KECK SCIENCE

FACULTY RESEARCH INTERESTS

Thesis Projects for 2011-2012 **Jennifer Armstrong**

My laboratory is interested in chromatin structure and gene expression. In the last few years, we have focused on a protein that is conserved in yeast, flies, and humans called CHD1 (named for its protein domains: chromodomains, helicase, DNA binding domain). Humans have nine CHD proteins, several of which are linked to various conditions including dermatomyositis, neuroblastoma, and CHARGE syndrome, making basic research on this protein subfamily a high priority. Our studies using the giant polytene chromosomes of Drosophila uncovered a role for CHD1 in the maintenace of global chromosome structure. CHD1 has recently become a topic of great interest as mouse CHD1 is required for stem cell pluripotency. Student projects in my lab include (but are not limited to):

Project 1: To understand how CHD1 functions, it would be useful to identify protein interaction partners. One approach to the identification of physical interaction partners is co-immunoprecipitation of HA-tagged CHD1 from embryonic protein extracts followed by identification of the proteins by mass spectrometry. This project is ongoing in the lab and involves basic biochemical techniques.

Project 2: To identify proteins that functionally interact with CHD1 we have developed a wing-based genetic assay. This project is ongoing in the lab, and candidate genes that interact with *chd1* are currently under investigation. I would like to see a student expand this project and conduct a pilot genetic screen. This project is completely genetics based.

Project 3: CHD1 is localized to all active genes on polytene chromosomes, but is not required for sustained transcription. Preliminary data suggests that CHD1 may be required during times of transcriptional stress, or for recovery following that stress. This is an ongoing project and involves basic genetics, immuno-fluorescent staining of chromosomes, and fluorescent microscopy.

Project 4: Many chromatin proteins alter gene expression through changes to chromatin structure. An assay for such an activity is PEV (position effect variegation). A gene placed in heterochromatin will be transcriptionally silenced. That transcription can be dominantly modified by mutation in genes encoding factors important for chromatin structure. There is one report in the literature that CHD1 affects PEV, and I would like to clarify and expland on that result, linking a transcriptional readout assay to our observed changes in chromosome morphology following loss or over-expression of CHD1. This is a new project and is genetics based.

Project 5: Our lab uses the technique of immunofluorescence of polytene chromosomes to examine levels of CHD1, RNA Polymerase II, chromatin proteins, histone marks, etc. To move from a qualitative evaluation of these data to a quantitative analysis, a student wrote a Matlab program to quantify fluorescently labeled chromosomes. This approach can be expanded to ask whether CHD1 affects the newly denoted five forms of chromatin: black (bound by H1), green (bound by HP1), blue (bound by Polycomb proteins), red (bound by the Brahma ATPase), or yellow (bound by MRG15). This is a complex project that could extend over several years and involve several members of the lab. It involves basic genetics, immuno-fluorescent staining of chromosomes, and fluorescent microscopy.

Bethany Caulkins Research Description

Nuclear magnetic resonance (NMR) is a powerful tool for the determination of molecular structure; never is this power more evident than when working with a biological system. NMR allows for a description of not just structures, but also dynamics and kinetics and a myriad of other useful pieces of information. My research employs the physical technique of NMR to probe the structure and function of biomolecules. Students in my lab gain experience in the overexpression and purification of proteins and fundamental bio-NMR structural techniques. There are three systems currently being studied in my lab: ubiquitin, serine racemase, and ornithine decarboxylase.

Ubiquitin is a small 76-residue protein that is found ubiquitously in human cells. Ubiquitination and other post-translational modifications play a major role in the proliferation of many neurodegenerative diseases, developmental disorders, and cancers, and as such are subjects of recently increased biochemical interest. The overall goal of the ubiquitin project is to probe and characterize the interaction of ubiquitin with a water-soluble, self-folding, negative cavitand. The synthetic cavitand used in these experiments has already proven useful in biosensing applications, and understanding the nature of its interaction with cationic proteins can shed light on both the binding properties of the protein and the cavitand. Initial experiments will utilize ^{15}N-^{1}H HSQC NMR experiments to determine possible sites of cavitand binding by analyzing the chemical shift changes of the protein's lysine and arginine motifs. Results may indicate the cavitand's viability as a biosensor for ubiquitination and other modifications, therefore possibly accelerating diagnostics and disease research as a result.

Serine racemase is a pyridoxal-5'-phosphate (PLP)-dependent, fold type II enzyme that catalyzes the transformation of L-serine to D-Serine. Previously thought to have no role in physiological events, it was later found that D-serine is a crucial co-agonist in the activation of N-methyl-D-aspartate (NMDA) receptors and that hypoactivity of these receptors is thought to play a role in brain conditions like schizophrenia and amyotrophic lateral sclerosis (ALS, Lou Gehrig's Disease). D-serine is degraded by the enzyme D-amino acid oxidase, but this enzyme is not found in the forebrain areas where D-serine is highly prevalent. Researchers have proposed that levels of D-serine are kept constant through a deleterious side reaction, in which PLP catalyzes the conversion of D-serine to pyruvate and ammonia via an α-aminoacrylate intermediate. This presents a tantalizing opportunity to explore the connection between protonation state and reaction specificity. What electrostatic differences in the active site encourage the racemization over the β-elimination reaction as directed by the PLP cofactor? To try and answer this question, stabilization of the intermediates that occur along the reaction pathway will be a major focus. Once stabilization conditions are determined, the different intermediates can be probed with both solution- and solid-state NMR to tease out the small details of the catalytic reaction.

Ornithine decarboxylase (ODC) is a 94 kDa homodimer and is responsible for polyamine metabolism in nearly all eukaryotic cells. As a pyridoxal-5'-phosphate (PLP)-dependent enzyme, ODC also joins an extensive class of enzymes reliant on this efficient cofactor. Despite a crystal structure (PDBID: 1D7K) for the wild type enzyme being solved, no NMR structural data have been deposited in the Biologic Magnetic Resonance Data Bank (BMRB). This leaves a gap in the understanding of the dynamic nature of this enzyme and limits studies on how the structure of this enzyme influences its function. We will express and purify ODC and perform ^{1}H-^{15}N HSQC experiments to begin the assignment of the amino acid sequence of the protein. This will pave the way for more detailed studies into the dynamics, relaxation, and mechanism of the enzyme.

In addition to our focus on ODC, we will look at other PLP-dependent enzymes to place ODC in its proper context in this enzyme family.

While this is the starting point, other projects are always popping up. Plans are already being made to expand the number of systems being studied and determine the structure and function of other biomolecules. The atomic-level details involved in proper function of proteins and enzymes is necessary for the development of treatments and diagnostic aids. More than that, though, the study of acid-base chemistry in the enzyme active site can lend information to more diverse systems that would benefit from a similar study.

M. Coleman - Lab Research Projects

My lab uses song birds as a model system to study several major questions in neuroscience. We study how neurons are connected and are involved in a learned behavior (song) and we study the neural basis of pair-bonding.

In the zebra finch songbird, song is a male-specific behavior that is learned from a tutor. In order to learn song, a young male bird must listen to his father's song, form a memory of that song and then match his own vocalizations with the memory of the father's song. Areas of the bird brain that are involved in auditory processing and song production have been identified. One project in the lab is to examine synaptic connections between areas involved in auditory processing and/or song productions. For these experiments, we use extracellular and intracellular electrophysiological recording techniques.

The second project in the lab examines the neural basis of pair-bond maintenance in finches. Zebra finches pair-bond, which means they co-parent and are monogamous. The best studied model system for the neural basis of pair-bond formation is prairie voles. In these rodents, it is thought that dopamine is released from 'reward pathways' in the brain and reinforce the olfactory signal from the mate. We hypothesize a similar mechanism in finches, but the dopamine pathway reinforces the auditory signal (song) of the mate. To test this idea we implant cannulae into the brain and block or add dopamine to a specific auditory area of the brain and examine the effect on the female's preference for her mate's song over other male songs. We hypothesize that dopamine will enhance her preference for her mate's song and dopamine antagonists will block her preference. In addition, we put dopamine or dopamine antagonists in the brain to examine directly their effect on the activity of neurons. For these experiments, we use extracellular recording techniques, anatomical and behavioral techniques.

Gretchen Edwalds-Gilbert, Ph.D.
Associate Professor of Biology
Thesis topics 2011-2012

I have two major projects in my lab. One examines the regulation of a specific conserved protein in gene expression. The other is a collaborative project with Dr. Irene Tang and others to determine toxin response pathways in two evolutionary divergent yeasts.

Project 1:
The overall goal of my research program is to understand the regulation of the RNA-dependent ATPase Prp43, a conserved member of the DExD/H-box protein family that is essential for both pre-mRNA splicing and ribosome biogenesis. Pre-mRNA splicing is an essential process in eukaryotic gene expression in which introns are removed and exons are ligated. While existence of both informational and non-informational sequences in genes was identified over twenty years ago, the precise mechanism by which introns are removed from pre-messenger RNAs is still under study. Mutations that cause errors in splicing are found in at least 15% of human genetic diseases; additional diseases are caused by mutations in trans-acting splicing factors. There are eight members of the DExD/H-box family of putative RNA helicases that play essential roles in different steps of pre-mRNA splicing, perhaps for resolution of RNA-RNA and/or RNA-protein interactions. Ribosome biogenesis involves assembly of the RNA-protein complex required for translation of mRNAs. Prp43 is required for spliceosome disassembly and for ribosomal RNA processing, and co-purifies with a wide variety of complexes involved in RNA metabolism. Residues essential for RNA-dependent ATPase activity *in vitro* have been identified; however, *in vivo* Prp43 acts within complexes, interacting with factors that may regulate its activity.

Projects 1 addresses the following specific aims:

1) Investigate whether mutations in Prp43 affect specific protein-protein interactions, which in turn affect either pre-mRNA splicing or ribosome biogenesis.
2) For Prp43 mutations that affect ribosome biogenesis, determine at what step in the pathway the mutants are blocked.

These projects involve both *in vivo* and *in vitro* approaches. *S. cerevisiae* has many advantages as a model system: the mechanisms of pre-mRNA splicing and ribosome biogenesis have been evolutionarily conserved between yeast and more complex eukaryotes, such as humans, and yeast are amenable to both genetic and biochemical studies. The mouse homolog of Prp43, mDEAH9, can substitute for the yeast protein *in vivo*.

Project 2:
This project is a comparative genomic study of the genetic networks for phenol-stress response in the evolutionary context of budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*. Living organisms in variable natural environments must respond to environmental changes; the ability of species to adapt to such environmental stresses is necessary for their survival under natural selection. The chosen phenol derivatives are naturally occurring and synthetic compounds that exert effects on organisms as stress factors. Intriguing biological effects of individual phenol derivatives include protective anti-oxidation, prooxidation, DNA-damage and apoptosis. However, little is known about the principal cellular processes affected and how eukaryotes cope with the

stress at a molecular level. Global characterization of the genetic networks required for phenol response will fill a gap in knowledge about organism-environment interactions at the systems level.

S. cerevisiae and *S. pombe* are evolutionarily divergent as eukaryotic models. They are both amenable to cell-based and genetic analysis, and are the only eukaryotes with collections of genome-wide gene deletions available, offering unique value for deciphering the phenol-stress networks. This research takes advantage of the yeasts' suitability for cell-based analysis, genetic tractability, and availability of deletion libraries to study the stress-response signaling pathways at cellular and molecular levels. Based on previous studies and our preliminary results, we hypothesize that specific genes are required to detect the phenol stress in and adapt to the environment; cell survival of the stress requires DNA damage/repair function and integral cell cycle regulation. The important questions are: What are the genes required for the phenol-specific and general stress responses? What components of the stress response networks are conserved through evolution? Are DNA checkpoint/repair and cell cycle control signaling critical for cell survival of the phenol stress?

Project 2 addresses the following specific aims:

Aim 1. To identify genes involved in the phenol response networks by genome-wide screening of the deletion libraries of both yeasts Deletion sensitivity profilings (DSP) of haploid/diploid homozygous strains and haploinsufficiency profilings (HIP) of diploid heterozygous strains in the presence of the phenols will be constructed.

Aim 2. To determine conserved elements in the phenol-stress networks by comparing the response profiles of both yeasts, and uncovering common components in general stress networks by analyzing responses under different conditions including temperature, osmotic, and oxidative stresses The genomic-scale comparisons will reveal the key components of the networks conserved through evolution, distinct between the two yeasts, common to general stresses, specific for phenols, and distinct to different types of phenols.

Aim 3. To evaluate the roles of DNA damage checkpoint/repair and cell cycle regulation in cellular survival of phenol stress The roles of the crucial genes in response pathways will be verified and their corresponding deletion strains will be tested for fitness including genomic stability and cell cycle progression under phenol stress conditions.

The robust and established experimental procedures of yeast biology ensure the feasibility of the project. The central rationale is that although nearly as distantly related as both are to humans, the yeasts share a similar DNA damage checkpoint/repair apparatus and cell cycle control machinery, which are probable targets of phenol stress. Consequently, the conserved phenol response elements are likely to be identified in these basic cellular processes through comparative genomic analysis of the two yeasts, thereby shedding light on the molecular mechanisms of stress response fundamental to all eukaryotes in changing environments.

Techniques used in the lab include PCR, cloning, DNA sequencing, protein expression and purification, RNA purification and analysis, electrophoresis (nucleic acids and protein), yeast growth and transformation, and other molecular biology/biochemical techniques.

Dr. Elise Ferree

My research in Animal Behavior addresses both theoretical and applied questions. Students work with me in the field around campus and the Bernard Field Station during the school year and in Costa Rica during the summer. Opportunities also exist for analysis-based research using previously collected data.

Avian ecology

The Bernard Field Station (BFS) is one of few remaining fragments of coastal sage scrub in our area, and I am interested in understanding the behavior of the birds that use it. One focus of my work are white-crowned sparrows (*Zonotrichia leucophrys*), a common wintering bird species at the BFS. They are small, seed-eating songbirds that migrate to southern California from their breeding grounds in Alaska in the fall and return in the spring. Quite a few white-crowned sparrows can be found at the BFS each winter, normally around 50 individuals estimated on a given day (eBird). I recently began to capture and color-band this population (each bird is given a unique combination of colored leg bands) so that I can better understand how this species uses the BFS during the winter and to monitor their migratory patterns. Specifically I want to determine whether individuals just pass through the field station, or if is it an over-wintering site for specific birds. I can answer this question using mark-recapture and re-sighting techniques. I am also interested in using this species to ask questions about migratory patterns. For example, I want to test whether the local population follows predicted patterns of sex-biased differential migration, in which males migrate shorter distances from the breeding ground than females (Morton 1984; Newton 2008). By taking a blood sample from white-crowned sparrows captured at the BFS, I can use PCR (polymerase chain reaction) to determine their sex and examine the sex ratio of the wintering populations. I predict that the BFS population will be strongly female-biased under the hypothesis of differential migration. Given the changing climate, I am also interested in determining whether over time sex-biased differential migration becomes less pronounced. Many bird species appear to be adjusting their migratory patterns in relation to climate change.

I also band other species that I capture (California towhees and California thrashers in particular), and data from these individuals can be used to address other questions.

Behavioral ecology of golden orb web spiders

Social behavior in spiders is exceedingly rare, with over 99.99% of spiders being solitary. The golden-orb web spider (*Nephila clavipes*) is one of few species that shows colonial behavior. Females build large webs that capture prey, provide a location for mating and harbor kleptoparasites, and in this species, webs can be found in isolation or clustered with other webs. To determine why colonial behavior is so rarely favored in spiders, our lab seeks to determine under what conditions grouping pays off. Specifically we investigate how the trade-offs of web clustering vary as spiders in our species grow and age, and how yearly variation in environmental factors influences clustering behavior. Two students each summer join me to study spiders at Pitzer's Firestone Center for Restoration Ecology in Costa Rica.

Bioacoustics

Current technology allows us to get good recordings of animal sounds and to extract detailed information about those sounds. I am interested in both the function of and variation in animal sounds. For example we are testing hypotheses about the function of a call that male black phoebes make when providing care to their young. In addition to having hours of recordings of local songbirds, from which students may develop projects, I have other bioacoustics topics that I am eager to explore.

RESEARCH INTERESTS – DR. PATRICK FERREE

EFFECTS OF GENETIC AND MICROBIAL PARASITES ON EUKARYOTIC HOST DEVELOPMENT

Higher eukaryotes are hosts of numerous genetic and microbial parasites. A sub-group of these agents can profoundly alter host development in order to selfishly increase their own frequencies in nature. I am interested in indentifying the specific host molecular targets in order to better understand the underlying mechanisms. On a broader level, I am interested in understanding the impact of these effects on host genome evolution.

Ongoing studies are revealing host chromatin as a general target. Research in my laboratory is divided into three main areas, which focus on parasitic effects on whole chromosomes, gene groups, and individual genes in the fruit fly *Drosophila melanogaster* and the jewel wasp *Nasonia vitripennis*.

1. INVESTIGATING THE IMPACT OF SELFISH SATELLITE DNA ON HOST CHROMOSOMES. Satellite DNA is arguably the most common form of 'junk DNA' in the typical eukaryotic genome. These highly repetitive, non-protein-coding sequences can accumulate to between hundreds and thousands of copies at certain chromosomal locations. Because of their abundance, satellite DNAs comprise as much as half of the genome in many plant and animal species, including *D. melanogaster*. They are packaged into an extremely condensed form of chromatin called heterochromatin. This process believed to be important for preventing the expansion of these sequences to harmful levels.

Despite the silencing effects of heterochromatin, some specific satellite DNAs have increased to extremely high copy number over short evolutionary periods. Such is the case for the 359-bp DNA, a large and highly complex satellite DNA present in multi-mega-base pair amounts on the *D. melanogaster* X chromosome. These high copy numbers are benign within the *D. melanogaster* species, but in hybrids produced from crosses between *D. melanogaster* and its sibling species, *D. simulans*, this satellite DNA causes striking chromosome segregation defects and early embryonic lethality. The genome of *D. simulans* contains only a very small amount of 359-bp satellite DNA. These observations together suggest a model whereby the *D. melanogaster* 359-bp satellite DNA is improperly packaged into heterochromatin by *D. simulans*-specific proteins. On a broader level, these results suggest that selfish satellite DNAs may effectively cause reproductive isolation between species and, therefore, may play an important role in speciation.

Why is the 359-bp satellite DNA, and none of the other satellite DNAs, not packaged properly into heterochromatin in hybrids? One obstacle to experimentally addressing this question is the inability to use genetic methods to map the genes involved; this stems from the fact that F1 hybrids are lethal and, therefore, cannot be used in genetic crosses.

As an alternative approach, my lab has been developing an intra-specific model (*i.e.*, solely in the *D. melanogaster* species) for investigating the observed 359-bp DNA effects. It turns out that abnormal circularized (ring) Y-chromosomes (normally they are linear) in *D. melanogaster* are lethal because of mitotic defects similar to those seen in hybrids. We have recently shown that these defects map to a large block of 359-bp DNA linked to the ring-Y chromosome. Our current hypothesis is that the 359-bp satellite DNA when on the Y chromosome in ring form fails to become packaged properly. Some important research directions include genetic experiments (1) confirming the role of 359-bp DNA, (2) linking further the observed defects to heterochromatin, and (3) identifying specific protein-coding genes involved. We are also using confocal and epifluorescence microscopy to image ring-Y chromosome dynamics in early-stage *D. melanogaster* embryos.

2. UNDERSTANDING HOW AN INVADER CHROMOSOME SELFISHLY CONVERTS FEMALES INTO MALES. In the jewel wasp *N. vitripennis*, the number of chromosomes determines the sex of an individual. Females have ten

chromosomes, five deriving from each parent. Males, however, have only five chromosomes, which originate solely from the mother (there is no Y chromosome in this insect group). Under normal circumstances, the mother controls the sex ratio of her offspring by regulating how many eggs are fertilized; fertilized eggs become diploid females while those not fertilized become haploid males. However, some individual wasps in nature harbor a foreign chromosome known as Paternal Sex Ratio (PSR), which is transmitted solely via the sperm. The origin of this foreign chromosome is unknown. PSR is considered to be the ultimate selfish genetic element because it completely destroys the five normal paternal chromosomes that it is transmitted with shortly after fertilization, while sparing itself from this fate. Thus, PSR converts a fertilized egg, which would normally become female, into male. This effect is believed to be advantageous to PSR because it is transmitted solely through males.

Although considerable research has focused on the evolutionary implications of PSR, the underlying cell and molecular mechanisms are currently unknown. Some intriguing questions are (1) how is PSR able to escape the fate of the destroyed paternal chromosomes immediately following fertilization? and (2) what is the molecular mechanism by which PSR destroys the paternal chromosomes? Interestingly, previous studies have demonstrated that PSR consists almost entirely of heterochromatic sequences, including satellite DNA. This observation leads to some interesting possibilities for the mechanism of lethality, including the mis-localization of heterochromatin proteins on the paternally derived chromosomes.

We have begun to use advanced microscopic imaging to follow the dynamics of PSR during the very first stages of embryonic development (*i.e.*, immediately following fertilization). Our hope is that careful cytological observations will provide important clues for understanding how PSR escapes the paternal chromosomes and protects itself from its chromosome-poisoning effects.

Other long-term plans include identifying and analyzing key heterochromatin proteins encoded in the *N. vitripennis* genome. This goal is now possible due to the recently sequenced *N. vitripennis* genome. Once these proteins are identified, we can begin to individually address whether they become mis-localized in the presence of PSR, and testing if PSR exhibits its own novel set of heterochromatin proteins.

3. INVESTIGATING THE NATURE OF SPIROPLASMA-INDUCED MALE KILLING. Many types of bacteria live inside the reproductive cells of insects and nematodes, where they can be easily transmitted to host offspring. Several different bacteria have evolved the ability to selfishly alter host reproduction for their own benefit. One such bacterium is *Spiroplasma pulsonii*, a natural pathogen of *D. melanogaster* that kills male flies during their development. This effect is believed to benefit female siblings (and thus the bacteria because they are maternally transmitted) by providing these females more resources. Previous genetic experiments suggested that *S. pulsonii* kills males by operating through the male-specific Dosage Compensation Complex (DCC). Some outstanding questions are (1) do these bacteria alter DCC activity directly or through downstream pathways and (2) specifically how are these pathways altered at the molecular level?

In one line of investigation, we are using microscopic imaging to test if *S. pulsonii* causes mis-localization of the DCC in order to kill males. Normally, the DCC binds solely to genes along the single X chromosome in males. Once bound, the complex recruits enzymes that modify chromatin so that transcription levels of these genes increase 2-fold and, therefore, equal to levels found in females (which have two X chromosomes). One possibility is that *S. poulsonii* prevents the DCC from properly binding to X chromatin. Alternatively, the bacteria may cause the DCC to abnormally bind sites on the non-sex chromosomes.

It is highly likely that *Spiroplasma*-induced male death involves gene mis-expression, regardless of the immediate target. We currently are making plans to analyze whole genome transcription using microarrays in order to identify individual genes across the genome that are mis-regulated by *Spiroplasma*.

Findley Finseth is an Assistant Professor of Genomics in the Keck Science Department at the Claremont Colleges.

Her research program investigates the evolutionary drivers of biodiversity. By complementing modern genomics studies of natural populations with classic genetics experiments, Findley's work offers novel insight into the maintenance of genetic variation, the processes of adaptation and speciation, and the evolution of the genome itself. Currently, Findley focuses on a group of California native wildflowers, *Mimulus*. Her work in *Mimulus* is broad, spanning studies of selfishly evolving genes to the genetic basis of thermally-adapted plants in Yellowstone National Park.

Sarah Gilman

My laboratory studies the ecological communities on rocky intertidal shores. I am interested in understanding how abiotic factors, such as temperature, influence an organism's success and the outcome of interactions with prey, predators, and competitors. Research projects in my lab can involve laboratory studies, field experiments, and/or computer modeling.

Past/current student projects include:

- Measuring the effect of low tide on the feeding rates of barnacles
- Measure the oxygen consumption of barnacles under different temperatures
- Testing the preference of predatory snails for native and nonnative oysters in Newport Bay
- Measuring the effects of air and water temperature on the feeding rates of predatory snails
- Hydro-acoustics: using sound to monitor the feeding behavior of predatory snails
- Modeling the growth and survival of intertidal barnacles under warm and cool temperatures

Dr. Scot Gould

RESEARSH INTERESTS:

1. Ultrastructure of spider silk
2. Surface properties of fluidized cracking catalysts
3. Synthetic polymer formation and liquid crystals
4. Characterization of surfaces using fractal techniques
5. Physics education: computer integration with Maple
6. Economics based: systemic risk in financial institutions

See Webpage: http://faculty.jsd.claremont.edu/sgould/

Mary Hatcher-Skeers – Biophysical Chemistry

The Hatcher-Skeers group studies the role of DNA structure and dynamics in recognition processes. In particular, we are interested how small perturbations in DNA sequence affect the local structure and dynamics of short oligomers. An important example of a sequence perturbation is cytosine methylation, a perturbation associated with heritable gene silencing which plays a major role in the development of cancer.

Solid-state deuterium NMR studies of methylated and unmethylated DNA binding sites have shown that cytosine methylation quenches DNA backbone dynamics. However, these experiments required extensive sample preparation, lengthy acquisition times and allowed the study of only one nucleotide step at a time. The Hatcher-Skeers research group recently published a phosphorus NMR methodology that allows for simultaneous investigation of the backbone structure and dynamics of each step in methylated and unmethylated DNA sequences. In addition, we demonstrated the efficiency and usefulness of FTIR spectroscopy in studying DNA structure using the example of altering sequence context. The ability to quickly study a large number of binding sites with different sequence perturbations provides important insights into the role of DNA structure and dynamics in recognition processes.

Current projects use and expand these recently developed FTIR and NMR methodologies to study the effects of sequence context and methylation on DNA structure and dynamics. In addition, we are studying the correlation of these effects to biological activity using fluorescence assays to study drug binding in native and methylated sequences in differing sequence contexts. Finally, we use solid-state deuterium NMR to carefully study the local dynamics of DNA sites with interesting drug binding and/or structural profiles.

Adam S. Landsberg

Professor of Physics

Main Research Area: Broadly speaking, my research centers on the mathematical modeling of complex systems. Past work has included projects in areas such as network analysis, nonlinear dynamics, and game theory, cultural dissemination ← e.g. how ideas/beliefs spread across a society

Potential thesis students should contact me directly to discuss currently available projects. This work is most suited to students with strong interests in mathematical and computer modeling.

To give you a flavor of some of the types of thesis projects that students have done with me in the past, I give two examples below. (Note: These are *not* necessarily currently available projects):

Analysis of networks: In its simplest form, a network is just a collection of points connected by lines. However, this simple concept has tremendous utility across many disciplines, from biology to sociology to economics to physics. The points (i.e., "nodes") in a network can represent people, companies, neurons, etc., while the lines (i.e., "edges") can represent various types of relationships or interactions between these points. Some well known examples of networks include the neuronal network of the brain, the internet, social networks, and food webs. In this project students will examine various aspects of networks (e.g., types of networks, network measures and metrics, and local/global network structure), and write and/or use computer code to numerically explore various network properties and their applications.

Background/Required Skills: At least one computer programming course (in Python or Matlab), and math though multivariable calculus (but linear algebra is ideal).

Combinatorial games: Combinatorial games are a class of two-player games with no chance elements in which players take turns moving (e.g., chess, checkers, go, nim, chomp). Recently, interesting connections have been uncovered between such games and some key ideas in physics relating to crystal growth and renormalization. In this project students will explore various facets of these games.

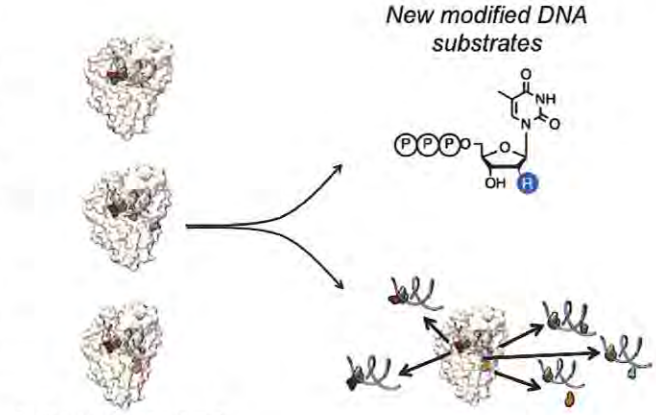
Background/Required Skills: At least one computer programming course (in Python or Matlab), and math though multivariable calculus (but linear algebra is ideal).

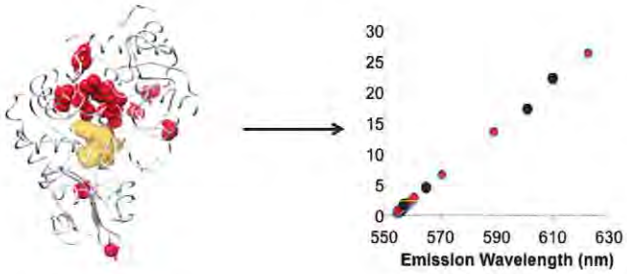
LECONTE GROUP

Please refer to our website for more (and more frequently updated) information:
http://faculty.kecksci.claremont.edu/aleconte/

Overview: In addition to being the basis for all known life, the process of evolution has been used in the laboratory to create a number of useful medicines and materials such as blockbuster drugs (*e.g.* Humira), basic science tools (*e.g.* GFP), and every day items (*e.g.* laundry detergent). The Leconte group focuses on understanding and harnessing the power of evolution to create new proteins that have useful or novel properties. We study two proteins, Taq DNA polymerase and Luciferase, both of which have broad-ranging applications including, but not limited to, enabling basic science discoveries and medical diagnostics.

Taq DNA Polymerase: Chemically modified forms of DNA have wide-ranging applications from medical diagnostics to therapeutics; however, their application is limited by the inability of DNA polymerases to amplify modified forms of DNA. While a number of research groups have attempted to use evolution to identify mutant enzymes capable of synthesizing modified DNA, these efforts have yet to succeed in creating a biotechnologically viable enzyme. To date, our efforts have been focused on biochemically and structurally characterizing the previously identified mutants. These studies have both elucidated the mechanism of these previous mutant enzymes as well as identified exciting new modified DNA-mutant polymerase combinations. In addition to these ongoing efforts, we are now directly applying our discoveries to actively engineer the enzyme.





library of 10^4 mutant enzymes

screening identifies mutant enzymes with improved properties

Luciferase: Bioluminescent imaging (BLI) couples *in vitro* and *in vivo* biological events to the output of the enzyme luciferase, which catalyzes a chemical reaction that emits light. BLI has exciting applications in whole animal and longitudinal imaging studies, and has potential for human medical imaging applications. While BLI holds great promise, there are a number of properties of luciferase that limit the application of luciferase in imaging. We are using evolution to identify novel mutants with improved properties.

Thesis Opportunities

Former Theses

Senior Thesis Opportunities with Dr. Donald McFarlane
Fall 2018 - Spring 2019

In addition to the projects outlined below, I am willing to consider well thought-out proposals in the area of general mammalogy, field-based ecology, ecological modeling, and paleontology. I will also co-supervise Geology/Earth Sciences projects developed in collaboration with colleagues in the Depts. of Geology (Pomona), or Environmental Analysis.

Uranium Leaching at an Abandoned Mine, Mojave Desert
The New Method mine is an small, abandoned uranium mine near Amboy in the Mojave Desert. This project would involved #D mapping of the mine talus by "drone" photogrammetry, followed by laboratory analysis of potentially leached uranium in surrounding desert plants and soil.(2 semesters)

Naturally Occurring Fluorine levels in Tea
The tea plant, Camelia sinesis, is know to preferentially accumulate fluorine ions. In areas of appropriate geology and soil chemistry, accumulated fluorine levels can reach levels of concern. This project will involve laboratory determination of fluorine in a variety of tea samples from different countries. (1 semester)

Assessment of a California Bat Population Size by Quantitative Video Analysis.
Very large bat populations in caves are notoriously difficult to quantify. This project will examine video footage of the exodus of bats from a roost in Riverside County, determine flight speed and "flux rate", to derive a statistically supportable estimate of total population size.
(1 semester; Fall only). Requires transport to make ~ 3 visits to the roost in September.

Professor Sarah Marzen

The Marzen lab is interested in the physics of squishy things-- anything squishy, we'd like to quantify. We might quantify its behavior; we might quantify what its thinking; we might quantify how these relate. Example projects include a treatment for schizophrenia, predictions for mutation and selection strengths in organisms based on the principle of maximal information transfer, optimal foraging behavior, quantification of learning to predict in sequence learning experiments, quantification of how much cultured neurons learn to predict about their stimulus, and so on.

JENNA A. MONROY

As an integrative physiologist, I am broadly interested in the sensory, mechanical, neuromuscular and energetic factors that influence animal movement. My current research focuses on the ways in which intrinsic elastic properties of muscles affect how animals move. Not only can muscles shorten to produce force and positive work but they also can stabilize motion at the joints, store and recover elastic energy, and absorb energy. These properties can contribute to movement instantaneously or with long-lasting effects. My research integrates aspects of muscle physiology, biomechanics, neurobiology, and animal behavior to study how the history- and time- dependent properties of muscle contribute to movement control.

I use frogs and mice to study how intrinsic muscle properties contribute to movement from the single cell to the whole organism. The elastic protein, titin is the third most abundant protein in muscle and spans an entire half sarcomere. Titin plays many important roles in muscle including passive force generation. However, its role in active muscle remains unclear. By investigating titin function in active muscle, I hope to better understand several facets of muscle function that are not well explained by the Sliding Filament theory. I use *in vitro* muscle preparations to describe the fundamental principles of intrinsic muscle properties. I also study prey capture in frogs to explore the role of intrinsic muscle properties in a natural behavior within an evolutionary framework. The neuromuscular control of tongue projection in microhylid frogs provides an excellent test of how the demands of the nervous and musculoskeletal systems change with the evolution of a novel behavior.

Senior Thesis Ideas
Marion Preest
AY 2015-2016

Feeding Energetics in Lizards

Previously I have been involved in studies quantifying the aerobic and anaerobic costs of feeding in lizards. I have been particularly interested in the effects of prey size and prey type on the costs of capturing, subduing, and swallowing prey. A senior thesis student could expand these studies and investigate other aspects of the feeding process from an energetic perspective. I have also been investigating chemical prey luring in chameleons and the ontogenetic development of feeding ability

Conflicting Energy Demands: Reproduction and Tail Regrowth

Animals have finite energy resources, and energy used for one "task" means less energy for another. Recently I began a project with a student investigating the kinds of "decisions" lizards make about energy allocation. i.e. if a pregnant lizard loses her tail, how does she allocate energy? Does she regrow her tail rapidly and produce fewer or smaller offspring or does she maintain the pregnancy and regrow her tail at a slower rate than a non-pregnant animal?

Effects of Incubation Temperature on Avian Development

I am interested in having a student investigate some aspects of the development of bird embryos in the absence of incubation, i.e. at temperatures below those maintained when a parent is sitting on a clutch of eggs. This work has ecological implications for the timing of initiation of incubation relative to clutch completion. The project will involve incubating bird eggs under a variety of experimental regimes and using established clearing/staining techniques and staging tables to assess development.

Miscellaneous, less well-developed (but interesting) ideas

1) Do tadpoles that hatch early in response to the presence of predators hatch at earlier stages of development? Could clear and stain specimens, run enzyme assays
2) Do eggs of tadpoles that are exposed directly (rather than through physical but permeable barrier) to predators hatch earlier than control or barrier-separated eggs?
3) There is a spillway just south of the Claremont Wilderness Park that acts like a giant pitfall trap. Who ends up in the trap, how likely are they to survival, what is the influence of season, what could be done to minimize captures and deaths, etc?
4) The larvae of wax moths produce a large amount of metabolic heat as they develop. Can we quantify this heat? How expensive is it to produce? What is the effect of the increase in ambient temperature on growth rate, digestive efficiency, etc?

Professor Purvis-Roberts Research Summary:

The Los Angeles basin contains many potential sources of air pollution, including power generating stations, factories, and automobiles. Gas phase pollutants emitted into the atmosphere, such as sulfur dioxides (SO_x), nitrogen dioxides (NO_x), ammonia, and various organic compounds, combine to form particulate matter (PM) in the atmosphere. PM can cause a variety of respiratory and cardiovascular health effects, especially in sensitive populations such as children and the elderly.

Although filter samples are an excellent means for determining the chemical composition and average concentration of particulate matter (PM) over several hours, peaks in outdoor PM intensity can occur due to weather patterns, pollution sources, time of day, etc. Maximum concentrations could cause adverse health effects for humans, so it is necessary to understand continual composition and concentration over shorter time scales. A Particle into Liquid Sampler (PILS), built by Dr. Purvis-Roberts and her students, based on Ion Chromatography, will be used to measure the concentrations of various ions in PM pollution. The PILS will take continual PM measurements every 15-45 minutes.

Chemical Mechanism for Particulate Matter Formation from Amines Utilized in Carbon Sequestration Technologies

Carbon Capture and Storage (CCS) is an important strategy for climate change mitigation, as it removes carbon dioxide from the atmosphere and helps to limit the impacts of warming. The largest source of CO_2 emissions to the atmosphere is the burning of fossil fuels, so the ability to capture post-combustion CO_2 from coal and natural gas power plants is a necessary step to limit greenhouse gas emissions. One of the most promising technologies for carbon capture is an amine-based solvent scrubbing system, but little has been done about the environmental and health impacts of amine emissions into the atmosphere from this source. Currently, none of these systems is installed on commercial power plants, but it is estimated that emissions of monoethanolamine (MEA) from a typical natural gas combined cycle 420 MW power plant would be ~8.0×10^4 kg yr^{-1}. Scientists are concerned about amine emissions from CCS facilities into water and air, but not much has been done to understand how the amines used in solvent systems react with oxidants in the atmosphere to form particulate matter. More research needs to be done to understand how potential alcohol amine candidates for these CCS solvent scrubbing systems can react in the atmosphere with different oxidants and varying humidity.

Specific Aim 1: Understand the chemical mechanism behind aerosol formation for oxidation of target amines for CO_2 sequestration technologies.

Specific Aim 2: Probe how aerosol formation differs for atmospherically relevant oxidants, O_3, OH, and NO_x, and under varying humidity.

In previous work described above, we have discovered that simple alkyl amine gases (i.e. trimethylamine, diethylamine, and butylamine) react to form amine salts, oxidized organics, or both in the aerosol, depending on reaction conditions and oxidant. We will extend this research further by studying the reaction mechanism of four different alkyl alcohol amines identified for potential use in amine-based solvent scrubbing systems. Monoethanolamine (MEA), diethanolamine (DEA), *N*-methyldiethanolamine (MDEA), and diglycolamine (DGA), are interesting candidates, because we will be able to study reaction mechanisms of primary (MEA), secondary (DEA & DGA), and tertiary (MDEA) amines. MEA is currently used in pilot CCS studies, but MDEA is actually more efficient at CO_2 capture. All of these amines have high enough vapor pressures that they will all be released in the atmosphere if used for CCS applications.

All experiments will be done at the Environmental Chamber at CE-CERT at UC Riverside in collaboration with Professor Cocker's research group. In order to identify and quantitatively measure the concentration of amine salts that form in the reactions, my research group will first develop an Ion Chromatography method for separation of the target alcohol amine compounds. Then we will perform experiments in the 12,500 L Teflon chamber, which has humidity and temperature control, focusing on each amine, different oxidants, and varying humidity.

Chemical Mechanism for Particulate Matter Formation from Amines & Reduced Sulfur Compounds Emitted from Agricultural Sources

This project will probe the chemical mechanisms for atmospheric aerosol formation from agricultural emissions, specifically, reduced nitrogen and sulfur gases. The main goals are to:

1. Characterize emissions of reduced nitrogen and sulfur compounds from animal production.
2. Assess secondary atmospheric aerosol formation through field, laboratory, and modeling studies.

Field studies during the summer will be completed on dairy farms and piggeries in Kentucky, and will focus on detection and quantification of reduced nitrogen and sulfur compounds. Previous studies by the Purvis-Roberts research laboratory have demonstrated that atmospheric amine chemistry is extremely complex as alkyl amines participate in both acid-base chemistry and atmospheric photochemical oxidation reactions. Laboratory and modeling studies will focus on the atmospheric fate of nitrogen and sulfur compounds emitted from these agricultural sources. Environmental fate experiments for aerosol formation potential will focus on the interaction of atmospheric oxidants with target nitrogen and sulfur compounds at varying relative humidity and temperature. Initial target nitrogen containing compounds will include trimethylamine, diethylamine, butylamine, a diamine (such as ethylene diamine, putrescine, or cadaverine), and ammonia. Sulfur compounds to be studied include methanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), and hydrogen sulfide.

Colin R. Robins
crobins@kecksci.claremont.edu
Assistant Professor of Environmental Science
***I am on sabbatical in Fall 2015.**

Research Interests and Student Opportunities

I use soil geomorphology, mineralogy, and geochemistry to study the ways in which landscape components record surface, climate, and ecosystem histories at scales ranging from the nanometer to the kilometer, and at time scales ranging from the present day to hundreds of millions of years ago. My chief research interests are currently: (1) applications of soil science and geochemistry to challenges in stratigraphy, species conservation, and/or land management efforts, and (2) the pursuit of quantitative, isotopic dates and conceptual models of arid soil minerals and landscape (geomorphic surface) formation. While I'm not taking new students in Fall, 2015, I would support independent (volunteer or independent study) and thesis research in Spring and Summer 2016 by science majors interested in the Earth and Environmental Sciences.

Projects include:

- Micromorphology & Microscopy - Optical and/or scanning electron microscope (SEM) analysis and interpretation of minerals or soil/paleosol microstructures (duration flexible)
- Experimental soil genesis - Devise/engineer lab experiments to replicate enigmatic mineral or biological soil structures found in a variety of field settings. (duration flexible)
- Clay mineralogy, micromorphology, and geochemistry - Clay minerals are among the most reactive components of any soil. How and why do clays change between structures or horizons in a soil profile? (2 semester thesis or indep. research)
- Soil-plant dynamics and/or Soil solution chemistry - To what extent do distinct plants influence soil chemistry? How do differences among standard method protocols influence understanding of short vs. long term changes in soil chemistry and soil-plant dynamics? (1-2 semester, lab-intensive thesis or indep. research).
- GIS mapping of soil-geomorphology and floral biogeography (1-2 semester)
- Soils on exo-planets? (1 semester library thesis) - what minerals might be common? In what types of solutions might reactions take place?

Lars Schmitz

"The Schmitz Lab studies organism-environment interactions and the evolution of vertebrate eyes. All students in our lab have a keen interest in evolutionary biology and functional morphology, and work on projects that fall in one of two major categories:

1) Analysis of morphological evolution. Students are measuring morphological traits in museum collections (e.g., LA County Museum of Natural History) and analyze the data from a functional and phylogenetic perspective.

2) Retina physiology and visualization. We can analyze retina fine structure with traditional histology, stereology, and immunohistochemistry. In addition to lab work, students are involved in developing new visualization techniques of spatial distributions of cells across the retina.

All projects have a strong computational component with programming in R."

Professor Tessa Solomon-Lane

Why and how do individuals behave the ways that they do? The Solomon-Lane lab group is interested in understanding individual variation in social behavior. Social behavior is one of the most intensely studied categories of behavior because interactions among members of a social group have important consequences, including for evolutionary fitness and health. There are diverse social species across the animal kingdom, from insects, reptiles, birds, and fish, to mammals, such as humans! To answer behavior questions in the lab, we study Burton's Mouthbrooder (*Astatotilapia burtoni*), a highly social species of African cichlid fish. They form naturalistic social communities in the laboratory and expresses a suite of social behaviors common across vertebrates, such as aggression, affiliation, courtship, reproduction, parenting, cooperation, and social learning. The neural mechanisms regulating these behaviors are also highly conserved evolutionarily. Adults regularly reproduce in the lab, making it feasible to study development and maintain a lab population. A substantial amount is already known about the brains and behavior of this species, and our research continues to build on this knowledge. Current projects in the lab focus on the development of behavior, the development of the neural and neuroendocrine mechanisms of behavior, early-life effects, reproductive maturation, and social group dynamics. The Solomon-Lane Lab is a welcoming, collaborative group, and we are committed to an inclusive, honest, and fair environment for research and learning, free from discrimination or harassment of any kind. We prioritize teamwork, respect, and positivity. This is a Lab where you are encouraged to be your whole self. Every voice and perspective matters. In fact, it's your different perspectives, interests, ideas, and identities that will make our science and our lab successful and unique!

Projects in Professor Tang's Laboratory

Research interests

1. Cell signaling for the interplay between cell-division cycle and gene expression events such as pre-mRNA processing, mRNA export, as well as heterochromatic silencing in eukaryotes involving several protein kinases including Dsk1 and Kic1.
2. Genes involved in the cellular sensitivity and resistance to platinum-based anticancer drugs
3. Conserved response networks to phenol derivatives as environmental stress factors

Model organism

A single-cell eukaryotic organism, fission yeast *Schizosaccharomyces pombe*

S. pombe has been particularly influential in studies of cell cycle regulation, DNA damage/repair mechanisms, and chromosome dynamics including RNA interference (RNAi). In addition, the fission yeast S. pombe shows no evidence for genome duplication and holds the smallest sequenced eukaryotic genome, which led to its rising popularity as a eukaryotic model in the last decade. Based on current information, out of 4981 protein coding genes in S. pombe, 3385 fission yeast proteins have one or more orthologs in humans. Therefore, investigators studying mammalian cell biology are increasingly using S. pombe to test their gene of interest, as it may be present in only one copy, bestowing fission yeast the nickname "micro-mammal."
(From a book Chapter by Zhaohua Tang and Gretchen Edwalds-Gilbert beingpublished by Springer)

Methods

Molecular genetic, cell biology, and biochemical approaches combined with genomic scale analysis

Projects

Student research projects and senior thesis projects conducted in my lab are derived from the following three aspects of the research.

1. To investigate the function of Dsk1 and Kic1 protein kinases in RNA processing and beyond. Since we have demonstrated that the kinase family is conserved through evolution, we thus use a single-cell eukaryotic organism, *S. pombe*, as a single-cell eukaryotic *in vivo* model to determine the mechanisms of the kinase function. We found that Dsk1 and Kic1 are required for pre-mRNA splicing. We also obtained preliminary evidence for their involvement in heterochromatic silencing, as well as the nuclear export of mRNA for specific genes in cell cycle regulation. Further understanding of the functions of the kinases will shed light on the complex gene expression processes fundamental to all eukaryotes.
   a). To genetically dissect the role of Dsk1 and Kic1 in heterochromatic gene silencing.
   b). To determine the potential interactions and kinase-substrate relationship of CK2 or Swi6/HP1 with Dsk1 or Kic1 in heterochromatin formation and maintenance.
   c). To study whether Dsk1 or Kic1 affects pre-mRNA splicing and nuclear export of mRNA of specific genes, especially those functioning in cell cycle regulation.
   d). To examine whether Dsk1 or Kic1 phosphorylates poly(A) binding protein, Pabp, thereby regulation the nuclear export of mRNA.

2. To dissect the cellular response pathways to the platinum-based anticancer drugs at genomic scales. Questions to address:
   a). What genes are required for the cellular resistance and sensitivity to cisplatin, carboplatin, oxaliplatin, and dicycloplatin, respectively?
   b). Are the response networks the same for various platinum-based drugs?
   c) What are the common components shared by the cell signaling pathways for different platinum drug?
   d). What are the key elements that make the networks distinct for individual platinum-based anticancer drugs?

3. To decipher response networks to phenol derivatives as environmental stress factors using comparative genomics (collaborative research with Professor Katie Purvis-Roberts and Professor Gretchen Edwalds-Gilbert at Keck Science Center, Professor Tina Negritto at Pomona College and Ruye Wang at Harvey Mudd College).

Living organisms including human are exposed to various chemicals in air, water, and food, as industrial by-products and pollutants in environment. This project is a genome-wide investigation of the genetic networks responsive to selected phenol derivatives as environmental stress factors. The selected phenol derivatives have been widely used as pesticides, food preservatives, and high volume industrial chemicals. In spite of the importance of such issues, it remains unclear what biological consequences the phenol derivatives cause and by which molecular signaling pathway living organisms survive the stress environment.

The important questions are: What are the genes required for the phenol-specific and general stress responses? What components of the stress response networks are conserved through evolution? Are DNA checkpoint/repair and cell cycle control signaling critical for cell survival of the phenol stress?

<u>Steps to address these questions</u>

a). To identify genes involved in the phenol response networks by genome-wide screening of the deletion libraries of both yeasts.
b.) To determine conserved elements in the phenol-stress networks by comparing the response profiles of both yeasts
c.) To evaluate the effects of the phenol derivatives on the cell fitness and genome stability, as well as the roles of DNA damage checkpoint/repair, cell cycle regulation systems in cellular survival of the phenol stress.

Diane Thomson – Thesis Research Projects

Conservation biology (especially for plants and insects), causes and effects of biological invasions, and pollination ecology. Thesis students in my lab carry out projects on a wide range of topics, but some examples of ongoing opportunities include research on:

Interactions between native annual plants and invasive grasses at the Bernard Field Station.

Effects of invasive herbivores and climate change on rare plant populations and communities of the California Channel Islands.

Changes in pollination biology of native plants resulting from habitat fragmentation and introduced bees.

Modeling extinction risk of rare species.

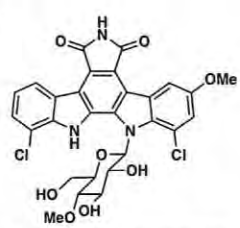
Summary of Research Plans *Ethan B. Van Arnam*

My research interests center on the chemistry of microbial symbiosis. I am interested in discovering new bioactive molecules and in understanding the role of antibiotics and other small molecules in nature through the lens of ecology and evolution. Microbial symbioses – bacteria or fungi engaged in specific relationships with animals, plants, or other microbes – are the underpinnings of virtually all life on earth. The function of microbes in symbiosis frequently boils down to chemistry; secreted molecules convey information or suppress unwanted intruders. My lab will focus on insect-microbe symbioses – ideal model systems to understand how chemistry underlies interspecies interactions. Projects will begin with a strong ecological framework but will ultimately center on chemistry: understanding the structures, biosynthetic origins, and activities of microbial small molecule natural products. Undergraduate research experiences in my lab will include field collections, the culturing and assaying of microbes, metabolomics of microbial cultures, extraction and purification of active compounds, chemical analysis to determine molecular structure, and bioinformatics to gain insight into molecular biosynthesis.

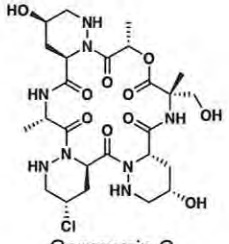
Project 1: Chemistry of defensive symbiosis in the fungus-growing ants

Fungus-growing ants harbor antibiotic-producing bacteria that defend their nests against fungal pathogens.[1] My postdoctoral work has uncovered new molecules from these bacteria that offer a unique window into antibiotic deployment in an ecological context.[2-4] Our studies also indicate that much more chemistry remains to be discovered in this underexplored system. I plan to extend this work to unexplored aspects of this fascinating symbiosis in a continuing collaboration with ecologist Prof. Cameron Currie at the University of Wisconsin. These studies will draw from a collection of symbiotic bacteria that I isolated from ants in Panama in 2015 and will be supplemented by future collecting trips with Prof. Currie. **Aim 1:** Characterize antibacterial molecules that mediate competition among bacterial symbionts. **Aim 2:** Uncover the molecular basis for nutrient acquisition in these specialized bacteria by characterizing their unusual iron-chelating siderophores.

C



9-Methoxyrebeccamycin

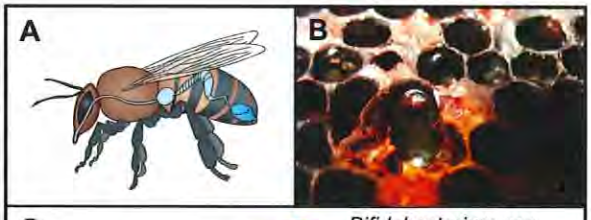


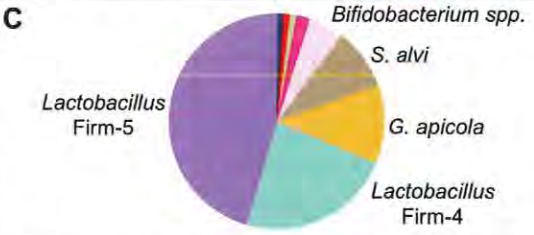
Gerumycin C

A,B. Fungus-growing leafcutter ants. **C.** New antibiotics discovered from bacterial symbionts of fungus-growing ants. Photos ©Alex Wild

Project 2: Chemical dissection of the honeybee microbiome

The honeybee gut, like that of all other animals, hosts a consortium of bacteria with important roles in processing the diet but is also broadly important in supporting organism health and disease through mechanisms that are poorly understood.[5] Unlike the dizzyingly complex human gut microbiome, however, the honeybee microbiome is a simple, experimentally-tractable community of defined composition in which all major members can be readily cultured in the lab.[6-8] I propose to study this ideal model microbiome from the honeybee, a species of global agricultural importance, to understand how chemical exchange among bacteria supports a stable bacterial community. **Aim 1:** Determine the molecules with which members of the honeybee gut microbiome chemically antagonize the causative agent of American foulbrood disease, *Paenibacillus larvae*. **Aim 2:** Using an unbiased phenotypic screen, identify instances of interbacterial communication by diffusible small molecules and determine the identities of those molecules.

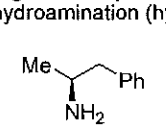




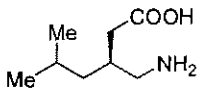
A. Schematic of the honeybee gut, modified from (5). **B.** Honeycomb affected by American foulbrood (*P. larvae* infection). **C.** Bacterial composition of the honeybee gut.[8]

I. Overview of Proposed Research. Chiral amines are a prevalent structural motif found in 40% of commercially available pharmaceutical products and 20% of crop-protection compounds. In addition to their crucial role as pharmaceutical building blocks (e.g., Figure 1), chiral amines are also sold annually on a multi-hundred-ton-scale as kinetic-resolution reagents. With the growing importance of chiral amines in the life-science industries, the development of efficient methodologies to access this compound class continues to be a topic of interest in organic chemistry and catalysis.

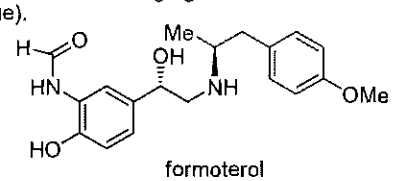
Figure 1. Representative chiral amine pharmaceutials and resolving agents that could be derived from asymmetric hydroamination (hydroamination motif shown in blue).



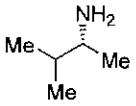
dextroamphetamine
ADHD



pregabalin (Lyrica)



formoterol
asthma/COPD



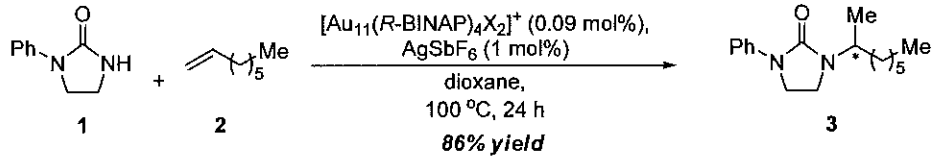
(*R*)-2-amino-3-methylbutane
resolving agent

Hydroamination reactions involve the formal addition of an N-H bond across a C-C multiple bond, and can serve as an efficient route towards the preparation of chiral amines. The complete atom economy of this reaction satisfies one of the main tenets of "green" chemistry. In particular, the enantioselective, intermolecular hydroamination of unactivated alkenes is a significant challenge..Reports from the Basic Energy Sciences Advisory Committee and others cite the hydroamination of olefins, particularly in the anti-Markovnikov sense, as one of the top ten catalysis targets for the 21st century. To date, there are only limited reports describing the enantioselective hydroamination of unactivated olefins. Of these, only one report—a 2014 seminal paper by Buchwald and Zhu—describes an enantioselective hydroamination of unactivated alkenes in an anti-Markovnikov sense. While a wide range of functional groups were tolerated in this report, enantioselectivities varied widely (7-98% ee). In addition, only poor-to-moderate conversion was observed for unactivated 1,2-disubstituted alkenes, and no conversion to product was observed for unactivated, trisubstituted alkenes and α-substituted acrylates. Undesired side reactions were also observed: free alcohols underwent silylation, and aldehydes and ketones were hydrosilylated. Thus, while significant progress has been made—which enables a benchmark for comparison—the opportunity for further reaction optimization exists.

The proposed research focuses on the preparation of chiral heterodonor phosphorous/thioether and phosphorous/amine ligands as alternatives to classically used bisphosphines for coordination onto palladium, ruthenium, and copper. Chiral bidentate ligands equipped with strong and weak donor heteroatom pairs (e.g., P/S or P/N) can capitalize on favorable electronics to strongly influence the stability and reactivity associated with the stereochemistry-defining intermediates of a catalytic cycle, yet the use of heterodonor catalyst complexes in the investigation of alkene hydroamination reactions remains relatively unexplored. Once prepared, the proposed systems will be applied towards effecting two hydroamination subclasses: an intermolecular, asymmetric, anti-Markovnikov hydroamination of unactivated olefins and the enantioselective, Markovnikov hydroamination of dienes to produce dihydroquinolines. In the latter case, organocatalysts will also be explored, as the use of nonmetal-based systems for the enantioselective hydroamination of alkenes and dienes is additionally an area where a high potential for future development exists.

II. Ongoing Projects.

Scheme 1. Gold nanocluster-catalyzed intermolecular hydroamination of 1-octene.

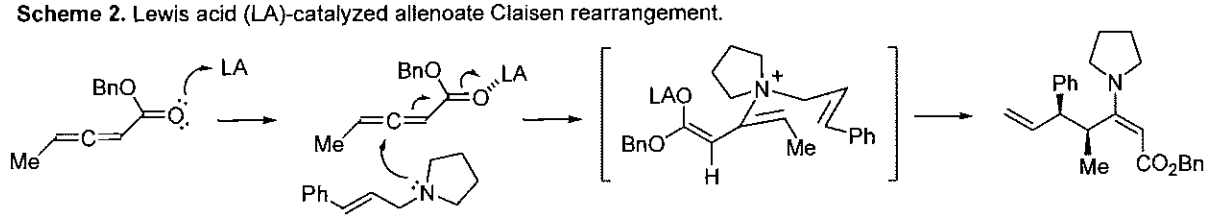


1 2 3

Gold Nanocluster Catalysis. "Catalysis in nanoscience" was recently identified as one of the top opportunities for scientific advances by a recent report from the Basic Energy Sciences Advisory Committee. To investigate the possibility of gold nanocluster catalysis, an undergraduate recently prepared $[Au_{11}(R\text{-BINAP})_4X_2]^+$ gold nanoclusters derived from Au_2X_2(BINAP). These nanoclusters were originally developed as chiroptical agents, but we envisioned that they could potentially promote the hydroamination of olefins. Ordinarily, these clusters are highly stable due to shielding from the attached ligands. However, similar to monoligated systems, we postulated that coordination sites on this nanocluster could be opened via halide abstraction using silver salts. For example, when $[Au_{11}(R\text{-BINAP})_4X_2]^+$ was treated with $AgSbF_6$ (Scheme 1; 0.09 mol% nanocluster, 1 mol% Au), it was found that the resulting nanocluster could catalyze the hydroamination of **1** in 86% yield. Enantioselectivity determination is in progress. These results require 5x's less gold, yet afforded a similar yield to the best results reported in the literature. No conversion was observed when this reaction was conducted solely in the presence of either $AgSbF_6$ (1 mol%) or $[Au_{11}(R\text{-BINAP})_4X_2]^+$ (0.09 mol%), indicating that both are required for this reaction to occur. It is important to note that—while gold nanoclusters have previously been reported to catalyze *intra*molecular hydroaminations—to our knowledge, this is the first example of a nanocluster-catalyzed, intermolecular variant of this reaction. Utilizing this hydroamination reaction as a model system, we are currently investigating the influence of phosphine modification on resulting nanocluster size and catalytic efficacy, as well as possible asymmetric induction. Solvent modification and the addition of base (e.g. Cs_2CO_3, etc.) as a reaction additive are also under investigation.

Asymmetric Allenoate Claisen Rearrangement. The [3,3]-sigmatropic rearrangement of allyl vinyl ethers, the Claisen rearrangement, is a prominent method for the rapid construction of carbon-carbon bonds between vicinal stereogenic centers. To date, more than 15 variants of this reaction exist, and several asymmetric, catalytic methods have been reported. Searching for a readily-accessible, modular version of the Claisen rearrangement, we came across a 2002 communication by Lambert and MacMillan. In it, the authors reported the use of zinc triflate (10 mol%) to catalyze the addition of tertiary allyl amines to allenic esters (Scheme 2). A subsequent Claisen rearrangement of the zwitterionic allyl-vinylammonium species **6** afforded the corresponding Claisen product tautomer (7).

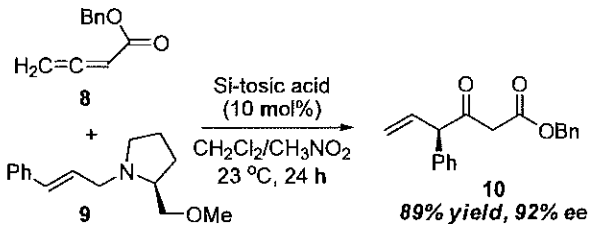
Scheme 2. Lewis acid (LA)-catalyzed allenoate Claisen rearrangement.



4 5 6 (±)-7

We initially postulated that the major factor hindering the development of an enantioselective allenoate Claisen rearrangement was the distal binding of the Lewis acid (LA) catalyst in **6**, thereby preventing facial selectivity in the stereo-determining step. Capitalizing on an observation reported by Pinho e Melo and coworkers in 2010, recent work in our laboratory has shown that the allenoate Claisen reaction can be made asymmetric. In the Pinho e Melo report, the authors observed that an aluminum-catalyzed allenoate Claisen reaction directly formed racemic ketone instead of the corresponding enamine 7 observed by MacMillan and coworkers, presumably the result of hydrolysis following the completion of the Claisen. Intrigued, we envisioned that this result could be developed into an asymmetric reaction via the use of a chiral amine as an auxiliary. Indeed, when allene **8** and amine **9** were reacted with $AlCl_3$ (10 mol%), ketone **10** was isolated in 60% yield and 76% enantiomeric purity after 24 h. Switching to silicated tosic acid (10 mol%) as the reaction catalyst, **10** was isolated in 89% yield and 96% enantiomeric purity after 24 h (Scheme 3; unoptimized). We have found that the chiral amine can be recovered via a simple extraction, and the catalyst readily removed via filtration. It is important to note that this is the first example of an enantioselective allenoate Claisen. We are currently in the process of optimizing this lead result via reaction condition modification, the investigation of bulkier auxiliary amines, and the preparation of diastereoselective variants in preparation for publication in a peer-reviewed journal.

Scheme 3. Asymmetric allenoate Claisen rearrangement.



Gold Aggregate Preparation and Characterization. Recent research in collaboration with Prof. Jacob Berlin at the Beckman Institute at the City of Hope has focused on the preparation of gold nanocluster aggregates. Gold nanoparticle aggregates are of interest for applications in material science, chemistry, and medicine. Current aggregation methods rely on the formation of micelles, charge-based interactions, or expensive bioactive molecules like DNA to assemble gold nanoparticles. Small molecules can also be used to assemble nanoparticles into aggregates. For example, gold nanoparticle aggregates (Figure 2) using a tetravalent crosslinker, pentaerythritol tetrakis (3-mercaptopropionate) (PTMP) has demonstrated proof-of-principle for this concept. We are currently interested in using other crosslinkers to control aggregate formation in order to determine the necessary components of a crosslinker in determining aggregate properties. Thus far, we have been able to assemble aggregates from a bivalent, trivalent and tetravalent crosslinker. The aggregates formed were found to differ in stability, the range of particles that can be assembled, and final aggregate size. Several, inter-related characteristics will be investigated: hydrophobicity, linker geometry, valency, linker rigidity, and length. For the purposes of this initial study, we are the most interested in learning how the rigidity and hydrophilicity

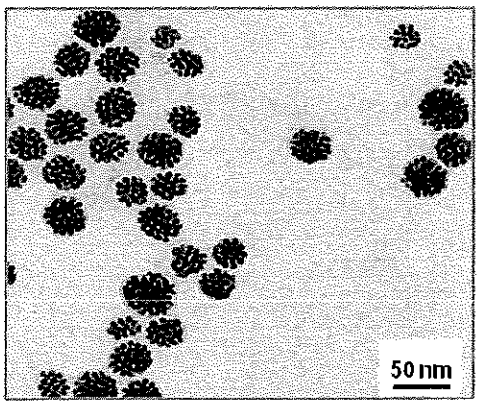
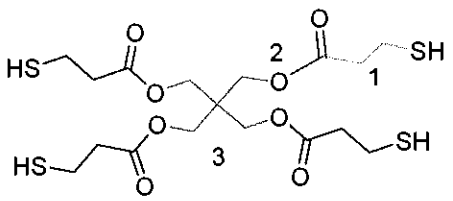


Figure 2: Aggregates formed from 5nm gold nanoparticles and PTMP



1. Linker Arm
2. Ester Bond
3. Core Scaffold

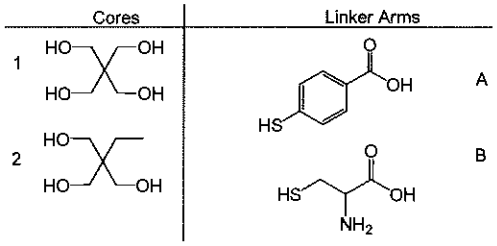


Figure 3: A) Linker components, using PTMP as an example. B) Linker components of interest.

of the linker effects aggregate formation and the characteristics of the aggregates.

In terms of design, the linkers can be thought of as two main components, a linker core and a linker arm (Figure 3A). Tetravalent and trivalent cores with differing arm hydrophilicity and rigidity (Figure 3B) are currently being prepared. Once the linkers are prepared, we will examine their ability to form aggregates according to our current methods, evaluate their physical properties, and test them in biological applications.

Emily Wiley
molecular biology, epigenetics

My lab does research in the field of epigenetics. We identify enzymes that make chemical modifications to histone proteins, and study how they impact gene expression, and regulate processes such as nuclear differentiation and nuclear death. These processes, which have important implications in development and disease, can be easily studied in the ciliated protozoan model system *Tetrahymena thermophila*. We also study the molecular basis of other emerging chromatin modifications, such as histone clipping, which likely impact genome differentiation.

Available projects:

1. Investigate roles of histone "clipping" in genome differentiation.

In 2008 it was shown that a small peptide from the amino terminal end of histone H3 is "clipped" off during stem cell differentiation. Scientists speculate that this clipping is an important mechanism the cell uses to regulate gene expression in different parts of the genome during development. Similar to mammalian stem cells, *Tetrahymena* cells also clip a fraction of their histone H3 molecules. This project examines the regulation of H3 clipping using a variety of molecular biology, cell biology, and biochemistry techniques.

2. Investigate the importance of erasing histone acetylation during chromatin development

We previously discovered a histone deacetylase enzyme that erases histone modifications on newly-made histones after they are deposited onto newly-replicated DNA. By studying chromatin phenotypes of cells lacking this enzyme, this project will elucidate the biological function of modification erasure, a phenomenon that is observed in all organisms examined. This project will utilize a range of cell biology and molecular biology techniques.

3. Investigate involvement of a sirtuin enzyme in regulated nuclear death.

Sirtuins are histone modifying enzymes linked to aging control in a range of organisms. We discovered a sirtuin in *Tetrahymena* that may promote the scheduled degradation/death of a nucleus during development, in a manner similar to apoptosis in mammalian cells. This project will examine the role of this sirtuin (called Thd14) in the degradation/death process using techniques in genetics, molecular biology, and biochemistry, which will possibly uncover novel important roles for these important sirtuin enzymes.

4. Investigate how "chromodomain" proteins bind to histones and compact chromatin.

Proteins that contain a chromodomain are known to bind to histones and affect gene expression in those regions where they bind. An important problem is how they are targeted to the correct genome regions to regulate expression. This project will explore mechanisms for chromodomain protein targeting and binding to the correct genome regions to promote compaction of the region into silent chromatin (heterochromatin). Techniques spanning biochemistry, cell biology, and molecular biology will be used.

Professor Branwen Williams
Assistant Professor of Environmental Science

I am an Environmental Scientist studying our climate and oceans. My research seeks to understand the human contribution to recent changes in our environment including global warming and coastal eutrophication. I use the corals and algae as tools to do this because these organisms capture ambient changes in their environment in their hard skeletons as they grow. By measuring the chemical and physical properties of the skeletons, we can thus recreate changes in the environment going back thousands of years into the past.

My research asks questions such as:
How have warming temperatures altered Pacific Ocean currents?
Can we create records of nitrogen concentrations in the oceans from coral skeletons?
How fast do deep sea corals grow and for how long?
Is the Arctic Ocean warming faster than the global average?
Is seawater pH decreasing faster than the global average in the Arctic Ocean?
Can we quantify terrestrial effluent in shallow and deeper water coastal habitats?

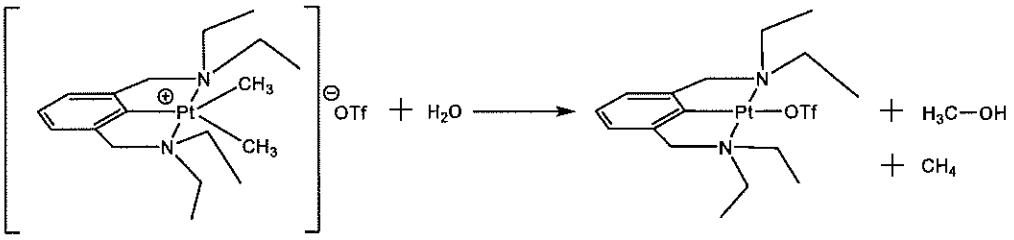
Since my research is interdisciplinary at its core, students from Physics, Biology, Chemistry, and Environmental Science may all be interested in research opportunities in my lab. Recent students have attended national conferences to present their research and submitted first-authored manuscripts. Please contact me at Bwilliams@kecksci.claremont.edu or stop by room 227 on the second floor of Keck if you are interested in discussing research opportunities.

Nancy Williams' Research Group

Organometallic Chemistry

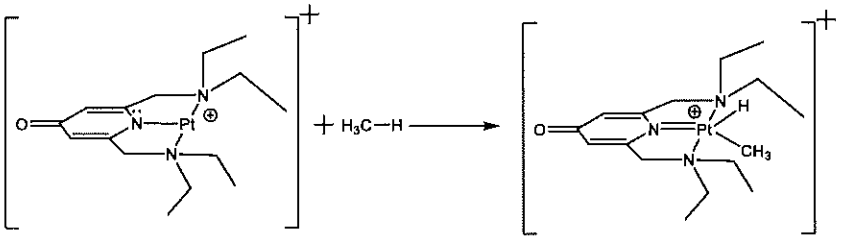
Project 1: Methanol Formation from Platinum (IV)

In our lab, we are interested in the making and breaking of strong bonds to enable the creation of cheaper, more environmentally friendly fuels. The last step in a putative methane-to-methanol cycle involves the nucleophilic attack by water on a Pt(IV) methyl group, but such a reaction has almost never been observed experimentally. However, in our lab, we see production of methanol from a Pt(IV) complex reacting with what we suspect is adventitious water from the glassware. We will characterize this reaction, positively identify the source of the water, and determine the mechanism of C-O bond formation.



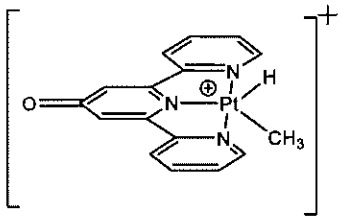
Project 2: Computational Investigation of a C-H Bond 'Wire Cutter'.

The key first step of any methane-to-methanol process is the cleavage of a C-H bond in methane. We have hypothesized that the molecular orbital structure of a pyridonate pincer complex on platinum (II) should be outstanding at cleaving even the strongest C-H bonds. Initial calculations suggest improvements in the bond breaking ability of these complexes by a factor of about a trillion over a more conventional complex.



Project 3: Synthesis and Metallation of a C-H Bond 'Wire Cutter' Ligand.

The final project involves the synthesis of just such a pyridonate ligand and its metallation to test the computational prediction.



Research Interests – Professor Sierra Williams

Multidrug-resistant bacteria are one of the leading causes of illness and death in the United States.[1-2] Despite the development of small molecule drugs to combat these harmful bugs, bacteria have evolved to escape death.[3] Thus, new methods to kill these microbes without triggering resistance mechanisms are needed. A promising alternative to synthetic drugs are protein therapeutics, including muralytic enzymes (Figure 1). These enzymes target and kill bacteria by breaking down their cell walls.[4] Thus, making muralytic enzymes attractive therapeutics because they cause cell death without triggering resistance pathways.[5] One family of enzymes that show promise as therapeutics are bacteriophage-derived enzymes, known as endolysins. However, before endolysins are approved for use in the general public, improvements to their specificity and activity are necessary. *In the Williams lab, we will address these issues by generating new classes of endolysins to help combat bacterial infections.* Future work will focus on expanding the therapeutic potential of other lytic proteins.

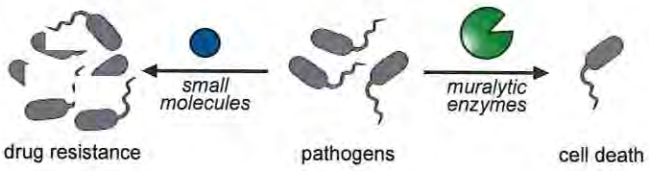


Figure 1. Combatting pathogenic bacteria. Drug therapeutics can lead to bacterial resistance and cell division. Use of lytic enzymes as alternative therapeutics can overcome resistance mechanisms and kill cells.

Students will use protein engineering and chemical synthesis to generate and characterize endolysins with novel activity. The foundation of projects in the lab will rely on enzyme mutagenesis, a common technique used in chemical biology. To assess the properties of the endolysin mutants, a fluorogenic assay with synthetic peptides will be developed. Students will also have the opportunity to design peptides for new cleavage recognition sites, while simultaneously engineering endolysins that recognize new cut sites. Through the projects outlined below, students will gain experience in organic synthesis, molecular cloning, protein engineering, and imaging. *The new endolysins developed in the Williams lab will advance the development of antibiotics against drug-resistant pathogens, and further studies will focus on optimizing activity.*

<u>Project Area 1:</u> Improving activity of endolysins at physiological conditions.

One of the most well-characterized endolysins, LysK, has been found to have the highest membrane cleavage activity against *Staphylococcus* strains. Disruption of the bacterial cell wall is dependent on three domains of the endolysin: cysteine, histidine-dependent amidohydrolase/peptidase (CHAP), *N*-acetylmuramoyl-L-alanine amidase (amidase-2), and cell wall binding domain (SH3b, Figure 2A).[5] However, the truncated LysK variant, $CHAP_K$, maintains activity *in vitro* and *in vivo*.[5] Despite the wide range of temperatures $CHAP_K$ operates, its activity is optimal at 15 °C.[6] To increase the potential of this endolysin as a therapeutic, the cleavage activity at 37 °C must be improved. **In this project, directed evolution will be used to improve the thermostability of $CHAP_K$ to boost its antibiotic activity.** To enhance the thermostability, a library of $CHAP_K$ mutants will be generated using random mutagenesis (Figure 2B). Loss in activity at elevated temperatures was shown to be linked to changes in secondary-structure. Since sites that influence structural changes can be hard to predict, error-prone PCR will be used to randomly change the identity of amino acids in the protein. After the libraries are generated, the mutants will be assessed for improved activity at physiological temperatures.

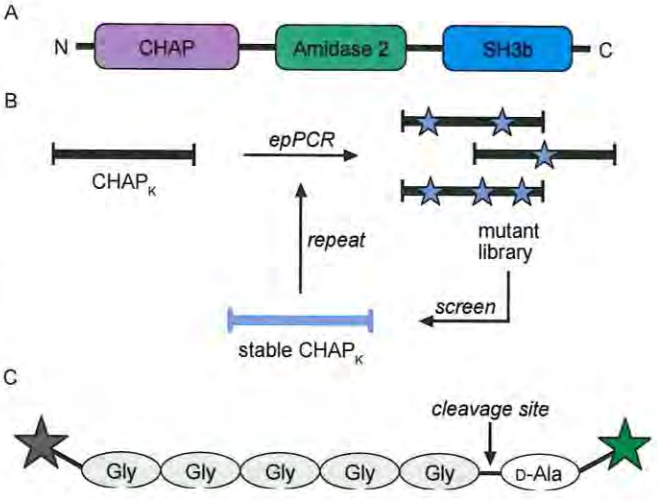


Figure 2. Engineering $CHAP_K$. (A) Structure of LysK endolysins. $CHAP_K$ only contains the CHAP domain. (B) Strategy for evolving a thermostable $CHAP_K$ using random mutagenesis. (C) Small peptide containing a fluorophore (green star) and quencher (gray star) to report on cleavage activity.

Research Interests – Professor Sierra Williams

When testing the activity of $CHAP_K$, assays involving treatment of the endolysin with the bacteria of interest are used. However, these methods are often low-throughput, making evaluation of mutant endolysin activity difficult. An optical readout in the absence of bacteria is necessary to test activity in a high-throughput manner. LysK is known to cleave the *S. aureus* peptidoglycan between the first glycine in the pentaglycine cross bridge and D-alanine.[7] To mimic the cut site for $CHAP_K$, a short peptide will be synthesized bearing a fluorophore and a quencher at each end (Figure 2C). In the absence of a functional $CHAP_K$, the full length peptide will produce no fluorescence signal. However, in the presence of the endolysin, the peptide will be cleaved and fluorescence can be measured. The amount of functional endolysin will directly correlate to the amount of fluorescence signal observed. This analysis will not only provide an endolysin with immediate therapeutic applications, but a more high-throughput method for directly assessing $CHAP_K$ activity in the absence of bacteria. This method can also be applied to assessing the activity of other properties of $CHAP_K$ and mutant endolysins of interest. Some examples include studying activity in biological environments or in the presence of complex bacterial mixtures.

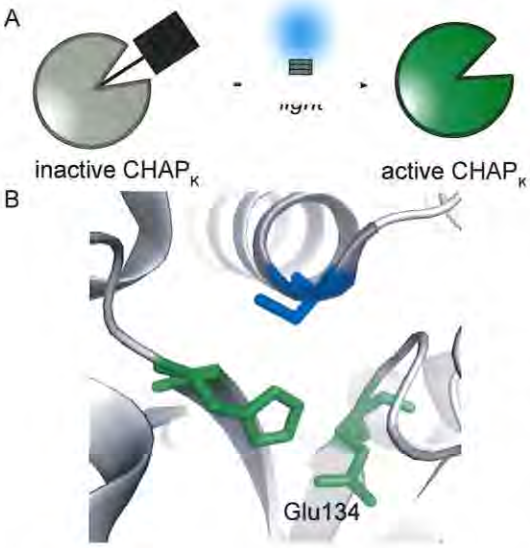


Figure 3. Light activated $CHAP_K$. (A) Strategy for temporal control of endolysin activity. (B) Proteolytic triad (Cys54-His117-Glu134) of $CHAP_K$ in the active site (PDB: 4CSH). The reactive cysteine is highlighted in blue, while the histidine and glutamine residues are shown in green.

Project Area 2: Targeted treatment of bacteria using $CHAP_K$. Traditional antibiotic treatments suffer from off-target effects that can kill both pathogenic and healthy cells in patients. Most endolysins have a domain that directs the enzyme to specific bacterial cell walls, thus making them naturally specific to cell targets. However, since $CHAP_K$ lacks the SH3b domain, it has the ability to kill a large range of bacterial cells. Previous work has focused on swapping cell wall binding domains of endolysins to control bacterial specificity.[8] However, this method can diminish lysis activity. An alternative method with additional control is engineering a light sensitive endolysin (Figure 3). Activation via light serves as a switch that can be used to turn the activity on.[9] This would allow targeted control of activity, and further reduce the chances of bacterial resistance. **In this project, genetic code expansion will be used to generate a light-activatable endolysin.** The activity of $CHAP_K$ is mediated by cysteine and histidine residues in the enzyme active site. Incorporating an unnatural amino acid at the reactive cysteine should block activity of the endolysin. Upon uncaging of the residue, activity will be restored and cell death can occur at the location of interest. This work can be further expanded to controlling activity of other protein therapeutic targets.

Project Area 3: Investigating the cleavage activity of $CHAP_K$.

$CHAP_K$ effectively lyses *Staphylococcus* and *Streptococcus* species due to its low specificity.[6] This lytic activity is largely due to the similarities in cell walls amongst these bacteria. However, considering the presence of other alanine residues in the peptide bridge, it is not clear why the endolysin only cuts between glycine and D-alanine. More specifically, is it the presence of the small side chains on the amino acids? Is the stereochemistry of the amino acids important? Or a combination of both? **In this project, a panel of peptides will be developed to assess the activity of $CHAP_K$ with different cleavage sites.** Understanding the mechanism of cleavage could be beneficial for engineering variants that can target other bacterial species. In addition, $CHAP_K$ could be mutated to further improve its activity for cleaving new sites. Using a similar screening strategy as Project Area 1, a panel of small peptides will be synthesized containing a quencher and a fluorophore at opposing ends. The first generation of peptide library will consist of D-amino acids, excluding alanine. The fluorescence signal will then be assessed in the presence of $CHAP_K$ to determine whether the endolysin can recognize a different peptide sequence. In tandem, a library of $CHAP_K$ mutants will be evolved and screened against the peptides to determine whether a new variant can recognize these cut sites.

Research Interests – Professor Sierra Williams

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July 2003

Project Ideas for the Bernard Field Station

Carry out/simplify/expand one of these, or use them as inspiration in designing a different project.

Physics/math

How do the tendrils of *Marah macrocarpus* curl? Is the direction always the same? What are the physical principles and constraints? How does the structure of a tendril change as it curls around an object? How much resistance is provided by the tendril coils when the wind blows? What is the tensile strength? How many would be required per unit mass of plant?

What are evaporation rates in different locations? How and why do they change over time? Do they correlate with changes in morphology of nearby plants? How might these reduce water loss? Can you model this? If you calculate the precipitation/evaporation ratios using monthly rainfall figures for Claremont, how do the values compare with published values for coastal sage scrub and other biomes?

Is there a correlation between leaf specific mass, thickness and density in coastal sage scrub plants under different conditions? What are the possible physical benefits/constraints?

Do plant allometric relationships (e.g. total leaf area versus height; number of branches/branchings versus height) change during development or in response to competition? Are they related to sexual dimorphism if that exists in a particular plant species?

Can you model growth characteristics of a chosen plant at different planting densities or under different environmental conditions?

How do loading conditions experienced by stems and leaves of elderberry (or other) change during growth?

Are there changes in material properties of Yerba Santa (or other) leaves/stems during maturation?

Does flexural stiffness change during development of a plant organ so that the ability of the organ to bend and deform also changes? Are any changes scaled to changes in self-loading?

How does the heterogeneous character of a plant stem affect its mechanical properties? Can this be modeled for different species? Does it vary with species?

What is the design factor (ratio of allowable stress to the working stress for normal, everyday load duration) for different plants? How great a margin of safety is there?

What are the forces that affect emergence of a seedling from the seed/soil? How does the structure of the seedling change in response to them?

How does the shape of a plant/pollen/wind-dispersed seed or fruit affect air flow around it? What are the characteristics of the flow?

What are the heating/cooling advantages of different shapes, surfaces, etc. What relation do they have to coping with environmental changes? Do leaf movements in plants function to exploit radiative heating or cooling at different times of day?

Chemistry

What is the composition of the volatiles given off by *Artemesia californica* or *Salvia apiana*, as determined by HPLC fitted with columns suitable for terpenoids, or GC-MS?

What secondary metabolites are produced by *Brassica* (or other species) and does the concentration of these change if the plants face competition/water stress/changes in light availability?

Is there allelopathy in any coastal sage scrub plant? What compounds are produced and in what quantities? Does production change in response to age/time of year/damage?

Why doesn't coastal sage scrub grow to the west of the BFS entrance road? Is there something in the soil?

What are the bacterial communities in soil, rhizosphere, and inside roots at the BFS as detemined by fatty acid methyl ester analysis?

What is the chemical constitution of plant cuticles? Do they differ by species, age, or environmental conditions? How easily do they break down in nature? Does their structure vary?

What is the chemical structure of a harvester ant pheromone?

Is there a change in the composition of the air/soil as you get farther from the edges of the BFS?

Is there a change in protein composition of a chosen plant under stress?

Does the composition of nectar vary at different times of day, on different plants, in different locations?

Molecular: genetics/evolution/other

Is there evidence for inbreeding in populations of some chosen species found at BFS, using restriction fragment length polymorphisms and gel electrophoresis to determine Wright's inbreeding coefficient?

Are there genetic differences among populations of a chosen species found at BFS and on the 5C campuses or in other nearby areas? (allozyme assays have been worked out for many local species)

Can you identify vesicular arbuscular mychorrhizal fungi ("VAMs") from different plants using PCR?

What shifts are there in the bacterioplankton community composition in pHake Lake over time, measured by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA genes.

How stable is the mating system for a chosen plant as determined using electrophoresis?

Is the endangered *Berberis nevenii* at the BFS genetically identical to the specimens grown at the botanic garden?

Animal behavior:

What evidence for habituation to human activity is there in lizards or birds?

How does Harvester ant (or other ectotherm) activity vary with temperature? A comparison between native and argentine ants would be particularly interesting. What patterns are related to body size and so on.

How does mosquito fish distribution and feeding rate vary with the presence of barriers to dispersal among feeding stations, barriers to visibility among stations, different levels and kinds of predation risks and so on.

How is predator switching affected by presence of non-prey species (a lab experiment)?

Is there territoriality in carpenter bees (or some other insect)?

What are the parental provisioning patterns in one of the bird species? How often do birds come back to feed chicks, how much food do they bring (body mass) and what are the relative contributions of each parent?

What interactions are there between Argentine and Harvester Ants?

Are there honeypot ants? If so, is there a shift to animal/plant parts as a food source in autumn?

What are the vertical migration patterns of zooplankton (eg, copepods) in pHake Lake? Do they correlate with changes in specific physico-chemical variables in the water?

What territoriality and aggression behavior do you see in different species of dragonflies at pHake Lake? Do these species have a fixed perch? What home range is defended? Will they defend a larger range against a different species than against their own?

How do grasshoppers avoid predators? Do they select variegated over uniform substrate patterns? Do they land so as to cast no shadow? Do they always fly an erratic course? Etc.

What characterizes thermoregulation behavior in grasshoppers? When undisturbed, do they switch between sun and shade? Is this related to air or ground temperature, age/size of animal, local relative humidity, etc?

What characterizes thermoregulation behavior in dragonflies? Is local perch temperature correlated with cooling/heating behaviors, etc?

Does perch height of lizards vary with local prey abundance, temperature, perceived risk, visibility, and so on?

Physiology/development:

Are water/light availability correlated with size/growth pattern/carbon or nitrogen assimilation, etc. for a plant species?

What mychorrhizal fungi are present and associated with development/health in different plants?

What is the effect of competition on plant allometric relationships?

What factors affect development in fairy shrimp or Western toads (or some other species)?

How are malaria infection rates of lizard related to squirrel burrow proximity, lizard density, etc? Lizard malaria is reported have a ~10% infection rate (Noah Levine HMC 02) with the vector being a fly that lives in ground squirrel burrows.

Do any of the BFS plants have seedlings which undergo developmental changes in response to the presence of nearby plants (modeled by a change in the characteristics of available light)?

How does limb morphology of lizards differ between individuals found on different perches or between populations (subpopulations) found in different habitats?

Is energy intake rate limited by capture rate or processing rate in lizards, spiders, etc. Examine energy intake rate vs. satiation or metabolic rates.

Are there any effects of increased soil nitrogen on germination and early growth of coastal sage scrub plants?

How does energy metabolism of an insect vary during rest and motion? Is this correlated with gender or with being parasitized?

How do the large *Epiphragmophora* snails native to the area around pHake Lake cope with the dryness of their native habitat? Can you characterize temperature conditions necessary to trigger summer hibernation? Will they form a plug (epiphragm) in the shell entrance in response to temperature?

What is the effect of mechanical perturbation (wind, handling) on growth and development characteristics in the field?

Ecology

What is the difference between two habitats (of your choice) in terms of species composition and environmental gradients? Can these be manipulated?

Are particular animal species in an area associated with all the plants in that area or only with some?

If you change the available microhabitats in an area, for instance by setting out logs or rocks, do you see a change in the distribution or abundance of organisms?

If you remove one species from an area, does another increase, or a new one replace it?

How do annual precipitation/evaporation ratios change for different regions of the BFS at different times of year and how do these relate to vegetational structure?

How does the insect fauna, species richness, etc. vary around different plants, or at different times of day, or caught by sweep netting as opposed to pitfall traps?

Can differences in insect activity over a 24 hour period be related to insect size, heat tolerance, or coloration/reflectance?

Is there a correlation between butterfly (or some other species) diversity and abundance and the degree to which a patch of coastal sage scrub is isolated by development?

Is plant structure or plant diversity more highly correlated with insect diversity (eg, Homoptera)?

Are better plant competitors simply large or only large in relation to their particular neighbors? A field and lab study, which could include the effects of ant predation.

What effect does herbivory have on the establishment and growth of seedlings of a chosen plant?

Does early germination lead to increased survival and reproduction? Is it related to seed size, parent plant, or competition?

Is there a relation between plant species number and culturable soil bacteria, and does this act as an indicator of biodiversity?

What organisms exploit the cattails on pHake Lake? How do they interact? Are any restricted to particular portions of the plant?

Are there any insects whose eggs or larvae use the specialized air-conducting tissues (aerenchyma) of *Typha* (or other emergent vegetation) as an oxygen source (by plugging spiracles into the stems)? Are these restricted to particular zones?

The willows around the lake are parasitized by a gall-forming sawfly and can be studied for insect development, leaf-selection criteria, larval survival in single versus multiple-galled leaves, etc.

Oaks: Why are there few or no oak seedlings along the road into the BFS?

> **Galls**– What types of galls are present on the oaks and in what numbers? What are the associated insects? Are any of the galls parasitized themselves? By what?
>
> Do the numbers of galls vary from tree to tree? Is this correlated with tree density or health? Are twig-borne galls located only on new growth? Is there a correlation between size of gall and number of exit holes? Between these and size of holes?
>
> **Acorns**--How does infection/predation affect the acorn crop? Do the oaks shed the damaged acorns early? Can the numbers of acorn borer weevils per acorn be predicted from a simple probability function if you know the incidence of infection, or are multiple infections more common or more scarce than expected?
>
> Can a standardized way be developed to quantify the acorn crop and compare it between trees, between years, between locations, and see if it correlates with climate variables or acorn borer infection?

Vernal Pools: Do different light, water and temperature regimes trigger emergence of organisms in mud samples from vernal pools?

What is the order of emergence of organisms from the vernal pool substrate? Are there any carnivorous species or are all essentially algal or bacterial grazers/filter feeders?

What cryptobiotic organisms can you germinate by hydrating vernal pool mud? Are adults of these species desiccation tolerant, or only the eggs? What maximum rates of drying are tolerated?

Pollination biology: If you water fall-blooming plants, does this correlate with differences in pollination or seed set?

How does light pollution affect plants normally pollinated at night? Is the distribution of night-pollinated species correlated with local light intensity at night?

Pollination/seed studies: for the chosen plant–to what extent does nectar build up, what is the composition of the nectar, what visits and when, timing of nectar and pollen production, effect of location on visitor types, pollen/seed set ratios, effectiveness of different pollinators, pollen germination rates, etc.

Does deposition of pollen from another species affect seed set in the plant of your choice?

What effect does heat/rainfall have on synchrony of flowering by conspecifics? Does this affect cross-pollination?

What are the characteristics and consequences of pollen deposition/pollen tube competition or seed size/ packaging variation in the plant of your choice?

More descriptive:

Census and develop a key to ants (or other species) of the BFS.

Is there a difference in bird species and abundance between the BFS, wilderness park, golf course, residential neighborhoods, office park and business district? Do they seem to be correlated with any differences in environmental conditions? If so, which?

What species of (choose plant or animal group, e.g. cacti, grasses, dragonflies, spiders, earthworms) are present at the BFS and where are they located? What environmental conditions are common to these areas?

What are the life history characteristics of the native *Epiphragmophora* land snail found under logs near the lake (or of another plant or animal species)?

What is the habitat use description for a particular species or group of species? This could be complemented with habitat selection experiments similar to Andi Renden's thesis, HMC 2002.