To better understand the acid-induced activation of HdeA, we sought to identify which of HdeA's acid-titratable residues were the key players in sensing environmental pH changes, and which residues' protonation would trigger HdeA's monomerization and unfolding.
Using protein sequence alignments, constant pH molecular dynamics calculations and predictions of pKa values, we identified several residues that are likely involved in maintaining the inactive dimer conformation at neutral pH and causing the unfolding and monomerization events upon shift to low pH. We substituted these residues with alanines and examined their dimer stabilities at neutral pH, their pH midpoints of monomer-dimer transitions and unfolding, and their pH-dependent activities. We identified several HdeA variants that are activated at higher pH values compared to the wild-type protein and are significantly destabilized already at neutral pH. By combining two mutations, we were further able to generate an HdeA variant that shows chaperone activity at neutral pH (where wild-type HdeA is completely inactive), making it a constitutively active variant of a normally acid-activated chaperone.
These mutants will help us to understand on a structural level which regions of HdeA need to be flexible or unstable for the protein to function, and will help to determine how pH-driven changes in HdeA flexibility drive its activation.

2932-Pos Board B87
Development of Fluorescence assays for Studying Protein Disaggregation by Molecular Chaperones
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The concentrated and complex interior of a cell presents a difficult challenge to folding of many essential proteins. Partially structured folding intermediates, populated during biosynthesis or upon environmental stress, are prone to assemble into large, non-functional aggregates. A network of specialized molecular chaperones evolved to deal with this problem. How aggregate disassembly is accomplished, its impact on disease progression, and how disaggregation is coupled to productive folding is not well understood. We have therefore initiated an effort to establish the enzyme RuBisCo as a model substrate for studying GroEL-dependent protein folding. We have engineered the sequence of RuBisCo so that exogenous fluorescent probes can be coupled to designed surface cysteine residues in a highly specific manner. We are applying fluorescence based techniques to the study of RuBisCO aggregate formation and disassembly by the DnaK-ClpB bi-chaperone system. Our preliminary studies with acid and urea denatured RuBisCo have shown that this protein populates at least two general aggregate assembly pathways, distinguishable by their distinctly different types of aggregate growth. We have also found that the DnaK system (DnaK/DnaJ/GrpE) is capable of altering the progression of RuBisCo aggregation on its own, without either fully arresting aggregation or fully disassembling RuBisCo aggregates. using both light scattering and fluorescence resonance energy transfer (FRET), we find that RuBisCo aggregates are good substrates for the full DnaK-ClpB system.

2933-Pos Board B88
Analysis of Myosin Motor Domain Interactions with its Chaperone UNC-45 at the Single Molecule Level
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Myosins are actin-based motor proteins that convert chemical energy from ATP hydrolysis into mechanical work. Movement of myosin heads along actin filaments is a result of structurally complex conformational changes in the myosin motor domain induced by ATP binding and hydrolysis. UNC-45, a member of the UCS family of proteins, acts as a chaperone for myosin and is essential for proper folding and assembly of myosin into muscle thick filaments *in vivo*. The molecular mechanism of the myosin-UNC-45 interaction in the promoting proper folding of the myosin head domain is not known. We have devised a novel approach to elucidate the interaction of the UNC-45 chaperone with the myosin motor domain utilizing single molecule atomic force microscopy (AFM). By chemically coupling a titin I27 polyprotein to the motor domain of myosin we synthesized a chimera protein that possesses the property of a ''molecular reporter''. Our new construct provides a specific attachment point and the well-characterized mechanical fingerprint of the titin octamer in the AFM measurements. Refolding experiments of the chimeric S1-I27 molecules showed that the myosin motor domain interfered with the refolding of otherwise robust I27 modules, presumably by recruiting them into a misfolded state. The presence of UNC-45 restored the folding of the titin I27 domains. We identify the canonical UCS domain of UNC-45 as the essential component of chaperone like activity. This approach enables the study the myosin-UNC-45 interactions at a single molecule level and their consequences for motor domain folding and misfolding in mechanistic detail.

2934-Pos Board B89
Interaction of RNase H^D and Sh3 Proteins with DnaK Molecular Chaperone
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Most proteins have DnaK binding sites. DnaK is an *E. coli* Hsp70 molecular chaperone which helps prevent protein aggregation by assisting co- and post-translational protein folding. How extensively and by what mechanism does DnaK interact with the proteins? We know very little about this important question. We used RNase H^D and SH3 as model protein substrates to study how DnaK (and its co-chaperones DnaJ and GrpE) interacts with nonobligatory clients (i.e. proteins capable of folding even without the assistance of chaperones) and to provide insights into mechanism of action of DnaK. Stopped-flow circular dichroism, size-exclusion chromatography and enzyme activity assays provide evidence for kinetic retardation of folding due to DnaK-substrate complex formation. Furthermore, multidimensional NMR and photo-CIDNP (photochemically induced dynamic nuclear polarization) provide atomic level details regarding DnaK-substrate interactions. Overall, a combination of various experimental techniques provides insights into how the DnaK chaperone assists protein folding within the cellular environment.

2935-Pos Board B90
Potentiated Hsp104 Variants Antagonize Diverse Protein Misfolding Events
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Aberrant protein folding is implicated in several devastating neurodegenerative diseases. Inclusions containing the proteins TDP-43 and FUS are implicated in some cases of amyotrophic lateral sclerosis (ALS), while amyloid fibers comprised of α-synuclein are implicated in Parkinson's disease. Hsp104, an AAA+ protein from yeast, functions in regulating the disassembly of amorphous aggregates as well as prions. There are no other proteins known that are capable of specifically disassembling and solubilizing amyloid. Though Hsp104 is highly conserved, it has no human homologue. Therefore, we have developed potentiated Hsp104 variants and applied them to disease models of TDP-43, FUS, and α-synuclein pathology. These potentiated Hsp104 variants dissolve the aggregates, return the proteins to their proper cellular location, and strongly suppress toxicity in each of these disease models at levels far greater than wild-type. Surprisingly, we have also found that at certain positions in Hsp104, generic mutations to nearly any class of amino acid yield a hyperactive protein capable of eliminating aggregates. using pure protein biochemistry experiments, we have probed the biochemical basis for these variants' potentiated activity and found that they have an enhanced ATPase and translocation rate, and are capable of dissolving aggregates without requiring co-chaperone collaboration. These results reveal important new insights into the mechanism by which Hsp104 dissolves amyloid, and demonstrate that proteins that misfold in neurodegenerative disease can be reactivated to their native state.

2936-Pos Board B91
Potentiated Hsp104 Variants Antagonize Diverse Proteotoxic Misfolding Events
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Aberrant protein folding is implicated in several devastating neurodegenerative diseases. Intractable inclusions containing the proteins TDP-43 and FUS are implicated in some cases of amyotrophic lateral sclerosis, while amyloid fibers comprised of α-synuclein are implicated in Parkinson's disease. Hsp104, an AAA+ protein from yeast, functions in regulating the disassembly of amorphous aggregates as well as prions. There are no other proteins known that are capable of specifically disassembling and solubilizing amyloid. Though Hsp104 is highly conserved, it has no human homologue. Therefore, we have developed potentiated Hsp104 variants and applied them to disease models of TDP-43, FUS, and α-synuclein pathology. These potentiated Hsp104 variants dissolve the aggregates, return the proteins to their proper cellular location, and strongly suppress toxicity in each of these disease models at levels far greater than wild-type. Surprisingly, we have also found that at certain positions in Hsp104, generic mutations to nearly any class of amino acid yield a hyperactive protein capable of eliminating aggregates. These ''gatekeeper'' residues reveal important new insights into the mechanism by which Hsp104 dissolves amyloid.