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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Keywords: MAP kinase, specificity

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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,

periplakin, and envoplakin. The N-terminus of plectin contains an actin binding domain (ABD) build up of a tandem pair of calponin homology domains (CH1 and CH2) and a ~1000 residue long region termed the plakin domain that is conserved among the protein family. The plakin domain of plectin contains protein-protein interaction sites that are important for the localization of plakins at junctional complexes. We have previously identified eight canonical Spectrin Repeats (SR) in the sequence of the plakin domain of plectin (SR1 to SR5 and SR7 to SR9), and an additional shorter SR-like domain (SR6). The SR2 and SR3 of plectin are connected by a ~20-residues long linker predicted to be non-helical, while repeats SR3 to SR9 occur contiguous in the plectin sequence. Despite the existence of several crystallographic structures of isolated pairs of spectrin repeats, there is not structural information on the global shape and conformation of the plakin domain of plectin. Here, we use Small-Angle X-ray Scattering (SAXS) to generate low resolution models of the whole plakin domain and smaller multi-repeat fragments. The available plectin crystallographic structures are further used to generate pseudo-atomic models, either by docking or rigid-body modelling procedures.

Keywords: SAXS, plectin, spectrin repeats

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Low resolution structure in solution and amyloidogenesis of human cystatin C

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Human cystatin C (HCC) is a cysteine proteases inhibitor which inhibits proteins belonging to the papain and legumain families. The general fold of monomeric form of cystatin has been defined by the three-dimensional structure of chicken cystatin. Crystallographic studies of native HCC (tetragonal [2] and cubic [1] crystal forms) have shown that the protein exists in crystal in the form of symmetric three-dimensional domain-swapped dimers. Human cystatin C is also strictly related to the occurrence of Iceland type amyloidosis. HCC Leu68Gln mutation causes protein oligomerization and deposition of amyloid fibrils.

Insoluble amyloid fibrils are found as deposits in patients with a range of conformational diseases, e.g. Alzheimer disease, reactive amyloidosis or cystatin C amyloidosis. [3-5]. Knowledge of the molecular mechanism causing the transition of physiologically normal and soluble proteins to toxic oligomers and insoluble fibrils is essential for the development of treatment strategies for this group of common, but currently incurable, diseases.

In this study we perform a small angle scattering experiment to probe the conformation and low resolution structure of native form of HCC and mutants Val57Pro, Val57Asn, Val57Asp to probe the effect of this mutations on cystatin C structure in solution. The aim of our study was also the characterisation of the formation of HCC oligomers and amyloids in different pH and temperature conditions. SAXS measurements were preformed on the X-33 EMBL beamline at DESY, Hamburg (Germany) using the Pilatus photon counting detector.

Using *ab initio* program DAMMIN [6] we created low resolution 3D models of native and mutated cystatin C in solution. HCC form a dimer in solution with elongated conformation as in tetragonal crystal form. The processes of formation of oligomers and fibrils were also monitored using SAXS in 1h time steps.

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Biological small angle scattering - A tool for gaining structural insight when other methods aren't enough

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As the number of protein structures increases so does the complexity of biological systems under study and the difficulty of studying more complex structural problems. Small angle X-ray scattering (SAXS) techniques have emerged as complementary tool for the structural biologist to extract structural information from biological systems. Though the comparative information gleaned from SAXS is of significantly lower resolution, SAXS methods offer an avenue to study macromolecules in near physiological conditions and to glean structural information where other methods, such as X-ray crystallography, fall short. In particular, SAXS has proven an ideal method to study macromolecules in partially disordered environments and to monitor structural changes, including small perturbations due to ligand binding or environmental.

Here we present data collected on the BioSAXS-1000 system, a small angle scattering system designed for macromolecular samples. These studies were aimed at identifying standard proteins for biological SAXS experiments and to evaluate good practices for collecting small angle scattering data on home laboratory systems. These results show that the BioSAXS-1000 system provides synchrotron-quality SAXS in as little as 15 to 30 minutes for most protein samples. The design inherits it strength from a combination of specially designed focusing optics coupled to a microfocus rotating anode and a high sensitivity hybrid pixel array detector. Unlike traditional Kratky systems, this system produces a point focused beam thus eliminating smearing issues common to traditional Kratky camera systems. This presentation will summarize the data collection and processing results for various standard samples collected on the BioSAXS-1000 system.

Keywords: bioSAXS, small angle X-ray scattering, structural biology

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Towards the three-dimensional structure of human small heat-shock proteins B1 and B6

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