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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Keywords: human cystatin C, SAXS, amyloid

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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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Small heat-shock proteins (sHSPs) are a family of evolutionary conserved ATP-independent chaperones. These proteins share a common architecture defined by a signature α-crystallin domain (ACD) flanked by highly variable N and C-terminal extensions. The ACD, which has an immunoglobulin-like fold, plays an important role in sHSP assembly, mediating dimer formation of individual protomers, that then may assemble into larger oligomers. In vertebrate sHSPs the dimer interface is mediated by the symmetrical antiparallel pairing of two β-strands (β7), resulting in the formation of an extended β-sheet on one face of the ACD dimer. Recent crystallographic studies of isolated ACDs from a number of vertebrate sHSPs suggest a variability in the register of the β7/β7 interface, which may, in part, give rise to the polydispersity often seen with the full-length proteins. To further analyse the structure of ACD dimers we have employed a combination of X-ray crystallography and solution small-angle X-ray scattering (SAXS) to study the ACD-containing fragments of human HSPB1 and HSPB6. Unexpectedly, the obtained crystal structure of the HSPB1 fragment does not reveal the typical β7/β7 dimers, but rather hexamers formed by an asymmetric contact between the β4 and β7 strands from adjacent ACDs [1,2]. Nevertheless, in solution, both ACDs form stable dimers via the symmetric antiparallel interaction of β7 strands. Using SAXS, we show that it is possible to discriminate between different putative registers of the β7/β7 interface, and that under physiological conditions there is only a single register of the strands for both proteins [2]. Furthermore, we have solved the crystal structure of a fragment of HSPB6 including a portion of its N-terminal extension, the ACD and the C-terminal extension. The structure reveals a stable tetramer, as also confirmed by the SAXS data in solution. The novel tetramer formation is possible due to specific 'patching' of the β4/β8 side of the ACDs by a short hydrophobic motif found in the N-terminal extension.

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Keywords: small heat-shock protein, crystallography, small-angle X-ray scattering

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Using raman and fluorescence spectroscopies in protein crystallography

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Structural biology increasingly relies on the application of complementary methods to the same protein crystals that are used during X-ray crystallography experiments. We present here a comprehensive ensemble of improved spectroscopic setups aimed at analysing nano-volume samples, such as protein crystals or small amounts of concentrated solutions, by UV/vis absorption, fluorescence or Raman spectroscopy. *In crystallo* spectroscopy allows to verify the state of protein crystals in comparison to its solution state and, in combination with crystallographic data, to correlate structure and function. These techniques can be directly carried out on synchrotron beamlines, yielding immediate complementary information during X-ray data collection.

On the one hand, this yields information about, *e.g.*, the redox state of a metalloenzyme, the binding of a ligand, or the state of a photoactive protein. On the other hand, the onset and progression of X-ray radiation damage can be accurately monitored, as spectroscopic techniques are significantly more sensitive to subtle radiation damage than global crystallographic indicators such as changes of B-factors or loss of diffraction quality. Raman spectroscopy yields a wealth of information about chemical bonds at sub-atomic resolution and has opened considerable perspectives as a complementary tool to X-ray diffraction [1], and can be employed, for instance, to observe and quantify X-ray induced reduction of disulphide bonds in proteins [2].

In another application, we have studied the structural bases for the improved fluorescence properties of various green [3] and cyan [4] mutants of Green Fluorescent Protein. In particular, UV/vis absorption and fluorescence spectroscopies were used to monitor the X-ray induced bleaching of these fluorescent proteins, while crystallographic data recorded in parallel gives insights into the underlying structural changes.

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Structure of the second pair of fibronectin type III repeats of the integrin β4

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The integrin α6β4 is a component of the hemidesmosomes (HD), protein complexes that mediate the stable anchoring of basal epithelial cells to the basement membrane [1]. The cytoplasmic domain of the β4 subunit is unique among the integrin family and it is responsible for most of the intracellular interactions of α6β4, including the interaction with other hemidesmosomal components. The cytoplasmic region of β4 contains four fibronectin type III domains (FnIII1 to FnIII4) arranged in two pairs separated by a region named the connecting segment. Upstream of the FnIII1 domain there is a Calx- β domain [2], while a 90-residue long C-terminal tail extends downstream of the FnIII4 domain. It is proposed that prior to HD assembly the cytoplasmic domain of β4 adopts a closed conformation stabilized by an intramolecular interaction between the connecting segment and the tail; binding to plectin would unleash β4 and favour the association of β4 with other components of the HD [3].

We have combined x-ray crystallography, small angle x-ray scattering (SAXS), mutagenesis, and biochemical analysis to characterize the second pair of FnIII domains of β4. The crystal structure of the FnIII3 was phased by molecular replacement and it was refined against data to 1.6 Å resolution. The crystal structure of the FnIII4 was phased by single isomorphous replacement with anomalous scattering using a mercurial derivative, and the structure was refined against native data extending to 1.8 Å resolution. The structure of the FnIII3-FnIII4 region was analyzed in solution by using SAXS.