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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Keywords: halogen bond, dna holliday junction, force field

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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,