ESRF	**Experiment title:** Time dependent small angle scattering on the refolding process of substrate protein mediated by the *E.coli* chaperonin system GroEL-GroES	**Experiment number:** LS-868
Beamline:B	**Date of experiment:** from: 1.07.1998-4.07.1998 to: June 1998	Date of **20.08.1998**
Shifts:12 W4/ID2	**Local** contact(s):O.Diat, T.Narayanan	*Received at* 01 SEP. 1998

Report:

The measurements of the last beamtime periode were aimed to follow the fast kinetics of formation of GroEL-GroES complex in presence of different nucleotides, i.e. ATP, ADP and non-hydrolysable ATP analog AMP-PNP. Therefore we rebuild the stopped flow apparatus and supply it with more powerfull stepping motors in order to gain shorter mixing times. Using this new mixing device a deadtime of less the 15ms could be reached.

A second approvement of the device was the introduction of a third syringe adapted to the mixing cell (see fig. I). This third syringe was filled with buffer and used to pump the already mixed solution through the beam. The flux of the third buffer syringe was varied in the way that only a small volume of the mixed solution was irradiated for a short time (depending on the total exposure time, but less than 5s). This procedure had to be applied in order to reduce radiation damage of the protein by the full x-ray flux of the ID2 beamline (see last report). The machine was easily installed at the beamline and worked satisfactorily.

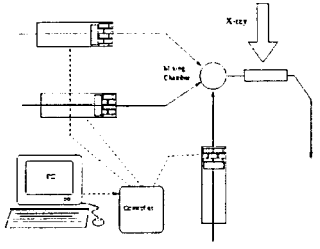

Fig. 1: Stopped flow apparatus with third syringe

Previous studies had shown that under ADP conditions the co-chaperonin GroES binds to one end of the GroEL cylinder. The binding kinetics of this reaction was investigated at II/97 at ESRF's ID2 beamline (see last report). The further steps of the refolding mechanism we have monitored by following the kinetics of the GroEL-GroES binding using other nucleotides like ATP and the non hydrolisable ATP analoge AMP-PNP. The experiment with AMP-PNP were performed in order to simulate the binding of nucleotides to the GroEL and to stall the ATP hydrolisis reaction.

Therefore different experiments have been performed:

I. GroEL with AMP-PNP
2. GroEL with ATP
3. GroEL + GroES + ADP
4. GroEL + GroES + ATP
5. (GroEL-GroES) + ATP

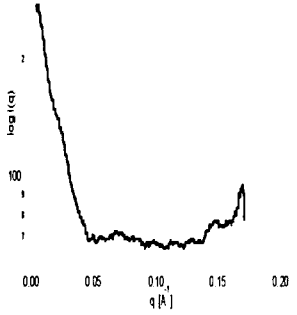

Fig. 2: Scattering function of the main chaperonin GroEL. The protein was measured as a reference. The statistical accuracy of the curve is sufficient after Is exposure time. Further analysis of the data using the Guinier approximation showed unfortunatly an error in the spatial correction of the raw CCD data.

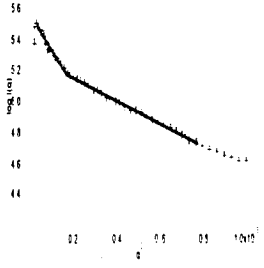

Fig. 3: Guinierplot of the scattering function from fig. 2.

It seems that the guinier plot had two different slopes. Normaly two different slopes indicate two different types of particles but in this case an error in the data treatment is responsable for this behavior.

Together with the local contact T. Narayanan, we are still in progress to overcome the problem with the spatial correction and we hope to finish the data evaluation in a few weeks.