bases should in principle allow data mining and machine learning in the hope to unearth statistically valid knowledge about how to select and optimize the best crystallization conditions for a given protein.

Indications have emerged that the process of knowledge-based predictions in protein crystallization is not going as smoothly as one would hope. The foremost reason lies in the complex and locally determined nature of the crystallization process, and the high dimensionality and sparse sampling of the multivariate crystallization parameter space [1]. Optimal experimental design, careful annotation, and robust machine learning methods have provided various reliable general rules - often affirming prior empirical suggestions - while specific predictions yet remain of limited statistical significance due to low confidence of the derived rules.

[1] Rupp B., Wang J., *Methods*, 2004, **34**, 390-407

Keywords: crystallization, data mining, predictive models

MS02 CHAMELEON PROTEINS

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The Amazing Versatility of Proteins – Structural Polymorphism and Evolution

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Structures of hydrophobic core residue mutants of the immunoglobulin binding domain B1 of streptococcal protein G (GB1), a universal model protein, were determined. Surprisingly, the oligomeric state and quaternary structure of several of these mutant proteins is drastically changed. A domain-swapped dimer and a symmetric tetramer, with inter-molecular strand-exchange involving all four units were discovered. These findings demonstrate that proteins are able to undergo substantial global rearrangements through the acquisition of very few point mutations. The domain-swapped dimer dissociated into a partially folded, monomeric species at low micromolar protein concentrations and we have characterized this monomeric, partially folded species by NMR. Extensive conformational heterogeneity for a substantial portion of the polypeptide chain exists and exchange between the conformers within the monomer ensemble on the micro- to millisecond timescale renders the majority of backbone amide resonances broadened beyond detection. Despite these extensive temporal and special fluctuations, the overall architecture of the monomeric mutant protein resembles that of wild-type GB1 and not the monomer unit of the domain-swapped dimer. Interestingly, this partially folded monomeric species seems to constitute the critical folding intermediate for amyloid fibril formation.

Our results suggest that destabilization of a monomeric protein can be compensated for by multimerization and that alternative structures (multimers or higher order oligomers) are accessible to proteins from long-lived partially folded intermediates that are capable of large scale conformational fluctuations.

Keywords: structural polymorphism, evolution, mutations

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The Mechanisms of Conversion of Proteins into Amyloid Fibrils

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In addition to folding to unique and well-defined three-dimensional structures that are relatively easy to crystallise and analyse with X-ray crystallography, proteins also have a tendency to misfold and self-assemble into stable fibrillar aggregates. These structures, known as amyloid fibrils, are responsible for over 20 human diseases including Alzheimer and Parkinson's diseases and various systemic amyloidoses.

Amyloid formation is not limited, however, to the few macromolecules associated with diseases but is a generic property of natural and synthetic proteins. An understanding of the process of amyloid formation is therefore essential not just for elucidating the pathogenesis of amyloid diseases, but also for the rational design of proteins with the ability to escape aggregation. Given the wide range of morphologies and structures that can be achieved by converting different proteins into amyloid fibrils, the rational and controlled formation of these structures can give rise to a number of materials with useful but yet unexplored properties.

I will describe the mechanism of amyloid formation of proteins, with particular emphasis on the structural and amino acid sequence determinants of this process. Experimental evidence suggests that amyloid formation follows rules that are rather general and applicable to various systems.

Keywords: amyloidogenesis, folding, protein assembly

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Structural Changes in the Bacterial Toxin Pneumolysin During Pore Formation

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The bacterial toxin pneumolysin is released as a soluble monomer that kills target cells by assembling into large oligomeric rings that form pores in cholesterol-containing membranes. Using cryo-EM and image processing we have determined the structures of both the prepore and membrane-inserted pore oligomer forms, providing a direct observation of the conformational transition into the pore form of a cholesterol-dependent cytolysin.

To form the pore structure the pneumolysin domains reorganize and double over into an arch, forming a wall that seals the bilayer around the pore. This transformation is accomplished by membrane deformation and the substantial refolding of two of the four protein domains. The pore structure supports the hypothesis that two regions of α-helices refold into β-hairpins that insert into the membrane to form the pore [1, 2]. These hairpins form the largest β-barrels observed; our largest reconstruction of the pore contains 44 subunits forming a 176 strand β-barrel around a 260 Å diameter channel.

[1] Shepard L.A., Heuck A.P., Hamman B.D., Rossjohn J., Parker M.W., Ryan K.R., Johnson, A.E., Tweten R.K., *Biochemistry*, 1998, **37**, 14563. [2] Shatursky O., Heuck A.P., Shepard L.A., Rossjohn J., Parker M.W., Johnson A.E., Tweten R.K., *Cell*, 1999, **99**, 293.

Keywords: cytolysin, toxin structure, electron microscopy

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Obeying Anfinsen: a Serpin that folds to the most Stable State

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Members of the serpin superfamily of protease inhibitors represent an exception to Anfinsen's conjecture and fold to a native metastable conformation, rather than the theoretically most stable "relaxed" conformation. Inhibitory serpins utilise metastability to inhibit target proteases. Unfortunately, as a consequence of metastability, serpins are conformationally labile, and vulnerable to mutations that promote the formation of inactive loop sheet polymers. Polymerisation of human serpins is the critical factor in the development of a number of degenerative diseases (serpinopathies).

Eukaryote serpins are sensitive to mild heating, however, to our surprise, we have identified serpins in thermophilic prokaryotes. A structural study on the serpin thermopin reveals that this molecule is able to adopt the native and cleaved state and inhibits a metastable α-