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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.

[1] Baranova et al *Acta Crystallogr Sect F* **2009,** 65, 1277-81. [2] Baranova, Weeks et al *J. Mol. Biol.* **2011,** in the press.

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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.

[1] J.E. McGeehan, D. Bourgeois, A. Royant, P. Carpentier, *Biochim. Biophys. Acta* **2011**, in press. [2] P. Carpentier, A. Royant, M. Weik, D. Bourgeois, *Structure* **2010**, *18*, 1410-1419. [3] A. Royant, M. Noirclerc-Savoye, *J. Struct. Biol.* **2011**, *174*, 385-390. [4] M. Lelimousin, M. Noirclerc-Savoye, C. Lazareno-Saez, B. Paetzold, S. Le Vot, R. Chazal, P. Macheboeuf, M.J. Field, D. Bourgeois, A. Royant, Biochemistry **2009**, *48*, 10038-10046.

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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.

The FnIII3-FnIII4 tandem has a radius of gyration (Rg), calculated from the SAXS data, of 21 Å, which is significant smaller than the Rg of the FnIII1-FnIII2 (~29 Å) indicating that the second pair of FnIII domains adopts a more compact structure than the first pair of FnIII domains. We have modelled the low resolution structure of the FnIII3-FnIII4 region by using the SAXS data and *ab initio* methods. This region has a heart-shaped structure. The limited resolution of the SAXS-based model hinders the unequivocal docking of the high resolution structures into the molecular envelope. Thus, we have used structure-based Cys-scanning mutagenesis combined with crosslinking experiments to obtain distance restraints that will help us to elucidate the relative orientation of the FnIII3 and FnIII4 in solution. Our results have implications for the organization of the integrin β4 subunit and for its mechanisms of auto-inhibition and activation.

[1] S.H. Litjens, J.M. de Pereda, A. Sonnenberg, *Trends Cell Biol* **2006**, *16*, 376-383. [2] N. Alonso-García, A. Inglés-Prieto, A. Sonnenberg, J.M. de Pereda, *Acta Cryst D.* **2009**, *65*, 858-871. [3] K. Wilhelmsen, S.H. Litjens, I. Kuikman, N. Tshimbalanga, H. Janssen, I. van den Bout, K. Raymond, A.J Sonnenberg, *Cell Biol* **2005**, *171*, 799-810.

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Raman assisted X-Ray crystallographic study of nitric oxide binding to deoxygenated hemoglobins

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Nitric oxide (NO) is a signaling molecule that regulates essential physiological processes, including neurotransmission, vasodilatation, and blood clotting [1].

The heme groups of deoxy hemoglobin (Hb) bind NO very strongly, almost irreversibly, with a $K_{diss} = 0.9 \times 10^{-12}$ M. The kinetic constant of NO dissociation from $Hb(NO)_4$ increases as the reaction progresses, indicating that partially NO-saturated T-state Hb has a lower NO affinity than fully bound, R-state, Hb [2].

Previous spectroscopic and crystallographic studies have shown that NO binding to the heme groups of T-state human hemoglobin (HbA) produces the breakage of Fe-proximal histidine bonds at the α-subunits but not at the β-subunits [3]. NO can also react with the thiol group of the Cys93β of HbA [4].

Until now there are very few crystallographic structures of nitrosyl-hemoglobin, due to the high and various reactivity of these species.

Here we report a Raman-assisted crystallographic study of the NO binding to the hemoglobin isolated from the Antarctic fish Trematomus bernacchii (HbTb). HbTb is endowed with the Root effect, i.e. a drastic drop of cooperativity at acidic pH [5,6]. The crystal structures of the nitrosylated form of T-state HbTb, crystallized at pH 6.2 and 8.4 (HbTb6NO and HbTb8NO), have been solved. These structures and the Raman spectra have been compared to those of nitrosylated HbA, reported in literature [3,7]. The main results of the analysis reveal a different behavior of α and β chains. In particular, in both HbTb6NO and HbTb8NO, the α-heme is nitrosylated and shows a six-coordination, whereas the iron ion at β-heme is clearly oxidized in high spin form.

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[1] S. Moncada, R.M. Palmer, E.A. Higgs, *Pharmacol Rev* **1991**, *43(2)*, 109-142. [2] E.G. Moore, Q.H. Gibson, *J Biol Chem* **1976**, *251(9)* 2788-2794. [3] N.L. Chan, J.S. Kavanaugh, P.H. Rogers, A. Arnone, *Biochemistry* **2004**, *43(1),* 118-132. [4] N.L. Chan, P.H. Rogers, A. Arnone, *Biochemistry* **1998**, *37*, 16459-16464. [5] A. Vergara, L. Vitagliano, A. Merlino, F. Sica, K. Marino, C. Verde, G. Di Prisco, L. Mazzarella, *J Biol Chem* **2010**, *285*, 32568-32575. [6] L. Mazzarella, A. Vergara, L. Vitagliano, A. Merlino, G. Bonomi, S. Scala, C. Verde, G. di Prisco, *PROTEINS: Structure, Function, and Bioinformatics* **2006**, *65*, 490–498. [7] D.M. Scholler, M.Y. Wang, B.M. Hoffman, *J Biol Chem* **1979**, 254(10), 4072-4078.

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Small Angle X-ray Scattering Studies of Effect of pH and Temperature on the Global Compactness on Cellobiohydrolase I from *Trichoderma harzianum*

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Cellulases produced by filamentous fungi are widely used in biotechnological applications, including biomass depolymerization and second generation bioethanol production [1]. To make possible the use of biomass it is necessary to degrade cellulose, a constituent of the cell wall, to fermentable sugars [2]. One form of degradation is the enzymatic hydrolysis. The complete enzymatic cellulose hydrolysis involves synergistic actions of endoglucanases (EC 3.2.1.4), exoglucanases/cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) [3]The structural knowledge of the enzymes as well as the interaction of these with the pre-treated bagasse from sugarcane during the hydrolysis is extremely important. However, stability of cellulases in defined pH and temperature ranges sets limits to their industrial utilizations. SAXS is able to give structural parameters about intact structures, as radius of gyration, maximum diameter, shape and relative position of the domains. SAXS studies have been done with cellobiohydrolase I from *T. harzianum* (*Th*CBHI). These study aims at understanding the molecular basis of the functioning of enzymes for application in the production of bioethanol from sugarcane bagasse. Our results show that pH and temperature perturbations affect *Th*CBHI stability by two different mechanisms. Variations of pH modify protonation of the enzyme residues, directly affecting its activity, but leading to structural destabilization only at extreme pH limits. Temperature, on the other hand, has direct influence on mobility, fold and compactness of the enzyme, causing irreversible unfolding of *Th*CBHI just above optimum temperature limit. Thus, our studies might provide insights into understanding, of the interplay between structure and activity of *Th*CBH1 at different pH and temperature conditions, which can be useful for possible biotechnological applications of the enzyme as bioethanol production.

[1] A.S. Bommarius, A. Katona, S.E. Cheben, Patel AS, A.J. Ragauskas, K. Knudson, Y. Pu *Metabolic Engineering* **2008**, *10,* 370-381. [2] K.A. Gray, L.S. Zhao, M. Emptage *Current Opinion in Chemical Biology* **2006**, *10,* 141-146. [3] Y. H. P. Zhang, M.E. Himmel, J.R. Mielenz. *Biotechnology Advances* **2006.** *24***,** 452-481.

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