	Experiment title: Solution structure and ligand induced changes in galectins and murein hydrolases	Experiment number: 16-02-60
Beamline: BM16	**Date of experiment**: From: 06-05-09 to: 09-05-09	**Date of report**: 6-04-09
Shifts:	**Local contact(s)**: Francois Fauth	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

F. Díaz*[1], M. Menéndez*[2], B. Pera*[1], E. Buzamet*[2], C. Gallego*[2]

[1]Centre for Biological Research (CIB), CSIC, Madrid (Spain)

[2] Institute of Physical-Chemistry Rocasolano, CSIC, Madrid (Spain)

Report:

We have examined the solution structure of several proteins exhibiting carbohydrate-binding activity (galectins) or proteoglycan hydrolytic activity (murein hydrolases) using SAXS. Many of them are modular proteins whose conformational plasticity might be relevant for biological activity and account for fails in the full-length protein crystallization. The spectra were collected on the BM-16 line in the high and low angle region of the SAXS profile using a 3.5m length camara coupled to a gas detector. Radiation characteristics allowed sample irradiation for 16 min without loss of the SAXS pattern. The spectra were used to generate three dimensional models at low/medium resolution of the protein envelopes using DALAI_GA (1). Identication of protein modules or subunits in the SAXS models was carried out by docking either structures available in the Protein Data Bank for the isolated modules or high resolution models generated by homology or threading methods. Compatibility of SAXS-based models with the structural and hydrodynamic information provided by other techniques is being examined to further validate the proposed structures.

Galectins:

Proteins studied were the two variants of the recently discovered chicken tandem-repeat-galectin **CG-8** containing linkers of different length (**CG-8I** and **CG-8II**) and its N-terminal module N-CG-8; **CG-3**, a "chimera" type galectin containing a C-terminal carbohydrate recognition domain (CRD), a collagen-sensitive stalk and a short *N*-terminated section acting as a target for phosphorylation; **CG-2** a "proto-dimeric" type galectin constituted by two identical CRDs linked by non-covalent interactions that shows an unusual hydrodynamic behaviour; and chicken **CG-1** as prototype of homodimeric galectins. Data of **CG-2** and **CG-3** were collected both in the absence and in the presence of lactose (a ligand for the CRD domain) while **CG-8** variants were only examined in the lactose loaded state. Figure 1 summarizes the most probable models derived from SAXS for different galectins. According to them, monomer disposition in the **CG-2** dimer (Fig. 1a) will differs from that seen in CG-1 crystallographic structure. Instead, it reminds the orientation adopted by monomers in crystals of galectin-7, which could explain the atypical hydrodynamics behaviour displayed by CG-2. On the other hand the three domains identified in **CG-3** sequence appear as differentiated regions in the full-length protein structure (Fig. 1b). Finally the two domains forming the tandem-repeat **CG-8** galectin adopt an extended disposition (Fig. 1c).

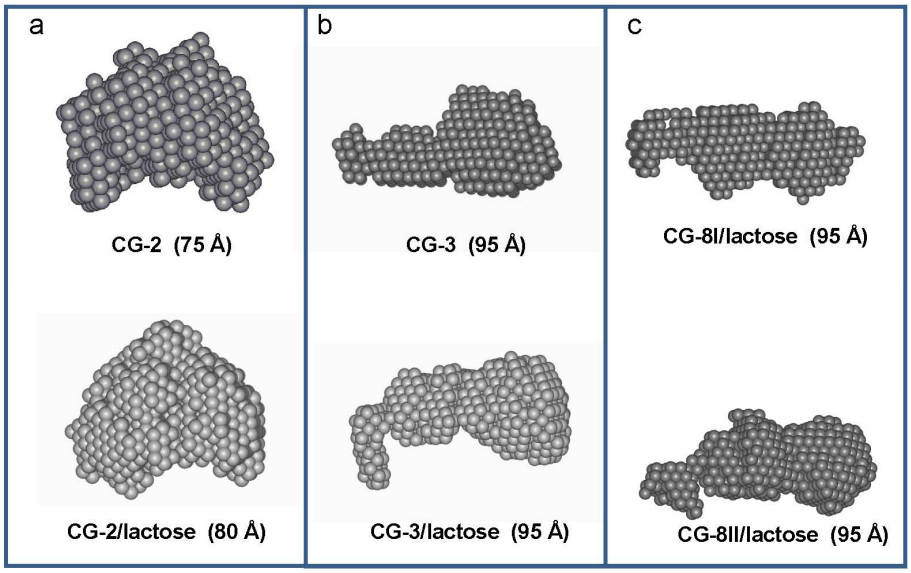

Figure 1. SAXS based models for a) proto-dimeric type **CG-2** galectin, b) chimeric-type **CG-3** galectin, and c) isoforms of the tandem-repeat type **CG-8** galectin. Figures in parenthesis are the maximum intraparticle distances calculated with GNOM (2).

Murein hydrolases:

Proteins studied were **LytC** (the pneumococcal lysozyme), **LytB** (the pneumococcal glucosaminidase), and **Skl** (bacteriophage encoded CHAP amidase). The two first were characterized in their choline loaded state while the solution structure of **Skl** amidase was examined both in the absence and in the presence of choline (specific ligand of the cell wall binding domain present in the three enzymes). Moreover, changes associated with **Skl** conversion from a low-activity E-state to a high-activity C-state upon incubation with choline or pneumococcal cell walls at low temperature was also explored by collecting data of the choline bound state before (37ºC) and after conversion (4ºC). The poor quality of SAXS data for **LytB** prevented its modelling. However good models compatible with the high-resolution structures generated, independently, for the two modules comprised in LytC structure were obtained (Fig.2 a). Moreover, data recorded in different solvents might indicate a certain conformational plasticity at the module interface in **LytC**. **Skl** profiles made evident that Skl dimerizes under choline saturating conditions both in its low- and high-activity states, although they might present differences at the dimerization surface (Fig.2 b-c). Finally, docking of the high-resolution models built for the catalytic module and the cell wall binding module of Skl were consistent with the size and shape of the monomer molecular envelope derived from SAXS (Fig. 2b).

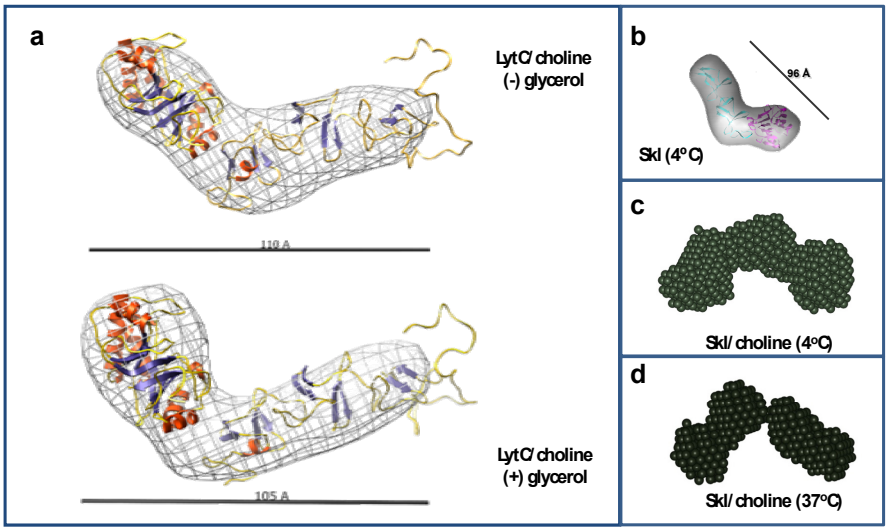

Figure 2. a) SAXS based models (grid repressentation) for the pneumococcal lysozyme **LytC** in phosphate buffer with and without glycerol. Homology models of the catalytic barrel and the cell wall binding module manually docked in the SAXS envelope are also depicted. b-d) models of **Skl** structure in the absence (b) and presence of choline (c-d) before and after conversion from E-form (37°C) to C-form (4°C). Homology models of the catalytic module (magente) and the cell wall binding module (cyan) appear docked in the monomer envelope (b). Bars indicate e maximum intraparticle distances.

Manuscripts on galectinc structures are in progress but additional experiments are required for **LytB** and to asses the structural changes evolved by **Skl** during conversion.

(1) Chacón et al., (1998). *Biophys. J.* **74**:2760-2775
(2) Svergun, D.I. (1992). *J. Appl. Cryst.* **25**:495-503