ESRF

	Experiment title: **Structure and kinetics of formation of aggregates of rituximab, a monoclonal antibody**	**Experiment number**: **SC-2860**
Beamline: BM26	**Date of experiment**: from: sept 28 2009 to: oct 2 2009	**Date of report**: Feb 15 2010
Shifts: 9	**Local contact(s)**: Giuseppe Portale	*Received at ESRF:*

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Report:

We have recently reported [1] that rituximab, a monoclonal antibody used as a drug, forms aggregate when temperature is increased above room temperature. To investigate the structure of these aggregates in solution we have performed a series of SAXS experiments at different conditions:

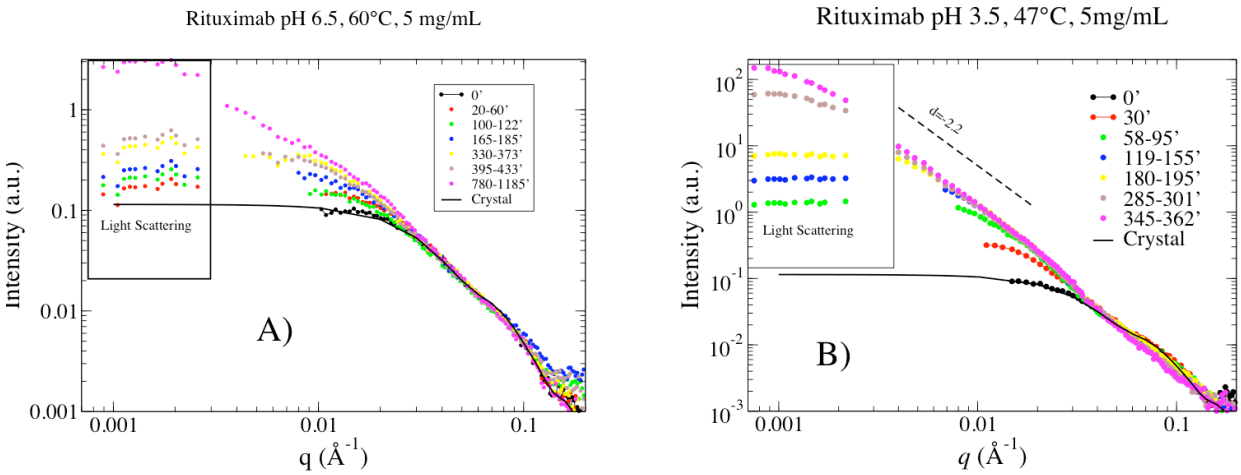
K1) T=60°C, pH=6.5, c=10mg/ml
K2) T=60°C, pH=6.5, c=5mg/ml
K3) T=47°C, pH=3.5, c=5mg/ml
K4) T=42.5°C, pH=3.5, c=5mg/ml

where we indicate the incubation temperature, the pH of the buffered solution and the concentration of rituximab, respectively.

We observed a change in scattering for a series of exposures of ??sec on a 10mg/mL , at room temperature and pH 6.5 rituximab sample Being aware of the effect of radiation damage on prompting aggregation even at RT, we incubated the sample in a series of a capillary to avoid reusing the exposed samples. We also found that it was possible to expose

different spots of the capillary to the beam for a limited amount of time, without producing further aggregates.
Thus the aggregation kinetics was followed by measuring samples that were incubated for an increasing amount of time. In this way it was also possible to follow more than one kinetics at a time, with almost no dead beamtime.

As the expected size of the aggregates is in the range 30-160 nm we pushed the setup to the low-q limit by using a camera length of 8m. A selection of the kinetics study was repeated with a shorter setup to catch the molecular features of the antibody under thermal stress. The kinetics were followed up to a time where no changes were oberved in the scattered intensity profile, i.e. from 5 to 20 hours.
In all cases the excess scattering was very close to the background signal making the subtraction a very critical step in the analysis of the data.



In Fig. A) we show the intensity profiles at several times along the kinetics K2 together with corresponding light scattering data measured in a separate experiment at our laboratory. A single scaling factor had to be chosen to find a good matching between the two set of data. The formation of the aggregates give rise to a growing contribution for $q<0.02$Å^{-1}. To extract the average structural features of the aggregate population we have to subtract the contribution due the the remaining isolated monomers. The task looks complicated because of the amount of signal and of the quality of the data. A more promising case is that of the kinetics K3 at a lower pH and temperature, illustrated in Fig. B). The acidic enviroment helps destabilizing the antibody and that in turn causes a more rapid and intense growth of the scattered intensity. In this case the influence of the monomer population can be ignored for times larger than one hour and $q<0.02$Å^{-1} and the main features of the aggregates can be directly measured from the I(q). In fact the average radius of gyration of the aggregates (not shown) was found systematically smaller (ca. 30%) than the hydrodynamic radius measured in a separate experiment run at our laboratory. Towards the end of the kinetics the I(q) behaves as a power law at low q, with an exponent -2.2. It is possible to join the light scattering and SAXS data (magenta points in Fig. B)) use a fractal aggregate model [2] to extract fractal size and a fractal dimension of 80nm and d=2.1 respectively.

1) Andersen at al., Protein Science, 2010, **19**, 279-290.
2) Gimel et al., Macromolecules, 1994, **27**, 583-589.