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

Chairpersons: Lynne Regan, Paul Curmi

MS02.24.1

Acta Cryst. (2005). A**61**, C10

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

MS02.24.2

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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

MS02.24.3

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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

MS02.24.4

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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-

lytic-like protease. In contrast, the high-resolution crystal structures of another prokaryote serpin, tengpin, reveals that the serpin domain of this molecule folds spontaneously and rapidly to most stable (i.e. relaxed) conformation. This is an exciting result, since tengpin represents the first serpin identified to date that obeys Anfinsen's conjecture. Furthermore, the X-ray crystal structures of tengpin reveals the structural basis for a novel mechanism for loop-C-sheet serpin-polymerisation. Analysis of the structural data provides striking insight into the mechanism of serpin metastability and the structural basis for serpin polymerisation.

[1] a) Irving J.A., et al., *Structure 2003*; b) Fulton K.F., et al., *J Biol Chem*, 2005.

Keywords: serpin, folding, polymerisation

MS03 CHIRAL AND NON-CENTROSYMMETRIC STRUCTURES

Chairpersons: Shiv P. Halasyamani, Reiko Kuroda

MS03.24.1

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Spontaneous Resolutions in Halogen Bonded Fluorinated Networks

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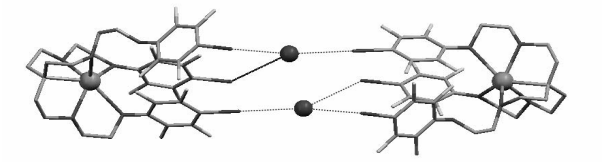
Halogen bonding is an efficient tool for self-assembling halo-perfluorocarbons (PFC) and hydrocarbons (HC) [1]. Its particular ability to control spontaneous resolution in hybrid PFC-HC systems has been discovered only recently [2]. Up to now we observed spontaneous resolutions in four cases affording chiral cocrystals, space group $P2_12_12_1$. Three of them involved long-chain iodo-PFC's (C_8-C_{10}) with either QUATS or *N,N,N',N'*-tetramethyl-*p*-phenylendiamine as bases. Their different features with regard to the segregation behaviour and the conformation of the PFC chains will be outlined. The X-ray structure of a chiral alkali halide complex **1** (Figure) involving a tripodand will also be presented.



1

[1] Metrangolo P., Neukirch H., Pilati T., Resnati G., *Acc. Chem. Res.*, 2005, *in press.* [2] Neukirch H., Guido E., Liantonio R., Metrangolo P., Pilati T., Resnati G., *Chemm. Commun.*, 2005, 1534.

Keywords: halogen bonding, chiral resolution, molecular cocrystals

MS03.24.2

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Invariom Modeling for Improving Absolute Structure Determination

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A reliable determination of the Flack parameter [1] for structures of organic molecules, containing only the elements H, C, N, and O usually fails. The reason for this is the very weak anomalous signal obtained from the light atoms [2]. Recently we have introduced invariom [3] and here we try to improve the absolute structure determination by replacing the independent atom model with the aspherical invariom scattering model. The determination of the Flack parameter was included in the program XDLSM [4]. Alternatively, its calculation has been attempted via a hole-in-one procedure. A precise data set on a steroid compound was collected using copper radiation and CCD detection, and first results are reported.

[1] Flack H. D., *Acta Cryst*., 1983, **A39**, 876. [2] Flack H. D., Bernardinelli G., *J. Appl. Cryst.*, 2000, **33**, 1143. [3] Dittrich B., Koritsanszky T., Luger P., *Angew. Chem. Int. Ed.*,2004, **43**, 2718. [4] Koritsanszky T., Richter T., Macci P., Gatti C., Howard S., Mallinson P.R., Farrugia L., Su Z.W., Hansen N.K., *XD*, Freie Universitat Berlin, Berlin, 2003.

Keywords: Invarioms, Flack parameter refinement, chiral structures

MS03.24.3

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Optical Topographies of Chiral Structures

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Can optical rotatory power, a phenomenon typically associated with chirality or handedness, be used as a contrast mechanism in microscopy? Chiroptical imaging techniques have not heretofore been implemented. This neglect has created a hole in the science of molecular chirality, particularly with respect to complex, heterogeneous, organized media. We built a circular extinction imaging microscope to examine chromophores in anisotropic hosts. With this instrument, images of crystals were made via two mechanisms, intrinsic circular dichroism (CD) and a new effect that was discovered and called anomalous circular extinction (ACE). Through these new chirality "spectacles" we have observed left and right handed twinning in crystals of a dye that was masked by all previous methods of analysis, Figure 1 [1]. However, when turned onto unusual dyed crystals, we observed optical effects that mimic those due to chirality.

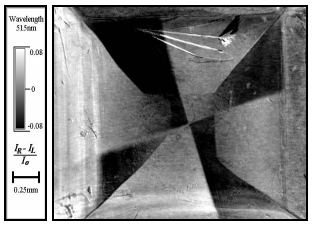

Figure 1. Circular Dichroism in 1-8-Dihydroxyanthraquinone.

[1] Claborn K., Puklin-Faucher E., Kurimoto M., Kaminsky W., Kahr B., *J. Am. Chem. Soc.*, 2003, **125**, 14825-14831.

Keywords: chirooptical properties, circular dichroism measurement, dyes

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Nonlinear Optical Properties of Chiral Polymers and Systems

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We present the nonlinear optical properties of different thin film films of chiral (conjugated) polymers. These systems exhibit large magnetic dipole nonlinearities, in some cases larger than the effects linked to electric dipole interactions. The nonlinear optical effects observed indicate the links between magnetic hypersusceptibilities and chirality. We also investigated supramolecular assemblies of helicenes where the nonlinear optical effects are exclusively described by electric dipole interactions. In the crystalline liquid state the chirality, as expressed by nonlinear CD effects, of these helicene assemblies could be switched by the application of an electric field.

Keywords: polymers, chirality, nonlinear optics

MS03.24.5

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Some Reminiscences of Non-centrosymmetric Structures

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In the "neanderthal" age of crystallography, a light atom non-centrosymmetric crystal was usually relegated to the skeleton collection of unsolvable structures. The development of MULTAN