Poster #1

Three dimensional Structure of the Anthrax toxin PA pore translocon-Lethal Factor complex by Cryo-Electron Microscopy

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Upon acidification of the endosome, the receptor bound anthrax toxin protective antigen (PA) moiety forms oligomeric pores and translocates the enzymatic moieties of the toxin-lethal factor (LF) and edema factor (EF) across the endosomal membrane into the cytoplasm of mammalian cells. The unfolding and translocation process of the LF and EF appears to be driven by a simple proton-motive force (pH 5.5 to pH 7.0) mechanism. At endosome pH (~5.5), LF or EF becomes protonated, unfolds and translocates into cytoplasm through the PA pore into the cell cytoplasm (pH 7.0). The translocated LF or EF then deprotonates and refolds perhaps with the aid of intracellular chaperones. In order to understand the mechanism behind this pH driven translocation, we need to understand the nature of the lethal factor-PA pore interactions and map the structural components of the PA pore lumen before and after the pore conversion. To this end, we constructed and visualized the N-terminal domain lethal factor LF₅-PA-pore translocon complex inserted into a model lipid bilayer (a lipid nanodisc) using Cryo-electron microscopy. Single particle image analysis in combination with 3D reconstruction yielded the structure at 16-Å. The heptameric mushroom shaped cap structure prominently displays seven extensions of domain three extending away from the central channel and extends 140 Å from the nanodisc surface. Our low resolution LF₅-PA structure possesses distinct densities in positions where LF₅ has been noted to bind to the PA subunit interfaces. Also, the interior pore region of the LF₅-PA structure is filled with additional protein density, consistent with the large unstructured region of the LF₅ extending towards the interior phe clamp region of the PA pore translocon lumen. The extra density observed in the interior of the LF₅-PA structure provides structural support to previous biochemical and biophysical studies¹ that examined the interaction of the unstructured N terminus of LF₅ with the PA phe clamp.

¹References:
Poster #2

The N-terminus of the VirG autotransporter destabilizes the entire passenger in vitro Implications for in vivo secretion

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VirG is a virulence-associated protein of Yersinia pestis, the causative agent of bubonic plague. VirG belongs to the autotransporter (AT) family of virulence proteins, and like most other ATs, the mature extracellular (passenger) domain is predicted to adopt β-helical structure, which we have proposed is important for AT biogenesis. Previous studies in our lab with two other ATs, pertactin from B. pertussis and Pet from a pathogenic strain of E. coli, have shown that the C-terminal β-helical portion of the passenger domain adopts a stable structure that is resistant to chemical and thermal denaturation, and extracellular folding of this domain could serve as a driving force for outer membrane (OM) secretion. This study aims to identify the features of the VirG passenger domain that are important for its biogenesis, including what keeps the passenger domain unfolded during its transit across the periplasm prior to OM secretion. We established a VirG expression system in E. coli and purified two VirG passenger domain constructs. Our preliminary results suggest that the shorter C-terminal passenger domain construct is more resistant to chemical and heat denaturation, and is more resistant to protease digestion, than the longer construct with an N-terminal extension. The N-terminal portion of the passenger domain could therefore be preventing premature folding of the entire passenger domain while in the periplasm maintaining a conformation compatible with OM secretion.

Poster #3

Investigating the conformation of an autotransporter protein in the bacterial periplasm

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Autotransporter (AT) proteins are virulence factors in Gram-negative bacteria. They are synthesized with an N-terminal signal sequence that is cleaved after inner membrane secretion, a central passenger domain (the mature protein), and a C-terminal outer membrane (OM) translocator domain. The translocator domain is required for OM secretion of the central passenger domain. Transport of the passenger domain across the OM does not require ATP nor a proton gradient, and therefore the driving force for efficient secretion remains unknown. Previous studies suggest that the AT passenger domain is secreted from C- to N-terminus across the OM, and that vectorial folding on the outside of the cell could drive secretion. This mechanism implies that the AT passenger domain must remain in an unfolded, secretion-competent conformation in the periplasm. To test this model, we are using a novel antibiotic resistance reporter assay (Mansell, T. et al., Protein Science, 2010) to measure the extent of folding of the passenger domain of pertactin, an AT from Bordetella pertussis, as it transits the
periplasm. We were able to confirm that pertactin adopts a soluble but unstable conformation in the periplasm, consistent with our proposed secretion mechanism. Moreover, while native pertactin is resistant to protease digestion, the periplasmic conformation of pertactin is susceptible to protease digestion, suggesting it adopts unfolded, non-native conformations that would facilitate subsequent vectorial secretion across the OM. Presently, we are using the same assay to screen a library of random pertactin mutants in order to identify mutants that adopt a stable conformation in the periplasm, in order to investigate how these mutations affect OM secretion.

Poster #4

Alterations in the Activity of the Yeast Peroxiredoxin Tsa1 Upon Modification by Alkylating Agents

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Organisms have evolved a variety of defense mechanisms to deal with adverse environmental challenges, including exposure to reactive oxidants and organic electrophiles. In *Saccharomyces cerevisiae*, one of the proteins involved in defense against oxidants is the peroxiredoxin thioredoxin peroxidase 1 (Tsa1). Tsa1 possesses two highly reactive active site cysteine residues that undergo oxidation to form a disulfide bond during catalysis. Here, we report that Tsa1 is a cellular target of electrophilic alkylating agents. In cells treated with the electrophilic protein cross-linkers diethyl acetylenedicarboxylate and divinyl sulfone, Tsa1 is cross-linked to itself and other proteins, including cytosolic thioredoxins. Cross-linking of Tsa1 is dependent on its active site cysteine residues, implying that these residues are subject to electrophilic modification. We tested whether pre-treatment of Tsa1 with the alkylating agent *N*-ethylmaleimide inhibited its peroxidase activity and found that alkylation of the protein prevents its oxidation by H$_2$O$_2$. Despite this, yeast strains lacking *tsa1* are hypersensitive to cell death induced by electrophiles and have an increased amount of proteins accumulating in insoluble aggregates under similar conditions. Since Tsa1 also functions as a molecular chaperone, our results suggest that its molecular chaperone activity may account for its protection against electrophiles.

Poster #5

Positive Selection for Rare Codon Clusters

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The genetic code is degenerate, but some synonymous codons occur less often than others, and these rare codons are associated with slower translation and lower translational accuracy. Surprisingly however, rare codons form clusters in both prokaryotic and eukaryotic ORFeomes at levels far greater than predicted by random chance, suggesting that rare codon clusters may serve a functional role. It has been suggested that rare codon clusters may promote co-translational protein folding by modulating the rate of protein synthesis. A rare-codon induced translational pause could potentially affect co-translational folding pathway and final protein conformation by limiting which regions of the sequence are outside the ribosome exit tunnel and available to interact, separating the folding of structural elements in a protein. If rare codon clusters are functionally significant, then the rareness of a coding sequence is expected to be under positive selection through evolution. To determine if rare codon clusters are subject to positive selection, clusters of homologous proteins from bacteria were identified and aligned. The %MinMax algorithm was used to quantify coding sequence rareness and locate rare codon clusters. The resulting alignments were analyzed to calculate the probability of positive selection. Preliminary results show that rare codon clusters are subject to positive selection in bacteria, with >10% of homolog clusters analyzed exhibiting co-occurrence (p ≤ 10^-5 that a rare codon cluster occurred in the same positions in the homologs by chance). Currently, clusters of homologous eukaryotic and archaeal proteins are being analyzed for positive selection of rare codons to determine if this phenomenon occurs across all three domains of life. Homologous bacterial proteins containing co-occurring rare codon clusters are being examined to determine if co-occurring rare codons can be correlated to structural or folding features of the encoded proteins.

Poster #6

Capturing the pH dependent kinetic transition of the Anthrax Protective antigen prepore to pore translocon using biolayer interferometry (BLI) and surface plasmon resonance (SPR)

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Under acidic conditions, the protective antigen (PA) prepore protein of Bacillus anthracis undergoes a conformational change from a near-globular prepore structure to a membrane-insertable pore structure. In vivo, this transition is critical for the delivery of the toxic enzymes lethal factor and edema factor as the endosomal environment is acidified. If the kinetics of the transition can be analyzed, it will provide a quantitative basis for studying the effect of potential small molecule inhibitors that could regulate the transition. Of particular interest in the case of a pathogenic microbe such as anthrax, one would like to ultimately identify agents that will inhibit pore formation, slowing or halting the progress of the disease. We used label free techniques, biolayer interferometry (BLI) and surface plasmon resonance (SPR), to observe this conformational change by PA while bound to a truncated form of the lethal factor (LFn) positioned on appropriate
sensor tips/chips. The techniques of BLI and SPR measures changes in the immobilized protein layer wherein changes in the distribution of matter thickness near the protein (in this case due to PA conformational changes) are monitored in real time. The large (85 Å to 180 Å) prepore to pore transitions (unfolding/refolding) showed distinct pH-dependent changes in both kinetics and amplitude. In the absence of a soluble receptor, these conformational changes were observable over a pH range of pH 7.0 to pH 5.0. From a more physiological standpoint, as expected, the presence of soluble physiological receptors bound to the prepore prior to acidification resulted in dramatically slower transition kinetics. For example, the receptor constrained transitions at pH 5.9 are slowed tremendously but show a progressive acceleration as the pH is raised to pH 5.5. As the pH is dropped to pH 5.0, a rapid complete transition to the pore is once again observed. Our ability to monitor these kinetic transitions with LFn- PA pore-receptor complexes in real time allows us to recapitulate events during the acidification of the endosome and will allow us screen for appropriate small molecule inhibitors at both extracellular and endosomal pH values.

Poster #7

Chemical genomics in Saccharomyces cerevisiae reveal functional roles of J proteins

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The molecular chaperone heat shock protein 70 (Hsp70) works with J protein co-chaperones to carry out its functions in protein quality control. There are at least 22 J proteins in Saccharomyces cerevisiae and more than 40 in humans. J proteins share a conserved J domain that stimulates the ATPase activity of Hsp70s but they are otherwise structurally diverse. A growing body of evidence suggests that some J proteins have specialized functions. To probe this possibility, we used S. cerevisiae deletion mutants for 11 cytosolic and nuclear J proteins and tested their response to a panel of 10 chemical inhibitors that act on known biological pathways, such as translation, cell wall biogenesis and kinase signaling. In two types of growth assays, we found that deletion of three J protein genes, YDJ1, ZUO1, and SWA2, conferred sensitivity to most of the compounds, while others, such as JJJ3, caused sensitivity to only a subset of the treatments. Moreover, other deletions did not cause sensitivity to any of the compounds, suggesting that either they are redundant with other J proteins or that they have specialized functions unrelated to these compounds. Together, these findings support the model that J proteins have both specific and general roles in the support of diverse cellular processes. Further, these results begin to identify pathway-specific activities of J proteins in yeast.

Poster #8

Are Transgenic Mice Valid Animal Models for the Study of Alzheimer’s Disease (AD)?

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Background: AD is characterized by dementia associated with extracellular plaque deposits of beta-amyloid (abeta). Most investigators believe that the dementia is due to abeta neurotoxicity. Yet, there is a poor correlation between plaque burden and cognitive decline. On the other hand, linkage studies have established that the familial forms result from mutations in three proteins, amyloid precursor protein and presenilins 1 and 2. Several investigators have developed mouse models transfected with the mutant forms of these human genes. Since these animals show cognitive decline and plaque deposition with age, they have been used to study the cell biology of AD and identify potential therapies for it.

Objective: Determine the outcomes of Phase 3 clinical trials of agents which were effective in transgenic mice to determine whether they are valid models for the study of the cell biology of AD and drug discovery.

Methods: Animal and phase 3 clinical trials were identified through web searches.

Results: To date there have been seven completed trials of agents which were effective in transgenic mice but had no clinical benefit. The most glaring example of these is the Elan vaccine trial in which patients received abeta. The vaccine induced antibodies which cleared plaque, but had no effect on the cognitive decline. It is difficult to reconcile the paradigm which posits that the dementia is due to abeta toxicity when a treatment which clears plaque had no effect on cognition.

Failures of other agents which were active in transgenic mice include: two γ-secretase inhibitors; MK-677, an agent which increases IGF-1 secretion; Ginkgo biloba; estrogen; and docosahexaenoic acid, an omega-3 fatty acid.

Conclusions: The repeated failure of agents which were effective in transgenic mice to show efficacy in phase 3 clinical trials raises a major issue for the development of therapies for sporadic AD. These failures suggest that the dementia is not due to abeta toxicity, but rather to more fundamental biochemical changes which probably underlie the aging process in general. Furthermore, if the two, current, large scale phase 3 trials of monoclonal antibodies from Elan/J&J/Pfizer and Lilly, which have been demonstrated to clear plaque, fail to halt the cognitive decline, it would seem difficult to continue to search for therapies based on the paradigm that the dementia is due to the neurotoxicity of abeta.

Poster #9

Ribosome-associated chaperones control aging-dependent decline of protein synthesis

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The production of newly synthesized proteins is a key process of protein homeostasis that initiates the biosynthetic flux of proteins. Protein synthesis is highly regulated on
multiple levels to adapt the proteome to environmental and physiological challenges. The proteome undergoes severe changes with aging, as proteins become prone to misfolding and hence lose function from an early stage of adulthood on. The loss of protein function could therefore be compensated by enhanced *de novo* synthesis to replace the proteins at risk. However, we show that the opposite scenario happens. We observed a strong decline of polysome formation, indicating a substantial decrease of protein synthesis with aging. This decline in protein biosynthesis is controlled by ribosome-associated chaperones such as the Nascent polypeptide Associated Complex (NAC). In addition to its functional association with the ribosome, NAC exhibits chaperone activity, and upon proteotoxic stress, shifts its localization from a soluble ribosome-associated state to the insoluble aggregated protein pool. This functional depletion of NAC from ribosomes reduces the levels of polysomes and greatly diminishes the translational capacity with aging and upon proteotoxic stress. This novel chaperone-mediated regulatory mechanism provides the cell with an additional point of control for ribosome activity and provides a rapid and efficient response to imbalance in proteostasis.

Poster #10

Development of Small Molecule Modulators of Small Heat Shock Protein Structure and Function

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Small heat shock proteins (sHsps) are a family of ubiquitous molecular chaperones that are essential to cellular protein homeostasis. These chaperones lack enzymatic activity but are thought to prevent the pathological aggregation of client proteins by holding them in stable complexes that are competent for refolding by the ATP-hydrolyzing chaperones. For at least one small heat shock protein, Hsp27, its chaperone functions are linked to changes in its quaternary structure, which can range from a dimer up to a globular oligomer containing 24-40 subunits. Point mutations that disrupt the distribution of this ensemble result in hereditary neuropathies, including Charcot-Marie-Tooth disease, suggesting that structural plasticity is required for chaperone functions. However, the detailed mechanism of action of this important sHsp remains elusive and its critical structure-function relationships are not known.

We hypothesized that small molecules able to modify the self-assembly of Hsp27 would be powerful chemical probes for understanding sHsp function. Towards that goal, we have carried out a fragment-based screen by HSQC NMR against the dimeric core crystallin domain of Hsp27. Fragment-based drug discovery (FBDD) is a powerful, emerging method for identifying low molecular mass compounds that are especially suitable for further synthetic elaboration. The advantage of FBDD by NMR is that the binding sites of the fragments are identified as part of the routine screening process,
providing simultaneous structural information that can be used to guide the syntheses. We screened 1,000 chemical fragments (<300 Da) by NMR and identified 4 fragments with affinity for Hsp27. We are currently working to improve the affinity of these screening “hits” and studying how they might perturb the distribution of quaternary structures. We anticipate that these studies will provide a starting point for elucidating the molecular roles of Hsp27 in disease.

Poster #11

Developing a Chaperonin-based Label-free real-time high-throughput assay platform that identifies lead compounds to stabilize misfolded proteins

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Protein misfolding diseases account for 30-50% of known human diseases. It is possible that chemical ligands that stabilize proteins can be used to treat a significant subset of these folding diseases. Discovery of such lead compounds is, however, hampered by slow & expensive screening. We have previously utilized an immobilized high affinity form of E. coli chaperonin GroEL as a tool to detect misfolded proteins and identify small molecule ligands to stabilize them (Naik et al, Biopolymers, 2010). Based on our proof of principle success, we have now developed a high-throughput platform using the GroEL chaperonin in conjunction with label-free protein interaction techniques such as Surface Plasmon Resonance (SPR) and Bio-layer interferometry (BLI) to detect general protein unfolding and stabilization. We demonstrate that the nucleotide free form of Cystic Fibrosis Nucleotide Binding Domain 1 (CFTR NBD1) undergoes partial unfolding reactions that can be detected with GroEL when the chaperonin is either immobilized or free in solution. These label-free techniques allow us to monitor direct binding of GroEL to the transient states of partially folded proteins, providing real time quantitative information on the specificity and affinity of binding as well as the kinetics of the interactions. Any ligands that bind to native protein will shift the dynamic folding equilibrium from their partially folded form toward the native protein fold, decreasing GroEL binding (decreased signal), which can potentially result in the further development and characterization of the potential lead compound(s). A limited set of CFTR correctors tested showed the characteristics of potential protein stabilizers (decreased GroEL binding) for both wild type and delta F508 CFTR variants. In conclusion, the chaperonin screening assay combined with SPR and BLI gives us a broad-range, rapid & real-time HTS system that can be used to screen and validate potential misfolding protein stabilizers.

Poster #12

Identification of factors involved in the biogenesis and transport of Kir2.1, a mammalian potassium channel
The inward rectifying potassium channel, Kir2.1, functions at the plasma membrane of muscle and neuronal tissues to maintain membrane potential. Gain or loss of function mutations in Kir2.1 cause diseases such as Anderson-Tawil syndrome and short QT syndrome, respectively. The spectrum of cellular determinants that assemble, deliver, and maintain channel plasma membrane residence remain largely unknown. Using yeast as a model system, we propose to identify and explore the factors involved in the biogenesis and trafficking of Kir2.1.

Using immunofluorescence and sucrose gradient centrifugation we demonstrated that the majority of Kir2.1 is localized to the endoplasmic reticulum, with a smaller population resident at the plasma membrane. Like other complex ion channels, Kir2.1 is subject to protein quality control. Cycloheximide chase assays revealed that a significant percentage of Kir2.1 is targeted for ER associated degradation (ERAD). Kir2.1 protein is stabilized in strains deficient in the ER associated E3 ubiquitin ligases Hrd1 and Doa10, and in the presence of the proteasome inhibitor MG132. Using temperature sensitive chaperone mutants we discovered that the cytoplasmic Hsp70 protein, Ssa1, was also involved in Kir2.1 ERAD. Studies to determine if Ssa1 is directly involved in the ubiquitination of Kir2.1 are currently in progress.

The presence of a population of Kir2.1 at the plasma membrane and its function as a potassium transporter allow us to use the yeast system to identify the spectrum of factors involved in the regulation and trafficking of Kir2.1 through the secretory pathway. Yeast express two potassium transporters (Trk1 and Trk2), that allow growth on low potassium media. In the absence of Trk1 and Trk2 the cells require high extracellular potassium to grow, but an exogenously expressed potassium channel can restore growth on low potassium media. Thus, yeast growth on low potassium media provides a direct readout for Kir2.1 plasma membrane residence. The trk1Δ trk2Δ strain expressing Kir2.1 was crossed to the yeast deletion collection and the resulting strains were screened on low potassium. Initial results from the screen uncovered nearly all members of the Endosomal Sorting Complex Required for Transport (ESCRT) and the entire retromer complex, as well as members of the transport protein particle (TRAPP) and Conserved Oligomeric Golgi (COG) complexes, indicating that trafficking of Kir2.1 to the plasma membrane is highly regulated. The effects of these proteins will be further examined to determine how they impact Kir2.1 trafficking through the cell.

**Poster #13**

**Development of high-throughput screening assay to identify small molecule inhibitors of molecular chaperones**

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Molecular chaperones are key regulatory molecules involved in various cell-signaling pathways and are extensively studied in the context of cancer, cardiovascular and neurodegenerative disorders as potential drug targets. Various compounds are known to
block the functioning of Hsp90 in vitro and in animal models but clinical efficacies are less than satisfactory. Therefore, there is an urgent scientific as well as a clinical need to identify novel small molecule inhibitors of Hsp90 chaperoning machinery. Progesterone Receptor (PR), a known physiological client of Hsp90, is dependent upon smooth functioning of the Hsp90 chaperoning machinery for its biological activity. With this idea, we developed a high throughput drug screening approach using the PR reconstitution as a model system. We immunopurify PR on a 96 well plate, strip away its associated endogenous proteins using stringent buffer conditions and then refold it to its hormone binding state with reticulocyte lysate (RL) and ATP regeneration system. The assay readout is the binding of 3H labeled-progesterone by PR. This in vitro reconstitution model very well mimics the physiological chaperoning of PR and thus offers a reliable tool to study the functional dynamics of Hsp90 chaperoning machinery. We successfully transformed PR chaperoning assay from Sepharose resin bead-based microtube assay to a 96 well plate system. This allowed increasing the throughput of the assay. The assay has a strong Z factor value (0.72) and 11% overall standard deviation which highlight the robustness of the assay. Partial automation using the robotic program specifically designed for this assay showed good reproduction of the data obtained during manual experiments. As proof of principal, we screened a small NIH library. We obtained few ‘hits’ that are further tested in a secondary assay, which utilizes 5 purified molecular chaperones (Hsp90, Hsp70, HOP, Hsp40 and p23) instead of RL system. The assay thus offers a powerful tool that generates highly reproducible data, which makes it suitable to screen larger and more diverse chemical libraries.

Poster #14

Importance of HSF1 in Ovarian Cancer Invasiveness

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HSF1 is frequently overexpressed in a variety of cancers and is vital to cellular proliferation and invasion. Upon analysis of mRNA expression data from The Cancer Gene Atlas, we found that HSF1 and many of its associated heat shock proteins are overexpressed in ovarian tumor samples; however, the effect of their increased levels on ovarian cancer development or progression is not known. Since the presence of HSF1 has been shown to be key in the growth and invasion of hepatocellular and breast cancer, it is reasonable to think that it is important in the growth and invasion of ovarian cancer as well. In order to determine the role of HSF1 in ovarian cancer, HSF1 knockdown cell lines were created by utilizing small hairpin RNA. The lines used were ovarian cancer-derived HEY and SKOV3 in addition to normal immortalized ovarian epithelia T80 cells. Changes in cell migration were determined using a wound healing assay and changes in invasion were measured by monitoring cell movement through a matrigel basement membrane matrix. Results show that ovarian cancer cell lines are more dependent on HSF1 to maintain migration and invasion than the normal epithelial derived cell line. Further investigation of the means by which HSF1 promotes invasion in ovarian cancer
will elucidate new mechanisms that contribute to cancer metastasis in ovarian cancer and other cancers with high HSF1 levels or activity.

**Poster #15**

**Biochemical and Functional Analysis of the Interaction Between Human Hsp70s and BAG Domain Co-Chaperones**

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Heat shock protein 70 (Hsp70) and its co-chaperones play a critical role in protein quality control. The Bcl-2-associated anthanogene (BAG) family of co-chaperones is an evolutionary conserved group of nucleotide exchange factors (NEFs) that helps to regulate Hsp70. Interestingly, while all BAG proteins (BAG 1-6) interact with Hsp70 through their highly conserved Bag domains, it is the additional regions of the BAGs that appear to dictate the specific activities of the Hsp70-BAG complexes. For example, BAG2 is associated with proteasomal degradation of Hsp70 substrates, while BAG1-Hsp70 is linked to reduced degradation and substrate refolding. These observations suggest that the formation of specific complexes will help dictate the fate of bound substrates and, moreover, that this decision could be influenced using chemical compounds that alter BAG binding. In this project, we wanted to characterize the various human Hsp70-BAG complexes using a suite of biochemical, biophysical and structural methods. Towards that goal, we have measured the affinities of Hsp70 for BAG1-3 using multiple platforms and found that they bind with mid- to high nanomolar dissociation constants. We have also measured the activity of these complexes in ATPase and *in vitro* refolding assays. These findings are revealing the specific characteristics of the various Hsp70-BAG complexes. The ultimate goal of these studies is to develop chemical inhibitors that could be used to better understand the biological roles for NEFs in cells and animals. Towards that goal, we have explored two emerging screening platforms, flow cytometry and capillary electrophoresis, which are well suited to measuring protein-protein interactions. Together, these studies are providing insight into the biochemical properties of the Hsp70-BAG complexes and they may establish the groundwork for new chemical screens.

**Poster #16**

**Heat Shock and Caloric Restriction have a Synergistic Effect on the Heat Shock Response in a sir2.1-Dependent Manner in Caenorhabditis elegans**

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The beneficial effects of caloric restriction (CR) and the importance of maintaining protein homeostasis via the heat shock response (HSR) are becoming increasingly clear.
While several studies have used *C. elegans* to link CR to the HSR, heat shock (HS) and CR have yet to be evaluated in unison. The induction of the HSR was visualized using an hsp70 promoter-GFP reporter strain (C12C8.1::GFP), which fluoresces upon HS. We show here that HS and CR have a synergistic effect on inducing GFP expression in this reporter animal. In order to measure this effect on endogenous gene expression, the mRNA levels of hsp70 family members were quantified with Q-PCR. We found a striking synergistic induction of gene expression for hsp70 family members upon HS and CR conditions. In addition, we have analyzed the cytoprotective effect that induction of the HSR has on the ability of *C. elegans* to survive a lethal heat stress and have shown that HS and CR synergize to promote increased survivability and fitness. Due to the fact that SIRT1, the *C. elegans* sir2.1 homolog, has been shown to impact both the HSR and CR in mammals, we investigated whether sir2.1 is required for the synergy between HS and CR and we found that it is. Furthermore, we found this effect is specific to sir2.1 and not other sirtuins. Finally, our findings show that CR and HS have a synergistic effect in the preservation of movement in a polyglutamine toxicity neurodegenerative disease model.

*Poster #17*

**Defining the protein quality control mechanisms of the nucleus**

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A barrage of internal and environmental stresses continuously challenges the native conformations of proteins, and cells employ a network of dedicated quality control pathways to alleviate protein misfolding and aggregation. The consequence of diminished or misregulated quality control is manifested in a variety of human maladies, including many neurodegenerative disorders, type II diabetes, and cancer. A notable pathological feature of some of these disorders is the accumulation of protein aggregates in the nucleus. Distinct compartments of the cell - the cytoplasm, endoplasmic reticulum, and mitochondria - utilize different pathways to govern protein homeostasis, and many of the responsible genes have been elucidated; yet, surprisingly little is known about what mediates protein QC in the nucleus. The goal of this project is to 1) Define the parameters of nuclear protein misfolding and aggregation at the cellular and organismal level, and 2) Identify the network of proteins that modulate the nuclear UPR. We are currently developing *in vivo* nuclear protein misfolding models in *S. pombe, C. elegans,* and mammalian cells to characterize misfolding, aggregation, and toxicity in this compartment. Ultimately, these models will be used to conduct proteomic and genetic screens to identify what modulates the fidelity of the nuclear proteome.

*Poster #18*

**Spergualin analogs as inhibitors of Hsp70-CHIP protein-protein interactions**
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The molecular chaperone, heat shock protein 70 (Hsp70), is an essential regulator of protein homeostasis that has roles in both primary folding and clearance of misfolded proteins. Thus, Hsp70 is thought to make triage decisions and direct the fate of its protein substrates. Several studies have suggested that it is the interaction between Hsp70 and a network of co-chaperone proteins that determines how its substrates are processed. For example, binding of Hsp70 to the E3 ubiquitin ligase, CHIP, is thought to guide Hsp70-bound substrates to the proteasome. However, the mechanism governing this fate decision remains unresolved. We have recently discovered that the natural product, Spergualin, directly antagonizes the protein-protein interaction between Hsp70 and CHIP. To improve the potency and stability of this promising chemical scaffold, we developed a new combinatorial synthetic route that facilitates access to spergualin analogs. These efforts have yielded a more stable analog that potently inhibits the Hsp70-CHIP interaction. We anticipate that these inhibitors will be useful for probing the roles of the Hsp70-CHIP complex in directing protein triage in vitro and in vivo.

Poster #19

Molecular Chaperones DnaK and DnaJ Share Predicted Binding Sites on Most Proteins in the E. coli Proteome

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In Escherichia coli, the molecular chaperones DnaK and DnaJ cooperate to assist the folding of polypeptides. These chaperones each bind to hydrophobic motifs in these proteins, while full pro-folding activity also demands that DnaK and DnaJ interact with each other. This system is thought to be sufficiently versatile to act on the entire proteome, which creates interesting challenges in understanding the large-scale, ternary interactions between DnaK, DnaJ and their thousands of potential substrates. To address this question, we computationally predicted the number and frequency of DnaK- and DnaJ-binding motifs in the E. coli proteome. This analysis revealed that nearly every protein is predicted to contain multiple DnaK- and DnaJ-binding sites, with the DnaJ sites occurring approximately twice as often. Further, we found that an overwhelming majority of the DnaK sites partially or completely overlapped with the DnaJ-binding motifs. These observations suggested that the relative stoichiometry of DnaK and DnaJ might partly determine whether they either bind synergistically or competitively. To test this idea, we measured the chaperone-assisted folding of two denatured substrates and found that the distribution of predicted DnaK- and DnaJ-binding sites was indeed a good indicator of the optimal stoichiometry required. These studies provide insight into how DnaK and DnaJ might cooperate to maintain global protein homeostasis.
**Poster #20**

**Cap structures reduce β-helix aggregation propensity**

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Examination of β-sheets in protein crystal structures has revealed that edge β-strands are protected from solvent by a variety of mechanisms. Protection of unpaired hydrogen bond donors and acceptors in β-sheet edge strands could prevent intermolecular associations and therefore reduce aggregation of these proteins. We investigated the role of β-sheet capping mechanisms in the folding and aggregation properties of pertactin, an autotransporter protein from *Bordetella pertussis*. Pertactin, a right-handed β-helix protein, folds extremely slowly *in vitro*, yet is surprisingly resistant to aggregation. Examination of the pertactin crystal structure suggests two different strategies might prevent multimerization. The C-terminus has irregular β-helical structure, avoiding solvent exposure of both the hydrophobic core of the β-helix and its edge strands. The N-terminal rung of the β-helix contains three charged residues. Removal of the C-terminal cap or mutation of two of the three N-terminal charged residues led to increased aggregation and formation of soluble oligomeric fibers as observed by size exclusion chromatography and transmission electron microscopy. We are creating a construct that lacks both the C- and N-terminal caps, to examine whether these mutations will lead to even greater aggregation and fiber formation. Results from these studies will allow us to determine to what extent pertactin, and by extension other β-helical proteins, employ capping mechanisms to reduce/prevent aggregation.

**Poster #21**

**A NETWORK OF UBIQUITIN LIGASES FUNCTION IN THE QUALITY CONTROL OF MISFOLDED CYTOSOLIC PROTEINS**

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Protein homeostasis is maintained by quality control pathways that involve molecular chaperones and ubiquitin ligases. Molecular chaperones promote folding of newly synthesized or misfolded proteins, while ubiquitin ligases promote degradation of misfolded proteins through the proteasome. Upon loss of function of molecular chaperones, due to mutations or small molecule inhibitors, or when the capacity of the ubiquitin/proteasome system (UPS) is exceeded, then misfolded proteins accumulate in aggregates that can be cleared by autophagy. This study is focused on the balance of degradation and aggregation of a protein kinase Ste11ΔN^K444R, when Hsp90 is inhibited with geldanamycin.

The mutant kinase, Ste11ΔN^K444R was tagged with GFP in order to visualize it and to follow its fate in live cells. Upon geldanamycin treatment, Ste11ΔN^K444R–GFP was prone to aggregation, with one or more aggregates forming outside the nucleus of treated
cells. Aggregate formation was dose-dependent and saturation occurred at 15 μM geldanamycin. In ubr1Δ cells, there was a two-fold increase in the number of aggregates compared to the wild type. Deletion of the UBR2 ubiquitin ligase not only did not increase the numbers of aggregates formed in comparison to wild type, but also suppressed the effect of deleting UBR1. We found that the suppression phenotype resulted from increased proteasome activity in ubr2Δ cells, which correlates with stabilized Rpn4, a transcription factor for proteasome subunits. Besides UBR1, protection against aggregate formation was also conferred by other E3 ligases, such as LTN1, UFD4, DOA10 and HUL5. Double mutants between these E3s and UBR1 displayed hypersensitivity to azetidine 2-carboxylic acid, a protein folding poison, suggesting a functional redundancy of their pathways. We also provide evidence that Ubr1 is involved in the clearance of aggregates, especially when the autophagic system is compromised. In conclusion, we demonstrate the existence of a network of E3 ligases that protect the cells against accumulation of protein aggregates and we show that UBR2 has a distinct role, due to its capacity to regulate Rpn4 stability.

Poster #22

Specific Chaperones and Proteases Control the Degradation of the Epithelial Sodium Channel

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The epithelial sodium channel (ENaC) is composed of 3 subunits, α, β, and γ, and helps regulate serum sodium concentration and osmotic homeostasis in the kidney. Mutations in ENaC that alter cell surface expression lead to diseases, such as Liddle’s syndrome and pseudohypoaldosteronism. ENaC levels are tightly controlled, and several mechanisms exist by which ENaC can be degraded, including endoplasmic reticulum-associated degradation (ERAD). During ERAD, molecular chaperones target misfolded proteins to the cytosolic proteasome where they are destroyed. We have found that ENaC is an ERAD substrate when it is constitutively expressed in the yeast, Saccharomyces cerevisiae. However, significant differences in degradation efficiency are seen between each subunit. In addition, when yeast lack the ER lumenal Hsp40s, Jem1 and Scj1, ENaC is stabilized. Surprisingly, ENaC degradation is Hsp70 (BiP) independent. To avoid potential toxicity due to constitutive expression and a possible adaptative response, a methionine repressible ENaC expression system was established. Cells with aberrant degradation pathways transformed with a methionine repressible ENaC expression construct were tested in cycloheximide chase experiments. Our results confirm that ENaC degradation is at least in part proteasome dependent, and we again observed differences in degradation between the subunits. Notably, a significant percentage of the degradation of the α subunit and γ subunit was proteasome independent, suggesting that these subunits are degraded by another mechanism. When cells lacked a functional vacuole, degradation was again proficient. Experiments in which cells lacked the cytosolic protease, calpain, indicate that this protease is also dispensable for each subunit’s degradation. Current experiments are investigating what accounts for the
distinct degradation requirements of each subunit, and ultimately we hope to better understand how ENaC is degraded and therefore how its function is modulated.

Poster #23

Cell-non-autonomous regulation of organismal proteostasis

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Cellular proteostasis in all cells and tissues of an organism is achieved by a repertoire of molecular chaperones that function to coordinate and protect against the deleterious consequences of physiological and environmental stress. HSP90 is a core component of the cytoplasmic proteostasis network that orchestrates folding and activities of diverse client proteins, while also being a potent regulator of the cellular heat shock response (HSR). Recent studies showed that in metazoans, the HSR is controlled by thermosensory neuronal circuitry, suggesting that organismal proteostasis is designed to prevent an imbalance of chaperone levels during stress. Here we show that whereas overexpression of HSP90 in intestine, muscle cells, and neurons is well tolerated during normal development, it severely compromises organismal fitness during both acute and chronic stress conditions. HSP90 acts epistatic to the gcy-8 regulated neuronal circuitry, through both neuronal and non-neuronal tissues, to control heat shock protein expression in all cells of the animal. This cell-non-autonomous negative regulation of the HSR by HSP90 is due to decreased DNA-binding of transcriptionally active HSF-1 homotrimers throughout the organism. Thus, a key aspect of metazoan stress responses is the necessity to maintain balanced chaperone expression across all tissues to coordinate organismal protection against proteotoxic damage. Cell-non-autonomous mechanisms that allow this level of tissue-to-tissue communication as a result of localized chaperone imbalance will be discussed.

Poster #24

Tau regulation by Hsp90 and FKBP51

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The microtubule associated protein tau is involved in a class of neurodegenerative diseases termed tauopathies, in which aggregated hyperphosphorylated tau is believed to play a major role in the progression of the diseases. Alzheimer’s disease (AD) is the
most prevalent tauopathy, and molecular chaperones have been shown to play a large role in the progression of the disease. Pin1 is a peptidyl-prolyl isomerase (PPIase) that alters the conformation of phosphorylated tau to promote dephosphorylation and has been shown to have a role in the progression of AD. We have previously shown FK506 binding protein-51 (FKBP51), which binds heat shock protein 90 (Hsp90) through a tetratricopeptide repeat (TPR) domain and also possesses a peptidyl-prolyl isomerase domain, is involved in regulating tau phosphorylation and stability in cells. However, little is known about how FKBP51 coordinates with Hsp90 to regulate tau. We therefore examined this role and how disruption of the complex affects tau biology. We found structural changes as a result of tau and FKBP51 interactions. In examination of how Hsp90 affects tau levels in conjunction with FKBP51, we found that FKBP51 reduction alone reduces tau levels, but reduction of FKBP51 and Hsp90 simultaneously preserves tau levels. This indicates Hsp90 is involved in the FKBP51-mediated turnover of tau. Finally, we found that FKBP51 on its own cannot influence tau aggregation in vitro. However, FKBP51 attenuated the ability of Hsp90 to modify tau aggregation in vitro. Further investigation into the interactions of FKBP51, tau, and Hsp90 should provide more specific targets for the treatment of tauopathies and Alzheimer’s disease, while minimizing effects on neurons that would occur through targeting Hsp90 directly.

Poster #25

Enhanced Toxicity of the Protein Cross-Linkers Divinyl Sulfone and Diethyl Acetylenedicarboxylate in Comparison to Related Monofunctional Electrophiles

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Protein alkylating agents have been used in biochemical and cellular research to study protein function and alter key biological processes. Protein cross-linkers make up a class of bifunctional alkylating agents that are commonly employed to stabilize and identify transient protein-protein interactions. Previously, we determined that diethyl acetylenedicarboxylate (DAD), a protein cross-linker, had significantly greater cellular toxicity when compared to structurally related alkylating agents that are incapable of cross-linking proteins (e.g., diethylmaleate (DEM)). We hypothesized that other molecules capable of causing protein cross-linking would exhibit a similar enhancement in their toxicities. We compared the toxicities of divinyl sulfone, a known cross-linking agent, to ethyl vinyl sulfone (EVSF), its monofunctional counterpart in human colorectal carcinoma (RKO) cells and Saccharomyces cerevisiae. In RKO cells, DVSF was approximately six times more potent than EVSF, and similar trends were observed in yeast. Both DVSF and DAD caused intermolecular cross-linking of the redox-active protein thioredoxin 2 (Trx2p) from S. cerevisiae in vitro, and cytotoxic concentrations of DVSF and DAD promoted the cross-linking of Trx2p to other cellular proteins in treated yeast. Our results suggest that cross-linking of proteins intracellularly is considerably more detrimental to cellular homeostasis than is simple protein alkylation.
DEFECTIVE TRANSLATIONAL CONTROL IN RESPONSE TO PROTEASOME INHIBITION IN HUMAN PANCREATIC CANCER CELLS

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Protein synthesis in mammalian cells is tightly regulated in response to a variety of stresses and plays an essential role in proteostasis. The hub of this translational control network is the eukaryotic initiation factor 2 (eIF2), which upon phosphorylation of the α-subunit at the S51 residue effectively attenuates cap-dependent protein synthesis. Proteasome inhibitors (PIs), a class of anti-neoplastic agents, are known to cause eIF2α phosphorylation, but conflicting studies implicate this phosphorylation in cell survival or death. We have found that human pancreatic cancer cells display heterogeneity in both basal and PI-induced eIF2α-phosphorylation. Importantly, this heterogeneity closely paralleled sensitivity to PI-induced cell death, in that sensitive cells failed to significantly increase eIF2α phosphorylation while resistant cells showed a robust increase in phosphorylation. Correspondingly, sensitive cells failed to down-regulate protein synthesis as measured by 3H-leucine incorporation. This ineffective translation attenuation lead to a toxic accumulation of ubiquitylated misfolded proteins, evident by anti-ubiquitin immunofluorescence microscopy and immunoblotting of detergent-insoluble fractions. Much of the current literature equates proteasome inhibition with ER stress, so we hypothesized that perhaps the differences in translational control lay at the level of the unfolded protein response (UPR). However, across both sensitive and resistant cells, we show that when compared with the classic ER-stressor thapsigargin, the proteasome inhibitor bortezomib induced minimal changes in Grp78 and CHOP mRNA levels, whereas Hsp70 (HSPA1A) mRNA levels rose dramatically. This suggests that cytosolic stress is a more significant consequence of proteasome inhibition in our cancer cell models. Overall, our study suggests that defects in translational control contribute to the heterogeneous cytotoxic effects of PIs in human pancreatic cancer cells and that inhibitors of cytosolic stress response pathways could be attractive within the context of combination therapy.

Poster #27

Drosophila melanogaster torsin loss-of-function model

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AAA+ ATPases (ATPases associated with various activities) are protein machines that use energy from ATP to induce conformational changes in macromolecules. Torsins are a sub-group of AAA+ ATPases found only in animal proteomes, which suggests that their function is essential for animal physiology. Torsins are the only known AAA+ ATPases targeted to the secretory pathway. The biological function of torsins is unknown, their
substrates or partners have not been fully identified, and the conformational rearrangements induced by torsins in other macromolecules have not been characterized. In humans, a mutation in the torsinA gene is linked to early-onset dystonia, a debilitating neurological disease. There are four torsin genes in each mammalian genome, but only a single gene in insects. We generated transgenic Drosophila that express torsin-targeted dsRNA. Ubiquitous expression of torsin dsRNA in adult flies using an Actin/Gene Switch promoter system resulted in efficient suppression of torsin mRNA in male, but not female flies with no apparent lethality. In contrast, the expression of torsin dsRNA in earlier developmental stages reduced the survival of flies by up to 40% at the pre-pupal stage. We also used dsRNA to suppress the torsin mRNA levels in Drosophila S2 cells. Through the differential microarray analysis of mRNA isolated from the torsin knockdown vs. control samples, we found 21 genes that were up- or down-regulated in the torsin knockdown larvae and 14 up- or down-regulated genes in S2 cells. Interestingly, the products of 9 up-regulated genes in the torsin knockdown larvae are structural constituents of the insect cuticle. Moreover, we identified three heat-shock proteins (Hsp67Bc, Hsp68, Hsp26) that were up-regulated in S2 cells upon the torsin knockdown. Our results suggest that the loss of torsin might induce stress-response in insects. Further characterization of the torsin loss-of-function models will allow us to explore the role of torsin in insect development.

Poster #28

The Role of YBR074 in Yeast Cell Wall Integrity: Genetic Interactions with the AAA+-ATPase, CDC48

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In the eukaryotic cell, one third of all proteins are targeted to the endoplasmic reticulum (ER) to fold and mature before they continue through the secretory pathway. However, under conditions of stress, or if these proteins harbor mutations, they may misfold and become targeted for degradation by the cytoplasmic proteasome. This requires the misfolded substrates to be retrotranslocated out of the ER, and this process is known as ER Associated Degradation, or ERAD. Sequestering the protease in the cytosol helps protect proteins in the ER that are on the correct folding pathway. Surprisingly, the yeast protease Ybr074 is predicted to have an ER luminal catalytic domain. This uncharacterized transmembrane protease also has a mammalian homolog, Fxna, which has been shown to function in ovarian development. By using yeast as a model system, we found that YBR074 interacts with CDC48 under conditions of cell wall stress. Cdc48 is a AAA+ ATPase involved in ERAD as well as in cell wall maintenance. Based on these data, we hypothesized that Ybr074 modulates the cell wall integrity pathway in parallel with CDC48. To test this hypothesis, YBR074 and CDC48 mutant strains were examined for chemical-genetic interactions under conditions of cell wall stress. In order to distinguish which of the many functions of Cdc48 are involved in modulating cell wall stress, we also analyzed various mutant alleles of CDC48. Some of the mutant alleles confer temperature sensitivity and inhibit ERAD whereas others are synonymous with mutations in a muscular degenerative disease known as Inclusion Body Myopathy associated with Paget’s Disease (IBMPFD). Thus far, we have identified differential
genetic interactions between YBR074 and various alleles of CDC48. Current work is focused on examining the composition and integrity of the cell wall in these strains to define the role of Ybr074 in cell wall integrity, an effort that may suggest why Fxna is required for ovarian development. Because most anti-fungal agents target the cell wall, our results may also be important for developing new methods to combat fungal pathogens associated with human disease.

Poster #29

**Protein-disaggregating activity of the AAA+ ATPase ClpB from Ehrlichia chaffeensis**

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ClpB, a member of the AAA+ family of ATPases associated with various activities reactivates aggregated proteins in cooperation with the co-chaperones DnaK/DnaJ/GrpE. It has been established that ClpB is upregulated during heat shock and is essential for survival of bacteria, yeast, and plants under severe stress conditions. Evidence has been also accumulating for the essential role of ClpB in the infectivity and virulence of a number of pathogenic microorganisms. The biological function of ClpB in pathogens during infection of host cells is currently unknown, but the lack of ClpB in animal cells makes it an attractive target for novel anti-microbial therapies. We performed a biochemical characterization of ClpB from *Ehrlichia chaffeensis*, a tick-transmitted intracellular pathogen causing human monocytotropic ehrlichiosis. The ClpB transcript is strongly upregulated within hours after infection of mammalian cells by *Ehrlichia*. We purified the recombinant ClpB from *E. chaffeensis* (EhClpB) and compared its biochemical properties with those of *E. coli* ClpB (EcClpB). We found that EhClpB forms ATP-dependent oligomers and catalyzes the hydrolysis of ATP, similar to EcClpB. However, while the basal ATPase activity of EhClpB is twice that of EcClpB, the ATPase of EhClpB fails to respond to the activators: casein and poly-lysine. EhClpB together with DnaK/DnaJ/GrpE from *E. coli* efficiently reactivates aggregated glucose-6-phosphate dehydrogenase and firefly luciferase. Interestingly, unlike EcClpB, which forms stable complexes with substrates in the presence of the non-hydrolysable ATPγS, EhClpB binds to the aggregates in the presence of the hydrolysable ATP. Altogether, our results demonstrate that ClpB from *E. chaffeensis* displays a protein disaggregase activity in vitro, but the mechanism of its interactions with substrates may be different from that of *E. coli* ClpB. This study sets the stage for assessing whether the disaggregase activity of ClpB is essential for survival of *Ehrlichia* inside the infected mammalian and tick hosts.