

- ***Discovering new therapeutic targets using natural products. Scott Bur (Chemistry), and Jeff Dahlseid (Biology and Chemistry)***

Naturally occurring small molecules (natural products) found in plant and animal extracts interact with biological systems in interesting ways. Many of today's therapeutically important drugs are based on the structure of natural products. We propose to analyzing natural products for biological activity by synthesizing natural product derivatives, which can be immobilized on a resin, and using affinity chromatography to discover proteins that interact with the natural product. The proteins that have affinity for the natural product could then be studied. If the proteins that bind are involved with known disease states, these will be potential therapeutic targets for which one already knows a drug-like structure that can interact.

In Dr. Bur's lab, the focus of research is on new methods to produce natural product derivatives. Students will generate small libraries of natural product derivatives that can be attached to a solid phase resin. The natural products that inspired the methodology studies have broad spectrum of biological activity including anti-bacterial and/or anti-fungal. Simple growth inhibition tests using the model eukaryote, *Saccharomyces cerevisiae*, will be used to determine if any of the derivatives created in Bur's lab may have anti-fungal activity. Those compounds identified in the assays can be further studied in Dahlseid's lab to determine with what cellular proteins they interact. Identifying these proteins may reveal new therapeutic targets for anti-fungal activity. Similar studies could be done using model bacteria in a similar way. Methods for identifying and expressing proteins that interact with the natural products can include traditional affinity chromatography of homogenized cell cultures, which would lead to protein sequencing, microbial genetic screens, which would uncover genes that are functionally important for resistance to the natural product derivatives, and eventually, viral phage display library systems, which would provide DNA sequences directly.

Program Goals

This project will allow students to merge the traditional fields of synthetic organic chemistry and biomolecular science (*i.e.* biochemistry, molecular biology, molecular genetics, etc.) to solve problems of general medicinal interest. This cross disciplinary approach to discovering therapeutic target systems integrates the strengths of both fields to model the cooperative problem solving efforts encountered in the pharmaceutical industry, specifically, and biotechnology industries in general.

- ***Characterization of γ -glutamylcysteine ligase (γ -GCL) —Brenda Kelly (Chemistry and Biology)***

The involvement of glutathione (GSH, tripeptide of glutamic acid-cysteine-glycine) in drug detoxication, steroid isomerization, chemotherapeutic drug resistance, and the storage and transport of cysteine has been well established in mammalian biological systems. This low molecular weight thiol is also essential for survival in many bacteria, including *Escherichia coli* (*E. coli*) and *Salmonella typhimurium*. Therefore, decreasing GSH levels in bacteria may provide a mechanism to exploit in the design of novel antibacterial agents. γ -glutamylcysteine ligase (γ -GCL) is an ideal target for rational design of an inhibitor that may decrease GSH levels because of its role as the first and rate-limiting enzyme of GSH synthesis. However, due to the presence of the same

essential enzyme with an identical function in humans, an inhibitor must be specific for the bacterial form of the enzyme. In addition, rational design requires some structural knowledge of the enzyme that one is attempting to inhibit (*E. coli*) or prevent inhibition of (human) and currently there is no available high resolution structural data for any form of γ -GCL. The goal of the Kelly lab is to characterize both human and *E. coli* γ -GCL using biochemical and biophysical techniques in order to establish structural differences which can be utilized in future design of a bacterial specific inhibitor.

In Kelly's lab, students will have the opportunity to purify *E. coli* and/or human γ -GCL from a recombinant system using anion-exchange, gel filtration, and affinity chromatography. Electron paramagnetic resonance (EPR) studies will be used to establish the active site environment of the essential divalent metal ion/s (# and types of interactions). Students will use UV/VIS and fluorescence spectroscopy to probe substrate binding sites by monitoring enzyme activity and binding properties in the presence of non-native substrate analogs of different sizes, shapes and chemical properties.

- ***Control of Nitric Oxide Synthesis in Macrophages – John Lammert (Biology) and Brian O'Brien (Chemistry)***

Macrophages contribute to inflammation in such autoimmune disorders as rheumatoid arthritis. A key inflammatory mediator released by these phagocytes is nitric oxide (NO). When cultured macrophages are given an inflammatory signal, the gene for the enzyme catalyst (inducible nitric oxide synthetase, or iNOS) of NO synthesis is activated and transcribed. The signaling pathway that leads to activation of this gene is of considerable interest because pharmaceutical dampening of unwanted transcription might be possible. Phenytoin (PHT), with a long history of use as an antiepileptic medicine, has been reported to have anti-inflammatory effects. Lammert and students have found that PHT inhibits NO production by macrophages in a dose-dependent manner.

PHT may interfere with a signaling protein that regulates expression of the iNOS gene. Recently, Lammert and O'Brien, a synthetic organic chemist, have begun collaboration, and are attempting, with students, to synthesize an amino-containing derivative of PHT, which will be coupled to resin to affinity purify any PHT-binding proteins from macrophage cell extracts. In Lammert's lab, students will measure iNOS mRNA levels by Northern blot analysis. Electrophoretic mobility shift assays will be used to gain information about any changes caused by PHT on signaling pathways that activate the iNOS gene. Alternatively, PHT may act to inhibit the enzymatic action of iNOS. Should PHT be found to affect iNOS, collaborative work with O'Brien could provide insight into inhibitory mechanisms. For example, PHT interacts with heme, an essential contributor to the active site of iNOS. O'Brien would provide the expertise for NMR spectroscopic experiments to address such interactions.

- ***Methylmercury Formation and Accumulation in Aquatic Food Chains in Voyageurs National Park – Jeff Jeremiason (Chemistry)***

Jeremiason provides expertise in environmental chemistry that will create opportunities for biology, chemistry, and environmental studies students interested in the fate, transport, and bioaccumulation of contaminants in the environment. Mercury is the contaminant of interest in this proposed project. Mercury (Hg) released into the

environment undergoes various chemical and biologically mediated transformations prior to conversion to methylmercury (MeHg) and subsequent accumulation in aquatic food chains. A basic understanding of these transformations and aquatic food web structure is necessary to assess mercury levels in game fish. A multi-disciplinary research project addressing elevated, but highly variable mercury levels in fish at Voyageurs National Park (VNP) in northern Minnesota is currently underway. Project cooperators include four government agencies, a university, and Gustavus Adolphus College.

The proposed project is a small, but integral part of the larger multidisciplinary project. Determination of aquatic food chain structure and MeHg formation rates in wetlands are primary objectives of this proposal. Students will participate by collecting, processing, and analyzing various aquatic biota samples from small lakes in VNP. Mercury and stable isotopes of nitrogen and carbon will be measured in each sample. Aquatic food chain structure will be inferred from nitrogen and carbon stable isotopes and related to mercury levels in biota. Wetlands are a known source of MeHg to aquatic systems. Cores from several different wetland types adjacent to VNP lakes will be collected and injected with stable Hg isotopes. After an incubation period, MeHg formation rates will be determined and related to MeHg levels in adjacent lakes.

- ***Post-transcriptional Control of Gene Expression: Roles of mRNA Processing and Stability in Model Genetic Systems – Jeff Dahlseid (Biology and Chemistry) and Colleen Jacks (Biology)***

With the arrival of Dahlseid this fall, there are now two laboratories with gene expression as their primary focus at Gustavus. Both laboratories utilize model genetic systems and share an interest in post-transcriptional control of gene expression involving processing and stability of mRNA. Dahlseid is interested in regulation mediated by a specialized biochemical pathway for mRNA degradation in the baker's yeast *Saccharomyces cerevisiae*. Upf proteins control the abundance of mRNA for at least 7% of wild-type yeast genes. This research aims to identify mRNAs that are degraded by the Upf-mediated pathway and to reveal the molecular basis for their recognition. Research is focused upon a subset of candidate mRNAs encoding proteins involved in chromosomal function. Jacks is interested in the regulation of genes involved in ribosome function and biogenesis in the plant *Arabidopsis thaliana*. The S15 ribosomal protein is encoded by a family of six genes that are differentially expressed during plant growth. RT-PCR analysis indicates the presence of a partially or alternatively processed *RPS15C* transcript, primarily in root tissue, and a knock-out mutant for this gene has been isolated. Research will focus on sequence characterization of this *RPS15C* transcript and analysis of the mutant for a molecular and morphological phenotype, with the ultimate goal of understanding the role of each family member in maintaining appropriate levels of S15 expression during plant growth. The Dahlseid and Jacks laboratories will work jointly to develop methods for quantifying mRNA levels using infrared fluorescence and detection with an automated DNA sequencer. Approaches will include RT-PCR and primer extension with fluor-labeled oligonucleotides.

- ***Physiological and Computational Studies of Neurotransmitters – Michael Ferragamo (Biology) and Jonathan Smith (Chemistry)***

This study continues the work Ferragamo has begun to exploit an adaptation found in anurans as a unique strategy towards unraveling the contribution of spectral and time domain processing in an animal where the contribution of the two codes

can be easily parceled. Anurans are the only vertebrates that possess three different organs in their inner ear to process airborne sounds. None of these peripheral organs contains an epithelial membrane homologous to that found in mammals; hence, the information emanating from the signal transduction apparatus is simplified to conveying the periodic structure of the waveform. Preliminary recordings from the midbrain in response to amplitude-modulated (AM) signals in quiet and at variable signal-to-noise levels reveal circuitry with an exquisite capacity to encode signals with temporal precision. Results predict that this computation is implemented by the interplay of synaptic inputs mediated by γ -aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors. The nonlinearities in the NMDA current make it ideally suited for the detection of coincidence of synaptic inputs. Immunocytochemical studies have shown that the 5-HT (serotonin) receptor and transporter is diffusely distributed throughout the entire auditory system. The influence of serotonin, often regarded as a slow, modulatory neurotransmitter, in a sensory system whose hallmark is speed and fidelity, is largely unexplored. Future *in vivo* studies will examine the role of each of these putative inputs to midbrain neurons by iontophoretic application of postsynaptic-receptor antagonists prior to delivery of biologically relevant AM signals. Ferragamo and Smith foresee the reciprocal benefits of having students from Smith's group contribute to the understanding of agonist-receptor binding interactions as part of a question that spans from the molecular level to the behavior of the organism.

Computational chemistry has been one focus of research for Smith and several students since coming to Gustavus in 1998. One of his projects has involved using quantum chemical calculations to determine the geometry and flexibility of the neurotransmitter serotonin (5-HT) and related compounds in order to understand their relative receptor binding affinity. The initial calculations have focused on 5-HT and trying to map out the relevant molecular conformations using high-level quantum chemical computations, including a polarizable continuum solvation model or other models that include several explicit waters. The proposed project will expand to include several related compounds that have been found to be agonists at certain 5-HT receptors (5-HT₃) and antagonists at other 5-HT receptors (5-HT₄). These calculations can be compared to the results of a recently developed 3D pharmacophore for these receptors. A second set of comparisons can be made for the 5-HT_{1D} receptor whose agonists have been shown to be related to migraine headaches. A third intriguing direction will be the study of related compounds that are agonists for the 5-HT_{3A} receptor, which has been cloned and sequenced.