From: Eric Long <long@chem.iupui.edu>
Date: Fri Aug 25, 2006 8:53:57 AM America/New_York
Subject:

Regioselective hydroboration-oxidation and -amination of fluoro-substituted styrenes and the synthesis of the corresponding amino acids

FLUO 3

P. Veeraraghavan Ramachandran[1], Satish Madhi[1], and Martin J. O'Donnell, odonnell@chem.iupui.edu[2]. (1) Department of Chemistry, Purdue University, Herbert C. Brown Center for Borane Research, 560 Oval Drive, West Lafayette, IN 47907-2084, (2) Department of Chemistry, Indiana University Purdue University Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202

Hydroboration of fluorinated styrenes with common hydroborating agents results in polymerization. However, regioselective hydroboration can be achieved by utilizing iodoborane-dimethyl sulfide. A series of fluorinated beta-phenethyl alcohols and amines were synthesized via this methodology. The corresponding alfa-phenethyl alcohols and amines were prepared via transition metal-mediated catalytic hydroboration with catecholborane. The organoborane intermediates were also converted to the corresponding alfa-amino acids using Schiff base esters.

Molecular dynamics simulations of the orientation of netropsin-DNA binding

BIOL 136

Ya-Yin Fang, yfang@howard.edu[1], Eric C. Long, long@chem.iupui.edu[2], Vernon R. Morris, vmorris@howard.edu[3], and William M. Southerland, wsoutherland@howard.edu[1]. (1) Department of Biochemistry & Molecular Biology, Howard University College of Medicine, 520 W Street NW, Washington, DC 20059, (2) Department of Chemistry & Chemical Biology, Indiana University-Purdue University Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202, (3) Department of Chemistry, Howard University, 525 College Street NW, Washington, DC 20059

A great deal of experimental research has focused on the origins of the sequence selectivity of netropsin-DNA binding. In the work described herein, we employed molecular dynamics simulations to investigate the dynamic behavior and factors controlling the preferred binding orientations of netropsin to a 16 base pair oligonucleotide, $d(CTTAATTCGAATTAAG)_2$. This oligonucleotide contains two A/T-rich netropsin binding sites in an asymmetric flanking sequence context and was co-crystallized recently with netropsin. We observe that netropsin is oriented distinctly in the available AATT binding sites with the guanidinium end adjacent to the dyad axis of the oligonucleotide. The implications of this work on the molecular recognition of DNA by netropsin and other minor groove binding agents will be discussed.

High-throughput, high-resolution strategy for the rapid structural elucidation of site-selective DNA binding agents

BIOL 122

Mark A. Lewis, malewis@iupui.edu[1], Kristie D. Goodwin, kdgoodwin@iupui.edu[2], Millie M. Georgiadis[2], and Eric C. Long[1]. (1) Department of Chemistry & Chemical Biology, Purdue School of Science, Indiana University-Purdue University Indianapolis (IUPUI), 402 North Blackford Street, Indianapolis, IN 46202, (2) Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indiana University-Purdue University Indianapolis (IUPUI), 635 Barnhill Drive, Indianapolis, IN 46202
A general strategy for the rapid structural analysis of DNA binding ligands is described. By combining a high-throughput fluorescent intercalator displacement (HT-FID) assay and a high-resolution (HR) host-guest crystallographic technique, a system was produced that is capable of determining detailed structural information pertaining to DNA-ligand interactions within ~ 3 days. This “HT-HR” strategy can

quickly reveal the binding site preferences for even an unstudied DNA-interacting ligand and, subsequently, oligonucleotides can be designed and the host-guest crystallographic method used to generate diffraction quality crystals, at times, overnight. Using the HT-HR strategy, we have examined the DNA interactions of: (1) RT-29, a new benzimidazole-diamidine compound that displays anti-trypanosomal activity; (2) netropsin; and (3) combinatorial libraries of minor groove binding compounds. Our analyses suggest that the HT-HR strategy can expedite the screening of novel DNA binding and damaging agents, including libraries of potential DNA-interacting compounds.

Acetylenic fatty acid biosynthesis: Cloning and expression of the first genes from a Basidiomycete

BIOL 89

Robert E. Minto, minto@chem.iupui.edu[1], Brenda J. Blacklock, blacklock@chem.iupui.edu[1], Brian Scheffler[2], and Naomi N. Jayasuriya[1]. (1) Department of Chemistry and Chemical Biology, Indiana University – Purdue University Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202, (2) USDA, ARS, CGRU, P.O. Box 38, Stoneville, MS 38776

The diverged fatty acyl desaturase enzyme family generally catalyzes a wide variety of lipid-oxidizing reactions. The É¢12-acyl phosphatidylcholine (PC) desaturases are particularly striking, carrying out important dehydrogenation and oxygenation reactions leading to cellular lipids and seed oils, intermediates for signaling molecules, and natural products. Like plants in the families Asteraceae, Apiaceae, and Araliaceae, fungi of the phylum Basidiomycota are teeming with unsaturated lipids and acetylenic natural products. We have recently cloned two É¢12-acyl PC desaturase homologs from the Pacific golden chanterelle (*Cantharellus formosus*), including the first fungal acetylenase gene. Functional expression of CfACET in the presence of linoleate yielded crepenynate ((*Z*)-9-octadecen-12-ynoate), an acetylenic fatty acid that is the originating metabolite of many acetylenic natural products. In the absence of linoleate, the 12-trans isomer of linoleate was accumulated by CfACET-expressing yeast. CfDES, possesses cis-desaturase activity and is able to catalyze the formation of the (9*Z*,14*Z*)-desaturated acetylene, dehydrocrepenynic acid.

microRNA detection based on in situ activation of a bioluminescent enzyme through protein reassembly

ANYL 169

Kyle A Cissell[1], Suresh Shrestha[2], and Sapna K Deo[2]. (1) Department of Chemistry, Indiana University Purdue University Indianapolis, 402 North Blackford Street, LD 326, Indianapolis, IN 46202, (2) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N Blackford St, LD 326, Indianapollis, IN 46202

MicroRNAs are short, 18-24 nucleotide base length RNAs that perform gene regulation in eukaryotes. Currently, there are few methods available for miRNA detection, including hybridization and Northern blotting, which have limited sensitivity and cannot be used *in-vivo*. Here we have investigated a novel miRNA detection method using an enzyme reporter and protein reassembly. In order to detect miR21, a known miRNA, *Renilla* luciferase was cleaved into two inactive peptide fragments which were chemically conjugated to two oligonucleotide probes, both complimentary to 5' and 3' portions of miR21. Upon hybridization, the inactive peptide fragments reassemble, thus forming an active enzyme. When the substrate coelenterazine is added, the luciferase oxidizes the coelenterazine, releasing light. The photon release will only occur upon hybridization of the oligos to the miRNA. This is a mix-and-measure type of assay suitable for intracellular analysis. Furthermore, the use of a bioluminescent enzyme reporter allows for detection of miRNA in the femto-attomole range.

Mapping of loops in red fluorescent protein for application in single-step assays

ANYL 168

Yasmeen Rahimi, yrahimi@iupui.edu[1], Suresh Shrestha[2], Tanushree Banerjee[1], and Sapna K Deo[2]. (1) Department of Chemistry & Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford Street, Indianapolis, IN 46202, (2) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N Blackford St, LD 326, Indianapollis, IN 46202

Availability of genes of a variety of reporters provides versatility for bioanalysis since they can be manipulated genetically and can be employed in the detection of analytes of interest in conjunction with antibodies or binding proteins for the respective analytes. A red fluorescent protein (DsRed) has become a popular fusion tag. We have demonstrated for the first time application of DsRed monomer as a quantitative label in single-step assays. To achieve that, we examined the X-ray crystal structure of DsRed monomer targeting loop regions of the protein. The introduction of a unique cysteine at different solvent-exposed loops of the protein allowed us to attach a maleimide derivative of biotin to the protein through sulfhydryl of the unique cysteine. The sites selected were such that the biotin attached through unique cysteine is structurally close to the fluorophore and any binding events occurring at this site will be transduced to the DsRed fluorophore. A change in the fluorescence of DsRed upon addition of avidin, which binds to biotin was evaluated.

Stimuli-responsive hydrogels based on hinge motion binding proteins as recognition elements

POLY 251

Jason D. Ehrick, jdehri0@uky.edu, Stephanie Bachas-Daunert, Sean Stokes, Elizabeth A. Moschou, Sapna K. Deo, Leonidas G. Bachas, bachas@uky.edu, and **Sylvia Daunert**, daunert@uky.edu. Department of Chemistry, University of Kentucky, 800 Rose Street, Lexington, KY 40506

Hinge motion binding proteins, like the periplasmic binding proteins of bacteria, undergo substantial conformational changes upon binding to their ligands. Therefore these proteins behave as stimuli-responsive molecular machines, and can be utilized for the development of stimuli-responsive hydrogels. The protein calmodulin (CaM), was selected as a model hinge motion binding protein, and was integrated along with a low-affinity ligand (phenothiazine) in the bulk of a porous hydrogel network. CaM binds to phenothiazine creating chemical crosslinks and causing the hydrogel to adopt a constricted (shrunken) configuration. Upon the addition of a high-affinity ligand, like chlorpromazine, the protein prefers to release phenothiazine breaking the chemical crosslinks, and bind to the free chlorpromazine. As a result the hydrogel undergoes a phase transition adopting a higher volume (swollen) configuration. We have taken advantage of this mechanism of actuation to incorporate it in high throughput screening systems, as well as in responsive drug delivery devices and smart microlenses.

Biochemical characteristics of red fluorescent protein variants with incorporated non-natural amino acid analogs

ANYL 153

Ann M Goulding, agouldin@iupui.edu, Suresh Shrestha, and Sapna K Deo. Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N Blackford St, LD 326, Indianapollis, IN 46202
Red fluorescent protein (DsRed) has become a popular fusion tag in biochemical studies. It is better suited for cellular and tissue studies, compared to GFP, because of its excitation and emission in the red region of the spectrum, high quantum yield, and tissue translucency. Several genetic modifications have been performed on this protein to overcome drawbacks such as obligate oligomerization and slow fluorescence maturation. In addition to this traditional way of preparing protein variants, non-natural amino acids can also be incorporated into proteins, imparting unprecedented properties. The effect of non-natural amino acid incorporation on the structure and properties of DsRed will be presented here. Specifically, properties of DsRed variants with incorporated non-natural amino acids 3-fluoro-L-tyrosine and 3-amino-L-tyrosine will be evaluated. A strategy of forced biochemical incorporation of non-natural amino acids will be utilized to create the variants. The non-natural analogues of the amino acid tyrosine are incorporated because tyrosine is involved in chromophore formation.

Biophysical studies on polymer-sandwiched lipid bilayers

PHYS 481

Sumit Garg, sgarg@iupui.edu[1], Jay X. Tang, jay_tang@brown.edu[2], Christoph A Naumann, naumann@chem.iupui.edu[1], and Juergen Ruehe, ruehe@imtek.de[3]. (1) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford St., LD 326, Indianapolis, IN 46202, (2) Department of Physics, Brown University, 184 Hope Street, Box 1843, Brown University, Providence, RI 02912, (3) Department of Chemistry and Physics of Interfaces, Institute for Microsystem Technology, University of Freiburg, Georges-Koehler-Allee 103, Freiburg, 79110, Germany

We report on epifluorescence and single molecule fluorescence microscopy studies on planar lipid bilayers containing raft-mimicking lipid mixtures, where the bilayer is sandwiched between two polymeric environments of different persistence length, the flexible poly(methyloxazoline) (PMOx) and the semiflexible F-actin. Several types of polymer-bilayer linkages are compared. Interestingly, our studies show that the domain size in the presence of F-actin is affected significantly if the bilayer contains some negatively charged lipids, but does not show measurable changes in the absence of such lipids. Domain sizes are also altered if PMOx is tethered to the bilayer using high tethering densities. Bilayer diffusion properties are only moderately influenced due to F-actin coupling, whereas a significant drop in lateral mobility is observed at elevated PMOx-tethering densities. The experiments presented provide new insight of how electrostatic and mechanical membrane properties in complex membrane architectures may affect domain size and membrane fluidity.

Coupling of obstructed diffusion in polymer-tethered bilayers

PHYS 242

Christoph A Naumann, naumann@chem.iupui.edu[1], Sumit Garg, sgarg@iupui.edu[1], Miranda A Deverall[1], Juergen Ruehe, ruehe@imtek.de[2], Rainer Jordan, Rainer.Jordan@ch.tum.de[3], and Karin Luedtke[4]. (1) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford St., LD 326, Indianapolis, IN 46202, (2) Institute for Microsystemtechnology (IMTEK), Chemistry and Physics of Interfaces, University of Freiburg, Institute for Microsystemtechnology, georges Köhler Allee 103, Freiburg, D-79110, Germany, (3) Lehrstuhl für Makromolekulare Stoffe, Technische Universität München, Lichtenbergstr. 4, Garching, 85747, Germany, (4) Lehrstuhl für Makromolekulare Stoffe, TU München, Lichtenbergstr. 4, Garching, 85747, Germany

Comparing single molecule tracking experiments on fluorescently labeled phospholipids (TRITC-DHPE) in the inner and outer leaflets of a polymer-tethered phospholipid bilayer are presented which show that individual polymer-tethered lipids exclusively located in one leaflet can create diffusion obstacles for non-bilayer-spanning tracer molecules in the opposite (tethered lipid-free) leaflet. Our data indicate that the coupling of obstructed diffusion between both leaflets is due to the polymer-induced local bending of the bilayer around tethering points, where the bending depends on bilayer elasticity, polymer stiffness, and tethering density. Experimental data on polymer-tethered bilayers of varying cholesterol content are shown, which verify the relevance of the membrane elasticity and which highlight the important role of cholesterol in the coupling/decoupling of obstructed diffusion. We hypothesize that such a biophysical mechanism could be relevant in plasma membranes if the cellular membrane is bent similarly by the cytoskeleton around cytoskeleton-associated lipids like PIP2.

Biophysical mechanism of raft domain coupling in planar lipid bilayers

PHYS 14

Sumit Garg, sgarg@iupui.edu[1], Christoph A Naumann, naumann@chem.iupui.edu[1], Juergen Ruehe, ruehe@imtek.de[2], Rainer Jordan, Rainer.Jordan@ch.tum.de[3], and Karin Lüdtke[3]. (1) Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford St., LD 326, Indianapolis, IN 46202, (2) Department of Chemistry and Physics of Interfaces, Institute for Microsystem Technology, University of Freiburg, Georges-Koehler-Allee 103, Freiburg, 79110, Germany, (3) Lehrstuhl für

Makromolekulare Stoffe, Technische Universität München, Lichtenbergstr. 4, Garching, 85747, Germany

The intermonolayer coupling of domains in a planar polymer-tethered phospholipid bilayer consisting of SOPC, egg SM, and cholesterol (molar mixing ratio of 1:1:1) is studied using epifluorescence microscopy. To correlate the degree of intermonolayer coupling of domains to the bilayer-substrate distance, this liquid-ordered/liquid-disordered phase-separating lipid mixture is investigated on three planar bilayer architectures distinguished by their bilayer-substrate distance d, including a solid-supported bilayer (d~15Å) and two polymer supported bilayers with d~30Å and d~58Å, respectively. We show that complete domain registration between inner and outer monolayer domains can be achieved only in the latter case. Our experimental data indicate that domain registration is caused by lateral rearrangement processes of lipids in the outer leaflet, which are influenced by pre-existing domains in the inner one. The observed biophysical mechanism could be of relevance for the understanding of raft-mediated transbilayer signaling in cellular systems, as suggested recently by Kusumi's group.

Sonochemical synthesis of highly fluorescent core/shell quantum dots
COLL 295

Michael J. Murcia and Christoph A Naumann, naumann@chem.iupui.edu. Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford Street, Indianapolis, IN 46202-3274

High quality fluorescent CdSe/ZnS core/shell quantum dots were synthesized via a novel sonochemical synthesis procedure. This method allows precise control of the nanocrystal growth rate, while maintaining a monodisperse particle size, making the synthesis of gram quantities of a specific size (and thereby color) straightforward. These fluorescent nanocrystals have been synthesized through the use of inexpensive, air safe reagents, while maintaining relatively low synthesis temperatures. The surface coatings of these quantum dots were modified for water solubility, colloidal stability and 1:1 conjugation to biomolecules for single molecule imaging. Two separate strategies were employed for the controlled 1:1 conjugation of these nanocrystals to biomolecules. Both have demonstrated that individually labeled quantum dot bioconjugates allow increased observation time over conventional fluorescent dyes at the single molecule level due to the enhanced photostability of the semiconductor nanocrystals.

Quantum dots as single molecule imaging labels on model membranes
PHYS 499

Michael J. Murcia and Christoph A Naumann, naumann@chem.iupui.edu. Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, 402 N. Blackford Street, Indianapolis, IN 46202-3274

Membrane biomolecules have been singly labeled with highly fluorescent CdSe/ZnS core/shell quantum dots for use in single molecule imaging on planar, solid-supported model membranes. Tagging methods have been developed enabling the trajectories of individual quantum dot-labeled phospholipids as well as other various membrane bound biomolecules to be traced. Two approaches have been utilized in the 1:1 labeling of quantum dots to membrane components. Both have shown that the enhanced photostability of these fluorescent nanocrystals over conventional fluorescent dyes enables the increased observation time of single molecules. This has allowed a more detailed analysis of fluorescently-labeled single molecule trajectories within fluid, two-dimensional membranes of varying compositions.

Apparatus and results for pressurized planar electrochromatography (PPEC)
ANYL 305

A. L. Novotny, allnovot@iupui.edu[1], D. Nurok, nurok@chem.iupui.edu[1], R. E. Santini, rsantini@purdue.edu[2], R. W. Replogle[2], and G. L.

Hawkins[2]. (1) Department of Chemistry, IUPUI, 402 N Blackford St, Bldg LD Rm 325, Indianapolis, IN 46202, (2) Department of Chemistry, The Amy Facility for Chemical Instrumentation, Purdue University, 560 Oval Drive, West Lafayette, IN 47907

Pressurized Planar Electrochromatography (PPEC) is a new separation technique that is both substantially faster and more efficient than classical Thin Layer Chromatography (TLC). The mobile phase is driven by electroosmotic flow, while the sorbent layer is pressurized in such a manner that the separation temperature is controlled. PPEC retains the attractive features of TLC, and this includes the ability to simultaneously separate multiple samples. For this reason the technique is very suitable for high-throughput analyses.
Our presentation will discuss the apparatus for PPEC, and the dependence of separation quality on variables that include the applied electric field, the applied pressure, the temperature of separation, the buffer concentration and the nature of the sorbent layer. Separations of different classes of compounds will be discussed. The separation of nine compounds in two minutes illustrates the speed of analysis. Under optimum conditions an extrapolated efficiency of over 100,000 plates per meter is obtained.

PLTL Leaders: Transforming students into scholars

CHED 403

David J. Malik, malik@chem.iupui.edu[1], **Shannon Sykes**, ssykes@iupui.edu[2], Shilpi Bhargav, shilpi_bhargav@hotmail.com[1], and Bonnie G. Stevenson, bosteven@iupui.edu[1]. (1) Department of Chemistry, Indiana University Purdue University Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202, (2) University College, Indiana University-Purdue University Indianapolis, 815 W. Michigan Street, Indianapolis, IN 46202-3274

Peer-Led Team Learning has several winners: students, leaders, and the institution. The focus of this presentation is to provide quantitative data on the impact on student peer leaders in the context of first semester general chemistry. Student leader gains have been identified as some of the most compelling data resulting from PLTL. Student leaders have long self-reported significant gains in a variety of professional development attributes. The Student Assessment of Learning Gains (SALG) is a helpful tool to identify the elements in the course that best support student learning and those that need improvement. While many interventions to enhance student learning have been introduced over the years, their role will be presented and assessed from the perspective of student leaders. Leader SALG evaluations will be evaluated and differences between the typical chemistry populations and leader groups assessed and contrasted. Other gains that will be measured include leadership development, sociability and communication skills, and improved thinking and critical skills. Several instruments will be described and their efficacy assessed, and primary gains identified.

ACS exam measures of the impact of PLTL

CHED 399

Jack A. Kampmeier, kamp@chem.rochester.edu, Department of Chemistry, University of Rochester, Box 270216, University of Rochester, Rochester, NY 14627, Ana Fraiman, A-Fraiman@neiu.edu, Chemistry, Northeastern Illinois University, 5500 North St. Loius, Chicago, IL 60625-4699, and David J. Malik, malik@chem.iupui.edu, Department of Chemistry, Indiana Univ Purdue Univ Indianapolis, 402 N Blackford Street, Indianapolis, IN 46202.

Some assume that course content is invariably compromised by team leaning methods such as PLTL. Performance on standardized ACS exams contradicts this assumption. In addition, we have tested students before and after their work as peer leaders. Non-leaders provided a suitable reference group. One year after completing a course in organic chemsitry, non-leaders showed a substantial decay in their percentile rank on the ACS exam compared to the rank attained at the end of the course. In contrast, before and after peer leader percentile scores were roughly comparable.