TRPV1 Expressed in HEK293T/17 Cells is Not Regulated by Plasma Membrane Cholesterol Content
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TRPV1 is an ion channel of great importance for pain and heat sensing. The EC50 (threshold dose) of the ion channel TRPV1 is highly variable depending on the cell type in which it is expressed. For example, the EC50 of TRPV1 in HEK293T/17 (a cultured mammalian cell line) is ~1 µM whereas the EC50 of TRPV1 in isolated DRG neurons is ~100 nM. Numerous explanations have been proposed for this phenomenon, with one possible explanation being the varying lipid contents of the plasma membrane regulating the EC50 of TRPV1. The cholesterol content of the plasma membrane is a candidate for regulating TRPV1, as it is known to vary among types of cells. We measured TRPV1 function with patch-clamp electrophysiology to determine the effect of removing cholesterol from the plasma membrane on channel function. Inside-out excise patches remain stable for tens of minutes, allowing for cholesterol removal and replacement by flowing a Methylated-β-Cyclodextrin, a ring of sugar molecules that bind cholesterol, across the patch. In intact HEK293T/17 cells, we estimated the cholesterol content to be about 30%. Thus, the Methylated-β-Cyclodextrin is expected to produce a very large change in plasma membrane properties as removes cholesterol. Recordings from these patches showed that both the EC50 for activation of TRPV1 by capsaicin and the capsaicin-induced maximum current were unaffected by patch treatment with Methylated-β-Cyclodextrin. These results are significant as they show that TRPV1 function is unaffected by a large reduction in plasma membrane cholesterol. Because removal of such an abundant component of the plasma membrane likely produces meaningful changes in the bulk properties of the bilayer, these data also indicate that lipid regulation of TRPV1 is caused by lipids which have specific, chemical interactions with the channel, rather than the bulk properties of the plasma membrane.

A Sticky Situation: How Protein Folding Impacts Infectious Disease
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Treatment resistant bacterial infections are a major public health concern. Staphylococcus aureus, in particular, is responsible for thousands of deaths and hospitalizations every year in the U. S. alone. S. aureus has many virulent characteristics, including its ability to subvert the immune system via neutrophil lysis and biofilm secretion. Biofilm secretion is a common bacterial defense mechanism in which a mixture of specific proteins and cellular debris are assembled into a protective matrix. This matrix serves to shield bacteria from antibiotic treatment and mechanical disruption. Interestingly, phenol soluble modulins (PSMs) – small, soluble, amphipathic proteins which are the main constituent of biofilms – also modulate neutrophil lysis. Neutrophils are one of the body’s primary defense mechanisms against infection. Unfortunately, S. aureus have evolved to evade the normal function of these cells by lysing them from the inside. PSMs’ membrane-lytic behavior largely contributes to this pathology. Despite their importance in the promotion of persistent S. aureus infection, little is known about the biophysical mechanism of action of PSMs. In this project, I look into these two interconnected pathways using a series of high-resolution spectroscopic techniques, including aggregation fluorescence assays, fluorescence correlation spectroscopy, high performance liquid chromatography, and electron microscopy. Greater understanding of these pathways will help lead to better treatments to combat the global burden of resistant bacterial infections caused by S. aureus.
Determination of Key Locations to Disrupt Aggregation in Light Chain Amyloidosis

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Light Chain Amyloidosis (AL) is a disease defined by the deposition of aggregated immunoglobulin light chains in the form of fibrils in several organs. While these fibrils are insoluble, the soluble oligomers that precede them in the pathway involving unfolding and then aggregation of the protein are toxic to the cell. A novel secondary structure, alpha-sheet, was hypothesized to be the cause of toxicity. This structure is rarely seen in normal proteins, but it is populated in all peptides and proteins involved in amyloid diseases studied so far in our lab. In addition, the correlation between thermal stability and fibril formation has led to the study of the impact of amino acid mutations on the unfolding pathway. As is the case for most amyloid diseases, Light Chain Amyloidosis is incurable and fatal. The determination of specific patterns and mutations that destabilize the protein and lead to increased toxicity is important to better understand how to intervene. To this end, we are comparing two patient-derived light chains, one non-amyloidogenic and the other amyloidogenic. By analyzing their differences, we can determine regions of the protein that contribute to aggregation. We performed molecular dynamics simulations of each protein and analyzed their unfolding pathways and characterized the structure of the amyloidogenic interemediate associated with toxicity. The docking of pre-existing amyloid inhibitors developed in the lab to the resulting structures was done to determine a lead compound. Through rational and evolutionary design, several iterations were done on the lead compound and an inhibitor with an overall better binding score was developed.

Application of Synthetic α-Sheet Peptides Rescues a Human Cell Model from Aβ42 Cytotoxicity

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Amyloid diseases like Alzheimer’s disease, type 2 diabetes, and Parkinson’s disease represent some of the most pressing concerns facing our healthcare system today. Human consequences aside, these disorders place an enormous economic burden on society; Alzheimer’s disease care in the United States alone costs upwards of $226 billion annually, and these expenses will continue to rise as the population ages. As such, substantial effort has been invested in the development of novel compounds that would halt or reverse the effects of these diseases. Our group’s previous research suggests that amyloid diseases, though caused by the selective dysfunction of different and unrelated proteins, share a common mechanism of pathogenicity, wherein misfolded proteins aggregate into toxic oligomers typified by novel α-sheet secondary structure. Synthetic peptides designed to adopt a structure complementary to this motif inhibit in vitro aggregation of several different amyloid systems. In this study, we show that the inhibitory effects of these molecules also extend into the realm of simple biological models, in this case inhibition of toxicity in neuroblastoma cells. We co-incubated IMR-32 cells with Aβ42 peptide (whose aggregation is associated with Alzheimer’s disease) and our synthetic α-sheet compounds for periods of twenty-four to forty-eight hours, then assessed cytotoxicity by a MTT cell viability assay. When compared with neat Aβ42, the designs were able to rescue cells from necrosis, significantly lessening detectable toxicity. While preliminary, these results suggest that our peptides may have promise as potential therapeutic agents for treating a broad spectrum of amyloid diseases.

Nutrient Parameters are Associated with Metabolomic Profiles across 11 Species of Drosophila

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Regulation of metabolic pathways is often implicated in many normal as well as disease processes in organisms. Our previous work discovered changes in the metabolome associated with longevity in eleven different species of fruit flies. The metabolic pathways associated with longevity included gluconeogenesis, the Krebs Cycle, and amino acid degradation. However, it is still unknown how basic nutrient parameters influence metabolic pathways in flies. We used mass spectrometry coupled with liquid chromatography to quantify the small molecules present in whole fly tissue. After finding metabolites associated with age, we completed nutrition assays to determine if there were associations between nutrient parameters and metabolomic profiles. The measurement of basic nutrient parameters consisted of colorimetric assays for total glucose and protein levels. Data analysis revealed correlations between the levels of each nutrient found in whole fly tissue and corresponding metabolomic profiles. In our future work, we plan to manipulate metabolic pathways or nutrient parameters found to be significantly associated with age and observe whether there are changes in longevity.