ESRF	**Experiment title:** OLIGOMERIC STATE AND CONFORMATIONAL FLEXIBILITY OF TIP49A PROTEIN COMPLEXES.	**Experiment number**: SC-3472
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Report: SAXS on TIP49A protein and its Y366A and G383A mutants was performed in the range of the scattering vector magnitudes 0.04 to 3 nm^{-1} at 20°C in the range of protein concentrations 0.5 - 4.5 mg/ml in Tris-HCl buffer (20 mM, pH 7.4 at 4°C). Where appropriate, $MgCl_2$ (5 mM) and double-stranded phage λ DNA were added. To reduce the degree of protein aggregation some of the samples were treated with Triton-X100 (0.05%) followed by centrifugation on a dialysis membrane 10 kDa.

For all samples the data show high degree of protein aggregation (Fig.1), pointing to formation of amorphous polydisperse aggregates with the size of 25 nm and larger. Decrease of the protein concentration from 4.5 to 0.5 mg/ml did not affect the aggregation (Fig.1). Treatment with Triton-X100 had some effect on the scattering spectrum but did not eliminate the aggregates.

Protein aggregation did not allow us to make any conclusions regarding the protein oligomeric state. However, the subsequent investigation of the aggregation of TIP49a protein aggregation by DLS have revealed the presence of two aggregate fractions with the hydrodynamic diameters of 16 nm (mass fraction ~70%) and 60 nm (~30%). An addition of Triton-X100 completely eliminated the fraction of the smaller aggregates with the reduction of the mass of the larger aggregates to less than 2% of the total mass. The average size of the larger 60 nm aggregates did not change, while the most of the

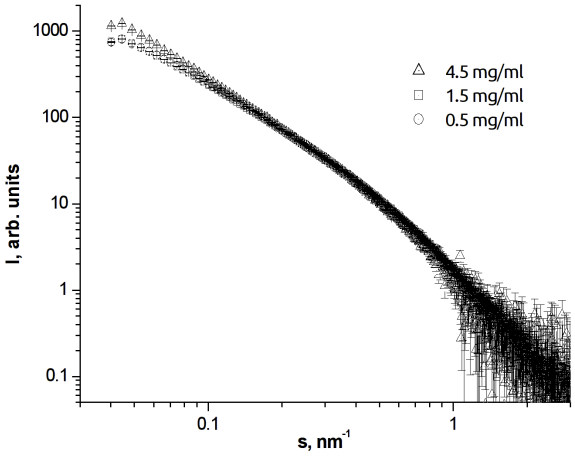

Figure 1: SAXS on TIP49A solution at three different concentrations.

protein was in the form of the particles with the diameter of 6.4±0,7 nm which is close to the protein hexamer (Fig. 2).

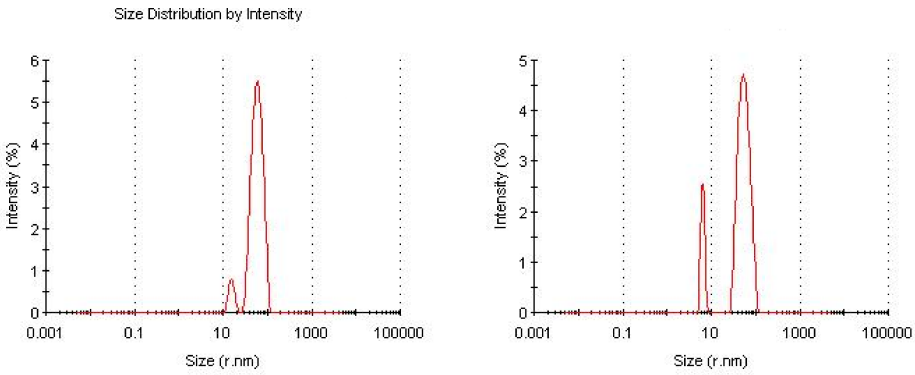

Figure 2: Dynamic light scattering of TIP49A solution in the absence of detergent (left) and after treatment with 0.05% Triton-X100 (right).

We therefore reevaluated our SAXS results to see if they can be explained as superimposition of the scattering of the aggregated form (prevalent in the absense of Triton-X100 detergent) and oligomeric form of the protein (which appeared when the detergent wass added). In the range 0.05 - 0.25 nm^{-1} the SAXS spectra in the presence of Triton-X100 were fitted to a linear combination of the corresponding spectrum of the sample without detergent and Guinier approximation for either globular or rod-like particles. Typical results of the fit are shown in Fig. 3. The analysis performed for all measured samples suggested that (1) Tip49a aggregation is Mg-dependent; (2) for the wild-type Tip49a and G383A mutant the data is consistent with rod-like filaments with the cross-sectional gyration radius of approximately 5 nm; (3) in the presence of double-stranded DNA the hydrodynamic radius of the rod like filaments decreases to 1.6 nm for the wild-type and to 4.3 nm for G383A mutant proteins; (4) for Y366A the data was

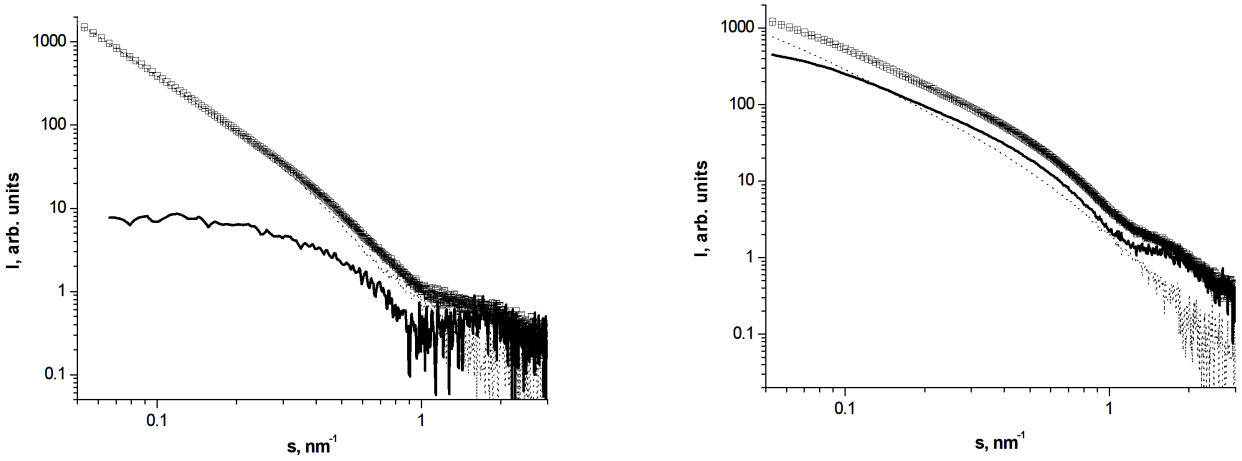

Figure 3: (left) SAXS on TIP49A mutant Y366A sqaures, fitted to the linear combination of the aggregated protein (dotted line) and Guinier approximation for monodisperse particles with R_g =4.7 nm (solid line), χ^2=3.7; (right) SAXS spectrum of wild type TIP49A fitted to the linear combination of the aggregated protein (dotted line) and Guinier approximation for monodisperse particles with R_c =4.8 nm (solid line), χ^2=4.3.

consistent with particles with the gyration radius of 4.7 nm. These results appear consistent with those obtained in the presious SANS measurements but due to scattering by the aggregates can not be considered reliable and further purification of the sample from the aggregates (which, according to DLS account for less than 2% of the protein mass) is required.

Results were recently presented on FEBS-2013 congress and included in: Lebedev D.V., Sokolova M.L., Fedorova Ya.V., Pobegalov G.E., Chervyakova D.B., Landa, S.B., Khodorkovskiy M.A. Supramolecular structures formed by TIP49A protein *in vitro*. St. Petersburg State Polytechnical University Journal: Physics and Mathematics, 2013, No. 2(170), pp. 156-162. (in Russian).