**Summer REU opportunities in the Department of Biochemistry and Biophysics at Oregon State University (2020)**

**Dr. Elisar Barbar, Ph.D.**, <http://biochem.science.oregonstate.edu/content/elisar-barbar>

**Structural biology of dynamic protein complexes** - The Barbar lab has an international, well-regarded reputation for groundbreaking work in the assembly and regulation of dynein cargo complex, related motor protein systems and dynamic protein complexes in general. How dynein knows when and what cargo to transport requires interactions with other proteins that control dynein function. These interactions are regulated by modification in the protein sequence in regions of dynein that lack regular unique three-dimensional structure and are referred to as intrinsically disordered proteins. New findings on dynein interactions with both the dynactin complex (a dynein activator) and a protein called NudE whose depletion in mice produces a small brain and mental retardation show that dynactin and NudE bind to a common segment of dynein that is intrinsically disordered. Elucidating differences in their binding modes explains how one regulator may be selected over the other even when both are present in the same cellular compartment. These results offer a novel role for protein disorder in cellular processes, and highlight the advantages of NMR spectroscopy in elucidating atomic level characterization of complex dynamic cellular assemblies. Understanding this system will ultimately be the basis for the design of synthetic nano-machines that can transport materials on demand.

**Maria C. Franco, Ph.D.,** <http://biochem.science.oregonstate.edu/content/maria-c-franco>

The Franco lab studies the role of oxidants and oxidized molecules in the development and growth of tumors of the nervous system. We showed that tyrosine nitration, an oxidative modification to proteins that occurs in pathological conditions, induces a metabolic reprogramming and supports survival of human schwannoma and glioblastoma multiforme cells. We uncovered that tyrosine nitration of the molecular chaperone Heat shock protein 90 (Hsp90) induces a pro-oncogenic gain-of-function, a new function that normal Hsp90 does not have. Because tyrosine nitration is very low or absent in normal tissues, new therapeutic approaches targeting nitrated Hsp90 in the tumor cells could be developed. We are currently studying how nitration affects Hsp90 structure to induce a new function with the goal of uncovering inhibitors specific to nitrated Hsp90. Nitrated Hsp90 is one of many proteins we found nitrated in tumors. We are currently identifying additional nitrated proteins playing pro-survival roles in tumor cells, and the signaling pathways they regulate. Nitrated proteins have the potential to be a novel category of tumor-directed targets for the development of effective, safer therapies for tumors of the nervous system.

**Dr. Michael Freitag, Ph.D.,** <http://biochem.science.oregonstate.edu/content/michael-freitag>

**A panel of histone H3 mutations to investigate centromere maintenance and gene silencing -** Histones are responsible for DNA packaging in chromosomes. They also affect expression of the DNA they bind. Other protein complexes remodel histones and determine which sections of DNA are not bound to histones and thus can be transcribed or “expressed”. Each of the four core histones has amino acids that can be modified by adding small chemical groups, for example, methyl, acetyl or phosphate groups.  Histone H3 has been studied in the model fungus, *Neurospora crassa,* as related to gene silencing and DNA methylation. Certain point mutations within H3 can abrogate DNA methylation [1].

One project will extend previous work by systematically mutating amino acids along the entire H3 protein and replacing the normal gene with the mutated copies in both *Neurospora crassa* and in *Fusarium graminearum*. This study will be much more comprehensive than previous studies in *Neurospora* or *Fusarium.* Many point mutations will center around lysine (K) amino acids that can be acetylated (usually correlated with expressed genes) or methylated (depending on position correlated with gene activation or silencing), specifically those not usually modified in either of the two commonly used yeasts, *Saccharomyces cerevisiae* and*Schizosaccharomyces pombe*. Hypothesis: *Point mutations in histone H3 will change the balance of H3K9 and H3K27 methylation and acetylation and lead to severe consequences in centromere maintenance and gene regulation.*

Point mutations will be made using an improved “QuickChange” method. Plasmids with the *N. crassa* *hH3* gene surrounded by either *N. crassa* flanks or *F. graminearum* flanks and introduced single or multiple point mutations will be validated by Sanger DNA sequencing, and integrated into the genome of the two fungi by targeted gene replacement of the normal *hH3* gene. All plasmids contain a selectable gene marker, coding for hygromycin phosphotransferase (*hph*), which confers resistance to the antibiotic Hygromycin B, selecting for colonies with the desired mutations. As both fungi have multinucleate spores, strains containing the mutations will be crossed to strains with centromere and gene silencing markers to create haploid progeny with the desired mutations. Assays for how H3 point mutations affect centromere function and DNA methylation in *Neurospora*, or gene expression by H3K27 methylation in *Fusarium* will be a combination of molecular (Southern and western blots, chromatin immunoprecipitation [ChIP]) and morphological assays. Some secondary effects that may be observed include changes in DNA repair and recombination, as well as the ability to undergo meiosis. Our expectation is that mutations in specific lysine residues (K4, K9, K14, K18, K23, K27, K36, K56, and K79) will decrease centromere maintenance and abolish normal gene regulation. Secondary mutations will restore some of these effects to normal. The outcome of this study will be a much improved understanding of the importance of individual histone lysine residues.

**Reference:** [1] Adhvaryu, KK *et al.* 2011. *PLOS Genetics*. 7: 1-13.

**Dr. Adrian “Fritz” Gombart, Ph.D.,** <http://lpi.oregonstate.edu/faculty-staff/adrian-gombart>

**Nutritional regulation of the cathelicidin antimicrobial peptide gene -** Our research focuses on understanding the regulation of antimicrobial peptide expression by vitamin D and other micronutrients. Vitamin D is required for induction of the cathelicidin antimicrobial peptide in cells when they encounter a pathogen. Humans and primates, but not in other mammals possess this mechanism for *CAMP* expression; therefore, we developed a transgenic mouse that carries the human cathelicidin gene. Using this model, we are testing the ability of vitamin D to protect against infection and promote barrier defense. Another focus of our research is to identify additional dietary compounds that regulate the expression of the cathelicidin gene. Working with Dr. Fred Stevens (LPI), we recently identified xanthohumol, a plant prenylated flavonoid, as another inducer of the cathelicidin gene via the farnesoid X receptor (related to the vitamin D receptor). The identification of new regulatory compounds may identify other nutrients that we can use to boost the immune system. With funding from the NIH, we are: 1) determining how xanthohumol alters the composition of the gut microbiota with the goal of treating obesity and metabolic syndrome and 2) developing nanofiber bandages (with Dr. Jingwei Xie, a materials scientist at University of Nebraska Medical Center) that release these compounds to boost immunity against skin infections and speed wound healing.

**David Hendrix, Ph.D.**, <https://biochem.oregonstate.edu/content/david-hendrix>

Hop (Humulus lupulus L. var Lupulus) is a diploid, dioecious plant with a history of cultivation spanning more than one thousand years. Hop cones are valued for their use in brewing, and around the world, hop has been used in traditional medicine to treat a variety of ailments. Efforts to determine how biochemical pathways responsible for desirable traits are regulated have been challenged by the large, repetitive, and heterozygous genome of hop.  Our haplotype-phased assembly and annotation of the Cascade cultivar genome is the most extensive to date. PacBio long-read sequences from hop were assembled with FALCON and phased with FALCON-Unzip. Using the diploid assembly to assess haplotype variation, we discovered genes under positive selection enriched for stress-response, growth, and flowering functions. Comparative analysis of haplotypes provides insight into large-scale structural variation and the selective pressures that have driven hop evolution. Previous studies estimated repeat content at around 60%. With improved resolution of long terminal retrotransposons (LTRs) due to long-read sequencing, we found that hop is nearly 78% repetitive. Our quantification of repeat content provides context for the size of the hop genome, and supports the hypothesis of whole genome duplication (WGD), rather than expansion due to LTRs. This project will involve the analysis annotation of the hop genome, leading to improved genomic resources provided at our web-accessible database [hopbase.org](http://hopbase.org). The student will write code in python and php, and learn about the identification of genes and gene families in the genomic sequence.

**Victor Hsu, Ph.D.**, <http://biochem.science.oregonstate.edu/content/victor-hsu>

**Biomolecular recognition and binding of receptor-drug complexes** - The research focus of the Hsu lab is on characterizing and understanding biomolecular interactions. We strive to answer questions such as: "What are the intrinsic properties of a protein or receptor domain that direct and influence binding to its interacting partners?"; and "Can we design drugs and predict whether they will productively bind to specific receptors?" In the lab, we use computational methods such as molecular simulations, computer vision and machine (deep) learning algorithms to identify potential drug candidates. We then utilize various spectroscopic techniques, including nuclear magnetic resonance (NMR), mass spectrometry, fluorescence spectroscopy and circular dichroism, to verify (and improve) our prediction algorithms. We collaborate on these projects with researchers in the Chemistry department and the Electrical Engineering and Computer Science department. We are currently working on antileukemic drugs and identifying compounds that might interact with a receptor implicated in metabolic syndrome diseases. If you are interested in these research topics and learning any (or all) of these experimental techniques, I would like to talk to you. If you have any ideas or hypotheses related to biomolecular recognition and interaction that you would like to test, I am especially interested in talking to you!

**Colin Johnson, Ph.D.**, <http://biochem.science.oregonstate.edu/content/colin-johnson>

**Wound repair, neurotransmitter release and neuronal signaling** - Our research is focused on a family of proteins known as the ferlins, which help regulate membrane trafficking events and have been linked to several human pathologies. Specifically, work currently focuses on the muscle protein dysferlin, which repairs damage in muscle tissue. Mutations in dysferlin have been implicated in several forms of muscular dystrophy, suggesting a key role for the protein in muscle physiology. Also of interest is otoferlin. Although still unclear, otoferlin may control the release of neurotransmitter and the encoding of sound in the brain. Mutations in otoferlin have been linked to deafness in human patients. We are currently characterizing both of these calcium sensing proteins in an attempt to understand the exact contributions of these proteins to membrane fusion, as well as the basis for why mutants in dysferlin and otoferlin result in muscular dystrophy and deafness.

**Afua Nyarko, Ph.D.**, [https://biochem.oregonstate.edu/content/afua-nyarko](https://biochem.oregonstate.edu/content/afua-nyarko%20)

**Specificity and selectivity in WW domain proteins**. Specific protein–protein interactions are essential for the precise transmission of information in eukaryotic cells. Surprisingly, only a small number of modular domains mediate the majority of protein-protein interactions. The WW domain, a modular domain found in 52 human proteins, mediates protein interactions with proline-rich-motif containing proteins. Currently 100 WW domains are reported to interact with ~2000 proline-rich motifs. With very few WW domains and so many proline-rich motifs, how is binding specificity achieved? In the Nyarko lab, we aim to understand how the structure and dynamics of WW domain proteins engenders them to specifically and selectively bind relevant proline-rich motif partners and assemble into functional complexes. We approach this fundamental question by combining molecular, structural and cell biology approaches. Currently, our prime focus is the WW domain and C2 (WWC) sub-family of WW domain proteins which regulate cell proliferation, are linked to various cancers and are prime targets for therapeutic intervention. The three structurally similar proteins in this family, KIBRA, WWC2 and WWC3 interact with the same proline-rich proteins. However, initial studies in the Nyarko lab show different binding affinities for the proline-rich targets. The goal of the project is to identify structural and dynamic features that give rise to the differential binding affinities. Proposed experiments include 1) generate mutations that alter the dynamics of the WWC proteins and 2) evaluate the effect of altered dynamics on binding affinities. Understanding the molecular origins of binding specificity can guide the design of new drugs to treat cancer.