De Novo Computational Design of Disordered Fg-Nucleoporins

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exhibits unusual pressure and temperature dependences. We observed that the low-complexity domain is pressure sensitive, undergoing a major conformational transition within the prescribed pressure range. Remarkably, this transition has the inverse temperature dependence of a typical folding-unfolding transition. Our results suggest the presence of a low-lying extended, and fully solvated state(s) of the low-complexity domain that may play a role in phase separation. This study highlights the exquisite sensitivity of solution NMR spectroscopy to observe subtle conformational changes and illustrates how pressure perturbation can be used to determine the properties of metastable conformational ensembles.

137-Pos

How do Evolutionarily Encoded Compositional Biases Influence the Phase Behaviors of Intrinsically Disordered Low Complexity Domains of Proteins?

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The stickers-and-spacers model describes phase transitions of archetypal multivalent macromolecules such as the intrinsically disordered low-complexity domain of hnRNPA1 (A1-LCD). Cohesive interactions among aromatic stickers determine the driving forces for phase transitions and the global dimensions of individual molecules in dilute solutions. Previously, we modeled Tyr and Phe as energetically equivalent stickers and all other residues were modeled as spacers. Bioinformatics analysis of orthologous LCDs reveals conserved compositional biases that place bounds on parameters such as the net charge per residue, the fraction of aromatic (Phe and Tyr) residues, and the Gly/Ser contents. We also find significant variations in the asymmetry between Tyr and Phe contents. Here, we ask if observed compositional biases have implications for the phase behavior of A1-LCDs? Using experiments and simulations we show that tyrosine is a stronger sticker than phenylalanine, indicating that the phase behavior of A1-LCDs might be evolutionarily tuned by the asymmetry between Tyr and Phe contents. Further, Arg is either a sticker or a spacer depending on its context whereas Lys weakens sticker-sticker interactions in general. Importantly, the coupling between single-chain and multi-chain behavior can be weakened by changes to the overall net charge whereby small changes to net charge, through changes to spacer residues, can have a significant effect on the multi-chain behavior without impacting the single-chain compaction. Phase diagrams, computed using the LASSI engine, are congruent with experimental measurements and our findings highlight the importance of sequence-encoded hierarchies of sticker and spacer interactions to overall phase behavior. Using results from simulations we uncover how changes to stickers and spacers impact the spatial organization of molecules within condensates and the distinct conformational preferences of LCDs within the condensate interior and interface.

138-Pos

Effects of Membrane Curvature on Amyloid-Beta Aggregation

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Membrane assisted misfolding and aberrant self-assembly of amyloid beta peptides have been associated with pathogenesis of Alzheimer's disease. While several reports have suggested that an increased membrane curvature can support faster peptide aggregation, a mechanistic explanation of this curvature dependence is missing. in this work, we have explored the effects of surface-tension as a proxy for membrane curvature on Amyloid-beta 16-22 (K-L-V-F-F-A-E) aggregation, using physics-based coarse grained molecular simulations of model membranes composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids. Our simulations also agree to experimental observation of a positive correlation between increased peptide aggregation and membrane curvature. The initial competition between peptide-peptide and peptide-membrane interactions result in three distinct regimes of peptide aggregation behavior, with low curvatures promoting peptides aggregation in solution and high curvatures on membranes. in addition, the membranes with high curvature have higher local defects in lipid packing that can engage peptide's hydrophobic groups and initiate a backbone driven ordered aggregation into beta-sheet rich structures. Higher curvatures can also promote faster rearrangement of lipid molecules to increase local solvent accessible hydrophobic surface area, that is necessary for membrane associated peptide assemblies, due to increased lipid diffusion. This work reveals essential physical insights on membrane-assisted aggregation pathways and the relevance of membrane curvature on peptide aggregation.

139-Pos

Bottom-Up Coarse-Grained Models for Intrinsically Disordered Proteins

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Intrinsically disordered proteins (IDPs) have recently become a popular topic of study in the protein science community due to their role processes such as cellular signaling and liquid-liquid phase separation (LLPS). A number of coarse-grained models for IDPs have been recently been developed. These models are typically of the top-down variety; they are parameterized to reproduce a set of experimental information. While these models have been effective for providing a computational basis for the study processes such as LLPS, they must rely on assumptions about the interactions between coarse-grained sites due to the lack of microscopic information. This is contrasted with bottom-up coarse-graining, which relies on data from a higher resolution source, like an atomistic molecular dynamics trajectory, to parameterize the model. Using high-quality atomistic data generated from recently developed force-fields, we have developed multiscale coarse-grained and relative entropy minimization coarse-grained models of several residues common among IDPs. These models are not limited to the standard Lennard-Jones and screened electrostatic functional forms typically used in top-down coarse-grained models. This model is also able to better capture the effects of solvation on the IDP. These new functional forms provide a basis to create a full interaction matrix that is based on mixing rules determined for a small number of residues. This new model creates higher fidelity coarse-grained model for the study LLPS as well as providing a basis-set for parameterizing interactions of new top-down models.

140-Pos

Quantification of the Onset of Condensation in Negative Elongation Factors

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Several biomolecules condensate in cells to form membraneless organelles via liquid-liquid phase separation, usually in response to changes in their environment. Recent studies suggest that biological phase separation for example regulates cell survival and pathology. The process of biological phase separation itself is not well understood. It is for example unclear how many proteins of a kind are necessary to induce a dense phase in cells – or if the local concentration is the limiting factor. Here we employ highly inclined and laminated optical sheet microscopy and single-molecule fluorescence methods to quantify early steps of phase separation of the negative elongation factor (NELF). NELF downregulates transcription and condensates upon heat and toxin stress. We treat the NELF as a model system to quantify the threshold concentration and rapidness of NELF condensation at low expression levels in fixed and live cells. by counting the number of NELF proteins in the dense phase in a time dependent manner we will contribute to understand the molecular mechanisms underlying liquid-liquid phase separation in cells.

141-Pos

***De Novo* Computational Design of Disordered Fg-Nucleoporins**

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The transport mechanism by which the Nuclear Pore Complex (NPC) governs transport from and to the cell nucleus in a size-selective manner remains elusive. intrinsically disordered FG-Nucleoporins (Nups), rich in motifs containing Phe and Gly (i.e., FxFG, GLFG) form a selective meshwork in the NPC's central channel: small molecules can freely translocate, whereas larger macromolecules are hindered unless bound to Nuclear Transport Receptors (NTRs) such as the Karyopherin protein family.
In earlier work[1] we highlighted how four simple design rules enabled the *de novo* design of an artificial 311-residue FG-Nup that reconstituted the NPC's size-selective barrier function in solid-state nanopores. Here, we go beyond this proof-of-concept and computationally design a collection of 800-residue long FG-Nups, where we systematically vary physiochemical properties such as the charged-to-hydrophobic (C/H) amino acid ratio and the spacing between

FG-motifs in a controlled manner. We assess the role of C/H ratio and FG-spacing in the phase behavior of our artificial FG-Nups through a combination of modeling and *in vitro* phase separation assays. Finally, simulations of nanopores coated with our artificial FG-Nups reveal that the permeability barrier function and NTR-mediated transport depend sensitively on the C/H ratio and FG-motif spacing. We experimentally verify this finding via transport measurements in zero-mode waveguides functionalized with the artificial Nups. Our method for *de novo* designing disordered proteins for tunable transport barriers can also find application in synthetic biology and protein filtration purposes.

1. A. Fragasso*, H.W. de Vries*, J. Andersson, E.O. van der Sluis, E. van der Giessen, A. Dahlin, P.R. Onck, and C. Dekker. BioRxiv: 2020.02.04.933994.

142-Pos

Integrating NMR, SAXS and Single-Molecule FRET Data to Infer Conformational Ensembles of the Yeast Sic1 Protein

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Intrinsically disordered proteins (IDPs) have fluctuating heterogeneous conformations, which makes structural characterization challenging. An accurate description of IDP conformational ensembles depends crucially on the amount and quality of the experimental data, how it is integrated, and if it supports a consistent structural picture. We used integrative modeling and validation to apply conformational restraints and assess agreement with the most common structural techniques for IDPs: Nuclear Magnetic Resonance (NMR) spectroscopy, Small-angle X-ray Scattering (SAXS), and single-molecule Förster Resonance Energy Transfer (smFRET). Agreement with such a diverse set of experimental data suggests that details of the generated ensembles can now be examined with a high degree of confidence.

Using the disordered N-terminal region of the Sic1 protein as a test case, we examined relationships between average global polymeric descriptions and higher-moments of their distributions. To resolve apparent discrepancies between smFRET and SAXS inferences, we integrated SAXS data with non-smFRET (NMR) data and reserved the smFRET data as an independent validation. Consistency with smFRET, which was not guaranteed a priori, indicates that, globally, the perturbative effects of NMR or smFRET labels on the Sic1 ensemble are minimal. Analysis of the ensembles revealed distinguishing features of Sic1, such as overall compactness and large end-to-end distance fluctuations, which are consistent with biophysical models of Sic1's ultrasensitive binding to its partner Cdc4. The results underscore the importance of integrative modeling and validation in generating and drawing conclusions from IDP conformational ensembles.

143-Pos

Effects of Familial Mutation and C-Terminal Truncation on Nucleation and Fibril Elongation of α-Synuclein

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Aggregation of α-synuclein (α-syn) into amyloid fibrils is closely associated with Parkinson's disease (PD). Familial mutations or posttranslational truncations in α-syn are known as risk factor for PD. An A53T mutation is most frequent and links to early-onset PD. in human brain with PD, α-syn is specifically truncated at N103 by asparagine endopeptidase, and the resulting C-terminal-truncated fragment Δ104-140 has high aggregation propensity and neurotoxicity. Here, we examined the physicochemical mechanism of aggregation of PD-related α-syn variants, A53T and Δ104-140, using kinetic and thermodynamic analyses.α-Syn aggregation was monitored by amyloid-specific dye, thioflavin T (ThT), and the kinetics of the ThT fluorescence increase were analyzed by Finke-Watzky 2-step model of a homogeneous nucleation followed by autocatalytic heterogeneous fibril elongation. At physiological condition (20 μM α-syn, pH 7.4, 37 ^{o}C), both A53T and Δ104-140 variants aggregated faster than WT α-syn, in which the A53T mutation markedly increases nucleation rate whereas the C-terminal truncation significantly increases both nucleation and fibril elongation rates. Analysis of the dependence of aggregation reaction of the variants on the monomer concentration suggested that the A53T mutation enhances conversion of monomers to amyloid nuclei whereas the C-terminal truncation enhances autocatalytic aggregation on existing fibrils. in addition, thermodynamic analysis of the kinetics of nucleation and fibril elongation of the variants indicated that both nucleation and fibril elongation of WT α-syn are enthalpically and entropically unfavorable. interestingly, the unfavorable activation enthalpy of nucleation is greatly reduced by the A53T mutation, and becomes reversed in sign for the Δ104-140 variant.Taken together, our results indicate that the A53T mutation and the C-terminal truncation enhance α-syn aggregation by reducing unfavorable activation enthalpy of nucleation, and suggest that the C-terminal truncation further triggers the autocatalytic fibril elongation on the fibril surfaces.

144-Pos

Real-Time Observation of Structural and Dynamical Changes of the FUS Low-Complexity Domain During Liquid-To-Solid Phase Transitions

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Many of the proteins found in pathological protein fibrils also exhibit tendencies for liquid-liquid phase separation (LLPS) both in vitro and in cells. The mechanisms underlying the connection between these phase transitions have been challenging to study due to the heterogeneous and dynamic nature of the states formed during the maturation of LLPS protein droplets into gels and solid aggregates. Here, we interrogate the liquid-to-solid transition of the low complexity domain of the RNA-binding protein FUS (FUS LC)-which has been shown to adopt LLPS, gel-like, and amyloid states-in real time using magic-angle spinning (MAS) NMR. We also apply our methodology to FUS LC G156E, a clinically relevant FUS mutant that exhibits accelerated fibrillization rates. We show that the final state of FUS LC fibrils produced through the maturation of LLPS FUS LC is distinct from that grown from fibrillar seeds, and begin to unravel the structural and sequence specific contributions to this phenomenon with computational studies of the phase separated state of FUS LC and FUS LC G156E.

145-Pos

Environment Dependent Secondary Structure of a Ribosome Stalling Peptide

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The Vibrio export monitoring polypeptide (VemP) is mechano-sensitive peptide which helps Vibrio bacteria survive in both salty and fresh water by inducing elongation arrest and promoting alternative gene expression under low salinity conditions. Normally, sodium-dependent membrane translocation exerts a mechanical force on the nascent VemP which ensures an uninterrupted protein synthesis. Cryo-electron microscopy (cryo-EM) showed that in the absence of this force VemP adopts a helix-turn-helix secondary structure (SS) near the ribosome catalytic site and stalls peptide synthesis. It is not known if VemP only adopts this structure within the ribosome, or it is maintained in different environments as well.

Here, we apply circular dichroism spectroscopy to study the structure of VemP in solvents of varying hydrophobicity, to determine how important ribosome specific interactions are for the observed helix-turn-helix structure. Bayesian SS estimates based on CD spectra indicate that under physiological conditions VemP is unstructured, but it readily adopts helical structures in more hydrophobic environments. However, the estimated overall helix contents suggest that specific interactions formed between VemP and the ribosome exit tunnel and catalytic center are necessary to stabilize the observed cyro-EM structure. Furthermore, a combination of CD-based SS estimates of truncated VemP constructs and molecular dynamics simulations allows us to identify key interactions of VemP in the ribosome.

146-Pos

Probing the Interactions of Intrinsically Disordered Protein with Metal Ions and Lipid Membranes by Fluorescence Spectroscopy

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The misfolding and aggregation of intrinsically disordered proteins (IDPs) are believed to play crucial roles in developing neurodegenerative diseases, such as AD and PD. Nevertheless, the roles of metal ions and lipid membranes in IDPs aggregation are not fully understood. We investigated the interactions of