ESRF	**Experiment title:** **Human S100A12 protein.**	**Experiment number**: LS-1532
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Names and affiliations of applicants (* indicates experimentalists):

Moroz, O.V.[1], Antson, A.A.*[1], Murshudov, G.N.[1], Maitland, N.J.[2] Dodson, G.G.[1,3], Wilson, K.S.[1], Skibshoj, I.[4], Lukanidin, E.[4] & Bronstein I.B.[1]

[1]Structural Biology Laboratory, Department of Chemistry and [2]Yorkshire Cancer Research Unit, Department of Biology, University of York, York, UK, YO10 5DD

[3]National Institute of Medical Research, Mill Hill, London, UK, NW7 1AA

[4]Department of Molecular Cancer Biology, Danish Cancer Society, Strandboulevarden 49, Copenhagen 2100, Denmark.

Report:

S100 proteins are small acidic proteins with a molecular weight in the range of 10-14 Kda, which form a subfamily of the EF-hand calcium-binding proteins. Almost all S100 proteins tend to form dimers, thought to be essential for their target binding and function (Réty *et al*, 2000). The name S100 was introduced because several proteins of this group were soluble in 100% ammonium sulphate (Moore, 1965). A12 belongs to the calgranulin subfamily; *i.e.* it is expressed exclusively in granulocytes and is relatively abundant in this type of leucocyte (Kerkhoff *et al*, 1998). The number of crystal structures of calcium-bound S100 proteins is growing rapidly. Structures of five S100 proteins have already been determined: bovine calbindin D9K, bovine S100B, human S100A7, human S100A10 and human S100A11.

For structural studies, S100A12 protein was isolated from human granulocytes. The crystals were grown by hanging-drop vapour diffusion with a protein concentration of 5.0 – 8.0 mg ml^{-1}(Moroz *et al*, 2000). The reservoir contained 0.1M sodium-cacodylate pH 6.5, 0.2M $CaCl_2$ and 20-25% PEG 5K monomethyl ester (Brzozowski, A. M., 1993). Crystals belong to the space group R3, with cell dimensions a = b = 99.6 Å, c = 64.2 Å. The initial data were collected in-house to 2.5 Å resolution. The same crystal was used to extend the resolution to 1.95 Å using synchrotron radiation at the ESRF beam line ID14-2. Several reflections in the synchrotron data had intensities above the dynamic range of the detector, therefore they were merged with the data collected in-house. The data were processed with DENZO and SCALEPACK. 48386 individual reflections were reduced to 17248 unique observations with an overall R_{merge} of 8.6 % (Table 3). The data are 99.3 % complete.

The structure was solved by molecular replacement with MOLREP (Vagin & Teplyakov, 1997) using the structure of S100B with a sequence identity of 38% (Matsumura *et al*, 1998, PDB code 1mho) as starting model. The resulting model was used for maximum likelihood refinement as implemented in the program REFMAC (Murshudov *et al*, 1997). In the early stages, rigid body refinement with each molecule as a single rigid group was employed. After convergence of rigid body refinement individual atomic refinement was

performed. In the first cycles, non-crystallographic symmetry (NCS) averaging using DM (Cowtan & Main, 1993) was used to improve the phases. The resulting phases were used for phased refinement (Pannu *et al*, 1999) with suitable blurring factors to optimise the fall of R and R_{free} values. Manual rebuilding was performed using the XFIT option (Oldfield, 1994) of the program QUANTA (Molecular Simulations). For further refinement, a bulk solvent correction as implemented in the program REFMAC was used and improved R and R_{free} values by around 2%. In the final stages, TLS refinement (Shomaker & Trueblood, 1968, Winn *et al*, 2000) was used to account for overall anisotropic motion of the molecules. TLS refinement gave a 3% drop of R/R_{free} (19.9%/24.0%), and individual atomic refinement improved R/R_{free} further to 18.3%/22.7%.

Like the majority of S100 proteins S100A12 is a dimer, with the interface between the two subunits being composed mostly of hydrophobic interactions. The fold of S100A12 is similar to the other known crystal and solution structures of S100 proteins except for the linker region between two EF-hand motifs. Sequence and structure comparison between members of the S100 family suggests that the target-binding region in S100A12 is formed by the linker region and C-terminal residues of one subunit and the N-terminal residues of another subunit of the dimer. The N-terminal region of the target-binding site includes two glutamates that are conserved in most of the S100 sequences. The comparison also provided a better understanding of the role of the residues important for intra- and inter-subunit hydrophobic interactions. The precise role of S100A12 in cell behaviour is yet undefined, as is the case for the whole family, though it is evident that the interaction of S100A12 with the RAGE receptor is implicated in inflammatory response.

References:

Brzozowski, A. M. (1993). *Acta. Cryst*. **D49**, 352-354.
Cowtan, K.D. & Main, P. (1993). *Acta Cryst.* **D49**, 148-157.
Kerkhoff, C., Klempt, M. & Sorg, C. (1998). *Biochem. Biophys. Acta*, **1448**, 200-211
Matsumura, C.H., Shiba, T., Inoue, T., Harada, S. & Kai, Y. (1998). *Structure*, **6**, 233-241.
Moroz, O.V., Antson, A.A., Dodson, G.G., Wilson, K.S., Skibshoj, I., Lukanidin, E. & Bronstein, I. (2000). *Acta Cryst*. **D56**, 189-191.
Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst*. **D53**, 240-255.
Oldfield, T. J. (1994). in Proceedeengs of the CCP4 Study Weekend (Eds Bailey, S., Hubbard, R. & Walker D.) 15-18 (Daresbury laboratory, Warrington, UK).
Pannu, N.S., Murshudov, G.N., Dodson, E.J. & Read, R.J. (1998). *Acta Cryst.* **D54**, 1284-1294.
Réty, S, Osterloh, D, Arie, J-P, Tabaries, S., Seeman, J., Russo-Marie, F., Gerke, V. & Lewit-Bentley, A. (2000). *Structure,* **8**, 175-184.
Schomaker, V. & Trueblood, K.N. (1968). *Acta Cryst*. **B24**, 63-76
Vagin, A.A. & Teplyakov, A. (1997). *J. Appl. Crystallog.* **30**, 1022-1025.
Winn, M.D., Isupov, M. & Murshudov, G.N. (2000) *Acta Cryst*., submitted for publication