of CRT and lays a structural groundwork for future studies of the role of CNX/CRT in diverse biological pathways.

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Fluorogen-activating proteins

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Using phage display technology, antibody V_L or V_H domains have been selected for their binding to non-fluorescent dye molecules. The dye molecules (fluorogens) then become fluorescent [1] while bound within a dimer of the antibody fragments. These fluorogen-activating proteins (FAPs) are being developed for use as tags for the fluorescent imaging of proteins. Antibody-based FAPs can utilize different arrangements of immunoglobulin variable light and heavy domains (V_L-V_H, V_L-V_L, or V_H-V_H), with different combinations of V_L and V_H domains causing significant differences in fluorescence output. Crystal structures have been determined for the FAP L5-MG(L89S), in the unliganded and malachite green-bound forms, to 1.93 and 2.45Å, respectively. The structure of the malachite green-bound L5-MG(L89S) reveals an unusual homodimeric arrangement of human lambda V_L domains with the malachite green fluorogen sandwiched in between V_L subunits. The dimeric arrangement of the V_L domains is different from any seen thus far for V_L dimers. A similar homodimer is found for in the crystal of the unliganded L5-MG(L89S), although this V_L domain exists as a monomer in solution. The structural results are being used as a guide for design and improvement of future FAP constructs.

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Crystal structure of an aminopeptidase important in antigenic peptide processing

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Two highly homologous endoplasmic reticulum aminopeptidases, ERAP1 and 2, trim antigenic peptide precursors down to the mature epitopes for binding onto nascent MHC class I molecules. The two enzymes operate in concert and have to process a vast variety of different epitope precursor sequences. In this study we report the structure of ERAP2 at 3Å resolution and compare it to recently solved structures of ERAP1. The structure of ERAP2 resembles the structure of ERAP1 in "closed" conformation [1]. Domain IV in ERAP2 is in close proximity to domains I and II, forming a closed large internal cavity. This cavity extends away from the active site and is large enough to accommodate even the longest of antigenic peptide precursors. Amino acid side chains lining the interior of this cavity form shallow specificity pockets with distinct patterns, which can account for different specificity patterns between ERAP1 and 2. A crystallographic dimer formed through the interaction of the N-terminal domains may constitute a model for a proposed ERAP1/2 heterodimer. Overall, the ERAP2 structure provides insights on how two homologous aminopeptidases have evolved to be able to cooperate in order to trim a very large variety of peptidic sequences of relatively long peptides, a function fundamental to their biological role in antigen processing.

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Oppermann *Proc. Natl. Acad. Sci. USA* **2011**, doi/10.1073/pnas.1101262108

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Structure-Energy Relationship of Biological Halogen Bonds: Development of Anisotropic Force Fields

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Halogen bonds (X-bonds) result from an electrostatic attractive interaction between the electropositive crown of a polarized halogen, X, and an electron-rich Lewis base or accepting atom, A, resulting in an X—A distance closer than the sum of their traditional van der Waals radii. X-bonds have been shown to direct protein ligand recognition and binding [1] as well as the conformation of biological molecules [2].We have demonstrated via high-resolution x-ray crystallography the ability of X-bonds to direct the isomeric conformation of DNA Holliday Junctions [2]. The stacked-X junctions can isomerize between two conformations; an X-isomer stabilized by X-bonding at the junction crossover, or the H-isomer stabilized by a hydrogen bonding (H-bonding) at the junction crossover leaving the halogen on the outside strand. The structures of DNA Holliday junctions incorporating fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) halogenated uracil were determined by single crystal x-ray diffraction from 1.6 to 2.2 Å resolution. DNA junctions that formed the X-isomer were found to have a junction stabilizing X-bond between the C5 halogenated uracil and a phosphate oxygen. The angle of approach of the oxygen towards the halogen was near linear with respect to the halogen sigma bond, consistent with current halogen polarization and sigma hole theory. These structures show X-bonding interactions primarily with the π-orbital electrons of the phosphate oxygen, similar to interactions seen with proteins. The ratio of each isomer observed in the crystal structure was determined via occupancy titration calculations in which the halogen occupancy are varied to determine the percent of junction in the X- vs. H-isomer. We have shown that this ratio is correlated with the isomeric concentrations present in solution and therefore an indication of stabilization energy provided by either the X- or H-bonding. We have supported this conclusion by differential scanning calorimetry of identical junctions in solution. We observe that halogen polarization, which increases from F > Cl > Br > I, affects both the X-bond structure

and strength. The resulting structure and energy relationships of observed X-bonding interactions will be employed in development and parameterization of an anisotropic force field to accurately model the electrostatic and geometric treatment of halogens in current modeling programs. This will facilitate the applications of X-bonding interactions as a tool for biomolecular design and engineering.

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Surface flexibility of Plk1 – implications in substrate binding and drug design

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Polo-like kinase 1 (Plk1) is a serine/threonine kinase, crucial for successful progression of the cell through mitosis. Its catalytic activities are regulated by an extensive array of phosphorylation-dependent protein-protein interactions mediated by its C-terminal polo-box domain (PBD). To date, the molecular mechanism explaining how a single phosphate-binding site can bind many different partners with exquisite spatial and temporal regulation remains unclear. Since Plk1 is overexpressed in a number of tumours, targeting these protein-protein interactions represents an attractive alternative to the application of ATP-competitive inhibitors in cancer therapy. [1]

To gain insight into the mechanism of its molecular recognition, we performed extensive crystallographic characterization of PBD interactions with known phosphopeptide ligands, leading to crystallization of the protein in several different crystal forms. We examined the crystal-packing interactions (biologically irrelevant interactions between the protein molecules in the lattice), identifying a region of protein flexibility adjacent to the phosphate binding site, forming a new potential binding pocket involved in a crystal contact. Consideration of the residues interacting with the pocket allowed us to speculate on the molecular recognition motif causing the surface rearrangement and identify potential ligands utilizing this newly discovered site in combination with binding to the phosphate pocket. A combination of bioinformatics, molecular dynamics simulations, biophysics, site-directed mutagenesis and protein crystallography validated the new binding pocket giving an insight to its molecular recognition.

Consequently, we have shown its importance in binding of polo-box interacting protein 1 (PBIP1), a mitotic scaffold protein responsible for the correct localization of Plk1 during the mitosis process. We believe that the conformational change and involvement of the hydrophobic pocket in this interaction allows PBD to achieve better selectivity towards PBIP1 over other ligands. [2]

The potential application of the new binding site and surface flexibility in the development of molecular therapeutics targeting PBD of Plk1 was subsequently explored. To assess the ability of particular chemical moieties to affect the conformation of the protein surface, a series of small molecules were fused to the anchoring peptides, designed to bring them close to the surface patch of interest. A combined biophysics and crystallography approach led to finding molecules utilizing the newly discovered binding site with unprecedented affinity (Kd = 20 nM). At the same time, a high-throughput biophysical assay for preliminary binding mode determination has been developed.

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The molecular basis of MAPK specificity and fidelity

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The p38 mitogen-activated protein kinase (MAPK) pathway directs the cellular response to environmental stresses and/or inflammatory cytokines. Thus, stringent control of p38 activity, orchestrated by activating kinases and inactivating phosphatases, is essential to maintain cell function. These critical regulators interact with p38 via a conserved ~15 residue motif (D-motif or kinase interaction motif (KIM)). Every KIM binds to a common docking groove composed of a hydrophobic pocket and a basic patch, the CD site. Nevertheless, while it is clear that the KIM is necessary for p38 association, we have demonstrated that additional interactions contribute to selectivity.

Hematopoietic tyrosine phosphatase (HePTP), a critical regulator of p38 and Erk2 activity in immune cells, contains a C-terminal tyrosine phosphatase domain and an N-terminal, flexible extension which includes a KIM. Here we present novel insights into how p38 achieves selectivity by interacting with residues outside the HePTP KIM. As this system is highly dynamic, we combined small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) spectroscopy, biochemical studies and now, x-ray crystallography, to determine, for the first time, how p38 interacts with HePTP. These studies have revealed: 1) the mechanism of KIM peptide binding and, more importantly, selectivity; 2) how the kinase specificity sequence (KIS), which is C-terminal to the KIM, generates specificity and 3) that inactive p38 and HePTP associate in a highly extended manner such that p38 does not interact with the HePTP catalytic domain, but instead only binds HePTP via its flexible N-terminal extension. In accordance with these results, we will present the first solution structure of a MAPK bound to a key regulatory protein.

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Structure of the plakin domain of plectin by SAXS

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Plectin is a member of the plakin family of high molecular weight proteins that interconnect elements of the cytoskeleton and tether them to membrane associated structures, also known as cytolinkers. Plectin (~500 kDa) has a tripartite structure consisting of N- and C-terminal regions separated by a central rod domain, a structure which is also found in other epithelial plakins such as BPAG1, desmoplakin,