ESRF	**Experiment title:** **Structure of a tubulin – kinesin complex**	**Experiment number**: MX-895
Beamline: ID14-3	**Date of experiment**: from: 18/6/09 to: 19/6/09	**Date of report**:
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Report:

Objective, strategy and materials:

Kinesins are molecular motors that use chemical energy arising from ATP hydrolysis to produce force. By contrast with motile kinesins which move on microtubules, depolymerizing kinesins (also called KinIs) promote the disassembly of tubulin from the ends of microtubules. Consistently, KinIs have better affinity with disassembled tubulin than their motile homologs (Desai et al; Cell. 1999; 96:69-78). Therefore, experimental conditions can be established to get homogenous complexes made of KinI and tubulin, a prerequisite for a detailed structural study of the interaction between these two partners. For this purpose, we used the following materials for the experiment MX-895:

-Tubulin (110 kDa) – extracted and purified from mammalian brain – was preliminarily sequestered by the stathmin-like domain of the RB3 protein (RB3-SLD; 17 kDa) so that it formed a stable and monodisperse complex (T2R) made of two tubulin heterodimers and one RB3-SLD. This complex is well characterized and its crystallographic structure has been determined (*e.g.*Gigant et al, Cell. 2000; 102:809-16, Ravelli et al, Nature. 2004; 428:198-202).

-KinI was a recombinant protein expressed in *E. coli*. It consisted of a minimal functional construction of Kif2C (45 kDa), *i.e.* the motor domain added by an N-terminal extension called the neck. Crystal structure of such construct of KinI has also been resolved (Ogawa et al., Cell. 2004; 116(4):591-602).

The expressed Kif2C was shown to interact with T2R, as its ATPase activity is stimulated by the latter. Additionally, preliminary experiments (spin-down, DLS), indicated that relatively high ionic strength (150-200 mM KCl) drastically diminishes the rate of aggregates when mixing Kif2C and T2R, encouraging us to perform SAXS experiments.

Exploitation of the resulting data will allow establishing a structural model of the complex T2R-Kif2C with the use of the available crystallographic structures of each component. As T2R contains two tubulins and profiting from the asymmetry of its structure, the stoechiometry of the complex as well as the relative positioning of Kif2C on T2R will constitute original results useful to a better understanding of the structural basis of the interaction between kinesins and tubulin.

Samples and data collection:

The measured samples are listed below:

1) T2R alone (29 mg/ml, 14.5 mg/ml, 5.8 mg/ml)
2) T2R + Kif2C (ratio 1:1) (12.6 mg/ml, 6.3mg/ml)
3) T2R + Kif2C (ratio 1:1) + RB3-SLD in excess (136 µM) (9.4 mg/ml, 4.7 mg/ml, 1.9 mg/ml)
4) T2R + Kif2C (ratio 1:2) + RB3-SLD in excess (136 µM) (8.1mg/ml, 4 mg/ml)

-Samples had been dialyzed against a buffer containing 200 mM KCl in order to limit aggregation and 5 mM DTT to limit radiation damage.
-One run of data collection consisted of collecting 10 frames of 30 sec exposure times. The sample-detector distance was 1.755 m and the beam attenuation was systematically checked to ensure that each data collected was inside the linearity range of the detector.
-Before and after measuring each sample at each concentration, the scattering of the buffer was measured.
-For experiments 3) and 4) the "buffer" considered contained 136 µM RB3-SLD; dilutions were made with this solution.

Results:

As a control, the data collected from T2R alone (expt 1)) arose as expected. Parameters calculated from the experience all agree with what is predictable from the crystallographic structure. Indeed, the values of Rg = 4.9 nm and Dmax =18 nm as well as the overall appearance of the pair distribution function (p(r)), typical of elongated objects (one maximum at r ~ 4 nm) (Fig 1), all are in accordance with what was calculated from the crystallographic structure (using Hydropro). By the same, the experimental data fits quite well with the theorical scattering curve (Chi=2, for momentum transfer q=[0.2-2] nm^{-1}). However a slight slipping appears at wide angles (q> 2 nm), maybe due to an internal flexibility of T2R in solution and/or the fact hat some parts of the complex are missing in the crystallographic structure.

For the samples where Kif2C were mixed with T2R (expts 2) to 4)), observation of all difference curves at very small angles proved the presence of large species indicating that some aggregation occurred in the medium (Fig 2), which is consistent with the fact that no plausible Rg could be estimated from the Guinier plots. The addition of RB3-SLD in excess (expts 3) and 4)) notably diminished the rate of these "aggregates", though this did not get rid of them. In addition, these samples appeared to be sensitive to radiation damage, so that only the two first data curves were averaged, in particular to get stable data at very small angles.

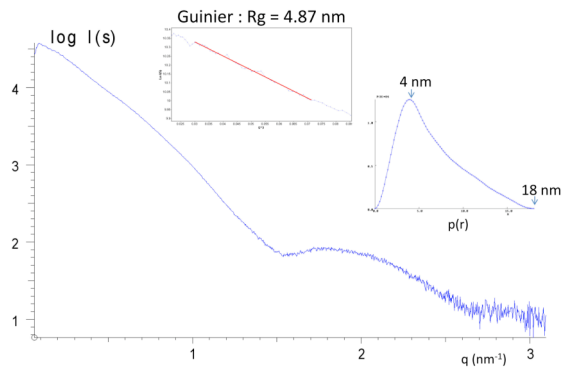

Figure 1

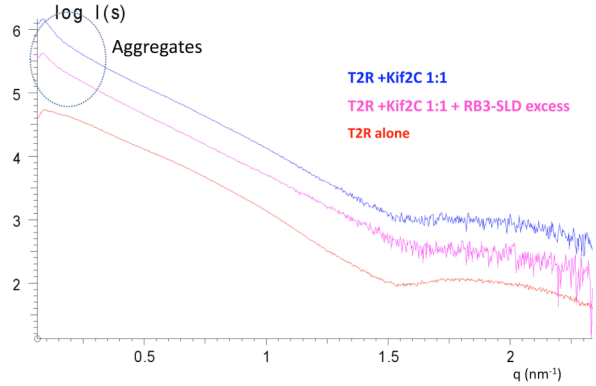

Figure 2

Usefulness of the data and perspectives:

It appears from these experiments that only the data from T2R alone is of sufficient quality to be well exploited. To our mind, by its own this data is of little interest with regard to the main objective, but is to be compared to subsequent data *i.e.* when mixing T2R with Kif2C.

As for the origin of large objects detected when adding Kif2C to T2R, several non-exclusive hypotheses can be brought up, considering the hereafter behaviour of Kif2C in the presence of tubulin:

(i) The positively charged Kif2C interacts with a negatively charged portion of tubulin (non-specific interaction) resulting to the formation of amorphous aggregates. This is limited by increasing the ionic strength.

(ii) KinIs, when interacting with tubulin, (relevant interaction) trigger the formation of assemblies of varying sizes, in which tubulin auto-associates as in protofilaments. RB3-SLD normally prevents such auto-assembly of tubulin.

In our system, on the one hand, the quantity of KCl in the medium might not be optimal to block enough the pathway (i). On the other hand, the pathway (ii) implies that Kif2C displaces RB3-SLD from tubulin; this seems the case to some extent, since the addition of an excess of RB3-SLD diminishes the rate of large objects.

Finally, we were not able to control the homogeneity of the samples we measured during the SAXS session MX-895. Clearly, more effort is needed to isolate the complex of interest (T2R-Kif2C). Several strategies are under investigation for this purpose. For example we are testing new constructs of Kif2C designed to limit the non-specific interaction with tubulin. We also are further biochemically characterizing the competition between Kif2C and RB3-SLD for tubulin binding.