

Glenn T. Seaborg
Medal



Since 1987, honoring scientists for their significant
contributions to chemistry and biochemistry

2015
Glenn T. Seaborg
Symposium

Honoring
Professor Stefan W. Hell

2014 Nobel Prize in Chemistry

Max Planck Institute for Biophysical Chemistry, Göttingen
German Cancer Research Center (DKFZ), Heidelberg

Poster Session

**The Seaborg Symposium and Medal
Award Dinner events are made
possible by the continued support of
our generous donors.**

Thank you.

Corporate sponsorship for this year's
events was provided by
Leica Microsystems:



Many thanks to Amgen for their continued
support of the Department of Chemistry
and Biochemistry.

Single-molecule assay development for studying human RNA polymerase II promoter-proximal pausing

Yazan Alhadid, Benjamin Allen, Sangyoon Chung, Dylan Taatjes and Shimon Weiss

Promoter-proximal RNA Polymerase II (Pol-II) pausing has been shown to play a significant role in transcription regulation of elongating Pol-II complexes in a large number of metazoan and mammalian genes (*1*). The traditional understanding of transcription regulation in mammals involved controlling Pol-II recruitment to promoters and controlling initial steps at the promoter, including pre-initiation complex formation and promoter escape. Most works investigating promoter-proximal PolII pausing have employed chromatin immunoprecipitation followed by sequencing to determine Pol-II localization or *in vitro* transcriptional assays using nuclear extracts analyzed with radioactive gel electrophoresis. In order to gain greater mechanistic insight into the regulation of promoter-proximal Pol-II pausing, we use single molecule ALEX spectroscopy to monitor RNA transcripts production as function of composition and order of addition of transcription factors to an *in vitro* reconstituted human Pol-II system. The RNA transcripts are detected by complementary doubly dye-labeled single-stranded DNA (ssDNA) probes. The human gene HSPA1B for heat shock protein 70 (Hsp70) is used as a model system due to its extensive characterization in *drosophila*. Our approach provides a rapid, sensitive and robust avenue for screening protein factors regulating promoter-proximal Pol-II pausing.

Towards unified access to ansa-bridged prodiginines: Exploring whether tactics employed in a recent roseophilin synthesis are adaptable to spirocyclic pyrrolophanes marineosins A & B

Tyler K. Allred, Hui Ding, James H. Frederich and Patrick G. Harran

Our laboratory has interests in complex prodiginines. Certain structures in this class of compounds are thought to potentiate apoptotic signaling mediated by Bcl-2 proteins. As a prerequisite to exploring this issue in detail, we sought a unified synthetic entry to the ansa-bridged series. Our plans were first validated in a total synthesis of roseophilin. Attention has now shifted to study how and if late stage ansa-bridge constructions related to that used in the roseophilin work can be exploited in the synthesis of marineosins A and B. The latter are recently isolated, bioactive spirocyclic pyrrolophanes thought to arise in nature via biosynthetic schemes related to those that generate roseophilin and its cogeners. Bridge constructions in that case are thought to occur via free radical additions to intact core motifs. Our roseophilin synthesis exploited a similar ordering of bonding events, yet relied upon phosphoryl transfer terminated aldolization in the macrocycle forming step. Attempts to adapt those procedures to marineosin targets will be discussed, as will a variety of alternate constructions en route to intermediates wherein installation of the defining spirocyclic tetrahydropyran motif can be examined.

Mixed quantum and classical simulation of the hydrated electron - temperature dependence in Resonance Raman Spectra and excited states relaxation

Chen-Chen Zhou, Erik P. Farr, William J. Glover and Benjamin J. Schwartz

The structure of hydrated electron has been studied by mixed quantum/classical (MQC) simulations in the past decades, but there's still massive controversy in its structure. Previous pseudopotentials (Schnitker et al. 1987; Turi et al. 2001) are generally highly repulsive and give a cavity-like structure where the electron interrupts the hydrogen bonding of the water molecules, 'digs out' an empty space and resides in it. Our model (Larsen et al. 2010) however suggests a different picture where wave function of the hydrated electron overlaps with several solvent molecules. The cavity and non-cavity models, being the results of different pseudopotentials, behave differently in response to a change of temperature. This work gives some definitive experimental predictions that can help distinguish between the two. Resonant Raman Spectra has been calculated with a semi-classical method, where non-cavity model exhibits a strong temperature dependence while the cavity model does not have significant variation with change of temperature. Non-adiabatic dynamics has also been done to study the excited state relaxation of hydrated electron. The non-cavity model shows a fast relaxation from the excited state and slow ground state cooling, while the cavity model gives a slow relaxation and fast cooling. These two mechanisms result in different features in transient absorption spectra that can be experimentally observed.

1,2-Cyclohexadiene as a useful synthetic building block

Joyann S. Barber, Michael M. Yamano and Neil K. Garg

Heterocycles are prevalent motifs seen in numerous biologically active natural products and pharmaceuticals. This presentation will describe a mild approach for the construction of heterocycles based on the trapping of in situ-generated 1,2-cyclohexadiene. In particular, trapping with nitrones provides an unconventional route to produce stereochemically-rich isoxazolidine products.

Effect of the secondary structure of long RNAs on their packaging by viral capsid protein

Christian Beren, Lisa Dreesens, Katherine Liu, Richard Sportsman, Charles M. Knobler and William M. Gelbart

A large class of viruses use (1000s-of-nucleotides) long single-stranded (ss)RNA molecules as their genomic material, and protect them in self-assembled nucleocapsids. These ssRNAs form large amounts of secondary structure (typically half of the nucleotides are base-paired in duplexes), making them more compact than they would be otherwise. We are interested in the consequences of these secondary structures on the packaging of long ssRNAs by cowpea chlorotic mottle virus (CCMV) capsid protein (CP), which has been shown to package, *in vitro*, a wide range of lengths and sequences of RNA. In particular, we compare viral-length polyU RNA molecules – which are largely free of secondary structure – with RNAs of normal nucleotide (nt) composition, i.e., comparable numbers of A, U, G, and C. We find that polyU RNAs ranging in size from 1000-10000 nts can be completely packaged by CCMV CP. But in every case they are packaged into virus-like particles (VLPs) with a diameter of about 21 nm, despite the fact that the diameter of the VLPs formed from long normal-composition RNA molecules is 28nm. We have also compared the relative packaging efficiencies of polyU and normal-composition RNA the same length, by mixing equal masses of them with an amount of capsid protein sufficient to package either all of one *or* all of the other, but not all of both. In particular, we compete RNA molecules that are 3000 nts long, the length of the viral genes packaged by this protein. We find that comparable numbers of each molecule are packaged, but the assembly products involve a mix of 21nm and 28nm particles containing polyU RNA and the normal-composition RNA.

Phosphine-mediated iterative arene homologation using allenes

Kui Zhang, Lingchao Cai, Xing Jiang, Miguel A. Garcia-Garibay and Ohyun Kwon

A phosphine-mediated multi-component reaction between *o*-phthalaldehydes, nucleophiles and monosubstituted allenes furnished the functionalized non- C_2 -symmetric naphthalenes in synthetically useful yields. Also, when the *o*-phthalaldehydes were reacted with 1,3-disubstituted allenes in the presence of diethylphenylphosphine, naphthalene derivatives were obtained in yields up to quantitative. The mechanism of the latter transformation is straightforward, involving aldol addition followed by Wittig olefination and dehydration. The mechanism of the former reaction has been established as a tandem γ -umpolung/aldol/Wittig/dehydration process through careful analysis of data based on the preparation of putative reaction intermediates and mass spectrometry. The current method can also be applied iteratively to prepare anthracenes and tetracenes when carboxylic acids are employed as pronucleophiles.

Knight shift investigations of charge carrier concentrations in topological crystalline insulators via β NMR studies

Orin Yue, Dimitrios Koumoulis, Thomas C. Chasapis, Gerald D. Morris and Louis-Serge Bouchard

Topological Crystalline Insulators (TCI) are a recently discovered class of topological insulator where the time reversal symmetry requirement is replaced with crystalline symmetry. Recent work done by our group has sought to characterize the topological properties by use of nuclear magnetic resonance (NMR). To probe such properties, we utilize ^8Li βNMR , an exotic form of NMR that detects the nuclear spin precession signal from a radioactive lithium-eight nucleus. In addition to the tenfold increase in sensitivity compared to a conventional NMR experiment, ^8Li βNMR allows for a controlled deposit of radioactive nuclei into our bulk sample. In so doing, we are able to perform highly accurate Knight shift measurements on bulk crystals of SnTe at multiple depths and compare the observations made with carrier concentration and temperature to name a couple. Here, we present an investigation of the charge carrier concentrations and the Knight shift of the topological crystalline insulator SnTe via βNMR conducted TRIUMF laboratories (Vancouver, Canada).

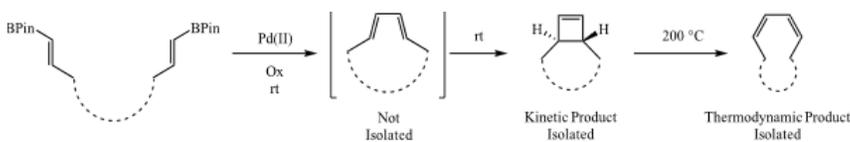
Self-amplifying RNA genes, and their delivery in in vitro reconstituted virus-like particles

Adam Biddlecome, Devin Brandt, Charles M. Knobler and William M. Gelbart

Most viruses have RNA genomes, and most of these are positive-sense RNA molecules that are directly translated to yield enzymes – RNA-dependent RNA polymerases (RdRps) – that make up to a million copies of the genome within one hour. Working at the RNA level, we fuse this replicase gene (from an insect virus) to a gene of interest, whose protein product we would like expressed to high levels in target cells, and package the resulting molecule in a virus-like particle (VLP) reconstituted *in vitro* with a plant virus capsid protein. Using Luciferase as a reporter gene of interest, we present preliminary results demonstrating a high level of protein expression in mammalian cells transfected either with the naked or packaged form of this RNA.

Using ring strain to control 4π electrocyclization reactions: torquoselectivity in ring closing of medium ring dienes and ring opening of bicyclic cyclobutenes

Byron A. Boon, Aaron G. Green, Peng Liu, Kendall. N. Houk and Craig A. Merlic



Syntheses of strained cyclic dienes were accomplished via palladium(II)-catalyzed oxidative cyclizations of terminal bis(vinylboronate esters). The reactions generated strained (E,E)-1,3-dienes which, due to instability at room temperature, underwent 4π electrocyclizations to bicyclic cyclobutenes. The strain driving the second cyclizations was set in place by thermodynamically favored reductive eliminations from palladium(II). Unique selectivity for cyclobutenes, as opposed to typically more stable 1,3-dienes, arises from strain in the intermediate medium-sized (E,E)-1,3-diene rings. Thermal ring opening of the cyclobutenes gives (Z,Z)-1,3-diene products, as opposed to the (E,E) products. DFT calculations verified the thermodynamic versus kinetic control of the reactions and kinetic studies were in excellent agreement with the calculated energy changes. Further exploration of the tandem coupling/electrocyclization pathway was demonstrated by a palladium(II) oxidative homocoupling/ 8π electrocyclization cascade.

Local dynamic range compression for high order Super-resolution Optical Fluctuation Imaging (SOFI)

Xiyu Yi, Xi Lin and Shimon Weiss

The addition of Superresolution Optical Fluctuation Imaging (SOFI)^{[1][2]} to the arsenal of superresolution methods offers a simple and affordable alternative to the more sophisticated techniques (such as STED, localization microscopies, and structured illumination). Calculations of higher order cumulants lead to a larger improvement in SOFI resolution, but also introduce a large nonlinear expansion in brightness dynamic range. Balanced SOFI (bSOFI)^[3] provides a way to correct for the expanded dynamic range, but at the same time it introduces artifacts in the corrected image. In this work, we introduce a novel local dynamic range compression method, where the brightness dynamic range of a high (up to 6th-) order SOFI image is locally self-calibrated by the brightness of the corresponding second order SOFI image. The method is implemented for both auto- and cross- correlation SOFI with 36 fold extra pixels, and is combined with deconvolution. Local dynamic range compression SOFI (ldrc-SOFI) is demonstrated for simulated data and for QD625 labeled alpha-tubulin in fixed 3T3 cells with a 4-fold resolution enhancement. We show that ldrc-SOFI suffers from fewer artifacts compared to bSOFI, while exhibiting faithful dynamic range compression. A large library of simulated data of filaments networks with variable filaments density, labeling density, labeling uncertainty, noise level, background level and nonspecific binding probability was generated and examined by both bSOFI and ldrc-SOFI. When the signal to noise ratio (SNR) and other sample conditions are favorable, both algorithms perform well. In fact, under very low filaments density bSOFI performs better. However, under challenging imaging conditions (high feature density, non-specific background, high noise level), ldrc-SOFI yields better performance with less artifacts.

Utilizing nickel catalysis to forge carbon–carbon bonds from amides

Nicholas A. Weires, Emma L. Baker, Bryan J. Simmons, Jacob E. Dander, Sarah M. Anthony and Neil K. Garg

The Suzuki–Miyaura and Negishi couplings have become some of the most important and prevalent methods for the construction of carbon–carbon bonds. Although palladium catalysis has historically dominated these fields, the use of nickel catalysis has become increasingly widespread due to its unique ability to cleave carbon–heteroatom bonds that are unreactive toward other transition metals. This presentation will focus on the first nickel-catalyzed Suzuki–Miyaura coupling of amides, which proceeds by an uncommon cleavage of the amide C–N bond following *N*-Boc activation. The methodology is mild, functional group tolerant, and can be strategically employed in sequential transition metal-catalyzed cross-coupling sequences to unite heterocyclic fragments. Current directions, including the development of a Negishi coupling to forge C–C bonds from amides, will also be discussed. These studies demonstrate that amides, despite classically being considered inert substrates, can in fact be harnessed as synthons for use in C–C bond forming reactions through cleavage of the C–N bond using non-precious metal catalysis.

Catalytic asymmetric total synthesis of (–)-actinophyllic acid

Lingchao Cai, Kui Zhang and Ohyun Kwon

(–)-Actinophyllic acid was reported as a potent inhibitor of the zinc-dependent carboxypeptidase U (CPU), with IC₅₀ of 0.84 μM. CPU is an endogenous inhibitor of fibrinolysis, the breakage of fibrin clots. Consequently, inhibitors of CPU can facilitate fibrinolysis and inhibit the blood clot formation that is a cause of various cardiovascular disorders. However, there has been no subsequent biological studies reported, presumably because of the scarcity of the natural product due to its low isolation yield (0.0072%). Therefore, an efficient de novo synthesis of this potent CPU inhibitor should provide a great tool for the exploration of its biomedical potential.

Structurally, (–)-actinophyllic acid contains cage-like scaffold of 1,2,3,5,6,7,8,10a-octahydro-1,7-methanopyrrolo[1,2-a]azocine with five contiguous stereogenic centers, one of which is quaternary carbon, bridged with tetrahydrofuran ring system, which is unique from other indole alkaloids. Due to these demanding structural features, only two racemic and one enantioselective synthesis of actinophyllic acid have been successfully accomplished by Overman's and Martin's group despite efforts by other groups.

Recently, our group finished a catalytic asymmetric total synthesis of (–)-actinophyllic acid by means of chiral phosphine-catalyzed [3 + 2] annulation between an imine and allenolate to forge the pyrroline ring. The synthesis features CuI-catalyzed coupling between ketoester and indole 2-iodide to form hexahydroazocine ring system, intramolecular alkylative lactonization, SmI₂-mediated intramolecular pinacol coupling between ketone and lactone to construct the complex skeleton of (–)-actinophyllic acid, and an unprecedented regioselective dehydroxylation.

Bacterial microcompartments: selective molecular transport through shell pores

Sunny Chun, Chiranjit Chowdhury, Allan Pang, Michael R. Sawaya, Sharmistha Sinha, Thomas A. Bobik and Todd O. Yeates

Bacterial Microcompartments (BMCs) are proteinaceous organelles that sequester key metabolic reactions to increase enzymatic efficiency or to prevent the loss of volatile or toxic intermediates. The mechanisms of small molecule transport and retention of toxic intermediates by BMCs remain poorly understood. Our biochemical and physiological studies of structure-guided mutants show that the hexameric PduA shell protein of the 1,2-propanediol utilization microcompartment (Pdu MCP) forms a selective pore favoring Pdu MCP influx of 1,2-propanediol, the substrate, and limiting the efflux of propionaldehyde, the toxic intermediate of 1,2-propanediol utilization. Crystal structures of the various PduA pore mutants, solved to atomic resolution (2-3.3Å), provide corroborating evidence of our biochemical and in vivo results. Importantly, the pore residues (Ser40) confer selectivity of the small molecule transport. Overall, these studies shed insight on our understanding of the BMC shell and the mechanisms of function for selectively permeable protein channels.

Ni-based electrochemical catalysts for water splitting

Yiliu Wang, Gongming Wang and Xiangfeng Duan

Low-cost catalysts with small overpotential are highly demand for electrochemical and photoelectrochemical water splitting systems. Here, we demonstrated ion implantation method is an effective and general strategy to modify the catalyst surface of Ni-based electrochemical catalysts and further improve its water oxidation performance. Compared to other chemical modification method, ion implantation process does not involve any elements other than the element of interest and could offer a much cleaner and more reliable method for introducing selected impurities into a solid state material. It is also a general approach for introducing different or multiple impurity elements for systematic studies. Under assistance of the co-implanted cobalt and iron, the catalytic performance of Ni(OH)₂ can be significantly improved, and overpotentials have been decrease by about 50 mV and about 100 mV for HER and OER respectively in basic solution. Besides, the prepared ion implanted nickel based catalysts also exhibited excellent electrochemical stability up to two weeks. The capability of making high performance electrochemical catalyst by ion implantation could open up new opportunities in the water splitting area and provide an alternative approach to develop new catalyst with various metal/non-metal functionalities.

Coaxing a viral RNA out of its shell: how does a viral RNA genome initiate contact with its host?

Richard W. Sportsman, Christian Beren, Benjamin Kartub, Rees Garmann, William M. Gelbart and Charles M. Knobler

A conceptual challenge in understanding the life cycles of all viruses is how a rigid protein shell – the “capsid” – can both protect the viral genome from nucleases and other insults and, at the appropriate downstream stage, make its genetic information available to the host cell machinery. For double-stranded DNA viruses, like bacteriophages or Herpes Simplex Virus, genomes are packaged into preformed empty capsids, and released by pressure-driven ejection, with the capsid remaining intact: work is done to package the genome, but its delivery is spontaneous. In contrast, for single-stranded RNA viruses, like polio or SARS, packaging of their genomes is spontaneous, with capsid protein organizing around the RNA in a self-assembly process. This immediately raises the question of whether work is needed to release the RNA from its protective capsid. In the case of positive-strand RNA viruses, whose genomes are directly translated following their entry into the host cell cytoplasm, ribosomes have been implicated in the delivery/release of the viral RNA. We report here on the synthesis of constructs designed to measure the force needed to pull RNA out of its capsid, using virus-like particles reconstituted *in vitro* from RNA and purified capsid protein from a particularly well-studied virus, cowpea chlorotic mottle virus (CCMV). We also investigate the extent to which the ends of a packaged RNA are accessible for interaction with probes impermeable to the interior of the capsids. These results are used to interpret relative translation efficiencies of naked and packaged forms of RNA genes in cell-free extracts, in an effort to determine how ribosomes are involved in this RNA uncoating process.

Unified synthetic strategy toward the tubingensin alkaloids

Michael A. Corsello, Junyong Kim and Neil K. Garg

Indole diterpenoids comprise a vast and architecturally intriguing class of alkaloids, which has long provided a fruitful area for scientific discovery. Tubingensin A is a complex alkaloid of this variety isolated from the fungus *Aspergillus tubingensis*. Structurally, it contains four contiguous stereocenters, two of which are vicinal quaternary centers. Our laboratory has accomplished a 9-step (longest linear sequence) synthesis of tubingensin A using a reactive heterocyclic aryne intermediate to construct the challenging vicinal quaternary centers. Additionally, we are pursuing the application of this strategy to build tubingensin B, which contains an additional stereocenter and a unique bicyclo[3.2.2]nonane core. Progress toward its total synthesis will be presented.

In situ monitoring electrochemical surface of the electrocatalytic materials using a nanoelectronic based on-chip electrical transport spectroscopy (ETS) approach

Mengning Ding, Qiyuan He, Gongming Wang, Hung-Chieh Cheng, Yu Huang and Xiangfeng Duan

In situ monitoring the electrochemical interfaces during electrocatalysis is crucial for the fundamental understanding and continued optimization of electrocatalysts, which will benefit the future energy technologies. To date, conventional spectroscopic techniques are generally employed for this purpose, yet it is considerably challenging. We have developed an alternative on-chip electrical transport spectroscopy (ETS) approach for directly probing the electrochemical surfaces of metallic nanocatalysts under *in operando* conditions. Exploiting the well-known electron scattering effect on the metallic surfaces, we show that electrical transport properties of ultrafine metal nanowires are highly sensitive to electrochemical surface states, and can be used to create a nanoelectronic signaling pathway that reveals electrochemical interface information during in-device voltammetry. Our results not only show a high degree of consistency with generally accepted conclusions in platinum electrochemistry, but also provide important insights on various technically important electrocatalytic reactions. This study defines a novel nanoelectronic on-chip characterization strategy for *in situ* electrochemical surface studies with high surface sensitivity and surface specificity.

Chemical fabrication of transparent gold-patterned polydimethylsiloxane

Liane S. Slaughter, Huan H. Cao, Qing Yang, Thomas D. Young, Kevin M. Cheung, Andrew C. Serino, Dominique Zosso, Jing An, James Stevick, Nicholas Takaki, Morgan Weiss, Andrea L. Bertozzi, Anne M. Andrews and Paul S. Weiss

We present a straightforward, contact-based, strategy to functionalize and to pattern polydimethylsiloxane (PDMS) with atomically thin gold over square millimeter areas. The pattern features span hundreds of nanometers to hundreds of microns. Functionalization depends on a recently discovered contact-based chemistry, chemical lift-off lithography (CLL), where PDMS first conformally contacts a gold surface with self-assembled hydroxyl-terminated alkanethiols, then removes molecules and gold from the surface upon separation. We have devised a suite of fabrication and characterization tools to pattern flat PDMS via CLL and to visualize these patterned features, which do not measurably influence the optical transparency or flexibility of the PDMS. We visualize the patterns directly through peak-force atomic force microscopy and scanning electron microscopy. We then demonstrate the chemical functionality of the lifted-off gold on PDMS through a simple DNA hybridization assay. Thiolated single-stranded DNA probes bind to the lifted-off regions on the PDMS, and the pattern becomes visible in fluorescence microscopy when the probes bind to their dye-labeled target strands. Compared with common strategies for patterning PDMS, the CLL mechanism is chemically selective, straightforward, replicable at ambient conditions, and enables nanometer to millimeter feature sizes. Further, this study forms the basis on which to interrogate the optical, electrical, and chemical properties for the morphologies of the ultrathin gold produced here.

Live cell thermometry using optically detected magnetic resonance

Hemal Semwal, Michael Lake and Louis-Serge Bouchard

Fluorescent nanodiamonds are carbon crystalline structures that contain nitrogen vacancy (NV) defects allowing temperature dependent fluorescence through coherent manipulation of quantum mechanical spin states. Thermal lattice strains create shifts in the energy levels of the unbound electron pair. A green laser (532 nm) excitation pulse initiates nitrogen fluorescence, accurately correlating specific temperatures ranging from 280-330 Kelvin to emission wavelengths between 650-800 nm. Our method uses microwave pulses to excite nitrogen vacancy centers between singlet and triplet states and detect emission spectra through a technique known as optically detected magnetic resonance (ODMR). Hence, phonon vibrations in the nanodiamond crystals are associated with local temperature and can be detected through fluorescence of NV spin states. This method, coupled with non-endocytotic delivery of fluorescent NV nanodiamonds into living HEK 293 cells, enables us to determine the local temperature environments by wide field confocal microscopy, providing thermodynamics insights. We show that whole cell and site-specific intracellular temperature mapping using fluorescent nanodiamonds could, in principle, determine the inherent relationship between temperature and organelle function.

10 ways this graphene supercapacitor could change your electronic devices

Maher F. El-Kady, Melanie Ihns, Mengping Li, Jee Youn Hwang, Mir F. Mousavi, Lindsay Chaney, Andrew T. Lech and Richard B. Kaner

Batteries run just about everything portable in our lives such as smartphones, tablets, computers, etc. While we have become accustomed to the rapid improvement of portable electronics, the slow development of batteries is holding back technological progress. But imagine charging your cell phone in just a few seconds, or what if you could run that same device for a week on a single charge? By combining graphene with a conventional battery material manganese dioxide, we produced hybrid supercapacitors that can store as much charge as conventional batteries, yet they can be recharged in seconds instead of hours. These hybrid supercapacitors use aqueous electrolytes and are assembled in air without the need for expensive 'dry rooms' required for building today's supercapacitors. What makes these supercapacitors even more interesting is that they are compact, reliable and energy dense, charge quickly, and possess both long cycle life and calendar life and are made of abundant and environmentally friendly materials. This remarkable performance holds great promise for the next-generation consumer electronics.

Stable and oxide-free gadolinium nanoparticles display large magnetic moments and record high per-particle t_2 NMR relaxivities

Yavuz N. Ertas, Nanette N. Jarenwattananon and Louis-Serge. Bouchard

Among the elements in the periodic table, gadolinium (Gd) has the highest number of unpaired electrons, magnetocaloric effect and neutron capture cross section. However, the potential of this rare-earth metal has not yet been fully realized due to challenges in its chemical synthesis, namely its high reduction potential, leading to the formation of oxides with suboptimal properties. This problem is also prevalent with other lanthanides, severely limiting their uses in industry. A non-synthetic fabrication approach along with a reduction process and appropriate capping have been developed to produce oxide-free, stable gadolinium nanoparticles. We demonstrate broad tunability of the particle size while maintaining remarkably narrow size distributions (< 5%). The nanoconstructs displayed the highest magnetization measured to date for Gd, 206 emu/g Gd at 2 K along with a record high per particle nuclear magnetic resonance (NMR) transverse relaxivity (r_2) of $2.7 \times 10^8 \text{ mM}^{-1}\text{s}^{-1}$, which corresponds to the highest per-particle r_2 relaxivity reported for any T_2 contrast agents to date. Unlike traditional approaches, this process can be extended to produce oxide-free nanoconstructs of other lanthanides, making them accessible for technological or biomedical applications.

Global control of RNA 3'-end processing by RNA polymerase II roadblocks

Kevin Roy, Jason Gabunilas, Abigail Gillespie, Duy Ngo and Guillaume Chanfreau

To properly regulate gene expression, cells must control where RNA polymerase initiates and terminates transcription throughout the genome. In eukaryotes, RNA polymerase II (Pol II) is responsible for transcribing the vast majority of genes, and its activity is controlled throughout the stages of initiation, elongation, and termination. Pol II termination and RNA 3'-end formation are tightly coupled, and depend chiefly on the recognition of specific sequences in the nascent RNA by *trans*-acting factors. In this work, we show that DNA-binding proteins downstream of Pol II elongation complexes play a major role in controlling 3'-end processing and termination, by pausing Pol II to enhance the efficiency of intrinsically weak RNA processing signals upstream. Specifically, we show that the transcriptional activator Reb1 and the RNA polymerase III transcriptional machinery enhance the transcription termination efficiency of small non-coding RNAs, and exert genome-wide control over sites of alternative 3'-end processing on protein-coding mRNAs. Furthermore, we show that these DNA-binding factors act as transcriptional roadblocks to halt cryptic Pol II transcription in intergenic regions in order to limit the extent of aberrant Pol II activity. Importantly, we show that these roadblocks regulate gene expression in a dynamic manner, as environmental conditions that trigger the release of TFIIIB from DNA relieve the roadblock effect on Pol II. This results in Pol II elongation into downstream regions to remodel gene expression in response to stress. Additionally, this work uncovers the first known example of an mRNA whose mature 3' end does not require any processing, but rather is generated by the collision between Pol II and a DNA-binding factor. Overall, our work underscores the importance of DNA roadblocks in controlling RNA processing, and shows that 3'-end formation mechanisms in eukaryotes are more diverse than previously appreciated.

Atomically precise hybrid nanoparticles

Elaine A. Qian, Alex I. Wixtrom, Jing Yang Wang, Sylvia Chow, Azin Saebi and Alexander M. Spokoyny

A current challenge in bionanotechnology involves the development of novel molecular scaffold platforms for particle-based therapeutics. This new class of scaffolds must incorporate key advantages such as multivalency and be customizable, while avoiding shortcomings including polydispersity and labile bonding interactions commonly found in hybrid metal-based nanoparticles. Our laboratory is currently investigating perfunctionalized derivatives of icosahedral dodecaboranes, $[B_{12}H_{12}]^{2-}$, a class of molecules which have garnered attention for their unique properties of three-dimensional aromaticity, exceptional stability, and tunable photophysical properties. In particular, we are engaged in the preparation of new classes of metal-free, rigid, versatile, atomically precise scaffolds that can be grafted with biomolecule-based receptors.

We have synthesized several novel *hypercloso*- $[B_{12}(OR)_{12}]^0$ molecules (R = aryl or alkyl). Facile functionalization of these molecules is possible via highly efficient and orthogonal S_NAr click-like transformation involving thiols and perfluoroaromatic functional groups to form well-defined nanomolecules ranging in sizes between 2-10 nm. In addition, the developed transformation can be followed *via* ^{19}F and ^{11}B NMR spectroscopic handles that these structures inherently possess. Such *in situ* tracking allows for rapid and efficient optimization of reaction conditions for specific substrates yielding the desired perfunctionalized species. We are currently investigating applications such as the protein-stabilizing and multivalent-binding capabilities of these perfunctionalized nanomolecules. Future work will elucidate the full scope of these novel nanoscale clusters and their potential to transform relevant biomedical and therapeutic technologies.

Optical control of cancer initiation in zebrafish

Zhiping Feng, Sophie Vriz, Michel Volovitch, Ludovic Jullien, Shuo Lin, Shimon Weiss and David Bensimon

Although cancer initiation and evolution have been extensively studied, they are not, as of yet, fully understood. Several models have attempted to answer how cancer arises from individual transformed cells. However, current probes of cancer development are restricted to the collective properties of many thousands of cells. In particular, two outstanding questions, the effectiveness of oncogenic transformation and the role of the local microenvironment on cancer initiation, require the study of the fates of individual cells and their progenies. Recently, we developed a technology that allows for the control of protein activity and gene expression in single cells through light activation. In this work, we utilize this method to activate in a small number of cells in a live zebrafish a typical oncogene, K-RasG12V, and investigate effects of these changes on tumorigenesis under varied genetic backgrounds. We successfully demonstrated the spatiotemporal control of oncogene expression in live zebrafish. Furthermore, we investigated different tumorigenic phenotypes by transiently or permanently activating K-Ras at varied developmental stages. We believe that our studies could shed new light on cancer initiation and growth and provide new tools for target validation and testing of novel anti-cancer drugs.

Characterizing competing mechanisms in HDAC8 with a variety of physiologically available divalent ions

Nathan M. Gallup, Michael R. Nechay and Anastassia N. Alexandrova

Histone Deacetylases (HDACs) are responsible for the removal of acetyl groups from histones resulting in gene silencing. Overexpression of HDACs is associated with cancer, and HDAC inhibitors are of particular interest as chemotherapeutics. The best studied in this category is HDAC8. The physiological metal utilized by HDAC8 is a matter of debate, but mechanistic studies have shown that a zinc-dependent HDAC8 is capable of shuttling protons to produce the desired products. We used QM/MM methods to test two conflicting mechanisms with Zn, Co, Fe, Mn, Mg, and Ni as the participating, bound divalent ion, and have found that previous studies omitted important pieces of the active site that assist in further elucidating the mechanism of proton shuttling.

Synthesis and characterization of a large-area graphene membrane

Nanetta Pon, Jaime Torres, Xinwei Huang, and Richard B. Kaner

We examine the feasibility of a proposed graphene desalination membrane. Graphene has been shown theoretically to be an excellent reverse osmosis membrane material due to its strength, atomic thinness, and the potential selectivity of functionalized nanopores. However, a practical graphene membrane has still never been produced. For this project, the salt rejection of large-area graphene with intrinsic defects was measured. Few-layer graphene was synthesized by chemical vapor deposition (CVD) onto nickel and copper catalysts. A method was developed to produce large-area graphene-polymer composite membranes by freeing from the growth substrate graphene sheets up to 50 cm² in area, and transferring them onto a polymer filter. The membranes were tested in a stop-flow cell with a 2 g/L NaCl solution (brackish water) at 150 psi. The composite membranes survived high-pressure conditions over several hours, and salt rejection rates of 20% were achieved. This performance supports graphene's promise as a desalination material for real-world applications.

Total syntheses of akuammiline alkaloids (+)-strictamine, (-)-2(*S*)-cathafoline, and (-)-aspidophylline a

Elias Picazo, Lucas A. Morrill, Jesus Moreno, Joel M. Smith and Neil K. Garg

The akuammiline alkaloids are a structurally diverse family of indole natural products. Their complex structures, coupled with their pharmacological properties, have made them attractive synthetic targets. Despite seminal studies, compounds that possess a methanoquinolizidine core have not yet succumbed to total synthesis. This presentation will describe the first enantioselective syntheses of aspidophylline A and methanoquinolizidine-containing akuammilines strictamine and 2(*S*)-cathafoline.

Biochemical characterization of the substrate specificity of two unique members of the mammalian protein arginine methyltransferase family, PRMT7 and PRMT9

Andrea Hadjikyriacou, You Feng, Yanzhong Yang, Alessandra Espejo, Mark T. Bedford and Steven G. Clarke

Protein arginine methylation is a widespread and important posttranslational modification in eukaryotic cells, shown to be involved in the activation or repression of transcription, modification of the splicing machinery, signaling, and DNA repair. Mammalian protein arginine methyltransferases include a family of nine enzymes that transfer methyl groups onto arginine residues, producing monomethylarginine only (MMA, type III), symmetric dimethylarginine (SDMA) and MMA (Type II), or asymmetric dimethylarginine (ADMA) and MMA (Type I). While the role and activity of the other members of the family have been well characterized, the two final members, PRMT7 and PRMT9, had been unclear and the substrates for these enzymes had been elusive. Both PRMT7 and PRMT9 contain two methyltransferase domains and also have acidic residues in the well-conserved substrate-binding motif, features not seen in the other PRMT enzymes. Our work confirmed PRMT7 as the only type III enzyme in the group, with an unusual low temperature optimum for activity, and preference for a basic stretch of residues in an R-X-R sequence for methylation. Mutations of the acidic residues in the substrate-binding motif results in a loss of the specific R-X-R activity and the appearance of a G-R-G specificity typical of many of the other PRMTs. The physiological substrate of PRMT7 has yet to be confirmed, although histone H2B is an effective *in vitro* substrate. PRMT9, on the other hand, had no reported activity, until immunoprecipitation from HeLa cells showed it pulled down two splicing factors, SF3B2 and SF3B4. Amino acid analysis showed that PRMT9 methylates SF3B2 to produce both MMA and SDMA, thus making it the second type II enzyme. PRMT9 knockdown results in modulation of alternative splicing events; the enzyme appears to be relatively specific for the SF3B2 protein and the position of the methylated arginine is important. Thus, PRMT7 and PRMT9 represent unique members of the mammalian PRMT family.

Conversion of amides to esters by the nickel-catalyzed activation of amide C–N bonds

Liana Hie, Noah F. Fine Nathel, Tejas K. Shah, Emma L. Baker, Xin Hong, Peng Liu, Yun-Fang Yang, K. N. Houk and Neil K. Garg

This presentation will describe the nickel-catalyzed activation of amide C–N bonds, which has been realized through the conversion of amides to esters. The reaction methodology proceeds under mild reaction conditions and avoids the use of a large excess of an alcohol nucleophile. These studies are expected to fuel the further use of amides as valuable building blocks for the construction of C–heteroatom or C–C bonds using non-precious metal catalysis.

Metabolite-driven modifications: lysine acylations elucidate substrate metabolism in syntrophic bacteria

Hong Hanh Nguyen, Phuong Nguyen, Robert Gunsalus, Michael McInerney, Joseph Loo, Rachel R. Ogorzalek Loo

Syntrophic bacteria play an indispensable and rate-controlling role in organic matter degradation in methanogenic microbiota to cycle carbon back to the environment. They activate substrates with coenzyme A and stepwise convert acyl-CoAs down to acetyl-CoA for ATP production. Syntrophs generate barely enough ATP to sustain life and the mechanisms how cells distribute and conserve energy are poorly understood. With the rising family of reversible conserved short-chain lysine acylations and their emerging regulatory function in coordinating metabolism with changes in nutrient availability, studying lysine acylations may elucidate substrate metabolism in syntrophs.

Using acyl-CoAs suggested from substrate degradation pathways in *Syntrophomonas wolfei* and *Syntrophus aciditrophicus* to predict which acyl-lysine modifications are hidden in MS/MS datasets, we detected most of the predicted modifications and semi-quantified levels of protein expression and acyl-specific lysine modification under different cultivation conditions, along with the stoichiometries of various acyl-modifications at individual sites. Overall levels of lysine acylation correlate with cellular levels of acyl-CoA. The presence of some acylations and thus acyl-CoAs suggests alternative reversible adaptive processes in *S. aciditrophicus* to separately control substrate degradation and electron disposal by parallel pathways and in *S. wolfei* to control a reversible branchpoint between substrate degradation and electron disposal depending on the availability of nutrient and the rate of electron disposal. The semi-quantitative data in combination with published research allows us to come up with a hypothesis: Part, if not most, lysine acylations in syntrophic bacteria happen spontaneously in a regulatable manner to provide a quick sensing and responding mechanism, with little overhead applied, to the metabolic flux to guide near-equilibrium conversions towards the optimal pay-out in ATP.

Mapping the energy landscape for second stage folding of a single membrane protein

Duyoung Min, Robert E. Jefferson, James U. Bowie and Tae-Young Yoon

Membrane proteins are designed to fold and function in a lipid membrane, yet folding experiments within a native membrane environment are challenging to design. Here we show that single molecule forced unfolding experiments can be adapted to study helical membrane protein folding under native-like bicelle conditions. Applying force using magnetic tweezers, we find that a transmembrane helix protein, *E. coli* rhomboid protease GlpG, unfolds in a highly cooperative manner, largely unraveling as one physical unit in response to mechanical tension above 25 pN. Considerable hysteresis is observed, with refolding occurring only at forces below 5 pN. Characterizing the energy landscape reveals only modest thermodynamic stability ($\Delta G = 6.5 k_B T$) but a large unfolding barrier ($21.3 k_B T$) that can maintain the protein in a folded state for long periods of time ($t_{1/2} \sim 3.5$ hrs). The observed energy landscape may have evolved to limit the existence of troublesome partially unfolded states and impart rigidity to the structure.

A multi-spot confocal platform for high-throughput freely diffusing single-molecule FRET studies

Antonino Ingargiola, Eitan Lerner, Sang Young Chung, Angelo Gulinatti, Ivan Rech, Massimo Ghioni, Shimon Weiss and Xavier Michalet

Since single-molecule FRET (smFRET) was demonstrated 20 years ago, tremendous progresses have been made in sample preparation and data analysis, allowing the study of increasingly complex systems and the extraction of precise structural information. However, technical improvements have been mostly confined to combining the technique with microfluidics or complementary techniques such as optical tweezers or atomic force microscopy.

Problematically, the approach remains a low throughput one, due to the fact that “single-molecule” FRET measurements are really “multiple single-molecule” measurements, requiring thousands of single-molecule transits to be recorded in order to accurately characterize a sample. Combined with the necessary low molecular concentration, this translates into several minute-long acquisition times, preventing the study of dynamic processes occurring on faster time-scales. In order to overcome these limitations, we have embarked in a long-term project to develop a high-throughput smFRET system using a parallel multispot confocal platform and new detector arrays, with the aim to reduce the acquisition time by close to two orders of magnitude compared to conventional single-spot systems. Here, we review the current performance of our system for smFRET and FCS measurements, benchmarked using different doubly-labeled DNA samples. In particular, we show that the different confocal spots probe independent volumes of the sample and individually obtain comparable results, allowing pooling of their data into a single higher-statistics result. As an illustration of the advantage of a faster data acquisition rate, we present results on the kinetics of the initial stage of *E. coli* RNA transcription, inaccessible by a conventional single-spot smFRET approach.

Single-molecule measurement of membrane protein stability

Robert E. Jefferson, Yu-Chu Chang, Eitan Lerner, Xavier Michalet, Shimon Weiss and James U. Bowie

Our understanding of membrane proteins has been severely hindered by a dearth of techniques to investigate membrane protein folding and stability in lipid bilayers. Our lab has developed a technique to drive membrane protein unfolding under native conditions called the steric trap method, which uses a protein that preferentially binds the unfolded state such that binding drives unfolding. Previously, this method has been reliant on the use of a functionally assayable protein, such as the chromophore-containing protein bacteriorhodopsin or the membrane enzyme diacylglycerol kinase. We propose generalizing the steric trap method using single-molecule fluorescence technology. By moving the system to infinite dilution in which there is only a single protein per vesicle we can eliminate aggregation problems that plague membrane protein studies. The sensitivity of single-molecule detection permits us to use much less protein and lipid material, while preserving the lipid bilayer environment instead of using solubilizing agents required for bulk spectroscopic assays. By circumventing the requirement for a protein-specific functional assay, we hope to expand the reach of membrane protein folding studies.

Metal-free, boron-rich cluster cationic styrene polymerization photocatalysts

Marco S. Messina, Alex Wixtrom, Paul Chong, Raymond Wang, Jonathan C. Axtell, Kent Kirlikovali, Brianna M. Upton, Stephanie Deshayes, Anastassia N. Alexandrova, Heather D. Maynard, and Alexander M. Spokoyny

We have developed a novel metal-free olefin polymerization photocatalyst based on perfunctionalized derivatives of icosahedral boron clusters. This catalyst was synthesized utilizing a new microwave-based synthetic route to produce derivatives of *closa*-[B₁₂H₁₂]⁻². This class of boron “closomers” has garnered attention due to their stability and unique properties including three-dimensional delocalization of the cage-bonding electrons and tunable photophysical properties. The perfunctionalized cluster species investigated in this study undergo photoexcitation with visible light, initiating cationic polymerization of olefin-containing monomers. Employing the *hyperclos*-[B₁₂(OCH₂C₆F₅)₁₂]⁰ cluster in as low as 0.005 mol% produced polymers in a variety of styrene substrates containing electron-withdrawing and –donating substituents with remarkably low PDI (polydispersity index). Our metal-free system offers lower catalyst loading over previously developed systems, improved monomer scope, ease of photo-catalyst preparation, and ease of polymer purification, as the photocatalyst is readily soluble in most organic solvents.

Strained alkynes as useful synthetic building blocks

Jose M. Medina, Tejas K. Shah, Robert B. Susick and Neil K. Garg

Heterocycles are prevalent motifs seen in numerous biologically active natural products and pharmaceuticals. This presentation will describe a mild approach for the construction of decorated heterocycles based on the trapping of in situ-generated strained cyclic alkynes. Additionally, the distortion / interaction model provides an explanation for the regioselectivities observed.

Stabilization of Pt nanoclusters for their use as catalysts

Elisa Jimenez-Izal, Jonny Dadras and Anastassia N. Alexandrova

Today, over 90% of all chemical manufacturing processes use catalysis, having an enormous impact in the world's economy. The development of nanotechnology has had a deep influence over the progress of this field. Indeed, heterogeneous catalysis nowadays is based on small metallic particles of catalyst, with a diameter of 1-10 nm. However, although surface-deposited metallic clusters can be really superb catalysts, they deactivate rapidly via sintering and coke deposition. In this work we studied MgO supported Pt clusters, that are interesting as catalysts for dehydrogenation of alkanes. We show that doping these clusters with boron leads to substantial stabilization of these nanoparticles against both means of deactivation. The non-stoichiometric boride cluster obtained via such alloying is found to anchor to the support via a covalent B-O bond, and the cluster-surface binding is much stronger than in the case of pure Pt clusters. Additionally, B introduces covalency to the intra-cluster bonding, leading to structural distortion and stabilization. The energy required to dissociate a Pt atom from a boride cluster is significantly larger than that of pure Pt clusters. These energetic arguments lead to the proposal that sintering via both Ostwald ripening and particle coalescence would be discouraged relative to pure Pt clusters. Finally, it is shown that the affinity to C also drops dramatically for borated clusters, discouraging coking and increasing the selectivity of potential cluster catalysts.

Bacteriophage P22 ejects all of its internal proteins before its genome

Yan Jin, S. M. Sdao, J.A. Dover, Natalia B. Porcek, Charles M. Knobler, William M. Gelbart and Kristin N. Parent

Double-stranded DNA bacteriophages are highly pressurized, providing a force driving ejection of a significant fraction of the genome from its capsid. In P22-like Podoviridae, internal proteins ("E proteins") are packaged into the capsid along with the genome, and without them the virus is not infectious. However, little is known about how and when these proteins come out of the virus. We employed an *in vitro* osmotic suppression system with high-molecular-weight polyethylene glycol to study P22 E protein release. While slow ejection of the DNA can be triggered by lipopolysaccharide (LPS), the rate is significantly enhanced by the membrane protein OmpA from *Salmonella*. In contrast, E proteins are not ejected unless both OmpA and LPS are present and their ejection when OmpA is present is largely complete before any genome is ejected, suggesting that E proteins play a key role in the early stage of transferring P22 DNA into the host.

Platinum nanoparticles catalysts for heterogeneous catalysts in para-hydrogen induced polarization for MRI signal enhancement

Jeffrey McCormick, Stefan Glöggler, Alex Grunfeld, Yavuz Ertas, Shawn Wagner and Louis-Serge Bouchard

Magnetic Resonance Imaging (MRI) is an incredibly powerful diagnostic tool in medicine and analytical chemistry, but it suffers from low sensitivity caused by poor population differences in nuclear spin states. Several hyperpolarization techniques have been explored for signal enhancement by use of singlet state hydrogen (*para*-H₂) attachment, though most are limited by high cost of equipment and inseparable transition metal catalysts such as rhodium and iridium required to label compounds with *para*-H₂. One technique known as ParaHydrogen-Induced Polarization (PHIP) has been explored using more practical sample preparation and relaxation timeframes yielding signal enhancements of >50,000 fold. However, these PHIP reactions require harsh solvents such as methanol and acetone, which limit their clinical application. PHIP labeling in water has proven difficult, as the polar nature of water and its interactions with metal surface coordination reduces signal enhancement achieved to impractical levels. Our group has developed innovative ligand-capped metal nanoparticles which serve as catalysts for *para*-H₂ labeling of biological compounds in water, and allow separation from solution within the timeframe of MRI acquisitions. Here, we demonstrate aqueous PHIP signal achieved using heterogeneous catalysts which can be removed and reused easily. The ability to spin label biological compounds of interest by PHIP could dramatically improve the sensitivity of MRI and its ability to detect diseased states in living patients.

Co-solvent exfoliation and suspension of hexagonal boron nitride

Kristofer L. Marsh, Mina Souliman and Richard B. Kaner

A simple method is presented for exfoliating and suspending hexagonal boron nitride (h-BN) using a simple co-solvent approach. Liquid exfoliation is a simple method to produce boron nitride nanosheets (BNNS) from bulk h-BN powder. Generally, h-BN powder is mixed with a solvent, and energy, usually ultrasonic energy, is introduced into the system. Studies have shown that h-BN disperses reasonably well in isopropyl alcohol, N,N-dimethylformamide, dimethyl sulfoxide, and N-methylpyrrolidone. However, many of these solvents are harmful and/or dangerous to work with. We show that a 60 w/w% concentration of *tert*-butanol in water is very effective at exfoliating boron nitride especially when compared to the individual components alone as indicated by UV-vis and transmission electron microscopy. Molecular weight and surface tension are found to play inverse roles in the exfoliation.

From UV to IR: Prodigious light absorption enhancement of titanium dioxide in a hybrid TiO₂/B₁₂-cluster material

Dahee Jung, Liban M. A. Saleh, Ekaterina Titarenko and Alexander M. Spokoyny

Metal and metalloid oxides have a ubiquitous presence in modern life. The most popular examples, silicon dioxide (SiO₂), iron oxide (Fe₃O₄), aluminium oxide (Al₂O₃) and titanium dioxide (TiO₂), are earth-abundant and have a wide range of diverse applications, which include the catalyst for the fixation of nitrogen (Fe₃O₄) and the fabrication of microelectronic components (SiO₂). The ability to control and tune the properties of earth-abundant oxides through mild and operationally straightforward methods is critical to the expansion of their applications.

In recent years, TiO₂ has been extensively studied to exploit its semi-conducting and light absorption properties. A particular area of interest is to increase the percentage of sunlight that TiO₂ can absorb as it is limited to the UV range, which makes up only 7 % of sunlight. It has been demonstrated that doping TiO₂ with other elements such as hydrogen, carbon and nitrogen can deliver subtle changes to the electronic and optical properties of the oxide. We recently discovered how the stable boron-rich cluster [B₁₂(OH)₁₂]²⁻ can be incorporated into the framework of a metal oxide *via* molecular synthetic methods to create TiO₂/B₁₂ hybrid materials. This dramatically changes the photophysical properties of TiO₂, producing a hybrid material capable of absorbing light beyond the UV regime. This method can also be used to access other hybrid metal oxides including Al₂O₃, B₂O₃ and ZrO₂. For the first time, our study shows how stable boron-rich clusters can be used as molecular precursors and can be efficiently interfaced with solid-state materials to enable new properties.

Determination of structural organization and morphology of β -amyloid *via* scanning tunneling microscopy

Diana Yugay, Lisa M. Kawakami, Dominic Goronzy, Jerome Gilles, Tze-Bin Song, Yang Yang, Ya-Hong Xie, and Paul S. Weiss

Alzheimer's Disease (AD) is a chronic neurodegenerative disease that involves the aggregation of β -amyloid ($A\beta$) peptides in the brain. Transition metal ions, such as Cu^{2+} and Zn^{2+} , are known to be abnormally concentrated in $A\beta$ aggregates and synaptic areas of the brain in people with AD. A cure for AD has yet to be discovered due to the limited knowledge about cause and effect in the disease, which may involve the binding of transition metal ions to $A\beta$; thus, elucidating amino acid coordination at the binding site(s) is an important step in understanding the disease. The binding site(s) of the $A\beta$ peptide in the presence of metal ions has been extensively studied theoretically, but there is a paucity of experimental data due to the mechanical and conformational flexibility of $A\beta$ before it organizes into β -sheet oligomers. Previously, we have demonstrated the ability to resolve sub-molecular structures of biological molecules and differentiate between side chains of individual amino acids and their orientations by using scanning tunneling microscopy (STM) and related spectroscopic imaging methods. In this study, we report structural elucidation of the first 16 amino acids of the full length $A\beta$ ($A\beta_{1-16}$) *via* STM and its structural progression in the presence and the absence of Cu^{2+} ions *via* circular dichroism (CD), atomic force microscopy (AFM), and surface-enhanced Raman spectroscopy (SERS). Though $A\beta_{1-16}$ is reported as a disordered region of $A\beta$ and, therefore, is often omitted from the computational studies, our findings indicate that upon the deposition of $A\beta_{1-16}$ on highly oriented pyrolytic graphite (HOPG), $A\beta_{1-16}$ laminates into structured β -sheet domains. Most importantly, based on the analysis of the length and position of protruding features of Cu^{2+} ions within $A\beta_{1-16}$ peptides from STM images, we determine that Cu^{2+} ions participate in inter-sheet $A\beta_{1-16}$ binding by coordinating with two neighboring Histidine (His) residues, His13 and His14.

Electric field induced strong enhancement of electroluminescence in multi-layer MoS_2

Dehui Li, Rui Cheng, Hailong Zhou, Chen Wang, Anxiang Yin, Yu Chen, Nathan O. Weiss, Yu Huang and Xiangfeng Duan

The layered transition metal dichalcogenides (TMDs) have attracted considerable interest due to their unique electronic and optical properties. Here we report electric field induced strong electroluminescence in multi-layer MoS_2 and WSe_2 . We show that $GaN-Al_2O_3-MoS_2$ and $GaN-Al_2O_3-MoS_2-Al_2O_3$ -graphene vertical heterojunctions can be created with excellent rectification behaviour. Electroluminescence studies demonstrate prominent direct bandgap excitonic emission in multi-layer MoS_2 over the entire vertical junction area. Importantly, the electroluminescence efficiency observed in multi-layer MoS_2 is comparable to or even higher than that in monolayers, corresponding to a relative electroluminescence enhancement factor of >1000 in multi-layer MoS_2 when compared to its photoluminescence. This striking enhancement of electroluminescence can be attributed to the high electric field induced carrier redistribution from low energy points (indirect bandgap) to high energy points (direct bandgap) of k -space, arising from the unique band structure of MoS_2 with a much higher density of states at high energy points. The electric field induced electroluminescence is general for other TMDs including WSe_2 , and can provide a fundamental platform to probe the carrier injection, population and recombination in multi-layer TMDs and open up a new pathway toward TMD based optoelectronic devices.

Pausing in *Escherichia Coli* transcription initiation

Eitan Lerner, Chung Sangyoon, Allen Benjamin, Shuang Wang, Jookyung J. Lee, Shijia Winson Lu, Grimaud Wilson Logan, Ingargiola Antonino, Alhadid Yazan, Borukhov Sergei, Strick Terence, Taatjes J. Dylan and Weiss Shimon

An essential and highly regulated step in gene expression is transcription initiation. After promoter binding and DNA unwinding ('bubble opening') and in the presence of nucleoside triphosphates (NTPs), the RNA polymerase (RNAP)-promoter initial transcribing complex (RPitc) engages in 'abortive initiation', a process in which RNAP cycles between synthesis and release of short RNA transcripts. In abortive initiation, RPitc is believed to undergo a sequence of transitions between different initiation sub-states. The kinetics of the production of a full RNA transcript starting at a late initiation sub-state is expected to be similar or faster than the kinetics measured from an earlier initiation sub-state. To test this hypothesis, we developed a novel *in vitro* single-run quenched kinetics transcription assay based on the detection and quantification of run-off transcripts. Using this assay and corroborating it with gel-based and magnetic tweezer assays, testing two different promoters, we surprisingly found that run-off transcription kinetics starting from late initiation sub-states is slower than kinetics starting from earlier initiation sub-states. When the same kinetic measurements were performed in the presence of the transcription elongation factor GreA, the kinetics starting from a late initiation sub-state was accelerated. Experimental results suggest that as a function of shortage in NTPs RPitc can enter an off-pathway state in which the nascent RNA is in a backtracked, paused position, awaiting incorporation of the missing NTP. Our findings suggest that pausing at distinct stages of transcription initiation could regulate gene expression under stressed conditions.

Enhancing pharmacokinetics and stability of protein drugs using trehalose polymers

Yang Liu, Juneyoung Lee, **Jeong Hoon Ko** and Heather D. Maynard

Proteins are important as therapeutic drugs, but their instability towards environmental stressors such as heat increases the cost and the risk of medical emergencies. We have previously reported glycopolymers based on trehalose, a natural protein-stabilizing sugar, that stabilizes various proteins to heat and lyophilization. In this work, we extended this platform for stabilization of insulin as a model therapeutic drug. The insulin was conjugated to trehalose polymer, and the conjugate exhibited longer half-life *in vivo*. The polymer added as an excipient showed increased stability towards heat. In addition, the trehalose polymer did not induce toxicity as assessed by toxicological experiments in mice. These results suggest that the trehalose polymer is promising as a reagent for enhancing pharmacokinetics and stability of protein drugs.

Sensing membrane potential by inorganic semiconductor nanorods

Kyoungwon Park, **Yung Kuo**, Volodymyr Shvadchak, Antonino Ingargiola, Xinghong Dai, Lawrence Hsiung, Wookyeom Kim, Z. Hong Zhou, Peng Zou, Alex J. Levine, Jack Li and Shimon Weiss

Unraveling emergent brain activities requires simultaneous recording of action potentials from a large number of neurons. Electrical recording methods such as patch clamp and optical recording by voltage sensing dyes and proteins have been developed for years and are widely utilized. However, such techniques have insufficient spatial and/or temporal resolutions and/or suffer from poor photostability, posing a need for probes that circumvent these limitations. Improved probes, with high sensitivity and photostability, could afford the study of large neural networks (in a large field-of-view) and/or at very high spatial resolution. Using bandgap-engineering and colloidal synthesis methods, we have synthesized seeded semiconductor (SC) nanorods (NRs) with Type-II heterojunctions that exhibit a large Quantum Confined Stark Effect (QCSE) at room temperature. For using these NRs as voltage sensors, however, one needs to impart them with membrane-protein like properties so that they can be stably inserted into the membrane. We report here spontaneous insertion of SC NRs into liposomes and cell membranes by functionalizing them with specially designed peptides. We provide evidences for insertion from cryo transmission electron microscopy (TEM) and polarized light microscopy. We also report on first attempts to sense membrane potential with these particles with single-particle sensitivity. With further improvements, SC NRs could potentially be used to study signals from whole neural networks in a large field-of-view. Moreover, successful implementation of SC NRs would allow for the analysis of voltage signals at the nano- (single synapse-) scale.

Direct write protein patterns using electron beam lithography

Uland Y. Lau, Sina S. Saxer, Juneyoung Lee, Erhan Bat and Heather D. Maynard

Direct write patterning of multiple biologically relevant molecules on surfaces has tremendous potential in applications of tissue engineering, diagnostics, proteomics and biosensors. Precise control over the position and arrangement of proteins, especially on the micro- and nano- scale level is a particular challenge towards the improvement of miniaturized devices. Here, a trehalose glycopolymer is utilized as a resist material that protects proteins during electron beam lithography, and thus enables direct write multiplexed protein patterns with micro- and nano- scale features and alignment capabilities. The trehalose glycopolymers behave as negative resists, crosslinking to the substrate upon electron beam irradiation, and provide stabilization to proteins against the harsh processing conditions. Patterns with user-defined shapes were generated with a variety of protein types, such as antibodies, enzymes, and growth factors, and were shown to retain their activity. To further demonstrate the technique, fabrication of antibody patterns for multiplexed cytokine detection from live cells was achieved. A sandwich immunoassay was developed for the detection of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF α) secreted directly from stimulated macrophage cells. Multiplexing with both IL-6 and TNF α on a single chip was demonstrated successfully with high specificity, in relevant cell culture conditions, and at monitoring different time points following after cell stimulation. Simultaneous detection of these extracellular signaling markers demonstrate the potential application towards disease profiling and diagnostics.