EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

	Experiment title: Solution structures and quaternary assembly of human guanylate-binding protein 1	**Experiment number**: MX-1399
Beamline:	**Date of experiment**: from: 17.06.2012 to: 18.06.2012	**Date of report**: 14.09.2012
Shifts: 2	**Local contact(s)**: Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Andreas Stadler, Forschungszentrum Jülich, Germany (*)

Clemens Sill, Forschungszentrum Jülich, Germany (*)

Christian Herrmann, Universität Bochum, Germany

Dieter Richter, Forschungszentrum Jülich, Germany

Report:

Human guanylat binding protein 1 (hGBP1) is a monomer under normal buffer conditions, in the presence of the GTP analog GppNHp the protein assambles as a dimer, and in the presence of GDP and AlF the protein is in the intermediate state between the conversion from GTP to GDP and is assumed to be a tetramer. Crystal structures of the hGBP1 monomer and the central region of the dimer exist. Biochemical analysis and single molecule FRET data indicate that the solution structure of the monomer differs from the Xtral structure, namely the so called α12/13 domains swings away from the rest of the protein. Small angle X-ray scattering was measured of hGBP1 in the different conformations. To maximise the measured signal and to correct for the effect of aggregation and interparticle effects, concentration series were measured between 1 and 30 mg/ml. The temperature was set to 10°C during the experiment.

Measured data of the monomer and the dimer are shown in Figure 1a) and 1b). The assumed tetrameric state of hGBP1 was found to have a molecular mass similar to that of the monomer.

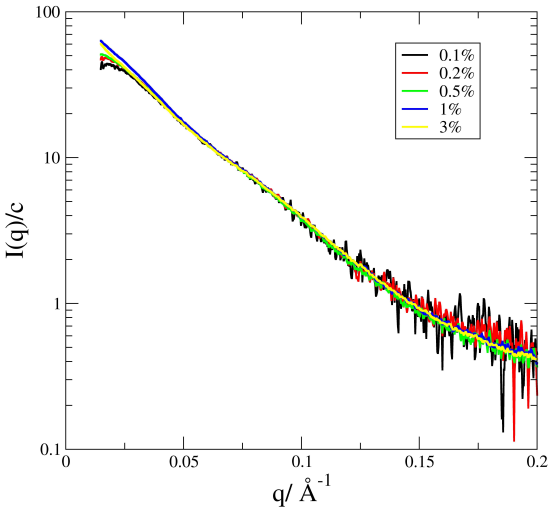

Figure 1a) SAXS data of the hGBP1 monomer.

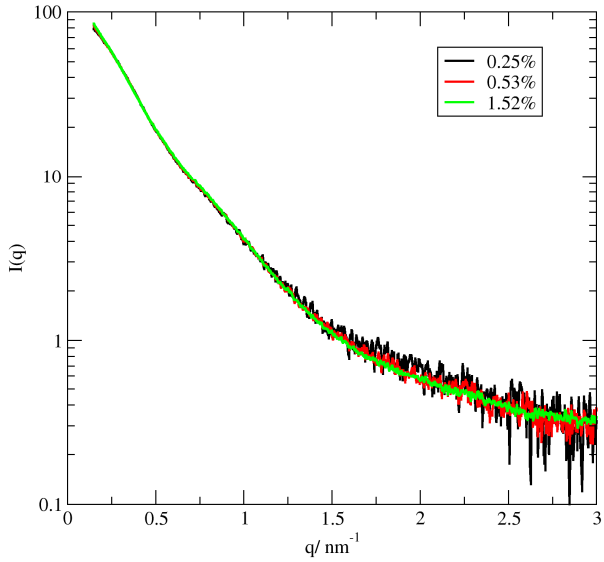

Figure 1b) SAXs data of the hGBP1 dimer.

The Xtal structure of the monomer and the calculated diffraction pattern are shown in Figure 2. The Xtal structure does not correctly describe the measured SAXS data. Rigid body refinement was used to generate a refined a solution structure. The protein was split into three domains and the *sasref* software from the Hamburg EMBL group was used to fit the rigid body domains against the measured SAXS data. The result of the rigid body refinement is shown in Figure 3. The results demonstrate that the solution structure of the monomer is more opened than the crystal structure, and that indeed the α12/13 domain swings away from the rest of the protein.

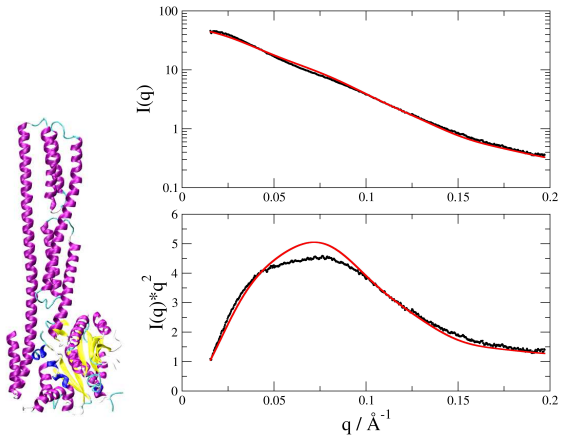

Figure 2: Crystal structure of hGBP1 monomer and the calculated diffraction pattern.

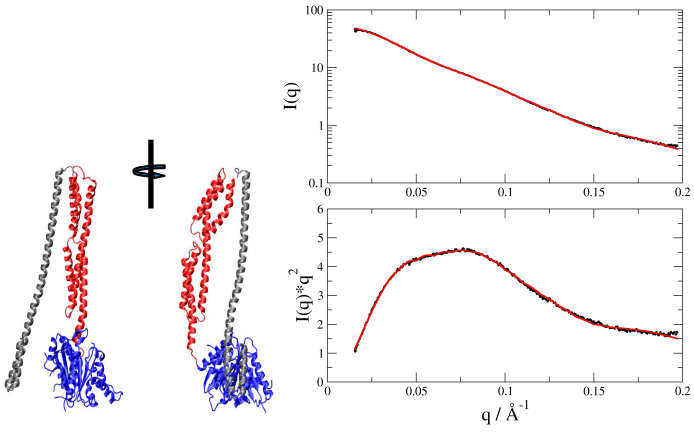

Figure 3: Solution structure of the hGBP1 monomer obtained by rigid body refinement. The calculated diffraction patteren from the solution structure agrees well with the measured data. The α12/13 domain is shown in grey.

The measured data of the dimer was interpreted using the bead modelling approach (*damif* software from the Hamburg EMBL group). The obtained low resolution shape of the dimer is shown in Figure 4. The crystalographic domains were just added to the known structure of the dimer interface and the whole crystallographic dimer structure was aligned to the bead model. The low resolution shape seams to validate the assumed crystal structure.

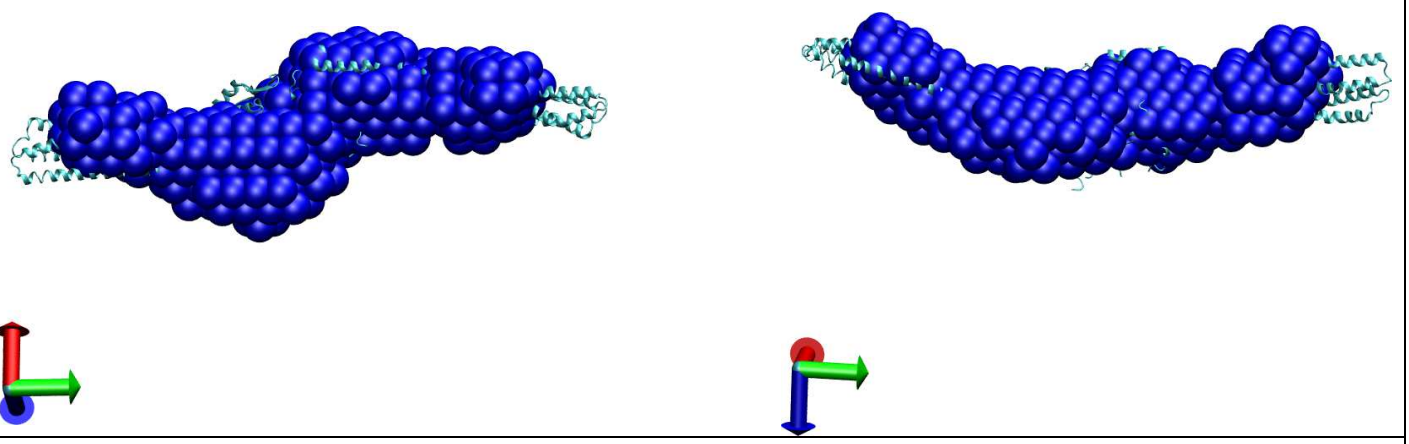

Figure 4: Bead models of the hGBP1 dimer together with the assumed crystallographic model of the dimer.

Our collaborator Prof. Claus Seidel has performed extended FRET measurements on the hGBP1 monomer and dimer. The FRET data using dye labels allowed the precise triangulation of intraprotein distances in the hGBP1 monomer and dimer.

The FRET data indicate that the ends of the α12/13 domains are in close contact in the dimer. This is stretically only possible when the α12/13 domains are disconnected from the rest of the protein. which is not the case in the low resolution structure shown in Figure 4. Importantly, such a configuration would be just rejected by the used bead modelling algorithm as the condition of compactness is violated in that case. At the moment we are using rigid body refinement to generate precise models for the hGBP1 dimer which are in agreement with the measured SAXS and FRET data

To interpret the FRET data of the hGBP1 monomer coarse grained computer simulations have been performed. The combination of FRET and SAXS measurements was found to increase dramatically the resolution of the coarse grained structures. We found that there exists an equilibrium between the closed crystal structure and the opened solution structure of the hGBP1 monomer. Interestingly, the relative populations of the opended and closed state depend on the environmental temperature. In a continuation on BM29 we would like to investigate that phenomenon in more detail.