ADE, further information on germline antibody binding to ZIKV and the affinity maturation process that gives rise to potently neutralizing antibodies is needed. Here, we compared mature and inferred-germline antibody binding to envelope protein domain III (EDIII) of ZIKV and other flaviviruses through binding assays, structural characterization, and neutralization and ADE studies. We showed that affinity maturation of the light chain variable domain is important for strong binding of the recurrent VH3-23/VK1-5 neutralizing antibodies to ZIKV EDIII and identified interacting residues that contribute to weak, cross-reactive binding to other flaviviruses. These findings provide insight into the affinity maturation process and potential cross-reactivity of VH3-23/VK1-5 neutralizing antibodies, informing precautions for protein-based vaccines.

Platform: Protein Prediction, Design, and Stability II

1332-Plat

Direct Observation of the Contraction of an Unfolded Protein with Temperature

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A fundamental tenet in protein folding is that the hydrophobic effect promotes chain collapse. For the unfolded state, however, we and others find that proteins adopt conformations that closely resemble a self-avoiding random walk (SARW). That an unfolded protein would remain expanded suggests that the hydrophobic effect is smaller than anticipated or other factors promote chain expansion such as backbone solvation. In a similar vein, NMR data on Gly-rich proteins and poly(Asn) consistently measure minimal protection factors across a variety of buffer conditions. Here we investigate the effect of temperature on the dimensions of the unfolded state by using SEC-SAXS to measure the R_g and Flory exponent, ν, where $R_g \propto N^{\nu}$. Consistent with the hydrophobic effect increasing with temperature due to the corresponding gains in solvent entropy upon water release, we find that PNt, the unstructured N-terminal domain of Pertactin, contracts by 10% upon changing the temperature from 10 to 60°C. Across this temperature range, the solvent quality of water decreases from that of a SARW ($\nu \sim 3/5$) and approaches that of a theta solvent ($\nu \sim 1/2$), where self-avoidance is balanced by intra-chain interactions. These data point to weak intra-chain interactions in the unfolded state but with hydrophobicity playing a larger role than hydrogen bonding.

1333-Plat

Probing the Impact of Molecular Chaperones on the Refoldability of the *E. coli* Proteome

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We have demonstrated that roughly half of the *E. coli* proteome is unable to refold to their native structures following denaturation with chemical denaturants. To accomplish these studies, we devised a limited proteolysis mass-spectrometry (LiP-MS) approach, in which a permissive protease that cleaves at flexible regions is used to probe the structural differences between the native and refolded forms of proteins in whole lysates. Here, we interrogate the ability of several molecular chaperone systems (trigger factor, HSP70, and the chaperonin GroEL/GroES) to rescue the refoldability of proteins that cannot refold on their own. We conducted these experiments by first globally unfolding *E. coli* lysates and then diluting them into native buffers containing the different molecular chaperone systems at their physiologically relevant concentrations. We then developed a strategy to deplete the chaperones *after* refolding, so that our results can focus on the effect the chaperones exercise on the structural outcomes of their clients. Our experiments show that some - but not all - proteins can be successfully guided to their native structure in a chaperone-dependent manner. These studies therefore shed light on which kinds of proteins can refold under a thermodynamic gradient versus which ones most likely require co-translational folding to properly assemble.

1334-Plat

Exploring Exhaustively the Conformations of a Tandem Domains Protein using a Discrete Distance Geometry Approach

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The optimization problem encountered in protein structure determination is undergoing a change of perspective due to the larger importance in biology taken by disordered regions of biomolecules. In such cases, the convergence criterion is more difficult to set up; moreover, the enormous size of the space makes it difficult to achieve a complete exploration. The interval Branch-and-Prune (iBP) approach, based on a reformulating of the Distance Geometry Problem (DGP) provides a theoretical frame for the exhaustive sampling of the conformations. An implementation of the iBP approach, oriented toward the sampling of protein structure, was recently proposed (Worley et al, 2018; Malliavin et al, 2019).

The development of structural biology conducted to the discovery of numerous tandem domains related by a flexible linker. Solution NMR, sensitive to the internal mobility, and SAXS, sensitive to the gyration radius, are quite often used in parallel to investigate such systems. Here, we propose a pipeline based on the iBP approach to determine the set of representative conformations of the tandem domains as well as the weights of these conformations. This pipeline has been applied on the tandem PDZ domains (Delhommel et al, 2017), playing an essential role in the function of whirlin, involved in the hearing and vision systems. The obtained conformations of the tandem PDZ domain along with their weights will be analyzed according to the biological context.

Delhommel et al. Structural Characterization of Whirlin Reveals an Unexpected and Dynamic Supramodule Conformation of Its PDZ Tandem. Structure 25, 1645 (2017).
Malliavin et al. Systematic exploration of protein conformational space using a Distance Geometry approach. J Chem Inf Model 59, 4486 (2019).
Worley et al. Tuning interval Branch-and-Prune for protein structure determination. J Glob Optim 72, 109 (2018).

1335-Plat

Emerging Features of Rheostat Positions

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Protein function can be progressively fine-tuned by substituting amino acids at ''rheostat'' positions. For example, at a rheostat position that modulates binding affinity, substitutions exhibit K_d values that span a wide range. Early studies of rheostat positions revealed outcomes that could not be explained by side chain chemical similarities or by evolutionary frequency. In ongoing efforts to catalog the prevalence of rheostat positions, we have identified them in proteins that evolved under different physical constraints: globular soluble, integral membrane, and intrinsically disordered proteins. However, the density of rheostat positions within a protein can vary widely: >40% of *E. coli* LacI comprises rheostat positions, whereas they have yet to be identified in *Z. mobilis* pyruvate kinase despite numerous selection strategies. Among these strategies, analyses of sequence alignments can identify sets of positions enriched for rheostat positions, but no single metric definitively identified rheostat positions. In contrast, a subset of ''neutral'' positions (all substitutions have wild-type function) were identified from combined sequence analyses. *In crystallo* and *in silico* structural studies of rheostat substitutions showed only local perturbations and modest effects on protein stability. Additional functional studies revealed new complexities: (1) The rheostat character of each position falls on a continuum between the all-or-none substitution outcomes of ''toggle'' positions and the mutational insensitivity of neutral positions. (2) Some rheostat positions have complex effects on ligand specificity. (3) ''Multiplex'' rheostat positions simultaneously modulate multiple functional parameters, such as K_d and allosteric coupling; these show intriguing overlap