mutation can switch one protein fold to multiple disparate conformations. Furthermore, cross-pair sequence identity levels are consistent with identity cut-offs determined from the PDB: pairwise sequence alignments of all G_A variants are >40% identical, while pairwise sequence alignments of the three alternative folds are <30% identical. Together, these results demonstrate that stepwise mutation can induce one parent fold to spawn multiple new folds in a way that is consistent with observations of experimentally determined protein structures.

1021-Plat
Resolving Conformational Switching of AAA+ Protease FtsH using Single-Molecule FRET
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FtsH is a homohexameric AAA+ protease embedded in the prokaryote membrane, where it recognizes, unfolds, translocates and lyses protein substrates to be degraded and is essential for cell viability. Structural data of Thermotoga maritima FtsH in an ADP and ATP-bound state showed a large conformational change between both nucleotide conditions. Current mechanistic models inferred from the structural data assume a coupled conformational change with ATP consumption and, by extension, protein unfolding. Interestingly, bulk assays indicated ATP-independent proteolytic activity. Here, we present single-molecule FRET data reporting in real-time conformational changes in FtsH. Single FtsH monomers were labeled with donor and acceptor fluorophores and co-encapsulated with unlabeled monomers in unilamellar vesicles. These vesicles were surface immobilized, such that single FtsH hexamer activity could be observed over tens of seconds. We present data on the cooperativity of the six subunits during conformational changes in the absence and presence of degradation substrates. Interestingly, conformational changes occurred in the presence and absence of ATP indicating a Brownian ratchet mechanism for FtsH. Point mutations including a proteolysis deficient variant and a single point mutant found in a human homolog paraplegin causing spastic paraplegia were studied.

1022-Plat
Anti-Prion Ligand Binding Promotes Native PrP Folding Over Misfolding at the Single Molecule Level
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Ligands that bind to the prion protein, PrP, and prevent the spread of the diseased state have been discovered, but their mechanism of action remains uncertain. Determining anti-prion mechanisms may provide insight into the still-unknown means by which native PrP is converted to the infectious form. To explore the effects of an anti-prion ligand at the molecular level, we used force spectroscopy to study how a tetrapyrrole known to have anti-prion activity, iron(III)meso-tetra(N-methyl-4-pyridyl-prophine) or Fe-TMPyP, alters the folding behavior of individual PrP molecules. Single PrP molecules were unfolded using optical tweezers in the presence and absence of Fe-TMPyP. Ligand binding to the native structure was found to significantly increase its unfolding force. Not only did Fe-TMPyP binding stabilize the native state as expected from ensemble binding studies, but analysis of the unfolding force distributions revealed that Fe-TMPyP binding altered the nature of the transition state for unfolding the native structure: the energy barrier moved closer to the native state, making the transition state more compact, and the barrier height increased. Unexpectedly, Fe-TMPyP was also able to bind to PrP when it was unfolded or only partially folded, thereby delaying the normally rapid refolding into the native state. Probing the effects of Fe-TMPyP on inter-molecular interactions by measuring PrP dimers revealed that ligand binding promoted the formation of the native structure in individual monomers, preventing the formation of a thermodynamically-stable misfolded dimeric state. The ligand thus promotes native folding by stabilizing the native state while at the same time suppressing interactions that drive the formation of stable aggregates. These results suggest parallels between pharmacological chaperones like Fe-TMPyP and cellular chaperones.

1023-Plat
What Computational Methods can Teach us about the Alzheimer-Protective Nature of A2V- and A2T-Mutant Amyloid-Beta Oligomers
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Unlike the large set of risk genetic mutations causing the familial form of Alzheimer's disease (AD), recent mutations in the Amyloid-β (Aβ) protein have been discovered to confer protection against AD.
The A2V mutation is such a protective mutation in its recessive form only (i.e. mixed with wild-type (WT) Aβ) by preventing the Aβ-WT peptide from forming fibrils and toxic aggregates while, surprisingly, the A2V mutation in its dominant form (pure Aβ-A2V) becomes more potent than Aβ-WT and displays an increased toxicity and self-aggregation propensity [1]. Accounts of another protective mutant, A2T, (in both its recessive and dominant form) have also been reported [2]. While these two mutants reduce the production of Aβ delivered in the brain, this is not enough to clearly explain their beneficial effects against AD.
We aim here to understand the impact of these mutations at a molecular level via all-atom molecular dynamics simulations to follow up on the promising results previously obtained computationally for the Aβ(1-28)-A2V monomer, providing a first look at the mechanism behind this protective mutation [3]. Now studying four dimer systems (WT-WT, WT-A2V, A2V-A2V and WT-A2T) and two tetramer systems (WT-WT and WT-A2T), we are able to explain, via extensive conformational searches and detailed free-energy calculations, how a single-point mutation at the position 2 of Aβ is sufficient to induce protective or potent properties compared to the Aβ-WT. These first computational results on the A2V and A2T mutations demonstrate that their protective (or potent) effects are encoded already at the dimer and tetramer level.
[1] Di Fede G et al. (2009). Science, 323(5920), 1473-1477.
[2] Peacock ML et al. (1993). Neurology, 43:1254.
[3] Nguyen,PH, Tarus, B and Derrreumaux, P. (2014). J Phys Chem B, 118:501-510

1024-Plat
NMR Structure Reveals Novel Interactions between Intrinsically Disordered PEP-19 and Calmodulin
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Background: PEP-19 has no known intrinsic activity other than binding predominately to the C-domain of calmodulin (C-CaM), yet it is implicated in numerous cellular processes. We showed that an acidic sequence in PEP-19 is required to greatly increase the rates of Ca^{2+} binding to C-CaM. Importantly, the acidic sequence is also required for PEP-19 to sensitize HeLa cells to ATP-induced Ca^{2+} release. **Goal:** The goal of the current study was to determine the high-resolution NMR solution structure of the PEP-19/apo C-CaM complex.
Results: Apo C-CaM adopts a semi-open conformation when bound to PEP-19, with the helices E and F of Ca^{2+} binding site III showing the greatest change in angle relative to free apo C-CaM. The conformation of Ca^{2+} binding loop III is similar to that in free apo C-CaM, but loop IV adopts a different conformation with increased conformational exchange when bound to PEP-19. Residues 1-29 in PEP-19 remain disordered when bound to C-CaM, and are thus accessible for potential interactions with other proteins. The IQ motif in PEP-19 adopts a well-defined alpha helix that binds to a hydrophobic groove in apo C-CaM. The C-terminal part of the acidic sequence is alpha helical, but the N-terminal portion (aa 28-36) forms loop and coil structures that are stabilized by interactions between Ile32 and Met34 in PEP-19 and hydrophobic residues in Ca^{2+} binding site III of C-CaM. This allows acid side chains in PEP-19 to extend toward the solvent to greatly increase negative charge density near site III of C-CaM.
Conclusions: The structure suggests that the acidic sequence in PEP-19 modulates Ca^{2+} binding to site III of C-CaM by direct interactions and/or electrostatic steering of Ca^{2+}, but that allosteric effects increase conformational exchange to modulate Ca^{2+} binding to site IV.

Platform: Protein-Nucleic Acid Interactions II

1025-Plat
Oligomerization Kinetics of ORF1p is Correlated with Line1 Retrotransposition
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ORF1 protein (p) is encoded by the long interspersed nuclear element-1 (LINE1) retrotransposon. LINE1 replicates by converting its transcript into genomic DNA, a mechanism that can also similarly process some host gene transcripts. LINE1 activity has thereby greatly expanded mammalian genomes