Arrestin experiments

The termination step in GPCR signaling involves a two-step mechanism -a rapid phosphorylation of the receptor followed by the arrestin binding that sterically blocks further interaction of G-protein with the receptor, resulting in termination of the primary signaling event. We have previously reported crystal structures and SAXS studies on constitutively active variants of visual arrestin (**p44**-a naturally occurring truncated variant; and **R175E**-a charge reversal arr-1 mutant in the polar core).

Currently, we are investigating the effect of synthetic peptides that mimic the phosphorylated C-terminus of rhodopsin on arr-1 variants. Our preliminary results using size-exclusion chromatography (SEC) show complex formation and a shift in elution profile, suggesting a change in oligomeric state.

In the SAXS experiment on BM29 we have investigated structural changes upon binding of a synthetic peptide that activates arrestines.

Below is a summary of the key results:

As controls we have measured the proteins p44 and R175E without the peptide being present (fig 1). The control experiments demonstrated that both proteins are present in solution. P44 shows absence of aggregation, R175E had a small correctable amount of aggregation.

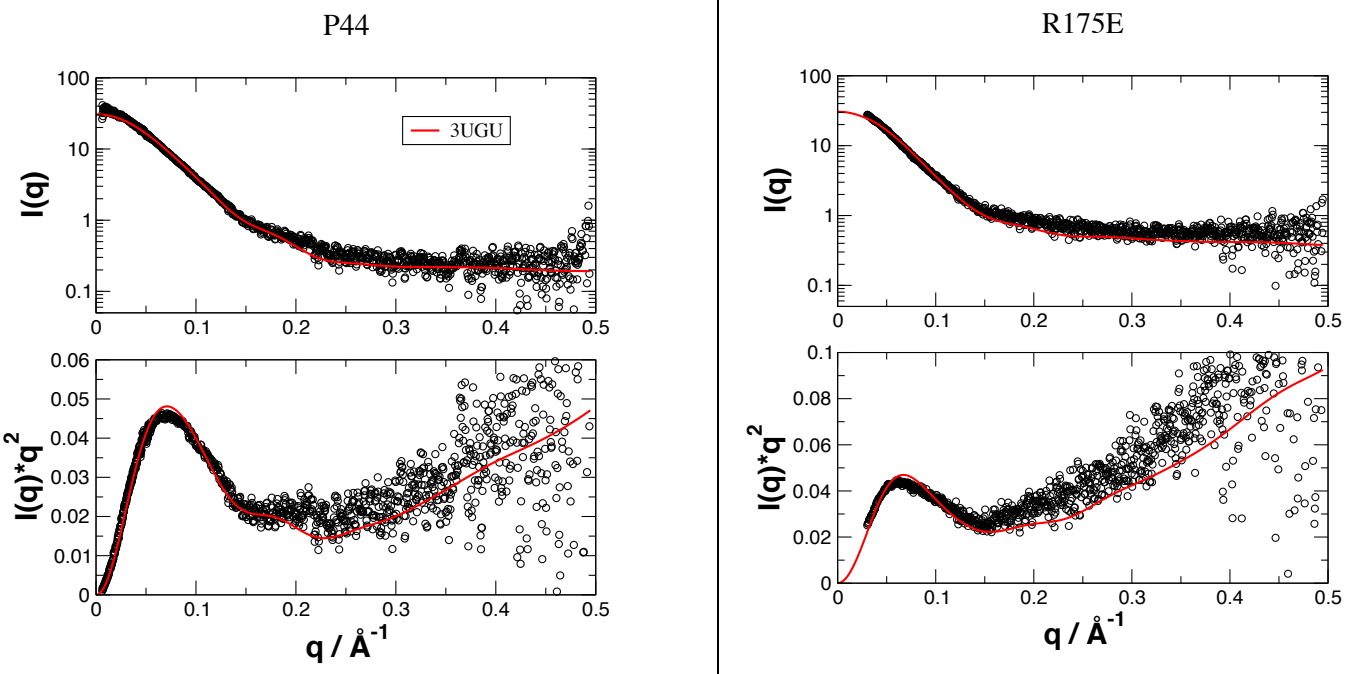

Figure 1: SAXS data of p44 and R175E in the absence of synthetic peptide. The red solid line are calculated theoretical scattering curves based on the available crystal structures of p44 and R175E, respectively.

In a second step we added the synthetic peptide to the p44 and R175E solutions and recorded SAXS data. The experimental data is shown in fig. 2.

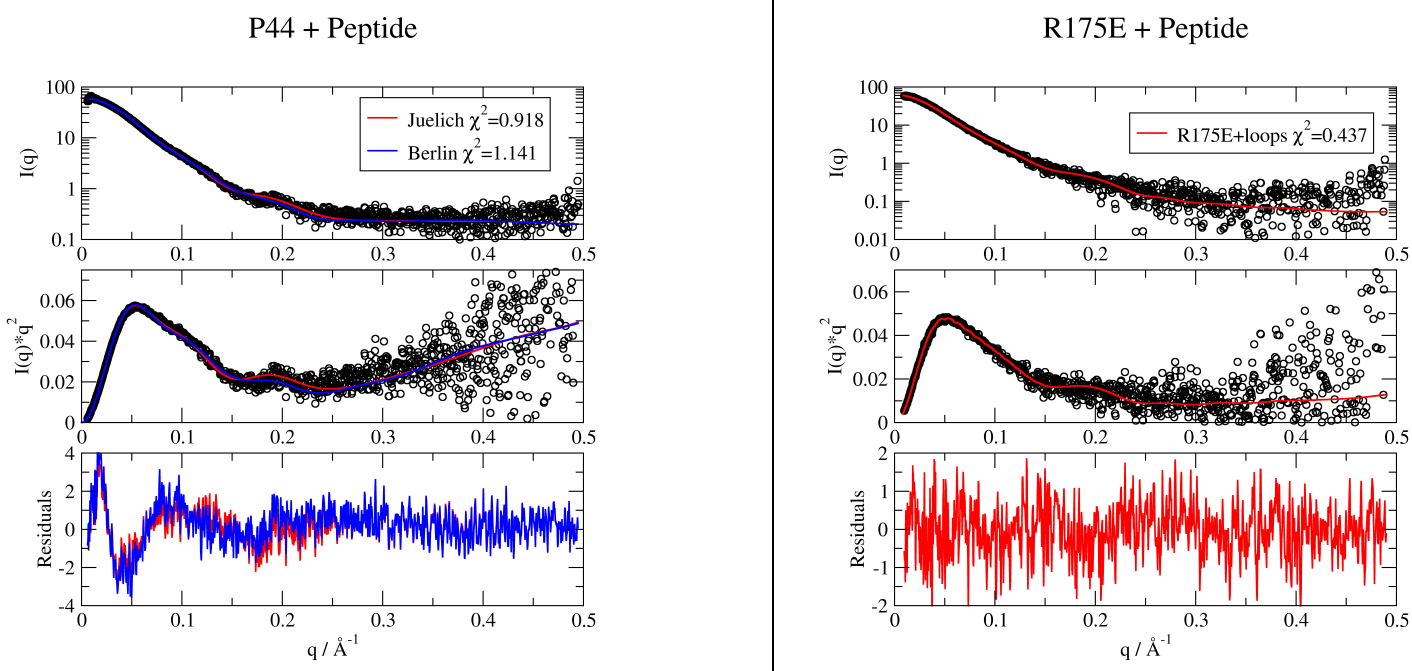

Figure 2: Measured SAXS data of p44 and R175 in the presence of peptide. The solid lines are models of dimeric proteins.

The measured forward scattering demonstrates directly that peptide binding induces the assembly of p44 and R175E dimers. Possible assemblies of dimers have been screened and we could determine to possible dimerisation assemblies, which are actually very similar in their overall shape, but differ in the rotation of the monomers around the long axis.

Kinetic experiments of LOV proteins after light-excitation

Our group members have determined crystal structures of, PpSB1-LOV, *Ds*LOV of *Dinoroseobacter shibae* DFL12^{T}, and more recently, dark state crystal structure of PpSB1-LOV. Comparison of the two states reveal remarkably large conformational changes including ~11 Å movement of the C-terminal helix Jα, disruption of hydrogen bonds in the dimer interface, and a ~29 ° rotation of chain B relative to chain A. Here, we would like to perform time-resolved kinetic measurements of dark recovery of PpSB1-LOV protein variants using SAXS.

We have performed kinetic SAXS experiments of PbSB1-R66I. The protein solution was illuminated by blue light and the recovering process in the dark was followed by kinetic SAXS experiments.

The difference SAXS spectrum (light illuminated at t=0 minus dark at t) at different times is shown in figure 3. A characteristic drop is found at 0.05 Å^{-1}, which was also observed in static SAXS experiment performed before on BM29.

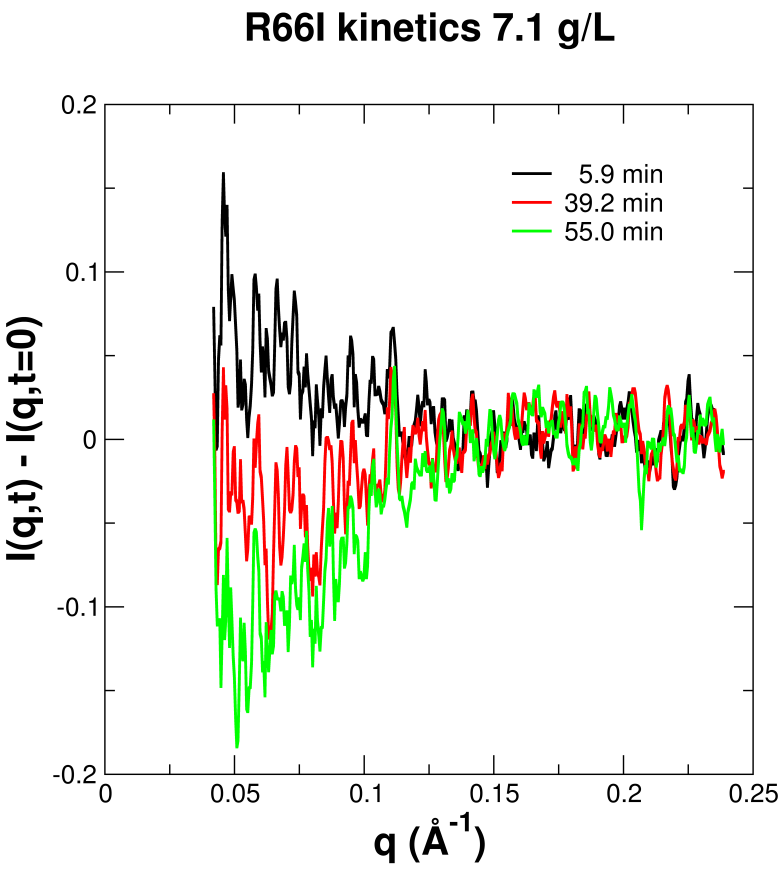

Figure 3: Difference SAXS spectrum of light-illuminated R66I minus protein in the dark at different time-points.

The difference data were integrated over the q-range and the integral is plotted as a function of time in figure 4. The solid line in is a exponential with the relaxation time of 24 min that has been determined with UV/vis absorption.

The kinetic experiments clearly show that structural changes in response to dark recovery are observed. The time dependence seems to follow the previously determined recovery time. The data, however, are noisy and do not allow an unbiased fit to determine the relaxation time with unbiased accuracy.

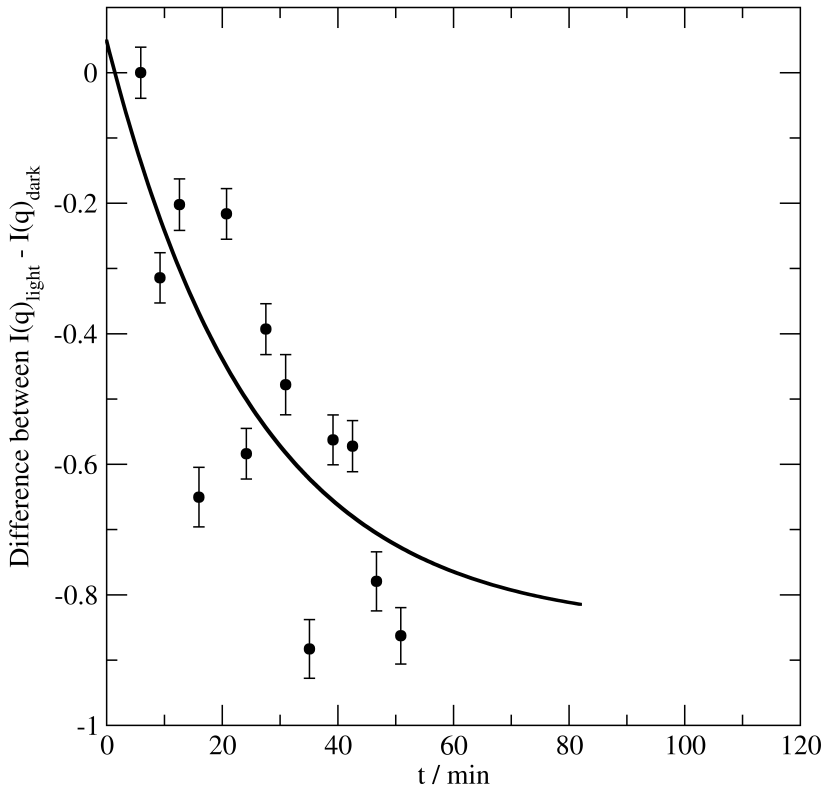

Figure 4: Changes in the difference SAXS signal (light – dark) as a function of time. The solid line is an exponential with relaxation time of 24 min.