Description of Research Projects of Faculty involved in the NSF REU Program

The following are general descriptions of on-going research projects of the faculty who are involved in the NSF REU Program in the Departments of Chemistry, Material Sciences and Biology.

Sudeep Bhattacharyay (Chemistry) *To Develope a Computational Inhibitor Screening Method for Quinone Reductases*

Quinone reductases (QRs) are cytosolic enzymes that are targets for anti-cancer and antimalarial drug development. QRs catalyze two-electron reduction of quinones to hydroquinones using a flavin adenosine dinucleotide cofactor.[12,13] The redox chemistry involves hydride transfer reactions between a substrate/co-substrate and the flavin ring of the cofactor *via* a classic one-site 'ping-pong' mechanism. This mechanism is unusual; the active-site appears to possess an oscillatory molecular recognition features (towards substrates/co-substrates) as it passes through its oxidative and reductive cycles. During a particular redox half-cycle, the observed changes in active-site properties (both in terms of geometric and dynamic) originate due to the charge separations between flavin ring atoms and the active-site. The redox state of the flavin and the resulting active-site changes are the primary determinants of molecular recognition. Therefore, a reliable model of selective inhibitor screening would require a precise determination of the QR-inhibitor interaction energies and the characterization of protein dynamics in the binding process. In the proposed study, a theoretical model of inhibition will be developed using mixed quantum/classical simulation. The study could provide accurate energetics for inhibitor binding and therefore, a reliable and faster computational inhibitor screening technique for this very important family of enzymes.

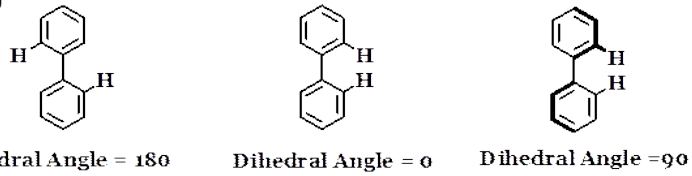
Patricia A. Cleary (Chemistry) *Atmospheric Chemistry: Urban Photochemistry and Chemical Kinetics*

This research group is involved in atmospheric measurement campaigns which target the spatial and temporal distribution of ozone over Lake Michigan. Air quality around Lake Michigan has been a target area of concern where shoreline counties beyond the urban corridor of Gary, IN-Chicago, IL-Milwaukee, WI have historically been in non-attainment of federal ozone standards. Students work with data collected from two complementary campaigns: Kenosha Harbor differential optical absorption spectrometry (DOAS) measurements from 2008-2009 and Lake Express ferry O_3 measurements from 2008-2010 and 2013-2014.[14,15] They will work on determining trends in the atmospheric data, comparisons between shoreline data and offshore data, the spatial distribution of ozone over Lake Michigan and the ability for the NOAA Air Quality Forecast model to adequately predict surface level ozone concentrations over Lake Michigan.[16] The program has expanded in the summer of 2014 to include O_3 measurements via unmanned aerial vehicle. Because the meteorological confinement of air masses over Lake Michigan from inversions and the complex meso-scale meteorological lake-breeze effect, we intend to evaluate the lake-breeze front barrier with respect to meteorological and chemical measurements. Other projects that we will explore involve chemical kinetics calculations of atmospheric interest by adopting the master equation calculation software developed at the University of Leeds, using a desktop model of the master equation program for collisional transfer and chemical reactions involving association, isomerization, disassociation and non-adiabatic hopping processes called MESMER (Master Equation Solver for Multi-well Energy Reactions). Mercury oxidation systems, most notably Hg + halogens, will be studied based on previous work on high temperature kinetics, reaction kinetics, and theoretical electronic structure and equilibrium geometries.[17] The previous theoretical and experimental work can be used to develop a global kinetics model dependent on pressure and temperature variations found in the atmosphere.

Bart J. Dahl (Chemistry) *Biphenyl and Terphenyl "Smart" Biaryls: Dihedral Angle Modulation*

1. *Biphenyl and Terphenyl "Smart" Biaryls: Dihedral Angle Modulation via pH and Redox conditions*

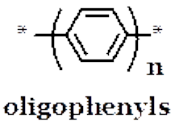
In this project we are synthesizing and studying bridged biphenyls and terphenyls.[18] The goal is to understand if we can control dihedral angle reversibly and whether or not there is some distinguishable output depending on the dihedral angle of the biphenyl. Fluorescence, color, and conductance are highly dependent on the dihedral angle of biphenyl compounds. We are primarily interested in the synthesis and study of the fundamental properties of these compounds. These "Smart" Biaryls could



Dihedral Angle = 180 Dihedral Angle = 0 Dihedral Angle =90

be useful broadly as sensors, molecular electronic components, molecular machines and in other nanoscience applications. There are several possible "bridged" compounds in which the dihedral angle could be modulated.

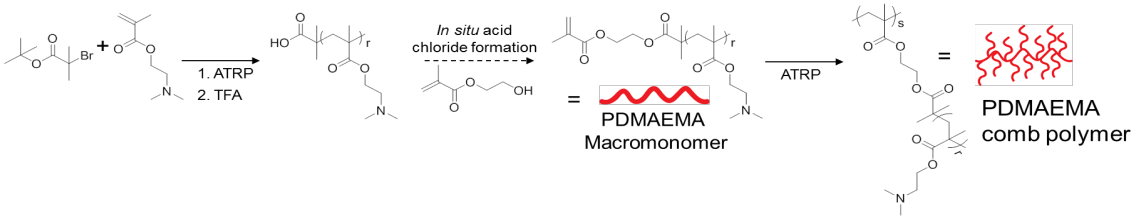
2. *Soluble Ladder and Star-Type Oligophenylenes*



Oligophenyl compounds are excellent candidates for organic-type molecular wires and semiconductors. However they do have many issues that need to be addressed, including solubility for processing purposes and poor conductance due to dihedral angles. Increasing the number of phenyl groups decreases solubility, and after n=3, oligophenyl molecules become nearly insoluble in all organic solvents. Additionally because their dihedral angles are > 0, their conductance actually falls off quite drastically. In this project we hope to sythesize a bridged oligophenyls with long alkyl chains which will increase their solubility and hold their dihedral angles at 0 degrees and thus increase their conductance. The goal is to develop a modular and step-wise synthesis of bridged oligophenyls that have both linear and dendritic shapes. Ultimately electrochemical studies will be done.

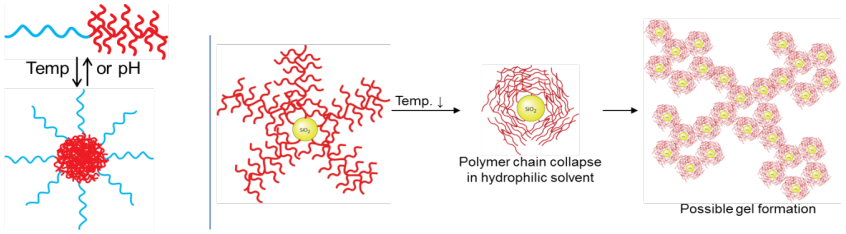
Elizabeth Glogowski (Materials Science) *Synthesis and Self-assembly of Novel Comb Polymers*

Our research program has multiple projects available for REU students that utilize a fundamental technique of polymerization to synthesize novel comb polymers for a variety of applications.[19,20] By using



atom transfer radical polymerization (ATRP), students will synthesize stimuli-responsive comb polymers that combine chemical functionality and architecture in order to create systems that reversibly self-assemble. Current undergraduate researchers are synthesizing stimuli-responsive macromonomers for comb formation and linear polymers for control samples.

REU students participating in research will be paired with current researchers in order to learn techniques, but REU students will be responsible for different aspects of each project, such as a different molecular weight macromonomer, in order to encourage ownership of their experiments. The first project focuses on the synthesis of stimuli-responsive block copolymers that reversibly form micelles as a function of pH and temperature. The synthetic method will allow for tuning of comb density, comb length, charge density, and polymer molecular weight to determine their impact on micelle formation for potential delivery applications. Using comb polymers will enable control of functional group density, micelle size, micelle stability, encapsulation efficiency, and stimuli responsiveness. The second project will involve the grafting of comb polymers from silica particles to tune the solution response of the particles for insight into the fundamental assembly properties of colloids and polymers. Polymer functionality will enable chain collapse and reversible particle aggregation to control interparticle interactions to achieve colloidal gels.



Reversible self-assembly of diblock comb polymers

Comb polymer grafting from silica colloids and reversible gelation

Sanchita Hati (Chemistry) *Coupling of Catalysis and Dynamics in Aminoacyl-tRNA Synthetases.*

My research is focused on the dynamic-function relationship in aminoacyl-tRNA synthetases (AARSs), a family of enzymes that are essential for protein synthesis. AARSs are large proteins with multiple

functional domains (Fig. 1), where cross-talking between domains is crucial for efficient catalysis. Our previous work suggested that inter-domain communications could be mediated by coupled and correlated motions between structural elements.[21] Recently, we have identified the probable pathways of inter-domain communications in *Escherichia coli* prolyl-tRNA synthetase (Ec ProRS, Fig. 1) using bioinformatics and molecular dynamic simulations.[22] These contiguous networks between two distant domains (aminoacylation and editing domains) (Fig. 1) are mainly comprised of highly conserved residues that are involved in correlated motions. Site-directed mutagenesis of some of these "on-pathway" residues (N305, E234, and F415, Fig. 1) had significant impact on both dynamics and function of Ec ProRS (Fig. 1).[22] It is still unclear how the interactions among individual pathway residues impact the dynamics that ultimately translated into reduced catalytic efficiency. In particular, it is not completely understood if the mutation of these residues had an impact on substrate binding (free energy of binding) or on catalysis (free energy of activation). Herein, we propose to probe the role of coupled dynamics (one of the highly debated topics of enzymology) on Ec ProRS function using site-directed mutagenesis and kinetic studies. In particular, steady-state kinetics will be performed and the free energy of binding ($\Delta G° = RTlnK_M$) and free energy of activation ($\Delta G^‡$, obtained from the van't Hoff plot of $ln(k^‡/T)$ vs 1/T, where $k^‡ = V_{max}/[E]_T$) will be compared for the wild-type and mutant proteins. The Michaelis–Menten constant (K_M) and maximum velocity (V_{max}) of the enzymatic reactions will be obtained using Lineweaver-Burk plot as described in our earlier work.[22,23] Comparisons of $\Delta G°$ and $\Delta G^‡$ values between wild-type and mutant proteins will help us understand if protein dynamics is important for substrate binding and/or for catalysis of Ec ProRS.

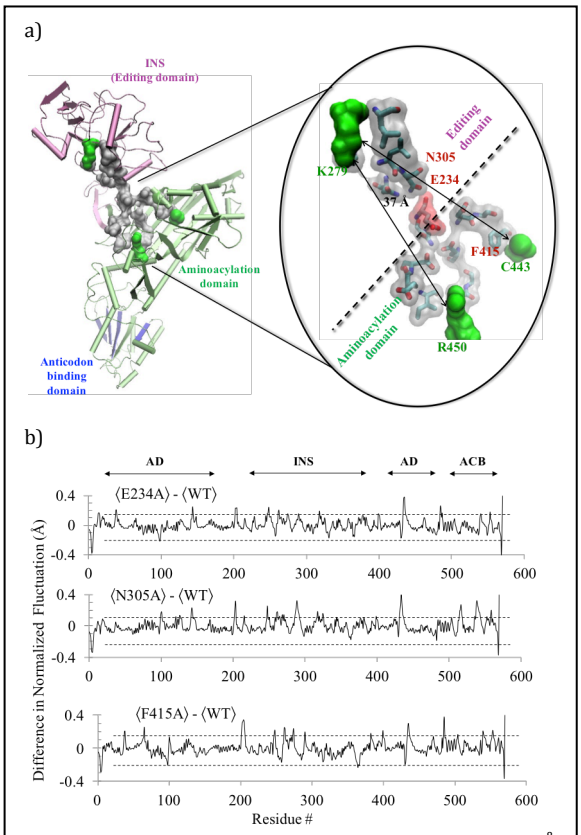


Fig. 1. a) Multiple pathways between two domains (> 37 Å apart) in Ec ProRS. b) Difference in backbone fluctuations between wild-type and ProRS mutants.

Matt C. Jewell (Materials Science) *Optimizing Superconducting Materials for High Energy Physics Applications*

Students in this REU will participate in our group's effort to understand the mechanical limits of bismuth-based high temperature superconducting composite round wires (Bi-2212). This material has realized significant gains in its ability to carry superconducting current in the last three years,[24] but its utility as a conductor in a particle accelerator magnet is limited by the brittle nature of the superconducting phase.[25] Our group is funded to investigate the mechanical performance of the material through the U.S. Dept. of Energy's *Early Career Research Program*, and the REU students will participate by developing metallographic polishing techniques to find and subsequently image defects such as current-blocking filamentary cracks in the wire. Of particular interest, the REU students will image the cracks using a three-dimensional technique enabled by our acquisition in fall 2014 of a scanning laser confocal microscope through the NSF's *Major Research Instrumentation* program.

David Lonzarich (Biology) *Fish Otolith Rings Relationship to their Survivability in Various Environments*

We are working on two university supported research projects in fish ecology. One project uses otoliths (fish ear stones) to answer questions about growth and survival of juvenile fishes in different freshwater environments.[26,27] Students work in the field collecting fish from lakes and streams, and in the lab measuring morphological traits and determining the age of fish from counts of otolith daily growth rings. Growth and survival patterns will differ among aquatic environments, species and individuals within species. My broad goal in this research effort is to use otoliths as a tool for characterizing patterns of juvenile fish growth and

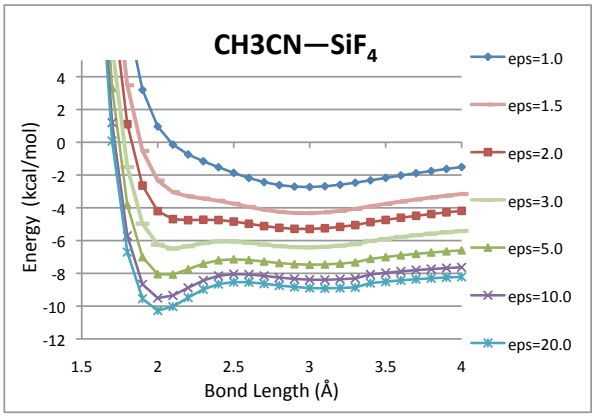
survival in different ecological contexts. The REU students involved in this project would work in the field and in the laboratory with a team of four UW-EC undergraduates. They would develop skills in fish sampling, in otolith microstructure preparation and analysis, and in fish morphological trait assessment. The second project focuses on characterizing and understanding population level variability in the occurrence of alarm cells in stream fish. Alarm cells are found in the epidermal tissue of minnows (Family Cyprinidae) and their close relatives.[28,29] Recent work concerning the function and evolution of these cells has focused on their potential importance in fish immunity. Students in this project will collect fish and habitat data from a variety of stream environments in northwestern Wisconsin for the purpose of identifying whether variability in the density of epidermal alarm cells of fish is linked to specific environmental conditions in these environments (e.g., parasite loads, stream degradation). As in the first project, the REU students involved in this research would work in the field and in the laboratory with a team of four UW-EC undergraduates. In the field they would develop skills in fish sampling and in stream habitat assessments. In the lab they would develop skills in histological preparation and microscopy.

Marc McEllistrem (Materials Science) *Characterization of Novel Nanostructured Materials*

There are two projects that are on-going in our lab. One (not described here) involves studies of graphene. The second involves the synthesis of metal nanoparticles (single-element and core-shell) using methanobactin (mb). Methanobactin is a 1154 Da "chalkaphore" (a copper ion chaperone molecule) made by methanotrophic bacteria. Our collaboration with Professor Alan DiSpirito at Iowa State has led student collaborators to determine the oxidation state of metal ions bound to mb using X-ray Photoelectron Spectroscopy (XPS), and has led to several publications (see McEllistrem biosketch). Less studied is the ease with which mb forms metal nanoparticles upon binding and reduction of some transition metal ions. To date, particle formation has been observed for Au, Ag, Pt, and Rh. Separate from these studies, metal-covered insulating nanoparticles (that is, "core-shell" nanoparticles) have received increasing interest for a variety of possible applications. Although gold covered particles are easily made, less attention has been devoted to other metals. Methanobactin can serve as an especially convenient aqueous reductant, but it is unclear whether ions reduced by mb will lead to core-shell particles or solid metal quantum dots. Students working on this project first prepare amino-terminated silica particles and reproduce literature results for gold and then explore other metal reduction (carried out in water). Reaction progress is followed at the bench by UV-Vis spectroscopy, with further characterization by XPS, SEM, powder X-ray diffraction, and AFM. A recent publication by us included 16 UW-EC undergraduate student co-authors (working on this research project as part of a course), in which many of the students used XPS to measure the oxidation state of copper ion bound to mb.[30,31]

Jim A. Phillips (Chemistry) *Condensed-Phase Effects on Nitrile-Group IV Lewis Acid Complexes*

This on-going project is concerned with the effects of bulk, condensed-phase environments on the structural and energetic properties of donor-acceptor complexes formed from nitriles and various Lewis acids.[32-34] Our primary tools are low-temperature infrared spectroscopy, which provides vibrational frequencies of these systems in inert matrices and solid thin films, and computations, which provide not only equilibrium structural properties, but also donor-acceptor bond potentials, both in the gas phase and in bulk dielectric media.[35,36] These data are key to assessing the condensed-phase response of any given system.



In the next phase of this project, we will work towards optimizing the condensed-phase response in a class of systems that shows great potential for medium effects: Nitrile complexes of Group IV tetrahalides (MX_4: M=Si, Ge, Ti; X=F, Cl) and their mono alkyl and mono aryl analogs (MX_3R). We have observed a propensity for medium effects in the CH_3CN complexes of the MX_4 Lewis acids, of which the best example is $CH_3CN–SiF_4$. A plot showing the Si-N bond potential, both in the gas phase and in bulk dielectric media is displayed above. The minimum in the gas-phase curve (top) occurs at about 3.0 Å, which reflects a weak, non-bonded interaction between CH_3CN and SiF_4. However, bulk dielectric media preferentially stabilize the inner region of the potential, and in turn, as the dielectric constant increases, a minimum develops near 2.0 Å, which ultimately becomes the global. The upshot is that the interaction with the medium

induces a major structural change, and now we will explore related complexes in search of similar behavior. The general strategy is to pair complexes of the weaker acids in this series with stronger nitrile donors (e.g. $(CH_3)_3CCN–GeCl_4$), and pair stronger acids with weaker nitrile donors (e.g. $FCH_2CN–TiCl_4$).

Thao Yang (Chemistry) *Synthesis of Mucin Peptides and Peptide-Antibody Interactions*

Our group has been studying linear mucin peptides interactions with specific monoclonal antibody (mAb) raised against the Variable Number Tandem Repeat (VNTR) region of MUC1 mucin (protein encoded by MUC1 gene). MUC1 mucin from healthy cells is a large glycosylated protein expressed normally on the apical surface of epithelial cells with thick carbohydrate chains compared to the one expressed in adinocarcinoma cells which is aberrantly glycosylated and overexpressed.[37] It is believed that the reduction of glycosylation in tumor MUC1 mucin results in exposure of the core peptide sequence which defines the epitope regions that elicit cellular immune responses. Thus, mucin peptides have been proposed to be used as antigenic agent against breast and colon cancers.[38] It has been discovered that the 5-amino acid (5-aa) sequence TRPAP at the 20-aa VNTR domain is the epitope required for recognition or binding to the specific monoclonal antibody SM3 expressed against MUC1 mucin in breast cancer cells.[39] There is evidence that the binding of mucin peptide to specific mAb is not of absolute specificity; for example, our recent peptide-mAb binding study, using Saturation Transfer Difference NMR (STD NMR) spectroscopy, showed that the sequence GVTSAPD (the sequence prior to TRPAP) resulted in binding and Pro6 is a critical residue required for binding, while the peptide lacking Pro at position 6 did not exhibit any binding to the antibody (see Figure 2).

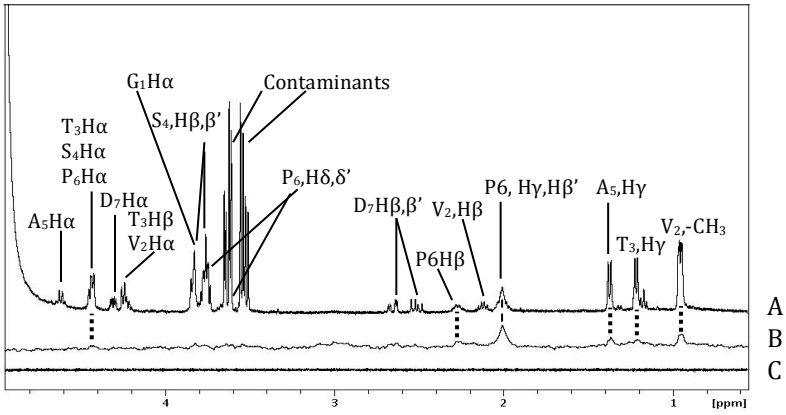
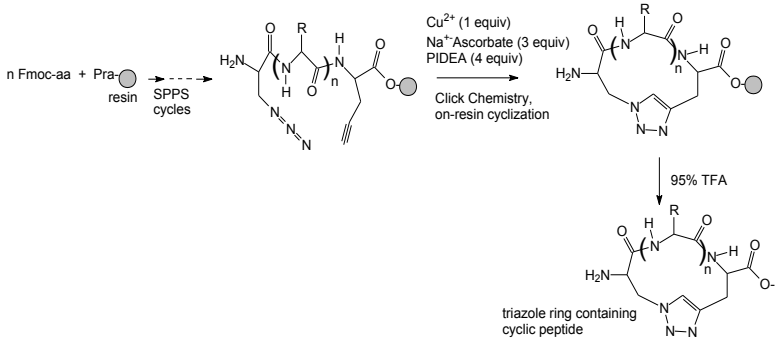


Figure 2. STD 1D ^{1}H NMR data showing which ^{1}Hs from the linear mucin peptides directly bind to mAb in phosphate buffer, pH 7 at 7 °C. The traces are: A). 1D ^{1}H NMR spectrum of a mixture of mAb and linear peptide (GVTSAPD) showing the ^{1}H resonances of the peptide on top of the unresolved resonances (broad-hump) of mAb (1000 μM peptide, 10 μM mAb); B). STD NMR spectrum of peptide plus mAb mixture showing mostly the STD effect of ^{1}Hs of Pro6 residue that are directly bound to mAb and some STD effect from the CH_3- groups of Val2, Thr3, and Ala5. C). STD NMR spectrum of GVTSADD peptide mixed with mAb; there is no binding, so no STD peaks. Note the huge contaminants in trace A do not bind to the mAb, thus are all subtracted out in trace B.

We will synthesize several MUC1 mucin peptides (both linear and cyclic peptides) with mutation at the binding epitope, follow by investigation of their binding properties to specific MUC1 mucin mAb. Students will use the Solid-Phase Peptide Synthesis (SPPS) method employing standard Fmoc chemistry to synthesize peptides;[40] they will use HPLC, LC-MS and NMR to collect and analyze data.[41] One type of cyclic peptides that Dr. Yang's group will synthesize contains the side chain-to-side chain five membered ring 1,4-disubstituted 1,2,3-triazole group, cyclized by the Cu(I)-catalyzed alkyne-azide cycloaddition (e.g. *Click reaction)*.[42] The reaction scheme shows the cyclization strategy employing the *Click* reaction.



Note: References to the articles are available upon request.