In the SAXS experiment MX 1493 on BM29 we performed a systematic study of active and non-active forms of arrestin at different buffer conditions. Arrestin is prone to aggregation at higher concentration. Therefore, data at small angles of samples below 3 mg/ml were combined with data at larger angles of samples with higher concentrations of 5 and 10 mg/ml. Up to now we focused on specific buffer conditions during data analysis which gave the best results in terms of protein stability and monodispersity.

Questions to be addressed were: (i) In solution, do the active truncated p44 and the active mutant R175E show oligomeric states like the non-active basal state of arrestin? (ii) What are the solution structures of the active and non-active forms of arrestin.

The key results are summarized below:

Non-active basal state of arrestin

Native arrestin crystalizes as a dimer of two dimers in the unit cell. Measured SAXS data of native arrestin are compared to the calculated scattering curves from the monomer, the dimer and the tetramer, see figure 1. Clearly native arrestin can be best described as dimer in solution, which is different to the situation in the crystal, where arrestin was found to be a tetramer. Rigid body refinement of the dimer was performed. The fit of the rigid body refinement to the SAXS data is given in figure 2 and the obtained dimer structure is shown in figure 3. The solution structure of the dimer is different from the corresponding arrangement in the crystal.

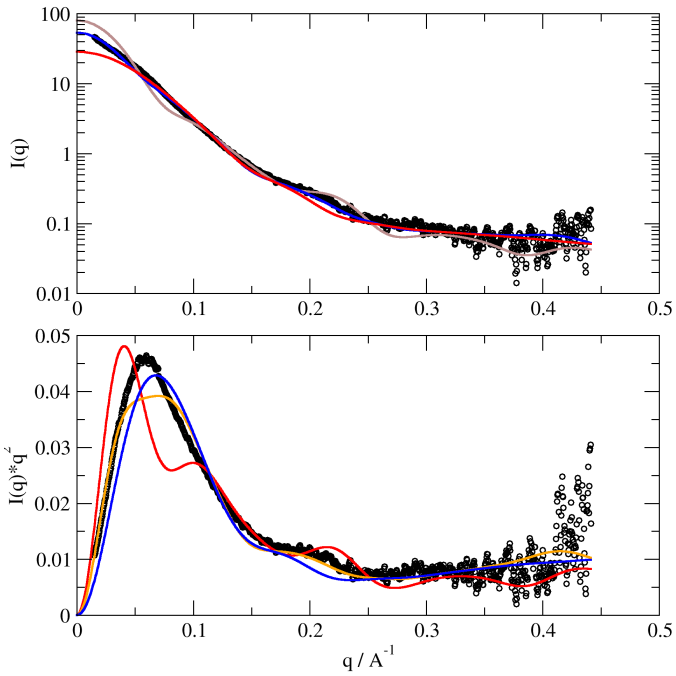

Figure 1: Measured SAXS data of arrestin and calculated theoretical curves from the crystal structures of the monomer (red line), dimer (blue line) and tetramer (orange). The dimer agrees best with the measured data.

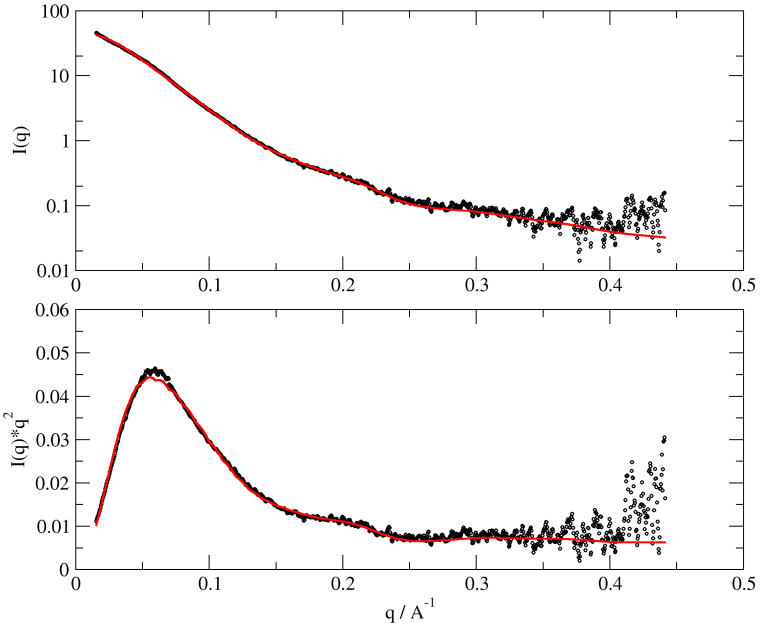

Figure 2: Measured SAXS data and calculate curves from rigid body refinement of th arrestin dimer.

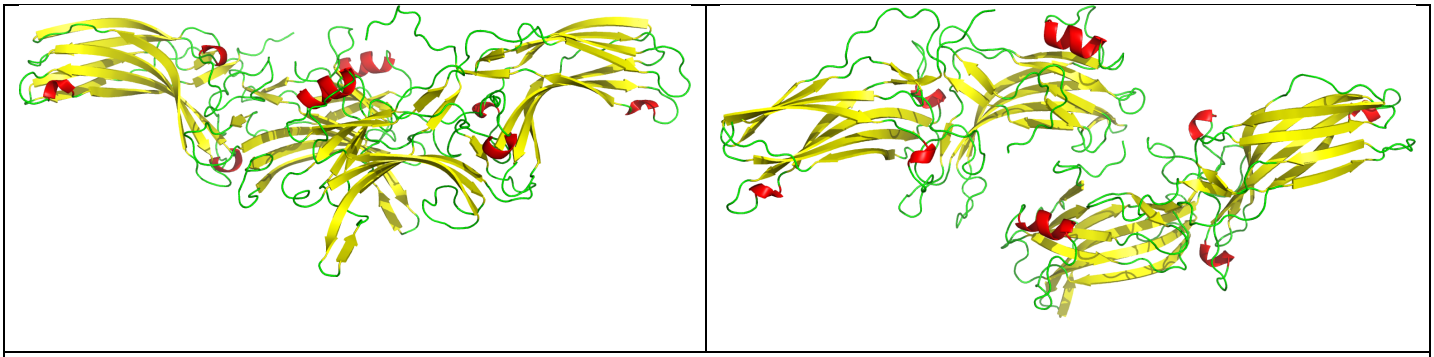

Figure 3: Model of the arrestin dimer as obtained from rigid body modelling. On the left from the side, on the right from the top.

Active states of arrestin p44 and R175E

The active states of arrestin crystallize as monomers. The SAXS data confirm that in solution the active and non-active differ in the oligomeric state. Low resolution *ab initio* models are shown in figure 4 and 5.

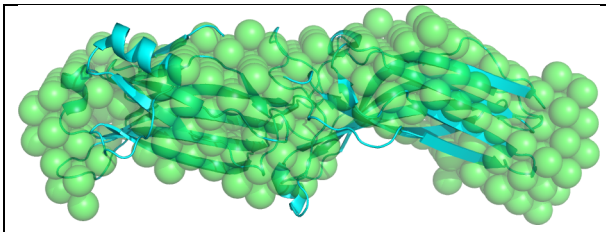

Figure 4: Bead model of the splice isoform p44 compared to the crystal structure.

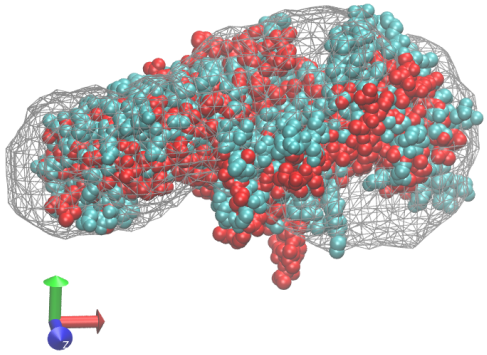

Figure 5: SAXS model of the active state mutant R175E. The green and red solid structures are two crystal structures, which differ in the loop positions at the binding site. The green closed structure agrees best with the model.

The C-terminal of the active state R175E mutant lacks electron density in the crystal structure. The structure of R175E including a flexible C-terminal was modelled and fitted to the measured SAXS data, see figure 6. The SAXS results demonstrate for the first time directly the flexible nature of the C-terminal of R175E, which plays an important role for the activation of the mutant. Aggregation of R175E is also visible at small scattering vecotrs, which was taken into account during data interpretation.

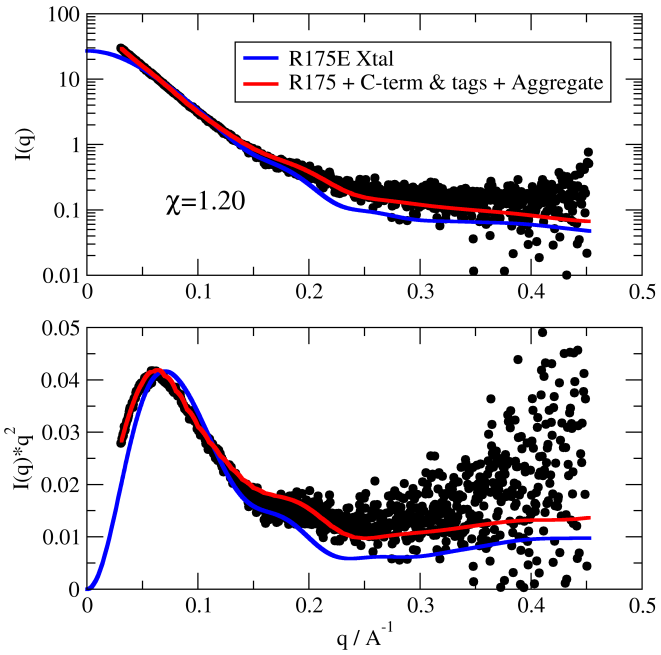

Figure 6: SAXS data of the active form R175E together with the calculated scattering curves from the crystal structure and from simulated structures including a flexible C-terminal plus a Gaussian term for protein aggregation.