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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

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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.

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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

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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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