FA1-MS06-T01

The Thermodynamic Influence of Trapped Water Molecules on a Protein–Ligand Interaction. Christian M. Stegmann[a,d], Daniel Seeliger[b], George M. Sheldrick[c], Bert L. de Groot[b], Markus C. Wahl[a,d]. [a]*Research Group X-ray Crystallography and* [b]*Computational Biomolecular Dynamics Group, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany.* [c]*University of Göttingen, Department of Structural Chemistry, Tammannstrasse 4, 37077 Göttingen, Germany.* [d]*Freie Universität Berlin, AG Strukturbiochemie, Takustrasse 6, 14195 Berlin, Germany.*
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The rational design of protein-binding substances requires an in-depth understanding of the energetics of protein–ligand interactions, including the free energy (ΔG), enthalpy (ΔH), entropy (ΔS), and the change of heat capacity at constant pressure (ΔC_p) upon binding. Previously, the ΔC_p of binding has been correlated with changes in the accessible surface areas of the interacting species. The effects of solvent (typically water) and solutes (for example ions) buried upon complex formation were mostly neglected in these considerations; yet it is well known that water can form non-covalent bonds to both the protein and the ligand. We investigated the thermodynamic consequences of trapping water molecules in the interaction of human cyclophilin G (CypG; an enzyme exhibiting peptidyl-prolyl *cis/trans* isomerase (PPIase) activity) and the immunosuppressive cyclic peptide cyclosporin A (CsA) [1].
Crystal structures at 0.75 Å and 0.80 Å resolution of the PPIase domain of CypG alone and in complex with CsA rationalize the differential thermodynamic contributions as determined by isothermal titration calorimetry. Molecular dynamics simulations give insight into the dynamics of this interaction and reveal prolonged residence times of water molecules in a cavity formed between the inhibitor and protein. Comparison of the CsA binding of the wild type CypG to a point mutant allowed us to single out the influence of trapped solvent molecules on the heat capacity change while excluding a significant differential contribution from buried surface areas.

[1] Stegmann, C. M., Seeliger, D., Sheldrick, G. M., de Groot, B. L. and Wahl, M. C. *Angewandte Chemie International Edition* (2009) 48[28]:5207-5210

Keywords: thermodynamics of biomacromolecules, molecular dynamics, drug design

FA1-MS06-T02

How Serpins Recognize and Inhibit Serine Proteases. James A. Huntington. *University of Cambridge, Department of Haematology, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Hills Road, Cambridge, CB2 0XY, United Kingdom.*

The serpins constitute a protein family whose primary role is the inhibition of serpin proteases. They utilize a unique conformational/topological change mechanism to bait and then trap proteases as the acyl-enzyme intermediate. This mechanism has several advantages over the static lock-and-key type mechanism shared by all other serine protease inhibitor families (e.g. Kunitz, such as BPTI). In this presentation I will describe the serpin mechanism, and two examples where a high level of regulatory control is afforded by the serpin fold and conformational plasticity. Antithrombin (AT) and heparin cofactor II (HCII) are plasma serpins that inhibit coagulation proteases. The circulating conformation of these serpins is of low activity in order to allow coagulation to occur where and when it is needed, but when bound to the cofactor heparin (or other glycosaminoglycans such as heparan sulphate or dermatan sulphate) the rate of inhibition is accelerated by 3-4 orders of magnitude. The molecular basis behind the regulation of AT and HCII has been established by solving crystal structures of the serpins, alone and in complex with cofactors and proteases, and illustrates why the serpins are in control of the tightly regulated processes critical for life.

FA1-MS06-T03

S. pombe Rpn12 and ubiquitin compete for binding to Rpn10. Jonas Boehringer[a], Christiane Riedinger[a], Jean-Francois Trempe[b], Jane Endicott[a]. [a]*Department of Biochemistry, University of Oxford, UK.* [b]*Department of Biochemistry, McGill University, CA.*
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Schizosaccharomyces pombe Rpn10 is a proteasomal ubiquitin (Ub) receptor located within the 19S regulatory particle of the proteasome where it binds to subunits of both the base and lid sub-particles. We have generated a model for full-length SpRpn10 by determining the crystal structure of the VWA domain and characterizing the full-length protein by NMR. We demonstrate that the SpRpn10 ubiquitin interacting motif (UIM) binds to SpRpn12. This is the first observation of a UIM binding a protein other than ubiquitin and suggests that SpRpn12 could modulate the activity of SpRpn10 as a proteasomal Ub receptor. We further show that the single UIM of SpRpn10 forms a 1:1 complex with K48-linked diUb, which it binds selectively over monoUb and K63-linked diUb. This selectivity results from steric contributions and additional interactions with a conserved linker between the SpRpn10 VWA domain and UIM motif.

Keywords: ubiquitin, proteasome, protein degradation

FA1-MS06-T04

Neutron diffraction structure of Antifreeze Protein leads to ice-binding model. A. Podjarny[a], M. Blakeley[b], M. Haertlein[b,c], I. Petit-Haertlein[b,c], I. Hazemann[a], A. Cousido[a], C. Mueller-Dieckmann[d] ,A. Popov[d], A. Mitschler[a], E. Howard[e]. [a]*IGBMC, CNRS, Université de Strasbourg, INSERM,1 rue Laurent Fries, Illkirch, France.* [b]*Institut Laue Langevin, 6, rue Jules Horowitz, Grenoble, France.* [c]*ILL-EMBL Deuteration Laboratory, Partnership, for Structural Biology, 6 Rue Jules Horowitz, 38042 Grenoble, France.* [d]*ESRF, 6 Rue Jules Horowitz, 38042 Grenoble, France,* [f]*IFLYSIB, CONICET, Calle 59, 789 La Plata, Argentina.*
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Recent advances in preparation of perdeuterated crystals for neutron diffraction and detectors have significantly decreased the required volume for measurement of useful data. This has opened the way to study biological systems with crystal volumes around 0.1-0.2 mm^3, as shown by our studies of type III antifreeze protein (AFP, 7 kDa). The highly homologous AFP sub-family shares the capability to inhibit ice growth in vivo at subzero temperatures. X-Ray crystallography studies have shown an Ice Binding Surface (IBS) through which the AFP binds the ice nuclei. The details of the antifreeze mechanism remain still unclear due to the known difficulty in identifying hydrogen atoms and disordered water molecules with X-ray diffraction data alone. Therefore, neutron studies were started. Perdeuterated crystals were produced and their qualities, together with the structural identity vs. the hydrogenated protein form, were checked by synchrotron X-ray data collection up to 1.05Å resolution at 293K. Neutron Laue diffraction data were collected up to a resolution of 1.85Å at 293K on the new Laue Diffractometer LADI-III at ILL in Grenoble with a "radically small" crystal of volume 0.13 mm^3 [1]. The structure has been refined using a joint X+N algorithm, the water molecules facing the IBS have been identified and a model of the IBS-ice interface has been built. Experimental details and current status of the project will be described. This example highlights the capability of Neutron Protein Crystallography to study biological systems at both the protonation and hydration levels with "tiny" perdeuterated crystals. This overcomes the major bottleneck of the large crystal volume needed so far for neutron diffraction, opening new perspectives to the structural biology community.

[1] I. Petit-Haertlein, M. Blakeley, E. Howard, I. Hazemann, A. Mitschler, M. Haertlein and A. Podjarny *Acta Cryst.*. F**65**, 406–409, 2009

Keywords: neutron crystallography, antifreeze proteins, macromolecular interactions

FA1-MS06-T05

Characterizing subtype specificity in nAChRs : hints to smoking cessation.

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Tobacco smoking is a major cause of mortality and accounts for around 30% of cancer related deaths in developed countries. Tobacco dependence arises from inhaled nicotine, an alkaloid which activates dopaminergic reward pathways by targeting nicotinic acetylcholine receptors (nAChRs) in the central nervous system. These nAChRs are members of the Cys-loop receptor family of ligand-gated ion channels, and form functional transmembrane protein assemblies composed of homo- or heteropentamers. Nicotine can target different nAChR subtypes but displays high affinity binding for the α4β2 nAChR subtype. Various nicotine replacement therapeutics have been devised to target the α4β2 nAChR subtype in order to help in smoking cessation. However, the variety of existing nAChR subtypes, together with the lack of high resolution structures for these proteins contribute to an as yet unresolved ligand selectivity issue.
Our goal resides in the structural and biophysical characterization of nicotinic acetylcholine receptor ligand binding sites and aims at defining those residues key to receptor subtype selectivity. We have used molluscan acetylcholine binding protein (AChBP), homologous to the extracellular ligand binding domain of nAChRs, as a surrogate to investigate the binding mode of different α4β2 selective compounds. We have solved structures of *Aplysia californica* AChBP in complex with cytisine and with varenicline, two anti-smoking compounds, and have compared the respective binding modes to that observed for an AChBP-nicotine complex. We have further investigated the importance of different binding site residues likely to account for subtype selective ligand binding in nAChRs.
Structural and biophysical data extracted from this study will allow us to better apprehend the nature of different nicotinic acetylcholine receptor binding sites, and particularly aspects that bring about ligand binding specificity. These data will be invaluable in terms of future drug design prospects.

Keywords: nicotinic acetylcholine receptors, ligand binding, x-ray structure