+</sup> in NaYF<sub>4</sub> codoped material was investigated. Yb<sup>3+</sup> in 4.40, 2.50, 1.35, 0.89, 0.71, 0.57 and 0.48 mol% were prepared by combustion synthesis. Samples of Lu<sup>3+</sup> in 0.63, 0.36, 0.19, 0.13, 0.10, 0.08 and 0.07 mol% were doped. The upconversion luminescence peak values were relatively 407, 476, 538, 653 nm. The rare earth Yb<sup>3+</sup>, Lu<sup>3+</sup> mol% influence on the intensity and purity of upconversion luminescence was obtained and influence of combustion synthesis temperature on influence intensity was investigated.

### Mass spectrometric approach to elucidation of singlet chemi-excitation of dioxetanes

H. K. Ijuin, M. Ohashi, mol/L. Tanimura, N. Watanabe and M. Matsumoto

Department of Chemistry, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa 259-1293, Japan.  
E-mail: ijuuh-01@kanagawa-u.ac.jp

1,2-Dioxetanes bearing an aromatic electron donor undergo intramolecular charge-transfer-induced chemiluminescence (CTICL). Although there has been some controversy regarding the mechanisms involved, there is little experimental evidence to support strongly any of the proposed mechanisms. In the course of our investigation to clarify these mechanisms, we tried to effectively ionize dioxetanes bearing a phenolic group, and found that poly(3-octylthiophene-2,5-diyl) was a promising matrix for

negative-mode MALDI-TOF-MS. Electron-transfer ionization was found to take place for dioxetanes bearing a hydroxyphenyl moiety substituted further with an aromatic group, which acted as an antenna to catch an electron from the matrix. Furthermore, the characteristic fragmentation of dioxetane, (5-*t*-butyl-1-[(4-benzo[d]thiazol-2-yl)-3-hydroxyphenyl]-4,4-dimethyl-2,6,7-trioxabicyclo[3.2.0]heptane), was thought to occur by the elimination of 2-methyl-1-propene (56 *u*) and pivalaldehyde (86 *u*) from deprotonated ion [M-H]<sup>−</sup> of dioxetanes, based on the results of (MS)<sup>n</sup> spectrometry measurements of dioxetanes. Based on a comparison of fragmentation in dioxetanes and the corresponding keto esters, fragmentation of dioxetanes were presumed to take place from the ketone part, which initially generated excited keto esters.

### Theoretical considerations on the roles of hydrogen bonding in thermal decomposition of peroxides

H. Isobe,<sup>1,2</sup> S. Yamanaka,<sup>1,2</sup> M. Okumura<sup>2</sup> and K. Yamaguchi<sup>1,2</sup>

<sup>1</sup>Centre for Quantum Science and Technology under Extreme Conditions, Osaka University, Toyonaka, Osaka 560-8531, Japan

<sup>2</sup>Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.

E-mail: isobe@chem.sci.osaka-u.ac.jp

Chemiluminescence and bioluminescence phenomena have recently attracted wide interest from the viewpoints of biological and biomedical applications. Despite considerable interest, why the dioxetane-based chemiluminescence is so inefficient in aqueous solution remains to be fully explained. Here, we present a simple theoretical analysis of the roles of water molecule(s) (or, more generally, hydrogen bonding) in the thermal decomposition of four-membered ring peroxides having a dioxetane or dioxetanone functionality and a phenoxide anion group, using the cluster-continuum approach, in which solvent molecules in the first coordination shell are treated explicitly and the remaining solvents are considered as a dielectric continuum. The results show that the presence of strong and specific solute-solvent interactions at the anion site of the peroxides brings about a mechanistic change in the decomposition when the dioxetane functionality acts as an electron acceptor, although this is not the case when the dioxetanone functionality is present.

### Theoretical studies of charge-transfer-induced luminescence in molecular and bimolecular systems

H. Isobe,<sup>1,2</sup> S. Yamanaka,<sup>1,2</sup> M. Okumura<sup>2</sup> and K. Yamaguchi<sup>1,2</sup>

<sup>1</sup>Centre for Quantum Science and Technology under Extreme Conditions, Osaka University, Toyonaka, Osaka 560-8531, Japan

<sup>2</sup>Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.

E-mail: yama@chem.sci.osaka-u.ac.jp

Theoretical studies of charge-transfer-induced luminescence (CTIL) in molecular and bimolecular systems have been carried

out at the B3LYP/6-31 + G(d) level, including the full Pauli-Breit spin-orbit coupling (SOC) interaction. The reductive activation for the <sup>3</sup>O<sub>2</sub> reaction is affected by the proton activity and solvent polarity of a surrounding reaction field. In a polar aprotic solvent, a base-prompted anionic substrate may react with <sup>3</sup>O<sub>2</sub> in a stepwise manner through complete electron transfer from the substrate anion to <sup>3</sup>O<sub>2</sub>, while the irreversible concerted <sup>3</sup>O<sub>2</sub> addition via intersystem crossing may become complete in a less polar solvent. SOC in the thermal decomposition of a resulting peroxide adduct can be controlled by the protonation state of the substrate. There exists an optimal protonation state for the suppression of SOC in the CTIL of the peroxide, which is closely related to the ability of a substituent to donate an electron. This will constitute a necessary condition for the high efficiency of chemi- and bioluminescence.

### Simultaneous multiplex bio- and chemiluminescent enzyme immunoassay for PCR products derived from genetically modified papaya

K. Ito,<sup>1</sup> Y. Tanaka,<sup>1</sup> M. Maeda,<sup>1</sup> K. Gomi,<sup>2</sup> S. Inouye,<sup>3</sup> H. Akiyama<sup>4</sup> and H. Arakawa<sup>1</sup>

<sup>1</sup>School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan.

E-mail: itok@pharm.showa-u.ac.jp

<sup>2</sup>Kikkoman Co., Chiba, Japan

<sup>3</sup>Chisso Co., Yokohama Research Centre, Japan

<sup>4</sup>National Institute of Health Sciences, Japan

We have developed a simultaneous luminometric assay for aequorin (Aq), firefly luciferase (Luc) and horseradish peroxidase (HRP). We had already established a simultaneous bioluminescent assay using Aq and Luc. In this study, the measurement of HRP with luminol and H<sub>2</sub>O<sub>2</sub> was followed by the Aq and Luc assay. The proposed assay was simultaneous measurement of three enzymes using luminometric detection. The measurable range of Aq, Luc and HRP were  $1.2 \times 10^{-19}$ – $4.8 \times 10^{-16}$  mol/assay,  $5.3 \times 10^{-18}$ – $2.2 \times 10^{-14}$  mol/assay and  $4.9 \times 10^{-16}$ – $3.1 \times 10^{-14}$  mol/assay, respectively. Furthermore, the proposed assay was applied to the detection of PCR products derived from genetically modified (GM) papaya genes. Two GM papaya-specific genes and the intrinsic papain gene of GM papaya were amplified by multiplex PCR. PCR products were simultaneously detected by bio- and chemiluminescent enzyme immunoassay (BCLEIA). The simultaneous BCLEIA was distinguishable between GM papaya and non-GM papaya.

### A novel fluorescent probe for Ag<sup>+</sup>:3,4-di(*N,N*-dimethylaminopropylamino)-7H-benzimidazo[2,1-*a*] benz[*d,e*]isoquinolin-7-one

L. Jia

Key Laboratory of Fine Chemicals (Qiqihar University), College of Heilongjiang Province, Qiqihar 161006, People's Republic of China. E-mail: jlh29@163.com

Silver ion is a very important transition metal ion which is both vital and toxic for many biological systems, so the detection of Ag<sup>+</sup> is attracting attention. Some silver ion probes



are based on UV absorption spectra, chemiluminescence or fluorescent band shift, and many of them are based on fluorescent quenching, whose determination is often strongly interfered with by  $\text{Hg}^{2+}$  and other heavy ions. Therefore, the development of novel selective and sensitive probes with fluorescence enhancement (FE) for  $\text{Ag}^+$  has become our target. Here, a new compound, 3,4-di(*N,N*-dimethylaminopropylamine)-7*H*-benzimidazo[2,1-*a*]benz[*d,e*] isoquinolin-7-one (**1**), was designed and synthesized as a novel fluorescent probe. The highly selective and sensitive response of FE for **1** towards  $\text{Ag}^+$  with four-fold FE and a blue shift about 16 nm was found in aqueous solution.

**Chemiluminescence of 9-benzylidene-*N*-methylacridans with electron-donating groups by chemically generated singlet oxygen. Application to metal ion sensing using aza-crowned compound**

M. Jiro, T. Toshimitsu, K. Motoki and Y. Nishii

Department of Chemistry, Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano 386-8567, Japan. E-mail: jmotoyo@shinshu-u.ac.jp

The singlet oxygenation of several 9-benzylidenacridans bearing electron-donating groups on the benzylidene aromatic rings and *N*-methylacridone (NMA), the latter of which was formed in the excited state and provided the chemiluminescence (CL) due to its fluorescence. Their CL quantum yields were in accordance with a Hammett relationship, with a negative rho-value depending on the electronic nature of the substituents, except for the dimethylamino group, which markedly decreased the CL quantum yield. The negative effect of this group is not due to an electron-transfer quenching of singlet oxygen but to a fluorescence quenching of MNA by shortening its fluorescence lifetime. Making use of this result, a benzylidenacridan with an azacrown ether group (aza-15-crown-5) was subjected to the CL reactions in the presence of various metal cations, among which calcium and magnesium ions enhanced the CL efficiencies much more effectively than other alkali-metal cations. The observed function of the azacrown moiety is explained by the large binding constant estimated between the azacrowned benzylidenacridan and a calcium ion, and by lengthening the fluorescence lifetime of NMA observed in the presence of the calcium ion-incorporated azacrowned benzaldehyde compared to the case without calcium ion. These results show a possibility of metal ion sensing using this CL system.

**Chemiluminescence determination of sparfloxacin using the  $\text{Ru}(\text{bipy})_3^{2+}$ -Ce(IV) system**

M. Karim,<sup>1</sup> J. H. Choi,<sup>2</sup> S. M. Alam<sup>1</sup> and S. H. Lee<sup>1</sup>

<sup>1</sup>Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: seikh\_alam@hotmail.com

<sup>2</sup>Department of Chemistry, Andong National University, Andong 760-749, Korea

A simple, sensitive and rapid chemiluminescence system was developed for the determination of sparfloxacin by a flow-

injection system. This technique is based on the enhancement effect of sparfloxacin on the CL peak from the reaction of  $\text{Ru}(\text{bipy})_3^{2+}$ -Ce(IV). The calibration curve was linear over the range  $7.0 \times 10^{-12}$ – $4.0 \times 10^{-8}$  mol/L, with a detection limit of  $1.2 \times 10^{-12}$  mol/L (S:N = 3). The relative standard deviation of  $1.0 \times 10^{-7}$  mol/L sparfloxacin was found to be 1.6% ( $n = 11$ ). The proposed method was successfully applied for the flow-injection determination of sparfloxacin in the real samples, with a minimum sampling rate of 120 samples/h.

**Fluorimetric determination of rutin, using rutin-Fe(III) system**

M. M. Karim,<sup>1</sup> C. W. Jeon,<sup>2</sup> S. H. Lee<sup>1</sup> and W. H. Kim<sup>1</sup>

<sup>1</sup>Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: shlee@knu.ac.kr

<sup>2</sup>Korea Institute of Geoscience and Mineral Resources, Daejeon 305-350, Korea

A new, simple and sensitive spectrofluorimetric method for the determination of rutin ( $\lambda_{\text{ex}} = 491$  nm,  $\lambda_{\text{em}} = 506$  nm) using Fe(III) as a sensitizing reagent has been investigated by measuring the increase of fluorescence intensity of rutin due to the complexation of Fe(III)-rutin at pH 9.0. Under optimum conditions, a significant relationship was obtained between the fluorescence intensity and rutin concentration. A linear calibration curve was obtained in the range  $1 \times 10^{-4}$ – $1 \times 10^{-6}$  mol/L, with a product-moment correlation coefficient ( $R$ ) of 0.9998 and a detection limit of  $2.5 \times 10^{-7}$  mol/L. The RSD is 1.35% ( $n = 5$ ). The method was applied successfully to the determination of rutin in pharmaceutical formulations.

**A new bright chemiluminescent reaction: interaction of acetone with solid-phase potassium monoperoxysulphate in the complex of europium nitrate**

D. V. Kazakov,<sup>1</sup> V. P. Kazakov,<sup>1</sup> F. E. Safarov<sup>1</sup> and R. Schmidt<sup>2</sup>

<sup>1</sup>Institute of Organic Chemistry, 71 Pr. Oktyabrya, 450054 Ufa, Russia

<sup>2</sup>Johann Wolfgang Goethe-Universität, Frankfurt am Main, Institut für Physikalische und Theoretische Chemie, Max-von-Laue-Strasse 7, D-60438 Frankfurt am Main, Germany

It is known that acetone-catalysed decomposition of potassium monoperoxysulphate leads to the formation of dioxirane – three-membered ring cyclic peroxide and singlet oxygen ( $^1\text{O}_2$ ). We found that the interaction of acetone vapour with a mixture of  $\text{KHSO}_5$  and europium nitrate hexahydrate powders at 90°C leads to bright chemiluminescence (CL) due to the formation of excited  $\text{Eu}^{3+}$ . Under appropriate conditions, luminescence may be observed in a slightly darkened room even with the naked eye. Excitation of the europium occurs in the metal coordination sphere as a result of energy transfer from the singlet-oxygen dimol or/and excited ester—a product of dioxirane intermediate isomerization. CL observed in this solid-phase system appeared to be a more general phenomenon, since light emission was

also revealed during interaction of methylethyl ketone and trifluoropropanone with europium nitrate, as well as of acetone with nitrates of terbium and samarium.

### Use of recombinant biotinylated acetate kinase in bioluminescent immunoassay

R. Ke,<sup>1</sup> W. Yang,<sup>1</sup> Y. Shen,<sup>1</sup> Y. Liao<sup>1</sup> and Q. Li<sup>1,2</sup>

<sup>1</sup>Molecular Diagnostics Laboratory, Department of Biomedical Sciences and the Key Laboratory of the Ministry of Education for Cell Biology and Tumour Cell Engineering, School of Life Sciences, Xiamen University, Fujian 361005, People's Republic of China

<sup>2</sup>Translation Medicine Centre, Institute for Biomedical Research, Xiamen University, Fujian 361005, People's Republic of China. E-mail: qgli@xmu.edu.cn

Firefly luciferase enzyme converts the substrate luciferin into oxyluciferin with high quantum yield light emission. Analytical chemists are trying to use this enzyme in ELISA as a labelled enzyme. However, this enzyme has some drawbacks that make it difficult for this purpose. It is heat sensitive and also loses its activity after chemical modification. To overcome these drawbacks, some studies have used kinase integrated with firefly luciferase to form a bioluminescent immunoassay system. These kinase enzymes can be chemically modified without losing activity and are more heat stable than firefly luciferase. One of these enzymes is acetate kinase, which generates ATP from acetylphosphate and ADP by the dephosphorylation of acetylphosphate. The ATP can be determined by the firefly luciferase–luciferin reaction. It would be advantageous if this enzyme could be expressed using a prokaryotic expression system. In the present study, the *ack* gene, encoding acetate kinase from a thermophile, *B. stearothermophilus*, was successfully cloned and inserted into expression vector pET-32a and functionally expressed in bacterium *Escherichia coli* BL21(DE3). The recombinant 6 × His tag acetate kinase was successfully purified to near-homogeneity. A bioluminescent enzyme immunoassay based on biotinylated recombinant acetate kinase was developed.

### Flow-injection analysis with chemiluminescence detection: determination of gatifloxacin using the KMnO<sub>4</sub>–formaldehyde system

M. A. Khan, S. M. Alam and S. H. Lee

Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: seikh\_alam@hotmail.com

A simple, rapid and sensitive flow-injection chemiluminescence (CL) method has been studied for the determination of gatifloxacin. A strong CL signal was observed when gatifloxacin was mixed with KMnO<sub>4</sub>–HCHO system. Under the optimum experimental conditions, the CL intensity was correlated linearly with the concentration of gatifloxacin over the range  $1.0 \times 10^{-8}$ – $3.0 \times 10^{-5}$  mol/L and the detection limit was  $1.1 \times 10^{-9}$  mol/L. The relative standard deviation (RSD) for 11 repetitive determinations of  $1.0 \times 10^{-7}$  mol/L gatifloxacin was 2.1%. The utility of this method

was demonstrated by determining gatifloxacin in pharmaceutical preparations.

### Determination of ciprofloxacin in pharmaceutical formulation by chemiluminescence method

M. A. Khan, S. H. Lee, S. M. Alam, S. M. Wabaidur and H. Y. Chung

Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: shlee@knu.ac.kr

For the quantitative determination of ciprofloxacin (CPLX) in pharmaceutical formulation, a batch-type chemiluminescence (CL) method is described. In this study, it was observed that CPLX could enhance the CL emission of the Ru(phen)<sub>3</sub><sup>2+</sup>–Ce(IV) system and that this enhancement effect was dependent on the concentration of CPLX. Based on this, a CL system was established for the determination of CPLX. Under the optimum experimental conditions, the linear range and detection limit are  $1.0 \times 10^{-7}$ – $1.0 \times 10^{-4}$  mol/L and  $2.0 \times 10^{-8}$  mol/L, respectively. The relative standard deviation (RSD) for 10 repeated measurements of  $1.5 \times 10^{-4}$  mol/L CPLX was 1.30%. The method was applied to the determination of CPLX in pharmaceutical formulations.

### Study on novel aryloxalate chemiluminescence reaction without addition of hydrogen peroxide

N. Kishikawa, K. Ohyama, K. Nakashima and N. Kuroda

Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.

E-mail: kishika@nagasaki-u.ac.jp

Peroxyoxalate chemiluminescence (PO-CL) is based on the reaction between hydrogen peroxide and aryloxalate, which produces strong luminescence in the presence of fluorophore through a chemically initiated electron exchange luminescence mechanism. The PO-CL is easily combined with HPLC and applied to the determination of fluorescent compounds. However, the addition of high levels of hydrogen peroxide causes an increase in the background noise and also the instability of hydrogen peroxide may affect the repeatability of the CL reaction. Recently, we found that CL was generated when specific fluorophores, which have 2-phenyl-6-dimethylaminobenzofuran structures, were mixed with aryloxalate without addition of hydrogen peroxide. It was found that there is a direct relation between the concentration of the fluorophores and CL intensity. We thus combined the proposed CL reaction with HPLC for the determination of biological thiols, such as cysteine, by using *N*-[4-(dimethylamino-2-benzofuranyl)phenyl] maleimide as a chemiluminescent labelling reagent. The labelled thiols were separated and detected by CL after mixing with aryloxalate only. The sensitivity of the proposed method in the absence of hydrogen peroxide was comparable to those of the conventional PO-CL method in the presence of hydrogen

peroxide and was sufficient to determine biological thiols in human serum.

### pH-tolerant mutants of *Luciola mingrelica* luciferase created by random mutagenesis

M. I. Koksharov and N. N. Ugarova

Department of Chemistry, Lomonosov Moscow State University, Moscow 119991, Russia. E-mail: mkoksharov@enz.chem.msu.ru

The majority of firefly luciferases demonstrate highly pH-sensitive bioluminescence spectra and undergo a large shift from green to red light when lowering pH from 7.8 to 6.0. A gene region coding the first 225 residues of *Luciola mingrelica* luciferase was subjected to random mutagenesis, using an error-prone PCR method. The mutant libraries were screened by *in vivo* bioluminescence assays to identify colour-shifted colonies. This approach mostly led to the isolation of pH-tolerant or red-shifted mutants. Several mutant enzymes with decreased pH sensitivity were purified and studied. We identified single and double substitutions which make bioluminescence spectra nearly pH-insensitive. The structural reasons for the effect of these mutations are discussed.

### Luminescent upconversion nanoparticle (ULNP) with photosensitizing functions to be used for the diagnosis and therapy of cancer

X. Kong,<sup>1</sup> H. Zhang,<sup>2</sup> Y. Sun,<sup>1</sup> Z. Zhao,<sup>1</sup> Y. Qu,<sup>1</sup> Y. Wang<sup>1</sup> and M. Aalders<sup>3</sup>

<sup>1</sup>Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Science, Changchun 130033, People's Republic of China. E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Faculty of Science, Van't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands

<sup>3</sup>University of Amsterdam, Faculty of Medicine, Academic Medical Centre, Laser Centre, University of Amsterdam, The Netherlands

The nanoparticle luminescence upconversion nanoparticle with highly effective upconversion luminescence is synthesized and assembled with the molecules of antibodies and photosensitizer to develop, validate and optimize a highly efficient, economic and convenient method for the early diagnosis and therapy of cancer, based on luminescence upconversion and singlet oxygen generation, and to understand the physical and chemical dynamics involved. For this purpose we employed luminescence upconversion nanoparticles (UN) doped with rare earth ions (UN:RE<sup>3+</sup>), coupled to these molecules of antibody and photosensitizer. Due to the specific interaction of the monoclonal antibody with the antigen, these particles enter selectively into cells with an antigen. Multi-photon IR absorption by RE<sup>3+</sup> results in the emission of light at various wavelengths. The infrared emission will be used to mark the cancer cell for diagnosis, and the upconversion emission will be employed to produce singlet oxygen in a sufficient amount to kill the cancer cell. The peculiar advantages of the proposed method are that cancer cells can be diagnosed and killed by the same UN: RE<sup>3+</sup> particles, while the use of IR excitation to ultimately produce singlet oxygen inside the cell by photosensitization avoids the damage of healthy cells. In addition, the UN into the cancer cell

can be used as cancer imaging via MRI. The results will provide sufficient experience with the technique to step into the *in vivo* situation.

### The interaction of bacterial luciferase with flavinmononucleotide activated by N-methylimidazole on the phosphate group

O. I. Krasnova, N. A. Tyulkova and I. O. Pozdnyakova

Institute of Biophysics, Siberian Branch of Russian Academy of Science, Krasnoyarsk, Russia. E-mail: biotech@ibp.ru

The substrate of bacterial luciferase–flavinmononucleotide was activated by *N*-methylimidazole on the phosphate group. The properties of the derivative of the FMN and its interaction with bacterial luciferase from *Photobacterium leiognathi* was investigated. The activated substrate in non-reduced form modify the enzyme on the SH group by means of increasing its reactionability, leading to irreversible inactivation. The reduced form of the activated FMN derivative possesses different properties, depending on the method of its reduction. When the activated FMNH<sub>2</sub> derivative is photoreduced it does not produce luminescence in reaction with luciferase. Prolonged luminescence of low intensity is observed by using the chemically reduced activated flavin derivative. The activated FMN derivative competes for an active site of enzyme with native FMN. The Michaelis constants and inhibition rate constants of reaction were calculated. It is assumed that differences in the behaviour of the activated flavin derivative are conditioned by intensification of the electronegativity of the phosphate group of the flavin.

### Immobilization of bioluminescent systems and their applications in biosensors

V. A. Kratasyuk,<sup>1,2</sup> and E. N. Esimbekova<sup>1,2</sup>

<sup>1</sup>Siberian Federal University, Pr. Svobodnyi 79, 660041 Krasnoyarsk, Russia. E-mail: VKratasyuk@yandex.ru

<sup>2</sup>Institute of Biophysics, Akademgorodok, 660036 Krasnoyarsk, Russia

A review on immobilization of luminous organisms and their enzymes is presented. Attention was focused upon the methods of immobilization of bacterial and firefly luciferases on sepharose 4B, agarose, nylon, collagen films, modified glass beads, polyacrylic acid, albumin gel and others. Chemical methods of immobilization give better yields of active immobilized luciferases than physical methods; the most effective solid supports have been proved. The immobilized bioluminescent systems as reagents and biosensors are discussed. The stability, sensitivity and precision and the effects of interfering substances and the microenvironment of the enzymes have been examined. Immobilized enzymes have greater stability, sensitivity and re-usability than the soluble forms. Co-immobilization of bacterial luciferase, oxidoreductase and their substrates into a starch gel is referred to as a 'multifunctional immobilized biosensor'. There is great possibility of application of immobilized bioluminescent systems as biosensors for toxicity bioassays or NADH, FMN and aldehydes. This work was supported by Grant No. 07-04-01340-a from the Russian Foundation for Basic Research.

## The gel model for the functioning of enzymes of luminous bacteria in the cell

V. A. Kratasyuk,<sup>1,2</sup> E. N. Esimbekova,<sup>1,2</sup> and I. E. Sukovataya<sup>1</sup>

<sup>1</sup>Siberian Federal University, Pr. Svobodnyi 79, 660041

Krasnoyarsk, Russia. E-mail: VKratasyuk@yandex.ru

<sup>2</sup>Institute of Biophysics, Akademgorodok, 660036 Krasnoyarsk, Russia

To determine the mechanisms that provide joining and functioning of metabolic chains in the cell, four prototypes of experimental modes were developed. They are based on sucrose, glycerol, starch gel and gelatin gel. Coupled systems of two enzymes of luminous bacteria (luciferase and NADH:FMN-oxidoreductase) were reconstructed in solutions of high viscosity (sucrose and glycerol) and also in a matrix structure of starch and gelatin gel. The models manifested bioluminescent activity and simulated the viscous micro-arrangement of enzymes in the matrix and their connections with the membrane structures. Comparative analysis of the characteristics (quantum output, residual enzyme activity, rate of decay of intermediate compounds, pH optimum, thermostability, activation energy, constant of thermoinactivation, and Michaelis constant and some other parameters) of the soluble and immobilized coupled enzyme system in the prototypes of experimental models revealed advantages and disadvantages of the models. The better characteristics of the sucrose and starch models led to their improvement. This work was supported by Grant No. 07-04-01340-a from the Russian Foundation for Basic Research.

## Bioluminescent monitoring of radiotoxicity in solutions of $\alpha$ -radionuclides

N. S. Kudryasheva, T. V. Rozhko, L. G. Bondareva, A. Y.

Bolsunovsky and G. A. Vydryakova

Institute of Biophysics SB RAS, Siberian Federal University, Krasnoyarsk 660036, Russia. E-mail: n\_qdr@yahoo.com

The chronic effects of  $\alpha$ -radionuclides on bacterial bioluminescent (BL) assay systems *in vivo* and *in vitro* were studied. Solutions of radionuclides of different specific activity—<sup>241</sup>Am(NO<sub>3</sub>)<sub>3</sub> and UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>—were used as sources of  $\alpha$ -radiation. BL intensity was used to evaluate the toxicity of the solutions. In americium solutions, the BL activation predominated for short-term exposure (55 h), and BL inhibition for longer-term exposure to radiation. The activation was up to 400% *in vivo* and up to 30% *in vitro*. BL inhibition only was observed in solutions of uranium. The effect of americium was observed under lower concentrations (down to 10<sup>-11</sup> mol/L), than that of uranium (>10<sup>-7</sup> mol/L). The influence of the radionuclides was compared to that of stable metals, e.g. europium and iron; the effects of americium were attributed to its radioactive properties; and the effect of uranium was due to its chemical properties. The possibility of using BL bioassays to evaluate the detoxification efficiency of humic substances in  $\alpha$ -radionuclides solutions is under discussion.

## Miniaturization of luminometric assays: optimizing the performance of common luminescent assays in 384- and 1536-well plate formats

J. Lampinen, M. Raitio and R.-R. Harinen

Microplate Instrumentation, Thermo Fisher Scientific Corp., Vantaa, Finland. E-mail: jorma.lampinen@thermofisher.com

The increasing use of small assay volumes with high-density plates is a clear trend in today's bioscience research. Most of the commercial assay kits are still designed for 96-well plates and all instructions are given for that plate format. This paper shows in general principle how luminometric assays can be adapted into standard 384-, 384- low volume or 1536-well formats and how assay performance can be optimized with these high-density plates. The effect of plate format on the central performance characteristics is discussed, based on, for example, the assay's detection limit, linear dynamic range and cross-talk. The results include data from the most common luminometric assays, such as flash-and-glow-type ATP quantitation assays, firefly and *Renilla* luciferase assays as well as *Aequorin* luminescence assay. In addition, the effects of assay volume and plate design are discussed. This paper also discusses the key requirements for the instrumentation used in these miniaturized luminescent assays.

## Chemiluminescence flow-through biosensor for hydrogen peroxide based on enhanced HRP activity by Au nanoparticles

D. Lan and B. Li

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China. E-mail: libaoxin@snnu.edu.cn

A novel chemiluminescence (CL) flow-through biosensor for hydrogen peroxide is described. It was prepared by immobilizing horseradish peroxidase (HRP) and Au nanoparticles with the sol-gel method in a flow CL cell. Hydrogen peroxide was sensed by the CL reaction of luminol-H<sub>2</sub>O<sub>2</sub>-HRP. In the presence of Au nanoparticles, the biosensor response is enhanced 50-fold. The calibration graph was linear in the range 1 × 10<sup>-8</sup>–1 × 10<sup>-6</sup> mol/L, and the detection limit was 4 × 10<sup>-9</sup> mol/L. A complete analysis could be performed in 1 min with a relative standard deviation of 4% for 5 × 10<sup>-7</sup> mol/L H<sub>2</sub>O<sub>2</sub> (n = 7). The proposed biosensor exhibited high sensitivity, easy operation, low cost and simple assembly.

## Improvement in carbaryl assay by fluorescence in micellar medium

S. H. Lee,<sup>1</sup> C. W. Jeon,<sup>2</sup> W. H. Kim,<sup>1</sup> H. Y. Chung,<sup>1</sup> S. M. Wabaidur,<sup>1</sup> H. W. Park<sup>1</sup> and Y. S. Suh<sup>1</sup>

<sup>1</sup>Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: whkim07@knu.ac.kr

<sup>2</sup>Korea Institute of Geoscience and Mineral Resources, Daejeon 305-350, Korea

Carbaryl (CA) is the common chemical name for an insecticidal molecule which was first introduced in 1956 under the registered trade name 'Sevin' by Union Carbide. CA, one of the carbamate pesticides, has been extensively used for pest control because of its low persistence and high effectiveness. It is used for the control of pests in fruit, vegetables, forage, cotton and other crops and domestic animals. Its wide use is due to its volatility, low solubility in water, relatively short half-life, and relatively low toxicity. However, its acute toxicity is of great concern and makes necessary the determination of CA residues



at low concentrations. Spectrofluorimetric methods were developed for the determination of CA. A simple, rapid and highly sensitive fluorimetric method for the determination of CA was described. The method was based on the formation of the micelle. Under the optimum conditions, the fluorescence intensity responds linearly to CA concentration in the range 0.1–20.1  $\mu\text{g/mL}$ , with a detection limit of  $2.3 \times 10^{-3} \mu\text{g/mL}$ . The method was successfully applied to the determination of CA in real samples.

### Micelle-enhanced fluorimetric determination of benserazide in pharmaceutical formulation

S. H. Lee,<sup>1</sup> W. H. Kim<sup>1</sup> and K. Meea<sup>2</sup>

<sup>1</sup>Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: whkim07@knu.ac.kr

<sup>2</sup>Department of Environmental Engineering, Andong National University, Andong 760-749, Korea

A fluorimetric method has been developed for the determination of benserazide, in pharmaceutical formulation. The influence of micellar medium on the fluorescent excitation and emission spectra character of benserazide was studied. The anionic surfactant of SDS showed a strong sensitizing effect for the fluorescence of benserazide in a pH 5.0 buffer. Possible reasons for this micellar-induced enhanced fluorescence were discussed. Under optimum conditions, the linear range was  $1 \times 10^{-7}$ – $1 \times 10^{-4} \text{ mol/L}$ . The detection limit was  $3.5 \times 10^{-8} \text{ mol/L}$  ( $\lambda_{\text{ex}} = 279 \text{ nm}$ ,  $\lambda_{\text{em}} = 318 \text{ nm}$ ). The proposed method can be applied to the assay of benserazide in real samples with good results.

### Sensitive fluorimetric determination of acetylsalicylic acid based on interaction with 18-crown-6

S. H. Lee, H. W. Park and M. A. Khan

Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: adsl789@knu.ac.kr

Acetylsalicylic acid (ASA), more popularly known as aspirin, is one of the most widely used drugs in the world. This drug has superior qualities as an antipyretic and general analgesic, but more specifically in the relief of headaches, muscular pain, colds and respiratory diseases, rheumatoid arthritis, etc. A large number of analytical methods for the determination of ASA in biological fluids and pharmaceuticals have been published. In this study, a sensitive fluorimetric determination of ASA is proposed. The developed procedure is based on the complexation of ASA with 18-crown-6, yielding an enhanced fluorimetric signal ( $\lambda_{\text{ex}} = 305 \text{ nm}$ ,  $\lambda_{\text{em}} = 421 \text{ nm}$ ). The calibration plot was linear in the range  $1 \times 10^{-6}$ – $3 \times 10^{-4} \text{ mol/L}$  ASA. The detection limit of the determination was  $1.5 \times 10^{-7} \text{ mol/L}$ . No interference was found from the excipients usually used in pharmaceutical formulations. The method was successfully applied to the determination of ASA in drug samples.

### Determination of enoxacin in pharmaceutical formulations by solid-phase fluorescence spectroscopy

S. H. Lee,<sup>1</sup> H. W. Park,<sup>1</sup> M. A. Khan,<sup>1</sup> H. Y. Chung,<sup>1</sup> Y. S. Suh,<sup>1</sup> S. M. Wabaidur<sup>1</sup> and J. H. Choi<sup>2</sup>

<sup>1</sup>Department of Chemistry, Kyungpook National University, Taegu 702-701, Korea. E-mail: adsl789@knu.ac.kr

<sup>2</sup>Department of Chemistry, Andong National University, Andong, 760-749, Korea

Enoxacin (Enx), a new generation of fluorinated quinolone is a new broad-spectrum antibacterial drug active against most Gram-negative and Gram-positive bacteria and some anaerobes. A rapid and simple solid-phase fluorescence spectroscopic batch procedure was developed for the determination of Enx. The procedure is based on the sorption of Enx on Sephadex DEAE A-25 anion exchanger gel (100 mg) by equilibration from an aqueous solution (25 mL) for 10 min; the equilibrated gel is transferred into a 1 mm quartz cell and the native fluorescence of Enx sorbed on it is directly measured at 416 nm when excited at 354 nm. Linearity was found in the  $1 \times 10^{-6}$ – $1 \times 10^{-4} \text{ mol/L}$  range for a 25 mL sample volume, with RSD of 2.75%. The procedure was successfully applied to the determination of Enx in pharmaceutical formulations.

### The structures of bioluminescence proteins

J. Lee<sup>1</sup> and E. S. Vysotski<sup>1,2</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA. E-mail: jlee@uga.edu

<sup>2</sup>Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Krasnoyarsk 660036, Russia

In the last decade, the spatial structures of a number of proteins involved in bioluminescence have been determined, from the bioluminescent bacteria, the non-fluorescent protein, luciferase, FMN: NADH reductase, firefly luciferases, many variants of green fluorescent proteins, the photoproteins aequorin and obelin, dinoflagellate luciferase, *Renilla* luciferase and its coelenterazine binding protein. In general, there is no structural relationship among any of these bioluminescence proteins; instead, structural homology is found with proteins that have unrelated chemical activities. This is consistent with the fact that the bioluminescence property arose independently many times in post-Cambrian evolution, after the evolution of vision and the neural processes providing responses to visual signals, and by adaptation and selection of accessible chemistry.

### Spectrometric studies of morphine chloride alkaloid binding to double-stranded DNA

J. Li and C. Dong

Institute of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: dc@sxu.edu.cn

The mode and mechanism of interaction of morphine chloride, an important alkaloid compound, with calf thymus deoxyribonucleic acid (ctDNA) was investigated by absorption and fluorescence titration techniques. The hypochromic effect was founded on the absorption spectra of morphine when the concentration of

ctDNA was increased. The decreased fluorescence study revealed non-cooperative binding of the morphine to ctDNA with an affinity of  $3.94 \times 10^3$  mol/L, and the stoichiometry of binding was characterized to be about one morphine molecule for each base pair. Stern–Volmer plots at different temperatures proved that the quenching mechanism was static. A ferrocyanide quenching study showed that the magnitude of Ksv of the bound morphine was lower than that of the free morphine. In addition, it was found that ionic strength could affect the binding of morphine and ctDNA. Fluorescence polarization and denatured ctDNA studies were also performed to prove the binding mode. As observed from all above techniques, the intercalation was supported as the binding mechanism of morphine and ctDNA, and it was estimated that the morphine molecules were partially intercalated between alternate base pairs of ctDNA.

### Luminescence studies of singlet oxygen production in ZnPcS<sub>2</sub>P<sub>2</sub>

B. Li, Z. Chen, L. Lin and S. Xie

Key Laboratory of OptoElectronic Science and Technology for Medicine, Fujian Normal University, Ministry of Education, Fuzhou 350007, People's Republic of China.

E-mail: bhli@fjnu.edu.cn

The major cytotoxic agent with most current photosensitizers used in photodynamic therapy (PDT) is widely believed to be singlet oxygen (<sup>1</sup>O<sub>2</sub>). Direct measurement of <sup>1</sup>O<sub>2</sub> from photo-irradiation of a new hydrophobic photosensitizer, disulphonated dipthalimidomethyl phthalocyanine zinc (ZnPcS<sub>2</sub>P<sub>2</sub>), in air-saturated methanol (MeOH) was performed by the detection of its near-infrared luminescence decay. The maximal <sup>1</sup>O<sub>2</sub> luminescence occurred at about 1277 nm, with a half-width of 20 nm. The <sup>1</sup>O<sub>2</sub> quantum yield (Φ<sub>A</sub>) value of ZnPcS<sub>2</sub>P<sub>2</sub> in MeOH, about  $0.16 \pm 0.02$ , was determined from a comparative study using a reference of Rose Bengal, which has the <sup>1</sup>O<sub>2</sub> quantum yield of 0.76 in MeOH. Additionally, the triplet-state and <sup>1</sup>O<sub>2</sub> lifetimes could be estimated by exponential fitting as  $0.36 \pm 0.07$  and  $10.49 \pm 0.90$  μs from the time-resolved <sup>1</sup>O<sub>2</sub> luminescence spectrum. This study suggests that the <sup>1</sup>O<sub>2</sub> could be the major cytotoxic species for ZnPcS<sub>2</sub>P<sub>2</sub>-mediated PDT applications.

### Magnetic bead-based chemiluminescence detection of sequence-specific DNA with HRP labelling

H. Li and Z. He

College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, People's Republic of China.

E-mail: zhkhe@whu.edu.cn

A new magnetic bead-based chemiluminescence (CL) method has been developed for DNA hybridization analysis with HRP labelling and the luminol–PIP–H<sub>2</sub>O<sub>2</sub> system. This method was used for the detection of the 31-base sequence related to the avian influenza A H1N1 virus, with a good linear correlation in the concentration range 0.3–300 pmol/L, along with a correlation equation of  $\log I = 1.241 + 1.075 \log C$  ( $R = 0.9990$ ) and a detection limit of 0.1 pmol/L. The sensitivity can be further enhanced through amplifying labels. Moreover, the complementary DNA target and the single-base mismatched DNA strand can be markedly differentiated by controlling the temperature, which indicates that the proposed protocol can be applied for single nucleotide polymorphism analysis. Also, the results can be determined by CL imaging and with high sensitivity, relying on the properties of the CL system.

### Development of dielectric barrier discharge induced chemiluminescent detector for gas chromatography

Y. Li,<sup>1</sup> Y. He,<sup>1</sup> X. Hou<sup>2</sup> and Y. Lv<sup>1</sup>

<sup>1</sup>College of Chemistry, Sichuan University, Chengdu 610064, People's Republic of China. E-mail: lvy@scu.edu.cn

<sup>2</sup>Analytical and Testing Centre, Sichuan University, Chengdu 610064, People's Republic of China

Dielectric barrier discharge (DBD) plasma, a typical non-equilibrium high voltage gas discharge, has been widely used in industry, owing to its attractive characteristics—excellent dissociation capability for molecular species and capability of working at atmospheric pressure. Recently, developments on an atmospheric pressure dielectric barrier discharge-induced chemiluminescent detector (DBD-CL) for gas chromatography have been carried out in our laboratory. The proposed detector is based on the phenomenon that the split products via DBD plasma react with luminol to produce intensive CL emission. Several typical volatile organic compounds (VOCs), including alcohol, aldehyde, chlorinated hydrocarbons, benzene hydrocarbons and ethers, have been investigated. The results showed that the CL signals and signal:noise (S:N) ratios greatly depended on the discharge intensity. Under optimized conditions, the system presented good performance for the investigated typical compounds, and limits of detection down to the sub-nanogram were achieved. This detector possesses the advantages of sensitivity to many VOCs, simple construction to fabricate in small size, and very low power consumption of less than 5 W, indicating that it is a promising methodology for gas chromatography.

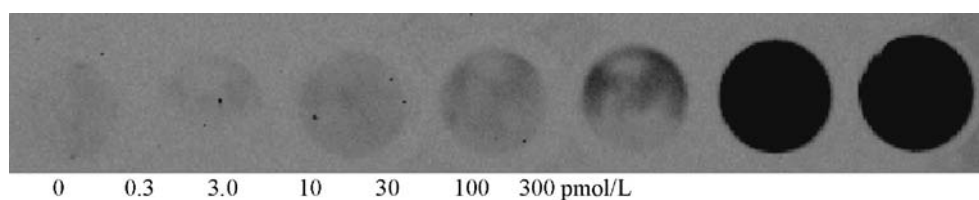


Figure 1. CL images of different concentrations of the target DNA.

### A novel method for the determination of furazolidone by flow-injection post-chemiluminescence

X.-G. Li, J.-W. Hu and H.-Y. Han

College of Science, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China. E-mail: hyhan@mail.hzau.edu.cn

The post-chemiluminescence (PCL) reaction was observed when furazolidone was injected into the reaction mixture after the completion of the CL reaction between luminol solution and hydrogen peroxide solution. A novel method for the determination of furazolidone was established by flow-injection PCL. The CL intensity responded linearly to the concentration of furazolidone in the range  $1.0 \times 10^{-7}$ – $1.0 \times 10^{-5}$  g/mL, with a linear correlation of 0.9949. The detection limit for furazolidone was  $2.7 \times 10^{-8}$  g/mL (S:N = 3). The relative standard deviation (RSD) was 6.4% ( $n = 11$ ,  $c = 1.0 \times 10^{-6}$  g/mL). The proposed method has been applied to the determination of furazolidone in pork tissue and the results were consistent with those obtained by the HPLC–UV method. A possible mechanism for the PCL reaction was also discussed by the investigation of CL kinetic characteristics, CL spectra and UV-Vis absorption spectra.

### Study on the interaction between human serum albumins and 7-ethyl-10-hydroxycamptothecin

G.-Z. Li and Y.-M. Liu

Chemical College, Yantai University; Yantai, Shandong 264005, People's Republic of China. E-mail: liuyongming100@126.com

The binding reaction between 7-ethyl-10-hydroxycamptothecin (EHC) and human serum albumins (HSA) were studied by fluorescence, UV-Vis absorption spectra and  $^1\text{H}$ -NMR spectra. The results indicated that their binding reaction was a single static quenching process; EHC strongly bound HSA and the binding equilibrium constant  $K_0 = 2.22 \times 10^3$  mol/L. The shortest binding distance ( $r$ ) and energy transfer efficiencies ( $E$ ) between donor (HSA) and acceptor (EHC) were obtained by Förster's non-radiative energy transfer mechanism ( $r = 2.94$  nm;  $E = 0.35$ ). The enthalpy change ( $\Delta H$ ) and entropy change ( $\Delta S$ ) were calculated at 25°C and 37°C. The results indicated that hydrophobic and dipole forces played a major role in the reaction. The results of the  $^1\text{H}$ -NMR spectrum indicated that the 10-OH and 20-OH of EHC binds with the amino acid residue of HSA.

### Kinetic spectrophotometric method determination of sulphite based on its additional reaction with pararosaniline

Z.-P. Li, X.-W. Ma, S.-M. Shuang and C. Dong

Research Centre of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: dc@sxu.edu.cn

A kinetic spectrophotometric assay method for the determination of sulphite was described. The method involved a reaction with pararosaniline (PRA) and sodium sulphite in phosphate buffer solution to form a stable complex. In the presence of sulphite, the reagent displayed an obvious change of absorbance at 540 nm. From the colour developing reactions of PRA and sulphite, the

kinetic reactions between both of them in aqueous solutions were studied, using spectrophotometry. Light absorbances in the visible spectral range were measured as a function of mole fractions of PRA at a fixed sulphite concentration and as a function of the mole fraction of sulphite at a fixed PRA concentration at set time intervals. Optimum conditions of the reaction were established as pH 6.0 at 540 nm and 25°C. When the reaction of pararosaniline was investigated, it was observed that the following rate formula and rate constant were found:  $v = k[\text{SO}_3^{2-}]^{1/2}[\text{PRA}]^{1/2}$ ,  $k = 2.33 \times 10^{-2}/\text{s}$ . The absorptions at 540 nm are linearly proportional to sulphite concentrations over the range 0.0126–1260  $\mu\text{g/mL}$ , with a detection limit of 0.08  $\mu\text{g/mL}$ , and the standard deviation for 12.6  $\mu\text{g/mL}$  sulphite was 1.2%. The interferences were studied by most common ions. The method has the advantages of wide determination range, good sensitivity and no noxious reagent (formaldehyde). The method is successfully applied to determine the sulphite contents of some beers. The results compare fairly well with data obtained using the standard method.

### Flow-injection chemiluminescence determination of thiamine by enhancement of the luminol–ferricyanide system

Y. Li,<sup>1</sup> Y. Yang<sup>1</sup> and J. Lu<sup>2</sup>

<sup>1</sup>School of Science, Xi'an Jiaotong University, Xi'an 710049, People's Republic of China. E-mail: liyh@mail.xjtu.edu.cn

<sup>2</sup>School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China

Thiamine is an essential nutrient for humans to maintain carbohydrate metabolism and normal neural activity and to prevent beri-beri. The use of chemiluminescence (CL) detection in pharmaceutical analysis involving the assay of active components in dosage forms represents a fairly selective and sensitive technique, which requires relatively simple instrumentation. However, the use of a CL method for the determination of thiamine is rather rare. In this study, a simple and sensitive FI–CL method for the determination of thiamine was devised. It was found that the CL emission generated from the oxidation of luminol with  $\text{K}_3\text{Fe}(\text{CN})_6$  could be enhanced significantly by thiamine. The experimental conditions that affected the CL reaction were carefully optimized. Under optimum conditions, the CL intensity was proportional to thiamine concentration over the range  $3.0 \times 10^{-8}$ – $1.0 \times 10^{-6}$  g/mL, with a detection limit of  $7 \times 10^{-9}$  g/mL. The RSD for  $5.0 \times 10^{-7}$  g/mL thiamine solution was 1.1% in 11 replicate measurements. The method was applied to the determination of thiamine in pharmaceutical preparations and compared well with the *Chinese Pharmacopoeia*.

### Photophysical and photochemical properties of photosensitizers in China

B. Li, S. Yu, L. Lin and S. Xie

Key Laboratory of OptoElectronic Science and Technology for Medicine, Fujian Normal University, Ministry of Education, Fuzhou 350007, People's Republic of China. E-mail: bhli@fjnu.edu.cn

Photodynamic therapy (PDT) is a modality for treating a variety of malignant and non-malignant conditions, based on the use of

light-activated photosensitizers. The photophysical and photochemical properties of newly developed photosensitizers in China, mainly including haematoporphyrin derivative (HpD), protoporphyrin IX (PpIX), photocarcinorin (PsD-007), haematoporphyrin monomethyl ether (HMME) and disulphonated diphtalimidomethyl phthalocyanine zinc ( $\text{ZnPcS}_2\text{P}_2$ ), were comparatively studied by measuring their molar extinction coefficients, fluorescence excitation–emission matrices and fluorescence quantum yields. The present results present the scientific basis for the selection of specific photosensitizers, and the determination of optimal excitation and emission wavelengths for photosensitizer-based fluorescence diagnosis.

### Study of the interaction between CdTe quantum dots and silver(I) ions with spectroscopic techniques in pure water solution

J. G. Liang, J. Wang and H.-Y. Han

College of Science, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China. E-mail: hyhan@mail.hzau.edu.cn

Study of the interaction between quantum dots (QDs) and metal (I) ions is not only helpful in developing the method for the determination metal (I) ions, but also provides good reference for the preparation of nanocomposites. In this paper, the interaction of CdTe QDs with silver (I) ions was investigated by fluorescence, UV-Vis and Raman spectroscopic techniques in pure water solution. The results showed that the fluorescence intensity of CdTe QDs was enhanced when the CdTe QD concentration was low. With increasing silver (I) ions concentration, an obvious quenching and red-shift was observed. The effect of QD size on the interaction was discussed. It was found that small sizes of QDs were more easily quenched by silver (I) ions, due to their higher surface energy. According to the above results and some reports in the literature, an interaction mechanism was also provided. When the silver ions were added to the CdTe solution, silver (I) ions could not only bind to the surface of CdTe QDs, but also coordinate with the thiols on the surface of CdTe QDs, which was further proved by the UV-Vis and Raman spectra.

### Fluorescence properties of haematoporphyrin monomethyl ether in cells *in vitro* and tissues *in vivo*

H. Lin, Y. Lv, B. Li and S. Xie

Key Laboratory of OptoElectronic Science and Technology for Medicine, Fujian Normal University, Ministry of Education, Fuzhou 350007, People's Republic of China. E-mail: bhli@fjnu.edu.cn

Haematoporphyrin monomethyl ether (HMME) is a novel and promising porphyrin-related photosensitizer that was developed first in China for photodynamic therapy and photodiagnosis. Steady-state and time-resolved fluorescence properties of HMME in phosphate-buffered saline (PBS), and in poorly differentiated human nasopharyngeal carcinoma CNE2 cells *in vitro* and nude mice bearing CNE2 tumour xenografts *in vivo*, were studied using an Edinburgh FLS 920 spectrofluorimeter. The fluorescence

emission peaks of HMME occur at about 625 and 690 nm *in vivo*, respectively, which has a red shift of 10 nm as compared to the emission peaks in solution and in cells. The fluorescence lifetime of HMME at 690 nm is about  $16.5 \pm 0.32$  ns in CNE2 cells and  $14.85 \pm 0.36$  ns in tumour model *in vivo*, respectively. These results provide basic reference information for optical diagnostic applications of HMME.

### Resonance Rayleigh scattering method for determination of alginic sodium diester with methylene blue

Y.-M. Liu and G.-Z. Li

Chemical College of Yantai University, Yantai 264005, People's Republic of China. E-mail: liuyongming100@126.com

Alginic sodium diester (ASD; more explicitly, propyl alginate and sulphate of sodium) is made from sodium alginate as the fundamental material extracted from seaweed. It is an important medicine developed from the marine economy in China. There is no report about the determination of ASD using the resonance Rayleigh scattering (RRS) method. We have investigated the interaction between methylene blue (MB), a kind of quinoneimine cationic dye, and ASD in acidity solution (pH 2.3). We found that when MB reacts with ASD to form an ion-association complex by virtue of electrostatic and hydrophobic interaction forces, the intensity of RRS enhances greatly and a new RRS spectrum appears. The intensity of RRS at 545 nm and ASD in the concentration range 0.5–5  $\mu\text{g/mL}$  obey a linear relationship. The method has high sensitivity and good selectivity, with a detection limit of 0.003  $\mu\text{g/mL}$ . Therefore, a simple, rapid, highly sensitive and selective method was developed to determine ASD. Meanwhile, we found that the formation of a MB–ASD ion-association complex causes the fading of MB solution, which can also be used to determination of ASD.

### Long-term direct and short-term indirect effects of ultraviolet-B radiation on photosystem II of spinach: a fluorescence spectrum analysis

X. Liu,<sup>1</sup> M. Yue,<sup>1</sup> Q. Ji<sup>1</sup> and J. He<sup>2</sup>

<sup>1</sup>The College of Life Science, Northwest University, Xi'an 710069, People's Republic of China. E-mail: little-ix@sohu.com

<sup>2</sup>State Key Laboratory of Transient Optics and Photonics; Xi'an Institute of Optics and Precision Mechanics; Chinese Academy of Sciences; Xi'an 710068, People's Republic of China

To clarify the influence of UV-B radiation on energy transformation of photosynthesis, the effects of UV-B radiation on photosystem II (PSII) from spinach was studied at room temperature, using a steady-state fluorescence spectrum. Deconvolution analysis was employed to better identify the spectral changes resulting from UV-B radiation. It was found that even a very low radiation dose (15  $\mu\text{W/cm}^2$ ) and shorter irradiance time (30 min) of direct UV-B irradiation could lead to clear fluorescence quenching of PSII and, simultaneously, the energy transformation pathway in the antenna system was also altered by direct UV-B irradiation. However, if plants were irradiated with UV-B for 7 days, no fluorescence quenching of



PSII separated from the leaves of irradiated plants was detected. It was presumed that plants under enhanced UV-B radiation could organize an adjustment mechanism in the growth process to guarantee that photosynthesis was carried on normally, such as increasing the fluorescence emission proportion of energy transmission to the reaction centre, changing the energy transfer pathway, especially the pathway carried by carotenoid. It was concluded that long-term direct or short-term indirect effects of UV-B radiation could change the energy assignment between PSII and photosystem I (PSI).

### Enhancement of thermostability of *Luciola mingrelica* firefly luciferase by mutagenesis of non-conservative residues Cys62 and Cys146

G. Y. Lomakina, Y. A. Modestova and N. N. Ugarova  
Department of Chemistry, Lomonosov State University, Moscow 119991, Russia. E-mail: lomakina@enz.chem.msu.ru

Mutant forms Cys146Ser and Cys62Ser of *L. mingrelica* firefly luciferases were obtained. Point mutations did not alter its catalytic and spectral properties but led to essential enhancement of the enzyme's stability. Investigation of thermoinactivation at 37°C demonstrated the two-step kinetics of the inactivation of the wild-type enzyme and its mutant forms. Dithiotreitol (DTT) decreased on  $k_{in}$  of the second step of the wild-type enzyme inactivation and did not change  $k_{in}$  of the mutant forms. It can be supported that free SH-groups of Cys62 and 146 located on the surface of the luciferase molecule are responsible for oxidative inactivation. Moreover, mutations resulted in the several-fold improvement of enzyme thermostability on both stages of the inactivation, independently of DTT presence. The enzyme stabilization was achieved not only by elimination of the oxidative inactivation effect but also due to the stabilization of the protein structure. Thus, the significance of the non-conservative Cys62 and 146 in *L. mingrelica* firefly luciferase inactivation was established.

### Effects of metal ions on peroxynitrite nitrifying protein

Y. Luo, S. Cui, L. Zhang and R. Zhong  
College of Life Science and Bioengineering, Beijing University of Technology, Beijing 100022, People's Republic of China

Many kinds of metal ions, existing in human organisms, have positive effects on the peroxynitrite nitrifying tyrosine residuals of protein, enhancing the risk of correlative illnesses. With a 3D-fluorescence spectra method and outputs of nitration products as targets, seven salts of metal ions ( $MgCl_2$ ,  $MnCl_2$ ,  $CaCl_2$ ,  $ZnSO_4$ ,  $CuSO_4$ ,  $CoCl_2$ ,  $NiCl_2$ ) were selected to assist in peroxynitrite damaging tyrosine and tryptophan. In the presence of Co(II) and Cu(II), the fluorescence absorption of the nitrated fibrinogen and bovine serum albumin decreased from 9900 to 1915 and from 9686 to 1101, respectively. This result was in accordance with the sharply increased nitrated products of tyrosine. As there were increased nitrated products and decreased dimer products, it was proved that the reactions leading to 3-NT

and di-Tyr had different reaction principles. On the basis of these experiments, we concluded that Cu(II) and Co(II) enhanced the modification of tyrosine induced by peroxynitrite, and led to conformation change of the protein. Also, the positive effects of nitration was dependent on Cu(II) and Co(II)'s concentrations.

### Study of the direct determination of lomefloxacin in urine with aluminium(III)-lomefloxacin-SDS system by synchronous fluorimetry

H. Ma, R. Tian, X. Sun and X. Liu  
College of Chemistry and Chemical Engineering, Yan'an University, Yan'an 716000, People's Republic of China.  
E-mail: Mahy6614@hotmail.com

Lomefloxacin is one of the synthetic antibacterial fluoroquinolone agents of the third generation. It has a good effect in clinical treatment. The determination of its content in various biological samples is of great importance. This work reports a simple and sensitive synchronous fluorescence method for the determination of lomefloxacin in human urine, based on the fluorescence characteristics of lomefloxacin-aluminium(III) in a micellar system. It has been found that the coexistence of aluminium(III) and sodium dodecyl sulphate (SDS) can enhance the fluorescence intensity of lomefloxacin remarkably in pH 3.8 HAc-NaAc buffer solution. When the wavelength interval ( $\Delta\lambda$ ) is 90 nm for scanning, the background interference from the urine blank can be effectively eliminated. The different experimental parameters that affect the fluorescence intensity were carefully studied. The linear range was 0.008–8.0  $\mu g/mL$ , with a detection limit of 0.0041  $\mu g/mL$ ; the RSD was 2.1%. The method was satisfactorily applied to the determination of lomefloxacin in human urine samples. Recovery was in the range 97.5–102%.

### A micellar enhanced-sensitive chemiluminescence method for the determination of ofloxacin by flow-injection analysis

H. Ma, Y. Zhang, L. Miao, X. Sun and Z. Zhang  
College of Chemistry and Chemical Engineering, Yan'an University, Yan'an 716000, People's Republic of China.  
E-mail: Mahy6614@hotmail.com

Ofloxacin is a synthetic fluoroquinolone antibiotic. It has a good effect in clinical treatment. The determination of ofloxacin has attracted much attention. In this paper, it was found that the chemiluminescence emission from the oxidation of luminal by  $H_2O_2$  in alkaline medium can be enhanced by ofloxacin. When the mixed solution of ofloxacin with sodium dodecyl sulphate in NaAc-HAc medium was injected into the above system instead of ofloxacin itself, the chemiluminescence signal can be apparently improved without any additional blank signal. Based on this, a highly sensitive flow-injection chemiluminescence method for the determination of ofloxacin in micellar medium was established. The different experimental parameters that affect the chemiluminescence intensity were investigated. The linear range was  $4.2 \times 10^{-12}$ – $3.6 \times 10^{-9}$  g/mL, with a detection limit of  $2.6 \times 10^{-12}$  g/mL; RSD was 2.0% ( $n = 11$ ).

Recovery was 98.5–101%. The proposed method has been applied successfully to the determination of ofloxacin in tablets and injections, and the results agree well with those obtained by an official method.

### Obelin mutants with altered affinity to calcium and bioluminescence colour

N. P. Malikova and E. S. Vysotski

Photobiology Laboratory, Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Krasnoyarsk 660036, Russia. E-mail: npmal@yandex.ru

Since the concentration of free calcium within cell compartments varies noticeably and calcium transients in those compartments run in different manners, it is desirable to have calcium indicators which would have various sensitivities to calcium and which would allow simultaneous measurement of  $[Ca^{2+}]$  in different cell compartments. Here we report the properties of some obelin mutants that simultaneously have the altered bioluminescence spectra and calcium affinity. All substitutions were done based on the 3D structures of obelin ligand-dependent conformational states. The mutants display a good bioluminescent activity and the physiological concentration of  $Mg^{2+}$  has no effect on their sensitivity to  $Ca^{2+}$ . Therefore, they hold much promise for the development of dual-wavelength methods for synchronous monitoring of  $Ca^{2+}$  transients in different cell compartments, to reveal how the local changes in  $[Ca^{2+}]$  switch the exogenous and endogenous stimuli to the corresponding cell response. Supported by: Grant No. Mκ-1963.2005.4 of the President of Russian Federation; CRDF Grant No. Y4-02-05; the Lavrentiev Grant for Young Scientists of the SB RAS; and RFBR Grant No. 06-04-08076.

### Distribution of luminescence in Ophiuroidea (Echinodermata)

J. Mallefet,<sup>1</sup> F. Quevy<sup>1</sup> and T. O'Hara T<sup>2</sup>

<sup>1</sup>Marine Biology Laboratory, Catholic University of Louvain, 3 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium.

E-mail: Jerome.mallefet@uclouvain.be

<sup>2</sup>Museum Victoria, GPO Box 666, Melbourne 3001, Australia

Although bioluminescence is well known in Echinoderms, it is still poorly understood in terms of phylogenetic and biogeographic distribution. This amazing capability has been mostly studied in the Ophiuroidea over the last 15 years, but on a limited number of species. Recently, a comparative study on brittlestar luminescence was initiated and analyses were conducted after a large sampling effort. The results indicated that, out of 195 species, the total number of known luminous species increased from 33 in 1995 to 64 in 2007. They were mainly collected on hard substrata in deep water, where luminescence appeared more intense. Although brittlestar luminescence was observed in tropical and temperate waters from shallow to abyssal depths, it was least prevalent on tropical coral reefs. Analysis is in progress to try to highlight a possible link between luminescence and ophiuroid phylogeny. New field surveys in a variety of marine regions and habitats will be organized to increase the number of ophiuroid species tested.

This is necessary also in order to understand why so many brittle stars glow in the dark.

### Nervous control of luminescence in *Ophionereis schayeri* (Ophiuroidea, Echinodermata)?

J. Mallefet,<sup>1</sup> J. Renard J<sup>1</sup> and T. O'Hara<sup>2</sup>

<sup>1</sup>Marine Biology Laboratory, Catholic University of Louvain, 3 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium.

E-mail: Jerome.mallefet@uclouvain.be

<sup>2</sup>Museum Victoria, GPO Box 666, Melbourne 3001, Australia

Among echinoderms, luminescence control mechanisms have been mainly studied in the class Ophiuroidea. Initial research performed on *Amphipholis squamata* and *Amphiura filiformis* suggested the preponderance of cholinergic control mechanisms. Within the framework of a comparative study of ophiuroid luminescence, a research programme was developed on *Ophionereis schayeri*, a common southern Australian luminous brittle star. Results show that  $\gamma$ -aminobutyric acid (GABA) is the main neurotransmitter involved in light emission. GABA triggers light emission through the activation of the GABA<sub>B</sub> receptor's sub-type located on the membrane of the luminous cells. Nevertheless, acetylcholine induced a weaker luminescence, probably via muscarinic cholinergic receptors. Furthermore, GABA, besides occupying the role of main neurotransmitter, seems to act as a positive neuromodulator of the cholinergic response. The results of this research reinforce the idea that many neurotransmitters and neuromodulators are involved in the control of ophiuroid luminescence.

### The function of conserved cystein residues in the bioluminescence of coelenterazine-dependent luciferase from *Metridia longa*; testing with site-directed mutagenesis

S. V. Markova, G. A. Stepanyuk and E. S. Vysotski

Photobiology Laboratory, Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia. E-mail: svetlana@ibp.ru

Recently, several secreted luciferases from the copepod *Metridia longa* (MLuc) were cloned and one of them was successfully used as a bioluminescent reporter enzyme in mammalian cells. MLuc is a small Cys-rich protein, which most likely holds intramolecular disulphide bonds. Here we report the results of mutational analysis of conserved 10 Cys residues of MLuc, which were revealed from the protein alignment. All cysteines are found within two non-identical 31 amino acid repeats (five in each). To estimate the role of these Cys residues in bioluminescence, each one was substituted by both Ala and Ser using site-directed mutagenesis. Practically all mutations shift the temperature optimum of MLuc bioluminescence to 0–4°C and result in decreasing bioluminescent activity to a variable degree. However, the replacements of the last Cys residues in the repeats led to almost complete loss of MLuc bioluminescent activity. This suggests the crucial role of these two Cys residues for enzyme catalytic activity. The possible function of cysteines in maintaining structural stability is discussed. Supported by RFBR Grant No. 05-04-48271 and RFBR-Taiwan NSC Grant No. 89502.

### Effect of nucleophilic acylation catalysts on luminol chemiluminescence

E. Marzocchi,<sup>1</sup> S. Grilli,<sup>1</sup> L. Della Ciana,<sup>1</sup> M. Mirasoli,<sup>2</sup> P. Simoni,<sup>3</sup> L. Prodi<sup>4</sup> and A. Roda<sup>2</sup>

<sup>1</sup>Cyanagen s.r.l., via Stradelli Guelfi 40/c, 40138 Bologna, Italy.  
E-mail: dellaciana@cyanagen.it

<sup>2</sup>Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

<sup>3</sup>Department of Internal Medicine and Gastroenterology, University of Bologna, via Massarenti 9, 40138 Bologna, Italy

<sup>4</sup>Department of Chemistry 'G. Ciamician', University of Bologna, Via Selmi 2, 40126 Bologna, Italy

A new chemiluminescence cocktail for horseradish peroxidase detection, based on the enhancer–luminol–oxidant system, has been developed by incorporating an acylation catalyst. This study demonstrates that the addition of the new component produces a very significant increase in light output, which is particularly evident in association with phenothiazine enhancers. This effect lowers the limit of detection of horseradish peroxidase from 50 to 8 amol, which can be translated into a corresponding improvement in sensitivity in immunoenzymatic chemiluminescent assays, such as Western blots, dot–blots and 'sandwich' ELISA.

### Web resource 'Bioluminescence and Luminous Organisms' of the IBSO culture collection

S. E. Medvedeva, D. A. Kotov and E. K. Rodicheva  
Institute of Biophysics of the Siberian Branch of the Russian Academy of Science, 660036 Krasnoyarsk, Russia.  
E-mail: ccibso@ibp.ru

The web resource 'Bioluminescence and Luminous Organisms' was developed to provide an important channel of information interchange in the global network on different aspects of bioluminescence and luminescent organisms. Bioluminescence is the basic attribute required to include the data on the phenomenon or organisms in this information system. The resource is intended for gathering all the analyses in one available source of full information on organisms with bioluminescent systems. The information system contains several sections: history of bioluminescence studies; fundamental knowledge about bioluminescence; practical applications; methods of bioluminescence investigation; a database, 'Biolumbase' of natural and transgenic luminous microorganisms; and a catalogue of cultures maintained in CCIBSO, and also information about reagents for bioluminescent analysis. It will provide searches and comparisons of various organisms and vectors possessing *lux* genes and will allow estimating their suitability for the decision of concrete fundamental and applied problems. The information on properties, functions, use of luminescent organisms and their bioluminescent systems is accompanied by bibliographic directories. The web resource can be used as a scientific–educational information resource. The information system is accessible at: <http://bl.ibp.ru>

### Excessive extracellular chemiluminescence and necrosis of neutrophils in bovine neonates and potentially supportive role of vitamin C

J. Mehrzad,<sup>1</sup> M. Mohri<sup>2</sup> and C. Burvenich<sup>3</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Ferdowsi University, Mashhad, Iran. E-mail: mehrzad@um.ac.ir

<sup>2</sup>Departments of Pathobiology and Clinical Veterinary Science, Ferdowsi University, Mashhad, Iran

<sup>3</sup>Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

To obtain a clearer insight into the oxidation–reduction reactions of blood neutrophils in bovine neonates, the kinetics of neutrophil chemiluminescence (CL) and viability were investigated in calves immediately after birth. In the first study, simultaneous analyses of CL kinetics and viability of neutrophils in the calves were measured within 12 h after birth ( $n = 10$ ). In the second study, at 10 days before anticipated calving, Holstein cows were divided into two groups; they were fed diets that provided 0 ( $n = 6$ ) or 50 ( $n = 6$ ) g/day of supplemental vitamin C. Within 12 h after birth, blood samples were collected for functional assays of the neutrophils. The CL profile was unexpectedly monophasic with very high intensity; the viability of the neutrophils was also markedly low in the neonates. Feeding vitamin C to the mothers indirectly improved viability and the superoxide anion production capacity of neonatal neutrophils. Our findings suggest that the subsequent intracellular myeloperoxidase–H<sub>2</sub>O<sub>2</sub> system is impaired, and may lead to a higher risk of infections in bovine neonates. Conversely, the slightly increased viability and superoxide anion production capacity in neonatal blood neutrophils with vitamin C could lead to better protection from infection in neonates. Further studies are in progress to explain these findings.

### The use of aequorins to image Ca<sup>2+</sup> signalling during zebrafish development: throwing light on embryonic pattern formation

A. L. Miller and S. E. Webb

Calcium Aequorin Imaging Laboratory, Department of Biology, HKUST, Clearwater Bay, Hong Kong, People's Republic of China.  
E-mail: almill@ust.hk

Through the injection or expression of aequorin, a Ca<sup>2+</sup>-sensitive bioluminescent protein, and the use of a custom-built photon imaging microscope (PIM), dynamic patterns of Ca<sup>2+</sup> signalling can be observed during the first 48 h of zebrafish development. These begin at egg activation and then continue to be generated throughout the subsequent zygote, cleavage, blastula, gastrula and segmentation periods of development. They are thus associated with all the major phases of early pattern formation: cell proliferation, differentiation, axis determination, the generation of primary germ layers, the emergence of rudimentary organ systems, and therefore the establishment of the basic vertebrate body plan. When Ca<sup>2+</sup> signals need to be transmitted across significant embryonic distances they take the form of waves, either intracellular waves when the cell size is large or, later in development when the cell size is reduced, intercellular waves. Both types of Ca<sup>2+</sup> signals are described, as well as (a) their integration into signalling networks and (b) their possible developmental function and significance. This work was supported by RGC-CERG Grants HKUST 6416/06M and HKUST 661707.

### Study on the bioluminescence mechanism of the luminous mushroom *Mycena chlorophos*; various factors affecting luminescence activity

K. Mori,<sup>1</sup> S. Kojima,<sup>1</sup> S. Maki,<sup>1</sup> T. Hirano<sup>1</sup> and H. Niwa<sup>1</sup>

Department of Applied Physics and Chemistry, University of Electro-Communication, 1-5-1 Chofugaoka Chofu, Tokyo, Japan. E-mail: moriken@hotaru.pc.uec.ac.jp

The luminous mushroom *Mycena chlorophos*, found in southern Japan, e.g. on Hachijo Island, emits pale green light from its whole fruiting bodies. The molecular mechanism of the light-emitting system has not so far been clearly elucidated. We investigated the effects of pH of immersion buffer and various additives for bioluminescence activity against the luminous mushroom pieces. We found that some additives were extremely effective for switching mushroom bioluminescence.

### Effects of surfactants on peroxyoxalate chemiluminescence reaction

K. Nakashima,<sup>1</sup> K. Abe,<sup>1</sup> S. Nakamura,<sup>1</sup> M. Wada<sup>1</sup>, S. Harada<sup>2</sup> and N. Kuroda<sup>1</sup>

<sup>1</sup>Department of Clinical Pharmacy, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan. E-mail: naka-ken@nagasaki-u.ac.jp

<sup>2</sup>Lumica Co., 65 Itogaura, Koga-city, Fukuoka 811-3136, Japan

The effects of surfactants on the peroxyoxalate chemiluminescence (PO-CL) reaction were examined. Surfactant solutions (0.5% or 2%) were spiked in: (a) bis(2,4,6-trichlorophenyl)oxalate (TCPO)–rhodamine B–H<sub>2</sub>O<sub>2</sub>–imidazole–HNO<sub>3</sub> buffer system; (b) TCPO–rhodamine B–H<sub>2</sub>O<sub>2</sub> system; and (c) TCPO–H<sub>2</sub>O<sub>2</sub>–imidazole–HNO<sub>3</sub> system, respectively. Fifteen commercially available surfactants, including anion, cation, dipolar and non-ionic types, were used in this study. CL measurement was performed at room temperature for 120 s, using a Luminescencer PSN AB-2200 (Atto Co., Tokyo, Japan). In system (a) the surfactants almost quenched CL intensity, while no quenching was observed without surfactant. This may be attributed to notable changes of pH caused by adding surfactant. On the other hand, in system (b) CL intensity was enhanced with several surfactants spiked. This result suggested that several surfactants can play a role as a catalyst. No remarkable inclination to enhance CL intensity could be observed among the types of surfactants. In system (c) CL intensity was also enhanced with some surfactants, due to the fluorescent impurities included.

### The mechanism of heavy atoms effect in bioluminescent reactions

E. V. Nemtseva,<sup>1,2</sup> T. N. Kirillova<sup>2</sup> and T. V. Brukhovskikh<sup>1</sup>

<sup>1</sup>Biophysics Department, Siberian Federal University, Krasnoyarsk 660041, Russia. E-mail: NEV@lan.krasu.ru

<sup>2</sup>Institute of Biophysics SB RAS, Akademgorodok, Krasnoyarsk 660036, Russia

The heavy atoms effect was registered in bioluminescent reactions of three types (bacterial, firefly and coelenterate). This effect was observed as a decrease of bioluminescent intensity in the presence of halide anions of different atomic weights. Two mechanisms of the effects of the halides were examined: (a) the

physicochemical effect of the external heavy atom, based on spin–orbit interactions in electron-excited structures; and (b) the biochemical effect, i.e. interactions with the enzymes resulting in changes of enzymatic activity. The physicochemical effect was evaluated by using photoexcitation of model fluorescent compounds (flavin mononucleotide, firefly luciferin, and coelenteramide)—structural analogues of the bioluminescence emitters. The bioluminescent and photoluminescent inhibition coefficients were calculated and compared to evaluate the contributions of the first mechanism. To characterize the second mechanism, the interaction of halide-containing molecules with bioluminescent enzymes was studied using fluorescence spectroscopy techniques. The biochemical mechanism was found to be dominant.

### Temperature-dependence of bioluminescence of the Australian glow-worm *Arachnocampa flava* Harrison (Diptera: Keroplatidae)

C. Niu,<sup>1</sup> C. Lei<sup>1</sup> and D. J. Merritt<sup>2</sup>

<sup>1</sup>Key Laboratory of Utilization of Insect Resources and Sustainable Control of Pests, Huazhong Agricultural University, Wuhan, Hubei 430070, People's Republic of China. E-mail: niuchangying88@163.com

<sup>2</sup>School of Integrative Biology, University of Queensland, St. Lucia, Queensland 4072, Australia. E-mail: d.merritt@uq.edu.au

*Arachnocampa flava* is a bioluminescent insect that inhabits rainforest areas of south-eastern Queensland. The larvae construct sticky fishing lines to capture prey attracted by their bioluminescence. Little is known about the relationship between their bioluminescence and temperature. We conducted experiments with fine temperature control on glow-worms that had been kept at constant darkness to render them arrhythmic. First, the larvae were exposed to a temperature change every 24 h within the range 10–23°C over 4 days. They reacted rapidly to the temperature changes. During the transitions between temperatures, light output tracked temperature change in a linear manner. Next, the larvae were exposed to a steady temperature decrease from 23°C to 6.7°C, followed by an increase to 22°C. The light output decreased in with decreasing temperature and increased as temperature increased. Finally, the larvae were exposed to cyclical changes of 6°C, with a range of 20–27°C. The first three temperature cycles produced corresponding peaks and troughs in light output and the following five cycles showed less variation from peak to trough. The data indicate that the bioluminescence of *A. flava* is temperature-dependent.

### Differentiation in HL-60 promyelocytic cell line induced by methacrylic compounds

G. Nocca,<sup>1</sup> F. De Palma,<sup>1</sup> P. Corsale,<sup>1</sup> M. Antenucci,<sup>1</sup> C. Rossi,<sup>1</sup> A. Lupi<sup>2</sup> and P. De Sole<sup>1</sup>

<sup>1</sup>Institute of Clinical Biochemistry, Catholic University, Largo A. Gemelli, 8-00168 Rome, Italy. E-mail: p.desole@rm.unicatt.it

<sup>2</sup>Institute of Molecular Recognition Chemistry, CNR, Rome, Italy

The polymerization of methacrylic monomers present in dental composite resins is never complete and this leads to a release of unreacted compounds in the oral cavity and in biological fluids,



causing possible local and systemic adverse effects. The aim of this study was *in vitro* investigation of the interactions of urethane dimethacrylate (DUDMA) and 1,4-butanediol dimethacrylate (BDDMA) with HL-60 cells, a cell line well able to simulate the behaviour of granulocytes. Chemiluminescence analysis shows the presence of oxidative burst in HL-60 cells treated with the monomer, indicating a differentiation process; furthermore, DUDMA and BDDMA induce a decrease of oxygen consumption, an alteration of glucose metabolism and a GSH depletion on this cell line. The differentiating activity of monomers could have significant implications in their cytotoxicity and can be therefore considered as an useful back-up for biocompatibility studies of dental materials.

### Determination of pyrogallol by imidazole chemiluminescence coupled to the peroxidase embedded organic monolith reactor

O. Nozaki,<sup>1</sup> M. Munesue<sup>2</sup> and H. Kawamoto<sup>3</sup>

<sup>1</sup>Department of Clinical Laboratory Medicine, Kinki University School of Medicine, Osaka-Sayama 589-8511, Japan

<sup>2</sup>Chemco, Osaka 530-0016, Japan

<sup>3</sup>Tottori University School of Medicine, Yonago 683-8503, Japan

A peroxidase embedded organic monolith reactor was developed by photopolymerization, and coupled to a flow-injection-chemiluminescence system for the determination of pyrogallol by horseradish peroxidase (HRP)-catalysed imidazole chemiluminescence (CL). Pyrogallol is a plant polyphenol and has antioxidant activity. Pyrogallol was assayed by the CL method by quenching light in the reference. The proposed method, however, assays pyrogallol by producing light. The method employed a flow-injection-CL system in which both pyrogallol and alkaline imidazole solution were merged into the translucent glass capillary reactor of an HRP-embedded monolith. Light was emitted through a wall of the reactor due to the CL reaction and detected using the photomultiplier of a CL monitor. The responsible intermittent molecule of the light emission was identified as imidazole hydroperoxide using a mass-spectrometer. The CL reaction showed good reproducibility and quantitative correspondence to the dose of pyrogallol, and is expected to be applicable to the determination of other polyphenols besides pyrogallol.

### Fluorescence and photo-induced electron transfer of complexes Trp with $\text{UO}_2^{2+}$ ions

I. O. Osina, S. S. Ostakhov and V. P. Kazakov

Institute of Organic Chemistry, Ufa Scientific Centre of the RAS, 71, Pr. Oktyabrya, Ufa, Russia. E-mail: chemlum@anrb.ru

The fluorescence (FL) of proteins is caused by the emission of tryptophan (Trp). We determined the influence of the excitation wavelength ( $\lambda_{\text{exc}}$ ) on the competition between fluorescence and photoinduced electron transfer (PET) from the highly excited states of tryptophan. We showed that the dependence of the intensity of FL under the conditions of selective excitation of the electron donor, Trp, in the complex  $[\text{UO}_2^{2+} \text{Trp}]$  on the concentration of  $\text{UO}_2^{2+}$  and the quenching of its FL occurs in the mechanism of reversible PET:  $\text{Trp} + \text{UO}_2^{2+} [\text{Trp}^* \cdot \text{UO}_2^{2+}] [\text{Trp}^{*+} \cdot \text{UO}_2^+]$ . The quenching constant of the Trp FL with uranyl nitrate

in the range 280–230 nm monotonically increases from 430 to 6500 L/mol. The monotonic change in the constant of quenching of the Trp FL with  $\text{UO}_2^{2+}$  in the range of excitation energies of the  $S_1$ – $S_2$  levels is probably due to intraspheric PET from 'hot'  $S_1^V$  states at a rate higher than that of rotation-vibration  $S_n^V \rightarrow S_n^-$  relaxation. Supported by the RFFI (Grant No. 08-03-00147) and the Division of Chemistry and Materials Science of the RAS in the framework of Programme 1-OKh.

### Fluorescence from $S_2$ -level of complexes of tryptophan with europium(III) in water-ethanol solution

I. O. Osina, S. S. Ostakhov and V. P. Kazakov

Institute of Organic Chemistry, Ufa Scientific Centre of the RAS, 71, Pr. Oktyabrya, Ufa, Russia. E-mail: chemlum@anrb.ru

This paper presents analysis results of fluorescence (FL) amino acid residue of protein tryptophan (Trp) in complexes with  $\text{EuCl}_3$  in water-ethanol solutions. In 90%  $\text{C}_2\text{H}_5\text{OH}$ , excitation into the second and shorter-wavelength absorption bands of Trp lead to the short-wavelength 'shoulder' that emerges in the FL spectrum of Trp. In the presence of  $\text{EuCl}_3$ , the 'shoulder' at 295 nm transforms into a peak, which is assigned to the  $S_2 \rightarrow S_0$  radiative transition. Quenching of the  $S_1 \rightarrow S_0$  FL of Trp ( $\lambda_{\text{max}} = 337$  nm) by  $\text{EuCl}_3$  makes it possible to clearly observe the short-wavelength  $S_2 \rightarrow S_0$  component ( $\lambda_{\text{max}} = 295$  nm). The latter becomes dominant at a  $\text{Eu(III)}$  concentration of  $6 \times 10^{-5}$  mol/L. At the same time, we observe the rise of  $S_2 \rightarrow S_0$  FL of Trp with an increase in the  $\text{Eu(III)}$  concentration. In the excitation wavelength ( $\lambda_{\text{exc}}$ ) range  $\Delta\lambda_{\text{exc}} = 210 - 260$  nm, the intensities of  $S_1 \rightarrow S_0$  FL and  $S_2 \rightarrow S_0$  FL of Trp are redistributed. At 210 nm, the  $S_1 \rightarrow S_0$  FL is dominant and the short-wavelength component is observed as a poorly pronounced peak. As  $\lambda_{\text{exc}}$  increases, the  $S_2 \rightarrow S_0$  FL intensity increases and achieves a maximum value at 245 nm. In the wavelength range 245–260 nm, the  $S_2 \rightarrow S_0$  FL decays, and at  $\lambda_{\text{exc}} \geq 260$  nm, only  $S_1 \rightarrow S_0$  FL of Trp is observed. Supported by RFFI (Grant No. 08-03-00147) and the Division of Chemistry and Materials Science of the RAS in the framework of programme 1-OKh.

### Mechanism of biophoton emission of protein molecules in life systems and its features

X.-F. Pang

Institute of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, People's Republic of China. E-mail: pangxf@mail.sc.cninfo.net

We think that biophotons emission is caused by the excitation of the vibrational quantum of amides of proteins, arising from the energy released in the hydrolysis of adenosine triphosphate. Thus, we calculate the energy spectra of proteins according to the theory of bio-energy transport. From the energy spectra we know that the proteins can both radiate and absorb biophotons with wavelengths of  $<3 \mu\text{m}$  and  $5\text{--}7 \mu\text{m}$ , which is basically consistent with experimental data obtained from the infrared absorption of collagen and acetanilide molecules and the luminescence of plasma and of person's finger hands, and from the laser-Raman spectra of acidity type I fibre collagen in lungs of mouse and metabolically active *Escherichia coli*. These results

affirm the validities of both the above mechanism and the theory of bio-energy transport, and give an account of the mechanism of the non-thermal biological effect of infrared rays absorbed, namely that infrared light can facilitate the vibrations of amides and the transport of bio-energy along the proteins. Thus we can explain the biological and medical function of infrared lights.

### The mechanism of biophoton emission of biotissues and its properties

X.-F. Pang

Institute of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, People's Republic of China. E-mail: pangxf@mail.sc.cninfo.net

We propose a model of photon emission of biotissues, in which we think that biophotons are emitted due to the transitions of energy levels of small biomolecules in the form and growth processes of bio-self-organization in living systems. According to the elementary processes of life activities, which can exchange energy, material and information with the environment, we have constructed a theory of form and growth of bio-self-organization by the quantum statistical theory of the non-equilibrium state, which is a dissipation structure of higher energy and lower entropy, having the functions of self-embellishment, self-reproduction, self-regulation and self-renewal. Thus, the bio-self-organizations can spontaneously emit biophotons after absorbing energy, materials and light from the environment. The distribution function of biophotons, in accordance with energy levels of:

$$n_i/W_i = f_i = \exp[B(n_i, \dot{n}_i, \varepsilon_i) - 1] \quad \text{or} \quad f_i = \exp[(1 - E' - I') - 1]$$

in living things, is consistent with the experimental results. We demonstrate that the intensity of biophoton emission may serve as a potential measurement of the degree of order and life activity of bio-self-organizations. Utilizing this theory, we explain further some interesting experimental phenomena of biophoton emission.

### Identification of developmental enhancers using targeted regional electroporation (TREP) of evolutionarily conserved regions

C. U. Pira, S. A. Caltharp, K. Kanaya, S. K. Manu, L. F. Greer and K. C. Oberg

Department of Pathology and Human Anatomy, Loma Linda University, 24785 Stewart Street, Evans Hall, Room B09, Loma Linda, CA 92350, USA. E-mail: koberg@llu.edu

During development, precise temporal and spatial regulation of critical genes is required to orchestrate morphology. Preservation of generalized body form across divergent species suggests that evolutionarily conserved regions (ECR) are likely candidates for regulatory elements. Identification of ECRs with enhancer activity has recently been described during the development of early chick embryos, by electroporation of ECRs linked to a GFP reporter. The major limitation of this technique is that the chick embryos only survive for about 48 h after electroporation and thus enhancers involved in later organogenesis cannot be determined. We have developed a protocol to overcome this

problem, using targeted regional electroporation (TREP) of ECR-GFP constructs. In this report, we demonstrate the activity of a limb-specific sonic hedgehog (*SHH*) regulatory region that enhances GFP expression within the native limb-specific *SHH* expression domain. We further show the utility of this technique using another ECR-enhancer associated with *Emx2* that exhibits shoulder/limb girdle activity typical of *Emx2* expression. Collectively, these data indicate that TREP can effectively screen for enhancer activity of presumptive regulatory regions at later stages of development.

### Synthesis of a novel fluorescence probe of $\beta$ -CD and pyridyl cuprous iodide inclusion complex and its application

J. Qiao,<sup>1,2</sup> R. Dong,<sup>1</sup> D. Li,<sup>1</sup> S. Shuang<sup>1</sup> and C. Dong<sup>1</sup>

<sup>1</sup>Environmental Science and Engineering Research Centre, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: smshuang@sxu.edu.cn

<sup>2</sup>Shanxi Medical University, Taiyuan 030001, People's Republic of China

The pyridyl cuprous iodide complex is a novel fluorescence probe but it was not stable in air. The shape of beta-cyclodextrin ( $\beta$ -CD) can be presented as a truncated cone which consists of seven D-glucopyranoside units linked by  $\alpha$ -1,4-glycosidic linkages. Its interior is hydrophobic and exterior hydrophilic, indicating that  $\beta$ -CD has the ability to include organic, inorganic and biological compounds and to increase stability and water-solubility. Therefore, this experimentation synthesizes the inclusion complex of  $\beta$ -CD and pyridyl cuprous iodide. The structure of the inclusion compound was confirmed by IR and <sup>1</sup>H-NMR. Investigations were performed into its fluorescence performance and the relationship between the structure of the compound and its fluorescence. The results indicated that the inclusion complex exhibited satisfactory solvent polarity and steady fluorescence characteristics in air. This novel fluorescence probe has not been reported previously. As the fluorescence of the complex can be quenched by methane, it can hopefully be used in the detection of methane.

### Determination of azithromycin by capillary electrophoresis with electrochemiluminescence (CE-ECL) detection

L. Qu<sup>1</sup> and H. Wang<sup>2</sup>

<sup>1</sup>Henan University of Technology, Zhengzhou, People's Republic of China. E-mail: qulingbo@zzu.edu.cn

<sup>2</sup>Henan Province Institute of Medical Appliances, Zhengzhou 450003, People's Republic of China. E-mail: dongdonggood@126.com

Azithromycin is an important antibiotic. Based on CE-ECL, a new method for the determination of azithromycin was investigated. We studied the CE and ECL behaviour of azithromycin with Ru(bpy)<sub>3</sub><sup>2+</sup> at the Pt electrode. The system responds linearly to azithromycin concentration in the range  $2 \times 10^{-6}$ – $3.0 \times 10^{-5}$  mol/L,  $R = 0.9998$ , with a detection limit of  $2.7 \times 10^{-7}$  mol/L. We have successfully applied this system to the determination of azithromycin in capsules. Supported by Henan Academic Foundation of Science and Technology (Grant No. 0512001400).

### **Chemiluminescent oxidative rearrangement of diketopiperazines involved in the biomimetic synthesis of marine pyrrole-2-aminoimidazole metabolites**

C. Ratinaud, C. Vergne, L. Ermolenko and A. Al-Mourabit  
Institut de Chimie des Substances Naturelles du CNRS-UPR2301, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France.  
E-mail: ali.almourabit@icsn.cnrs-gif.fr

Marine sponges belonging to the families Agelasidae and Axinellidae are remarkable for their ability to produce an interesting and increasingly large group of biologically active pyrrole-2-aminimidazole (P-2-AI) metabolites. Since our observation that the skeleton of the natural debromodispacamide A could be formed from proline diketopiperazine (DKP) and guanidine by spontaneous air oxidation, we presumed an important role of proline DKP as a key precursor in marine metabolites. The reaction depends on molecular oxygen, which reacts easily with the DKPs to give natural products. Importantly, the same reaction leads to a chemiluminescent decarboxylation of a dioxetanone intermediate. Considering that the natural amino acids derived from DKP could be key structures involved in the biogenesis of a large number of marine metabolites, an extensive mechanistic study and the isolation of guided metabolites from phylogenetically related sponges were conducted. Beyond the discovery of new reactions, biomimetic studies of molecular reactivity can also suggest explanations of the ecological purposes related to chemiluminescence. Mechanistic studies of a spontaneous oxidation of selected DKPs by atmospheric oxygen and their new chemiluminescent properties are discussed.

### **Electrochemiluminescence analysis in miniaturised disposable polymer microdevices**

Z. M. Redha, S. J. Baldock, P. R. Fielden, N. J. Goddard and B. J. Treves Brown  
School of Chemical Engineering and Analytical Science, University of Manchester, PO Box 88, Manchester M60 1QD, UK.  
E-mail: z.mohammed-redha@postgrad.manchester.ac.uk

Miniature electrochemical flow cells have been fabricated by injection moulding and used in a novel determination of heavy metal ions in aqueous solutions using stripping electrochemiluminescence (ECL). High-impact polystyrene was used for moulding the top plate containing the flow channel and the fluidic connectors, and 40% carbon fibre-filled polystyrene was used for moulding the electrodes. The electrodes were then incorporated into the flow cell using an overmoulding procedure to form the base plate of the device. Ultrasonic welding was used to bond both plates together, thus forming a well-sealed microdevice. The complete device not only has the advantages associated with miniaturization but is also disposable, thus eliminating problems such as drift, ageing and contamination. Methods using this device were developed for the analysis of four different metals. All were preconcentrated on a gold-coated carbon working electrode (WE) in an alkaline solution at a controlled potential and then stripped into a solution containing luminol. Copper ions were stripped at a constant potential and detected by applying a symmetrical double-step (negative and positive) potential to the WE. For both lead and cadmium a medium exchange method was used

for stripping and then detected by applying a single anodic step to the WE. Cobalt ions were detected by applying differential pulse anodic stripping voltammetry, albeit with lower sensitivity. All the investigated metal ions except cobalt inhibited the ECL. The linear concentration range varied with the preconcentration time. The detection limits were 2.7, 16 and 3.9 ng/mL for copper, cadmium and lead, respectively. Reliable measurements could not be made using the ECL signal of cobalt because the highly negative preconcentration potential induced bubble formation.

### **Combined chemiluminescent immunohistochemical quantitative determination of p16<sup>INK4A</sup> and fluorescent papillomavirus DNA *in situ* hybridization for assessment of cervical intra-epithelial neoplasia**

M. Rizzoli,<sup>1</sup> M. Mirasoli,<sup>1</sup> M. Guardigli,<sup>1</sup> P. Simoni,<sup>2</sup> S. Venturoli,<sup>3</sup> S. Ambretti,<sup>3</sup> M. Musiani<sup>3</sup> and A. Roda<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, Bologna 40126, Italy

<sup>2</sup>Department of Internal Medicine and Gastroenterology, University of Bologna, Via Massarenti 9, Bologna 40138, Italy

<sup>3</sup>Department of Clinical and Experimental Medicine, University of Bologna, Via Massarenti 9, Bologna 40138, Italy

Persistent infection with oncogenic high-risk human papillomavirus (HR-HPV) is considered the principal factor in the development of cervical cancer. However, since most infections with HR-HPV regress spontaneously without causing preneoplastic lesions, biomarkers able to stratify women regarding their risk for progression to cancer are required. To distinguish different grades of cervical intraepithelial neoplasia (CIN) and to allow accurate and objective evaluation of the risk of progression of early CIN, a chemiluminescent immunohistochemical (CL-IHC) procedure was developed for the objective p16<sup>INK4A</sup> quantitative evaluation and topographical localization in cervical biopsy sections. In addition, a fluorescent *in situ* hybridization (FL-ISH) procedure was performed on the same tissue section to obtain HPV DNA concomitant localization. The quantitative evaluation of p16<sup>INK4A</sup> expression combined with the localization of HR-HPV DNA showed a statistically significant discrimination among different lesions (non-neoplastic, low-grade and high-grade CIN), thus offering an accurate and objective diagnostic test providing important information for counselling, selection of therapy, follow-up and vaccine monitoring.

### **Analytical challenges for luminescence-based point-of-care testing devices in biomedical diagnostics**

A. Roda,<sup>1</sup> M. Guardigli,<sup>1</sup> M. Mirasoli,<sup>1</sup> E. Micheli,<sup>1</sup> L. S. Dolci<sup>1</sup> and M. Musiani<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, University of Bologna, Bologna 40126, Italy. E-mail: Aldo.roda@unibo.it

<sup>2</sup>Department of Clinical and Experimental Medicine, University of Bologna, Bologna 40138, Italy

Diagnostics is moving towards point-of-care testing (POCT), in which integrated portable analytical devices allow the performance of testing on biological fluids (blood, serum, saliva) at the site where the analysis is required or clinical care must be delivered.

A POCT device should fulfil requirements such as portability, need for minimum sample pretreatment, high sensitivity and the possibility of performing multiplexed assays to detect panels of biomarkers of given pathologies. Such analytical challenges could be satisfied by microfluidics-based devices employing biospecific (antigen–antibody, nucleic acid hybridization, etc.) recognition reactions involving immobilized probes and highly sensitive luminescence detection techniques (photo-, bio-, chemi-, electrochemiluminescence). In such devices, multiplexing would be achieved by combining ‘position encoding’ and ‘signal encoding’ detection, i.e. the recognition reactions corresponding to the different analytes will take place in different positions (thus being revealed by 2D luminescence imaging) or will be associated with different luminescence reporting processes and/or labels (thus being separately monitored by selective signal triggering or spectral resolution of light emission).

### Respiratory burst of peripheral blood leukocytes (PMNs): analysis by means of a microtitre plate luminometer

C. Rossi and P. De Sole

Institute of Clinical Biochemistry, Catholic University, Largo A. Gemelli, 8–00168 Rome, Italy. E-mail: p.desole@rm.unicatt.it

Measurement of chemiluminescence (CL) activity is a widely used test to monitor the respiratory burst of phagocytic cells, either isolated or directly in small amounts of blood. Commonly, CL is measured in small vials containing as few as  $10^3$  polymorphonucleocytes (PMNs). In the last 35 years many thousands of papers have appeared reporting CL data obtained with highly sensitive luminometers, while the use of luminometers for microtitre plates is very limited. The low sensitivity of these instruments makes it mandatory to use a large number of cells, rendering it impossible to measure CL activity directly in a whole blood system. The CL response obtained using a microtitre plate luminometer (Victor, Perkin-Elmer) is linearly dependent on the number of phagocytes only for a small cell range; in the present paper, a careful analysis of the factors that can cause the lack of CL linearity is presented and better experimental conditions for measuring the CL of PMNs are shown.

### Bioluminescence monitoring: detoxification of $\alpha$ -radioactive solutions by humic substances

T. V. Rozhko and M. A. Aleksandrova

Institute of Biophysics SB RAS, Siberian Federal University, Krasnoyarsk, Russia. E-mail: gutniktv72@mail.ru.

Bacterial bioluminescence assay systems were used to monitor the detoxification of  $\alpha$ -radioactive solutions by humic substances (HS). Solutions of americium-241 and uranyl nitrates were used as a source of low-level  $\alpha$ -radiation. Bioluminescent intensity of the systems in the solutions of the radionuclides has been shown to depend on radionuclide concentration, level of organization and integrity of the bioluminescent assay systems. It was shown that HS change bioluminescence kinetics in radioactive solutions, bring them close to control: bioluminescence activation and inhibition decreased in the presence of HS. Electron microscopic images of the bacterial cells in the

radioactive solutions in the presence and absence of HS were shown to be different: HS were found to decrease the damaged effect of radiation. Redistribution of the radionuclides inside the bacterial cells was studied in the presence of HS.

### Theoretical analysis on the absorption spectra of intermediates of firefly luciferin in deoxygenated dimethyl sulphoxide

H. Sakai and N. Wada

Faculty of Life Sciences, Toyo University, 1-1-1, Itakura, Gunma 374, Japan. E-mail: dx0600016@toyonet.toyo.ac.jp

In order to understand the chemiluminescence mechanism of firefly luciferin (Ln), the observed absorption spectra of two intermediates, M440 and M420, with absorption peaks at 440 and 420 nm, which are detected only if DMSO is evacuated before adding *t*-BuOK and Ln, are interpreted by the theory of *ab initio* calculations at the level of B3LYP/6-31 + G(d,p) and TD-B3LYP/6-31 + G(d,p) for geometrical optimization and optical absorption, respectively. Although ionized states of these intermediates were supposed by H-NMR, it is necessary to clarify the electronic properties of those intermediates which successively lead to the excited oxyluciferin via dioxetanone (DOX). The purpose of this study was to determine what kind of ionized states of Ln can be theoretically assigned to M440 and M420, based on a model in which the ionized states of the carboxylic group (COOH), the proton at C4 in the thiazolinone ring and the phenolic group (OH) in the benzothiazolyl ring are essential for determining the optical properties of these intermediates. It is thus found that M440 can be identified as  $\text{Ln}^{-2}$  deprotonated at the sites of both COOH and OH, but M420 is with difficulty assigned to  $\text{Ln}^{-3}$  derived from deprotonation of  $\text{Ln}^{-2}$  at the C4 site.

### Development of a novel bioluminescent assay for nitric oxide using soluble guanylate cyclase

Y. Sano,<sup>1</sup> M. Seki,<sup>1</sup> S. Suzuki,<sup>2</sup> K. Ito<sup>1</sup> and H. Arakawa<sup>1</sup>

<sup>1</sup>School of Pharmaceutical Science, Showa University, Tokyo 142-8555, Japan. E-mail: ysano@pharm.showa-u.ac.jp

<sup>2</sup>Research and Development Division, Kikkoman Corporation, Chiba 278-0037, Japan

Nitric oxide (NO) has been known to be involved in many biological processes, including vasodilatation and neuronal communication. Soluble guanylate cyclase (sGC) is well known as a ‘NO-sensor’, plays an essential role of signal transduction, initiating enzyme conversion of GTP to cGMP in the NO signalling cascade, pyrophosphate (PPi) being produced simultaneously. It is reported that the cyclase activity is activated by as much as 200-fold upon NO binding to sGC. We previously reported highly sensitive bioluminescent assay for PPi utilizing the PPDK–luciferin/luciferase reaction. In this study, we developed a novel bioluminescent assay for NO using sGC, based on the PPDK–luciferin/luciferase reaction. The measurable range of NO obtained by the proposed method was 100 nmol/L–10  $\mu\text{mol/L}$ , detection limit 2 pmol/assay. This method is sensitive and specific for NO.



### Spiropyran-based fluorescent probes for biological species

N. Shao,<sup>1</sup> J. Jin,<sup>1</sup> R. Yang<sup>1</sup> and W. Chan<sup>2</sup>

<sup>1</sup>College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, People's Republic of China.  
E-mail: yangrh@pku.edu.cn

<sup>2</sup>Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, People's Republic of China

Spiroyrans are one of the most fascinating photochromic compounds. To date a number of spiroyrans derivatives have been designed and applied in optical detection of metal ions by UV-visible spectroscopic methods, while development of new spiroyrans probes capable of fluorescent sensing and recognizing new targets remains a challenge. We designed and synthesized several spiroyrans compounds (SP) and applied them as fluorescent probes in the detection of biologically important substrates. First, based on the inner filter effect between zinc porphyrin and SP<sub>1</sub>, a fluorescent sensor for copper(II) ion was developed. Second, SP<sub>2</sub> was found to have a rationing fluorescent response to copper(II) ion, which could be used to detect Cu<sup>2+</sup> in serum. Moreover, the Zn<sup>2+</sup> complex of SP<sub>3</sub> was a good ratiometric fluorescent probe for pyrophosphate in urine. Finally, confocal microscopy experiments established that interaction of the intracellular GSH with SP<sub>4</sub> drives it to penetrate the cell membrane and produce photochromism in living cells.

### Preparation of novel biotinylated firefly luciferase-labelled nanoparticles and their application in bioluminescent enzyme immunoassay

Y. Shen,<sup>1</sup> R. Ke,<sup>1</sup> Y. Liao<sup>1</sup> and Q. Li<sup>1,2</sup> and W. Yang<sup>1</sup>

<sup>1</sup>Molecular Diagnostics Laboratory, Department of Biomedical Sciences and the Key Laboratory of the Ministry of Education for Cell Biology and Tumour Cell Engineering, School of Life Sciences, Xiamen University, Fujian 361005, People's Republic of China

<sup>2</sup>Translation Medicine Centre, Institute for Biomedical Research, Xiamen University, Fujian 361005, People's Republic of China.  
E-mail: qqli@xmu.edu.cn, weiyang@xmu.edu.cn

Firefly luciferase is well known for its high bioluminescence efficiency. It is widely used to monitor gene expression in mammalian cells and to detect ATP concentration, because of its high sensitivity and wide detection ability. However, the enzyme is too weak to be labelled and is therefore limited to applications in bioluminescent enzyme immunoassay (BLEIA). Here we made recombinant biotinylated firefly luciferase, which was expressed in *Escherichia coli* and successfully purified by Ni column. The biotinylated luciferase was labelled onto the surface of silica nanoparticles through the streptavidin-biotin system. The stability of the luciferase-labelled nanoparticle was remarkably improved. Novel complex nanoparticles were used in a model study of the sandwich immunoassay for hepatitis B surface antigen (HBsAg). The linearity of this immunoassay was in the range 2–110 ng/mL HBsAg. Our results show that firefly luciferase labelled nanoparticle is a sensitive and versatile bioluminescent reporter.

### Chemiluminescence study on the upregulation of NADPH oxidase activity by thioredoxin reductase in vascular endothelial cells

X. Shen and Z.-B. Liu

Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China. E-mail: shenxun@sun5.ibp.ac.cn

Vascular endothelial cells have a well-established role in cardiovascular homeostasis and the initiation of atherosclerosis. The upregulation of some inflammatory genes by reactive oxygen species (ROS) is considered to be the molecular mechanism of early atherosclerosis. A major source of ROS in vascular cells is NADPH oxidase, which consists of the membrane subunits gp91phox and p22phox, the cytosolic subunits p67phox, p47phox and the small GTPase rac1. However, the mechanisms that control the activity of this multi-subunit enzyme complex are not completely understood. Thioredoxin reductase (TrxR) is an antioxidant enzyme that participates in thiol-dependent cellular reductive processes. Since the enzyme regenerates reduced thioredoxin, which serves as a reducing equivalent and may also directly scavenge ROS, TrxR has been considered as an enzyme to reduce the ROS level in cells. However, our chemiluminescence study on ROS generation in endothelial cells (EAhy926) either overexpressing or silencing TrxR1 showed that TrxR1 enhanced intracellular ROS generation by upregulating NADPH oxidase activity. In the present study, intracellular ROS was measured by photon emission from cells suspended in HBSS buffer after the addition of luminol–HRP chemiluminescent solution. It was found that: (a) 30% overexpression of TrxR1 resulted in about two-fold increase of intracellular ROS; (b) TrxR1-enhanced chemiluminescence can be completely suppressed by the addition of 10 μmol/L DPI, the inhibitor of NADPH oxidase, indicating that the TrxR1-enhanced ROS might be due to activation of this enzyme; (c) Western blot analysis showed that overexpression of TrxR1 upregulated, while knockdown of the endogenous TrxR1 downregulated, the expression of p22phox. Correspondingly, the chemiluminescence-detected ROS in the cells having their p22phox knocked down decreased. The results suggest that TrxR1 upregulates the activity of NADPH oxidase via upregulation of the expression of its subunit p22phox, and that chemiluminescence detection is a good method for monitoring the activity of NADPH oxidase.

### Improved direct chemiluminescent imaging detection of serum proteins in polyacrylamide gels using organic dyes

S. Shen,<sup>1</sup> Z. Wang,<sup>1</sup> J. Ouyang<sup>1</sup> and W. R. G. Baeyens<sup>2</sup>

<sup>1</sup>College of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China.  
E-mail: jinoyang@bnu.edu.cn

<sup>2</sup>Department of Pharmaceutical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

An improved chemiluminescent imaging detection method for the detection of serum proteins after polyacrylamide gel electrophoresis was developed, employing organic dyes such as amidoblack; low-abundance proteins such as haemopexin were identified. The method offers enhanced sensitivity with a low

detection limit. The mechanism of this improved chemiluminescent imaging detection is discussed.

### Fluorescence spectral study on the inclusion complex of cryptophane-E and $\text{CHCl}_3$

W. Shen,<sup>1</sup> C. Zhang,<sup>1</sup> R. Fan, C. Dong<sup>1</sup> and S. Shuang<sup>1</sup>

<sup>1</sup>Centre of Environmental Science and Engineering Research, School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: smshuang@sxu.edu.cn

The design of hosts capable of binding neutral substrates has attracted a great deal of attention in supramolecular chemistry, and in this context the capture of the smallest aliphatic hydrocarbons still remains a challenging problem. The fluorescence spectra of a synthetic host, cryptophane-E, and its inclusion complex with chloromethane are reported. Cryptophane-E was synthesized from vanillin by a three-step method and its fluorescence spectroscopic properties were determined. A fluorescence maximum emission peak of cryptophane-E was obtained at 320–325 nm and the maximum excitations were about 294–298 nm in different organic solvents. When the chloromethane was added to cryptophane-E solution, fluorescence quenching occurred and the emission peak had no changes in definite concentration range. The results showed that cryptophane-E was well suited for inclusion of  $\text{CHCl}_3$  to form a stable 1:1 host–guest inclusion complex. The binding constant ( $K$ ) showed obvious solvent effect: it was comparatively larger in a bulky molecular organic solvent (ethyl acetate;  $K = 106 \pm 9$ ) than in a small molecular organic solvent.

### Phosphorescent properties of 2-bromoquinoline-3-boronic acid in sodium deoxycholate aggregates and potential application in recognition of carbohydrates

Q.-J. Shen,<sup>1</sup> W. S. Zou,<sup>1</sup> W. J. Jin<sup>1,2</sup> and Y. Wang<sup>1</sup>

<sup>1</sup>School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, People's Republic of China

<sup>2</sup>College of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China. E-mail: wjjin@bnu.edu.cn

We studied the RTP properties of the aromatic boronic acid 2-bromoquinoline-3-boronic acid (BrQBA) and anticipated its application in recognition of carbohydrate, which is more suitable for biological assay. RTP of BrQBA was induced in sodium deoxycholate (NaDC) aqueous solution without deoxygenation. It was found that 4.0 mmol/L was a proper NaDC concentration to form aggregates, which provided a rigid hydrophobic domain for BrQBA and protected BrQBA from being quenched. RTP intensity decay was fitted to a biexponential model and the lifetimes of long-lived and short-lived components were 6.99 and 1.39 ms, respectively. The fractional contribution of the long-lived component was > 73.5%, indicating that a majority of BrQBA molecules were protected well by NaDC aggregates. The RTP intensity of BrQBA increased continuously with standing time, and heating could accelerate the interaction between BrQBA and NaDC aggregates. As expected, the RTP of BrQBA was enhanced upon binding of carbohydrates. The stability constants of BrQBA–carbohydrate complexes were  $2.58 \times 10^3$  L/mol for D-fructose,  $1.84 \times 10^3$  L/mol for D-galactose,  $1.55 \times 10^3$  L/mol

for D-glucose and  $1.29 \times 10^3$  L/mol for D-glucose, respectively, indicating a good selective sequence.

### Enhanced luminol– $\text{O}_2$ ECL on the iron(II) phthalocyanine-modified electrode by imidazole

Q. Shu, L. Zheng, Y. Dong, Y. Chi and G. Chen

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), and College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, Fujian 350002, People's Republic of China. E-mail: y.w.chi@fzu.edu.cn

The combination of a luminol– $\text{O}_2$  electrochemiluminescent (ECL) system with a FePc– $\text{O}_2$  electrocatalytic system is of considerable interest in ECL, electrocatalysis and molecular biology studies. In the present study, luminol– $\text{O}_2$  ECL behaviours at a FePc-modified electrode and their interaction with imidazole (Im) were reported. The presence of FePc on the electrode inhibited luminol– $\text{O}_2$  ECL; however, further addition of Im or its derivatives could restore the ECL. The electrochemical and ECL behaviours of systems including luminol– $\text{O}_2$ , luminol– $\text{O}_2$ –FePc and luminol– $\text{O}_2$ –FePc–Im were studied in detail. On this basis, the mechanism of enhanced luminol– $\text{O}_2$ –FePc ECL in the presence of Im was demonstrated, and a novel and sensitive ECL method for detecting Im and its derivatives was established.

### Purification and characterization of recombinant luciferase from the click-beetle *Pyrearinus termitilluminans*

A. J. Silva Neto, F. G. C. Arnoldi and V. Viviani

Laboratório de Bioluminescência e Biotecnologia (UNISO), Universidade Federal de São Carlos, Sorocaba, Brazil.

E-mail: ajsn@rc.unesp.br

The expression, purification and characterization of recombinant luciferase from the larval click-beetle *Pyrearinus termitilluminans* is reported. The bioluminescence spectrum of the purified enzyme ( $\lambda_{\text{max}} = 538$  nm) is the most blue-shifted among all known coleopteran luciferases. The kinetics studies show a slow-type decay similar to green-emitting railroad worm luciferase (PxGR), while the red-emitting railroad worm luciferase (PxRE) presents a flash-like decay. The affinity constants for ATP and luciferin were also determined and compared with those from red- and green-emitting railroad worm luciferases. Modelling studies show a hydrophobic environment at the active site, as expected by structural studies from *Photinus pyralis* and *Luciola cruciata* luciferases and confirmed by TNS probe assays.

### Study on the interaction between methyl blue and HSA in the presence of $\beta$ -CD–HP– $\beta$ -CD by molecular spectroscopy

S.-M. Song, X.-L. Hou, S.-M. Shuang and C. Dong

Research Centre of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: dc@sxu.edu.cn

Research on the supramolecular interactions of organic dyes and biological molecules is important to understand the structure and function of biological macromolecules, which can

be used to simulate biophysical processes. In this paper, the interaction between methyl blue (MB) and human serum albumin (HSA) in the presence and absence of  $\beta$ -cyclodextrin ( $\beta$ -CD)–HP– $\beta$ -CD were studied, based on UV-vis and fluorescence methods. The experimental results showed that the fluorescence of MB can be enhanced by CDs because it can enter the hydrophobic cavity of CDs and the radiant transition from  $S_1$  to  $S_0$  was protected. Meanwhile, CDs can strengthen the binding between MB and HSA by changing the micro-environment of MB and forming a MB–CDs–HSA ternary supramolecular system. The inclusion ratio and inclusion constants of MB–HSA, MB– $\beta$ -CD–HP– $\beta$ -CD and MB– $\beta$ -CD–HP– $\beta$ -CD–HSA were calculated.

### Expression and purification of the recombinant luciferase of *Metridia longa*: solving the homogeneity problem

G. A. Stepanyuk,<sup>1,2</sup> H. Xu,<sup>2</sup> C.-K. Wu,<sup>2</sup> S. V. Markova,<sup>1</sup> J. Lee,<sup>2</sup> E. S. Vysotski,<sup>1,2</sup> and B.-C. Wang<sup>2</sup>

<sup>1</sup>Institute of Biophysics, Russian Academy of Sciences, Siberian Branch, Krasnoyarsk 660036, Russia.

E-mail: galchonok\_s@yahoo.com

<sup>2</sup>Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA

The luciferase of the marine copepod *Metridia longa* (MLuc) is a secreted protein which uses coelenterazine as a substrate to produce blue light ( $\lambda_{\text{max}} = 480 \text{ nm}$ ). MLuc is a very powerful and convenient reporter protein. The main advantage in applying a secreted luciferase as a reporter is the opportunity to measure intracellular events without destroying cells or tissues, and it is well-suited for the development of high-throughput screening technologies. However, because MLuc contains 10 Cys residues, *Escherichia coli* expression systems produce incorrectly folded protein that does not allow its physicochemical characterization. Therefore, MLuc has been expressed in *Spodoptera frugiperda* (Sf9) insect cells, using a baculovirus vector. The fully active recombinant MLuc was purified to homogeneity from insect cell culture medium with a yield of ca. 2–3 mg/L. The properties of this MLuc have been compared to those of MLuc expressed and purified from *E. coli*. MLuc from insect cells is a correctly folded monomer protein with activity four times higher than that of luciferase from *E. coli*. Supported by RFBR Grant No. 06-04-89502, Taiwan NSC, the University of Georgia Research Foundation and the Georgia Research Alliance.

### Kinetics of bacterial coupled enzymatic system NAD(P)H: FMN-oxidoreductase–luciferase catalysis in solvents of increased viscosity

I. E. Sukovataya, E. V. Kaykova, N. S. Buka and L. Zadorozhnaya  
 Department of Biophysics, Siberian Federal University, Pr. Svobodnyi 79, 660041 Krasnoyarsk, Russia.  
 E-mail: irinas@lan.krasu.ru

Luciferase activity has been shown to be regulated by solvent viscosity, induced by the addition of viscous solvents. Steady-state kinetic studies of bacterial coupled enzymatic systems, NAD(P)H:FMN-oxidoreductase–luciferase, were made in glycerol/water and sucrose/water solutions of increased viscosity in order

to determine the effect of diffusion control on the decay rate of excited emitter (light decay rate constant). The stabilization of the excited intermediate observed in the presence of sucrose was greater in the case of glycerol. The catalytic activity of the bacterial bioluminescence system was noticeably enhanced in the presence of sucrose, whereas glycerol was found to inhibit the coupled enzyme system in all concentration ranges used. This study demonstrates that solvent viscosity regulates the stabilization of the excited intermediate of the coupled reactions catalysed by FMN:NADH-oxidoreductase. Supported by Grant No. 07-04-01340-a from the Russian Foundation for Basic Research.

### Lecture course on photobiophysics based on bioluminescence

I. E. Sukovataya,<sup>1</sup> V. A. Kratasyuk,<sup>1,2</sup> E. N. Esimbekova<sup>1,2</sup> and I. V. Sviderskaya<sup>1</sup>

<sup>1</sup>Siberian Federal University, Pr. Svobodnyi 79, 660041 Krasnoyarsk, Russia. E-mail: VKratasyuk@yandex.ru

<sup>2</sup>Institute of Biophysics, Akademgorodok, 660036 Krasnoyarsk, Russia

All aspects of photoreactions, from the elementary phenomena and laws, through reactions and apparatus review to practical aspects of photochemistry, photobiology and photobiophysics, are presented in the course on photobiophysics. This course consists of the following sections: 1. Introduction to photobiophysics. Primary and secondary photochemical processes; 2. Bio-, photo-, electro-, sono- and chemiluminescence, fluorescence, phosphorescence, absorption of electromagnetic radiation; 3. Kinetics of photoreactions. Stern–Volmer equation. Chain reaction in nature. The types of photoreactions with examples: photodissociation, photocyclization, photoisomerization, photosubstitution; 4. Chemiluminescence; 5. Bioluminescence; 6. Photosynthesis: photobiochemistry and photobiophysics; 7. Spectroscopical methods: X-ray crystallography; NMR spectroscopy; molecular modelling; computational methods in biochemical systems; protein–DNA interactions and others. Through this course the student would obtain knowledge about the main principles of photobiophysics and would acquire the ability to understand and interpret different aspects of bioluminescence in the natural environment. Supported by Grant Nos 07-04-01340-a from the Russian Foundation for Basic Research and 06-06-00229a from the Russian Humanitarian Foundation.

### Measurement of the binding constants of hexasulphonated calix[4]arene and amino acids by capillary electrophoresis

S. Sun  
 Xuzhou Air Force College, Xuzhou 221000, People's Republic of China. E-mail: sunshian2006@163.com

Capillary electrophoresis is a useful approach for studying receptor–ligand interactions and determination of the binding constants of the complexes formed. An attractive feature is its unique ability to assess the study of receptor–ligand interactions, with only small amounts of samples required. Purification prior to injection is not necessary so long as the component to be analysed in the sample matrix can be separated from other

species. Simultaneous determination of binding constants for each individual component within a sample mixture is also possible. Therefore it can be used not only in macromolecular systems but also in biology. In this work, the binding constants between hexasulphonated calix[4]arene and the three amino acids L-Val, L-Leu and L-Phe were obtained by capillary electrophoresis. Under the conditions of 10 mmol/L phosphate buffer, the values  $\log K$  were 1.81, 2.29 and 2.51, respectively. Using the molecular mechanism method to study their interactions, we found that the Vander force was the main force in forming host–guest inclusion complexes.

### Assembly of semiconductor nanocrystals and interfacial recognition for DNA

X. Y. Sun, H. Liu, C.-X. Yang, G.-X. Yang and B. Liu  
College of Materials Science and Engineering, Huaqiao University, Quanzhou, Fujian 362021, People's Republic of China. E-mail: liumy@hqu.edu.cn

CdTe semiconductor nanocrystals were synthesized in aqueous solution, using thioglycolic acid as the stabilizer. The effects of conditions on the synthesis of CdTe were studied. CdTe was assembled onto the alkylated silica supports by electrostatic interaction. By spectroscopy techniques, characterizations of the membrane were carried out, microscope technique were employed to depict the film's surface structure. The interactions between DNA and CdTe quantum dots were investigated. The sensor-based self-assembly-monolayers (SAMs) were regenerable and extremely highly sensitive in detecting DNA.

### A novel multiplex biosensor based on fluorescent self-assembly multilayers

X. Sun, Z. Wang, F. Li, Y. Zhang and B. Liu  
College of Materials Science and Engineering, Huaqiao University, Quanzhou, Fujian 362021, People's Republic of China. E-mail: liumy@hqu.edu.cn

Quantum dots CdTe and other fluorescent reagents were assembled indirectly onto the surface of a glass substrate via their electrostatic interaction. A novel fluorescent biosensor based on multiplex assays without complex instrumentation and processing was designed. The effects of pH and ionic strength on this process were investigated initially. The mechanism was also studied. The developed multilayer films could be used to detect multiple analytes with good regeneration and sensitivity.

### Studies and application of the fluorescent behaviour of the interaction between porphyrin and enoxacin

S.-T. Sun, Y.-Y. Xu, X.-P. Zheng, Q. Liu, Y.-M. Yang, S.-Y. Liu and Q. Wei  
School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, People's Republic of China. E-mail: sdjndxwq@263.net

The binding reaction of meso-(3-methoxy-4-hydroxyphenyl) porphyrin and enoxacin was studied using a fluorescent

method. The porphyrin compound is a large link conjugated aromatic system and is widely used in the determination of metal ions, but nowadays the analytical applications of porphyrin have turned to biochemistry. Based on the fluorescence enhancement of meso-(3-methoxy-4-hydroxyphenyl) porphyrin by enoxacin, a new method for the determination of enoxacin was established. The reaction conditions of the system were studied in a series of experiments. At pH 5.70 and in the presence of AOT micro-emulsion, the excitation and emission wavelength were set at 421 nm and 650 nm, and the fluorescence intensity could be effectively enhanced by enoxacin. The linear range of the working curves was 1.00–12.00  $\mu\text{g/mL}$  for enoxacin, with a detection limit of 0.315  $\mu\text{g/mL}$ . The method is sensitive, stable and relatively free from interference of co-existing species. The binding number was also studied by the molar ratio method; the stable complex of porphyrin and enoxacin was formed in the molar ratio of 1:3. The analytical results for commercial enoxacin powder injection were satisfactory.

### Effects of photosensitizer and goat anti-human IgG on upconversion and downversion luminescence of $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ nanocrystals

Y. Sun,<sup>1</sup> Y. Yu,<sup>1</sup> Y. Zhang,<sup>1</sup> Y. Yang,<sup>1</sup> X. Liu,<sup>1</sup> Q. Zeng,<sup>1</sup> X. Kong<sup>1</sup> and H. Zhang<sup>2</sup>

<sup>1</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China. E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

Well-dispersed, small-sized, uniformly distributed and water-soluble  $\text{NaYF}_4:\text{Yb}, \text{Er}$  nanocrystals with highly effectively luminescence upconversion were synthesized directly by a polymer system via a hydrothermal process. Enhancement of upconversion luminescence and the changes of relative intensity among different emitting peaks of the nanocrystals coupled with goat anti-human IgG and photosensitizer was observed. Changes in the relative intensity of downconversion luminescence are dependent on the concentration and kinds of biomolecules, which perhaps could be used as a method of biomolecule identification and diagnosis of malignant and benign cells in tumours. The single oxygen results in the death of a cancer cell 20 min after the photosensitizing effect of a luminescence upconversion nanoparticle excited under a 980 nm laser, which indicates the feasibility for cancer therapy.

### Solvent-promoted chemiluminescent decomposition of bicyclic dioxetanes bearing a 4-(benzothiazol-2-yl)-3-hydroxyphenyl

M. Tanimura, N. Watanabe, H. K. Ijuin and M. Matsumoto  
Department of Chemistry, Kanagawa University, Tsuchiya, Hiratsuka, Kanagawa 259-1293, Japan. E-mail: matsumo-chem@kanagawa-u.ac.jp

When bicyclic dioxetane **1**, 4,4-dimethyl-5-alkyl-2,4,6-trioxabicyclo[3.2.0] heptane, bearing a 4-(benzothiazol-2-yl)-3-hydroxyphenyl moiety were heated in an aprotic polar solvent,



such as DMF and NMP, they underwent decomposition with the accompanying emission of green light. This solvent-promoted chemiluminescent decomposition (SPCD) was as effective ( $\Phi^{\text{CL}} = 0.25$ ) as those of base-induced chemiluminescent decomposition of **1** by a charge-transfer-induced chemiluminescence (CTICL) mechanism. Kinetic analysis revealed that the SPCD proceeded through considerably regulated hydrogen bonding of hydroxyphenyl group in **1** with solvent molecule(s), and was suggested to be an entropy-controlled reaction different from the usual base-induced chemiluminescent decomposition of phenolic dioxetanes.

### **Bioluminescence imaging of intracellular calcium dynamics by the photoprotein obelin**

M. M. Thet, T. Sugiyama and H. Suzuki

Research and Development Division, Olympus Corporation, Tokyo, Japan. E-mail: hirobumi2\_suzuki@ot.olympus.co.jp

We developed a luminescence microscope and applied it for imaging reporter assay of c-fos promoter. The c-fos promoter contains calcium response element region and responds to numerous environmental changes outside the cell. We tried to visualize the process from calcium signalling to gene expression of c-fos at the single-cell level. HeLa cells were co-transfected with apo-obelin- and c-fos promoter-luciferase vectors, and were incubated with coelenterazine to reconstruct obelin, a calcium-regulated photoprotein. Intracellular calcium dynamics triggered by ATP and ionomycin was visualized, and c-fos promoter activity regulated by calcium signal was also monitored sequentially in the same cell using a luminescence microscope. Calcium and c-fos signal lights emitted from obelin and luciferin were separated by an optical filter. As a result, heterogeneous responses of calcium signalling and c-fos activity were observed among individual cells. Such results can not be obtained by conventional luminometric assays. It is expected that the imaging promoter assay will contribute to further understanding of signal transduction, gene expression and other cellular activities.

### **Study on the interaction of kaempferol with human serum albumin, using spectroscopy and a molecular modelling method**

J. Tian,<sup>1</sup> J. Liu,<sup>3</sup> Y. Zhao,<sup>1</sup> Z. Hu<sup>2</sup> and X. Chen<sup>2</sup>

<sup>1</sup>College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004, People's Republic of China. E-mail: tianjn58@yahoo.com.cn

<sup>2</sup>Department of Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

<sup>3</sup>Mianyang Teacher's College, Mianyang 621000, People's Republic of China

The binding of kaempferol with human serum albumin (HSA) was investigated by fluorescence, circular dichroism (CD), Fourier transform infrared spectroscopy (FT-IR) and a molecular modelling study at pH 7.40. The binding parameters were determined by Scatchard's procedure, which were approximately consistent with the results of the Stern-Volmer equation and the molecular modelling study. The thermodynamic parameters were calculated according to the dependence of the enthalpy change on

temperature. On the basis of all the experimental results and the molecular modelling study, it was considered that kaempferol binds to site I (subdomain II) of HSA mainly by hydrophobic interaction and there are hydrogen bond interactions between kaempferol and residues Arg-222, Leu-260, His-242 and Arg-257 of HSA.

### **Binding of puerarin and bovine serum albumin using fluorescence and circular dichroism spectroscopy**

J. Tian, X. Liu, Y. Zhao and S. Zhao

College of Chemistry and Chemical Engineering, Guangxi Normal University, Guilin 541004, People's Republic of China.

E-mail: tianjn58@yahoo.com.cn

The interaction of puerarin with BSA was studied using the spectroscopic method. The experimental results indicated that the binding site of puerarin is in the region close to Trp212. From thermodynamic parameters it was revealed that hydrophobic and electrostatic interactions all play important roles in the binding of puerarin to BSA. According to the Förster energy transfer theory, the separate distance  $r$ , the energy transfer efficiency  $E$  and Förster radius  $R_0$  were calculated. Changes in the environment of the fluorophore groups and the conformation of protein and the effect of other conditions, such as ionic strength, metal ions, pH and surfactants, on the binding of puerarin and BSA were researched.

### **Ca<sup>2+</sup>-dependent coelenterazine-binding protein of *Renilla* provides higher bioluminescence efficiency than free coelenterazine**

M. S. Titushin,<sup>1</sup> S. V. Markova,<sup>1</sup> L. A. Frank,<sup>1</sup> N. P. Malikova,<sup>1</sup> J. Lee<sup>2</sup> and E. S. Vysotski<sup>1,2</sup>

<sup>1</sup>Photobiology Laboratory, Institute of Biophysics, SB RAS, Krasnoyarsk 660036, Russia. E-mail: puzyakin@mail.ru

<sup>2</sup>Department Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA

The *Renilla* bioluminescence *in vivo* involves three proteins – the luciferase, green-fluorescent protein and Ca<sup>2+</sup>-dependent coelenterazine-binding protein (CBP). CBP belongs to EF-hand Ca<sup>2+</sup>-binding superfamily of proteins, with only three of the EF-hand loops having the Ca<sup>2+</sup>-binding consensus sequences. The full-size cDNA encoding CBP from the soft coral *Renilla muelleri* was cloned and expressed in *Escherichia coli* cells as inclusion bodies. There is a weak sequence homology with the Ca<sup>2+</sup>-regulated photoproteins, primarily as a result of the necessary Ca<sup>2+</sup>-binding loop structures. The recombinant protein was refolded and purified. The apo-CBP is quantitatively converted to CBP by simple incubation with coelenterazine. In combination with *Renilla* luciferase, addition of only one Ca<sup>2+</sup> is sufficient to make coelenterazine available for the luciferase. The luciferase with CBP as a substrate generates bioluminescence with higher reaction efficiency than using free coelenterazine alone. The increased quantum yield, the difference of bioluminescence spectra, and markedly different kinetics, implicate that a CBP-luciferase complex might be involved. Supported by Grant No. 05-04-48271 from the RFBR.

### Simultaneous isolation/concentration and bioluminescent detection of *Escherichia coli* using bacteriophage-based biosorbents

M. Tolba,<sup>1</sup> O. Minikh,<sup>1,2</sup> L. Y. Brovko<sup>1</sup> and M. W. Giffiths<sup>1</sup>

<sup>1</sup>Canadian Research Institute for Food Safety, University of Guelph, Guelph, ON N1G 2W1, Canada.

E-mail: lbrovko@uoguelph.ca

<sup>2</sup>Department of Chemical Enzymology, Lomonosov Moscow State University, Moscow 117899, Russia

Recombinant T4 bacteriophages were engineered to display affinity tags on their heads, thus ensuring orientated immobilization of the phage on the respective active surfaces whilst retaining maximum infectivity. As affinity tags, both biotin carboxyl carrier protein (BCCP) and cellulose binding module (CBM) were introduced through fusion with the small outer capsid (SOC) protein. Phage-based biosorbents were constructed, using streptavidin-coated magnetic beads, microcrystalline cellulose and filter paper as the solid support. The resulting biosorbents showed fast and effective capture of target *E. coli* cells, followed by cells lysis. The ATP-bioluminescent assay was used to monitor the cell lysis. Conditions for bacterial capture and lysis were optimized to achieve the highest possible bioluminescent signal. The resulting experimental protocol for simultaneous isolation/concentration and detection of *E. coli* was tested using inoculated water samples. The obtained detection limit was at least one order of magnitude better than that reported previously for a biosorbent based on chemically biotinylated bacteriophages. The advantages of the proposed approach for construction of highly effective and specific bacterial biosorbents and possibilities for extending this detection method to other bacterial pathogens are discussed.

### The theoretical studies of light emitters in bioluminescence of Ca<sup>2+</sup>-regulated photoprotein obelin

E. N. Tomilin,<sup>1</sup> L. U. Antipina,<sup>1</sup> S. G. Ovchinnikov<sup>1</sup> and

E. S. Vysotski<sup>2</sup>

<sup>1</sup>The Kirensky Institute of Physics of SB RAS, Krasnoyarsk 660036, Russia. E-mail: felixnt@gmail.com

<sup>2</sup>Photobiology Laboratory, Institute of Biophysics SB RAS, Krasnoyarsk 660036, Russia

The photoproteins consist of a single polypeptide chain having a hydrophobic cavity in which is bound 2-hydroperoxycoelenterazine. Bioluminescence is triggered on adding Ca<sup>2+</sup>, the binding of which to the protein induces a decarboxylation of the 2-hydroperoxycoelenterazine with generation of the protein bound product, coelenteramide, in its excited state. The crystal structures of different conformation states of obelin that yielded important information about its active site have been determined. However, the crystal structure is only a 'snapshot' of the predominant conformation. What events occur during decarboxylation and emitter formation, and the function of amino acids in these processes are still unknown. The quantum chemical calculations might account for these questions. Here we report the quantum chemical modelling of the excited states of coelenteramide (~50 atoms) embedded in the active site of the protein (~500 atoms). We demonstrate that the fluorescence wavelength crucially depends on the position of the proton

between the oxygen atom of the 5-*p*-hydroxyphenyl group of coelenteramide and the nitrogen atom of His22. Supported by Grant Nos 07-04-00930 from the RFBR and 06-04-89502 from RFBR-Taiwan NSC.

### Generation of high-energy chemiluminophores in the ambient light

Y. B. Tsaplev, R. F. Vasil'ev and A. V. Trofimov

Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Ul. Kosygina 4, Moscow 119334, Russia.

E-mail: avt\_2003@mail.ru

We report studies on the novel phenomenon named light-created chemiluminescence (LCCL), which constitutes the conversion of usual 'dark' reactions into chemiluminescent ones upon exposure of the reagents to ambient light. The pertinent examples of the LCCL are furnished by the reactions of pre-irradiated anthrone and salicylaldehyde hydrazone with bases, in which the intermediate photodimers and photoisomers intervene as high-energy chemiluminophores. We discuss the kinetic and the temperature behaviour of the LCCL processes, the observed photochromic effects, the modelled reaction energy profiles and the first manifestations of the LCCL in living nature. The observations imply that the list of known chemiluminescent reactions needs to be 'revisited' to disclose systems that may be particularly influenced by ambient illumination when the reaction mixtures are being prepared. Supported by the RFBR, RAS and RSSF.

### Efficient MXT-QDs fluorescence off-on switch probe for DNA detection in aqueous solution

X. J. Tu, J. O. Du, W. B. Chen, X. F. Hao and X. Q. Guo

Department of Chemistry, MOE Key Laboratory of Analytical Sciences and Key Laboratory for Chemical Biology of Fujian Province, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, People's Republic of China. E-mail: xqguo@xmu.edu.cn

The potential of high quenching efficiency of a quencher/receptor to a fluorophore to induce a sensitive signal transduction upon analyte recognition through a off-on fluorescence switch has remained largely unrealized. A dark quencher/receptor-fluorophore conjugate is proposed. In the conjugate, the quencher/receptor mitoxantrone (MXT) binds to mercaptoacetic acid (MAA)-capped CdTe quantum dots (MAA-CdTe QD) by electrostatic interaction in aqueous solution. Electron transfer was confirmed as a cause for the emission quenching of QDs by steady-state fluorescence spectra, time-correlated single-photon-counting lifetime spectra, absorption spectra and cyclic voltammetry spectra. QDs emission was suppressed in a way that was unprecedentedly efficient. This dark quencher/receptor-fluorophore has been explored as a DNA sensor. As expected, the QDs emission was readily switched on in the presence of the analyte-binding-to-receptor process, due to the high affinity of MXT to DNA. The photon-induced electron transfer between MXT and QDs was interdicted and thus turned on the emission of QDs. The turn-on emission of QDs responded in good linearity to DNA over the ranges 0.4–24 (ctDNA) and 0.6–22 (fsDNA) µg/mL, and the determination limits (3σ) were 0.12 µg/mL for ctDNA and 0.18 µg/mL for fsDNA.

### New method of measuring bacterial bioluminescence

N. A. Tyulkova, O. I. Krasnova and I. O. Pozdnyakova  
Institute of Biophysics, Siberian Branch of Russian Academy of Science, Krasnoyarsk, Russia. E-mail: biotech@ibp.ru

A new method of measuring the activity of bacterial luciferase, in which flavinmononucleotide is reduced in the presence of dithiothreitol or nicotinamidinucleotide, is proposed. The kinetic parameters of the bioluminescence reaction under the pH and molarity of the phosphate buffer condition were investigated. The dependence of luminescence intensity on FMN concentration follows Michaelis–Menten kinetics. The values of  $K_m$  are close to respective ones in the reaction with photoreduced FMN. The bioluminescence reaction is a prolonged glow and proceeds as the first-order reaction at the rate constants of  $4.7 \times 10^{-5}/s$  with 40  $\mu\text{mol}$  of DDT, and  $1.5 \times 10^{-3}/s$  with 10–12  $\mu\text{mol}$  NADH in the reaction mixture. With DTT the reaction can run for several hours; in the first 30–40 min the luminescence intensity is constant.

### Mechanism responsible for the spectral differences in firefly luciferase bioluminescence

N. N. Ugarova  
Chemistry Faculty, Lomonossov Moscow State University, Moscow, 119991, Russia. E-mail: unn@enz.chem.msu.ru

Several models have been proposed to explain the spectral differences in firefly luciferase bioluminescence and the pH sensitivity of bioluminescence spectra, which were often considered as opposite to one another. In this report we consider these models in the frame of one united mechanism by analysing the literature and our own experimental data. A luciferase protein makes up the microenvironment of the emitter at an active site (rigidity of environment, micro-pH value, polarizability and so on), which controls the energy of emitter and therefore the bioluminescence  $\lambda_{\text{max}}$ . The more rigid a protein structure is around the excited oxyluciferin molecule, the higher is the emission energy and the lower the  $\lambda_{\text{max}}$  of the bioluminescence spectra. When the pH value gradually drops, or the emitter environment becomes more flexible, the emitter energy decreases, new states of the emitter can be realized with lower energy and new bands appear in the bioluminescence spectra. As a result, we observe a superposition of at least two different excited states that differ in energy level.

### Illuminating molecular processes in living cells

Y. Umezawa  
Department of Chemistry, School of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.  
E-mail: umezawa@chem.s.u-tokyo.ac.jp

For imaging molecular processes in living cells, we developed novel intracellular fluorescent and bioluminescent indicators for second messengers, protein phosphorylation, protein–protein interactions and protein localizations that work in single living cells. Key molecules and steps of cellular signalling pathways were visualized in target live cells under fluorescent microscopy using developed the fluorescent indicators.

### The origin of pH sensitivity in beetle luciferases

V. R. Viviani,<sup>1</sup> F. G. C. Arnoldi,<sup>2</sup> A. J. Silva Neto,<sup>2</sup> A. A. Barbosa<sup>3</sup> and Y. Ohmiya<sup>4</sup>

<sup>1</sup>Laboratório de Bioluminescência e Biotecnologia (UNISO), Universidade Federal de São Carlos, Sorocaba, Brazil.

E-mail: viviani@ufscar.br

<sup>2</sup>UNESP-Rio Claro, Brazil

<sup>3</sup>LNLS, Campinas, Brazil

<sup>4</sup>Hokkaido Graduate School of Medicine, Sapporo, Japan

Beetle luciferases produce a wide range of colours, varying from green to red. In firefly luciferases, the bioluminescence spectrum displays a remarkable red-shift at acidic pH, higher temperatures and other denaturing conditions, a condition that has been called 'pH sensitivity'. Other beetle luciferases do not display this spectral shift and for this reason have been called 'pH-insensitive' luciferases. Despite many studies about the structure and function of firefly luciferases, the structural origin of pH sensitivity was until recently unclear. Through a comparative site-directed mutagenesis and modelling studies using a set of pH-sensitive (*Macrolampis* and *Cratomorphus* spp.) and pH-insensitive (*Pyrearinus* and *Phrixotrix* spp.) luciferases cloned in our laboratories, we found that the loop between residues 223–235 and the associated network of hydrogen-bonded residues may constitute an alternative solvent gate to the active site in pH-sensitive luciferases, modulating bioluminescence colours. Studies with luciferyl–adenylate chemiluminescence support the idea that red bioluminescence, in the case of pH-sensitive luciferases, arises from the polarization of the active site or the release of luciferyl–adenylate from the active site. Despite the pH-dependent red-shift having been considered the result of denaturation, it could be a vestige of the early stages of evolution of bioluminescence in firefly luciferases.

### CCD imaging of basal bioluminescence in larval fireflies: clues on the anatomical origin of beetle bioluminescence

V. R. Viviani, F. M. Okawachi, V. Scorsato and F. C. Abdalla  
Laboratório de Bioluminescência e Biotecnologia (UNISO), Universidade Federal de São Carlos, Campus de Sorocaba, Sorocaba, SP, Brazil. E-mail: viviani@ufscar.br

The origin of beetle bioluminescence remains a mystery. Following CCD imaging, we report that larvae and pupae of the Brazilian fireflies *Aspisoma lineatum* and *Cratomorphus* sp. emit a continuous low level of bioluminescence throughout the entire body during all stages. This luminescence is especially developed after feeding, ecdysis and the in the pupal stage, gradually disappearing as the cuticle becomes sclerotized. This weak glow arises from the fat body, which is arranged as round bodies spread all over the body cavity. According to their pigmentation, these round bodies can be divided in whitish and pinkish categories which display different luciferase isozymes. These studies provide the first evidence that the ventral lanterns in the 8th abdominal segment of larval fireflies directly evolved from these white bodies, confirming the early proposal by Hess, and providing a rationale for the widespread location of lanterns in the larvae of different bioluminescent beetles. The biological and biochemical function of this weak diffuse bioluminescence is discussed in the context of the larval life-history. Supported by CNPq and FAPESP.

## Biodiversity of bioluminescent beetles in the Brazilian Atlantic rainforest

V. R. Viviani, R. M. Yamazaki, P. Tanioka, V. Scorsato and R. A. Prado

Universidade Federal de São Carlos, Campus de Sorocaba, Sorocaba, Brazil. E-mail: viviani@ufscar.br

Bioluminescence occurs predominantly in the marine environment. In the terrestrial environment, the tropical forests are the richest ecosystems. Among terrestrial organisms, the insects are the richest group of luminescent species, especially the order Coleoptera (beetles), which accounts for about 2000 described species in the superfamily Elateroidea, which include the families Lampyridae (1800), Phengodidae (150) and Elateridae (100). The Brazilian Atlantic rainforest is one of the richest and also more threatened ecosystems. During the past 20 years, we have done continuous prospection of luminescent species occurring in the Atlantic rainforest of São Paulo state. We have studied different habitats, mainly in the municipalities of Campinas, Salesópolis, Rio Claro and, more recently, Sorocaba and the Southern region of Atlantic rain forest in São Paulo state. A total of 49 different species were catalogued (Lampyridae, 34; Phengodidae, 6; Elateridae, 7; Staphylinidae, 1). The preserved area of the biological station of Boraceia Salesópolis accounted for the largest number of species (30), followed by Campinas (25), Rio Claro (22) and Sorocaba (20). Besides the scientific and biotechnological interests, some bioluminescent species may constitute new important environmental bioindicators, mainly for assessing nocturnal environments, water courses and marshy environments. Supported by FAPESP-Biota, Grant No. 0651911-0.

## Luminous mushrooms

G. A. Vydryakova,<sup>1</sup> N. V. Psurtseva,<sup>2</sup> N. V. Belova,<sup>2</sup> A. A. Gusev,<sup>1</sup> N. V. Pashenova,<sup>3</sup> S. E. Medvedeva,<sup>1</sup> E. K. Rodicheva<sup>1</sup> and J. I. Gitelson<sup>1</sup>

<sup>1</sup>Institute of Biophysics SB RAS, Krasnoyarsk, Russia.

E-mail: vydryakova@yahoo.com

<sup>2</sup>Komarov Botanical Institute Russian Academy of Sciences, St. Petersburg, Russia

<sup>3</sup>Institute of Forest SB RAS, Krasnoyarsk, Russia

There is a great variety of living things able to emit light. Fungi are among them; about 60 species of luminous fungi are known, mostly found in the tropics. *Armillaria mellea* is one of the most widespread among bioluminescent fungi, being a common root rot and wood decay fungus found across North America, Europe and Asia. Luminous fungi emit light during different stages of their life cycles. *Armillaria*'s mycelium and rhizomorphs are luminous; *Mycena rorida* produces only luminous spores; in a number of *Mycena* species luminescence presents in mycelia; *Panellus stipticus* and *Omphalotus olearius* have luminous mycelia and fruiting bodies; *Collybia tuberosa* produces only luminescent scleroti; while *Omphalotus af. illudent* is able to emit light in filament and fruitbody form (its whole body – pileus, stem, gills, spores – emits light). The mechanism of fungal luminescence is still unknown; the structure of natural luciferins has not been determined and luciferase has not been detected. Nevertheless, different species of naturally bioluminescent fungi have been used to develop bioluminescence-based bioassays for toxicity testing.

## The diversity of coelenterazine-dependent bioluminescent proteins

E. S. Vysotski,<sup>1,2</sup> S. V. Markova,<sup>1</sup> G. A. Stepanyuk,<sup>1</sup> L. A. Frank<sup>1</sup> and J. Lee<sup>2</sup>

<sup>1</sup>Photobiology Laboratory, Institute of Biophysics, Russian Academy of Science, Siberian Branch, Krasnoyarsk 660036, Russia

<sup>2</sup>Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA.

E-mail: eugene\_vysotski@ibp.ru or vys@uga.edu

Although the phenomenon of bioluminescence is widespread, most luminous species are marine inhabitants. Among several luciferins that have been identified in marine organisms, one well-characterized type is represented by imidazopyrazinone (3,7-dihydroimidazopyrazin-3-one) derivatives, such as coelenterazine and *Cypridina* luciferin. Coelenterazine and its derivatives have been identified as luciferins in various luminous organisms (corals, shrimps, medusas, squids, copepods, etc.). From such a diversity of organisms using these luciferins, it seems reasonable to assume that there could be many other luminous organisms as yet uninvestigated which also use these luciferins. Why so many bioluminescent proteins having no apparent similarity between the primary structures and most likely the spatial structures utilize the same luciferin for bioluminescence is still an unanswered question. Here we report some structural features and properties for available coelenterazine-dependent bioluminescent proteins, as well as discussing the mechanism of coelenterazine-dependent bioluminescence. Supported by Grant No. 06-04-89502 from the Russian Foundation for Basic Research and the Taiwan National Science Council.

## A novel electrochemiluminescent biosensor based on cationic polymer/chitosan for ultrasensitive detection of hydrogen peroxide

Y. Wang, H. Dai, X. Wu and G. Chen

Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and

Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350002, People's Republic of China. E-mail: wapple@fzu.edu.cn

In this paper, the electrochemiluminescence (ECL) behaviour of luminol and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) system on a poly(diallyldimethyl ammonium chloride) (PDDA)–chitosan-modified galssy carbon electrode (GCE) was studied. It was found that PDDA could greatly enhance the ECL intensity of luminol. When PDDA itself was used for fabricating the modified electrode, however, low reproducibility and poor signal stability was shown. Chitosan was introduced to improve the stability of the modified electrode, due to its excellent film-forming ability and high mechanical strength. It was observed that an increase in the ratio between chitosan and PDDA in the modified film induced more stability but less ECL of the system. Based on the enhancement of ECL intensity of luminol, a new ultra-sensitive and simple ECL method for the determination of  $\text{H}_2\text{O}_2$  was developed. Under the optimized conditions, the enhanced ECL intensity vs.  $\text{H}_2\text{O}_2$  concentration was linear in the range  $1.0 \times 10^{-9}$ – $5.0 \times 10^{-5}$  mol/L, with a detection limit of  $8.5 \times 10^{-10}$  mol/L, and the RSD for  $1.0 \times 10^{-7}$  mol/L  $\text{H}_2\text{O}_2$  was 0.97% ( $n = 12$ ). The method exhibited good reproducibility and sensitivity. It could be potentially used for bioanalytical purposes.



### Electrochemiluminescence determination of fentanyl citrate with a novel glassy carbon paste electrode

Y. Wang, H. Dai, X. Wu, H. Chen, L. Xu, Y. Chen and G. Chen  
Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University) and Department of Chemistry, Fuzhou University, Fuzhou, Fujian 350002, People's Republic of China. E-mail: wapple@fzu.edu.cn

Fentanyl citrate is a powerful narcotic analgesic for treating pain; however, it has been associated with many overdose accidents and deaths. To control its dosage, many methods, such as GC, HPLC, GC-MS and FIA, have been used for its quantitative assay. In this study it was found that fentanyl citrate could increase the electrochemiluminescence (ECL) intensity of  $\text{Ru}(\text{bpy})_3^{2+}$ , hence a new sensitive method based on ECL was developed for the determination fentanyl citrate. The ECL sensor was prepared by N-octylpyridium tetrafluoroborate (OPFP) ionic liquid and glassy carbon power, which is an isotropic form of carbon and has good electrical conductivity. The experimental results illustrated that a higher ECL intensity of  $\text{Ru}(\text{bpy})_3^{2+}$  is obtained with a GC/OPFP paste electrode than that with a GC/oil paste electrode. The conditions for the ECL determination of fentanyl citrate were optimized. Under the optimized conditions, the enhanced electrochemiluminescent intensity vs. fentanyl citrate concentration was linear in the range  $1.0 \times 10^{-8}$ – $1.0 \times 10^{-4}$  mol/L, with a detection limit of  $8.5 \times 10^{-9}$  mol/L, and the RSD for  $1.0 \times 10^{-6}$  mol/L fentanyl citrate was 1.90% ( $n = 10$ ). The method exhibited good reproducibility and high sensitivity and could be used for a potential ECL bioanalysis.

### In situ primary study on typical PAHs on mangrove leaves

P. Wang,<sup>1</sup> K. Z. Du,<sup>2</sup> Y. X. Zhu<sup>2</sup> and Y. Zhang<sup>1</sup>

<sup>1</sup>State Key Laboratory of Marine Environmental Science, Environmental Science Research Centre of Xiamen University, Xiamen 361005, People's Republic of China

<sup>2</sup>Department of Chemistry, Xiamen University, Xiamen 361005, People's Republic of China. E-mail: fruitful@xmu.edu.cn

A solid surface fluorimetry (SSF) method for direct determination of anthracene (An) adsorbed on mangrove fresh leaves was established. The effects of exposure concentration of An, exposure time and the different quantities of leaf-wax content among four kinds of mangrove leaves were investigated for their capacity to adsorb An. Under the same experimental conditions, the adsorption of An on the upper and lower sides of the same mangrove leaf and different regions on the upper side of the same mangrove leaf were also studied. The results showed that different kinds of mangrove leaves had their own capacities to adsorb An and also contained different quantities of leaf-wax. With the same exposure conditions of An, the quantity of leaf-wax was a significant positive correlation with the adsorbed An on the mangrove leaves. It was demonstrated that the SSF method was simple, rapid and it could be used for *in situ* determination of total An adsorbed on mangrove leaves. Therefore, SSF can directly reflect the adsorption of An on the mangrove leaf surface and roughly differentiate the distributions of adsorbed An in different regions of the same leaf surface without a necessity for sample pretreatment with a large amount of organic solvent.

### Luminol-reduced gold nanostructures: synthesis and chemiluminescence sensing

W. Wang, C.-F. Duan, T. Xiong, X. Yang and H. Cui  
Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, People's Republic of China. E-mail: weiw7@mail.ustc.edu.cn

The fabrication of chemiluminescence (CL) and electrochemiluminescence (ECL) sensors for  $\text{H}_2\text{O}_2$  was investigated using luminol-reduced gold nanostructures. First, luminol was used to synthesize gold nanoparticles (lumAuNPs) by acting as both reductant and stabilizer. The surface state of as-prepared lumAuNPs were investigated in detail and luminol was confirmed to be attached to the surface of lumAuNPs via Au–N interaction, which therefore offered lumAuNPs ECL activity. The functional lumAuNPs were subsequently immobilized on a gold electrode through a bridge-molecule cysteine to fabricate an ECL sensor for  $\text{H}_2\text{O}_2$ . In a follow-up study, hydrophilic polymer chitosan was introduced to the reaction system above and monodispersed three-dimensional gold nanoflowers (lumAuNFs) were obtained instead of spheric lumAuNPs. By virtue of the film-forming property of chitosan solution, a chitosan film containing lumAuNFs was easily fabricated on the surface of glass. This functional film exhibited linear CL responses to  $\text{H}_2\text{O}_2$  and could work as a CL sensor for its determination. The synthesis and CL/ECL sensing of luminol-reduced gold nanostructures described above show a novel way for the immobilization of CL reagent, which was thus of significance for the development and application of CL sensors.

### Rapid-mixing stopped-flow fluorimetric method for the determination of DNA and kinetic studies

R.-Y. Wang,<sup>1</sup> M.-N. Ji,<sup>2</sup> R. Wang<sup>2</sup> and J. Shi<sup>1</sup>

<sup>1</sup>Department of Chemistry, Zhengzhou University, Zhengzhou 450001, People's Republic of China. E-mail: rywang97@163.com

<sup>2</sup>School of Nursing, Zhengzhou University, Zhengzhou 450052, People's Republic of China

DNA quantification is critical for many biological studies, since it is often used as a reference for measurements of other biologically active components in biological fluids and genetic diagnosis. Fluorescent methods, which have the merits of high sensitivity, high selectivity and no radioactivity, play an important role in nucleic acid analysis. Rapid mixing techniques are widely used in kinetic studies of fast reactions. A simple, rapid, and sensitive stopped-flow kinetic fluorimetric approach was established for the assay of DNA in synthetic and real samples by using the measures of initial reaction rate. The increased initial reaction rate is in proportion to the concentration of DNA in the range  $1.0 \times 10^{-8}$ – $6.0 \times 10^{-6}$  mol/L. The optimum conditions for various parameters on which the binding of  $\text{Ru}(\text{bipy})_2(\text{dppz})^{2+}$  to DNA depends were investigated. The influence of various surfactants on the interaction was discussed. Furthermore, stopped-flow techniques were employed to determine kinetic parameters of  $\text{Ru}(\text{bipy})_2(\text{dppz})^{2+}$  binding to DNA under pseudo-first-order conditions. It was found that the interaction of  $\text{Ru}(\text{bipy})_2(\text{dppz})^{2+}$  with DNA was very fast. A two-step reaction mechanism, a fast phase followed by a slow phase, was proposed.

### Determination of diethylstilboestrol by flow-injection chemiluminescence immunoassay

S. Wang, L. Li, Y. Liang and Y. Zhao

Institute of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, People's Republic of China.

E-mail: shuhaowang@sohu.com

A novel approach to detect diethylstilboestrol using a flow-injection system coupled with enhanced chemiluminescent immunoassay was developed, based on non-competitive immunoassay formats. A conjugated diethylstilboestrol-ovalbumin immobilized immunoaffinity column was inserted into the flow system to trap the unbound horseradish peroxidase (HRP)-labelled antibody after an off-line incubation of diethylstilboestrol and HRP-labelled anti-diethylstilboestrol antibody. The trapped enzyme conjugate was detected by the injection of chemiluminescent substrates to produce an enhanced chemiluminescence. The linear range for the determination of diethylstilboestrol was 10.0–500 ng/mL, with a correlation coefficient of 0.993 and a detection limit of 5.0 ng/mL. The total time for the sampling and chemiluminescent detection of one sample was 400 s after 30 min of preincubation. The assay results for pregnancy serum samples obtained by this method were in good agreement with those obtained by ELISA.

### Synthesis of ytterbium and erbium co-doped sodium yttrium fluoride upconversion fluorescence materials

M. Wang, F. Li and S. K. Xu

Department of Chemistry, Northeastern University, Shenyang 110004, People's Republic of China.

E-mail: xushukun46@126.com

Nanocrystalline infrared-to-visible up-conversion phosphors, ytterbium and erbium co-doped sodium yttrium fluoride, were synthesized. Nanoparticles with narrow distribution and high luminescent intensity were prepared by a co-precipitation method in the presence of diethylenetriaminepentaacetic acid (DTPA) followed by heat-treatment at elevated temperature. Nanoparticles of different sizes in the range 25–120 nm could be obtained simply by varying the molarity of DTPA to  $\text{RE}^{3+}$  ( $\text{Y}^{3+}$ ,  $\text{Yb}^{3+}$ ,  $\text{Er}^{3+}$ ) and the pH value. Several parameters, such as pH value, concentration of the reactant, sequence of precursor addition, reaction time and annealing temperature, which have strong impacts on the structure, morphology and up-conversion fluorescence intensity of the nanoparticles, were investigated. Besides the strong emission at about 524 nm, a much more intense emission at about 541 nm was observed under 980 nm IR excitation. This strong NIR emission is favourable for the highly sensitive label used in biological detection.

### Determination of diethylstilboestrol by flow-injection chemiluminescence immunoassay

S. Wang, L. Li, Y. Liang and Y. Zhao

Institute of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, People's Republic of China.

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immunoassay was developed, based on non-competitive immunoassay formats. A conjugated diethylstilboestrol-ovalbumin immobilized immunoaffinity column was inserted into the flow system to trap the unbound horseradish peroxidase (HRP)-labelled antibody after an off-line incubation of diethylstilboestrol and HRP-labelled anti-diethylstilboestrol antibody. The trapped enzyme conjugate was detected by the injection of chemiluminescent substrates to produce an enhanced chemiluminescence. The linear range for the determination of diethylstilboestrol was 10.0–500 ng/mL, with a correlation coefficient of 0.993 and a detection limit of 5.0 ng/mL. The total time for the sampling and chemiluminescent detection of one sample was 400 s after 30 min of preincubation. The assay results for pregnancy serum samples obtained by this method were in good agreement with those obtained by ELISA.

### Selection of salt-tolerant rice variety using light-induced delayed fluorescence

J.-S. Wang,<sup>1</sup> W.-H. Xu,<sup>1</sup> D. Xing<sup>2</sup> and L.-R. Zhang<sup>2</sup>

<sup>1</sup>College of Information Technology, Dalian Maritime University, Dalian 116026, People's Republic of China.

E-mail: wangjsh@newmail.dlmu.edu.cn

<sup>2</sup>Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China

A method and biosensor was presented for the selection of salt-tolerant rice varieties, based on quantitative measurement of delayed fluorescence (DF). The integral characteristics of the DF decay dynamic curve of rice leaves under a single saturating light pulse was used to represent the salt-tolerance capacity of rice. The biosensor was composed of a hermetical leaf chamber, excitation light, fibre- and light-detection module, environmental controller, data acquisition and processing, keyboard and display, and so on. Rice (*Oryza sativa* L. var. *indica*) of known salt-tolerance capacity was used as the experimental material. Contrast experiments were accomplished between the biosensor and LI-6400. The results show that DF intensity can be rapidly and accurately used to represent the salt-tolerance capacity of a rice variety and it would be a powerful method in the selection of salt-tolerant plants.

### Alkaline metal ion-enhanced chemiluminescence of bicyclic dioxetanes bearing a 3-hydroxynaphthalen-2-yl group

N. Watanabe, F. Kakuno, N. Hoshiya, H. K. Ijuin and

M. Matsumoto

E-mail: sukesan\_3@hotmail.com

Chemiluminescence yields for base-induced decomposition of dioxetanes substituted with a hydroxynaphthyl group have been known to vary depending on the position of hydroxy group ('odd/even' relationship) on the aromatic ring. Thus, dioxetane 1 bearing a 3-hydroxynaphthalen-2-yl group ('even' pattern) displayed luminescence of yellow light with low efficiency, as expected, in TBAF/THF. However, the chemiluminescence

efficiency from dioxetane **1** was found to increase markedly (~200 times) when treated with *tert*-BuOM in THF. The chemiluminescence efficiencies increased in the order:  $K^+ < Na^+ < Li^+$ . Mechanistic investigation suggested that an alkaline metal ion coordinated most likely with an oxygen of dioxetane O–O as well as an oxidonaphthyl anion.

### Protein conformation changes study, based on room-temperature phosphorescence

Y. Wei, C. Dong and S. Shuang

Research Centre of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: yanli\_wei@yahoo.com.cn

Protein phosphorescence comes from tryptophan residues buried within its cores, which can be used to explore its conformation. In the present study, room-temperature phosphorescence of bovine (BSA) and human serum albumin (HSA) was developed, based on the iodide as 'heavy atom' and sodium sulphite for chemical deoxygenation. Various factors, including iodide concentration, sodium sulphite concentration and pH, were investigated in detail. The phosphorescence has better repetition and stability under the experimental conditions. The phosphorescence locates at 443 nm when excited at 287 nm, and the lifetimes are  $5.032 \pm 0.164$  and  $3.986 \pm 0.119$  ms, respectively. The phosphorescence is enhanced, the emissions are blue-shifted from 443 to 430 nm, and phosphorescence lifetime is prolonged with the addition of SDS. When the SDS concentration reaches 35  $\mu\text{mol/L}$ , the phosphorescence begins to decrease. It can be inferred that negatively charged SDS molecules can be aggregated around the protein and provide a dehydrated and anti-oxygen microenvironment. The CMC value of SDS can be decreased by the protein, and SDS micellar ball form to clubbed form cause protein folding, and the phosphorescence is quenched.

### Alleviation by exogenous salicylic acid and $\text{LaCl}_3$ of cadmium toxicity in maize seedlings; an ultra-weak bioluminescence study

Z. Wei,<sup>1</sup> C. Jiao,<sup>1</sup> Y. Su<sup>2</sup> and Z. Tian<sup>1</sup>

<sup>1</sup>Biology department, DeZhou University, DeZhou 253023, People's Republic of China. E-mail: wzl19741028@163.com

<sup>2</sup>Life Science School, HeBei University, HeBei 071002, People's Republic of China

Salicylic acid (SA) and  $\text{LaCl}_3$  were separated or mixed to alleviate Cd-stressed maize seedlings. To observe the difference in the alleviation capacity at 48 h, ultraweak bioluminescence (UBL) intensity, superoxide dismutase (SOD) and malonaldehyde (MDA) content were determined under different conditions. The UBL determination results showed whether 20 mg/L SA and 60 mg/L  $\text{LaCl}_3$  were separated or mixed and the delayed luminescence curve intensities were similar to control; with increased SA and  $\text{LaCl}_3$  concentration, curve intensity was similar to Cd treatment. SOD and MDA content roughly decreased as compared to Cd treatment, but a mixture of 20 mg/L SA and 60 mg/L  $\text{LaCl}_3$  was more significant than each alone. The SA and  $\text{LaCl}_3$  mixture was more significant to alleviate Cd-stressed maize seedlings at 48 h.

There is a close relationship between UBL and physiological parameters. The results suggest that plant UBL is more sensitive and more convenient than determination of physiological parameters.

### Effect of LMWOA on the biodegradation of phenanthrene by fluorimetry

X. Y. Wei,<sup>1</sup> L. Z. Sang,<sup>2</sup> Y. X. Zhu<sup>2</sup> and Y. Zhang<sup>1</sup>

<sup>1</sup>State Key Laboratory of Marine Environmental Science (Xiamen University), Environmental Science Research Centre, Xiamen University, Xiamen 361005, People's Republic of China

<sup>2</sup>Department of Chemistry, Xiamen University, Xiamen 361005, People's Republic of China.

E-mail: weixingyuan2006@xmu.edu.cn

Microbial degradation of polycyclic aromatic hydrocarbons (PAHs) is believed to be one of the principal means of successfully removing PAHs from polluted environments. Low molecular weight organic acids (LMWOA) have a potential effect on the biodegradation of PAHs in aqueous solution. However, studies on this have been reported rarely. In the present study we developed a fluorimetry method to investigate the effects of LMWOA on the biodegradation of phenanthrene in aqueous solution. Malic acid was selected as a model compound of LMWOA and three different concentrations of malic acid was set up for our research. The results indicated that the degradation rate of PAHs increased with the increment concentration of LMWOA. According to the study by Nehal, it can be hypothesized that the polarity of malic acid solvent affects the surface properties of bacteria and thus affects the interaction of PAHs and bacteria as well as the biodegradation of PAHs. The reason for the positive effect of malic acid on the biodegradation of PAHs and the mechanism of the biodegradation process await further research.

### Quantitative detection of singlet oxygen with chemiluminescence probe during photodynamic reaction

Y. Wei,<sup>1</sup> D. Xing,<sup>1</sup> S. Luo,<sup>1</sup> W. Xu<sup>1</sup> and Q. Chen<sup>1,2</sup>

<sup>1</sup>MOE Key Laboratory of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China.

E-mail: xingda@scnu.edu.cn

<sup>2</sup>Department of Radiation Oncology, University of Colorado at Denver, and Health Sciences Center, Aurora, CO, USA

Chemiluminescence probes are often used to detect microelements and free radicals by qualitative or quantitative methods. Although the detection method by chemiluminescence probes is highly sensitive and selective, quantitative measurement still can not be sufficiently precise for the concentration self-influence to luminescence efficiency of Probe, e.g. the depletion of concentration. Thus, the singlet oxygen chemiluminescence probe fluoresceinyl *Cypridina* luciferin analogue (FCLA) was studied in solution to achieve precise detection during photodynamic reaction. By analysing the light absorbance and self-quenching of FCLA, we deduced that not only concentration depletion but also self-quenching caused by concentration can affect the luminescence efficiency of the probe. In order to measure singlet oxygen more precisely using a chemiluminescence probe in photodynamic reaction,

a corrected measurement was proposed to minimize the influences.

### Detection of *Xanthomonas oryzae* pv. *oryzicola* by an electrochemiluminescence–polymerase chain reaction method

J. Wei, D. Xing, D. Zhu and X. Zhou

MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China.

E-mail: xingda@scnu.edu.cn

An electrochemiluminescence–polymerase chain reaction (ECL–PCR) method for the specific detection of pathogen *Xanthomonas oryzae* pv. *oryzicola* in rice has been developed. In the assay, primers were designed to amplify the 16s–23s rDNA spacer regions from this bacterium. At the end of the 3' terminal of the primer, a universal sequence was added so that all PCR products contain this sequence. These PCR products were used to hybridize with TBR-probe and Biotin-probe, which were complementary to the universal sequence. Through the specific interaction between biotin and streptavidin, the hybridized products were captured by magnetic beads coated in streptavidin. After magnetic separation, the samples were mixed with TPA and detected by ECL. The experiment results showed that healthy and infected rice leaf samples and infected ones can be distinguished easily using this ECL–PCR method at a high signal:noise ratio. The method described is simple and highly sensitive and could significantly reduce costs by employing universal probes.

### Optimization of a luminescent biosensor for intracellular cAMP

K. V. Wood

Promega Corporation, 2800 Wood Hollow Road, Madison, WI 53711, USA. E-mail: keith.wood@promega.com

The essential challenge for observing molecular processes within living systems is two-fold: providing sufficient discrimination to cope with the immense complexity of the intracellular environment, and minimizing perturbation to the system in the measurement process. Bioluminescence affords the ability to incorporate molecular probes at very low concentrations within cells, thereby minimizing their effect on normal cellular physiology. Detection specificity, however, has traditionally been confined largely to gene expression by coupling to genetic reporters, or selected physiological processes by coupling to the native luminogenic substrates or cofactors (e.g. calcium detection using aequorin). Currently, a greater role for bioluminescence is being enabled through the modification of enzyme structure to create novel biosensors. On this principle, our laboratory has been developing a biosensor of intracellular cAMP by incorporating a conformationally modulated nucleotide-binding site into the luciferase structure. Allosteric modulation upon binding cAMP is coupled to the catalytic efficiency, thereby altering light emission. This allows real-time monitoring of dynamically changing intracellular

cAMP in response to physiological stimuli. Various strategies for structural optimization have further optimized the response characteristics of the luminescent biosensor, yielding exceptional signal amplitudes relative to other intracellular sensor technologies.

### Application of the sea-firefly *Cypridina* bioluminescent system for bioassays

C. Wu,<sup>1</sup> K. Kawasaki,<sup>2</sup> S. Ohgiya<sup>2</sup> and Y. Ohmiya<sup>1</sup>

<sup>1</sup>Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology, Osaka 563-8577, Japan. E-mail: chun-wu@aist.go.jp

<sup>2</sup>Research Institute for Genome-based Biofactory, National Institute of Advanced Industrial Science and Technology, Sapporo 062-8517, Japan

The *Cypridina* bioluminescent system is a typical luciferin–luciferase reaction. The quantum yield of *Cypridina* luciferin in the bioluminescent reaction is about 0.3. The turnover rate of *Cypridina* luciferase is 1400/min. The secretory *Cypridina* luciferase is more stable than many other luciferases because it has high content of disulphide bonds. In 1989 the cloning of the luciferase gene of *Cypridina hilgendorffii* (*Vargula hilgendorffii*) and the expression of the gene in mammalian cells was reported. Recently another luciferase gene was cloned from *Cypridina noctiluca*. So far these luciferase genes have not been widely used, because the chemical synthesis of the luciferin is not easy. Since 2005 our research group has tried to optimize the synthesis of *Cypridina* luciferin and apply the *Cypridina* bioluminescent system for various bioassays. The results on this study are discussed.

### Interaction of a fluorescent probe with DNA and its application in the determination of DNA

F.-Y. Wu, Y.-L. Xiang, F.-Y. Xie and X.-F. Wan

Department of Chemistry, Nanchang University, Nanchang 330031, People's Republic of China.

E-mail: fywu@ncu.edu.cn

We report a metal complex, 1-Zn (2,5-di-[2-(3,5-bis (2-pyridylmethyl)amine-4-hydroxy-phenyl)-ethylene]-pyrazine-Zn), for fluorescence probe sensing for DNA. All experimental results, such as competitive experiment of EB (EB) with the probe to binding DNA, absorption spectral change and polarization changes in the presence and absence of DNA, revealed that interaction between the probe and DNA was via intercalation. An ionic strength experiment showed the existence of electrostatic interaction as well. Scatchard plots also confirmed the combined binding modes. The fluorescence enhancement of the probe was ascribed to a highly hydrophobic environment while it bound macromolecules such as DNA, RNA or denatured DNA. The binding constant between the fluorescent probe and DNA was estimated as  $3.13 \times 10^7$  mol/L. The emission intensity increase was proportional to the concentration of DNA. Based on this, the probe has been used to determine the concentration of calf thymus DNA (ctDNA). The corresponding linear response was in the range  $2.50 \times 10^{-7}$ – $4.75 \times 10^{-6}$  mol/L and the detection limit was  $1.93 \times 10^{-8}$  mol/L for ctDNA.



### Study on the interaction between silver nanoparticles and nucleic acids in the presence of cetylpyridine bromide and its analytical application

X. Wu,<sup>1</sup> J. Zheng,<sup>1</sup> L. Zhao,<sup>1</sup> J. Yang<sup>1</sup> and Q. Yang<sup>2</sup>

<sup>1</sup>Key Laboratory of Colloid and Interface Chemistry (Shandong University), Ministry of Education, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, Shandong, People's Republic of China. E-mail: wux@sdu.edu.cn

<sup>2</sup>School of Chemistry and Chemical Engineering, Jinan University, Jinan 250022, People's Republic of China

Surfactants play an important role in the synthesis of noble metal nanoparticles. Gold and silver nanoparticles have been utilized for DNA identification, biosensors and biochips. In this study it was found that nucleic acids could remarkably quench the RLS intensity of the silver nanoparticles (nanoAg)–cetylpyridine bromide (CPB) system. Based on this, a simple and sensitive method for the determination of nucleic acid had been established. Under optimum conditions, there were linear relationships between the RLS quenching extent at 351 nm and the concentration of nucleic acids in the range  $1.0 \times 10^{-8}$ – $4.0 \times 10^{-6}$  g/mL for fish sperm DNA (fsDNA),  $4.0 \times 10^{-8}$ – $4.0 \times 10^{-6}$  g/mL for calf thymus DNA (ctDNA) and  $1.0 \times 10^{-8}$ – $2.0 \times 10^{-6}$  g/mL for yeast RNA (yRNA). The detection limits (S:N = 3) of fsDNA, ctDNA and yRNA were  $1.7 \times 10^{-10}$ ,  $4.8 \times 10^{-10}$  and  $4.8 \times 10^{-10}$  g/mL, respectively. The study on the interaction mechanism of the system indicated that fsDNA could respectively react with nanoAg through chemical affinity and CPB through electrostatic, hydrophobic and  $\pi$ – $\pi$  stack forces, resulting in the dissociation of the nanoAg–CPB complex and the RLS quenching of the system.

### Rhodamine B-quinoline-8-amide as a fluorescent 'ON' probe for Fe<sup>3+</sup> in acetonitrile

Y. Xiang, Z. Li and A. Tong

Department of Chemistry, Tsinghua University, Beijing 100084, People's Republic of China. E-mail: tongaj@mail.tsinghua.edu.cn

A new fluorescent probe, rhodamine B-quinoline-8-amid (1), which showed selective response to Fe<sup>3+</sup>, was synthesized and characterized. A solution of 1 (10  $\mu$ mol/L) in acetonitrile was completely colourless and non-fluorescent, while a significant red fluorescence enhancement was observed upon the addition of two equivalents of Fe<sup>3+</sup> into this solution. This remarkable fluorescence 'on-off' switch was suggested to be a result of chelating-induced opening of the rhodamine B spirolactam ring by Fe<sup>3+</sup>. A metal:ligand ratio of 2:1 was calculated for the 1–Fe<sup>3+</sup> complex, according to Job's method. Other metal ions, such as Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Ag<sup>+</sup>, Hg<sup>2+</sup> and alkali and alkali earth metal ions, displayed very little fluorescence response or interference.

### Luminescence methods for non-invasive detection of plant physiology

D. Xing, L. Zhang, C. Gao and X. Liu

MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China. E-mail: xingda@scnu.edu.cn

Luminescence methods based on the registration of a light signal emitted by intrinsic biological luminescence or exogenously

added fluorescence probes are powerful tools in current plant biology to study the stress responses and physiological events of plants. Delayed fluorescence (DF), emitting from photosystem II through inverse photochemistry reactions, is an intrinsic fluorescence label of the photochemical efficiency of photosynthesis. Recently, monitoring DF emissions from leaves using a custom-made portable biosensor has been successfully used to ascertain the plant senescence process *in vivo* and evaluate the extent of plant senescence quantitatively. Moreover, studies performed in our laboratory within the past 5 years have also demonstrated that the DF technique holds tremendous potential for applications in real-time monitoring of the effects of salt stress, heat stress, herbicide toxicity, UV-B radiation and acid rain pollution on plant growth and development. Because DF detection can be carried out on intact plants and does not require an exogenously added probes or sample preparation (except adaptation to darkness), it should be extremely useful not only for non-invasive and rapid screening of the potentially interesting mutants, but also for real-time monitoring of plant responses to environmental stressors in the field. Finally, we discuss our recent studies on the applications of exogenously added fluorescence probes in monitoring the early events of plant programmed cell death and detecting seed vigour during early imbibition.

### PIG11 sensitizes cells to As<sub>2</sub>O<sub>3</sub>-induced apoptosis

X.-F. Xiong and E.-H. Cao

Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China. E-mail: caoeh@sun5.ibp.ac.cn

Previous studies indicated PIG11 as a candidate tumour suppressor could induce cell apoptosis and obviously enhanced the chemosensitivity of cells to As<sub>2</sub>O<sub>3</sub>. Exploration of the role of PIG11 may contribute to the elucidation of this novel mechanism of apoptosis. In this study, the role of PIG11 protein during apoptosis or inhibition of cell growth was investigated by a GFP marking technique, chemiluminescence and fluorescence labelling. The results indicated that the percentage of apoptotic cells in GFP–PIG11 transfectants was higher than that of GFP-alone transfectants. Treatment with As<sub>2</sub>O<sub>3</sub> resulted in a significant increase of apoptotic cells in the expression of GFP–PIG11 transfectants. Furthermore, PIG11 overexpression remarkably enhanced the generation of intracellular reactive oxygen species and aggravated the loss of mitochondrial membrane potential induced by As<sub>2</sub>O<sub>3</sub>. On the other hand, As<sub>2</sub>O<sub>3</sub> decreased intracellular reduced glutathione (GSH) and increased the GSH efflux rate, leading to an increase of extracellular superoxide generation. These findings suggested that overexpression of PIG11-sensitized cells to As<sub>2</sub>O<sub>3</sub> induced apoptosis through the accumulation of intracellular reactive oxygen species. This might be a novel candidate to influence chemosensitivity to As<sub>2</sub>O<sub>3</sub>.

### Morphology and optical properties of poly(vinylidene difluoride)–(Y<sub>0.97</sub>Eu<sub>0.03</sub>)<sub>2</sub>O<sub>3</sub> rare-earth nanocomposite

C. Xu

Department of Materials Science and Engineering, Shanghai Institute of Technology, Shanghai 200235, People's Republic of China. E-mail: chhxxu@sit.edu.cn

This communication reports the morphology and optical properties of the poly(vinylidene difluoride) (PVDF)–(Y<sub>0.97</sub>Eu<sub>0.03</sub>)<sub>2</sub>O<sub>3</sub> rare-earth

nanocomposite prepared by a simple co-precipitation method. The results indicate that  $(Y_{0.97}Eu_{0.03})_2O_3$  nanoparticles are dispersed as nanoparticle aggregates in the PVDF matrix, while the finely dispersed  $(Y_{0.97}Eu_{0.03})_2O_3$  nanoparticles are hard to see. The PVDF- $(Y_{0.97}Eu_{0.03})_2O_3$  nanocomposite shows a characteristic red emission at 612 nm corresponding to the intrinsic luminescence of the  $(Y_{0.97}Eu_{0.03})_2O_3$  nanoparticles. The optical band gap of the nanocomposite shows a decreasing trend with increasing  $(Y_{0.97}Eu_{0.03})_2O_3$  content. Due to the multi-application of the rare-earth material in fluorescent probes and organic light-emitting diodes (OLEDs), and the multifunction of PVDF in non-linear optics, the prepared luminescent nanocomposite shows promise for use as an optical material in the future.

### Flow-injection chemiluminescence determination of human serum albumin based on the fluoresceinyl *Cypridina* luciferin analogue(FCLA)- $^1O_2$ reaction

W. Xu,<sup>1</sup> Y. Wei,<sup>1</sup> D. Xing,<sup>1</sup> S. Luo<sup>1,2</sup> and Q. Chen<sup>1,2</sup>

<sup>1</sup>MOE Key Laboratory of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China.

E-mail: xingda@scnu.edu.cn

<sup>2</sup>Department of Radiation Oncology, University of Colorado at Denver and Health Sciences Centre, Aurora, CO, USA

A flow-injection chemiluminescence method using reactive oxygen species (ROS)-specific CL probe-fluoresceinyl *Cypridina* luciferin analogue (FCLA) for low-level human serum albumin (HSA) detection is proposed. The technique is based on the phenomenon that low-level HSA can effectively enhance CL emission of the FCLA- $^1O_2$  system. In comparison with the CL quenching technique used by others, the proposed method significantly improves the detection sensitivity (ca. 100-fold higher). After optimizing the current apparatus, we achieved a reliable detection range of HSA of  $1 \times 10^{-10}$ – $1 \times 10^{-8}$  mol/L. The limit of detection was  $4.5 \times 10^{-11}$  mol/L (S:N = 3) for HSA, and the RSD ( $n = 27$ ) was 3.08% for  $6 \times 10^{-9}$  mol/L HSA. The results are in excellent agreement with those from an established clinical technique. The possible mechanism is discussed briefly. Energy transfer in chemiluminescence system and changes of microenvironment are likely to be main factors for the enhancement of CL intensity by the addition of HSA.

### FRET between quantum dots and gold nanoparticles with enzyme as bridge

S. Xu and D. Yang

Department of Chemistry, Northeastern University, Shenyang, 110004, People's Republic of China.

E-mail: xushukun46@126.com

Based on the characteristics of quantum dots (QDs), gold nanoparticles (NPs) and the sensitivity of fluorescence resonance energy transfer (FRET), much research work has been carried out. However, there have been few attempts employing two FRET assemblies in one system as well as luminescence quenching by gold NPs with an enzyme as a bridge. First, two FRET assemblies between luminescent QDs and chymotrypsin, QDs and gold NPs in one system were studied. Using a set of QDs-gold NPs pairs with substantial spectral energy overlap

(gold NPs maximum absorbance at 525 nm and CdTe QDs emission at 525 nm), a visible quenching of the fluorescence intensity of CdTe QDs was outlined. Furthermore, the conjugated assemblies were excited at 280 nm (where tryptophan and QDs absorb) and longer wavelength 350 nm (where only the QDs absorb); an energy transfer between QDs and chymotrypsin was also demonstrated. This new configuration with two FRET assemblies revealed some important and valuable information about the interactions among QDs, NPs and macromolecules.

### Determination of matrine in pharmaceuticals by capillary electrophoresis with tris (2,2'-bipyridyl)ruthenium(II) electrochemiluminescence detection

R. Yang,<sup>1</sup> L. Chen,<sup>1</sup> H. J. Zeng<sup>2</sup>, L. Qu<sup>1,3</sup> and J. Li<sup>1</sup>

<sup>1</sup>Department of Chemistry, Zhengzhou University, Henan, Zhengzhou, People's Republic of China. E-mail: qulingbo@zzu.edu.cn

<sup>2</sup>College of Pharmacy, Zhengzhou University, Henan, Zhengzhou, People's Republic of China

<sup>3</sup>Henan University of Technology, Henan, Zhengzhou, People's Republic of China

Matrine (MT), a major active component from the root of *Sophora. avescens* Ait, has been extensively used in China for the treatment of viral hepatitis, cancer, cardiac diseases and skin diseases. In this study, a novel and rapid electrochemiluminescence (ECL) method based on the tris(2,2'-bipyridyl) ruthenium(II) system was developed for the determination of matrine in several pharmaceuticals. Parameters influencing separation and detection were optimized in detail. Under optimum conditions, matrine has a linear calibration graph in the range  $2.0 \times 10^{-6}$ – $4.0 \times 10^{-4}$  mol/L, with a limit of detection (S:N = 3) of  $3.2 \times 10^{-7}$  mol/L. The correlation coefficient was 0.9960 ( $n = 5$ ) with a RSD of 4.1% for 10 continuous injections of  $2.0 \times 10^{-4}$  mol/L matrine. The utility of this method was demonstrated by determining matrine in several pharmaceuticals.

### Fluorescence tuning of nanoparticles with different doping levels

G. Yang, X. Li, S. Li and Y. Qian

Key Laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, People's Republic of China. E-mail: gqyang@iccas.ac.cn

Luminescent organic nanoparticles consisting of a green-emitting (ESIPT) compound, (DHBIA), doped with a new red-emitting dye, (MAFN), have been prepared by a reprecipitation method. It was found that the emission of nanoparticles can be tuned gradually from green to red by increasing doping levels. These nanoparticles could be analysed as a system of densely packed chromophores that showed efficient energy transfer from DHBIA to MAFN. The energy transfer in these systems was dominated by the Förster resonance mechanism. The bathochromic of guest emission was presumably due to solid-state solvation effects (SSSE). The combination of high fluorescence brightness and tunable fluorescence of these blended nanoparticles is promising for ultrasensitive fluorescence-based assays.

### **A novel synergistic agent to the enhancers for HRP-H<sub>2</sub>O<sub>2</sub>-based chemiluminescence and its application in immunoassay**

X. Yang<sup>1</sup> and X. Sun<sup>2</sup>

<sup>1</sup>People's Hospital of Peking University, Beijing 100044, People's Republic of China. E-mail: xiaolin.yang@yuande.com

<sup>2</sup>China Medical Technologies Inc., Beijing 100176, People's Republic of China

Several chemicals, such as the phenols and phenylboric acids with special *para*-substituents, as well as some derivants of benzothiazole, are well-known enhancers of HRP-luminol-H<sub>2</sub>O<sub>2</sub> chemiluminescence. Owing to their extremely high enhancements, they are widely applied in immunoassays as an essential component of the commercialized chemiluminescence substrates sensitive to HRP, which is a commonly used label to the antibodies and antigens. However, no synergy for enhancement among them has so far been found; in other words, for the reaction with two kinds of enhancers, the signal never exceeded the mixture in which only the stronger one of the two enhancers above was involved. In our recent work a common substance, aminopyrine, was discovered to play a synergistic role with common enhancers in HRP-catalysed luminol-H<sub>2</sub>O<sub>2</sub> chemiluminescence. In optimized experiments, when aminopyrine was added in a HRP-luminol-H<sub>2</sub>O<sub>2</sub> mixture with one of the above enhancers, the light emission was always two to three times higher than in its absence; furthermore, the background was also largely depressed. The same phenomenon was observed when aminopyrine was employed in HRP-luminol-H<sub>2</sub>O<sub>2</sub> chemiluminescence without any of the enhancers. Therefore, aminopyrene may perhaps be regarded as a unique minor enhancer with a major synergistic effect on previous enhancers. In tests performed by immunoassay, the substrate comprising aminopyrine and a common enhancer also clearly represented better sensitivity and stability, the latter caused by mitigated time-shift of chemiluminescence.

### **Spectrofluorimetric determination of hydrogen peroxide and glucose based on the catalytic effect of haemin coupling with glucose oxidize**

M. Yang,<sup>1,3</sup> Y. Xi<sup>2,3</sup> and H. Qiu<sup>3</sup>

<sup>1</sup>Research Centre of Analysis and Measurement, Donghua University, Shanghai 200051, People's Republic of China. E-mail address: yangming@dhu.edu.cn

<sup>2</sup>College of Environmental Science and Engineering, DongHua University, Shanghai 201620, People's Republic of China

<sup>3</sup>Faculty of Materials Science and Chemical Engineering, China University of Geosciences, Wuhan 430074, People's Republic of China

A spectrofluorimetric method for determining hydrogen peroxide and glucose, based on the fluorescence velocity of the mimetic enzyme, haemin-catalysed oxidation of L-tyrosine by hydrogen peroxide, which coupled with the oxidation of glucose catalysed by glucose oxidase, was proposed. The optimal conditions were determined. The influence of different buffers and concentrations on the relative fluorescence velocity was tested, which showed that the enhancement effect was most evident in NH<sub>3</sub>-NH<sub>4</sub>Cl solution, especially at high pH. The effect of interferences on the determination of H<sub>2</sub>O<sub>2</sub> was

investigated. The reaction product was monitored fluorimetrically, with a 3 $\sigma$  detection limit of  $1.41 \times 10^{-7}$  mol/L glucose. A linear calibration graph was obtained over the glucose concentration range  $0.0\text{--}2.0 \times 10^{-4}$  mol/L, with a correlation coefficient of 0.998 (*n*D18). The RSD at a glucose concentration of  $6.0 \times 10^{-6}$  mol/L was 4.82% (*n*D9). The proposed method for spectrofluorimetric determination of glucose was successfully applied to the determination of glucose in fruit tissue fluid.

### **Assembly and chemical luminescence of porous nano-silica with luminol**

Y. Yang,<sup>1</sup> Y. Yu,<sup>1</sup> Y. Sun,<sup>1</sup> Y. Zhang,<sup>1</sup> Q. Zeng,<sup>1</sup> X. Liu,<sup>1</sup> L. Tian,<sup>1</sup> X. Kong<sup>1</sup> and H. Zhang<sup>2</sup>

<sup>1</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China. E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: h.zhang@uva.nl

Porous nanosilica smaller than 30 nm was synthesized and assembled with molecules of luminol. The effective bioluminescence was observed under HRP catalysis. The bioluminescence peak was at about 440 nm. The bioluminescence intensity and peak are highly correlated with the distance between the luminol and HRP molecules. The death of Hela cells was observed when the nanocomplex coupled with Hela cells under HRP catalysis. There are potential applications for cancer therapy and the bio-optical switch to release medicinal molecules *in vivo*.

### **The superweak luminescence of plants and its ultraweak photon image**

Q. Yang,<sup>1</sup> H. Zhou<sup>2</sup> and Z. Liu<sup>1</sup>

<sup>1</sup>Department of Biotechnology, Beijing Agricultural College, Beijing 102206, People's Republic of China. E-mail: yangqijian@hotmail.com

<sup>2</sup>Institute of Grassland Science, China Agricultural University, Beijing 100094, People's Republic of China

Living cells are able to emit light of the spectral range covering ultraviolet, visible and infrared light. It is known that superweak luminescence (SL) exists in all animal and plants. The luminescence phenomenon shows the important information of metabolism and energy transformation of the living biological organism. We studied the relationship between superweak luminescence and resistance to sprouting on the wheat ear at maturity, which is related to the activity of hydric groups of enzyme complexes in the seed coats. Conventional biophoton imaging is carried out with a two-dimensional (2D) photon-counting technique, using a highly sensitive CCD charge-coupled device camera. We have studied the metabolism change of germination seedlings using a conventional photon-counting technique and 2D imaging with a photon-counting image acquisition system (PIAS). It will be accurate and direct to observe and characterize the space-time dynamics of the emission properties. This is regarded as a good method that continuously measures the dynamics of plant metabolism and

growth without destroying the regular growth of plants in biological research work.

### **A novel chemiluminescent immunoassay of total thyroxine, using acridinium ester of 2',6'-dimethyl-4'-(*n*-succinimidylloxycarbonyl) phenyl-10-methyl-acridinium-9-carboxylate methosulphate as label**

D. Yin, C. Xie, L. Zhang, B. Liu, X. Zhou, P. Wang and M. Wu  
College of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, People's Republic of China.  
E-mail: dongguang\_yin@yahoo.com

A novel chemiluminescent immunoassay (CLIA) of total thyroxine (TT4), using acridinium ester of 2',6'-dimethyl-4'-(*n*-succinimidylloxycarbonyl) phenyl-10-methyl-acridinium-9-carboxylate methosulphate (DMAE-NHS) as label, has been developed. Microwells were coated with anti-T4 monoclonal antibody (McAb) and DMAE-NHS synthesized by our laboratory was conjugated with streptavidin (SA). T4-BSA was conjugated with biotin-N-hydroxysuccinimide (B-NHS). T4-BSA-B-NHS and the T4 in the standard or sample competitively react with anti-T4 McAb. Streptavidin-biotin separation and enhancing techniques were applied in this assay. The presented approach shows many excellent characteristics, such as a rapid assay process, with two incubations totalling 30 min, and high detection sensitivity, with 0.56 ng/mL of low detection limit.

### **Synthesis and characterization of a chemiluminescent compound of 9-[2',6'-dimethyl-4'-(succinimidoxycarbonyl)phenoxy carbonyl]-10-methylacridinium monomethyl sulphate**

D. Yin, C. Xie, L. Zhang, B. Liu, X. Zhou, P. Wang and M. Wu  
College of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, People's Republic of China.  
E-mail: dongguang\_yin@yahoo.com

The acridinium salt of 9-[2',6'-dimethyl-4'-(succinimidoxycarbonyl)phenoxy carbonyl]-10-methylacridinium monomethyl sulphate was synthesized successfully. The synthesis involves seven steps. All the intermediate compounds and the final product were identified by IR, <sup>1</sup>H-NMR, MS and elemental analysis and the chemiluminescent characters of the synthesized compound were investigated. The results show that the compound has desirable characteristics, such as flash light with 0.9 s of decay half-time,  $6.11 \times 10^{18}$  cps/mol chemiluminescent intensity and high stability.

### **Separation and detection of amino acids with a novel capillary electrophoresis chemiluminescence system**

D. Yin, C. Xie, L. Zhang, B. Liu, X. Zhou, P. Wang and M. Wu  
College of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, People's Republic of China.  
E-mail: dongguang\_yin@yahoo.com

A versatile peroxyoxalate-based chemiluminescence (CL) detection following capillary electrophoresis (CE) separations for

assaying amino acids is described. To achieve high signal stability and sensitivity, a novel peroxyoxalate CE-CL system was established, based on the design of a new interface including a new mixing mode and a new grounding electrode mode. The results show that this new system is quite effective for separating and detecting amino acids with high stability, resolution and sensitivity. The detection limits were 1.1 nmol/L for dansyl-leucine and 2.0 nmol/L for dansyl-aspartic acid. The RSDs of peak height and migration time were in the ranges 2.3–3.8% and 1.2–1.5%, respectively.

### **Fluorescence characteristics of novel chlorophenyl-arsenoxylphenylazo rhodamines and application in the determination of thallium(I)**

J. Yu, X. Cheng, S. Ge, B. Li and Y. Tan  
School of Chemistry and Chemical Engineering,  
University of Jinan, Jinan 250022, People's Republic of China.  
E-mail: yujinghua@vip.sina.com

Three novel reagents of 5-*o*-arsenoxylphenylazo rhodamines were synthesized. Structural and spectral characteristics of three novel 5-*o*-arsenoxylphenylazo rhodamines, each of which has a chlorophenyl group at a different location, are discussed. Both the quantum chemistry calculation and fluorescence spectra results indicate that, as a fluorescence reagent, rhodamine with the chlorophenyl group at the *-p* location is superior to rhodamines having the chlorophenyl group at the *-m* or *-o* location. Based on the reaction using thallium(I) to catalyse the oxidation of the rhodamine with *p*-location chlorophenyl group by hydrogen peroxide, a new method of catalytic fluorimetric determination of trace thallium(I) is described. The optimal conditions and allowed concentrations of foreign interference ions in the determination test were obtained. The linear range was 0–0.01 µg/mL and the detection limit was  $2.64 \times 10^{-9}$  g/mL. The developed method has a high selectivity and accuracy in analysing trace thallium(I).

### **Study on determination of deoxyribonucleic acid by second-order scattering with new-type rhodanine**

J. Yu, B. Li, Y. Zhu, X. Cheng and L. Zhang  
School of Chemistry and Chemical Engineering, University of Jinan, Shandong, Jinan 250022, People's Republic of China.  
E-mail: yujinghua@vip.com.cn

Second-order scattering and resonance Rayleigh scattering spectra of interaction between deoxyribonucleic acid (DNA) and 3-(4'-methylphenyl)-5-(2'-sulphoph-enylazo) rhodanine in the environment of the surface-active substance sodium dodecyl sulphate was reported for the first time. Second-order scattering used to be considered as a kind of interference in the study of spectrofluorometry, and it has infrequently been reported as a analytical method. It was found in the experiments that the second-order scattering intensity and resonance Rayleigh scattering intensity could be greatly enhanced by sodium dodecyl sulphate, based on which, two new methods for



determination of DNA were established. The relationship between second-order scattering and resonance Rayleigh scattering was discussed and it was found that the intensity of second-order scattering is relevant to the change of resonance Rayleigh scattering. Furthermore, second-order scattering was found to be more sensitive than resonance Rayleigh scattering. The possible mechanism was studied, which showed that the type of active force was converted by the presence of sodium dodecyl sulphate.

### Effects of goat anti-human IgG on ultraviolet-visible upconversion luminescence of $\text{NaYF}_4:\text{Yb}^{3+}, \text{Tm}^{3+}$ nanocrystals

Y. Yu,<sup>1</sup> Y. Sun,<sup>1</sup> Y. Zhang,<sup>1</sup> Y. Yang,<sup>1</sup> X. Liu,<sup>1</sup> Q. Zeng,<sup>1</sup> X. Kong<sup>1</sup> and H. Zhang<sup>2</sup>

<sup>1</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China. E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: h.zhang@uva.nl

Well-dispersed, small-sized, uniformly distributed and water-soluble  $\text{NaYF}_4:\text{Yb}^{3+}, \text{Tm}^{3+}$  nanocrystals with highly effectively UV-vis luminescence upconversion were synthesized directly by a polymer system via a hydrothermal process. Enhancement of UV-vis upconversion luminescence and the changes of relative intensity among different emitting peaks of UV-vis upconversion luminescence of the nanocrystals coupled with goat anti-human IgG was observed. The changes of relative intensity of UV-vis upconversion luminescence upconversion are dependent on the concentration and kinds of biomolecules. UV-vis upconversion luminescence results in the death of the cancer cell from the effect of UV-vis upconversion luminescence of nanoparticles excited under a 980 nm laser, which shows that it is possible that UV-vis luminescence upconversion can be used in the phototherapy of cancer.

### Determination of ascorbic acid by flow-injection chemiluminescence method

J. Yu, C. Zhang, Y. Tan, S. Ge, P. Dai and Y. Zha

School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, People's Republic of China. E-mail: yujinghua@vip.sina.com

A new kind of flow-injection chemiluminescence method for the determination of ascorbic acid has been developed, based on potassium periodate oxidating 3-(2'-nitrylphenyl)-5-(2'-sulphonicphenylazo) rhodanine in acid condition. Under optimum conditions, a linear calibration graph was obtained over the range 0.2–10  $\mu\text{g/mL}$ , and the detection limit was 0.02898  $\mu\text{g/mL}$ , with a relative standard deviation of 1.5% ( $n = 11$ ). The method was successfully applied to the determination of ascorbic acid in samples and the obtained results were consistent with those obtained by an official method. The CL mechanism of the proposed system was analysed.

### Determination of $\beta$ -methasone residues in chicken tissue by fluorescence-linked immunosorbent assay with quantum dots

Y. Yuan, C. Xu and C. Peng

School of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu Province, People's Republic of China. E-mail: XCL688@yahoo.com.cn

A new indirect competitive fluorescence-linked immunoassay (FLISA) for the detection of  $\beta$ -methasone (BET) was developed. The polyclonal antiserum was produced against BET linked to bovine serum albumin. The ZnS-coated quantum dots ( $\text{ZnS/CdSe}$  QDs) were conjugated to secondary antibody. Assay conditions, including concentrations of antiserum and QD-secondary antibody conjugates, were optimized. The optimized FLISA system had a low limit of detection of 0.1 ng/mL. The specificity was investigated using other nine steroids, and the cross-reactivities for dexamethasone, flumethasone were high, indicating a class-specificity of the antibody. Preliminary evaluation of assay performance through specificity, sensitivity and precision showed that this FLISA method can be applied to the practical detection of BET. In the sample analysis, the amounts of BET residues quantified by FLISA were compared with a liquid chromatography-mass spectrometry (LC-MS) method. Results from the analysis of incurred residues in chicken tissue samples showed that the methods can be applied to monitor BET and other corticosteroid residues in foods.

### Resistance to antibiotics among different species of luminous bacteria from CCIBSO 836

N. V. Zakharova,<sup>1</sup> S. E. Medvedeva<sup>1</sup> and L. Y. Popova<sup>2</sup>

<sup>1</sup>Institute of Biophysics, SB RAS, Krasnoyarsk 660036, Russia

<sup>2</sup>Krasnoyarsk Science Centre, SB RAS, Krasnoyarsk 660036, Russia

Multiple resistance to various classes of antibiotics among three species of luminous bacteria was investigated in order to use this attribute at an estimation of man's influence on the marine ecosystem. Research on the resistance to antibiotics among luminous bacteria has shown that there is a tendency of increase resistance to antibiotics in the line *Photobacterium phosphoreum*, *Photobacterium leiognathi*, *V. haweyi*. This dependence was characteristic for both the Indian and the Pacific oceans. The greatest number of *V. haweyi* strains resistant to the studied antibiotics was revealed among free-living inhabitants of the coastal waters of the two oceans, and multiple resistance to antibiotics was most typical for them. Among *P. leiognathi* strains (symbiotic and free-living forms) dominating in superficial waters, the number of resistant strains was lower than among *V. haweyi* strains. The lowest number of resistant strains was revealed among *P. phosphoreum*; these were isolated from depths <20 m. Resistance to the majority of antibiotics among bacteria for *P. leiognathi* and *P. phosphoreum* was more expressed in Pacific strains. However, the share of strains that showed resistance to antibiotics was much lower in the *P. phosphoreum* population. At the same time, strains from Indian Ocean were found to show resistance to doxycycline.

### Effects of coupled biomolecules on the luminescence properties of type II CdTe–CdS core–shell quantum dots in aqueous solution

Q. Zeng,<sup>1</sup> Y. Zhang,<sup>1</sup> Y. Yang,<sup>1</sup> Y. Yu,<sup>1</sup> Y. Sun,<sup>1</sup> X. Liu,<sup>1</sup> X. Kong,<sup>1</sup> and H. Zhang<sup>2</sup>

<sup>1</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China. E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: h.zhang@uva.nl

MPA-stabilized CdTe–CdS core–shell quantum dots (QDs) were prepared in an aqueous solution, following the synthetic route of successive ion layer adsorption and reaction. Different properties of enhanced luminescence of QDs were observed when QDs coupled with different biomolecules. The differences in properties of enhanced luminescence were strongly reliant on the species of biomolecule and coupling approaches, such as the physical and covalence coupling. The bioassay specificity and sensitivity are markedly improved when the QD is first modified by a specific biomolecule and then coupled antibody. These results have great potential for application in immunoassays and immunotherapy, biochips, fibre sensitizers and nanobiomedicine.

### Study on the protection of polysaccharide and flavone in six anticancer Chinese herbs to DNA with fluorescence probe

H.-R. Zhang, J.-E. Chen and Y.-F. Hao

Laboratory of Biochemical Analysis, Xinzhou Teachers' University, Shanxi 034000, People's Republic of China. E-mail: biochem@xztc.edu.cn

The protection of polysaccharide and flavone compounds in six kinds of anticancer Chinese herbs (*Radix isatidis*, root of cochinchinese, root of two-tooth *Achyranthes*, *Ophiopogon japonicus*, fruit of glossy privet and root of Indian pokeberry) for DNA was comparatively investigated. The fluorescence integration intensity of DNA, ethidium bromide (EB) and extracts containing polysaccharide and flavone compounds from six kinds of anticancer Chinese herbs were determined, with EB as a sensitive fluorescence probe. *D* can directly denote the degree of interaction of drug molecules with DNA. According to the size of *D*, we can discuss the influence of polysaccharide and flavone compounds on DNA's protective action. The results showed that the extracts of anticancer Chinese herbs could interact with DNA but the degree of interaction was different. The bigger the *D*, the stronger the interaction of drugs with DNA. The order of interaction of polysaccharide in extracts with DNA was as follows: fruit of glossy privet > root of two-tooth *Achyranthes* > *radix isatidis* > glucose(contrast) > root of Indian pokeberry > *ophiopogon japonicus*; The order of interaction of flavone in extracts with DNA was as follows: quercetin (contrast) > root of two-tooth *Achyranthes* > root of Indian pokeberry > *Ophiopogon japonicus* > *Radix isatidis* > root of cochinchinese asparagus.

### Water-soluble fluorescent zinc sensor based on carboxamidoquinoline

Y. Zhang<sup>1</sup> and X. Guo<sup>2</sup>

<sup>1</sup>State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, People's Republic of China. E-mail: xfguo@163.com

<sup>2</sup>Key Laboratory of Fine Chemicals (Qiqihar University), College of Heilongjiang Province, Qiqihar 161006, People's Republic of China

The design and synthesis of fluorescent sensors for zinc with high sensitivity, selectivity and reliability is a vibrant field of supramolecular chemistry. Here, a novel water-soluble fluorescent Zn<sup>2+</sup> sensor, **1**, based on carboxamidoquinoline with a di(2-hydroxyethyl)amino group as receptor, was reported. In aqueous solution, **1** showed about a 10-fold increase in fluorescence quantum yield in conjunction with a 70 nm red-shift of fluorescence emission with the addition of Zn<sup>2+</sup>. Importantly, an obviously blue-green emission of the solution was easily observed by the naked eye. Both UV-vis and fluorescence titrations implied the formation of a complex with 1:1 stoichiometry of **1** and Zn<sup>2+</sup>, and the association constant was  $3.2 \times 10^6$  mol/L. Moreover, a wide pH span (5–12) for Zn<sup>2+</sup> determination made it promising to determine Zn<sup>2+</sup> in aqueous solution for practical analysis.

### Photo-oxidation of protein induced by triplet 1,4-naphthoquinone: a mechanism study

Z. Zhang,<sup>1,2</sup> S. Hao,<sup>1,2</sup> H. Zhu<sup>1,2</sup> and W. Wang<sup>1</sup>

<sup>1</sup>Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, People's Republic of China. E-mail: wfwang@sinap.ac.cn

<sup>2</sup>Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Photoprocesses of 1,4-naphthoquinone (NQ) and its photoreactions with lysozyme in acetonitrile:water (3:1 v/v) solution were studied, using a 355 nm laser flash photolysis technique. Transient spectra of NQ were observed and transient species were assigned. It has been found that triplet NQ can photo-oxidize lysozyme via abstracting hydrogen atom from lysozyme with a rate constant of  $k_t = 2.4 \times 10^{10}$  mol/L/s. Furthermore, the photo-oxidation of lysozyme under UVA irradiation in the presence of NQ was studied using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and turbidimetric assay. The results showed that the mechanisms and products of oxidative damage were relative to the concentration of NQ, the time of irradiation and the ambience in the continuous gas saturation system. The type I process was examined in the nitrogen-saturated system, whereas in an aerobic system the mechanism was found to be that cooperation is mainly due to a type II photodynamic process with a small contribution of type I process.

### Molecular recognitions of amino acids by haematoporphyrin and metallohaematoporphyrin receptors

Y. Zhang, Y.-C. Lei and D.-S. Liu

Department of Chemistry, Shanxi University, Taiyuan 030006, People's Republic of China. E-mail: zhangyong@sxu.edu.cn

Molecular recognition interactions of amino acids with haematoporphyrin (HP) and metallohaematoporphyrin (MHP)

by means of fluorescence and absorption spectra. The optimal conditions of recognition between amino acids and metallohaematoporphyrin were investigated in detail. The results show that metallohaematoporphyrin exhibits significant recognition toward amino acids in phosphate buffer solution at pH 7.4. The order of the binding constants between amino acids and metallohaematoporphyrin was  $KZn(II)-HP > KCu(II)-HP \geq KNi(II)-HP > KCo(II)-HP$ , and the binding constants of the laevo-isomer were larger than those of the dextro-isomer or no optical amino acids. The mechanism of recognition was further discussed. Recognition was achieved by the cooperative functions of three recognition groups: carboxyl groups, hydroxy groups and aromatic group stack.

### Determination of trace nitrite by catalysis fluorescence of acridine yellow

T. Zhang and C. Li

Department of Chemistry, Tangshan Teacher's College, Tangshan 063000, People's Republic of China.  
E-mail: tielizhang@126.com

Acridine yellow could be oxidized by potassium bromate in phosphoric acid medium with its fluorescence quenching, but the rate of the reaction was very slow. The presence of trace nitrite was able to catalyse this reaction and made the fluorescence intensity of the system decrease greatly. According to this observation, a new catalysis-fluorescence method for the determination of trace nitrite was developed, in which acridine yellow was used as a fluorescence probe. Under the optimal detection conditions, the linear range and detection limit ( $3\sigma$ ) for nitrite were  $8.00-1.80 \times 10^2 \mu\text{g/L}$  and  $4.3 \mu\text{g/L}$ , respectively. The method was successfully applied to the assay of trace nitrite in sodium nitrate with analytical grade and ham meat samples. The results were in good agreement with those obtained using a spectrophotometric method. The kinetic parameters of the reaction were also estimated.

### Application of bacterial luciferase for serral CEA

F. Zhang,<sup>1,2</sup> S. Luo<sup>1</sup> and X. Chen<sup>2</sup>

<sup>1</sup>Beihua University, Jilin 132001, People's Republic of China  
<sup>2</sup>Jilin University, Changchun 130021, People's Republic of China.  
E-mail: fneqchun\_zhang@yahoo.com.cn

Bioluminescence immunoassay is a new advanced technique combining bioluminescence with an immunological reaction using antigen-antibody. A luminescent substance is used instead of radioisotopes and luminescence intensity is detected. This method is sensitive, specific and without isotope hazards. Carcinoembryonic antigen (CEA) is an ideal marker in diagnosing carcinoma and has important value in the diagnosis of malignant tumours of the digestive tract. Luciferase was extracted from luciferin marine bacteria and fixed to CNBr-activated Sepharose-4B. CEA was labelled with G6PDH. Bioluminescence immunoassay for serral CEA was established. Serral CEA was detected in 30 patients with carcinoma of the colon, 30 patients with chronic ulcerative colitis and 30 normal patients as controls by bioluminescence immunoassay. The results indicated that the CEA content in patients with carcinoma of the colon was 100 times higher than controls

and was obviously higher than that chronic ulcerative colitis ( $p < 0.05$ ). The bioluminescence immunoassay for detection antigen (as CEA) is sensitive, specific, simple, rapid, accurate and without isotope hazards. It is well-placed to replace radioimmunoassay in clinical testing.

### Detection of celecoxib by high-performance liquid chromatography with on-line electrogenerated Ag(II) chemiluminescence detection

Y. Zhang,<sup>1</sup> H. Ma,<sup>1</sup> G. Qi<sup>1</sup> and Z. Zhang<sup>2</sup>

<sup>1</sup>College of Chemistry and Chemical Engineering, Yan'an University, Yan'an 716000, People's Republic of China.  
E-mail: zhyt1819@hotmail.com

<sup>2</sup>College of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China.  
E-mail: zzj18@hotmail.com

The determination of celecoxib (CEL) in pharmaceutical and biological samples by means of high-performance liquid chromatography (HPLC) with on-line electrogenerated Ag(II) chemiluminescence (CL) detection was proposed. The method was based on the direct CL reaction of CEL and Ag(II), which was on-line electrogenerated by constant current electrolysis. The chromatographic separation was carried out on a Nucleosil RP-C<sub>18</sub> column (250 mm  $\times$  4.6 mm i.d., 5  $\mu\text{m}$  pore size, 100 Å) at 20°C. The mobile phase consisted of acetonitrile:methanol:distilled water 45:10:45 v/v/v, containing 0.2% acetic acid, pH 3.5, at a flow rate of 1.2 mL/min. The effects of several parameters on the HPLC resolution and CL emission were studied systematically. Under optimal conditions, a linear range of 10–1000 ng/mL ( $R^2 = 0.9990$ ) and a detection limit of 1 ng/mL (signal:noise ratio = 3) for CEL were achieved. The RSDs for 20 ng/mL CEL were 2.8% within a day ( $n = 11$ ) and 3.5% on five consecutive days ( $n = 6$ ), respectively. The recovery of CEL from human serum samples was >93%. The applicability of the method for the analysis of pharmaceutical and biological samples was examined.

### Molecular imprinted polymer-based chemiluminescence imaging sensor

Z. Zhang and L. Wang

Department of Chemistry, Shaanxi Normal University, Xi'an 710062, People's Republic of China. E-mail: zzj18@hotmail.com

Chemiluminescence imaging technology, which combines the advantages of high sensitivity of chemiluminescence and high resolution of the imaging assay, has become a simple, economical and speedy method for chemical and bio-analysis. However, it suffers from the disadvantage of weak selectivity of CL analysis, which has limited its usage widely. Molecularly imprinted polymer (MIP) can produce artificial binding sites in macroporous polymer particles, on surfaces or in membranes; these artificially generated recognition sites have their shapes, sizes and functionalities complementary to the analyte, and are capable of rebinding the analyte molecules in preference to other closely related structures. A precipitation polymerization method for preparing MIP microspheres with uniform shape and low-disperse phase distribution in good yield was used. The imprinted polymer microspheres were immobilized in 96-well microtitre plates. The analyte was selectively adsorbed in the MIP

microspheres. After washing, the bound fraction as a fluorophore was quantified based on bis(2,4,6-trichlorophenyl)oxalate (TCPO) reacted with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to emit chemiluminescence. The signal was detected and quantitated using a highly sensitive intensity charge-coupled device (ICCD) camera with Alpha Ease FC software. A new molecular imprinted polymer (MIP)-chemiluminescence (CL) imaging sensor approach towards chiral recognition of dansyl-phenylalanine (Phe) is presented. Influencing factors were investigated and optimized in detail. Control experiments using a capillary electrophoresis method showed that there was no significant difference between the proposed method and the control method. The proposed method has been also applied for the determination of *trans*-resveratrol in wine samples and dipyrindamole in human urine samples. The present or similar approaches could provide useful analytical systems in many instances.

### Synthesis and photoluminescence of green-emitting $\text{X}_2\text{-(Y,Gd)}_2\text{SiO}_5\text{:Tb}^{3+}$ phosphor under VUV excitation

Z. H. Zhang<sup>1,2</sup> and Y. H. Wang<sup>2</sup>

<sup>1</sup>School of Materials Science and Engineering, Wuhan Institute of Technology, 430073 Wuhan, People's Republic of China.

E-mail: zzhzhu@163.com

<sup>2</sup>Department of Materials Science, Lanzhou University, 730000 Lanzhou, People's Republic of China

Recently, special attention has been paid to phosphors under vacuum ultraviolet (VUV) excitation, due to the demands of plasma display panels (PDPs) and the possible generation of mercury-free fluorescent lamps. As far as green-emitting phosphors for PDP application were concerned, the most widely used one is  $\text{Zn}_2\text{SiO}_4\text{:Mn}^{2+}$ , but its decay time is so long that it is difficult to exploit the fast response of PDPs. Therefore, it is urgently necessary to find new green-emitting phosphors with a short decay time under VUV excitation. In this study, single phases of  $\text{X}_2\text{-(Y,Gd)}_2\text{SiO}_5\text{:Tb}^{3+}$  were synthesized by a co-precipitation process. Based on the systemic investigation of their photoluminescence under VUV excitation, the maximum emission peak was observed at about 542 nm in the emission spectra, and the  $f \rightarrow d$  transitions of  $\text{Ln}^{3+}$  ( $\text{Ln} = \text{Y, Gd, Tb}$ ), the charge transfer bands of  $\text{Ln}^{3+}\text{-O}^{2-}$  and the absorption of Si-O bonds were assigned in the excitation spectra. The sample of  $\text{X}_2\text{-Y}_{1.6}\text{Gd}_{0.2}\text{Tb}_{0.2}\text{SiO}_5$  had the strongest emission intensity, which was 196% of that of commercial  $\text{Zn}_2\text{SiO}_4\text{:Mn}^{2+}$ , and had a much lower  $1/e$  decay time (2.9 ms) than that of  $\text{Zn}_2\text{SiO}_4\text{:Mn}^{2+}$  (5.1 ms). Mechanisms of concentration quenching and energy transfer in the VUV region of this phosphor were proposed.

### Applications of delayed fluorescence and laser confocal scanning microscope techniques in monitoring artificial acid rain stress on plants

H. Zhang, F. Wen, L. Zhang and D. Xing

MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China. E-mail: xingda@scnu.edu.cn

Acid rain is one of the important environmental stress problems worldwide. With *Arabidopsis thaliana* as the testing mode, we

investigated the responses of higher plants to artificial acid rain stress using delayed fluorescence (DF) and laser confocal scanning microscope techniques. *In vivo* detection of the DF emissions from *Arabidopsis* seedlings with a custom-made DF biosensor enables DF parameters of samples to be rapidly produced. Results obtained at the whole-seedling level showed that the DF intensity declined with decreasing pH values of simulated acid rain. Moreover, the DF intensity had a significant decrease after only 15 min simulated acid rain stress, well before any visible damage could be found. Measurements of gas exchange and chlorophyll content demonstrated that the DF intensity correlated with net photosynthesis rate ( $P_n$ ) but not with chlorophyll content under artificial acid rain stress, indicating that the early decline in photosynthesis might be attributed to the reduction in the photochemical efficiency, not to direct chlorophyll degradation. At the single cell level, artificial acid rain treatment caused the enhanced accumulation of reactive oxygen species (ROS), which could be observed by means of a ROS-sensitive probe under a laser confocal scanning microscope (LCSM). These data suggest that the reduction in the photochemical efficiency caused by artificial acid rain might be through an oxidative stress-dependent mechanism. Summarizing, DF could be used an early marker for monitoring acid rain stress. The DF technique combined with LCSM imaging could provide a new strategy for elucidating the adaptation mechanisms by which plants cope with environmental stresses.

### Delayed fluorescence and optical molecule imaging techniques for detecting the stress response of plant to high temperature

L. Zhang and D. Xing

MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China. E-mail: xingda@scnu.edu.cn

High temperature is a key limiting factor for plant growth and productivity. To understand in depth the response of plants to high temperatures, we investigated the effects of heat stress on the heat-shock transcription factor knockout (*KoHsfA2*) mutant and wild-type (WT) of *Arabidopsis* plants, using delayed fluorescence (DF) and optical molecule imaging techniques. At the whole-seedling level, *in vivo* detection of the DF emissions with a custom-made biosensor showed that the DF intensity declined more markedly in the *KoHsfA2* mutants than in WT plants after high-temperature treatment. Moreover, the DF intensity could recover fully at the normal growth temperature condition after 2 h heat stress in WT plants but not in the *KoHsfA2* mutants. Measurements of gas exchange demonstrated that the changes in DF intensity were in line with those in photosynthesis rate ( $P_n$ ). Investigations performed at the single-cell level under a laser confocal scanning microscope (LCSM) showed that the production of reactive oxygen species (ROS) occurred much more rapidly and more intensively in the *KoHsfA2* mutants compared with WT plants after heat stress. Conversely, pre-incubation with ascorbic acid (AsA, antioxidant molecule) or catalase (Cat) reduced the accumulation of ROS and reversed the declines in the DF intensity and  $P_n$ . These results revealed that *HsfA2* plays an important role in regulating redox balance and DF is an excellent marker for detecting the response to oxidative stress caused by high-temperature



treatment. The DF technique combined with LCSM imaging could provide a powerful approach to elucidate the mechanisms by which plants response to environmental stresses.

### **A highly sensitive flow-injection chemiluminescence method for the determination of hydrazine in water**

J. Zhang and M. Yang

Department of Chemistry, Liaoning Normal University,  
Dalian 116029, People's Republic of China.

E-mail: yangmeils@163.com

A highly sensitive flow-injection chemiluminescence method for the determination of trace amounts of hydrazine has been developed. The procedure is based on the chemiluminescent reaction of luminol with hydrogen peroxide in alkaline solution, where hydrazine acts as a catalyst and strongly enhances the CL intensity. Under optimum conditions, the calibration graph was linear with the concentration of hydrazine in the range  $1.6 \times 10^{-8}$ – $1.6 \times 10^{-4}$  mol/L. A detection limit of  $5.1 \times 10^{-9}$  mol/L, along with RSDs of 5.4% and 0.82% ( $n = 11$ ) for  $1.6 \times 10^{-8}$  mol/L and  $1.6 \times 10^{-6}$  mol/L hydrazine, respectively, were obtained. The interferences resulting from ions potentially coexisting in water can be removed by the use of an ion-exchange column combined in the sample stream. The method is simple, rapid and has been successfully applied to the determination of hydrazine in water, with recoveries of 85.4–99.5%.

### **Luminescence studies on the interaction of CdSe–ZnS quantum dots with G-protein and goat anti-human IgG**

Y. Zhang,<sup>1</sup> Q. Zeng,<sup>1</sup> Y. Yu,<sup>1</sup> Y. Sun,<sup>1</sup> Y. Yang,<sup>1</sup> X. Liu,<sup>1</sup> X. Kong<sup>1</sup> and H. Zhang<sup>2</sup>

<sup>1</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China.

E-mail: xgkong14@ciomp.ac.cn

<sup>2</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: h.zhang@uva.nl

An unexpected observation on QDs with G-protein and goat anti-human IgG is the enhancement of PL efficiency that occurs when the G-protein and goat anti-human IgG couple with QDs, the enhancement factors increasing with increasing coupled protein concentration. Steady-state fluorescence measurements show that the enhancement of PL efficiency results from the long-range interaction between quantum dots and protein, instead of from fluorescence resonance transfer between the quantum dots and proteins, according to the results of excitation spectra. Time-resolved measurements of the fluorescence decay reveal bi-exponential kinetics. The bioassay specificity and sensitivity are markedly increased via fibre sensitizer detection, compared with the QDs coupled with goat anti-human IgG only, when the QDs are first modified by specific biomolecule and then coupled antibody. These results have great potential of application in immunoassay and immunotherapy, biochips, fibre sensitizers and nanobiomedicine.

### **Charge and energy transfer dynamics between nanoparticles and the influence of organic surroundings**

H. Zhang,<sup>1</sup> Y. Zhang,<sup>1,2</sup> J. Zhao,<sup>2</sup> Y. Sun,<sup>1,2</sup> Y. Wang<sup>1,2</sup> and X. Kong<sup>2</sup>

<sup>1</sup>Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

<sup>2</sup>Key Laboratory of Excited State Process, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, People's Republic of China

Functionalization of nanoparticles by conjugation with antibody (fragments) or proteins allows targeting to specific cells or molecules. By either using the inherent physical properties or coupling to therapeutic agents, functionalized nanoparticles could be used to treat diseases such as cancer and viral infections. To attain this goal, an understanding of the interactions, such as electron/hole and energy transfer processes, between the nanoparticles and their surrounding organic/biological systems is essential. We report our recent studies employing various spectroscopic techniques to cover the full time range above 300 fs, on serous nanoparticle systems in which the effect of shell layers, organic groups and excitation conditions, etc., on the process have been elucidated. Our results show that while the charge/energy transfer process is based on relevant energy configuration, other parameters, such as shell properties, etc., also play a role.

### **Determination of nucleic acids based on fluorescence enhancement effect of quercetin–Ag nanoparticles–nucleic acids system**

L. Zhao,<sup>1</sup> X. Wu,<sup>1</sup> J. Yang<sup>1</sup> and Q. Yang<sup>2</sup>

<sup>1</sup>Key Laboratory of Colloid and Interface Chemistry (Shandong University), Ministry of Education, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, Shandong, People's Republic of China.

E-mail: wux@sdu.edu.cn

<sup>2</sup>School of Chemistry and Chemical Engineering, Jinan University, Jinan 250022, People's Republic of China

Silver nanoparticles (nanoAg) have an important function in anti-inflammation, anti-virus, anti-AIDS and especially anticancer therapies. The interaction between nanoAg and nucleic acids has aroused broad interest and attention. It was found that nanoAg could further enhance the fluorescence intensity of the quercetin (Qu)–nucleic acids system. Based on this, a new fluorimetric method of determination of nucleic acids was proposed. Under optimum conditions, the enhanced fluorescence intensity was proportion to the concentration of nucleic acids in the range  $8.0 \times 10^{-9}$ – $2.0 \times 10^{-6}$  g/mL for fish sperm DNA (fsDNA);  $2.0 \times 10^{-8}$ – $8.0 \times 10^{-6}$  g/mL for salmon serum DNA (smDNA); and  $5.0 \times 10^{-8}$ – $5.0 \times 10^{-6}$  g/mL for yeast RNA (yRNA). The detection limits ( $S:N = 3$ ) of fsDNA, smDNA and yRNA were  $4.2 \times 10^{-9}$  g/mL,  $1.2 \times 10^{-8}$  g/mL and  $6.6 \times 10^{-9}$  g/mL, respectively. Investigation of the interaction mechanism of the system indicated that there existed cooperation of groove binding and electrostatic interaction between Qu–nanoAg and nucleic acids and that a complex of Qu–nanoAg–nucleic acids was formed.

### ciprofloxacin in biological fluids

X. Zhou, D. Xing, D. Zhu and L. Jia

MOE Key Laboratory of Laser Life Science and Institute of Laser Life Science, South China Normal University, Guangzhou 510631, People's Republic of China. E-mail: xingda@scnu.edu.cn

A simple and rapid capillary electrophoresis (CE) method with electrochemiluminescence (ECL) detection for the determination of ciprofloxacin (CIP) has been developed. The method is based on CE separation and the end-column ECL reaction between tris(2,2-bipyridyl)ruthenium(II) and aliphatic secondary amino moieties. Under the optimized conditions (detection potential, 1.15 V; separation voltage, 14 kV; 15 mmol/L phosphate running buffer, pH 8.5; 10 kV injection voltage; 20 s injection time), the calibration function of CIP was linear in the range 0.05–1.2 µg/mL, with a detection limit of 15 ng/mL. The RSDs of the peak height and the migration time for six consecutive injections of CIP were <4%. The developed method was successfully applied to determine CIP in human serum and urine samples.

### Study on superweak luminescence and salt tolerance in alfalfa

H. Zhou,<sup>1</sup> Q. Yang<sup>2</sup> and Q. Ji<sup>1</sup>

<sup>1</sup>Institute of Grassland Science, China Agricultural University, Beijing 100094, People's Republic of China. E-mail: zhouhe@cau.edu.cn

<sup>2</sup>Department of Biotechnology, Beijing Agricultural College, Beijing 102206, People's Republic of China

Alfalfa (*Medicago sativa* L.) is a important cultivated forage plant. In the study of an evaluation method for salt resistance of alfalfa, one method should be selected that can quickly and exactly evaluate the salt resistance of a plant. Various biophoton emission phenomena have been investigated widely by researchers in the many countries, based on highly sensitive photon detection. We report the relationship between superweak luminescence (SL) and salt resistance in alfalfa. Under stress conditions, seed SL values show obvious changes. The results indicate that the values of luminescence are not significantly different among the varieties of alfalfa in distilled water, while the difference among the varieties of alfalfa is distinct in NaCl solution. Under the same salt stress, the different varieties of alfalfa emitted different SLs in germinating seed. According to these differences, we can assess the degree of salt tolerance in alfalfa. As shown in the experiments, we attempt to look for the metabolizing indicator by means of measurement of SL. This method of measuring bioluminescence will probably become an indicator of salt resistance of alfalfa. It was designed to analyse the oxidative mechanism of the plant.

### Luminescent properties of Na<sub>2</sub>Ca<sub>4</sub>Mg<sub>2</sub>Si<sub>4</sub>O<sub>15</sub>:Tb<sup>3+</sup> nano-sized phosphor

L.-Y. Zhou, L.-H. Yi, J.-L. Huang, J.-S. Wei and F.-Z. Gong  
School of Chemistry and Chemical Engineering, Guangxi University, Nanning 530004, People's Republic of China. E-mail: zhouliyatf@163.com

A novel green emitting nano-sized phosphor, Tb<sup>3+</sup>-doped Na<sub>2</sub>Ca<sub>4</sub>Mg<sub>2</sub>Si<sub>4</sub>O<sub>15</sub>, was synthesized with a precursor prepared

by a sol-gel method at relatively low temperature. X-ray powder diffraction (XRD) analysis confirmed the formation of Na<sub>2</sub>Ca<sub>4</sub>Mg<sub>2</sub>Si<sub>4</sub>O<sub>15</sub>:Tb<sup>3+</sup>. Field-emission scanning electron microscopy (FE-SEM) observation indicated a narrow size-distribution of about 80–100 nm for the particles with spherical shape. Upon excitation with near-UV light, the phosphor showed strong green emission peaking at around 545 nm, corresponding to the <sup>5</sup>D<sub>4</sub> → <sup>7</sup>F<sub>5</sub> transition of Tb<sup>3+</sup>, and the highest PL intensity at 545 nm was found at a content of about 8 mol% Tb<sup>3+</sup>. As the Tb<sup>3+</sup> concentration increases, the Tb–Tb distance decreases, the fast diffusion of energy among terbium ions toward traps or impurities resulting in a decrease of the lifetime. The coordinates of Na<sub>2</sub>Ca<sub>4</sub>Mg<sub>2</sub>Si<sub>4</sub>O<sub>15</sub>:Tb<sup>3+</sup> were found to fall in the yellowish-green region of the CIE chromaticity diagram and, with a decrease of Tb<sup>3+</sup>, the phosphor exhibited deeper green emission.

### Metal-enhanced room temperature phosphorescence on solid surface

Y. X. Zhu,<sup>1</sup> Y. Q. Li<sup>1</sup> and Y. Zhang<sup>2</sup>

<sup>1</sup>Department of Chemistry, College Chemistry and Chemical Engineering, Xiamen University, Xiamen, People's Republic of China. E-mail: yzhang@xmu.edu.cn

<sup>2</sup>State Key Laboratory of Marine Environmental Science (Xiamen University), Environmental Science Research Centre, Xiamen University, Xiamen, People's Republic of China

For clinical applications, the photodynamic property of a lumophore has potential in the diagnosis and treatment of several diseases, such as diabetes, peripheral vascular diseases and cerebrovascular and cardiovascular events. However, modern photodynamic therapy is for the most part still limited by insufficient quantity of singlet oxygen when reacting with biological targets. An alternative method to resolve this problem is to populate high-lying triplet excited states of several dyes which produce oxygen-independent damage. Photoluminescence of molecules near metal surfaces can be increased both by absorption enhancement due to increases in the incident field experienced by the molecule and by emission enhancement. In this study, metal-enhanced room temperature phosphorescence properties of fluorescein in the presence of silver nanoparticles were studied on filter paper surface in the first time. We obtained remarkable enhancement of phosphorescence. The realization of metal-enhanced room temperature phosphorescence should supply potential applications in photodynamic therapy.

### Determination of trace lead in environmental water by a fluorescence quenching method

X. Zhu and L. Shi

College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, People's Republic of China. E-mail: xszhu@yzu.edu.cn

A new and sensitive fluorimetric method for the determination of lead was established, based on the quenching effect of lead on the fluorescence of sulphosalicylic acid (SSA) in pH 7.0 citric acid–Na<sub>2</sub>HPO<sub>4</sub> buffer solution. The maximum excitation and emission wavelengths of SSA were 303.0 and 402.0 nm, respectively. The main factors affecting the fluorescence quenching intensity were investigated in detail and the other ion interferences were

studied. Under optimal experimental conditions, the line arrangement of the determination was  $1.0 \times 10^2$ – $3.5 \times 10^3$   $\mu\text{g/L}$  and the detection limit of lead was  $3.8 \times 10^1$   $\mu\text{g/L}$ . The correlation coefficient ( $R$ ) was 0.998, the precision for six replicate determinations at  $1.0 \times 10^3$   $\mu\text{g/L}$  Pb was 3.5% RSD. The proposed procedure was applied to the analysis of standard water samples (GB 00486). Comparison with certified values was performed for accuracy evaluation, resulting in good agreement according to the  $t$ -test for a 95% confidence level. This method was used to determine trace lead in environmental water with satisfactory results.

### Capillary electrophoresis with electrochemiluminescence detection of Novel $\beta$ -cyclodextrin-modified organic polymeric monolithic substrate for solid-phase extraction–room temperature phosphorescence

R. H. Zhu and W. T. Xu

Department of Chemistry, Capital Normal University,  
Beijing 100037, People's Republic of China.  
E-mail: zhurh@mail.edu.cn

An organic polymeric monolithic substrate was prepared by *in situ* polymerization of glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) in a binary porogenic solvent consisting of 1-propanol and 1,4-butanediol, then modified by a reaction with  $\beta$ -cyclodextrin ( $\beta$ -CD). The  $\beta$ -CD-modified substrate can be used as solid substrate and solid phase extraction membrane for solid-phase extraction–room temperature phosphorescence (SPE–RTP). The room temperature phosphorescence (RTP) behaviours of 14 organic compounds on the  $\beta$ -CD modified substrate were examined. The results indicated that phenanthrene, 7,8-benzoquinoline, carbazole, fluorene, 3-indoleacetic acid and 1-naphthylacetic acid can emit strong RTP, and the limits of detection (LOD) were found to be  $6.45 \times 10^{-7}$ – $2.98 \times 10^{-6}$  mol/L. Used as solid phase extraction membrane, the modified disc could selectively enrich phenanthrene, 7,8-benzoquinoline, carbazole and fluorene. After SPE, the LODs decreased to  $4.04 \times 10^{-9}$ – $6.40 \times 10^{-8}$  mol/L. The  $\beta$ -CD modified substrate has low background, good permeability, high selectivity and extraction ability. The method was applied to the determination of fluorene and phenanthrene in tea, coffee, soil and environmental water samples with satisfactory results, and the recoveries for fluorene and phenanthrene were 84.6–101.7% and 76.7–92.0%, respectively.

### Determination of BSA with 3-(4'-methyl phenyl)-5-(4'-methyl-2'-sulphophenylazo) rhodanine by a new method of second-order scattering

Y. Zhu,<sup>1</sup> J. Yu,<sup>2</sup> P. Dai,<sup>2</sup> C. Zhang<sup>2</sup> and B. Li<sup>2</sup>

<sup>1</sup>School of Material Science and Engineering, University of Jinan, People's Republic of China.  
E-mail: mse\_zhuyn@ujn.edu.cn

<sup>2</sup>School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, People's Republic of China.  
E-mail: yujinghua@vip.sina.com

Second-order scattering (SOS) used to be regarded as a kind of interference in spectrofluorometry. The characteristics and principles of second-order scattering have not been studied deeply. In this study, second-order scattering was found to be a

new method to determine trace substances with good potential of analysis. The reaction between 3-(4'-methyl phenyl)-5-(4'-methyl-2'-sulphophenylazo) rhodanine (M4MRASP) and bovine serum albumin (BSA) was investigated by the method of second-order scattering. It was found that the second-order scattering intensities of 3-(4'-methyl phenyl)-5-(4'-methyl-2'-sulphophenylazo) rhodanine and BSA were weak in Britton–Robinson (BR) buffer solution at pH 1.62 with the presence of SDS microemulsion, but sharply enhanced when the two were compounded, based on which a new method for determination of BSA was established, which has high sensitivity and calls for simple operation. The concentration of BSA was linear, with 3-(4'-methyl phenyl)-5-(4'-methyl-2'-sulphophenylazo) rhodanine in the range 0–2.5  $\mu\text{g/mL}$ . Second-order scattering is found to be more sensitive than resonance Rayleigh light scattering (RRS). The new method was applied to the determination of BSA in real samples with satisfactory results.

### Development and optimization of a fast and sensitive ELISA for polio D-antigen using a GZ11-based signal reagent

G. Zomer and M. Hamzink

Unit Research and Development, Netherlands Vaccine Institute,  
PO Box 457, 3720BA Bilthoven, The Netherlands.  
E-mail: Bert.Zomer@nvi-vaccin.nl

The potency of inactivated polio vaccine (IPV) is traditionally determined by the measurement of the protective D-antigens present in the vaccine. Mostly, a sandwich ELISA test is used for this purpose. During production of the polio virus and during downstream processing of the vaccine there is a need for a reliable indication of the concentration of D-antigens present in the different sample types. Customarily, the ELISA test involves several incubations: first, the binding of the antigens using a type-specific antibody-coated microtitre plate; secondly, the antigen is bound by a type-specific monoclonal antibody; and third, the monoclonal antibody is bound by an HRP-labelled second antibody. Each incubation step is followed by washing steps. For these reasons, this assay is rather time consuming. In order to speed up the assay, the second and third incubation steps were combined. Furthermore, because of the high sensitivity of detection of the HRP conjugate using the GZ-11 signal reagent, much less antibody could be used, resulting in a rapid (within 2 h) and sensitive ELISA. The effects of other assay parameters, e.g. incubation temperature and agitation during incubations, are discussed.

### Development and optimization of a quantitative Western blot and dot-blot procedure for the determination of residual host cell protein present in inactivated polio vaccine, using a GZ11-based signal reagent

G. Zomer, M. Hamzink, A. De Haan, G. Kersten and K. Reubsat  
Unit Research and Development, Netherlands Vaccine Institute,  
PO Box 457, 3720BA Bilthoven, The Netherlands.  
E-mail: Bert.Zomer@nvi-vaccin.nl

Inactivated polio vaccine (IPV) contains as the active ingredient protective D-antigens. Because the polio virus is grown on a

host cell system, residual host cell proteins (HCPs) may be present in the bulk product. For registration purposes, the amount of these host cell proteins needs to be determined. In order to quantify these proteins, two approaches were used. First, the HCPs were separated on gel and, after incubation with rabbit polyclonal antiserum directed against the HCP-mix,

followed by incubation with detecting antibody (HRP-labelled mouse anti-rabbit), subsequently visualized and quantified using a GZ11-based signal reagent. Second, a dot-blot approach using the same two incubation steps and detection with GZ11 signal reagent was followed. Differences and analytical figures of merit are discussed.