Session XIV - Ionic Liquids in Protein Crystallization

Thursday, July 7 - afternoon

S14-L1

IONIC LIQUIDS (ILs) - WATER INTERPLAY IN PROTEIN CRYSTALLIZATION. FROM ADDITIVES TO NUCLEANTS TO...

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In our study we explore the potential of *ionic liquids (ILs)* to advance the field of protein crystal growing. ILs are a class of compounds composed of two oppositely charged species, the cation and the anion, that are liquid near room temperature. The number of possible combinations of composing ions is immense and theoretically it is possible to design a given ionic liquid that best suits a specific, desired purpose. However, one first needs to know how the ionic liquid properties relate to that particular purpose, in this case protein crystallization.

We started from identifying the variables and mechanisms behind the effect of different *water soluble ILs* used as *additives* in protein crystallization [1]. It has been recognized that, the same IL ions, which at low ionic strength (IS) conditions bind to the protein surface and screen the charges (promote salting-out and crystal nucleation), at high IS reduce the protein–water interfacial tension by remaining hydrated while attached to the surface (induce salting-in) (Fig. 1). Furthermore, it has to be pronounced that it is necessary to take into account the mutual affinity of the ions and their counterions in order to properly rank the respective (IL) ion pairs as salting-in/out agents.

As a next step, we have immobilized *hydrophobic ILs* at the surface of hydrophilic solid sub-micron particles [2]. This enabled the dispersion in aqueous solution of otherwise water-immiscible ILs and their first use in protein crystallization as heterogeneous *nucleants* [3] (Fig. 2). It has been recognized that their nucleating potential is determined by the protein-solid affinity based on short range H-bond donating/accepting interactions that can be tuned by changing the ILs structure.

Our on-going research is focused on the use of a remote physical trigger – the non-ionizing electromagnetic radiation to induce and control protein crystal growth. We showed the effect of infrared light (IR) on protein crystal nucleation and periodic self-assembly resulting from modulation of protein interfacial water (dipolar medium) by means of IR [4]. Now we want to use *ILs as polar and polarisable interfacial solvents* prone to respond to electromagnetic stimuli.

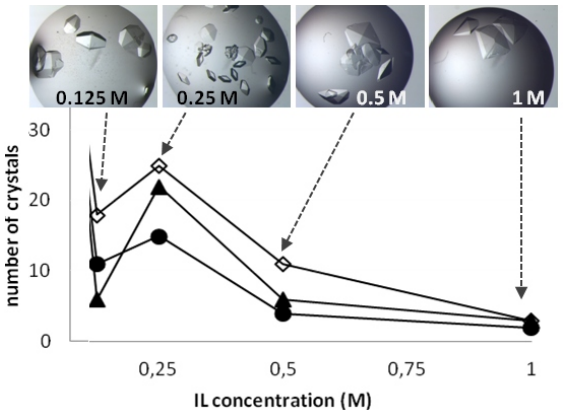

Figure 1. Concentration-dependent salting-in/out behaviour of ILs

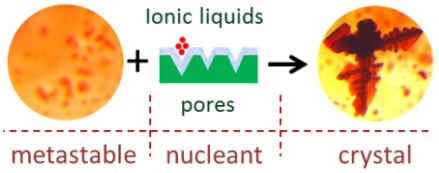

Figure 2. Schematic action of IL-functionalized nucleants

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S14-L2

IONIC LIQUIDS AS PROTEIN CRYSTALLIZATION ADDITIVES

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Ionic liquids (ILs) are typically defined as salts composed of separate cations and anions having melting points below 100 °C. ILs typically have chaotropic anions and surfactant cations, have low vapour pressure, have a wide liquidus range, and have depressed melting points as a result of low-symmetry ions. The cationic and anionic components can be individually tailored for various applications, which characteristic has resulted in a rapid proliferation in their use for a wide range of applications. This ability to finely tune their properties also makes ILs attractive materials for use in biochemical applications. Several investigations have been carried out using IL's in protein crystallization [1-3], with no conclusions that could guide one in their use. We are currently working to remedy this, testing a panel of 23 commercially available ILs, with dH_2O as a control, as protein crystallization additives. The ILs are used as 1M solutions in dH_2O, at final concentrations of 0.1, 0.2, and 0.4 M in the precipitant solution. The proteins employed are not the usual models, but those being worked as part of other ongoing crystallization projects in our group. Optimization with ILs has resulted in 3D faceted crystals being obtained from all types of starting outcomes tested, including non-faceted crystals (dendrites, urchins, spheroids, etc.), bright spot leads [4], and, most surprising, precipitate. The use of trace fluorescent labeling is critical to these experiments as the ILs often also crystallize from solution. Not every tested lead condition resulted in crystals, and some gave crystals with several ILs. In initial experiments the pattern of hits changes with the IL concentrations employed. Results from experiments currently under way, including diffraction analysis of crystals from IL+ and IL- conditions, will be reported.

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S14-L3

THE ARTIFICIAL PROTEIN OCTARELLIN CHALLENGES CRYSTALLOGRAPHERS AND MODELLERS

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The aim of *de novo* design proteins, often called the inverse protein folding problem, is to find amino acid sequences compatible with a given protein tertiary structure. Solving the inverse folding problem questions our understanding of sequence-structure relationships in proteins. Despite impressive successes in *de novo* protein design, designing a well-folded protein of more than 100 amino acids remains a challenge.

We will discuss the artificial protein Octarellin designed to adapt the TIM-barrel fold [1, 2]. Crystallization was only successful after the creation of stable complexes with antibodies from camelids (nanobodies) and alfa-repeat (Rep) proteins [3, 4]. As it turns out, the experimental X-ray structure deviates considerably from the idealized design, failing even at fold level. The experimental ( ) sandwich architecture bears some resemblance to a Rossman-like fold instead of the intended TIM-barrel fold. This surprising result gave us a unique and attractive opportunity to test the state of the art in protein structure prediction. We tested 13 automated webservers for protein structure prediction and found none of them to predict the actual structure. More than 50% of them predicted a TIM-barrel fold.

Note that the expected strand 2 (S2), helix 1 (H1), and the majority of the helix 8 (H8) are missing in the X-ray structure.

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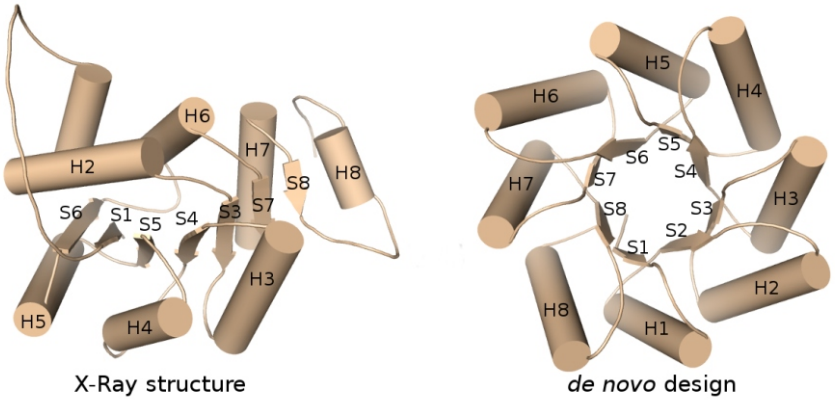

Figure 1. Comparison of the *de novo* designed and the X-ray structure from Octarellin.

S14-L4

WATER IN PROTEIN CRYSTALS

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Water is the ubiquitous solvent of biomolecules. Even protein crystals can contain up to 80% water by volume [1]. Yet water-protein interactions remain fairly poorly characterized. The probabilistic nature of solvation and the rich geometry and physical chemistry of the protein surface make this environment especially challenging to probe. Interestingly, X-ray diffraction of macromolecules also contains structural information about the solvating water [2,3], and recent computational and methodological advances provide new tools extracting that information.

In this work we compare protein diffraction data for which experimental phasing is available to constrained molecular dynamics (MD) simulation results. By correlating the resulting spatial density maps, we find that MD water models capture the radial extent of water fairly well, but fail to describe its orientation around certain protein atoms. Density correlations are higher for carbons than oxygens and nitrogens, and can even distinguish between different protonation states. We also confirm that water's distribution depends strongly on the protein surface environment, providing an explanation for the weakness of generic solvation models [4-6].

The structural performance of different water models – TIP4P, TIP3P, SPC/E, and SPC – is found to be nearly identical. Markedly improving the agreement between model and experiment would thus require models that are trained differently, or that include other aspects of water solvation. The relative robustness of existing solvent descriptions nonetheless paves the way to treating water's contribution to protein refinement more accurately.

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S14-L5

USE OF X-RAY CRYSTALLOGRAPHIC DATA FOR COMPUTATIONAL MODELLING OF RECEPTOR-LIGAND INTERACTIONS: DESIGN OF STEROIDAL INHIBITORS OF BREAST AND PROSTATE CANCER CELL GROWTH

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Hormone-sensitive tumours, such as breast or prostate cancers, are leading causes of death, and manipulation of steroid signaling is an effective treatment. X-ray structures of steroid receptors have been solved in complex with anti-cancer drugs (e.g. tamoxifen); and steroid modifying enzymes have been solved in complex with steroidal anti-tumour drugs (e.g. exemestane, Abiraterone). Although X-ray crystallography is essential for structure-based drug design, the vast chemical space prevents experimental analysis of all interacting compounds. To design novel anti-tumour steroidal compounds, we use X-ray crystallographic data from protein-ligand complexes as templates for molecular dynamics, Monte Carlo and molecular docking simulations using freely available resources. X-ray structures of proteins in complex with steroidal drugs used to treat hormone-dependent cancers were chosen: estrogen receptor, androgen receptor , aromatase (CYP19), 17 -hydroxylase (CYP17), 17 -HSD family enzymes and aldo-keto reductases (AKR1Cs) [1]. We use *in silico* methods to ask: Is it possible to reliably model new receptor-ligand complexes using X-ray structural data from known receptor-ligand structures. Simulation results are correlated with *in vitro* and anti-proliferation tests using human cancer cells. In general, *in silico* computational methods appear to predict the molecular targets of steroidal compounds, and can refine protein X-ray crystallographic data to model new ligand binding geometries (Fig 1) [2-4]. However, modelling ligand binding starting from *apo* structures is likely to fail. Moreover, for flexible ligand sites, X-ray structures of chemically similar ligand complexes appear necessary, combined with refinement by molecular dynamics or Monte Carlo. Thus, additional X-ray structures of proteins bound to diverse steroidal ligands are necessary for design of improved anti-cancer steroidal compounds.

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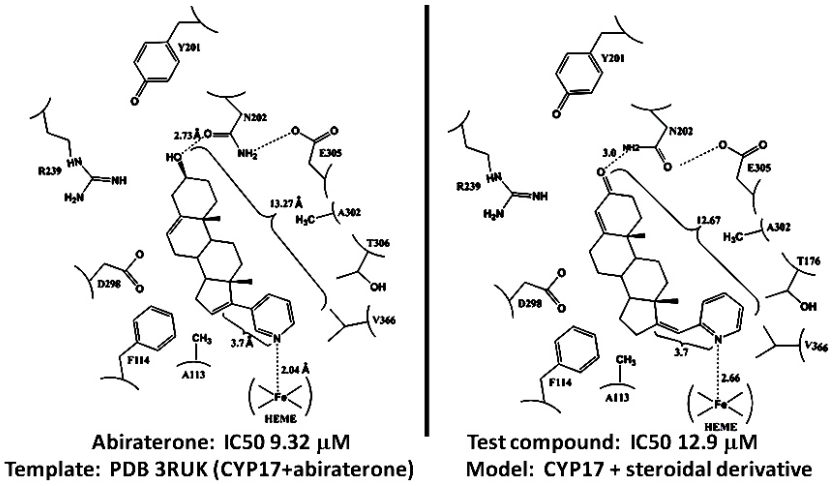

Figure 1. Example: modelling binding by new steroidal derivatives using X-ray structural data (CYP17 + anti-cancer drug Abiraterone). Predicted binding energies correlate with anti-proliferative activity.