unstructured regions in proteins are known for mediating many protein-protein or protein-nucleotide interactions during regulation of transcription, translation, and cellular signal transduction. The question arises how conjugated glycans influence protein and peptide conformational dynamics and by that modify their biological activity. We compare the conformational dynamics of unstructured polypeptides consisting of eight glycine-serine repeat units with and without glycosylated serine units. We synthesized glycine-serine repeats with O-conjugated beta-galactose at every serine residue by solid-phase synthesis with glycosylated dipeptides as building blocks. Introducing an organic oxazine dye and tryptophan at either end of these peptides allows measurements of end-to-end contact kinetics. Upon van-der-Waals contact between dye and tryptophan fluorescence is quenched by photo induced electron transfer (PET). Fluorescence intensity fluctuations are analyzed using fluorescence correlation spectroscopy (FCS) and contact formation rate constants are determined. We studied influences from solvent viscosity and temperature on end-to-end contact formation rates and found a decrease of rate constants upon glycosylation. Arrhenius analysis of end-to-end contact rates yields enhanced activation energy for the glycosylated sample. The viscosity dependence of the relaxation rates shows that contact formation still is viscosity controlled. This study confirms previous reports that glycosylation has a significant influence on peptide dynamics mostly through steric hindrance.

50-Plat

Transient Tertiary Contact Formation in the CGRP Neuropeptide Revealed by Nanosecond Laser Spectroscopy

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Calcitonin gene-related peptide (CGRP) is an intrinsically disordered, 37 residue neuropeptide that acts as a potent vasodilator, which is of considerable interest in migrane research. It is a member of the calcitonin peptide (Ct) family, together with amylin, calcitonin and adrenomedullin. These are genetically and structurally related intrinsically disordered hormone peptides that are able to bind to each other's receptors, though with varying degrees of affinity. They contain highly conserved sequence elements that have been experimentally shown to affect the secondary structural preferences of these peptides. The effect of such conserved elements on tertiary structure has not been experimentally explored to the same extent. Detecting tertiary structural properties of IDPs is considerably more challenging due to fast reconfigurations of the backbone over a wide range of possible conformations. High resolution time-resolved techniques are needed. We use a nanosecond-resolved spectroscopic technique based on tryptophan triplet quenching by cystine to detect tertiary contact formation in CGRP under varying solvent and temperature conditions. Using this technique, Vaiana et al.[1] have previously shown that conserved structural elements of amylin induce compact states characterized by short end-to-end distances, and that compaction is not driven by hydrophobic side-chains. Using triplet quenching we explore the effect of these conserved elements on the conformation and dynamics of CGRP, a peptide with higher mean hydrophobicity and net charge per residue than amylin. We discuss our findings in relation to secondary structural preferences of these peptides and discuss their possible functional role.

Footnote

[1] Vaiana S.M. et al. Biophys. J. 97 2009.

51-Plat

Single Molecule Study of the Intrinsically Disordered FG-repeat Nucleoporin 153

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The megaDalton sized nuclear pore complexes (NPCs) are among the largest molecular machines in eukaryotic cells. They span the nuclear envelope and constitute the only transport conduit between nucleoplasm and cytoplasm. Multiple copies of roughly a dozen different natively unfolded proteins form a selective permeability barrier inside the NPC and take a central role in the vital nucleocytoplasmic transport mechanism. Other than reoccurring phenylalanine-glycine elements (FG-repeats), these Nucleoporins (FG-Nups) have only limited sequence similarity among each other and across species. Still, they constitute a complex and distinct non-random amino acid (AA) architecture of FG-repeat clusters and intra-FG linkers. How such heterogeneous sequence composition relates to function and could actually give rise to a transport mechanism is still unclear. Currently a better understanding is largely hampered by our limited ability to study such highly flexible and intrinsically disordered proteins/protein domains. Here we describe a combined chemical biology and single molecule fluorescence approach to study the large human Nup153 FG-domain. in order to obtain insights into the properties of this domain beyond the average behavior, we probed the end-to-end distance (R_E) of several, approximately 50 residues long FG-repeat clusters in the context of the whole protein domain. Despite the sequence heterogeneity of these FG-clusters, we detected a reoccurring and consistent compaction from a relaxed coil behavior under denaturing conditions ($R_E/R_{E,RC} = 0.99 \pm 0.15$ with $R_{E,RC}$ corresponding to ideal relaxed coil behavior) to a collapsed state under native conditions ($R_E/R_{E,RC} = 0.79 \pm 0.09$). We then analyzed the properties of this protein on the supramolecular level, and determined that this human FG-domain was in fact able to form a hydrogel with physiological permeability barrier properties, i.e. nuclear transport cargos readily partition into the gel, while inert cargos do not.

52-Plat

Electrostatics and Intrinsic Disorder: A Single-Molecule Study of the Sic1 Protein

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Intrinsically disordered proteins (IDPs) play critical yet often poorly understood roles in a variety of cellular processes. Despite an apparent antagonism between structural disorder and protein recognition, the large number of IDPs involved in protein regulation suggests that they could in fact provide advantages in recognition over well-folded proteins. Sic1 is an IDP inhibitor of a cyclin dependent kinase (CDK) in yeast, which interacts with a single site on its acceptor Cdc4 only upon phosphorylation of its multiple dispersed CDK sites. The multiple phosphorylation events can in principle be the basis for ultrasensitivity in protein-protein binding, however its molecular basis remains elusive[1].

We performed a systematic study of the Sic1's fluctuating conformations in different salt and denaturant concentrations using single-molecule Förster energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS). smFRET data suggests that Sic1 protein is comprised of a continuum of conformers with varying end-end distances. Denaturant titration measurements suggest that these conformers are characterized by non-cooperative interactions. From FCS experiments, the exchange between Sic1 conformational states was found to occur on both ultrafast (10-100 ns timescale) and slow (10-100 ms) timescale. Burst smFRET experiments show that Sic1's end-end distances do not vary significantly upon addition of salt, which suggests that charge-shielding by salt may only affect the structure locally, around charged amino acids. Our single-molecule data resolves conformational heterogeneity and dynamics in a model IDP protein and represent the first step towards the validation of the polyelectrostatic model of the Sic1-Cdc4 interaction[2].

(1) Nash, P., Tang, X., Orlicky, S., Chen, Q., Gertler, F. B., Mendenhall, M. D., Sicheri, F., Pawson, T., Tyers, M. Nature 2001, 414, 514.
(2) Borg, M., Mittag, T., Pawson, T., Tyers, M., Forman-Kay, J. D., Chan, H. S. Proc. Natl. Acad. Sci. USA 2007, 104, 9650.

53-Plat

Integrating Theory, Simulations and Experiments to Reveal the Recognition-Specific Pathways in the Nuclear Co-Activator Binding Domain Ensemble

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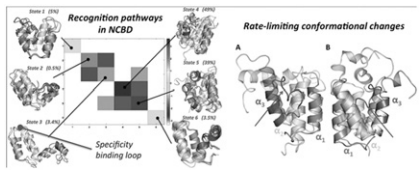
The nuclear receptor co-activator binding domain (NCBD) selectively recruits transcription co-activators (TCAs) during the formation of the transcription pre-initiation complex. However, the biophysical mechanisms of NCBD:TCA recognition remain unclear as both NCBD and several of its corresponding TCAs are intrinsically disordered. We therefore probed the conformational diversity of the apo- and holo-forms of NCBD using all-atom, explicit solvent molecular dynamics simulations (~100 μ1/4s) and small-angle neutron scattering experiments. We integrated theory, simulations and experiments into a unified framework called anharmonic conformational analysis (ACA). ACA identifies a hierarchy of conformational sub-states, intermediates and pathways that play a key role in NCBD:TCA recognition. The transitions between sub-states can be modeled by a bent paperclip, whose arms correspond to helices, α1-α2-α3. The pathways reveal that α1 and α2 adopt conformations



close to the holo-form and α3 undergoes extensive conformational changes in response to different TCAs (Fig. 1). The specificity binding loop in NCBD adopts intermediates that enables the bending and twisting of α1 and α2 into its final orientation with the target TCA. We hope this quantitative view of NCBD:TCA landscape can aid the design of novel cancer therapeutics.

54-Plat
Inferring Aggregation Mechanisms of Polyglutamine Through Quantitative Studies of Phase Behavior
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Many proteins associated with neurodegenerative diseases are intrinsically disordered, i.e. they lack a stable, folded structure under physiological conditions. It is currently believed that the aggregation of these proteins plays a causative role in neurodegenerative disease pathogenesis. Therefore, understanding how and why these proteins aggregate is crucial to understanding and possibly suppressing disease.
Proteins are polymers and protein aggregation is akin to phase separation. We employ techniques and theories borrowed from polymer physics to help understand the driving forces and mechanisms of protein aggregation. Our system of interest is polyglutamine, as expansions of polyglutamine are thought to be causally linked to the development of at least nine different neurodegenerative diseases. In this work, we measure the saturation concentrations of aqueous polyglutamine solutions containing 30 and 40 glutamines and either 2 or 4 lysines. We use classical polymer physics theory to construct the entire (soluble-insoluble) phase diagram from the measured saturation concentrations. The low-concentration arm of the phase diagram provides a thermodynamic basis for assessing aggregation propensity. For a given chain length of polyglutamine, we find that aggregation propensity increases with fewer lysines, and, for a fixed number of lysines, the aggregation propensity increases with increasing chain length. Although the phase diagrams are thermodynamic in nature, they still provide insights regarding the kinetic mechanisms of phase separation. For the concentrations used in most in vitro experiments, the phase diagrams predict that intrinsically disordered monomers first form disordered, higher-order oligomers or clusters which then undergo a nucleated structural conversion into an ordered, insoluble form. These predictions are supported by detailed atomic force microscopy studies. This work highlights the prominence of intrinsic disorder even in multimolecular complexes and its role in facilitating conformational conversion to ordered supramolecular aggregates.

55-Plat
Comparison of α-Synuclein and Amyloid Beta Membrane Interactions
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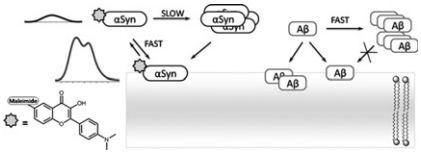
α-synuclein (αSyn) and amyloid beta (1-42) peptide (Aβ) are the main constituents of pathological deposits in the midbrain of individuals affected by Parkinson's disease and Alzheimer's disease respectively. Defining the interactions of αSyn and Aβ with membranes is essential for understanding the neurotoxicity caused by mutations and/or overexpression.



We performed a systematic *in vitro* study of the effects of membrane charge, phase, curvature, defects and lipid unsaturation on αSyn and Aβ binding using model vesicles and proteins labeled with a new solvatochromic fluorescent probe. The probe's emission spectrum strongly depends on the membrane properties, allowing clear discrimination of the protein bound to vesicles of different composition that enables measurements of the kinetics of αSyn migration between membranes of different compositions [1]. The interaction of αSyn with vesicular membranes is fast and reversible while the membrane binding of Aβ is mainly kinetically controlled and competes with aggregation. By introducing the probe at different positions of αSyn and Aβ we were able to estimate the relative immersion of different protein domains into membrane, and its changes depending on membrane composition and lipid-to-protein ratio.
[1] Shvadchak, et al., *J. Biol. Chem.*, (2011).

56-Plat
Protein Disorder and Degradation: Is Ubiquitin the Missing Link?
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The ubiquitin-proteasome system is responsible for degradation of numerous proteins. How the proteasome successfully degrades such a large variety of proteins is not well understood. It is thought that in order to be degraded, proteins must have a disordered region (an unstructured stretch of residues of a certain length) that can be used by the proteasome to initiate degradation. We have assembled a dataset of proteins that undergo ubiquitination and degradation, and characterized their structural properties (**Hagai et al., JMB, 2011**). Surprisingly, we observed that ~25% of the proteins that are successfully degraded lack the needed disordered region. Therefore, an additional mechanism should somehow provide a disordered region to enable degradation. Since the majority of proteins are ubiquitinated prior to their degradation, it is possible that the ubiquitin chain itself can influence the structure and thermal stability of the protein.
Using molecular dynamics simulations we showed that ubiquitination can significantly alter the protein's structure and stability (**Hagai and Levy, PNAS, 2010**). Ubiquitination of the substrate Ubc7 at the residues that are modified in vivo prior to degradation uniquely results in significant thermal destabilization and a local distortion near the modification site. These effects are specific to these sites, while other lysine residues which are not used in vivo, display diverse behavior upon ubiquitination. This indicates that ubiquitination can facilitate the unfolding process and create disordered regions, and therefore can assist degradation.
Our findings suggest that in addition to its signaling role, ubiquitination may alter protein biophysics to support degradation.
References:
Intrinsic disorder in ubiquitination substrates.Hagai et al. ***JMB***, 2011.
Ubiquitin not only serves as a tag but also assists degradation by inducing protein unfolding. Hagai and Levy. ***PNAS***, 2010.

Platform: Molecular Mechanics & Force Spectroscopy

57-Plat
Highly Covalent Ferric-Thiolate Bonds in Rubredoxin Exhibit Surprisingly Low Mechanical Stability
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Depending on their nature, different chemical bonds show vastly different stability with covalent bonds being the most stable ones that rupture at forces above nanoNewton. Studies revealed that Fe-thiolate bonds in metalloprotein are highly covalent and are conceived to be of high mechanical stability. Here we used single molecule force spectroscopy techniques to directly determine the mechanical strength of ferric-thiolate bonds in rubredoxin. We observed that the ferric-thiolate bond ruptures at surprisingly low forces of ~200 pN, one order of magnitude lower than that of typical covalent bonds, such as C-Si, S-S and Au-thiolate bonds. And the mechanical strength of Fe-thiolate bonds is observed to correlate with the covalency of the bonds in different protein systems. Our results shed new lights on the nature of Fe-thiolate bonds and suggest that highly covalent Fe-thiolate bonds are mechanically labile and clearly distinguish themselves from typical covalent bonds.

58-Plat
Towards a General Platform to Study Single-Bond Chemistry Under Force
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The application of force to reagents that participate in a chemical reaction can probe the transition state of the reaction with sub-Angstrom resolution. Using single-molecule force-clamp spectroscopy, this approach has been extensively applied to the cleavage of disulfide bonds in proteins. However, to date there is no methodology that can expand this class of experiments to bonds not naturally present in proteins. Here, we introduce an experimental platform with the potential to fulfill the requirements to perform single-bond rupture determinations on any covalent bond. We engineered polyproteins based on the I27 domain of titin including two cysteine residues. Bifunctional crosslinking molecules specific for thiol groups were then used to generate intradomain covalent bridges between the engineered cysteines, much similar to the manner disulfides link polypeptide chains. Different bismaleimide reagents containing cleavable covalent bonds were tested for their ability to crosslink the I27 domain. We used single-molecule force- spectroscopy to pull from the modified polyproteins. Successfully crosslinked domains gave rise to lower increments in contour length after mechanical unfolding, consistent with the number of amino acids protected in the protein loop formed by the covalent bridge. Using our new strategy, virtually any covalent bond can be used to generate intradomain crosslinks in proteins. Our method can be adapted to produce hybrids