ESRF	**Experiment title:** SAXS studies on mRNP components from yeast and human	**Experiment number**: MX-879
Beamline:	**Date of experiment**: from: 25.07.2009 to: 26.07.2009	**Date of report**: 24.02.2010
Shifts: 3	**Local contact(s)**: Petra Pernot	*Received at ESRF:*

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Report:

This second of two experiments was mainly used to improve data quality of sub-project 1 (the RNA-binding protein She2p), but also for testing proteins and buffer conditions for potential other projects.

We could successfully interpret data from the RNA-binding protein She2p and combine these information with results from size-exclusion chromatography and analytical ultracentrifugation. Our SAXS analysis on She2p were chosen by the ESRF as Research Highlight (Oct. 2009) and constributed important information to a publication:

Publication:

Müller, M., Richter, K., Heuck, A., Kremmer, A., Buchner, J., Jansen, R.-P., and Niessing, D.: Formation of She2p Tetramers is Required for mRNA Binding, mRNP Assembly, and Localization.
RNA **15**: 2002-2012 (2009).

Abstract from publication:

In eukaryotic cells, dozens to hundreds of different mRNAs are localized by specialized motor-dependent transport complexes. One of the best-studied examples for directional mRNA transport is the localization of *ASH1* mRNA in *Saccharomyces cerevisiae*. For transport, *ASH1* mRNA is bound by the unusual RNA-binding protein She2p. Although previous results indicated that She2p forms dimers required for RNA binding and transcript localization, it remained unclear if the dimer constitutes the minimal RNA-binding unit assembling *in vivo*. By using analytical ultracentrifugation we found that She2p forms larger oligomeric complexes in solution. We also identified a point mutant that shows impaired oligomer formation. Size-exclusion chromatography suggests that She2p forms defined tetramers at physiological concentrations. **Subsequent structural studies by small-angle X-ray scattering confirmed this finding and demonstrated that the previously observed She2p dimers interact in a head-to-head conformation to form an elongated tetrameric complex. This She2p tetramer suggests the generation of large**

continuous RNA-binding surfaces at both sides of the complex. Biochemical studies and immunostaining of cells confirmed that She2p tetramer formation is required for RNA binding, efficient mRNP assembly, and mRNA localization in vivo. Our finding on She2p tetramerization resolves previously raised questions on complex formation and mRNP function.

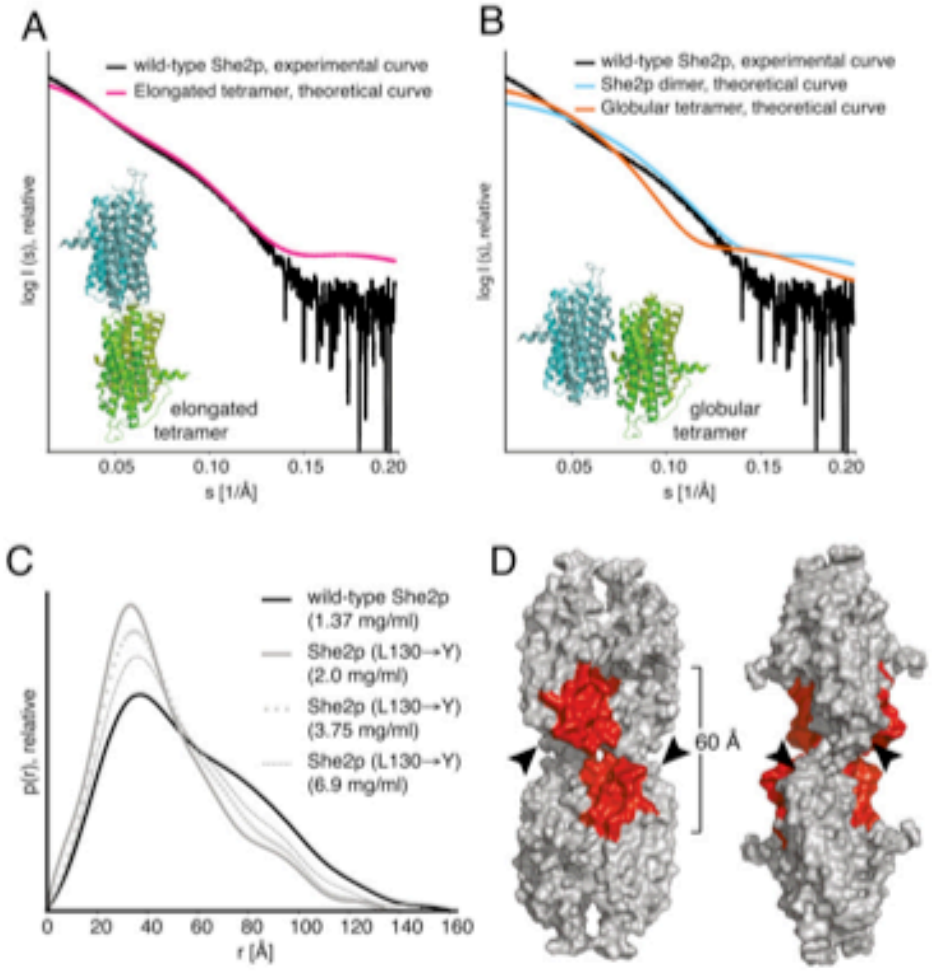

FIGURE 5. Evaluation of crystallographic tetramers for their presence in solution. (*A*) The theoretical scattering curve of She2p forming the elongated tetramer via the upper uncharged surface (see inset, and also Fig. 4A–C) shows a good fit with the experimental scattering curve of wild-type She2p (shown is the curve measured with 2.8 mg/mL She2p). (*B*) In contrast, neither the theoretical scattering curve of the She2p globular tetramer (inset, and also Fig. 4A) nor the theoretical curve of a She2p dimer matches the experimental curve of She2p. Both tetramer models were directly generated from the published crystal structure (PDB-ID: 1XLY; Niessing et al. 2004) and show, therefore, the original difference in completeness. (*C*) The pair-distribution functions of wild-type She2p suggest an elongated particle, whereas for She2p (L130→Y) a rather globular molecule is assumed. With increasing protein concentration, the pair-distribution functions for She2p (L130→Y) approach the shape of the wild-type She2p p(*r*)-distribution, suggesting a concentration-dependent oligomerization of the mutant protein. (*D*) Surface representation of the elongated tetramer depicted from the front (*left*) and rotated by about 90° (*right*). The basic-helical hairpin as RNA-binding motif is highlighted in red. The positions of the C-terminal tails, which were absent in the crystal structure, are indicated by arrowheads. This structural model has been derived by substituting the less complete monomer from the crystal structure by its more complete counterpart. For further details, see Niessing et al. (2004). The indicated distance shows the dimension of the continuous RNA-binding surface on both sides of the tetramer.

(Taken from Müller et al. 2009)