



# TIME-RESOLVED SAXS STUDY OF CONFORMATIONAL CHANGES IN THE CHAPERONIN SYSTEM GroE

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Time-resolved small-angle solution scattering was performed on the chaperonin system GroE from *E. coli* in order to obtain structural and kinetic data along the reaction pathway of a chaperonin system.

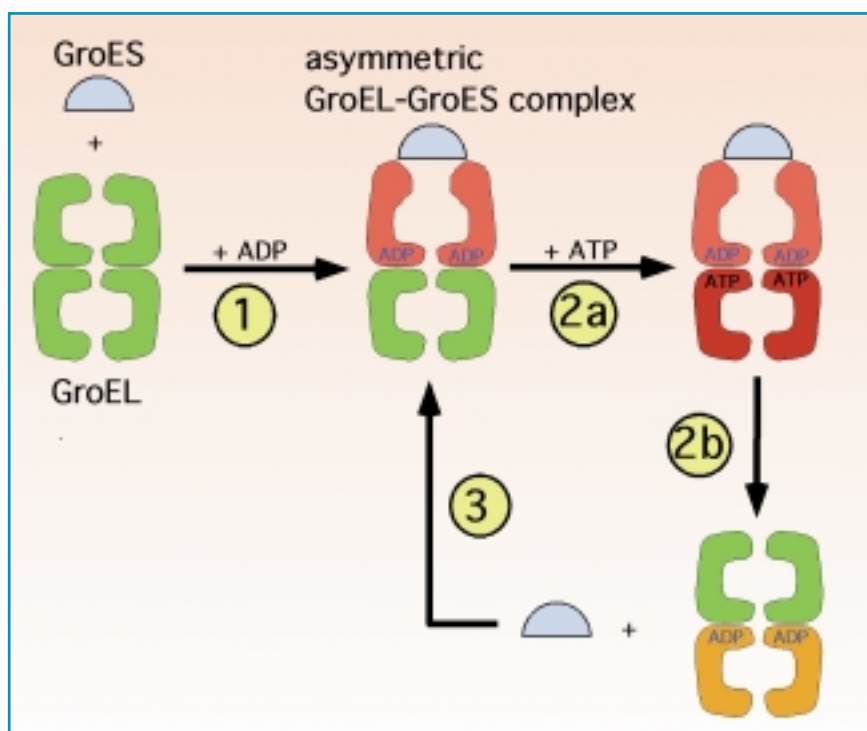
This investigation was made possible owing to the high brilliance of a third generation synchrotron source and the development of fast 2-D detectors, permitting real-time small-angle scattering measurements in the millisecond regime even on weakly scattering samples.

The biological functions of proteins are directly connected to the dynamics and the conformational changes within the domains of protein as well as the association-dissociation processes between interacting proteins. Many of these processes occur only in the aqueous physiological environment. Conformational changes of proteins in the millisecond to second time range can be followed by relatively low resolution methods, such as time-resolved small-angle x-ray scattering

(TR SAXS), by exploiting the high flux available at a synchrotron source like the ESRF. The application of this method requires rapid mixing of the components as well as fast image acquisition. By using a stopped-flow device for fast mixing (mixing time < 20 ms), we have investigated the chaperonin system GroE from *E. coli*. Molecular chaperonins are helper proteins which are essential for living cells to repair incorrectly folded polypeptides, such as denatured

proteins. The chaperonin system GroE consists of the main chaperonin GroEL (800 kDa) and the co-chaperonin GroES (70 kDa), which are proteins that assist the refolding of unfolded proteins [1]. GroES, a heptameric ring, binds during the reaction cycle to one end of the 14meric GroEL hollow cylinder [1,2]. The dissociation of GroES as well as the re-folding process are facilitated by the ATPase activity of GroEL [2], whereby ATP is hydrolyzed to ADP (Figure 1).

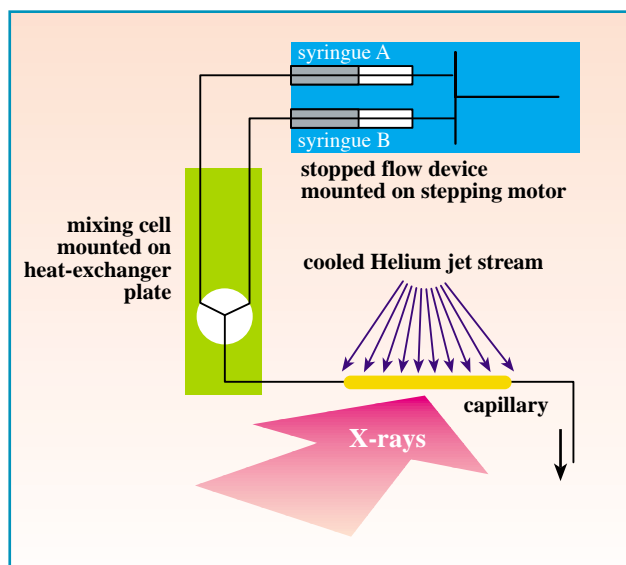
The stopped-flow device schematically shown in Figure 2 consists of two syringes driven by a remote-controlled stepping-motor system and a fast mixer. The data acquisition is triggered by the signal from this device which becomes active once the injection of the two syringes is completed. The protein denaturation due to radiation damage ( $\sim 2 \times 10^{13}$  ph/s/mm<sup>2</sup> at 12.5 keV) and



**Fig. 1:** Reaction cycle of the GroE system without denatured substrate protein. The asymmetric GroEL-GroES complex shows a high affinity for substrate protein and is therefore an important starting structure in the re-folding cycle.



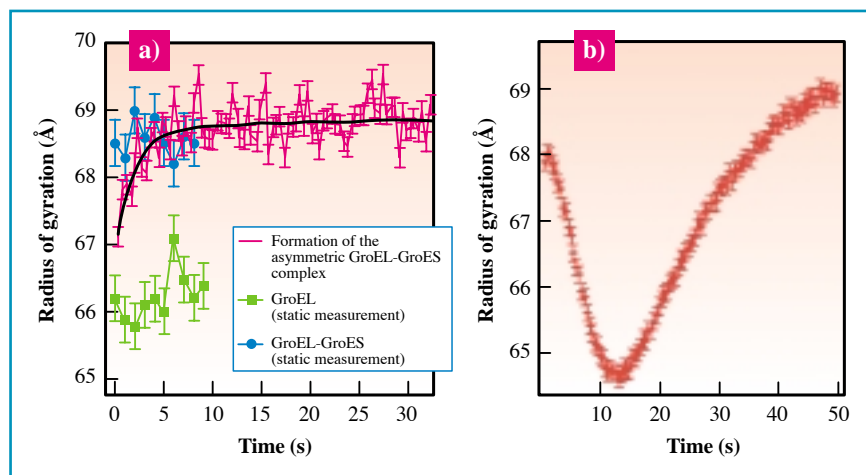
**Fig. 2:**  
*Schematic of the stopped flow device used for the time-resolved small-angle x-ray scattering. The full details of the sample environment box are not shown.*



beam heating were avoided by using glycerol as a radical quencher and a jet stream of cooled He-gas directed on to the sample capillary at the beam position. Despite these precautions, radiation damage could not be fully eliminated for long exposures (> 30 s). 2-D SAXS patterns were acquired using the fast XRII-FRELON CCD detector system. This detector system is capable of acquiring 10 images per second with a resolution of 1024x1024 pixels with a dynamic range of 14 bit. By adjusting the typical exposure time (150 – 200 ms) required for these samples which scatter weakly, it was possible to take about 110 time slices within a total acquisition time of 33s.

As a starting point, we have investigated the binding kinetics of GroES to GroEL in the presence of ADP - the first step in the chaperon cycle shown in Figure 1. In this experiment, GroEL (syringe A) and GroES together with ADP (syringe B) were mixed together. The subsequent steps of the chaperonin cycle, steps 2a to 3 in Figure 1, have been analyzed in a second experiment where the pre-formed GroEL-GroES complex (syringe A) was mixed with ATP (syringe B). The radius of gyration ( $R_g$ ), which provides sensitive information about the shape of the particles (deduced from the measured scattering curve), was used to follow the reaction. Reference measurements of the proteins mixed with buffer solutions containing no nucleotides were also performed.

The resulting data are shown in Figures 3a and 3b. The increase of the  $R_g$ -values in Figure 3a indicates the formation of the asymmetric GroEL-GroES complex. The reaction was finished within about 8 s after mixing. The time evolution of  $R_g$ -values in the second experiment, which probed the change of the pre-formed GroEL-GroES complex after mixing with ATP (Figure 3b), revealed an unexpected



**Fig. 3:**  
*Results of the real-time analysis along the reaction pathways of the chaperonin system GroE:*  
*a) Association of the GroEL and the GroES in the presence of ADP.*  
*b) The reaction of the GroEL and GroES in the presence of ATP.*

behavior. The measured radius of gyration immediately after mixing decreases and reaches a minimum after 13 s. Then, the value increases again for about 40 s. This variation suggests that the binding and hydrolysis of ATP, mediated by the GroE-associated ATPase is a multi-step process. The decrease of the  $R_g$ -value can be attributed to the dissociation of GroEL and GroES upon ATP binding and hydrolysis. After about 13 s, the dissociation process is completed and the second step begins, which is the rebinding of the GroES, is started.

In conclusion, our results indicate

that the interaction between GroEL and GroES is a multi-step process in the presence of ATP while it is a fast single-step process in the presence of ADP. ■

#### REFERENCES

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