2019 Life Sciences Symposium
Western Washington University
Hosted by the Chemistry and Biology departments
October 18, 2019

Schedule:
9:30 – 10:00 am: Coffee and breakfast  

10:00 – 10:10 am: Welcome by Assistant Professor of Chemistry Jeanine Amacher

10:10 am – 11:45 am: Session I
10:15 am – 10:30 am: Sam Witus (Klevit lab, University of Washington)
10:30 am – 10:45 am: Warren Anderson (Rawling lab, Seattle Children’s Research Institute)
10:45 am – 11:05 am: Dr. Kelly Hvorecny (Kollman lab, University of Washington)
11:05 am – 11:25 am: Dr. Pearl Magala (Klevit lab, University of Washington)

11:45 pm – 1:15 pm: Lunch and networking round-tables

Tentative table themes –
Tips for Applying to Graduate School
Networking
Deciding Where to Go to Graduate School and/or What to Do after College
Communication and Interviewing Skills
Diversity & Inclusion
Science Writing (including funding!)
Work/Life Balance (i.e., maintaining mental health!)

1:15 pm – 3:10 pm: Session II
1:15 pm – 1:30 pm: Hayley Waterman (Hamerman lab, Benaroya Research Institute)
1:30 pm – 1:45 pm: Lincoln Lewerke (Dandekar lab, University of Washington)
1:45 pm – 2:05 pm: Dr. Mitchell Lee (Promislow lab, University of Washington)
2:05 pm – 2:20 pm: Ellie Labuz (Theriot lab, University of Washington)
2:20 pm – 2:40 pm: Dr. Ping Mamiya (UW Institute for Learning and Brain Sciences)
2:40 pm – 3:00 pm: Dr. Tim Mackie (Gardner lab, University of Washington)

3:00 pm – 3:15 pm: Break

3:15 pm – 4:25 pm: Session III
3:15 pm – 3:30 pm: Hannah Ledvina (Mougous lab, University of Washington)
3:30 pm – 3:45 pm: Joseph Harman (Harms lab, University of Oregon)
3:45 pm – 4:05 pm: Dr. Katherine Reiter (Klevit lab, University of Washington)
4:05 pm – 4:25 pm: Dr. Kristine Deiber (Baker lab, UW Institute for Protein Design)

4:30 pm – 6 pm: Western Washington University Research Poster Session

“How to get into grad school:” Dr. Rich Gardner (University of Washington), SL 140
Informal Q&A about biotech industry: Dr. Aaron Moss (Certera), SL lobby
Speaker Abstracts and Affiliations

1. Sam Witus (Rachel Klevit lab, UW graduate student) - WWU alum! BIOCHEMISTRY

Structure and function of the BRCA1/BARD1 nucleosome complex

Sam Witus1, Anika Burell1, Justin Kollman1, Rachel E. Klevit1

1Department of Biochemistry, University of Washington, Seattle, United States

Deleterious mutations in the RING domain of the E3 ligase BRCA1 have long been implicated in familial breast and ovarian cancers. More recently, mutations in its heterodimeric RING partner, BARD1, have also been identified, further implicating the ubiquitin ligase activity of BRCA1/BARD1 in tumor suppression. We recently reported that while cancer-associated missense mutations in the BRCA1 RING are ligase-dead to all substrates due to the loss of E2 binding, cancer-associated mutations in the BARD1 RING show specific loss of function towards nucleosomal histone H2A (Stewart et al. PNAS. 2018). BRCA1/BARD1 selectively monoubiquitylates lysine residues on the extreme C-terminal tail of histone H2A in nucleosomes. This epigenetic modification is important in DNA repair and transcriptional regulation. The mechanism by which BRCA1/BARD1 engages the nucleosome to confer specific modification remains unknown, as attempts to crystallize the complex have proven unsuccessful. Here we present a cryo-EM structure of the BRCA1/BARD1 nucleosome complex, that suggests a mechanism for site-specific ubiquitylation on histone H2A. Our work provides insight into the underlying mechanisms of specificity for a growing number of E3s that monoubiquitylate distinct residues on the nucleosome in E3 ligase-mediated chromatin regulation.

2. Warren Anderson (David Rawling lab, UW graduate student) IMMUNOLOGY

Efficient CRISPR/Cas9 disruption of autoimmune-associated genes reveals key signaling programs in primary human T cells

Risk of autoimmunity is associated with multiple genetic variants. Genome wide association studies have linked single nucleotide polymorphisms in the phosphatases PTPN22 (rs2476601) and PTPN2 (rs1893217) to increased risk for multiple autoimmune diseases. Previous mouse studies of loss-of-function or risk variants in these genes revealed hyperactive T cell responses, while studies of human lymphocytes revealed contrasting phenotypes. To better understand this dichotomy, we established a robust gene editing platform to rapidly address the consequences of loss-of-function of candidate genes in primary human CD4+ T cells. Using CRISPR/Cas9, we obtained efficient gene disruption (>80%) of target genes encoding proteins involved in antigen and cytokine receptor signaling pathways including PTPN22 and PTPN2. Loss-of-function data in all genes studied correlated with previous data from mouse models. Further analyses of PTPN2 gene disrupted T cells demonstrated dynamic effects, whereby hyperactive IL-2R signaling promoted compensatory transcriptional events, eventually resulting in T cells that were hypo-responsive to IL-2. These results imply that altered phosphatase activity promotes evolving phenotypes based on antigen-experience and/or other programming signals. This approach enables the discovery of molecular mechanisms modulating risk of autoimmunity that have been difficult to parse in traditional mouse models or cross-sectional human studies.

3. Kelli Hvorecny (Justin Kollman lab, UW postdoc) BIOCHEMISTRY

Defining the divergent structural and cellular mechanisms of the actin cytoskeleton in Giardia

K.L. Hvorecny1, J. Quispe1, W.R. Hardin2, G.C. Alas2, A.R. Paredez2, J.M. Kollman1; 1Biochemistry, University of Washington, Seattle, WA, 2Biology, University of Washington, Seattle, WA

Cells from organisms as diverse as amoeba, dandelions, and humans contain actin, a filament-forming protein responsible for maintaining cell shape and forming cellular structures. This conservation underscores actin’s fundamental role in cellular processes. However, actin from the unicellular, intestinal parasite Giardia lamblia may be an exception to this conservation rule. Actin from Giardia has the most divergent protein sequence
yet identified in eukaryotes. Further, drugs used to target actin in other organisms are ineffective in Giardia. Additionally, the parasite lacks all of the canonical proteins that control actin function; these regulatory proteins are essential in other organisms. This suggests that actin in Giardia avoided the evolutionary constraints faced by other eukaryotes, thereby providing a natural experiment to probe fundamental actin properties. The divergence also makes actin and its regulatory network a potential therapeutic target in Giardia, which infects over 300 million people annually, with 20% of those cases resistant to front-line treatment. Current treatments for Giardia target all anaerobic organisms in the gut, decimating the commensal flora of patients and leading to other pathologies, including irritable bowel syndrome. Using fluorescent microscopy, we have visualized actin in the ventrolateral flange, a membrane protrusion resembling a lamellipodium involved in attachment by Giardia. In addition, knockdown of actin decreases the width of the flange and attachment by Giardia to a surface. I have recently begun biochemical and cryo-electron microscopy studies to explore how the large amount of variation in actin from Giardia affects actin filament structure and assembly dynamics, as well as electron cryotomography studies to examine the cytoskeletal architecture of the flange. Identifying functional properties that distinguish the actin cytoskeleton of Giardia from its vertebrate homolog will provide opportunities for new drugs that specifically target the parasite without harming the host. This work will also generate fundamental insights into core, conserved actin properties.

4. Pearl Magala (Rachel Klevit lab, UW postdoc) BIOCHEMISTRY

The role of Conformational Dynamics in Shear-Enhanced FimH-mediated Bacterial Adhesion.

Pearl Magala, Dagmara Kiesela, Angelo Ramos, Wendy E. Thomas, Evgeni V. Sokurenko, Rachel E. Klevit

Pathogenic bacteria, such as Escherichia coli (E. coli) establish infections in the intestinal and urinary tracts by adhering to host epithelial cells via the adhesion protein FimH. However, the intestinal and urinary tracts constantly undergo flow conditions that generate shear forces, which could serve to pull bacteria off the host cell. Instead, bacterial adhesion strengthens in the presence of shear forces to prevent elimination from the host. FimH is located at the tip of the bacterial pili and mediates the adhesion of bacteria to host cells by specifically recognizing and binding to mannose ligands in host glycoprotein receptors—the first step in establishing infections. In contrast to most biomolecular interactions that weaken when pulled on by force, interactions between FimH and mannose strengthen under shear force conditions. FimH binds ligands via its lectin domain and is anchored to the rest of the bacterial pili by its pilin domain. Ligand binding by FimH is accompanied by substantial structural rearrangements, in which its two domains dissociate from each other and its binding pocket closes around the ligand. Existing crystal structures of FimH do not provide clarity regarding how the two conformational alterations are coupled allosterically. We are using solution Nuclear Magnetic Resonance spectroscopy to examine the conformational dynamics and solutions structures of FimH to define the pathways and intermediate states that the adhesin adopts to promote ligand binding and shear enhanced FimH mediated bacterial adhesion.

5. Hayley Waterman (UW graduate student, Hamerman lab) - WWU alum! IMMUNOLOGY

Exploring the role of IgA and FcaR in plasmacytoid DC (pDC) immune complex activation in systemic lupus erythematosus (SLE)

Auto-reactive anti-nuclear antibodies (ANAs) are pathogenic in autoimmune diseases such as systemic lupus erythematosus (SLE). Nucleic acid-containing ANA immune complexes facilitate nucleic acid entry to the endosome where Toll-like receptors (TLRs) can promote cytokine production upon binding nucleic acids. Type I interferons (IFNs) are cytokines produced in response to endosomal TLR sensing of nucleic acids. These cytokines are important for clearing viral infections during normal immunity, but are pathogenic during SLE and correlated with disease activity. Plasmacytoid dendritic cells (pDCs) are strongly implicated in SLE disease pathology and produce major quantities of type I IFNs, especially IFNα, upon ANA immune complex stimulation. Most studies have focused on IgG ANA immune complex activation of pDCs despite the fact that SLE patients often have IgA ANAs. pDCs are known to express the IgG recognizing surface receptor, FcγRII, however, no studies have reported pDC expression of the IgA specific receptor, FcαR. Here I report
the novel finding that FcαR is expressed on human pDCs. Additionally, I observed increased FcαR expression (p=0.0152) in a small cohort of SLE patients (n=6) compared to matched healthy controls. To test the contribution of IgA ANAs on pDC stimulation in vitro, I generated IgG-containing immune complexes from IgA-depleted serum. pDCs stimulated with IgA-depleted immune complexes produced less IFNα compared to immune complexes with both IgA and IgG. In addition, reconstitution of IgA to IgA-depleted serum restored IFNα production. Combined these data suggest that IgA ANAs have a pathogenic role in SLE and future experiments will expand on these findings.

6. Lincoln Lewerke (Ajai Dandekar lab, UW graduate student) MICROBIOLOGY

Quorum Sensing in Pseudomonas Aeruginosa

Quorum sensing (QS) is a form of cell to cell communication used by bacteria to coordinate cooperative behavior. Pseudomonas aeruginosa QS displays a complex, hierarchical organization responsible for regulating the activity of dozens of genes. The master QS regulator is LasR which responds to the acyl-homoserine lactone (AHL) 3OC12-HSL. The signal 3OC12-HSL is produced by the signal synthase LasI. A second AHL QS circuit involves the transcriptional regulator RhlR which responds to the signal C4-HSL, produced by RhlI. In the lab strain PAO1, RhlR and RhlI are under the transcriptional control of LasR. When P. aeruginosa is grown in minimal media containing casein as the sole carbon and energy source, it requires QS activation to produce elastase to break down casein into amino acids for use as a carbon source. When the lab strain PAO1 is grown on minimal casein broth, LasR mutants emerge. Interestingly, mutations that result in a nonfunctional LasR are commonly observed in clinical isolates. Our group has recently shown that these isolates with nonfunctional LasR still participate in AHL QS through RhlR. In these isolates, the Rhl system has been rewired – transcriptionally freed from LasR through an unknown mechanism. This highlights the selective pressure placed on quorum sensing during infection and implies that quorum sensing is required in chronic infections. My project is focused on RhlR QS in a LasR-null P. aeruginosa clinical isolate, E94, which is “rewired” and uses RhlR as the primary QS transcription factor. Like the lab strain PAO1, E94 requires QS activation to grow in casein broth, but unlike PAO1, QS-mutant cheaters do not emerge in these populations. Instead, another type of QS-competent cheater emerges. I am working to characterize the mechanism of cheating in E94 QS-competent cheaters as well as investigating the mechanism by which RhlR mutants are restrained.

7. Mitchell Lee (Daniel Promislow lab, UW postdoc) - WWU alum! BIOLOGY

Pharmacogenomic and metabolomic predictors of healthspan interventions in natural populations

Mitchell B. Lee, Kenneth Han, Vanessa Paus, Jiranut Sukomol, Shufan Zhang, David Kim, Sarina Tran, Daniel E. L. Promislow, Department of Pathology, University of Washington School of Medicine

Age is the single greatest risk factor for most major causes of mortality in the US. There is growing interest in utilizing pharmacological interventions that delay age-related disease and extend healthy lifespan, or healthspan. These interventions target known longevity-related pathways, like the mechanistic Target Of Rapamycin (mTOR) signaling pathway. However, such interventions are not well-studied in genetically variable organisms like humans, leaving a critical gap in our understanding. Other non-pharmacological interventions that extend lifespan, like dietary restriction (DR), show a genotype-dependent response where some individuals within a population benefit and others do not. My research goal is to understand the broad utility of the mTOR inhibitor rapamycin as a healthspan intervention and identify biomarkers predictive of rapamycin efficacy by identifying genetic variants and metabolic pathways that regulate rapamycin response. I am modeling rapamycin efficacy using the Drosophila Genetic Reference Panel (DGRP), a collection of genetically variable fruit fly populations. Currently, I (with the help of my research group) am assessing rapamycin sensitivity among the DGRP by screening developmental timing, an easily measurable phenotype that is regulated, in part, through mTOR signaling. In future work, developmental rapamycin effect will be compared to rapamycin efficacy as a lifespan-extending intervention to identify connections that exist between development and aging; two very different molecular programs. These studies will establish the efficacy of a healthspan intervention in a model of natural genetic diversity and relate outcome to genome
and metabolome. Ultimately, these pharmacogenomic and metabolomic analyses advance a precision medicine approach where interventions are tailored to genetic background and metabolomic profile to maximize individual healthspan.

8. Ellie Labuz (Julie Theriot lab, UW graduate student) BIOLOGY

2D confinement of isolated zebrafish epidermal cells as a model of cell migration within skin

EC Labuz, AS Kennard, JA Theriot

Wound-healing fish epidermal cells are one of the fastest migrating cell types known, reaching speeds of several hundred nanometers per second in the embryonic zebrafish tail. Using a combination of microscopy techniques, our lab has observed that these cells are very thin and undergo many deformations during wound-directed migration, perhaps because they are confined to the lower of two layers in the developing zebrafish epidermis. A number of studies have shown that basal cell migration relies on the actomyosin cytoskeleton, but it is unknown how these proteins organize in order to transmit the forces required to move through the epidermal environment.

In order to investigate the cytoskeletal processes that underlie basal cell migration, we have developed a culture-based approach that provides control over the extracellular environment. Basal cells are isolated by trypsin digest and plated on glass coverslips, where they also display rapid migration, but with much fewer deformations compared to cells in vivo. Importantly, isolated cells are ~8 µm tall, almost 3 times taller than their counterparts in the thin zebrafish epidermis. We hypothesized that confinement to a thin layer is an important factor in basal cell migration in vivo; in support of this hypothesis, confining isolated cells with an agarose overlay causes their migratory behavior to look much more like that in vivo. We are developing genetic methods to directly test whether confinement by the skin environment is responsible for the thin shape and deformations we observe for in vivo migrating cells.

The agarose-confined isolated cells have proved to be a useful ex vivo model for how migrating cells interact with their environment. The cells respond to changes in confinement height nearly instantaneously, stop moving at high stiffnesses of agarose, and show an increased dependence on myosin in order to move their cell body. We are implementing force measurement methods to learn how the cells transmit forces differently in order to move in confining versus open environments.

9. Ping Mamiya (UW Institute for Learning & Brain Sciences Research Scientist)

The effects of gene-brain interactions on second language learning: Implications for critical periods

By Ping C. Mamiya, Ph.D., Research Scientist, Institute for Learning and Brain Science, University of Washington

Why is learning a new skill so much easier at a young age? Evidence from psychological studies supports the idea that there are critical periods for skill learning. For example, children acquiring a second language under age seven fare better than the ones learning later in life. Using second language learning as a model system, my research aims to identify factors that regulate the closing of critical periods. Because the same factors may also affect people suffering from learning difficulties, this investigation will help us understand the biological obstacles these patients have to face during learning. With this information, we can help design individualized treatment plans that improve learning.

To accomplish this goal, my research utilizes state-of-the-art brain imaging and genetic sequencing techniques to understand how young adult brains learn a second language. My research also aims to understand what brain structures enable second language learners to control attention and inhibit distractions. This information will help us understand how human brains control which language to use.

Thus far, my research has shown that Chinese college students learning English as a second language show different levels of English proficiency, and their learning outcomes can be predicted by brain and genetic factors. Specifically, their brains made adaptive changes during second language learning, with greater changes associated with better learning outcomes (1). These differences in brain changes depend on individuals’ COMT genotypes. My research also found that these students used the same neural pathways
when processing words printed in their 1st or 2nd language (2). My future work will continue to explore other genes that also contribute to brain adaptations during learning. By combining genetics, brain imaging, and language learning, my research will uncover the biological basis underlying critical periods for learning. Findings from this investigation can help design individualized treatment programs targeting genetic predispositions that affect brain structures in people with learning disabilities.

References

10. Tim Mackie (Rich Gardner lab, UW postdoc) BIOLOGY/PHARMACOLOGY

Defining hyperosmotic stress-induced liquid-liquid phase separation in young and aged yeast nuclei

Timothy D. Mackie and Richard G. Gardner, University of Washington, Dept. of Pharmacology

Stress can damage cellular macromolecules, and accumulated genomic and proteomic damage is associated with cellular aging. To understand how aging functions at the subcellular level, it is necessary to understand the mechanisms of stress response and how aging affects them. Hyperosmotic stress in budding yeast drives coalescence of the yeast transcriptional repressor Cyc8 into subnuclear puncta upon hyperosmotic stress. Concomitantly, Cyc8 is covalently modified with the small ubiquitin-like modifier (SUMO) peptide. Cyc8 is deSUMOylated and reverts to a pan-nuclear distribution when yeast activate intracellular glycerol production as an adaptive countermeasure against hyperosmotic conditions. Presently, we do not know if Cyc8 puncta coalescence and SUMOylation are causally related or how these dynamics change in aged cells. Fluorescence recovery after photobleaching reveals that hyperosmotic shock-induced Cyc8 puncta are solid-like with minimal solvent exchange. Given that these puncta readily dissolve upon resolution of intracellular osmolarity, it is likely that they represent a heretofore undescribed case of liquid-liquid phase separation. Future work will establish the precise osmotic conditions upon which Cyc8 phase separates using purified protein. It is currently unknown how stress-induced phase separation dynamics change in aged cells. My preliminary findings indicate that both hyperosmotic stress-induced Cyc8 nuclear puncta and heat shock-induced cytoplasmic stress granules become smaller and more fragmented in old cells. In the future, I will define the features necessary for hyperosmosis-induced nuclear puncta coalescence. I will focus on the low complexity regions of Cyc8, deleting them to determine the minimal disorder requirements necessary for HOS-induced nuclear puncta coalescence. Furthermore, I will use proximity biotinylation to capture additional nuclear proteins that colocalize with Cyc8 in puncta to further define the characteristics of this novel membraneless organelle.

11. Hannah Ledvina (Joseph Mougous lab, UW graduate student) - WWU alum! MICROBIOLOGY

Surviving and thriving on the inside: mechanisms employed by Francisella tularensis for promoting intracellular growth

The survival of pathogenic intracellular bacteria relies on their ability to establish and maintain a permissive niche. For Francisella tularensis, the causative agent of Tularemia, this involves both escaping the degradative endocytic pathway and the acquisition of essential nutrients from the host cell once in the cytosol. The work I will be presenting at this conference investigates mechanisms employed by F. tularensis at both stages of intracellular infection. We and others have previously established that escape from endosomes is mediated via the action of a set of effector proteins secreted by the F. tularensis subsp. novicida pathogenicity island-encoded secretion system. At this conference, I will present the discovery that a substrate of this secretion system, OpiA, represents a previously undescribed, widespread family of bacterial phosphatidylinositol (PI) 3-kinase enzymes. I will describe how OpiA is recruited to endocytic membranes and acts on the Francisella-containing phagosome to promote bacterial escape into the cytoplasm.
Furthermore, I will share our finding that the phenotypic consequences of OpiA inactivation are mitigated by arresting endosomal maturation. Once in the cytoplasm, the ability of *F. tularensis* to grow is dependent upon nutrients derived from the host. Critical amongst these is glutathione (GSH), a tripeptide whose catabolism provides a source of cysteine that *F. tularensis* requires for growth. I will present our discovery that *F. tularensis* encodes two distinct, yet essential pathways for the utilization of extracellular GSH. Additionally, my data will demonstrate the unique roles for each of these pathways during the course of infection. In total, this work highlights novel mechanisms utilized by *F. tularensis* to both manipulate and exploit host cell pathways to promote bacterial growth.

12. **Joseph Harman (Mike Harms lab, University of Oregon graduate student)**

*S100A9s evolved enhanced proinflammatory activity and lost proteolytic resistance through a single large-effect substitution*

Joseph Harman, Andrea Loes, Jeremy Anderson, Gus Warren, Maureen Heaphy, Kirsten Lampi, Michael Harms

Multifunctional proteins are an evolutionary puzzle: how do new functions evolve without perturbing existing ones? Here we use S100A9 (A9), a key innate immune protein, as a model for the evolution of multifunctionality. A9 activates inflammation by interacting with TLR4 and is antimicrobial as part of calprotectin (CP). These two functional states are regulated by proteolysis; A9 is readily degraded, while CP is resistant. Here we show that A9s both gained potent proinflammatory activity and lost proteolytic resistance through a single substitution (F63M) early in mammals. Reverting this residue in A9 (M63F) ablates its proinflammatory activity, stabilizes the protein, and renders it proteolytically resistant. M63F has no effect on CP. We thus propose that mammals evolved enhanced A9 proinflammatory activity and a proteolytic “timer” to regulate it, all without affecting CP. These findings reveal how a slight alteration to a basic biophysical property like protein stability can result in the evolution of multifunctionality and critical changes in mammalian innate immunity.

13. **Katherine Reiter (Rachel Klevit lab, UW postdoc) BIOCHEMISTRY**

RING acrobatics: how dynamics affect protein turnover

Katherine H. Reiter, Rachel E. Klevit, University of Washington, Seattle WA

Protein quality control is essential for cell viability and thus requires multiple checkpoints to ensure faithful protein turnover. Terminally damaged proteins are targeted for degradation by the ubiquitin proteasome system, yet the mechanisms by which substrates are recognized and triaged is unclear. As final sensors of protein damage, E3 ubiquitin ligases are kept under tight regulation to prevent aberrant protein removal. RING-Between-RING (RBR) ubiquitin ligases comprise a distinct class of auto-inhibited E3s that regulate mitophagy, organogenesis, and inflammation. The activation of RBRs requires large conformational rearrangements that must be coordinated prior to catalysis. Our understanding of these dynamic transitions has been limited to static crystal structures of the inactive and active states, but how these transitions occur remains unclear. To monitor the structural dynamics associated with activation, we are employing in-solution methods, such as NMR, hydrogen-deuterium exchange mass spectrometry, and crosslinking mass spectrometry. By allowing the proteins to adopt native conformations in-solution, we are able to capture a spectrum of structures that more fully describe the step-wise mechanisms involved during activation. Together, this work broadens our understanding of how higher order conformational changes contribute to the maintenance of faithful protein turnover, and how defects in dynamics can lead to associated pathologies.

14. **Kristine Deibler (David Baker lab, UW postdoc) BIOCHEMISTRY, PROTEIN DESIGN**

*De novo* Lariat Based Cyclic Peptide Design for Targeting GPCRs: An Approach Toward the Apelin/APLNR peptide/protein System
G protein-coupled receptors (GPCRs) are the largest family of cell surface receptors and are important human drug targets. Of the 826 human GPCRs, 118 of them recognize endogenous peptide or protein ligands. Many of the GPCRs that remain unexplored as therapeutic targets are peptide- or protein-binding receptors. In these cases, identification of binders has been more challenging due to the difficulties involved in developing small-molecule binders based on the endogenous ligand, which is a major obstacle for discovery of novel GPCR drugs. I have utilized Rosetta based computational methods to design stable cyclic and lariat-based peptide structures for targeting GPCRs. I have also utilized peptide design as a framework for incorporating non-selective small molecule binders into selective peptide motifs; thus, increasing the affinity of the peptide and the selectivity of the small molecule. Specifically, I have been working on targeting the apelin receptor (APLNR). APLNR naturally binds linear apelin and apela peptides of varying lengths. APLNR has recently been linked to regulating cardiac contractility and be essential for heart development. APLNR is thought to be a new target for the treatment of pulmonary arterial hypertension with decreased side effects. Here in I discuss progress towards targeting this select GPCR via de novo peptide design and optimization.