SYMPOSIUM AA
Applications of Novel Luminescent Probes in Life Sciences
April 13 - 14, 2004

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* Invited paper
SESSION AA1: Novel Luminescent Probes in Life Sciences
Tuesday Morning, April 13, 2004
Room 3018 (Moscone West)

8:30 AM *AA1.1
Fluorescence Lifetime Imaging of Sensor Arrays.
Michael Schäferling, Ming Wu, Zhihong Lin and Otto S. Wolfbeis,
Institute of Analytical Chemistry, Chemio- and Biosensors, University of Regensburg, Regensburg, Germany.

Optical imaging methods have attracted strong attention in life sciences since they find numerous applications in bioanalysis and medical diagnostics. Sensor probes for the imaging of oxygen partial pressure or pH in biological samples have been developed, which can be used for the detection of skin tumor cells. Principle applications of these new fluorescence-based ratiometric imaging methods with immobilized probe molecules, e. g. ruthenium complexes, will be demonstrated. In the second part we present different examples of the applicability of europium(III) tetracyclen(II) as a molecular sensor. Based on the fact that the [Eu{Tc}3] complex undergoes a 15-fold increase in fluorescence intensity on exposure to hydrogen peroxide (HP) and this effect is reversible, this probe can be used as molecular sensor for the detection and imaging of H2O2. This sensitivity and the long fluorescence decay time of this complex can be utilized for several applications, all working at neutral pH:

- Imaging of glucose via planar sensor foils, prepared by coadsorption with glucose oxidase on polymer hydrogels
- Determination and direct visualization of the activity of oxidases (e. g. glucose oxidase), catalases or peroxidases
- Detection of HP on colloidal particles leading to partial quenching of the luminescence. This loss is reversible

Our self-developed imaging system, basically consisting of a LED array and a CCD camera, triggered by a pulse generator, permits a fast data acquisition and evaluation process. The sensor probes can be adopted in microfluidic formats for read-out of multiplexed samples. It can be applied as immobilized sensor membranes as well as in solution. Different ratiometric and intrinsically referenced time-resolved imaging methods have been carried out, e. g. Rapid Lifetime Determination (RLD) or Phase Delay Rationing (PDR).


9:00 AM AA1.2
Frequency upconversion and imaging using rare-earth doped colloidal nanoprobes, M.A. de Dood1, B.J. Berkhout1, C.M. van Kats2, A. van Blaaderen2, Albert Polman3, FOM-Institute AMOLF, Amsterdam, Netherlands; 2Debye Institute, Utrecht University, Utrecht, Netherlands; 3Delft University of Technology, Delft, The Netherlands.

We have fabricated colloidal silica particles doped with optically active terbium (emitting at 650 nm), europium (920 nm) and erbium (1535 nm) ions that combine high photosensitivity and thermal stability, luminescence in a wide range of environmental conditions, have narrow spectral emission width (4 % of center wavelength), and high quantum efficiency (70 %). The particles were made using an acid-based hydrolysis and condensation reaction of tetraethoxysilane in the presence of rare-earth chlorides. Rare earth concentrations were in the range 0.1-1.0 at. %.

The colloidal silica host provides the proper coordination for the rare earth’s optically active trivalent state, and shields the luminescent 3+ from traces of the quenching effect of vibrational states that often limit the use of (organic) rare earth probes in solution. The rare earth doped colloids can serve as an optical probe in a variety of scientific fields. In this presentation, we will first illustrate the use of erbium as a probe of infrared optical modes in miniature photonic integrated circuits. By taking advantage of frequency upconversion effects in the rare earth ions (under continuous-wave laser excitation), the optical mode field can be imaged at a resolution 3 times smaller than the diffusion limit. By coupling the Er3+ ions to Si quantum dots that act as sensitizers, the effective excitation cross section for Er is enhanced by four orders of magnitude. Then, based on knowledge derived from our work on rare earths in nanophotonic materials we propose several applications for rare earth probes in biology, sensing, and related fields. New developments come to mind: one is the use of infrared pumping of terbium or europium doped colloidal probes, thus taking advantage of the small lateral resolution of SEM, and the high sensitivity of optical detection techniques. The fact that colloidal particles are non-toxic and can be functionalized with a wide range of biological entities makes these rare earth doped colloidal probes interesting for a broad range of applications. Due to their millisecond lifetimes, the rare earth emission can be readily separated from background signals using time-gated (TCID-imaging) with a wide range of analogies between photonic integrated circuits and microfluidic devices, and propose several geometries in which their integration, using rare earth ions as probes, can be used in a variety of novel sensing concepts.

9:15 AM AA1.3

Colloidal solutions and dispersible powders of lanthanide-doped nanocrystals have been prepared in high-boiling coordinating solvents. This synthesis yields gram amounts of highly crystalline nanoparticles with a narrow particle size distribution and a mean particle diameter below 10 nm. Upon UV-excitation of the nanocrystals, the luminescence line spectrum of the dopant ions is observed. The luminescence lifetimes are in the range of milliseconds. Depending on the dopant concentration, up to three different europium bulk sites are observed. Moreover it is shown that europium sites at the surface are converted into bulk sites by growing a shell of pure, i.e. undoped host material around the nanoparticles. The resulting core-shell nanosites have been studied by site-selective spectroscopy, i.e., a luminescence line-narrowing exhibiting 100% efficiency. In the case of europium-doped core and core-shell particles, we are able to distinguish between dopant sites in the interior of the nanocrystals ("bulk sites") and those located at the particle surface. Depending on the dopant concentration, up to 3 different europium bulk sites are observed. Moreover it is shown that europium sites at the surface are converted into bulk sites by growing a shell of undoped material around the nanoparticles. Similarly, only surface europium sites are observed if pure (undoped) nanoparticles are synthesized and subsequently reacted with europium ions. These materials are promising candidates for biolabeling applications because they combine the high chemical stability and photosubject of an inorganic host lattice with the narrow emission lines and the long luminescence lifetimes of the lanthanides.

9:30 AM AA1.4
Efficient multicolor upconversion emission in colloids of lanthanide doped NaYF₄ nanocrystals. Stephan Heer1, Karsten Koempe2, Markus Haase2 and Hans-Ulrich Giedda1, 1Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland; 2Institute of Physical Chemistry, University of Hamburg, Hamburg, Germany.

Organic dyes and semiconductor quantum dots play an important role in the search for new luminescent probes in life sciences. Recently, upconversion excitation of semiconductor quantum dots for in vivo imaging has been demonstrated. Upconversion (UC) is the generation of visible light by near-infrared excitation. This has distinct advantages compared to conventional UV or blue excitation of fluorescent probes in biological systems, e. g. greatly reduced autofluorescence, light scattering and photodegradation. By far the most efficient UC systems known are based on insulating phosphor materials doped with lanthanides, e. g. NaYF₄ doped with Er3+ and Tm3+ in combination with Yb3+, as a sensitizing agent. The underlying UC mechanisms are intrinsically many orders of magnitude more efficient than the two-photon absorption processes in semiconductors and organic dyes under similar excitation densities. Furthermore, the emission of the lanthanide ions consist of sharp lines and broad emission bands, respectively, offering the possibility to build luminescent probes with different colors, i. e. red, green and blue, by varying the dopant ions. Together it was a challenging task to synthesize tunable UC nanocrystals. We present the synthesis, characterization and structural and spectroscopic characterization of lanthanide doped NaYF₄ nanocrystals. The synthesis was performed in high boiling coordinating solvents using simple precursors reactions. Characterization by X-ray diffraction (XRD) shows the cubic...
α-NaYF ₄ phase with an average particle size of 15 nm. This value is in accord with the transmission electron microscopy images (TEM) of the nanomaterial. The microstep conversion particles as luminescent probes is to disperse them in a fully transparent colloidal solution and then to induce UC in the liquid medium. This is a critical test of the particle quality since the temperature dependence of UC is very sensitive to high energy vibrations of the solvents which can efficiently quench excited states. Characterization by dynamic laser scattering (DLS) shows well separated nanoparticles. Intense visible emissions can be excited in Yb⁺⁺/Er⁺⁺ (blue) and Yb⁺⁺/Er⁺⁺ (green, red) co-doped samples of NaYF₄ with low CW excitation at 14.7 μW, which can be achieved with high efficient and inexpensive laser diodes. The visible light output of the new nanomaterials is eight orders of magnitude larger than the previously investigated colloids of lanthanide doped phosphate nanocrystals. The high emission efficiency is an extremely efficient sensitizer for both the Tm⁺⁺ and Er⁺⁺ UC systems. Power dependent measurements show the nonlinear character of the processes. In conclusion, we report on an attractive new class of dispersible luminescent quantum dots (QDs) with tunable size, high optical quality, and easily processable by standard co-doping strategies of producing highly luminescent stable QDs, especially water-soluble luminescent quantum dots, has the potential to open a new field of highly sensitive, good reproduction with high reliability, and readily tunable spectral properties. Nanocrystals composed of II–VI semiconductors (e.g., CdSe, and InAs) have emission spectra that span the visible to the near IR. Nanocrystals made of silicon have photoluminescence that reportedly emit the UV to the visible. Silicon, which is abundant, cheap, and non-toxic, would be an ideal material while the element of cadmium in CdSe nanocrystals has the potential toxic problem for in-vivo cells. A key requirement for the successful use of nanocrystal bio-applications is aqueous solubility. Therefore, it needs to modify the surface of quantum dots with biocompatible molecules having functional groups (-COOH, -OH, or -SH) to confer water solubility. We report here a chemical approach to produce water-soluble silicon nanocrystals using NaSi in organic solvent. We chose the micropipet probe so that the focused ultrasound energy could be applied into the reaction solution at high intensity. This speedy route produces the silicon nanocrystals in the size range from 1 to 3 nm at room temperature and ambient pressure. For biocompatible surface modification, alkyl-amine was added to couple directly to the prepared silicon quantum dots with a covalent bonding (Si-C) during simple preparation. The product yield of nanocrystals was estimated to be higher than 60% based on the initial NaSi weight. HRTEM images show that silicon nanocrystals have a spherical shape with the size range from 1 nm to 3 nm. The mass transfer image clearly shows that silicon nanocrystals of which d spacing (-1.9 nm) is equivalent to a strong (220) silicon plane. FTIR data was collected to characterize the amine-terminated silicon nanocrystals. Emission spectra of our silicon nanocrystals typically span UV-blue spectrum, which might be resolved into narrow spectrum via a gel permeation chromatography (GPC). When samples are irradiated with the commercial low-intensity UV lamp (300 nm), each colored image is observable with the naked eyes in room light. We will discuss coupling properties of silicon quantum dots with biomolecules like streptavidin, etc.

11:00 AM AA1.7
Water-Soluble Silicon Quantum Dots by Ultrasound-Induced Solution Route. Soojin Lee1,2, Woon Jo Choo2, Chong Shik Chin2, Il Ki Han2, Young Ju Park2, Won Jun Choi2, Jin Dong Song2 and Jung Il Lee2, 1Dept. of Chemistry, Sogang Univ., Seoul, South Korea; 2Nano Device Research Center, Korea Institute of Science and Technology, Seoul, South Korea.

Labeling of biological molecules using colloidal semiconductor nanocrystals, referred to as “quantum dots”, has the potential to open a new field of highly sensitive, good reproduction with high reliability, and readily tunable spectral properties. Nanocrystals composed of II–VI semiconductors (e.g., CdSe, and InAs) have emission spectra that span the visible to the near IR. Nanocrystals made of silicon have photoluminescence that reportedly emit the UV to the visible. Silicon, which is abundant, cheap, and non-toxic, would be an ideal material while the element of cadmium in CdSe nanocrystals has the potential toxic problem for in-vivo cells. A key requirement for the successful use of nanocrystal bio-applications is aqueous solubility. Therefore, it needs to modify the surface of quantum dots with biocompatible molecules having functional groups (-COOH, -OH, or -SH) to confer water solubility. We report here a chemical approach to produce water-soluble silicon nanocrystals using NaSi in organic solvent. We chose the micropipet probe so that the focused ultrasound energy could be applied into the reaction solution at high intensity. This speedy route produces the silicon nanocrystals in the size range from 1 to 3 nm at room temperature and ambient pressure. For biocompatible surface modification, alkyl-amine was added to couple directly to the prepared silicon quantum dots with a covalent bonding (Si-C) during simple preparation. The product yield of nanocrystals was estimated to be higher than 60% based on the initial NaSi weight. HRTEM images show that silicon nanocrystals have a spherical shape with the size range from 1 nm to 3 nm. The mass transfer image clearly shows that silicon nanocrystals of which d spacing (-1.9 nm) is equivalent to a strong (220) silicon plane. FTIR data was collected to characterize the amine-terminated silicon nanocrystals. Emission spectra of our silicon nanocrystals typically span UV-blue spectrum, which might be resolved into narrow spectrum via a gel permeation chromatography (GPC). When samples are irradiated with the commercial low-intensity UV lamp (300 nm), each colored image is observable with the naked eyes in room light. We will discuss coupling properties of silicon quantum dots with biomolecules like streptavidin, etc.

11:15 AM AA1.8
Highly luminescent semiconductor nanocrystalline nanoscale and high-throughput analysis of biomolecules, Mingyong Han1, 1Institute of Materials Research and Engineering, Singapore, Singapore; 2Department of Materials Science, National University of Singapore, Singapore, Singapore.

Colloidal semiconductor nanocrystals, also known as quantum dots (QDs), are of tremendous fundamental and technical interest due to their applications as light-emitting devices, lasers, and biological labels. A major problem encountered over the years in fabricating high-quality QDs is associated with materials issues, primarily the tendency to form defects and surface-trap states under the employed growth conditions, resulting in low luminescence efficiency and stability deficits. Surface-passivation of the nanocrystals with suitable organic or inorganic materials can minimize this problem by removing the nonradiative recombination centers. Organic passivation is often incomplete or reversible. Effective inorganic-passivation can form coated structured QDs that are more resistant than the organic-coated QDs against chemical degradation or photooxidation. However, for the largely mismatched core-shell structures, the interfacial strain accumulates dramatically with increasing shell thickness, and eventually can be released through the formation of misfit dislocations, degrading the optical properties of the QDs. Consequently, it remains a major goal to develop new synthetic strategies of producing highly luminescent stable QDs.

10:45 AM AA3.6
Carrier Dynamics in Biologically Compatible CdSe Nanocrystal Quantum Dots. Rosa Leal1, Jay Nadkarni1, Jeremiah Kloepfer1, Saulius Marcinkewicz2 and Joerg Siegenthaler2, 1Jet Propulsion Laboratory, Pasadena, California; 2Royal Institute of Technology, Kista, Sweden.

Time resolved photoluminescence (TRPL), and temperature dependence of luminescence were evaluated in CdSe nanocrystals. Temperature dependent PL spectra shows increase in intensity and spectral shift with increasing temperature. Carrier dynamics were investigated in "core shell" CdSe/ZnS nanocrystals coated with trioxyporphine oxide (TOPO) and CdSe nanocrystals coated with mercapt-ocatic acid (MAA) and results were compared. MAA allows binding to proteins, nucleic acids, and other organic molecules, making these nanocrystals suitable for several bio-sensing applications. PL spectra and decay times were also obtained for the CdSe/ZnS/CdSe core/shell nanocrystals with core/shell excitation and detection. The most significant finding was that decay times could be fitted by two components, a very fast decay time (30 to 150 ps) and a much slower component (20 to 80 ns). Photo induced oxidation enhanced the PL intensity from the nanocrystals and also caused some changes in carrier lifetimes.
was one of the most promising organic CL marker in fluorescent markers, even if the samples preserve the moisture and are stronger CL intensity in low-vacuum than that in high-vacuum, which spatial resolution of 10 nm. Such a low vacuum pressure suppresses specimens and to detect the weak intensity of CL from organic other materials. Among them, an organic europium complex controlled up to about 100 Pa, keeping high vacuum in the complex with biological samples, such as proteins, chromosomes and core with donor and acceptor moieties at opposite ends of the Tsukuba, Ibaraki, Japan; 2Hitachi Science Systems, Ltd., Eu(dbm)e(phen) J. Twieg 3 ; 'Chemistry, Stanford University, Stanford, California; the system, the air pressure in the specimen chamber can be

pected for these purposes in the micrometer or sub-micron scale. Recently, the higher spatial resolution is needed for new techniques such as the fluorescent in – situ hybridization (FISH) method. Scanning electron microscopy (SEM) has been applied to observe the morphology of cell organelles and its spatial resolution is high enough to detect the fluorescence of the electron beam irradiation (cathodoluminescence). However, it has not been applied so far, because several technical obstacles such as the electron beam damage vacuum and CL breaching have blocked the application of CL to life science. Thus, we have tried to apply CL for the study of biomaterials. First, we have developed environmental SEM operating under lower vacuum at high resolution. The vacuum pressure of the specimen chamber can be controlled from 10^{-4} to 100 Pa keeping the spatial resolution of 10 nm. Such a low vacuum pressure suppresses the charging of the specimen and reduces the electron beam damage. Then, we have tried to find the optimum fluorescent markers for FISH-CL. In the present work, we report the development of the high-resolution and low-vacuum SEM equipped with a CL detector, and then introduce practical CL imaging applications for the biological specimens. This experimental system makes it possible to observe specimens and to detect the weak intensity of CL from organic fluorescent markers, even if the samples preserve the moisture and are kept without metal coating. The Schottky-emission tip was used as an electron-beam source of the high-resolution low-vacuum SEM system. In the system, the air pressure in the specimen chamber can be controlled to about 100 Pa, keeping high vacuum in the electron-gun and the lens sections. We measured the CL images as well as SE images of over a dozen kinds of organic dye in high- (10^{-4}-10^{-2} Pa) and low-vacuum (40 Pa) conditions; for example, perylene derivatives, oxadiazole compounds, cyanine dyes, vat dyes, metal complex dyes etc. as a result, almost all the substances indicated stronger CL intensity in low-vacuum than that in high-vacuum, which means the usefulness of the low-pressure SEM-CL. We also found that the metal complex dyes were more than two times as strong as organic dyes as CL source. Among them, an organic europium complex Eu(dbm)3(phen) was one of the most promising organic CL marker in our present experiment. To chemically combine the organic europium complex with biological samples, such as proteins, cells, membranes and lipids, it should be modified so as to have both amine-, maleimide- or diazo-groups. These modifications are now in progress.
employed in a single assay. For any new biolabel system this is an important condition for a broad use outside market niches.

3:30 PM **AA2.4**

**Engineering Phytochromes: Biliproteins that Switch & Glow.** Amanda J. Fischer1, William J. Coleman2, Mary M. Yang2, and J. Clark Lagarias1. 1Chemistry, University of California, Davis, California; 2KAIROS Scientific Inc., San Diego, California.

Phytochromes are biliprotein photoreceptors which exist in two phototransformational states - a red light absorbing Pr form and a far-red light absorbing Pfr form. Substitution of their native linear tetrapyrrole (bilin) prosthetic group with an unnatural bilin analog was shown to yield strongly fluorescent adducts of apophytochromes that can be reconstituted and activated in living cells [Murphy & Lagarias 1997 Curr. Biol. 7:870]. These fluorescent apophytochrome adducts, a.k.a. phytofluors, hold great promise for numerous cell biological applications; however, unlike the green fluorescent protein (GFP), exogenous bilin precursors are needed for phytofluor formation in cells. In the present studies, a directed evolution approach was undertaken with the goal of creating novel fluorescent and spectrally altered phytochrome mutants. Our strategy employed error-prone PCR to generate point mutations at random positions within the domain adjacent to the bilin binding domain of a cyanobacterial phytochrome, a domain that has been shown to be critical for its native spectroscopic and photochemical properties [Wu & Lagarias 2002 Biochemistry 39:13487]. We hypothesize that alterations in this domain will result in spectrally shifted and red/far-red fluorescent holophytochrome mutants ‘locked’ in either the Pr or Pfr form. Apophytochrome mutant libraries, expressed in different strains of E. coli engineered to synthesize different bilin precursors [Gambetta & Lagarias 2001 PNAS 98:10506], were screened using digital imaging spectroscopy [Bylina et al 2000 ASM News 66:211], fluorimaging and fluorescent activated flow cytometric methodologies. After identification of fluorescence, single mutants were constructed and characterized biochemically and spectroscopically.

4:00 PM **AA2.5**

**Affinitychromic Polyphosphenes: a Novel Bio-Photochromic Tool for High-Throughput Screening and Diagnostics.** Mario Leclerc1, William J. Coleman2, Mary M. Yang2, and J. Clark Lagarias1. 1Chemistry, University of California, Davis, California; 2KAIROS Scientific Inc., San Diego, California.

This presentation will describe the thermochromic, ionochromic, and affinitychromic properties of various neutral polyphophene derivatives. These optical features based on a conformational modification of the conjugated backbone induced from side-chain order/disorder transition have led to interesting colorimetric and/or fluorimetric transducers for diverse chemosensor and biosensor applications. In particular, the recent development of cationic, water-soluble, chromophoric polyphosphenes has allowed the easy, rapid, specific, and ultra-sensitive (as few as 250 molecules) detection of various nucleic acids and proteins in aqueous media. Clearly, this new platform that combines variable triggers and an optical transducer should create a new generation of useful tools in the areas of diagnostics, therapeutics, and drug discovery.

4:15 PM **AA2.6**

**Fluorescent PNA Probes Amplified by Conjugated Polymers for the Detection of Single Base Mutations Linked to Neurodegenerative Disorders.** Brent Stephen Gaylord1, Michelle Massie1, Shu Wang2, Bin Liu2 and Guillermo Bazan2. 1Materials, UC Santa Barbara, Santa Barbara, California; 2Chemistry and Materials, UC Santa Barbara, Santa Barbara, California.

Amplifying standard, singly modified, fluorescent DNA and PNA hybridization probes has recently been achieved using light-harvesting water soluble conjugated polymers. A single DNA detection scheme based on fluorescence resonance energy transfer (FRET) utilizing these materials was devised and comparisons in sensitivity to classic dual labeled probes, such as molecular beacons, have been demonstrated. In this work we evaluate how varying the length of human DNA targets generated by polymerase chain reaction (PCR) influences the energy transfer process and thus the measurable fluorescence signals produced by conjugated polymers and PNA probes. Minimizing any signal variability was achieved by the introduction of a standard nucleic. Since PNA probes are resistant to nucleases they can be used to protect the target DNA from enzymatic digestion in the region in need of amplification. The novel detection scheme was employed for identifying single base pair mutations in the genetic sequence of the protein Tau. Such defects result in protein dysfunction and cause frontotemporal dementia and parkinsonism linked to chromosome 17 (FTD-P). This initial work demonstrates the utility of conjugated polymers in fluorescence detection of specific genetic sequences and mutations at heightened levels of sensitivity using standard laboratory protocol such as PCR.

8:30 AM **AA3.1**

**Tailoring nanostructures for enhancing spectroscopies below the diffraction limit.** Naomie Halas, Electrical and Computer Engineering, Rice University, Houston, Texas.

The enhancement of nanoscale imaging of chemical species with detailed spectroscopic information about the molecules of interest is a major research goal of nanoscience. Exploiting the plasmon resonance of metallic nanostructured colloids and using localized surface plasmon spectroscopies has been a key focus. Modifying the geometry of nanoshells, spherical multilayer nanoparticles consisting of a dielectric core and a metallic shell, in order to systematically optimize the surface enhanced Raman scattering (SERS) properties of molecular spectroscopies has been pursued. In this geometry SERS can be optimized for a specific pump wavelength of interest, and the Stokes or anti-Stokes modes can be enhanced selectively. Nanoparticles designed to optimally enhance surface enhanced Raman scattering at their surfaces.

9:00 AM **AA3.2**

**Surface-enhanced Raman scattering (SERS) using metal nanoparticles and their applications.** Chaed E. Talley1, Thomas Huser2,3, Christopher W. Hollars1, and Stephen M. Lane2. 1Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; 2Physics and Advanced Technologies, Lawrence Livermore National Laboratory, Livermore, California.

Surface-enhanced Raman scattering (SERS) uses metal nanoparticles to provide a powerful tool for investigating the chemical microenvironmental conditions in biological systems. We are currently developing chemical sensors using functionalized metal nanoparticles combined with SERS. The sensors consist of gold or silver nanoparticles (50-100 nm in diameter) which are coated with a functional group that will react with the target analyte. Changes in the observed SERS spectrum upon analyte binding are then used to identify and quantify the analyte molecule. This approach improves the specificity of the technique and reduces the background resulting from non-specific adsorption to the metal surface. The region of enhancement is confined to within a few nanometers of the particle surface providing a highly localized signal. Additionally, the sensors are extremely robust allowing measurements to extend over long time periods without signal degradation. Here we present steps toward utilizing functionalized metal nanoparticles combined with SERS as chemical sensors. The results from pH measurements using the functionalized nanoparticle sensors will be presented as well as progress toward the development of sensors for other analytes of interest. Finally, progress toward incorporating these nanoparticle sensors into living cells for localized measurement will be presented. This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

9:30 AM **AA3.3**

**A Novel Synthesis of Metal Nanoparticles/Aggregates and Their Applications for Surface Enhanced Raman Scattering.** Jin Z. Zhang1, Adam M. Schwartzberg1, Abraham Wolcott1, Chaed E. Talley1, and Thomas Huser2,3. 1Chemistry, University of California, Santa Cruz, California; 2Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California.

We report a novel synthesis of metal nano-materials, including silver, platinum, copper, and gold nanoparticle aggregates (GNA), and the application of this GNA system for surface enhanced Raman scattering (SERS). The synthesis of the GNA’s has been reported previously as involving sodium sulfide as the reducing agent for gold. Aging of the sodium sulfide is required for the reaction to proceed, however, it was not clear how this ageing facilitated the reduction of the gold. Recently we have discovered that in the presence of oxygen, the sodium sulfide is oxidized to form thiosulfate (S2O32-), which is believed to be what carries out the reduction of the metal salt. Realizing this, thiosulfate has been directly utilized as a reducing agent for several metal salts in the first successful synthesis of silver, platinum, and copper nanostructures as well as GNA’s. The GNA’s have been further studied as substrates for SERS. SERS is a powerful technique for low concentration molecular detection with high specificity. This is due to a large field enhancement at the surface of metal surfaces, greatly increasing Raman scattering. These unique GNA’s show a large Raman enhancement factor on the order of 10^9, which is on the order of or greater than SERS enhancement factors reported recently elsewhere. SERS activity has been demonstrated for...
G protein coupled receptors (GPCRs) represent the largest group of membrane-bound receptors which regulate the cellular response via intermediary role of G proteins. GPCRs are the target in the human body for the majority of clinically used drugs. It has become increasingly evident that GPCRs can interact with a number of signalling and regulatory proteins beyond heterotrimeric G protein subunits thus leading to a great variety of biochemical responses in the cells. Because of the biological and pharmacological relevance of GPCRs, we have engaged ourselves in the effort of downsampling cellular signalling reactions into microarrays which can be used to screen efficiently the function either of different receptors, of different ligands or of different signaling molecules. For this purpose we have developed a procedure to immobilize either planar membranes, native vesicles or live cells comprising GPCRs in microarrays on solid supports. In addition, we were successful in fluorescent labeling by a variety of different approaches the a1-adrenergic, the NK1 and a number of taste and odorant receptors, as well as G protein subunits. Ensemble and single molecule spectroscopies and imaging methods are used in our laboratory to observe the various important signalling reactions in membranes in fragments//vesicles and live cells: Examples are the detection and quantification (thermodynamics and kinetics) of oligomerization of receptors, ligand binding to the receptor, subsequent conformational transitions of the receptor and finally investigate the structure and dynamics of molecular interaction between the receptor and effector molecules downstream the signal transduction cascade. This approach will contribute to understand the role of receptors in normal physiology and human disease. The selective (pulse) labeling with fluorescent probes of interacting partners allows us to monitor and quantify biomolecular reactions in real time in live biological cells or cell-derived vesicles. Labeling reactions which will be discussed are: fusion with fluorescent human alkalinephosphatase, site-selective insertion of non-natural amino acids into the sequence of a protein by suppressor t RNA technology, quantum dots, to mention a few. The developed miniaturized bioanalytical techniques are of interest for efficient (time consuming, cost reducing) probing of cellular signalling reactions both in fundamental research and for drug screening.

11:00 AM *AA3.5

Patterned Supported bilayers provide a new opportunity to present distinct and independently manipulated membrane environments within a single contiguous membrane sharing well-defined diffusive fronts. Such constructs can be derived by combining UV photolithography or micro-contact printing of a patterned first bilayer followed by targeted delivery of secondary bilayers in the lipid-free part of the pattern. These constructs are inherently non-equilibrium and display temporal evolution toward their equilibrium structure. Time-resolved Fluorescence characterization of the dynamics of probe molecules across these diffusive interfaces enable direct characterization of probe preference, partitioning, and equilibration characteristics in heterogeneous membranes, as well as provide a useful measure for developing their detailed phase diagrams.

11:15 AM *AA3.6
Highly-Sensitive Glucose Probes for Continuous Ocular Fluid Monitoring: Application to a Glucose Sensing Contact Lens. Chris D Geddes, Joseph R Lakowicz and Ramachandran Badugu; Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland. 2Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, Maryland.

We have developed a range of highly sensitive glucose binding fluorescent probes, based on the Quinolinium nucleus and the phenylboronic acid moiety. The new probes have sugar-bound pH < 7, affording for a fluorescence based glucose response when immobilised in off-the-shelf, disposable contact lenses, where tear glucose levels are known to track blood levels approximately 10 fold. In addition, the positive charge on the quaternary nitrogen heterocyclic nucleus affords for the charge stabilized borate ester form, providing for a modest glucose response. Our new approach enables glucose levels to be continuously monitored non-invasively within disposable plastic contact lenses, with a 50% response time of approximately 10 mins and a measured shelf-life in excess of 3 months. In addition the new probes have no visible absorption, providing for clear doped contact lenses.