Determination of oligomerization during GTP hydrolysis in human Guanylate Binding Protein 1

The oligomerization properties of nonfarnesylated-(nf-)hGBP1 after nucleotide addition were reported first from size exclusion chromatography (SEC) and dynamic light scattering (DLS) experiments. Without nucleotides, nf-hGBP1 was reported to elute as a monomer, whereas after addition of non-hydrolyzable GTP analogs like GppNHp it was classified as dimer. Furthermore, in the presence of GDP and aluminium fluoride ($GDP{\cdot}AlF_x$), which mimicks the transition state of hydrolysis, the apparent size was further increased and proposed to appear as tetramer.
When the purification of farn-hGBP1 was first described by Fres et al., it was proposed that hGBP1 undergoes structural changes after farnesylation and the surface therefore gets less hydrophobic, as can be seen from hydrophobic interaction chromatography. It was proposed that the C terminus is probably internally hidden after farnesylation.
In this SEC-SAXS experiment on BM29, we want to compare the structural differences between the ground states (nucleotide free) of the unmodified and farnesylated protein in solution. We found differences for farn- and nf-hGBP1 in SAXS measurements and did further investigations using AUC, DLS and analytical SEC to clarify the origin of the differences. During further investigations, also using SEC-SAXS, we are able to distinguish a purely monomeric appearance of farn-hGBP1 in solution opposed to the monomer-dimer mixture of nf-hGBP1 in solution. With all different techniques, we found a concentration dependent equilibrium with a dimer fraction of around 17% under standard conditions (2 mg/ml or 30 µM in Tris buffered solution at pH 7.9) for the commonly used nf-hGBP1 even in nucleotide free solution.

Results

SEC-SAXS was measured on BM29 using different protein concentrations of farnesyl (farn) hGBP1 and non-farnesylated (nf) hGBP1. NF-hGBP1 was measured nucleotide free (NF) condition and in the presence of nucleotides GppNHp and AlFx-GDP that induce formation of higher order oligomers.

In figure 1 the SEC profiles of nf-hGBP1 and farn-hGBP1 are compared. Farn-hGBP1 elutes as monomeric species, while dimers and tetramers are observed for nf-hGBP1.

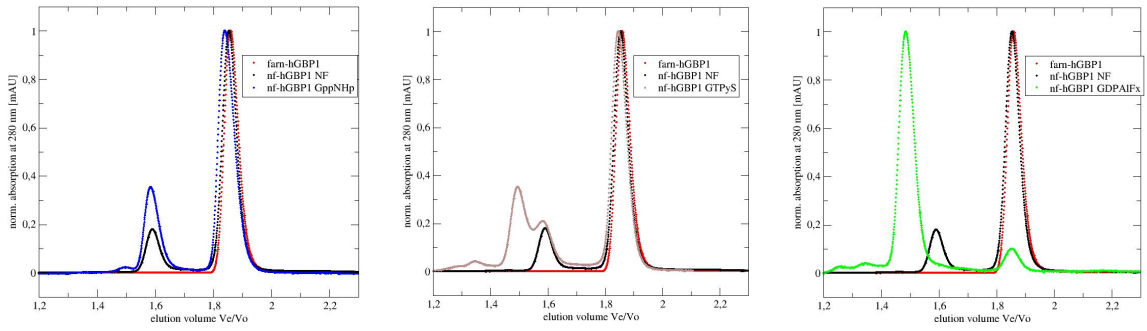

Figure 1: SEC elution profiles of hGBP1 with bound farnesyl (farn) and non-farnesylated (nf) state. Farnesylated protein elutes as a single peak from the column, whereas nf-hGBP1 elulte as monomer and higher order oligomer (dimer and potentially tetramer).

Measured SAXS date of batch mode and SEC-SAXS of farn-hGBP1 is shown in figure 2.

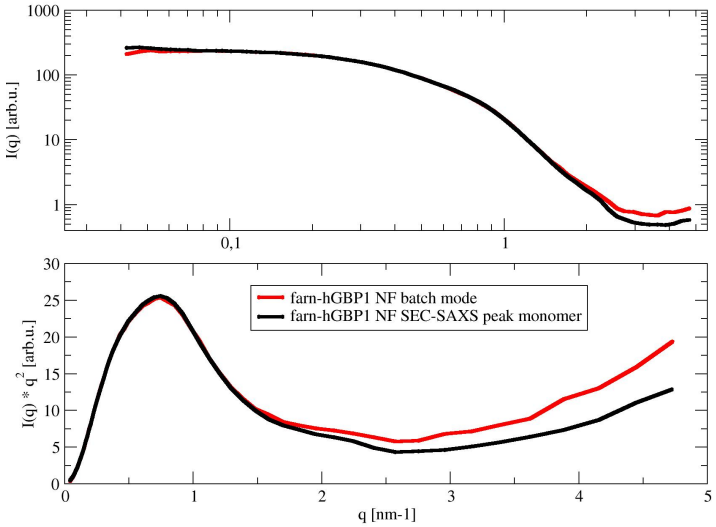

Figure 2: Farn-hGBP1 measured in batch mode SAXS and SEC-SAXS. Both SAXS curves are very similar due to the fact that the protein is in the purely monomeric state.

SEC-SAXS and batch mode SAXS data of nf-hGBP1 in the presence of the different solvent conditions is presented in figures 3 to 6.

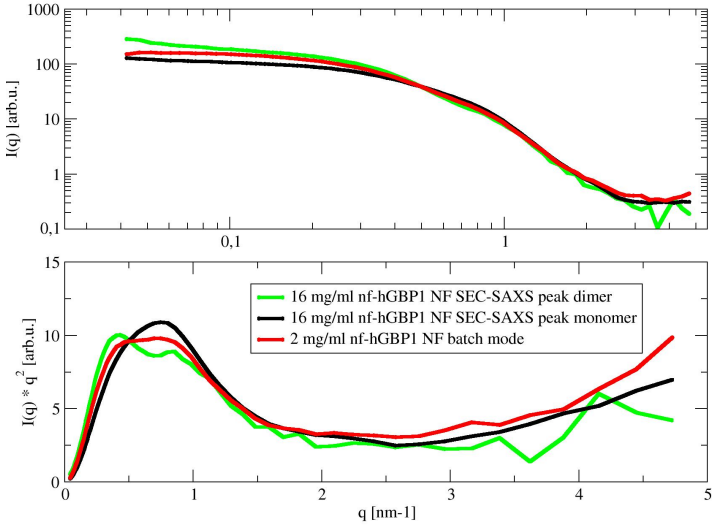

Figure 3: nf-hGBP1 under nucleotide free conditions. Compared are batch mode SAXS data and SEC-SAXS data of the monomer and dimer. Clear differences between monomer and dimer are observed by SEC-SAXS, whereas batch mode detected only the average of monomer and dimer.

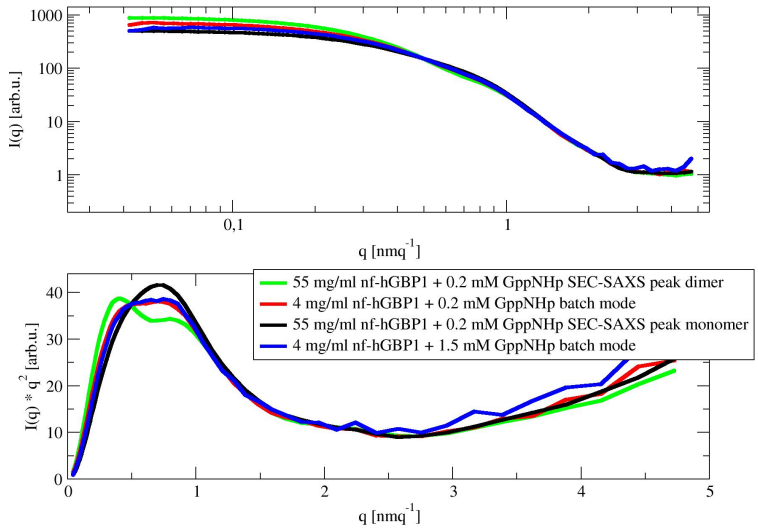

Figure 4: nf-hGBP1 in the presence of the nucleotide GppNHp. As in the nucleotide free state we observe monomer and dimer by SEC-SAXS.

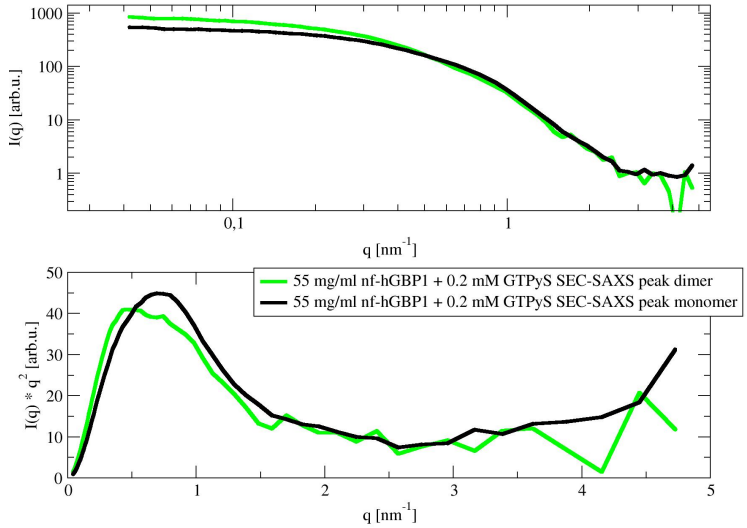

Figure 5: nf-hGBP1 in the presence of the nucleotide GTPλS. As in the nucleotide free state we observe monomer and dimer by SEC-SAXS.

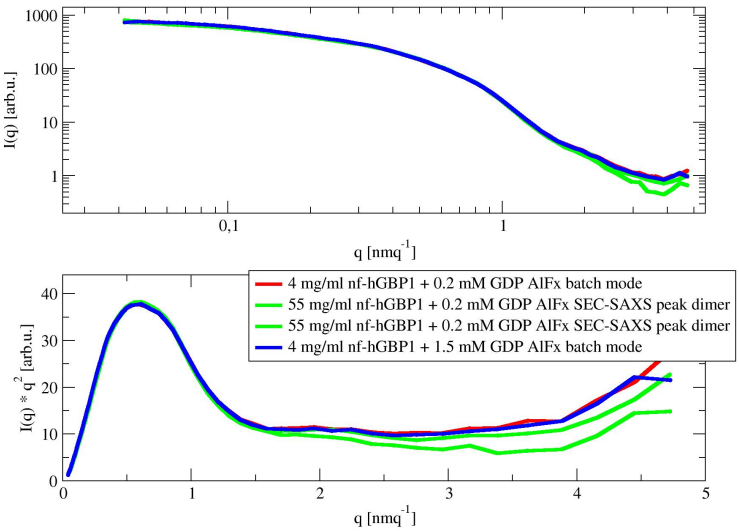

Figure 6: SEC-SAXS of nf-hGBP1 in the presence of the nucleotide AlFx-GDP. SAXS curves of the different elution peaks are very similar and not very elusive.

Interpretation of SEC-SAXS data using structural modelling

The calculated SAXS profile of nf-hGBP1 monomer is compared to measured SEC-SAXS data of nf-hGBP1 monomer in figure 7. Although theoretical curves first seem to agree with experimetal data, a close inspection of the residues shows systematic deviations from the measured data. A systematically better interpretation is obtained, when multiparameter set of experimental FRET & EPR & SEC-SAXS data is used for model building, see figure 8. The obtained model gives a significantly better agreement with experimental data as observed in the residuals.

Rigid body modelling was done to get a structural model of the nf-hGBP1 dimer. The result of the modelling approach is shown in figure 9.

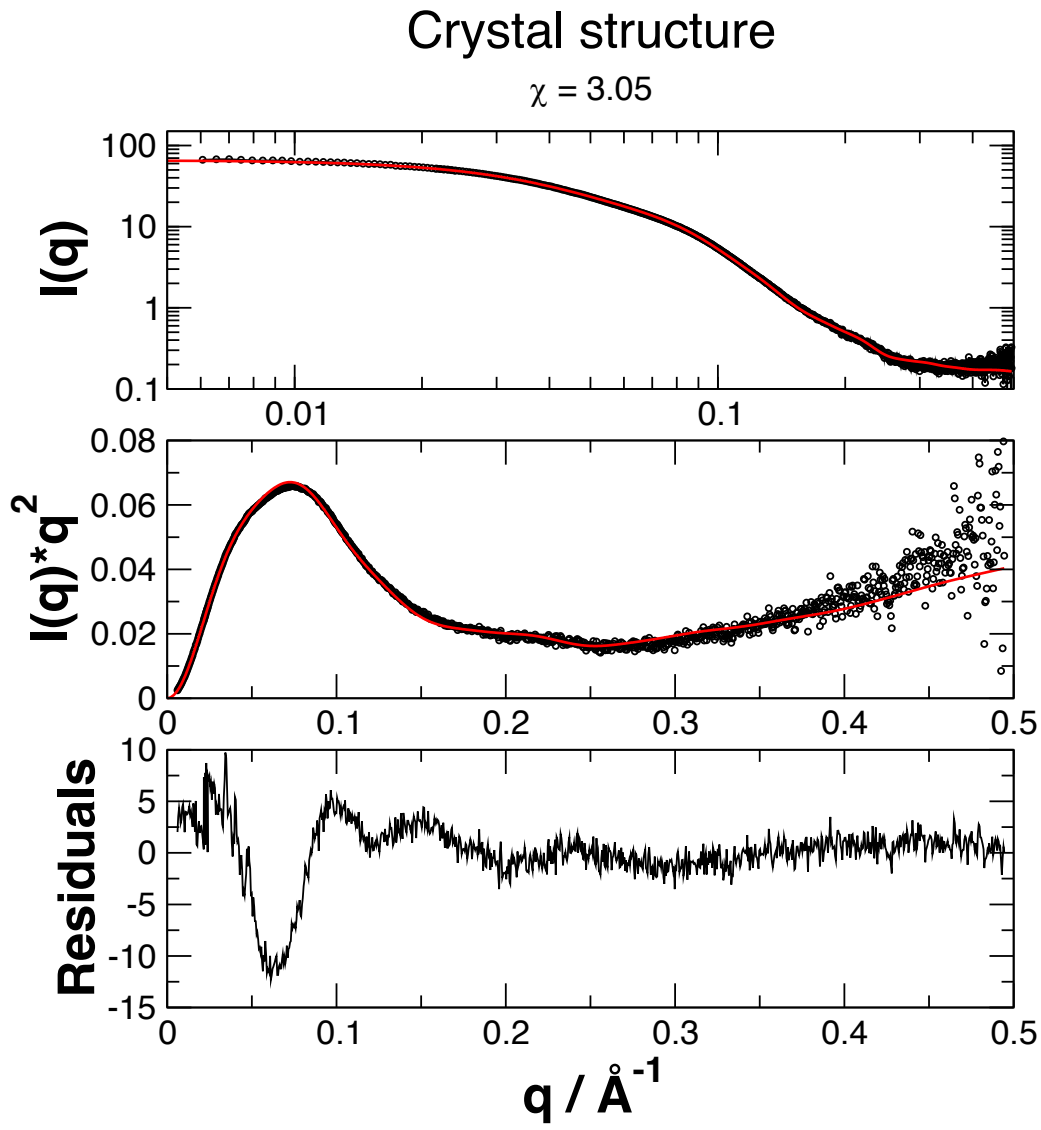

Figure 7: Theoretical SAXS curve of nf-hGBP1 crystal structure compared to SEC-SAXS data.

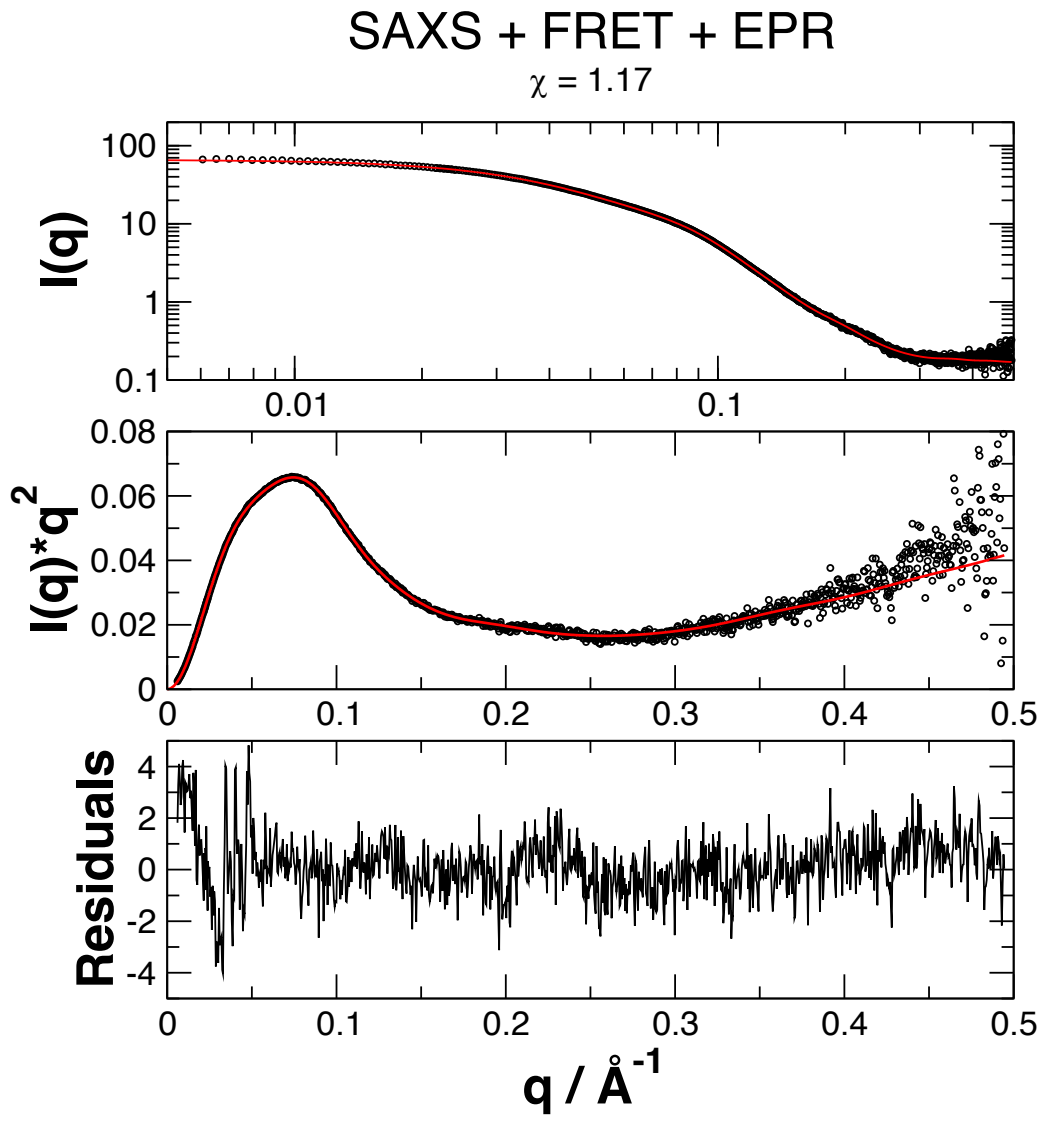

Figure 8: Theoretical SAXS curve of refined nf-hGBP1 monomer structure.

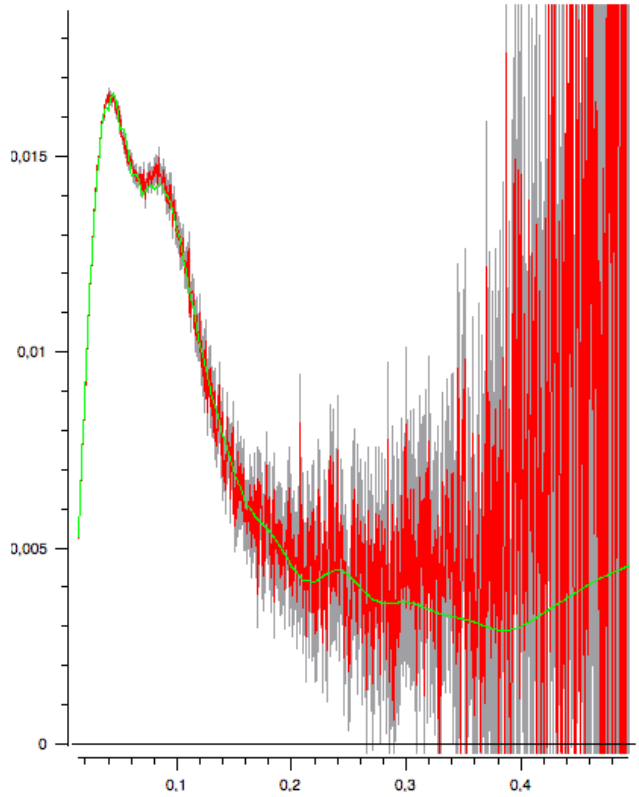

Figure 9: Rigid body modelling of the nf-hGBP1 dimer gave a good fit against the measured SEC-SAXS data.