SYMPOSIUM AA

Applications of Novel Luminescent Probes in Life Sciences

April 13 - 14, 2004

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*Invited paper
8:30 AM *AA1.1 Fluorescence Lifetime Imaging of Sensor Arrays. Michael Schäferling, 1 Meiping Wu, 2 Zhongqing Lin 1 and Otto S. Wolfbeis* 1Institute of Analytical Chemistry, Chemnitz and Biosensorn, 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 in biological samples have been developed, which can be used for the detection of skin tumor cells. Principle applications of fluorescence lifetime imaging (FLIM) are immobilized probe molecules, e.g. ruthenium complexes, will be demonstrated. In the second part we present different examples of the applicability of europium(III) terbincyanine [Eu(Tc)] as a molecular sensor in chemo- and biosensor probe. Based on the fact that the [Eu(Tc)]-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 H$_2$O$_2$. This sensitivity and the long fluorescence decay time (~60 µs) of this complex can be utilized for several applications, all working at neutral pH.

* Imaging of glucose in 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 glucose with coaggregated antibodies which enables the time-resolved fluorescent imaging of enzyme-linked immunosorbent assays (TRF-ELISA) through consumption of the strongly fluorescent [Eu(Tc)] complex and formation of the weakly fluorescent [Eu(Tc)].

*Time-resolved imaging of citrate and other main intermediates of the citrate cycle which is now possible for the first time. The assay offers their straightforward detection without the need for multi-enzyme systems.

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 multiplexed imaging 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 Ratiometry (PDR).


9:00 AM AA1.A2 Frequency upconversion and imaging using rare-earth doped colloidal nanoprobe. M.J.A. de Dood1, B.J. Berkhout1, C.M. van Kats*, A. van Blaaderen1 and Albert Polman* 1FOM-Institute AMOLF, Amsterdam, Netherlands; 2Debye Institute, Utrecht University, Utrecht, Netherlands.

We have developed colloidal silica particles doped with optically active terbium (emitting at 550 nm), europium (920 nm) and erbium (1535 nm) ions that show a high stability 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 hydrosol and condensation reaction of tetra-ethoxysilane 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 coordinating solvents using simple precursor reagents. The resulting host lattice with the narrow emission lines and the long luminescence lifetimes of the lanthanide ions consist of sharp lines and is very promising candidates for biolabeling applications because they combine the high chemical stability and photostability of an inorganic host lattice with the narrow emission lines and the long luminescence lifetimes of the lanthanide ions.

9:30 AM AA1.3 Synthesis and Site-Selective Spectroscopy of Lanthanide-Doped Core-Shell Nanocrystals for Bioimaging Applications. Olaf Lehmann, Karsten Koempe, Andreas Kornowski and Markus Haase* Dept. of Physical Chemistry, University of Hamburg, Hamburg, Germany.

Colloidal solutions and redispersible powders of lanthanide-doped nanocrystals have been prepared in high-boiling coordinating solvents. The synthesis yields gram amounts of highly crystalline nanocrystals 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 nature of the dopant. The nanocrystals display a variety of energy transfer phenomena like sensitized luminescence or even upconversion luminescence. A less desired process is energy transfer to the particle surface leading to partial quenching of the luminescence. This loss process is significantly reduced by growing a shell of pure, i.e., undoped host material around the nanoparticles. The resulting core-shell nanoparticles have been studied by site-selective spectroscopy, i.e., a luminescence line-narrowing technique. 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 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 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 bioimaging applications because they combine the high chemical stability and photostability of an inorganic host lattice with the narrow emission lines and the long luminescence lifetimes of the lanthanide ions.
α-NaYF₄ phase with an average particle size of 15 nm. This value is in accord with the transmission electron microscopy images (TEM) of the nanocrystals, which show that the absorbing particles as luminescent probes 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 phenomenon 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 Yb³⁺/Tm³⁺ and (blue) and Yb³⁺/Er³⁺/Tm³⁺ coded samples of NaYF₄, with low CW density excitation around 1 µm. This can be achieved with highly efficient and inexpensive laser diodes. The visible light output of the new nanomaterials is eight orders of magnitude larger than the previously investigated UC in chloride-doped phosphates nanocrystals. [1] The Yb³⁺ ion acts as an extremely efficient sensitizer for both the Tm³⁺ and Er³⁺ UC systems. Power dependent measurements show the nonlinear character of the processes. In conclusion, we report on an attractive new class of transparently dispersible luminescent molecules with a wide potential, which can be excited in the near-infrared by photon upconversion. [1] Angew. Chem. In. Ed. 2003, 42, 3170-3182.


The advantages of using stable water-soluble luminescent quantum dots (QDs) have been demonstrated in several recent biomolecular investigations. However, due to the process in which these materials are synthesized, the resulting surface properties have limited the ability to manipulate them in aqueous environments. We have designed and prepared a new series of multidentate oligo- and polyethylene glycol (PEG) terminated ligands for capping of QDs. These new ligands were prepared in simple esterification schemes, using thioctic acid and various oligo- and PEG precursors to produce multi-gran quantities of capping substrates. QDs capped with these PEG-terminated ligands are stable in a wide range of buffers ranging from strongly acidic to strongly basic. We will also present results of time-resolved photoluminescence (TRPL), and temperature dependent fluorescence experiments, where changes in the QD donor lifetime upon interacting with the acceptor dye were measured as a function of the degree of spectral overlap and the QD-conjugate architecture. Excellent agreement between the data sets was found. These findings were then exploited to develop a prototype FRET-based nanoscale sensing assembly that employed QDs as energy donors and used them to detect soluble analytes. This sensor design scheme can be applied to other receptor proteins or bio-recognition units and may facilitate development of a new generation of hybrid QD-based biosensors. 1. A.R. Clapp et al., J. Am. Chem. Soc., in press (2003); 2. I.L. Medintz et al., Nature Materials 2, 620-628 (2003).

10:45 AM • AA1.6 Carrier Dynamics in Biologically Compatible CdSe Nanocrystal Quantum Dots, Rosa Leon, Jay Nadeau, Jeremiah Kloepfer, Saulius Marcinevicius and Joerg Siegert, Jet Propulsion Laboratory, Pasadena, California; 4 Royal Institute of Technology, Kista, Sweden.

Time resolved photoluminescence (TRPL), and temperature dependence of luminescence were evaluated in CdSe nanocrystals. Temperature dependent PL spectra show increases in intensity and spectral shifts with increasing temperature. Carrier dynamics were investigated in “core shell” CdSe/ZnS nanocrystals coated with trioxylphosphine oxide (TOPO) and in CdSe nanocrystals coated with mercapto-acetic 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 CdTe/MAA nanocrystals of photoluminescence excitation and emission tuning. 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 the emission 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 toxic potential problem for in vivo use. A key requirement for the successful use of nanocrystals in bio-applications is aqueous solubility. Therefore, it needs to modify the surface of quantum dots with biocompatible molecules having functional groups (-COOH, -NH₂, -SH) that confer water solubility. We report here a chemical approach to produce water-soluble silicon nanocrystals using NaSi in organic solvent. We chose the micropipette tip 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 nm to 5 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 click reaction (Si-C) and simple preparation. The 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 5 nm. The magnified image clearly shows that nanocrystals of which d spacing (1.9 nm) is equivalent to a strong silicon crystal 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 (360 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 streptavidine, etc.
spectral width is the narrowest one for QDs ensembles at room temperature. On the other hand, we focus on developing new biological nanotechnology by using nano-sized semiconductor quantum dots and fluorescent organic molecules as tools for high-resolution measurements in the micrometer or sub-micron scale. The use of low-pressure SEM-CL has been applied to observe the morphology of cell organelles and its spatial resolution is high enough to detect the fluorescence from the electron beam irradiation (cathodoluminescence; CL). However, CL 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. 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 the FISH-CL. In the present work, we report the development of the high-resolution and low-vacuum SEM equipped with a CL detector, and the introduction of organic fluorescent makers suitable for biological applications. This experimental system makes it possible to observe specimens and to detect the weak intensity of CL from organic fluorophores. Even if the sample possesses 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 up to about 100 Pa, keeping high vacuum in the electron-gun and the lens section. We measured the CL images as well as SE images of over a dozen kinds of organic dyes in high-(10⁻⁴ Pa) and low-vacuum (40 Pa) conditions; for example, perylene derivatives, cyanines, anthracenes, fluorescein, rhodamine, and other luminous complexes 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 compounds, such as MoO₃, improved the sensitivity more than ten times as strong emission as other materials. 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, chromosomes and lipids, it should be modified so as to have biotin-, maleimide- or diazo-groups. These modifications are now in progress.

**SESSION AA2**

**Tuesday Afternoon, April 13, 2004**

**Room 3018 (Moscone West)**

**1:45 PM **

**AA2.1**

**Single-Molecule Fluorophores from Nonlinear Optical Chromophores, W. E. Moerner**, K. A. Willets, P. Callis, and R. J. Twieg; Chemical Physics, Stanford University, Stanford, California.

Nonlinear optical chromophores typically include a conjugated central core with donors and acceptors bound at opposite ends of the molecule, and such molecules have been widely used for second harmonic generation, phase modulation, optical switching, and other applications. We have shown that a particular class of nonlinear optical chromophores can satisfy the stringent requirements for optical imaging at the single-molecule level at room temperature, which include strong absorption, high fluorescence quantum yield, weak triplet bottlenecks, and high photostability. These molecules contain an amine donor and a dicyanodihydrofuran (DCDHF) acceptor linked by a conjugated unit (benzene, thiophene, styrene), and the energy levels of the donor and acceptor are well matched to optimize the efficiency of singlet and triplet energy transfer.

**2:15 PM **

**AA2.2**

**New Biosensor Strategies Based on Light Harvesting Water Soluble Conjugated Polymers and Related Macromolecules, Guillermo Bazan, Brent Gaylord, Shu Wang and Bin Liu; Materials and Chemistry, University of California-Santa Barbara, Santa Barbara, California.**

Homogeneous biosensor assays can be designed which take advantage of the optical amplification of conjugated polymers and the self-assembly characteristic of aqueous polyelectrolytes. For example, a ssDNA sequence sensor comprises an aequorin solution containing a cationic water soluble conjugated polymer such as poly(9,9-bis(2-N,N,2'-trimethylamino)-9H-fluorene) (PEN) with a peptide nucleic acid (PNA) labeled with a dye (PNA-C*). Transduction is controlled by hybridization of the neutral PNA-C* probe and the negative ssDNA target, resulting in favorable electrostatic interactions between the hybrid complex and the polymer. Distance requirements for Förster energy transfer are thus met only when ssDNA of complementary sequence to the PNA-C* probe is present. Signal amplification by the conjugated polymer provides fluorescence emission 350-500 times higher than that of the directly excited dye. Transduction by electrostatic interactions followed by energy transfer is a general strategy. Examples involving other biomolecular recognition events, involving structure pairs such as protein/RNA, DNA/DNA and RNA/RNA, will be provided. The mechanism of biosensing, with special attention to the varying contributions of hydrophobic and electrostatic forces will be discussed. Novel polymer and macromolecular structures can be designed which provide control over the frequency of emission and the level of optical amplification.

**2:45 PM **

**AA2.3**

**Nanophosphors as fluorescent multiplex labels for in-vitro diagnostics, Werner Hohesel, Karlheinz Hildenbrand, Haubold Barbara, California.**

In recent years, polymer-capped quantum dots and semiconductor nanowires (1-100 nm in size) have been developed as luminescent tools. These nanophosphors can be functionalized with biomolecules like antibodies or DNA-strands which participate as donors in fluorescence energy transfer (FRET) processes to adjacent dyes. Hence, the sensitized fluorescence of a fluorophore can be used as an indicator for a successful probe-DNA-hybridization event with high sensitivity and specificity. Multiplexed homogeneous DNA-hybridization assays are possible with the proposed system if multiple types of nanophosphors were
 employs 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 Lagarias, University of California, Davis, California; ¹KAIROS Scientific Inc., San Diego, California.

Phytochromes are biliprotein photoreceptors which exist in two phototransducer states - a red light absorbing Pr form and a far-red light absorbing Pfr form. Substitution of their native linear tetrapyrole (bilin) prosthetic group with an unnatural bilin analog was shown to yield strongly fluorescent adducts of apophytochromes that can be detected in living cells [Gambetta & 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 analogs are needed for phytochrome 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 39:13467]. 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 express different bilin precursors [Gambetta & Lagarias 2001 PNAS 98:10560], were screened using digital imaging spectroscopy [Bylina et al 2000 ASM News 66:211], fluorimaging and fluorescent activated flow cytometric methodologies. After identification of promising strains, single mutants were constructed and characterized biochemically and spectroscopically.

**4:00 PM AA2.5
**Affinitychromic Polythiophenes: a Novel Bio-Photonic Tool for High-Throughput Screening and Diagnostics. Mario Leclerc, Laval University, Quebec City, Quebec, Canada.

This presentation will describe the thermochromic, ionochromic, and affinitychromic properties of various neutral polythiophene 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 catonic, water-soluble, chromic polythiophenes 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 the 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 Massie², Shu Wang², Bin Liu² and Guillermo Banzà³,¹Materials, UC Santa Barbara, Santa Barbara, California; ²Chemistry and Materials, UC Santa Barbara, Santa Barbara, California; ³NRI, 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 simple DNA detection scheme based on fluorescence resonance energy transfer (FRET) utilizing these materials was devised and compared 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 fluorescent signals produced by conjugated polymers and PNA probes. Minimizing any signal variability was achieved by the introduction of a standard nucleas. Since PNA probes are resistant to nucleases they can be used to protect the target DNA from enzymatic digestion in the region in which the FRET is occurring. This modified 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 (FTDP-17). This targeted method will 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. Nomi 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 nanostructure [Taylor & Lagarias 1997 Curr. Biol. 7:870] and optimizing the surface enhanced Raman scattering (SERS) of vicinal molecules, 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 described in this presentation can be spectrally monitored chemical reactions at their surfaces.

**9:00 AM **AA3.2
**Nanoparticle Sensors Based on Surface-Enhanced Raman Spectroscopy. Chad E. Talley², Thomas Huser¹-², Christopher W. Hollars² and Stephen M. Lane³,¹Chemistry and Materials Science, Lawrence Livermore National Laboratory, Livermore, California; ²Physics and Advanced Technologies, Lawrence Livermore National Laboratory, Livermore, California.

Surface-enhanced Raman scattering (SERS) using metal nanoparticles provides a powerful tool for investigating the chemical microenvironments 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 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. Schwartzberg², Abraham Welcot³, Chad Talley² and Thomas Huser¹,¹Chemistry, University of California, Santa Cruz, California; ²Department of Chemistry 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 chloride. Aging of the sodium sulfide is required for the reaction to proceed, however, it was not clear how this aging facilitated the reduction of the gold. Recently we have discovered that 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 nanoparticles 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 nanoparticles, greatly increasing their sensitivity. This unique GNA’s show a large Raman enhancement factor on the order of 109, 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 α1-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 membrane 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 alkalitranse, site-selective insertion of non-natural aminoacids into the sequence of a protein by suppressor tRNA 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 Geddes1,2, Joseph R Lakowicz3 and Ramachandram Badugam2; 1Medical 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 phenyl boronic acid moiety. The new probes have sugar-bound pKₐ < 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 stabilised boronate 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 90 % 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.

11:45 AM AA3.7
New Time Resolved Assays for Highly Sensitive Screening in Pharma Research. Thilo Enderle1, Werner Nau2, Doris Roth3, Hugues Matile4 and Hans-Peter Jolesz; 1F. Hoffmann-LaRoche Ltd, Basel, Switzerland; 2School of Engineering and Science, International University of Bremen, Bremen, Switzerland; 3Rare Reagent Development, Roche Centralized Diagnostics, Penzberg, Switzerland.

Today’s drug discovery process requires efficient high-throughput screening (HTS) with a steady improvement in sample throughput and data quality. In HTS a library of several hundred thousands of potential drug compounds is tested in an automated fashion against a specific biological target. Identifying a chemical lead structure in such a library resembles finding a needle in a haystack. The development of robust, homogeneous ‘mix-and-measure’ assays with optical readout plays a central role to enable this process. The goal of the assays is to probe minute biological effects and translate them into strong signals of physico-chemical measurements. However, the drug compounds often show absorbance and fluorescence which interferes with the assay readout. In addition the background signal of the assay environment, reagents and micrometer test plate, needs to be suppressed efficiently. These challenges call for optimized labels and the use of sensitive detection schemes such as the measurement of fluorescence lifetime (FLT) measurements and time-resolved fluorescence (TRF). The presentation covers two new optical probes with lifetimes around one microsecond and the instrumentation that can be used to run lifetime-based assays in 384 and 1536 well microtiter plates. It will be demonstrated how the combination of new probe technology with state-of-the-art instrumentation allows to carry out “Fast-TRF” time resolved fluorescence in the nanosecond range. Assay applications for different target classes are included: An enzymatic assay to screen for inhibitors of a protease. An immunassay to detect second messenger molecules in cell signaling of G-protein coupled receptors.

12:00 AM *AA3.8
Novel SERS Probes for Highly Sensitive Detection of G Protein Coupled Receptors. Michael Young, Horst Vogel, Swiss Federal Institute of Technology, CH-1028 Lausanne, Switzerland.

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.

12:15 AM *AA3.9
Highly Sensitive Glucose Probes for Continuous Ocular Fluid Monitoring: Application to a Glucose Sensing Contact Lens. Chris D Geddes1,2, Joseph R Lakowicz3 and Ramachandram Badugam2; 1Medical 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 phenyl boronic acid moiety. The new probes have sugar-bound pKₐ < 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 stabilised boronate 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 90 % 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.