ESRF	**Experiment title:** Tracking the molecular mouse trap mechanism in human neuroserpin with time-resolved wide-angle X-ray scattering	**Experiment number**: SC-3512
Beamline: ID02	**Date of experiment**: from: 28/02/2013 to: 01/02/2013	**Date of report**: 25/02/2014
Shifts: 9	**Local contact(s)**: Gudrun Lotze	*Received at ESRF:*

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Report:

The aim of experiment SC-3512 was to investigate the structural events taking place during the interaction of a serine protease with a serpin. Serine proteases are enzymes that catalyze the hydrolysis of peptide bonds in proteins; they contain a highly reactive serine residue directly participating in the catalysis process. Serpins are serine proteases inhibitors. They undergo profound structural changes that lead to the entrapment of their target protease in a kind of molecular mouse-trap mechanism (Huntington et al., Nature, 407, 923-926, 2000). As a result of this inhibitory mechanism, typically a very stable protease-serpin complex is formed. The idea of the experiment was to use the stopped-flow apparatus available at ID02 to mix a protease solution with a serpin one (so as to rapidly trigger the inhibition reaction) and to use time-resolved X-scattering to monitor, in real-time, the induced structural events taking place in the resulting solution. Originally, we wanted to focus our investigation on human neuroserpin (hNS), a serpin molecule produced mainly by neurons and secretory cells of neuronal origin, and on its main physiological target, i.e. tissue-type plasminogen activator (tPA). Unfortunately, we were not able to produce recombinant hNS and tPA in the large quantities and high purity needed for the time-resolved X-ray scattering experiment at ID02. For most of the experiments performed during the beamtime we thus used alpha1-antitrypsin (AAT), the prototypical member of the serpin family, and the protease chymotrypsin (CHY). The production and purification of AAT and CHY in large quantities is a more standard procedure; moreover, we had already used these proteins for preliminary experiments performed at ID02 (Exp. SC-3243).

During the new available beamtime, we were able to expand on experiment SC-3243 by taking full advantage of the "WAXS detector" available at ID02 in order to monitor the scattered intensity of our samples at q-values as high as 2.5 $Å^{-1}$; this enabled us to monitor also the scattering contribution from the solvent during our kinetic experiments, which proved to be important in order to take into account small residual fluctuations in the X-ray incident intensity. Typical absolute scattering patterns measured after mixing of the AAT and the CHY solutions are reported in Fig. 1: the top panel shows the (azimuthally averaged) signal measured, between approximately 0.025 and 0.46 $Å^{-1}$, with a FReLoN2000 detector at 1.4 m from the sample, while the bottom panel shows a plot of the signal measured, between 0.41 and 2.5 $Å^{-1}$, with an off-axis AVIEX-2 detector at 0.1 m from the sample. Scattering difference patterns averaged over the set of different acquisitions performed are reported in Fig. 2 as q*ΔI vs. q plots; these difference patterns have been calculated using as a reference the scattering signal measured at the longest time-delay (~1 min) from mixing. Fig. 2 shows that a clear signal evolution in the investigated time-window is evident both in the SAXS and in the WAXS regions. A preliminary analysis of the reported data in terms of possible kinetic models that have been hypothesized for the reaction between AAT and CHY shows that our data clearly fit one kinetic model in preference over several others, in agreement with recent FRET-based experiments from Maddur et al. (J. Biol. Chem., 288, 32020, 2003).

The last few hours of our beamtime have been dedicated to an exploratory thermally-induced AAT polymerization experiment. After raising the sample temperature to 55 °C (by means of a thermostated cell available on the beamline), the AAT polymerization kinetics were monitored with X-ray scattering. The resulting experimental data are quite promising since they show, not only a SAXS signal evolution, but also the development of significant changes in the WAXS region due to protein tertiary structure rearrangements accompanying the polymerization process.

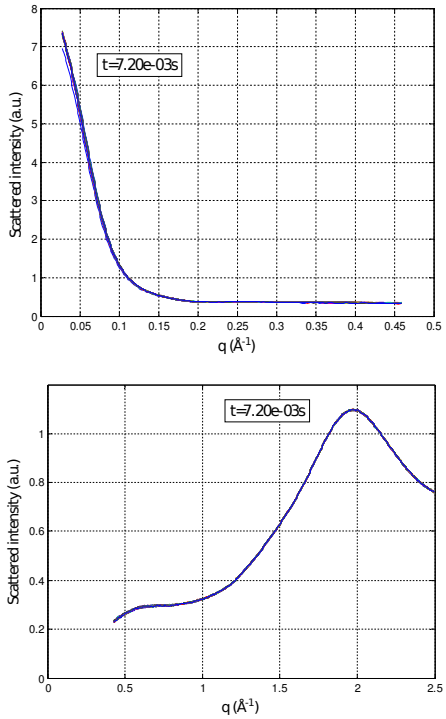

Figure 1 – Intensity of X-rays scattered by the sample in the SAXS (top panel) and WAXS (bottom panel) regions; several acquisitions at the same time-delay from mixing are reported.

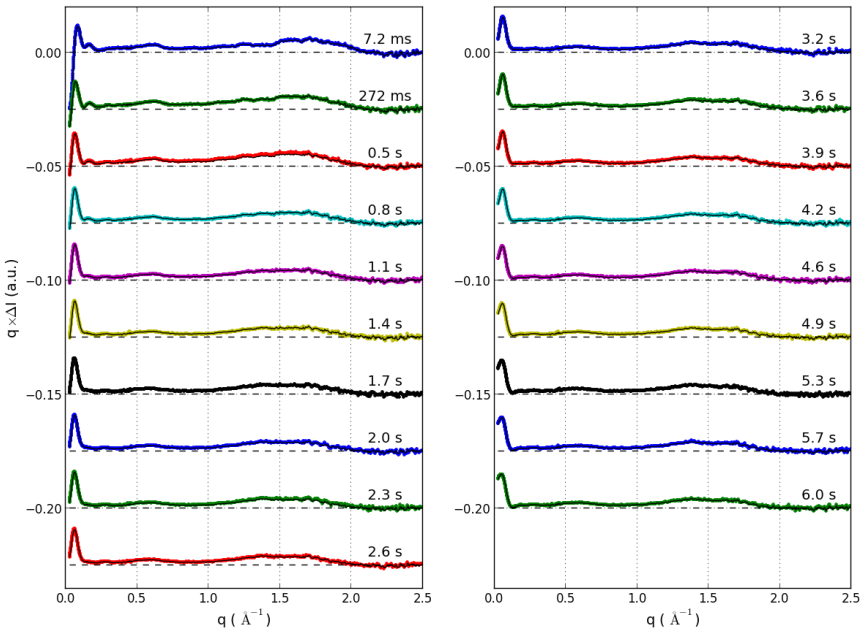

Figure 2 – Time-resolved X-ray scattering difference patterns obtained after averaging over the different acquisitions and merging of the SAXS and WAXS data. The scattering pattern measured at the longest time-delay has been used as a reference for the calculation of difference signals. Black lines are fits of the experimental data obtained in terms of the kinetic model that best describes the data.