2019-2020

UNDERGRADUATE RESEARCH PROGRAM

Brigham Young University
Department of Chemistry and Biochemistry
2019-2020

UNDERGRADUATE
RESEARCH PROGRAM

Department of Chemistry and Biochemistry
Brigham Young University

The Department of Chemistry and Biochemistry has a long tradition of undergraduate involvement in research with our faculty. Students gain valuable experience as they join graduates and undergraduates in ongoing programs.

For more information about the research described in this booklet, talk directly to the professor or visit chem.byu.edu/faculty.
Contents

Undergraduate Research Awards (URA) 4
Y-Chem Society 5
Research Facilities 7

FACULTY RESEARCH PROFILES

Joshua L. Andersen, PhD 9
Merritt B. Andrus, PhD 11
Matthew C. Asplund, PhD 13
Daniel E. Austin, PhD 15
Steven L. Castle, PhD 16
Kenneth A. Christensen, PhD 18
Daniel H. Ess, PhD 23
Steven R. Goates, PhD 24
Jaron C. Hansen, PhD 28
James Harper, PhD 31
Roger G. Harrison, PhD 33
Jeremy A. Johnson, PhD 35
Matthew R. Linford, PhD 40
David J. Michaelis, PhD 42
Dr. James D. Moody 45
J.C. Price, PhD 55
Joshua L. Price, PhD 57
Paul B. Savage, PhD 61
Eric T. Sevy, PhD 63
Kara J. Stowers, PhD 65
Pam M. Van Ry, PhD 67
Richard K. Watt, PhD 69
Barry M. Willardson, PhD 72
Brian F. Woodfield, PhD 75
Undergraduate Research Awards (URA)
The Department of Chemistry and Biochemistry has a long tradition of undergraduate involvement in research. Students gain valuable experience as they join graduates and undergraduates in ongoing research programs. Any student currently working in a research group has the opportunity to apply for an Undergraduate Research Award. A student may apply for an Undergraduate Research Award for Fall and/or Winter Semester and for Spring/Summer Terms.

How to Apply
- Be currently working in a research group.
- Attend a grant writing workshop:
  - First time applicants are required to attend the workshop; other applicants are also welcome. Faculty will be available to lead a discussion on how to write a high-impact aims page and how to incorporate any preliminary data you already have into the proposal. You are expected to already have a rough draft of your proposal by the time of this meeting. The day/time for the workshop will be advertised.
- Prepare a grant proposal aims page:
  - The URA application process will teach students to write a one-page overview of an NIH-style grant. This overview is called an “aims” page.
  - Why write a grant proposal? Prior to performing research, an investigator must secure funding. Funding covers the cost of research associates (postdocs, graduate students, and undergraduate students), supplies, and all other necessary items. Generally, funding is secured through a grant application to a foundation or a government agency such as the National Institutes of Health (NIH), National Science Foundation (NSF) or Department of Energy (DOE). Because funding has become increasingly competitive to secure, it is critical to learn excellent grantsmanship—the art of writing a grant.
- Application Process: Follow the instructions on the Chemistry and Biochemistry Website (www.chem.byu.edu) On the top ribbon, go to Undergraduates, then Undergraduate Research Awards. There you will find the three step process to apply for a URA.
  1. Complete "My Profile" (Includes uploading a photo, preferably of you working in your lab) at http://mentoring.byu.edu
  2. Complete "Application" (found on the dashboard) for each semester/term you apply, at http://mentoring.byu.edu
  3. Complete "URA Cover Page" and upload your proposal at https://goo.gl/forms/IPW0reZP5YqT2ryh2

Proposals will be read and evaluated by the Undergraduate Research Award Committee. Students will be notified of the outcome by email. If you have questions, please see the Administrative Assistant in C104 BNSN.
Y-Chem Society

Y-Chem is the student chapter of the Central Utah Section of the American Chemical Society and is designed to help BYU students succeed in a challenging scientific environment. Though the focus is on chemistry and biochemistry, students of every major are welcome.

Y-Chem is run by a group of students who are passionate about chemistry. They strive to share their love of science with others, while helping them with challenges they may encounter along the way. In addition to their efforts in planning events, they are good resources for questions that students may have. They also work closely with several professors.

One of Y-Chem’s main purposes is to help students succeed in their chosen discipline. Accordingly, many of the activities are directed to this end. Some examples of past activities include fundraising to sponsor students attending national meetings, graduate school preparation, and tours of academic and industrial science laboratories.

Another important purpose of the club is community outreach. The students are passionate about science and want to help kindle that flame in others. Y-Chem members receive the opportunity to be trained to perform chemistry “magic shows.” Once trained, these members perform dazzling chemistry demonstrations both on and off campus for a variety of audiences. They also participate in judging science fairs, as well as Undergraduate Research Night and the BYU major fair. In addition to these activities, Y-Chem also puts on an annual community outreach event called Open Lab Day. During Open Lab Day, Y-Chem members engage with junior high and high school students by helping them perform exciting science experiments.

The international chemistry community is relatively tight-knit. It is quite possible that today’s classmates will become tomorrow’s colleagues and employers. Y-Chem sponsors several social activities each year in order to promote networking amongst peers. In addition to meeting and associating with people on similar career paths, Y-Chem offers its members opportunities to interact with professors. These professors often become valuable contacts and can offer excellent career advice.
Y-Chem’s activities aren’t all business. Y-Chem also seeks to have activities that are just plain fun—like the annual Nerd Dance.

You are invited to join Y-Chem. It provides a great opportunity to associate with peers and professors as well as to learn, grow, and serve. Y-Chem strives to be as beneficial as possible and they are always excited to hear what suggestions members have so that they can better serve them.

To join or to find more information, please visit ychem.byu.edu or contact me at myna_reader@yahoo.com.

Sincerely,

Megan Porter, President
Research Facilities

Research activities occupy more than 50 percent of a 192,000-square-foot building. The University library, where the science collection includes more than 500,000 volumes and about 9,000 journal subscriptions, is located about 150 yards away.

Major equipment available in the department includes NMR (200, 300, and 500 MHz); mass spectrometry (high-resolution, quadrupole, ion cyclotron resonance, ToF-SIMS, and MALDI); X-ray diffraction (powder and single crystal); spectrophotometry (IR, visible, UV); lasers (YAG, gas, excimer, Ti-sapphire and dye); separations—including capillary column GC/MS, ion, and supercritical fluid chromatography; capillary electrophoresis; particle size analyzers; environmental chambers; ICP; thermodynamics (calorimeters of all types, including temperature and pressure scanning, titration, flow, heat conduction, power compensation, combustion, and metabolic); and molecular biology (DNA synthesizer and sequencer, phosphorimager, tissue culture facility, recombinant DNA facility, fluorescence activated cell sorter, and ultracentrifuges).

All computing facilities are fully networked, including computational chemistry and laboratory workstations as well as office personal computers, with convenient connection to supercomputing facilities and the internet. Fully staffed shops for glassblowing, machining, and electronics also serve research needs.
FACULTY RESEARCH PROFILES
Nearly half of us will have cancer in our lifetime and for the majority of patients, cytotoxic chemotherapy is the primary treatment option. The goal of these treatments is to induce tumor cell death. However, these therapies are often ineffective because tumor cells possess the dynamic ability to subvert cell death and become chemoresistant. With this in mind, our research combines molecular and proteomics approaches to understand the mechanisms by which tumor cells gain resistance to cell death and chemotherapy, with the ultimate goal of developing therapeutic approaches to overcome chemoresistance and improve clinical outcomes for patients.

Our recent work has focused on the dynamic phospho-binding protein 14-3-3ζ and its role in promoting tumor cell growth and survival. As part of this effort, we have harnessed 14-3-3ζ proteomics as a tool to identify novel tumor cell survival mechanisms. This work uncovered a mechanism by which tumor cells “switch on” a catabolic process called autophagy, which allows the tumor cell to survive through periods of stress commonly encountered in vivo. Another interest of our lab is to understand how acetylation of non-histone proteins regulates apoptotic/survival signaling in tumor cells. Since the experimental tools to study acetylation are limited, a critical part of our early effort has been in developing new tools to understand acetylation. These include comparative acetyl-proteomics approaches, biotin-switch methods to identify deacetylase substrates, and site-specific acetyl-lysine antibodies. Using these tools, we recently uncovered a mechanism by which the pro-tumor activity of 14-3-3ζ is governed by acetylation. We now have the ability to “turn off” 14-3-3ζ activity in tumor cells by modulating the enzymes that govern 14-3-3ζ acetylation, which we are now evaluating as a potential therapeutic approach in breast cancer.

Your research in my laboratory will expose you to current issues in the cancer field, and will teach you the molecular and biochemical tools to solve pressing research questions. Dedicated students will also get the chance to be a part of the larger scientific community through research presentations at
international meetings, and preparing and submitting research manuscripts to peer-reviewed journals. I encourage interested and highly motivated undergraduates to apply for research opportunities in our laboratory.

References


Merritt B. Andrus, PhD

*Organic & Biomolecular Chemistry*
C410 BNSN, 422-8178
Email: mbandrus@chem.byu.edu

**Natural Product Synthesis**

Efforts in our lab are focused on methods for the synthesis of biologically-active natural products that possess unique structures and potential for combinatorial library construction and screening. New methods include metal-catalyzed couplings and condensations to assemble key intermediates. Libraries of structural variants are then made and used to probe receptor binding and improve activity.

Recent work includes the synthesis of the polyene stipiamide, a new agent to treat multidrug resistance (MDR); geldanamycin A, a large anticancer macrocycle; englerin A, a terpene based anticancer agent; and resveratrol, a small disease preventative stilbene.

Resveratrol, a simple, yet very important target, is the suspected causative agent of the “French Paradox.” Diets rich in foods that contain this material, grapes in particular, lead to lower rates of cancer and heart disease. New coupling methods and strategies developed to produce this material will now be used to produce structural variants for various screens. New targets now include F4-4, an antiviral lignin
natural product that inhibits herpes and shingle infection, and simplified analogs of englerin A. General synthetic methods with broad application are also under development using new ligands for asymmetric styryl Diels-Alder and aldol transformations.

Dedicated undergraduate students including beginning students are welcome to participate in all aspects of the work.

References


The development of short-pulsed lasers, from 10 femtoseconds (10 x 10^{-15} s) to nanoseconds (10^{-9} s) has allowed for unprecedented information into the dynamics of chemical reactions. With pulses of light this short we can easily measure the spectra of chemical intermediates in condensed phase (primarily liquid solution) chemical reactions. A first photon (usually in the visible or UV region of the spectrum) begins the reaction, and the intermediates can be monitored on a number of time scales in the infrared to give structural detail. We have used this instrumentation to study a class of organometallic intermediates important in chemical catalysis. The reaction begins when a photon of UV light causes one ligand to dissociate from a metal center to form a metallic radical. On a very short time-scale, this unsaturated metal center forms a complex with a neighboring solvent molecule. Over time, this complex exchanges with other solvent molecules until it finally decomposes after 5-10 seconds. By following the infrared spectrum of the complex, we can measure the dynamics and binding energy of these weak complexes, and compare them with quantum chemical calculations.

**Model Ring Formation Reactions**

One area of particular interest in my lab is reactions involving organometallic species involved in the formation of new carbon-carbon bonds and the formation of rings. An interesting class of reactions is labeled Pauson-Khand reactions. In its most general form, it is the reaction of an alkene, and alkyne a carbonyl to form a 5-membered cyclopenteneone ring.
The reaction proceeds thermally, and in order to follow the reaction with time-resolved spectroscopic methods, we use a variant of the reactant that combines the alkene and alkyne in the same molecule.

The reaction mechanism shows that the first step is the removal of a CO from the Mo(CO)$_6$, followed by formation of a complex between the Mo(CO)$_5$ and the complex, followed by formation of the ring. We are trying to establish which part of the ligand attaches to the metal first.

**Bi-metal catalyst systems**

One of the difficulties in current catalytic systems is that they usually require use of a rare and expensive metal atom. There is tremendous interest in using bimetallic systems where the two atoms act cooperatively to give reactions that are similar to rare metals. While there are many catalytic reaction studies that have established the viability of this approach, there is little known about the details of the reactions. We are applying our transient infrared spectroscopy to these bi-metallic systems to try to understand how these cooperative systems drive chemistry.
Chemistry and Biology of High-Velocity Impacts: Simulating Space Processes

We are developing experimental tools to explore high-velocity impacts—particularly those of molecules, sub-micron particles, and microorganisms—on surfaces. In such impacts, the kinetic energy of the impacting species is converted to vibrational modes, which can drive both physical/biological changes and chemical reactions. As an example of a project in this area, we are building an ultra-fast rotor that allows molecule-surface impacts at 2-4 km/s. This is faster than can be studied using any other technique. The resulting molecular fragmentation is studied using gas chromatography mass spectrometry. This project has application to the study of planetary exospheres using mass spectrometers on flyby or orbiting spacecraft. We are also studying the impact survivability of bacterial spores, with the goal of exploring possible transfer of bacteria in space. Finally, we are looking at high-velocity impacts of aerosols and icy grains to gain understanding of space measurements of particulates.

Undergraduate students receive training in building scientific equipment, particularly vacuum systems and mass spectrometers. Any chemistry, physics, or engineering students who have completed their first two years of undergraduate study are invited to join.
Our research focuses on the total synthesis of complex bioactive natural products and peptides. The structures of such compounds serve as inspiration for the invention of new organic reactions and processes. Additionally, studies of their bioactivity can increase our understanding of their modes of action, potentially leading to the design and development of new therapeutic agents. Some of our recent synthetic targets are shown below.

The new reactions that we develop in the course of synthesizing a target compound are fully investigated with respect to scope and mechanism. It is our aim to develop widely applicable processes that deliver complex products from simple starting materials in a minimum number of steps. We also believe that it is important to understand how these processes operate.

We frequently synthesize structural analogues of the target natural products or peptides. This allows us to elucidate the modes of action of these compounds, often in collaboration with biological and biochemical research groups. We are also engaged in finding new ways to stabilize peptides to proteolytic degradation, thereby increasing their potential as drugs.

Students in our group receive rigorous training in the techniques of organic synthesis and structure determination. In addition, they learn the more general, widely applicable skills of strategic planning and problem solving. Furthermore, in the course of presenting their research in verbal and written formats, they acquire valuable communications skills. Prior to joining our group, students should have completed Chem 351, 352, and 353/354 (concurrent enrollment in 352 and 353/354 is acceptable).
References


My lab works in the fields of biochemistry and bioanalytical chemistry. We develop methods that apply optical spectroscopy, flow cytometry, time-lapse microscopy, and other current analytical and biophysical techniques to questions in biochemistry, biophysics, cell and microbiology.

A current area of research in my lab grew out of our discovery several years ago that the anthrax toxin receptors capillary morphogenesis gene protein 2 (ANTRX2/CMG2) and tumor endothelial marker 8 (ANTRX1/TEM8) were involved in angiogenesis in pathological conditions in the eye and in tumor models. Our current work focuses on identifying and characterizing critical extracellular ligands of the CMG2 and TEM8, which are thought to be extracellular matrix proteins. In addition, we are also identifying intracellular interactors via proteomics using mass spectrometry. Together, the lab is trying to address a critical barrier to progress in this field by identifying the role these cell surface receptors play in angiogenesis and developing a model that can be tested using traditional biochemical approaches. In parallel with these efforts, we also have some small molecule and peptide scaffolds that are being tested for activity in a corneal neovascularization animal model and for other pathologies of angiogenesis.

A second project focuses on measuring and monitoring the dynamics of metabolism in eukaryotic parasites. For example, in Trypanosoma brucei (the causative agent of Human African Trypanosomiasis), the sole source for generating ATP during the infectious lifecycle stage of the African trypanosome occurs exclusively in a unique peroxisome-like compartment called the glycosome. We are developing and using both recombinant protein-based FRET sensors and peptide-targeted small molecule sensors to quantitatively measure intraglycosomal pH, glucose, and ATP levels in live parasites. We are interested in the mechanisms the organism uses for regulation of pH, glucose, ATP production, and other important metabolites. Since glycolysis is key to parasite survival, inhibiting glycolysis in the glycosome could be an excellent targeted therapeutic approach for treatment of African Trypanosomiasis. Other parasites of interest are Leishmania donovonii and Trypanosoma cruzi.
Note: I am willing to work with beginning students; however, I am not accepting students for Fall 2019. Please contact about possibilities for joining the lab in Winter or Spring/Summer 2020.

**Selected References**


Back to top
Our group uses one of the most powerful types of mass spectrometry, combined with molecular modeling using high-end supercomputers, to develop methods for characterizing molecule-sized devices. My goal is to give students a real taste of the kind of work done by researchers on the cutting edge of science, culminating in publication and/or presentation of the results. Our group has an excellent track record placing undergraduates who desire additional training in some of the world’s top graduate programs. Projects will be selected based on the student’s level of preparation. All of our work includes strong possibilities for collaboration with other groups working in related areas.

**Tertiary Structure from Mass Spectrometry: A “CRAFTI” New Method**

Tertiary structure (the way a molecule is folded, resulting in its overall shape) is extremely important to molecular function in such diverse areas as biochemistry, catalysis, and the assembly and function of molecular nanomachines. Therefore, it is important to develop ways to determine tertiary structure, and to do so with very small samples. Although mass spectrometry is a powerful, sensitive technique for characterizing the atomic composition and connectivity of atoms within molecules, it usually yields no information about tertiary structure. We recently invented a new technique for obtaining tertiary structural information using Fourier transform ion cyclotron resonance mass spectrometry; we call the technique "CRAFTI" (from cross sectional areas by Fourier transform ion cyclotron resonance). Interested students will explore the strengths and limitations of this new technique, and develop supramolecular chemistry applications for it, supported by funding from the National Science Foundation. Students who are at least concurrently registered for Chem 461/462 will be most successful in these projects, although motivated students with less preparation (as little as Chem 111 or Chem 105) can also do excellent experimental work. Commitments of about 10 hours per week are generally required to make meaningful progress on experimental projects.
Visualization and modeling of molecular systems is an essential part of our research. Software packages such as SPARTAN, ECCE, NWChem, and IMoS will be used to model the same host-guest complexes we are studying experimentally. Most of these software packages have intuitive, graphical user interfaces that make them easy to operate. Goals of the modeling projects include determination of low-energy structures and energies for the complexes, dynamic simulation of the complexes, calculation of vibrational frequencies that can then be used as input to statistical mechanics programs, and calculation of collision cross sections for comparison with experiments. Much of this work is computationally very demanding and will require use of campus supercomputers. No prior knowledge of either modeling methods or computer operating systems is needed, but students will need to learn to be comfortable with UNIX. Students taking Chem 351/352 have sufficient background to carry out these projects successfully, and motivated students who are at earlier stages of their preparation will also be
able to make important, significant contributions. Again, to make meaningful progress on these projects students will need to commit to about 10 hours per week.

The undergraduates involved in this work will have full access to our state-of-the-art equipment. We have a well-equipped research lab centered around a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer with a 4.7-Tesla superconducting magnet and an external ion source equipped with electrospray and sonic spray ionization modules (Bruker model APEX 47e). All of this equipment is computer-interfaced and script-controllable, allowing very versatile experiments to be designed and performed. For students with good mechanical or programming skills interested in building instruments, we currently have ongoing needs for instrument control and data analysis software development.

References


If you like computers and chemistry, my group is the place to make discoveries. My group uses and develops quantum-chemistry and molecular dynamics methods to discover mechanisms, reactivity principles, and selectivity for experimentally important chemical reactions related to catalysis and energy. In practice this means using supercomputers and software to accurately model chemical reactions. My group emphasizes making predictions and designing catalysts that are then realized in the laboratory. This naturally leads to close collaboration with experimental groups in academia and industry. My group publishes several top-tier publications each year and undergraduates are very often co-authors. Current areas of research involve: (1) Computational catalyst design with industrial application. (2) Computational studies of alkane C-H functionalization reactions. (3) Computational studies on multinuclear catalysis. (4) Developing programs and running molecular dynamics simulations of organometallic reactions. See my webpage for a video and project descriptions: https://www.chem.byu.edu/faculty/daniel-h-ess/.

Undergraduate chemistry, biochemistry, engineering, physics, biology, and computer science students in my lab have a range in backgrounds, from a computer science minor to no programming experience. To be successful, you need to have a desire to learn inorganic and organic chemistry, develop computer skills, and work hard (15-20 hours per week in Fall/Winter and 30-40 hours per week in Spring/Summer).
Lasers have opened up new realms of study in virtually every field of science. My students and I employ lasers and spectroscopic techniques in a variety of studies, some of which are described below. The work is challenging, but I do not expect that students will enter my program with any background in this field of research. An eagerness to learn, self-motivation, and a readiness to tackle unfamiliar problems are the primary qualifications.

**Spectroscopy at surfaces and interfaces**

Unusual effects can be achieved with lasers. Among these effects are *non-linear wave generation* in which new beams are generated when two or more laser beams converge on a sample. An example is sum frequency spectroscopy (SFS) pictured in Figure 1. Another experiment of this type is coherent anti-Stokes Raman spectroscopy (CARS). Not only are such experiments impossible to do with ordinary light, but they reveal details about chemical structure at surfaces and interfaces (where much of the action in chemistry takes places).

**Spectroscopy for Medical and other Diagnostics**

Surface-enhanced Raman spectroscopy (SERS) is another technique we are exploring as a means to monitor biological species, such as insulin. An inexpensive method for measuring insulin would provide for early detection of diabetes, for example.

---

*Figure 1. Schematic depiction of an SFS experiment. Two laser beams at different colors are mixed at the sample, generating a new beam at a third color.*
Raman and Laser-Induced Fluorescence Spectroscopy of Mobile Phase Behavior

We have long had an interest in understanding the physical processes involved in chromatographic separations. At present we are particularly interested in what happens to the mobile phase in solvating gas chromatography (SGC) during the course of a separation. SGC is a high-speed separations method in which the mobile phase is in a supercritical state (i.e. above its critical pressure and temperature) at the head of the column, but converts to a gas at the end of the column. To take full advantage of this separation method it is important to determine how the mobile phase changes along the column and at what point it converts to a gas. We are using Raman spectroscopy to answer these questions because Raman allows the mobile phase to be probed without disrupting its flow. Beyond this particular study, we believe Raman will prove to be a valuable probe of a number of mobile and stationary phase changes in real chromatography columns.

Environmental Research

Collaborative projects with Dr. Jaron Hansen involve studying formation of air particulates and investigating capture and conversion of carbon dioxide.
Steven W. Graves, PhD

Biochemistry
C212 BNSN, 422-2148
Email: swgraves@chem.byu.edu

Serum Proteomics, Peptidomics and Lipidomics of Various Diseases, Including Preterm Birth, Preeclampsia, Alzheimer’s Disease, and Endometriosis

For several years those working in my laboratory have developed and applied one or more serum “omics” methods that typically incorporate capillary liquid chromatography coupled to mass spectrometry. Using this approach we can typically interrogate ~7,000-8,000 molecules in a small volume of blood. We have found quantitative differences in low abundance serum peptides, small proteins and lipids in pregnant women who later experience complications compared with women having uncomplicated pregnancies. We have identified several molecules, weeks prior to preterm labor, which are quantitatively different in women who will deliver early. Different sets of “markers” allow us to predict 85-90% of those pregnant women who will develop this complication. We have conducted other studies using this same approach and have found several biomarkers that predict which pregnant women will later develop a very serious complication of pregnancy termed preeclampsia. Using combinations of our biomarkers we can predict ~90% of women who will later develop preeclampsia. More recently, we used our serum proteomic approach to study Alzheimer’s disease (AD) and have found dozens of biomarkers that allow for the correct diagnosis of AD and even allow us to distinguish very early stage disease. Some of these biomarkers may even allow for the prediction of those who might develop this serious disease. Other markers appear to be specific to women or men with AD which may help to explain gender differences in AD. Currently, we are evaluating serum peptide and lipid markers for endometriosis, a complication affecting up to 10% of women of child bearing age that results in chronic or intermittent severe pain and frequently infertility in these women. Today, the diagnosis requires surgery but preliminary data from our “omics” approach suggests that biomarker panels can be found that diagnosis endometriosis biochemically without surgery.
References


Research in the Hansen group is divided into three elements: (1) Kinetics and Spectroscopy of Environmentally Important Reactions (2) Air Sampling Campaigns (3) Biofuel/Alternative Energy. Our group couples together computational and experimental studies to investigate the kinetics and mechanisms of important atmospheric reactions. Laboratory studies are complimented by air sampling campaigns designed to investigate the sources of air pollution. We utilize an environmental chamber to aid in the interpretation of our air sampling campaign studies. We also have an active research element in our group that studies the conversion of biomass into energy. Details about his group’s efforts in element 1 are included below. Interested students are encouraged to contact Dr. Hansen with questions about his research in Air Sampling Campaigns and Biofuel/Alternative Energy. Undergraduate students with at least two years of classwork are often utilized as research assistants in his group.

Kinetics and Spectroscopy of Earth’s Atmosphere
Aerosols are defined as solid or liquid particles suspended in air. Aerosols affect visibility, human health, and climate. Primary aerosols are released directly into the atmosphere from both biogenic and anthropogenic sources. Secondary aerosols form in the atmosphere as a result of physical and chemical processes. Formation of secondary aerosol particles is frequently modeled with classical nucleation theory (CNT). The first step in CNT is the nucleation step where molecular clusters first form and then grow in size until they reach the critical cluster size. The critical cluster size is defined as the maximum in the Gibb’s free energy curve (Figure 1). The second step in CNT is growth of the critical cluster through nucleation.
coagulation or condensation. Using this model, current predictions of atmospheric aerosol content underestimate measured concentrations of aerosols in the atmosphere. This discrepancy highlights our lack of understanding of the sources and formation mechanisms for aerosol particles in the atmosphere.

In the Hansen lab we expand on our previous computational and experimental work by using a variety of amines and carboxylic acids that are found in the atmosphere in the presence of water vapor to measure aerosol formation rates as a function of temperature, water vapor, amine, and carboxylic acid concentrations. These systems are explored both computationally and experimentally.

Experimentally we use an in-house designed instrument to measure the kinetics of particle formation. Figure 2 shows a schematic of the instrument. The instrument is modeled after the instrument described by Karlsson et al., but with expanded capability. The heart of the instrument is a 180 cm long Pyrex slow-flow reactor cell (i.d. 5.1 cm) coupled to two scanning mobility particle sizer (SMPS) detectors on the downstream end of the cell. Gases are introduced at the top of the reaction cell, Figure 2. Amines are introduced at varying points in the flow cell by use of a Teflon-coated shower ring attached to a stainless steel sliding rod. The shower ring (i.d. 4.5 cm) with pin holes allows for movement up and down the flow cell, Figure 3. This method of introducing amines allows for reaction times varying between 8 s to 48 s. The concentration of gases in the cell is varied by controlling the flow of gases using mass flow controllers. Their concentrations are measured by UV absorption spectroscopy to determine carboxylic acid and amine concentrations and NIR spectroscopy for detection of water vapor.

At both ends of the tube, aluminum blocks serve to support the reaction tube and provide holders for CaF$_2$ windows to introduce UV and IR light coaxially with the flow of gases into the reaction cell. Optics collimate the light from a 100 W D$_2$ lamp and direct it through the reaction cell and into a temperature-
regulated CCD detector. The concentration of particle precursors, like carboxylic acids and amines, in the cell is accomplished by UV absorption spectroscopy with published absorption cross-sections.

The carboxylic acid reacts with water vapor and an amine in the flow cell to form a carboxylic acid-water-amine complex (figure 4) that serves as the first step in aerosol formation. This complex further reacts with water molecules and/or other complexes/clusters in the flow cell to form particles. The number of particles formed depends on both the reaction time available and the concentration of precursors in the reaction cell. Both the size distribution and absolute number densities of particles is measured at the exit of the flow cell by two SMPSs.

Figure 4: Optimized geometry of formic acid-water-trimethylamine complex computed at the M062X/aug-cc-pvdz level. The principle hydrogen-bonds in the complex are shown (dashed lines). Bond lengths are reported in angstroms.
The primary aim of research in our laboratory is the NMR characterization of structure in solids that are difficult or impossible to examine by more conventional techniques. The relevance of our methods is, perhaps, best demonstrated by our recent characterization of the complete crystal structure of (+)-catechin, a ubiquitous and extensively studied antioxidant that had defied characterization for nearly a century.¹ This NMR work emphasizes 13C and 15N shift tensor measurements and relies on computational chemistry methods to tie these results to structure. This area is now being call “NMR crystallography”. A secondary area of emphasis involves the search for novel bioactive fungal products with a special focus on identifying unusual antioxidants. Work is each of these areas is described below.

**NMR crystallography** – prediction of complete crystal structures for intractable materials. Conventional crystallographic techniques are often unable to provide structure in materials. The typical limitation lies in difficulty in growing suitable crystals. We have recently demonstrated that accurate crystal structure can often be established without single crystals or even diffraction data if a combination of solid-state NMR and a theoretically crystal structure prediction process are used.² Remarkably, the structures now being solved in our lab with this methodology and powdered solids rival or surpass the accuracy of single crystal diffraction data.³ This approach promises to open new areas of structural analysis in challenging materials and studies are underway with the goal of characterizing high profile pharmaceuticals that have long defied traditional analysis.

**Design of superior free radical scavengers.** Some effort in our lab involves searching for bioactive fungi within higher plants (i.e. endophytic fungi). These “bioprospecting” studies have now identified several fungal products that have superb antioxidant activity.⁴ Some of these molecules have unique modes of action as indicated by mechanistic studies involving computational modeling. Presently the products we have examined are unsuited for pharmaceutical use, but it appears that the structural features creating the activity can be reproduced in more favorable products. Computational testing is
now underway on a wide variety of candidate antioxidants with the goal of identifying superior radical scavengers with more favorable biocompatibility.

Citations.

Molecular Binding and Encapsulation

The supramolecular interactions between a host molecule and a guest molecule allow for such interactions as selective binding, chiral recognition, and separation. Our introduction into the field of host-guest complexes came with the discovery in our group of a metal-assembled capsule, consisting of two synthesized cup-shaped molecules brought together by metal ions. Along with the capsules, we have formed metal-resorcinarene complexes with various metal ions such as iron, cobalt and copper. We are now pursuing with interest host molecules that will selectively bind one enantiomer preferentially over another. We are also exploring the synthesis and properties of larger host molecules and their ability to encapsulate water contaminants. Students working on this project learn to synthesize and characterize organic and inorganic compounds and use them to bind or encapsulate other molecules.

Cavitand molecule with alanines along its upper rim.

Separations of Contaminants Using Macroyclic Compounds

Another related area of research we are pursuing is the application of cavitands in separations. Small quantities of molecules are harmful to us as water contaminants or unwanted substances in our body. Students in our group use ion chromatography to detect and quantify anions, cations, pharmaceuticals and biological compounds. To do this they pack columns with cavitands and perform separations using an ion chromatography instrument. Students become experts in separation techniques and use their skills to analyze molecules in tap water, river water, and biological fluids.
**Metal Compounds Designed to Combat the Flu**

The flu virus has a protein channel used for proton movement that is essential for viral replication. Students in our lab design, synthesize, characterize and test metal containing compounds for blocking the protein channel. We have found neutral copper compounds are able to block the channel and inhibit viral growth. Students working on this project learn organic synthesis techniques and how to characterize compounds by NMR, MS, ICP, and IR. This is a collaborative project with the Busath lab, whose students test the compounds for viral activity.

**Nanomaterial Synthesis and Properties**

Materials with subunits in the nanometer range are being studied for their semiconductor and energy transfer properties. Members of our group synthesize nanoparticles, nanoprisms, and nanoplates made of ZnO and investigate their light absorption and emission properties, as well as gas adsorption. Students on this project synthesize new nanomaterials and while characterizing them, learn to operate many instruments, such as XRD, SEM, TEM, ICP, Uv-vis and NMR.

**References**


2. T. Panahi, D. J. Weaver, J. D. Lamb, R. G. Harrison “A new approach for trace analysis of guanidine compounds in surface water with resorcinarene-based ion chromatography columns” *Analyst*, 2016, 141, 939-946.


Jeremy A. Johnson, PhD

*Physical Chemistry*
C312 BNSN, 422-0245
Email: jjohnson@chem.byu.edu

*Creating Ultrafast Spectroscopy*

Light can be a wonderful tool to measure all sorts of fascinating material properties, but there is one important truth all spectroscopists keep in mind: light only cares about the optical properties of a material! In order to use light to learn about a whole host of material properties, the radiation must couple to the material property of interest. But oftentimes the optical properties are coupled to many material properties and understanding what we see can be difficult. Therefore, making measurements more “selective” to the property or dynamics of interest is crucial.

“Selectivity” in spectroscopy can be achieved in a number of ways. Perhaps the most straightforward is by simply changing the wavelength (color) of electromagnetic radiation we use, from x-rays to radio waves. In the Johnson Spectroscopy Lab, we focus on experiments using ultraviolet, visible, and infrared radiation. In addition, we have a strong emphasis on using terahertz (THz) radiation, an exciting region of the electromagnetic spectrum that lies just beyond the infrared, with wavelengths from 3 mm to 30 μm corresponding to frequencies from 0.1 to 10 THz (1 THz = 10¹² Hz). These frequencies are associated with the time scales of atomic vibrations in solids, the lifetimes of excited electronic carriers in some materials, electronic spin which gives rise to magnetism, and other dynamic properties we can study in solids, liquids, and gases. New high field THz sources are under development.

In typical “pump/probe” experiments to measure time-dependent laser-induced dynamics, thousands to even millions of laser shots are used to record the sample response. This requires the sample to return to exactly the same state after every single laser shot. In the Johnson spectroscopy lab
we are also developing what is called "single-shot probe" measurements, where all dynamics are recorded in a single laser shot. This opens up new possibilities to study irreversible dynamics central to laser processing of surfaces, light induced damage, and ultrafast phase transitions. Additionally, single-shot measurements can even expedite the collection of typical pump/probe data in normal, reversible measurements.

**Using Ultrafast Spectroscopy**

Ultimately, spectroscopy is a tool to study and control systems of interest. We study materials and processes that have promise to be used as ultrafast switches in the next generation of computing devices, as well as nanoparticles and layered hetero-structures with interesting properties relevant for energy production and catalysis.

We use high field THz pumping in tandem with single-shot probing to excite and control quantum mechanical modes coupled to macroscopic properties. We also use excitation light with wavelengths from the UV to IR to investigate and influence carrier dynamics, surface states, and energy flow in nanomaterials, which we can probe with optical light or THz radiation.

Note: I am willing to work with beginning students.

**References**


Mass spectrometry (MS)-based proteomics and metabolomics analyses enable the quantification of hundreds or thousands of biomolecules within biological system, providing critical information for understanding cellular structure, function and pathology. However, due to limitations in analytical sensitivity, samples comprising thousands or millions of cells are typically required for such in-depth biochemical measurements, which can lead to a blurry picture of the biological system that fails to differentiate multiple cell types, tissue structures and their microenvironments. In addition, each measurement can take hours or days to complete, which leads to a high cost per analysis.

Our research group focuses on developing improved methods and instrumentation for MS-based biochemical measurements. Specifically, we strive to extract the maximum amount of biochemical information from the smallest samples possible to address questions in biology that cannot be answered using existing approaches. This requires overcoming shortcomings and minimizing sample losses across the entire workflow, including sample isolation, preparation, separation, ionization and mass spectrometry.

**Tools of the trade**

Some of the instruments and techniques that we use and/or strive to improve are:

Sample isolation – Using laser capture microdissection, fluorescence-activated cell sorting and microfluidics approaches to isolate tissues or cells of interest while excluding unwanted background material

Sample preparation – Developing microfluidic and robotic systems such as our recent Nanodroplet Processing in One Pot for Trace Samples (nanoPOTS) system to efficiently convert raw biological material from ultrasmall samples including single cells into ready-to-analyze biomolecules

Separations – Miniaturizing and improving nanoscale liquid chromatography and capillary electrophoresis separations to effectively deliver biomolecules to the mass spectrometer
Ionization – Optimizing nanoelectrospray ionization to efficiently convert solution-phase biomolecules into gas-phase ions for analysis by MS

Mass spectrometry – Ensuring optimal performance for commercial and custom MS instrumentation

Applications

We collaborate with researchers at a variety of institutions to address otherwise intractable problems in biology and biomedicine. For example, we are working with Prof. Rosalie Sears, Co-director of the Brendan-Colson Center for Pancreatic Care at the Oregon Health & Science University, to understand what causes certain cells within pancreatic ductal adenocarcinoma tissues to undergo a transition from epithelial to neuroendocrine-like phenotype, and why these changes are associated with increased resistance to treatment. This requires us to map protein expression across tissues with high spatial resolution, and we are funded by the National Cancer Institute to develop the required technology.

We are also working to isolate and analyze extremely rare circulating tumor cells from the blood of cancer patients to track disease progression and responses to therapies with a minimally invasive assay.

Relevant Publications


Research in Synthetic and Analytical Chemistry on Surfaces

Students who work in my group have the opportunity to learn about many different areas of science while they focus on our primary interests: surface functionalization and characterization. We currently have projects that involve the development of new materials for chromatography (separation science) and chromatography sample preparation, i.e., new materials for solid phase microextraction (SPME), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC). We are also doing advanced surface characterization of glass surfaces, and developing new coatings for an industrial partner.

One of the reasons that students are exposed to many different ideas while they work in my group is because my work overlaps two different regions of chemistry: the synthetic side as well as the analytical side. On the synthetic end we have prepared surfaces with different reactive functional groups, such as epoxides or carboxyl groups, and attached DNA to them. We are also using or are planning to use different polymerization methods, including ring opening metathesis polymerization, atom transfer radical polymerization, and conventional radical polymerization to grow polymers from surfaces. This polymer work should fit in nicely with the new methods we have developed for patterning silicon surfaces with micron and even nanometer sized features. It should allow us to create polymeric features on surfaces with these tiny dimensions for nanotechnology.

On the analytical end, my students use a number of instruments and methods to characterize our new materials, and also to characterize other materials we get by collaboration. Tools that we use include X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), ellipsometry, wetting, scanning electron microscopy (SEM), and atomic force microscopy (AFM). While most undergraduate students are not familiar with these methods before they join my group, within a few months they have usually developed a good sense for the type of information that these tools can provide and have become users of more than one of them. We have also developed an increasingly strong emphasis in chemometrics in my group. An important branch of chemometrics uses advanced data
processing/statistical tools to extract information from large data sets. Two such tools we use are Principal Components Analysis (PCA) and Partial Least Squares (PLS). These tools are important for ToF-SIMS characterization of fuels (coal and biomass) and cancer tissue.

References


Nature often uses metals such as iron, copper, or cobalt in the active sites of enzymes in order to enable difficult reactions. In many instances, two or more metals are present that can cooperate to lower the barriers for reactions and enable faster reactivity. In organic synthesis, however, catalysts containing only a single transition metal are generally employed. In our laboratory, we are designing transition metal complexes containing two different metals as catalysts for organic synthesis. The second metal is specifically designed to interact with the catalytically active metal in such a way as to accelerate the overall rate of the reaction. Using this strategy, we are developing catalysts with unprecedented reactivity and exploring the development of new types of reactions that don’t work with traditional single-metal catalysts. Many of the complexes that we synthesize are air and water sensitive, and thus much of the chemistry performed for this project takes place in an inert atmosphere glove box. Students on this project learn organic and inorganic synthesis, air-free reaction techniques, and spectroscopic techniques such as NMR, Mass spectrometry, and X-ray crystallography.

**α-Helical Peptide Scaffolds as Modular, Tunable, Enzyme-Like Catalysts for Multistep Synthesis**

The enormous breadth of chemical reactions performed in biological systems can be attributed to nature’s ability to construct highly ordered arrangements of catalytic functional groups, or enzyme active sites. In addition, many organisms have evolved the ability to assemble polyketide synthases (PKSs) or multienzyme complexes that are capable of performing multistep synthesis in a linear fashion. Chemists have tried to mimic nature’s efficiency by constructing multifunctional catalysts or by designing multicomponent reactions. In this project, we are investigating the use of short helical peptides to scaffold multiple organic catalysts (transition metals, organocatalysts, Lewis acids) in close proximity in order to facilitate enzyme-like catalysis. This template approach will provide a new strategy for designing and optimizing catalysts that takes advantage of substrate preorganization and proximity to improve catalytic activity. The helical scaffold will also make possible the design and construction of
multifunctional catalysts capable of performing multistep synthetic processes. Our catalysts are
synthesized using microwave-accelerated solid phase peptide synthesis, and students on this project
learn to analyze their catalysts and reactions using HPLC, NMR, mass spectrometry, and circular
dichroism. We also perform kinetic experiments to quantify the reactivity of our enzyme-like catalysts.

Polymer-Incarcerated Nanoparticle Catalysts for Organic Synthesis

A third focus of our research program seeks to design, synthesize, and utilize polymer-supported
transition metal nanoparticles for catalytic applications. In these studies, we seek to modify the reactivity
and selectivity of the catalysts by changing the structure of the polymer support. Polystyrene is a highly
versatile polymer that can be easily modified to control the strength of the contact between the
nanoparticle and the polymer in order to influence nanoparticle reactivity. We desire to modify this
interaction by changing the steric and electronic properties of the polymer to rationally influence the
magnitude of interaction and hence finely tune the electronic properties of the metal particles. Early
published results from our laboratory have confirmed that the electronic structure of the supporting
polymer can have a significant impact on catalytic activity in nitroarene reduction reactions. One of the
main goals of this project is to understand the nature of the polymer-nanoparticle interaction in order to
rationally design new supports that enhance catalytic activity in organic reactions. We are also seeking to
employ these catalysts broadly in organic synthesis where they can serve as highly selective, tunable, and
recoverable/recyclable heterogeneous catalyst.

References

   “Synthesis of Chiral Titanium-Containing Ligands for Enantioselective Heterobimetallic Ti–Pd

   Duval, J. S.; Masino, B. M.; Cahoon, S. B.; Flansburg, R. R.; Conder, C. J.; Price, J. L.; Michaelis, D. J.
   “Proximity-Induced Reactivity and Selectivity with a Rationally Designed Bifunctional Helical


Protein engineering to accelerate scientific discovery

Currently we are working to develop generalizable protein engineering-based methods to facilitate protein structure determination by X-ray crystallography. We are also working to develop methods to re-engineer radical SAM enzymes to catalyze arbitrarily-chosen radical-mediated chemical reactions.

Moody laboratory approach

X-ray crystallography allows us to determine the structure of proteins at the atomic level, helping us to understand how protein dysfunction causes disease, develop new treatments, and engineer new protein-based tools. Unfortunately, X-ray crystallography is only useful for those proteins that can be induced to form ordered crystals; about 20-30% of all known proteins.\textsuperscript{1} Recently we engineered a variant of pyruvate formate-lyase activating enzyme (PFLAEH, a radical SAM enzyme), for facile crystallization.\textsuperscript{2,3} We observed that while PFLAEH formed at least 4 different crystal packing arrangements (lattices), all of these shared a conserved screw axis. Screw axes can be thought of as ordered fibers composed of stacked copies of the protein. Since this screw axis is common among 4 different crystal lattices, we
propose that it forms first during crystal nucleation and serves to dictate the packing arrangement of the rest of the crystal. Currently we are investigating fusing proteins of interest to engineered screw axis fibers to pre-order them and nucleate crystal formation. It is our hope that new protein crystallization methods like this one will enable structure determination of a much greater percentage of known proteins to greatly accelerate scientific discovery and disease treatment.

In addition to PFLAEH, we are also investigating the sterile alpha motif (SAM) domain of human translocation Ets leukemia protein (TEL). The SAM domain of TEL spontaneously forms a 6-fold helical polymer. In 2007, researchers re-engineered the polymer subunits to form 3-fold helical polymers and to only polymerize at low pH. They then fused proteins of unknown structure to these polymer subunits and were able to reproducibly obtain crystals at low pH. Unfortunately, most of the crystals were too disordered to give usable X-ray diffraction data. We aim to reengineer the TEL-SAM subunits so that polymerization occurs in a more controlled manner, allowing the formation of ordered crystals.
While many other types of enzymes have been designed or re-designed through computational or experimental enzyme engineering, radical SAM enzymes have yet to be explored as rationally-engineered synthetic catalysts. We are computationally engineering radical SAM enzymes to accept novel substrates. Radical SAM enzymes create highly reactive organic radicals and use them to accomplish a huge variety of high-energy chemical transformations in substrate molecules, nucleic acids, and other proteins.\(^5\)

**Radical SAM activation mechanism**
In the Moody lab you will learn computational protein modeling and design, molecular biology techniques, protein biochemistry, and macromolecular X-ray crystallography. If you’re interested, I’d love to talk with you! We welcome dedicated, hardworking students with all levels of experience, including beginning students. Be prepared to dedicate at least 10 hours per week to the research to make meaningful progress.

References

Soft Nanotechnology. My research involves i) understanding the self-assembly of stimuli-responsive polymers into dynamic nanostructures. Materials like this can form assemblies that respond to environmental stimuli – like changes in temperature, pH, or chemical potential. We’re going to use these materials to ii) control the chemical and electronic properties at liquid-solid interfaces and iii) use biocatalysis – enzymes or membrane proteins – to change the properties and morphologies of these self-assembly nanomaterials.

Designing and Controlling the Self-Assembly of Stimuli-Responsive Polymers. Amphiphilic molecules, like surfactants and lipids, spontaneously organize into aggregated nanostructures, like micelles and vesicles. Polymer amphiphiles offer a rich library of self-assembling molecules that may be finely tuned for specific applications. Incorporating functional groups that are responsive to pH, temperature, or chemical potential makes it possible to change the properties and shape of these assemblies. Students interested in this area will develop skills designing, synthesizing, and interrogating stimuli-responsive nano-structures, which will be useful for a range of applications from sensors to drug delivery.

Tuning the Chemical and Electronic Properties of Interfaces. Some polymer amphiphiles readily adsorb and fuse to hydrophilic surfaces to form layered structures with tunable thicknesses on the nanometer scale. These adsorbed layers may be used as models of cell surfaces and also allow tuning the chemical properties of interfaces. Understanding how these adsorbed layers change the electronic properties of interfaces can be especially important for developing new kinds of sensors. Students interested in this area will develop expertise in forming supported polymeric and hybrid (lipid/polymer) bilayers and measuring their electronic properties using electrochemical methods, like cyclic voltammetry.

Integrating Biological Function in Stimuli-Responsive Vesicles and Bilayers. We are also interested in creating materials that change their shape to favor the dynamic transformation between micelles, bilayers, and other structures depending on their local environment. Stimuli-responsive polymers coupled to hetero-geneous catalysts, including enzymes, make it possible to translate chemical signals
into physical changes to self-assembled nano-structures. Clever designs of such systems would enable the development of biohybrid materials that respond via catalysis to stimuli that are otherwise unrelated to the normal stimuli these materials are sensitive to. Such materials would find tremendous value as self-actuating nanomaterials.

References.
Materials Science is a very important area of current research. This interdisciplinary field involves aspects of chemistry, physics, and engineering. Our interest lies mainly in establishing the molecular basis for bulk properties of materials and how those properties are affected by external stresses. In many cases, the properties of the material that are the most important are the surface and interface properties. This is particularly important with composite materials, where two different materials are directly in contact with each other, and in mechanical processes such as lubrication and adhesion.

**Response of Materials to Mechanical Stress**

We are also interested in how materials respond to mechanical stress at the molecular level. We use nonlinear spectroscopy, specifically sum-frequency generation (SFG) and second harmonic generation (SHG), to probe the surfaces of materials before and after they are subjected to mechanical deformation. With these techniques, we are able to identify spectroscopic signatures of mechanical stress due to molecular-level changes at the surface of the material. These approaches have great potential for use in nondestructive testing and materials state awareness applications.

**Molecular Basis for Adhesion**

Because SFG can probe buried interfaces as well as free surfaces, we can investigate the molecular structure of bonded and composite materials. In a bonded system, two surfaces are held in mechanical contact by a layer of adhesive. Unfortunately, a full molecular basis for adhesive interactions has not been developed, primarily because of a lack of molecular level information on such systems. We want to systematically investigate bonded systems, such as polymers on solid substrates, industrial adhesive materials, and composite materials, to understand how changes in the molecular structure affect the strength of the adhesive interactions and other material properties. This investigation includes both static and dynamic experiments.
We also want to understand the formation of adhesive bonds. Scientific questions we want to address are: What are the chemical and structural changes that take place as an adhesive cures? How do changes in the environment affect this bond formation? We also want to investigate aging phenomena. How does the structure of the adhesive interface change over time, leading to bond failure? The results of this research program will be applicable to other fields such as materials science and mechanical engineering.

**The Interface of Science**

Our research focuses on interfacial systems, but we are also interested in exploring interfaces of science. Other fields we could explore include mechanisms of chromatographic separation, biocompatible materials, interfacial properties of nanomaterials, heterogeneous atmospheric chemistry, lubrication and others. Such projects will most likely involve collaboration with other members of the department and groups in other departments both on campus and at other universities. With the spectroscopic tools available to us, we are excited at the prospects of exploring a wide variety of interfacial systems. Our group is open to beginning students who have done well in their freshman courses, as well as more advanced students.

**References**

Nucleoside-Based Enediyne

Naturally occurring enediyne are among the most potent antitumor compounds currently known. Unfortunately, selectivity levels are low, and general cytotoxicities are a major problem. We have been intrigued by the possibility that nucleoside-fused or nucleoside-linked enediyne might selectively target rapidly dividing tumors due to the tumor’s enhanced need for building blocks for DNA or RNA. The enhanced uptake of nucleosides by rapidly dividing tumors could potentially lead to a more selective targeting of tumor cells and thereby reduce problems associated with general cytotoxicity. We have successfully synthesized numerous analogues, and a majority of the compounds tested demonstrate promising activities (IC\textsubscript{50} values in the low \textmu M range) against murine models of leukemia, mammary carcinoma, and several lines of human T-lymphocytes.

Transition State Analogue Inhibitors

Cytidine triphosphate synthetase (CTP synthetase; EC 6.3.4.2) and cytidine deaminase (CDA; EC 3.5.4.5) are two key enzymes involved in de novo synthesis and salvage pathways in pyrimidine biosynthesis. CTP synthetase catalyzes the conversion of uridine triphosphate (UTP) to CTP. This is the key step in the only de novo pathway for the biosynthesis of cytidine. CTP may be incorporated into RNA or undergo dephosphorylation to give cytidine diphosphate (CDP), a substrate for ribonucleotide reductase. The product of reduction (dCDP) is converted to dCTP which is incorporated into DNA. Salvage pathways for uridine include hydrolysis of cytidine and cytidine monophosphate. These hydrolyses are catalyzed by CDA and CMP deaminase respectively. The activity of CTP synthetase has been shown to correlate with cellular proliferation in malignant tissues and may be linked to the process...
of malignant transformation. High levels of CTP synthetase activity have been reported in several malignant tissues including rat hematoma, human renal cell carcinoma, Hodgkin’s disease, non-Hodgkin lymphomas, and acute lymphoblastic leukemia. Efficient CTP synthetase inhibitors could act as potent antineoplastic agents in these and other forms of cancer. CDA has been implicated in the development of resistance to commonly used cytosine-based antitumor agents such as arabinosyl cytosine (araC), 5-azacytidine (AZA), and gemcitibine. Inhibitors of CDA could potentiate the activities of these currently used antitumor agents by prolonging their lifetimes in vivo. Compounds IV mimic the transition states involved in the CTP synthetase—and CDA-catalyzed reactions and could act as competitive inhibitors of these important enzymes.

**HIV Integrase Inhibitors**

HIV integrase (IN) is one of three enzymes encoded by HIV. IN is a 32 kDa protein consisting of three distinct domains (N-terminus, catalytic core, and C-terminus), and plays a key role in incorporating viral DNA into host DNA. This is accomplished via a three-step process referred to collectively as integration. The first step in integration involves cleavage of a GT dinucleotide from both 3'-ends of double stranded viral DNA and is called 3'-end processing. The 3'-end processed duplex next undergoes a concerted transesterification reaction with a phosphodiester linkage in host DNA, a process referred to as strand transfer. In the final step, the unmatched AC dinucleotides on both 5'-ends and associated gaps are repaired by a cellular enzyme. The first two steps, 3'-end processing and strand transfer, are catalyzed by IN. IN is a magnesium metalloenzyme, and an active site Mg$^{2+}$ is involved in both 3'-end processing and strand transfer. Although IN was identified as a promising antiviral target over a decade ago, only recently has a viable candidate for clinical inhibition of IN been discovered. The relentless emergence of drug-resistant HIV strains demands continual addition of antiviral compounds to our arsenal of chemotherapeutic agents. We have recently embarked on a drug discovery program aimed at discovering inhibitors of HIV integrase. We utilize state-of-the-art molecular modeling software (FlexX and CombiFlexX, Sybyl 6.9, Tripos, Inc.) to perform high-throughput screening of vast (> 50,000) compound libraries in order to identify promising leads for laboratory synthesis. Vast combinatorial libraries are docked at strategic points indicated by the X-ray crystal structure of IN. Compounds identified by the docking studies share in common a nucleoside(tide) scaffold and should bind tightly to critical residues at or near the active site of IN. We will utilize our expertise in nucleoside chemistry to synthesize the most promising compounds indicated by the *in silico* high-throughput screen.
My research explores mechanisms used by living cells to control the quality and concentration of each molecule of our body. The complexity of this task is really astounding. For example, there are ~20,000 different protein types in each cell of your body. The quality and concentration of each one is carefully controlled in a condition generally referred to as protein homeostasis. When protein homeostasis fails, we quickly develop diseases like Alzheimer’s, diabetes, and cancer. Similar problems occur when the homeostasis of lipids or other metabolites fails. A big effort in our lab is developing the tools to study the processes supporting homeostasis in vivo and understand what changes as homeostasis is lost. Specifically, we use stable isotopes to label newly-synthesized molecules with a time-dependent tag. With a mass spectrometer, the time-dependent stable isotope enrichment and relative concentration of many molecules can be measured even within a complex mixture. This allows us to calculate synthesis and degradation rates simultaneously for many molecules in the body as it responds to stimuli. This allows us to perform experiments that survey broad sections of the proteome, and compare against DNA, RNA or small molecules produced by enzymes within the cell. We have successfully used this technique in many different biosynthetic systems from "cell free" environments to humans. Currently, we are focused on understanding post-transcriptional control of the proteome composition within cells, especially on the changes associated with aging or disease as well as how protein degradation is regulated to maintain homeostasis. If you find these research questions interesting please come talk to me, I am always open to working with motivated students.

Regional control of metabolism

One method of controlling chemical reaction rates is to compartmentalizes enzymes and substrates in side of the tissue or the cell. We are incorporating metabolic labeling, metabolomics and proteomics with surface imaging mass spectrometry to understand how regional regulation of metabolism within a tissue changes with disease and aging.
**Maintenance of proteome homeostasis through protein degradation**

Many of today’s most devastating diseases can be identified as diseases of protein homeostasis. Parkinson’s, Alzheimer’s, Huntington’s, diabetes and other diseases all exhibit cellular deposits of aggregated protein. These aggregates are often highly resistant to degradation and may indicate a dysfunction within the catabolic machinery of the cell. Continuous protein catabolism is critical in the presence of constitutive transcription and translation, yet these processes are poorly understood. The cell employs thousands of proteins (ubiquitin ligases, targeted proteases, proteasome, etc.) to guide the process of protein degradation. Thus, the complexity of the regulatory structure for removing a protein from the cell may be comparable to producing the protein in the first place. Our current work is focused on identifying the substrates for cellular proteases and understanding how targeted proteolytic processing is used by the cell.

**References**


We are broadly interested in how proteins and polypeptides fold and adopt the beautiful three-dimensional conformations that ultimately give rise to their diverse functions. We want to understand how modifying protein side-chains (via glycosylation, phosphorylation, or with unnatural polymers like polyethylene glycol) changes the thermodynamic stability and folding kinetics of the modified protein.

Our motivation for this work derives from the increasing promise of therapeutic proteins as treatments for conditions that are difficult to address with conventional small molecule therapies (cancer, chronic inflammatory and auto-immune disorders, anemia, neutropenia, etc.). Despite many recent successes, several problems continue to limit the usefulness of proteins as drugs: (1) they must be injected to avoid digestion by gastrointestinal proteases; (2) they are quickly cleared from blood via kidney filtration and proteolysis by serum proteases; and (3) they can adopt non-functional unfolded or misfolded conformations, which can then self-associate to form aggregates, sometimes leading to undesired side effects, including immune responses.

Increasing protein thermodynamic stability could address these problems because thermodynamic stabilization increases the population of the pharmacologically-active folded state, while decreasing the populations of the

Figure 1. (A) Ribbon diagram of the WW domain from the human protein Pin 1 (PDB: 1PIN, ref. 22). β-strands are shown in blue, reverse turns in gray. (B) Stick representation of reverse turn 1 in the WW domain. Main-chain hydrogen bonds represented by black dashes; the $i$, $i+3$, and $i+5$ positions are highlighted in red. Protein 6-F, T, and PEGylated protein 6PEG-F, T differ in the group attached to Asn_{i+3}. These differences affect the melting temperature $T_m$ of each protein as shown. $T_m$ values are given as mean ± standard error for 10 μM protein in 20 mM sodium phosphate buffer, pH 7 (ref. 19). All structures were rendered in Pymol.
protease-sensitive unfolded ensemble and/or aggregation-prone misfolded states. My research group is interested in developing reliable strategies for increasing protein stability.

**PEGylation Project**

One potentially useful strategy is to attach an ethylene oxide oligomer (i.e. polyethylene glycol or PEG) to a protein, typically by reacting a functionalized PEG electrophile with one or more nucleophilic side chain groups on the protein surface (this approach is hereafter called PEGylation). The bulky size of the attached PEG can block proteins from self-associating to form aggregates, can shield immunogenic epitopes on the protein surface, and can prevent the PEGylated protein from being filtered out of the bloodstream by the kidneys. We believe these beneficial effects could be further enhanced if PEGylation consistently led to increases in protein thermodynamic stability. However, little is known about the conditions under which PEGylation of a protein is energetically favorable.

We are currently working on uncovering the fundamental principles that allow PEGylation to increase protein thermodynamic stability. We are working to understand which secondary structures (sheets, turn, or helices) are most amenable to PEG-based stabilization and whether favorable interactions between PEG and nearby protein side-chains can increase this stabilizing effect. We are always willing to talk about research with undergraduate students; beginning students are welcome to apply!

**PEGylation Project**


Ground-breaking studies from physical organic chemistry have provided important insights about the key role played by non-covalent interactions in protein folding and conformational stability. These include the hydrophobic effect, along with non-covalent interactions, including salt bridges, hydrogen bonding, cation-π, anion-π, π-π, CH-π, and n to π* interactions. Understanding these interactions is crucial to developing methods for predicting protein secondary, tertiary, quaternary structure from primary sequence, especially for proteins with no known function and with little homology with known proteins. Computational chemistry and structural biologists have made substantial progress toward this end, but much remains to be done. Many investigations have evaluated non-covalent interactions between two amino acid residues via double mutant cycle analysis, in which the two residues of interest are mutated to a non-interacting surrogate (typically Ala) first individually, and then in combination. Comparing the stability, binding affinity, or some other measurable property of each variant then provides an estimate of the strength of the interaction between the two residues, independent of the intrinsic effect of individually mutating each residue to Ala. This approach has yielded important insights into the impact of non-covalent interactions on protein folding and stability but is limited by the assumption that the two residues of interest interact exclusively with each other and not with any other nearby amino acids.

This assumption is useful to a first approximation but seems unlikely to be generally true given the structural complexity of most proteins. Instead, one might expect the strength of some binary non-covalent interactions to be substantially influenced by one or more additional nearby amino acid side chains. Indeed, computational predictions, bioinformatic analyses, and experimental work on small-molecule model systems suggest that this kind of cooperative or synergistic coupling of three groups (i.e., a ternary interaction) is feasible within the complicated architecture of proteins. Experimentally assessing the impact of such ternary interactions involves measuring the strength of a binary non-covalent interaction when a third residue of interest is present vs. when the third residue is replaced by a non-interacting surrogate. This approach combines two double mutant cycles and is sometimes called triple mutant box analysis.

Previous experimental studies have used triple mutant boxes or analogous approaches to assess the impact of nearby residues on salt-bridges, hydrogen-bonds, and carbohydrate-π interactions within proteins. Despite these promising precedents, there are few studies aimed at identifying cooperativity and ternary interactions involving other important non-covalent interactions within proteins, including anion-π, cation-π, π-π, and n to π* interactions. Our lab has extensive training in assessing the impact of non-covalent interactions and is dedicated to developing new methods for predicting protein structure based on these insights.
covalent interactions on protein conformational stability using and triple mutant box analysis, which uniquely positions us to pursue a detailed fundamental understanding of these ternary cooperative effects, thereby providing important insights that are essential for further development of computational tools to predict and analyze protein structure and folding; for design of new protein-based catalysts, therapeutics, and nanostructured biomaterials; and for discovery of small-molecule inhibitors of enzymatic activity, receptor function, and protein-protein interactions. Our recent progress toward these ends is described in the publications cited below.

**Noncovalent Interactions Project**


We are always willing to talk about research with undergraduate students; beginning students are welcome to apply!
The ability to synthesize complex molecules enables organic chemists to influence, and in some cases control, biological processes. Our research group prepares new compounds and studies their interactions in multiple biological settings.

**Development of Antibacterial Agents, Control of Their Cell Selectivity**

Continuing emergence of drug-resistant bacteria has become a major health concern and may lead to untreatable infections in a vast number of people and animals. As a means of controlling bacterial growth without causing bacterial resistance, organisms ranging from bacterial to mammals produce peptide antibiotics that disrupt bacterial membranes. We have been interested in mimicking the antibacterial activities of these peptides using cationic steroid antibiotics developed in our laboratory. This research has led to preparation of multiple series of new potent antibiotics (e.g. structures 1 and 2).

These compounds rapidly kill a broad spectrum of bacteria (both Gram-negative and -positive), demonstrate selectivity for prokaryotic cells, and are unlikely to induce formation of resistant strains. We are currently using these compounds to study how small molecules can be used to disrupt bacterial membranes. We are also working to improve the potency and cell selectivity of the antibiotics. Research on this project spans a number of disciplines. Studies involving titrations to determine binding constants are performed, new compounds are synthesized, and bacterial susceptibilities are measured.
**Stimulation of Natural Killer T Cells and Generation of Conjugate Vaccines**

As the immune systems of higher organisms become better understood, the abilities of relatively small molecules to cause potent immunological responses become clear. An aspect of innate immunity in mammals governed by interactions with glycolipids is currently being elucidated. Association of glycolipids with a protein, termed CD1d, on antigen presenting cells is followed by binding of the glycolipid-CD1d complex with a T cell receptor on natural killer T (NKT) cells. Depending upon the structure of the glycolipid, the NKT cells can release a variety of potent chemical messengers. Release of these chemical messengers, called cytokines, can cause a strong up-regulation of the immune system (T helper 1 mediated). Responses from stimulation of NKT cells can be harnessed to improve the effectiveness of vaccines. We are preparing carbohydrate-based vaccines containing bacterial antigens, conjugating these on self-assembling protein nanoparticles and using NKT cell responses to give strong memory responses to the bacterial antigens.

**Recent Papers**


We are interested in studying events involving highly excited molecules with a “chemically significant” amount of energy. Highly excited molecules are of great importance, due to their reactivity; however, they are often extremely difficult to study as a result of their complexity. Reactants are produced using laser pumping techniques after which we observe the outcome of either a bimolecular collisional energy transfer event, or a unimolecular or bimolecular reaction. The goal of our studies is to understand these chemically significant events in a quantum state resolved fashion with detail that was, until recently, only dreamed of. We use novel high resolution spectroscopic techniques (~0.0003 cm⁻¹) to study the amount of energy distributed in the various energy states (vibration, rotation, and translation) of molecules after a reaction or collision. Current projects can be divided into three general categories:

**Collisional Energy Transfer**

Collisional Energy Transfer is one of the key steps in the Lindemann mechanism for unimolecular reactions. Collisional deactivation competes with chemical reaction by removing enough energy to bring the reactant species below threshold. By studying the final rotational and vibrational quantum states as well as the translational energy distributions of simple collision partners, we can establish the probability of transferring a specific amount and type of energy. The results from this quantum state picture can be converted into a probability distribution function, which provides information about the transition state and potential energy surface of the interaction.

**Photo-Induced Chemical Reaction Dynamic and Kinetics**

Using similar techniques, it is possible to track the products of a photodissociation process with quantum state resolution. Because the molecules used to study collisional energy transfer have such a large amount of energy (~5 eV), they are literally ready to explode into molecular and atomic fragments when the collision event takes place. Unimolecular decomposition is thus in competition with collisional energy transfer. By probing the molecular fragments, it is possible to follow the course of these photo-
induced chemical reactions with detail never before observed. It is possible to extract not only the reaction rate, but also learn a great deal about fundamental properties of chemical reactions.

**Combustion Chemistry**

The combustion of methane is of considerable importance in the generation of energy; thus, it has received considerable attention. This apparently simple chemical reaction is actually not so simple. The kinetics of the reaction of methyl radicals with oxygen atoms, the key step in the overall combustion process, has been studied extensively; however, a consensus has yet to be reached in our understanding of this important reaction. Some of the controversy is potentially tied to methyl radical production. Understanding the photodissociation dynamics of methyl radical precursors, particularly the partitioning of energy among the various quantum states, is of utmost importance if a completely clear picture is to be obtained for the reaction of CH3 with O (3P). It is highly improbable that various methods of CH3 production produce radicals with the same characteristics; thus, the outcome of subsequent reactions will also, most likely, be different. In addition to performing a detailed quantum state resolved study of methyl radical formation, we are also interested in studying the subsequent chemical reactions.

**References**


Welcome to the Stowers Laboratory!

We expect students to be hardworking and dependable, committed to learning to do hard things, and enthusiastic about research. It’s okay if you feel like you don’t know enough about chemistry to work with us - many students felt the same way when they got started. It would be great if you could attend group meetings in order to get to know the lab and the projects. We typically like students who are going to commit to being around over a summer. Chemistry students are preferred, but we’ve had mechanical and chemical engineers, and other majors who have worked with us. The following are three active areas of research in the group:

1) **Inorganic Synthesis of Heterogeneous Catalysts**

We have found that many catalysts are sensitive to the types of preparations and additives in order to increase efficiency for a particular reaction. Synthesizing and characterizing nanoparticle* catalysts and supported nanoparticle catalysts will allow us to determine how molecules interact with the surface and ultimately lead to designing better selectivity and reactivity. Students on this project will synthesize inorganic catalysts and determine the catalyst structure by a variety of techniques including microscopy and spectroscopy. We use these catalysts in a flow reactor where gases pass over the surface and are recombined into new chemicals, which are detected by an online spectrometer.

2) **Mechanistic Studies on Catalyst Surfaces**

We use a stainless steel chamber at ultra high vacuum in order to probe how organic molecules react at a metal surface without the competition of air or water molecules that usually cover surfaces. By using X-ray photoelectron spectroscopy we can find out information regarding concentration, oxidation state and elemental composition of intermediates on the surface. A heating ramp allows us to find out how the reactions react and desorb from the surfaces to form or break bonds. We use this model catalyst as a means for designing new catalysts, designing new reactions, or better understanding known reactions at
a metal surface. Students working on this project will operate and become familiar with an ultra-high vacuum chamber, in-situ XPS and temperature-programmed reaction spectroscopy, as well as computational analysis.

3) Fine Chemical Synthesis using Heterogeneous Catalysts

The interface between solids and liquids are interesting and can be tuned with the inclusion of heterogeneous surfaces that can act as acids or bases. We are interested in what kinds of chemical bonds can be broken or formed at the liquid solid interface in the context of fine chemicals. Catalysts that we have currently used include silver and copper nanoparticles and molybdenum-based metal oxides. Students working on this project will learn bench-top isolation techniques and characterize products using NMR or gas chromatography coupled with a flame ionization detector or a mass spectrometer.

Sample publications:


Identification of Protein Therapies for Muscular Dystrophy

The muscular dystrophies are a group of progressive degenerative muscle wasting diseases that vary in age of onset, phenotype, cause, severity and life span. Many of the treatment options for these diseases have not resulted in substantial quality of life treatment options desperately needed for patients and families. The goal of my lab is to identify protein therapies for several different types of muscular dystrophies. Once a therapy is identified, known endpoint markers for specific muscular dystrophies will be used to determine efficacy and mechanism.

Dysferlinopathy is caused by mutations in the DYSF gene (encoding dysferlin protein) and is characterized by the following: delayed removal of necrotic muscle fibers, loss of calcium sensitivity leading to signaling mis-regulation, increased inflammatory infiltrate, muscle atrophy, malformation of transverse tubule structure and defective membrane repair. The role dysferlin plays in membrane resealing is well established, however research shows that dysferlin has multiple roles in skeletal muscle, many of which still need to be elucidated. The lack of treatment options for dysferlinopathy patients requires increased research to decrease disease progression.

Galectin-1 is expressed in diverse tissues intra and extracellularly with tissue and pathogenic specificity. Galectin-1 is known to modulate the immune system, tumor progression, regeneration of central nervous system after injury, hypoxia, and vascularization. Research has shown that mdx mice treated with recombinant Galectin-1 display improved sarcolemma stability, reduced muscle pathology, improved muscle repair, and increased angiogenesis. These results indicate Galectin-1 is an exciting, new biologic therapy for the treatment of DMD. Since Galectin-1 has been shown to possess a diverse set of beneficial activities, we believe this biologic could be used to modify disease progression not only in DMD but also in other types of muscular dystrophy. My lab will test the hypothesis that Galectin-1 will improve the removal of necrotic muscle fibers, provide a network for the stabilization of stress-induced calcium...
signaling and formation in transverse tubules membrane, improves muscle strength and modulate immune response in mouse models of dysferlinopathy and other muscular dystrophies.

To accomplish the goals of my lab, students will learn to use a variety of histological, biomolecular, biochemical and physiological techniques, including immunohistochemistry, bright field skeletal muscle histology, polymerase chain reactions, cell and tissue culture, protein purification and quantification, along with evaluation of cardiac and skeletal muscle physiological techniques. As a student in my lab you will learn about current issues in the field of muscle disease. By learning the techniques used in my lab, students will be able to formulate and solve important research questions which will have translational impact on patients and families. Students showing dedication will also get the opportunity to present data at local and international meetings, prepare, write and submit manuscripts to peer-reviewed journals. Research experience is a valued tool for any student to obtain setting them apart from their colleagues. I encourage undergraduate students to apply for an opportunity to gain research experience in my laboratory. I welcome all motivated undergraduates showing an interest in gaining research experience.

References

Biological systems require trace amounts of transition metal ions to sustain life. Transition metal ions are required at the active sites of many enzymes for catalytic activity. In fact, transition metals catalyze some of the most energetically demanding reactions in biology. Unfortunately, these highly reactive metal ions also catalyze reactions that are dangerous for biological systems, especially if the metal ion is free in solution. For this purpose, biology has evolved elaborate transition metal ion handling systems to bind and sequester transition metal ions in non-reactive environments to prevent these dangerous reactions from occurring. The Watt lab focuses on how iron is properly moved throughout the body.

A healthy individual possesses iron trafficking systems to absorb iron from the diet, transport iron in the bloodstream and deliver iron to cells that require iron. The failure or inhibition of these iron trafficking systems results in free iron that is a potent catalyst to form reactive oxygen species or oxidative stress.

The Watt lab studies diseases where iron trafficking is disrupted and oxidative stress is elevated. Such conditions include Alzheimer’s disease, Parkinson’s disease, kidney disease, Diabetes along with other conditions.

**Anemia of Chronic Inflammation Caused by Hepcidin**

Hepcidin is an iron regulatory hormone induced by inflammation that degrades the iron transport protein ferroportin. Hepcidin causes a condition known as anemia of chronic inflammation. Ferroportin is required to transport iron into the bloodstream from the intestinal cells that absorb iron from the diet. Ferroportin also exports iron from the liver, and spleen into the bloodstream where transferrin binds iron and delivers iron to the bone marrow for red blood cell synthesis. The Watt lab has identified hepcidin inhibitors that prevent hepcidin production and stabilize ferroportin. Studies in rats show that iron delivery to the bone marrow is restored using these hepcidin inhibitors.

**Caption** — Inflammation produces hepcidin that binds to and degrades ferroportin. This stops iron delivery to the bone marrow. Inflammation also blocks EPO production and secretion from the kidneys. Combined these effects decrease red blood cell synthesis.
Caption—FerroMobilin Drug effect. FerroMobilin drugs block hepcidin production so ferroportin is not degraded. Iron is released from the liver and loads transferrin for iron delivery to the bone marrow. Depending on the cytokines that triggered the inflammatory process EPO may be present or ESA drugs may be required to stimulate red blood cell synthesis.

Inhibitors of Iron Binding Proteins

The Watt lab has focused on metabolites that build up in diseases with oxidative stress. We identified metabolites that disrupt iron loading into ferritin and transferrin. In Chronic kidney disease, serum phosphate levels increase because the kidneys are not properly filtering phosphate from the bloodstream. We demonstrated that elevated phosphate inhibits iron loading into ferritin and transferrin by forming insoluble iron phosphate complexes. We are now focusing on other elevated metabolites to determine if they also disrupt normal iron loading or release of iron from ferritin or transferrin.

Caption: As Fe$^{3+}$ is exported from the cell into the bloodstream it encounters a variety of serum molecules that can react with Fe$^{3+}$ and form complexes that are not substrates for loading into apo transferrin. This work shows that citrate and albumin can prevent these dangerous side reactions and mediate iron delivery to apo transferrin to prevent the formation of non-transferrin bound iron.
**Alzheimer’s Disease**

Iron dysregulation is intimately connected to Alzheimer’s disease (AD) but the direct connections are not clear. A new hypothesis relating to homocysteine disrupting iron loading into ferritin might explain the elevated cytosolic iron and oxidative stress. The inability to load iron into ferritin results in elevated cytosolic iron which upregulates expression of the Amyloid Precursor Protein (APP). Homocysteine also inhibits the phosphatase that dephosphorylates tau leading to elevated hyper-phosphorylated tau and tau tangles. In collaboration with Dr. Jonathan Wisco in the BYU PDBio department, we are testing this hypothesis.

**Diagnostics**

For each of the situations outlined above, we are developing point of care diagnostic methods to evaluate known biomarkers. The goals of the diagnostics research are two-fold. First, we are modifying and developing new methods related to antibody detection methods to provide increased sensitivity for this type of analysis. We also focus on particular biomarkers that give diagnostic information to aid clinical practitioners identify the most beneficial and effective treatment.

**Selected Publications**


Barry M. Willardson, PhD

Biochemistry
C204A, 104C BNSN, 422-2785
Email: bmwillardson@chem.byu.edu

Mechanisms of Assembly of Signaling Complexes

Most cellular functions are performed by proteins associated together into complexes. In fact, many proteins cannot exist in the cell without their binding partners. These protein complexes often require the help of other proteins, called chaperones, to bring the complexes together. This is certainly the case for protein complexes involved in cell signaling processes. Our work has focused on the mechanism of assembly of two types of signaling complexes, the G protein heterotrimer and the mTOR kinase complexes. It is through the G protein complex and its associated receptors and effectors that cells detect hormones, neurotransmitters, chemokines and sensory signals such as odorants, taste molecules and even photons of light. G proteins regulate almost every aspect of cellular physiology and as a result more than a third of current therapeutic drugs target G protein signaling pathways. The two mTOR complexes, mTORC1 and mTORC2, are also high-value drug targets because of their role in orchestrating cell survival, growth and metabolism in response to growth hormones and nutrient levels.

Both G protein and mTOR complexes are assembled with the help of the cytosolic chaperonin CCT (also called TRiC), a large protein folding machine with a double-ring structure of eight different chaperonin subunits in each ring. The center of each ring creates a protein folding chamber in which nascent proteins with intricate folding trajectories bind and are assisted in the folding process. One such protein fold is the β-propeller, which commonly has seven β-sheets that form the blades of a propeller-like circular structure. β-propellers have a unique folding trajectory that requires the C-terminus to interact with the N-terminus to make the last β-sheet that closes the β-propeller. CCT is believed to facilitate this process. We have found that the β-propellers of the G protein β subunit (Gβ) and the mLST8 and Raptor subunits of mTOR complexes are folded by CCT prior to their assembly into complexes.

The process of G protein heterotrimer assembly begins with the association of the G protein β subunit (Gβ) with the G protein γ subunit (Gγ) into the Gβγ dimer. Gβγ is an obligate dimer, meaning that neither subunit is stable in the cell without the other. As a result, Gβ and Gγ must be brought together by
chaperones. At some point during or after translation, the nascent Gβ subunit binds CCT and is folded into its β-propeller structure. However, the β-propeller is not stable in the absence of the Gγ subunit, and Gβ cannot associate with Gγ until it is released from CCT. This conundrum is resolved by the CCT co-chaperone, phosducin-like protein 1 (PhLP1). PhLP1 binds Gβ in the CCT folding cavity and initiates the release of Gβ from CCT. Once released, Gγ is able to bind Gβ in the PhLP1-Gβ complex and form the stable Gβγ dimer. The G protein α subunit then associates with Gβγ, forming the active Gaβγ heterotrimer and simultaneously releasing PhLP1. All four of the typical Gβ subunits are assembled with their 12 associated Gγ subunits by this same mechanism involving CCT and PhLP1.

The atypical Gβ5 subunit forms a dimer with regulators of G protein signaling (RGS) proteins of the RGS7 subfamily. These dimers have a different function than Gβγ dimers. They turn off G protein signaling in neurons by accelerating the rate of GTP hydrolysis on the Gα subunit. We have found that CCT and PhLP1 also assist in the assembly of these Gβ5-RGS complexes. In fact, the conditional deletion of the PhLP1 gene in the rod photoreceptor cells of mice results in the loss of the Gβ5-RGS9 dimer from these cells in addition to the loss of Gβγ dimers. Consequently, G protein-dependent responses to light by rod photoreceptors were diminished and their recovery was slow. These findings have confirmed the importance of PhLP1 in Gβγ and Gβ5-RGS dimer formation in vivo.

In the case of mLST8 and Raptor, both of their β-propeller domains are folded by CCT. They then release from CCT independently of PhLP1 to associate with mTOR. Cryo-EM structural studies of the Gβ-CCT and mLST8-CCT complexes, done in collaboration with the lab of Jose M. Valpuesta at the Centro National de Biotecnologia in Madrid Spain, show that the β-propellers of both proteins have reached a near-native state while bound to CCT, but they associate with CCT very differently despite their structural similarity (Fig. 1). Gβ binds the CCT apical domains at the top of the CCT folding chamber similar to actin, another CCT substrate, while mLST8 binds CCT at the bottom of the folding chamber between the CCT rings, which has not been previously seen with any CCT substrate. These positions explain the effects of PhLP1, which can
interact with Gβ at the top of the chamber and mediate its release, but it cannot access mLST8 between the rings. These structural studies provide the molecular details needed for structure-based therapeutic design to control the folding and thereby the function of these important CCT folding substrates.

My lab typically brings on undergraduates at the beginning of their junior biochemistry courses, but talented sophomores have occasionally joined in the lab as well. Successful students can expect to co-author a top-tier publication and compete for acceptance in the best graduate or medical schools.

References


While commercial specific heat apparatuses using relaxation methods exist, our custom designed and built instruments are capable of accuracies and precisions approaching, and even exceeding, 0.1%. This type of accuracy and precision allows us to study a wide range of interesting and relevant topics in solid-state physics and chemical thermodynamics. Shown below is an example of our measurements on a bulk sample of MnO and a sample of the colossal magnetoresister La1-xSrxMnO3.

Currently, our primary research interest is in the Energetics of Nanomaterials, which is funded by the Department of Energy. Our focus in this research project is to understand the fundamental driving forces governing the stability of materials as their particle sizes reach the nanoscale. We have done extensive work on high quality samples of the TiO2 polymorphs of anatase and rutile with sizes of 7 nm and on the magnetic material CoO.

Fisher-Tropsch Catalysis

We have created a Fisher Tropsch research focus in collaboration with the Catalysis Group in Chemical Engineering. We have applied our proprietary solvent deficient precipitation method to
synthesize a series of industrial viable and state-of-the-art alumina catalyst supports and Fe and Co Fisher Tropsch catalysts. These supports and catalysts have tunable properties and perform better than any catalysts currently reported in the literature. We continue to focus our work on innovating in the catalysis area using our proprietary solvent deficient method.

**Synthesis of Nanoparticles**

We have recently developed an elegantly simple process that allows us to make a nearly unlimited array of well-defined inorganic nanoparticles that have controlled sizes from 1 nm to bulk. The particles are highly crystalline with well-defined shapes (usually spherical but also rods). We can synthesize them with chemical and phase purities as high as 99.9999%, we can control the particle size distribution to approximately ±10%, and we project with confidence that we can make industrial size quantities with manufacturing costs significantly less than any other current technique. The types of particles we can make are, in general, metal oxides, but the process allows us to control the oxidation state so we can make high, medium, and low oxidation state oxides and metals. We can make oxides of all of the transition metals, lanthanides, and actinides, and any stoichiometric combination of any number of these metals. We can include group I and group II metals in combination with the transition metals. Consequently, we have the ability to make an almost innumerable array of nanomaterials (single metal and multi-metal) with well-controlled physical properties, purity, oxidation state, size and size distribution using a process that is fast, reliable, and inexpensive. Table 1 gives examples of some of the materials we have synthesized, and below are some representative TEM images for NiO, Y2O3, and CoO powders.
Adam T. Woolley, PhD

Analytical/Materials Chemistry
C305 BNSN, 422-1701
Email: awoolley@chem.byu.edu

My group works at the interface between chemistry, engineering and biology. Thus, students receive broad technical training and are well positioned to contribute in these key research fields. A common theme in my research is the interrelationship between biological molecules and miniaturization. We are utilizing miniaturization tools to analyze for clinically relevant biomolecules, and we are also applying DNA in forming nanoscale materials.

3D Printed Integrated Microfluidic Systems for Preterm Birth Risk Assessment

Preterm birth (PTB) is a serious issue, with approximately 10% of pregnancies resulting in a preterm delivery, frequently coupled with complications that lead to poor outcomes and increased medical costs. We are developing 3D printed microfluidic systems that integrate various analytical processes in a single microchip (Fig. 1). These devices will provide high-throughput, point-of-care screening from a finger prick quantity of blood to assess risk of a preterm delivery, weeks before contractions begin.

Biotemplated Nanofabrication of Electronics

My group is leading an interdisciplinary team whose objective is to explore bottom-up methods for the fabrication of nanoscale electronic systems. We fold DNA into controlled nanoscale designs that can be converted into functional electronic elements after metallization (Fig. 2). We are presently applying these
methods in making conductive metal nanowires and metal-semiconductor junctions with linewidths as small as 10 nm.

**Rapid Blood Infection Determination**

We are developing methods for detecting bacterial infections in blood in less than one hour, in collaboration with a group of biologists and engineers. A schematic of the proposed system is shown in Figure 3. Our focus is on the capture and fluorescent labeling of nucleic acid material from bacteria. We are developing microfluidic systems with solid supports designed to selectively capture nucleic acid sequences from pathogenic organisms in blood. The retained nucleic acids will then be labeled fluorescently for subsequent single-molecule detection.

![Figure 3. Schematic of integrated pathogen detection cartridge showing each component.](Image)

**References**