ESRF	**Experiment title:** SAXS studies on mRNP components from yeast and human	**Experiment number**: MX-879
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Shifts: 3	**Local contact(s)**: Adam Round	*Received at ESRF:*

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Report:

This first of two experiments was on one hand used for testing optimal buffer conditions for SAXS analysis. On the other hand, high quality data sets were collected for sub-projects 1 and 2.

Both sub-projects dealt with the analysis of mRNA-binding proteins and their oligomeric state and shape in solution. For both projects, high-resolution crystal structures of sub-domains were available from our group.

Data collection for sub-project 2 (the Pur-alpha RNA-binding protein) was particularly successful. It allowed us to determine the oligomeric state and shape of this protein in solution and contributed important information to the following publication:

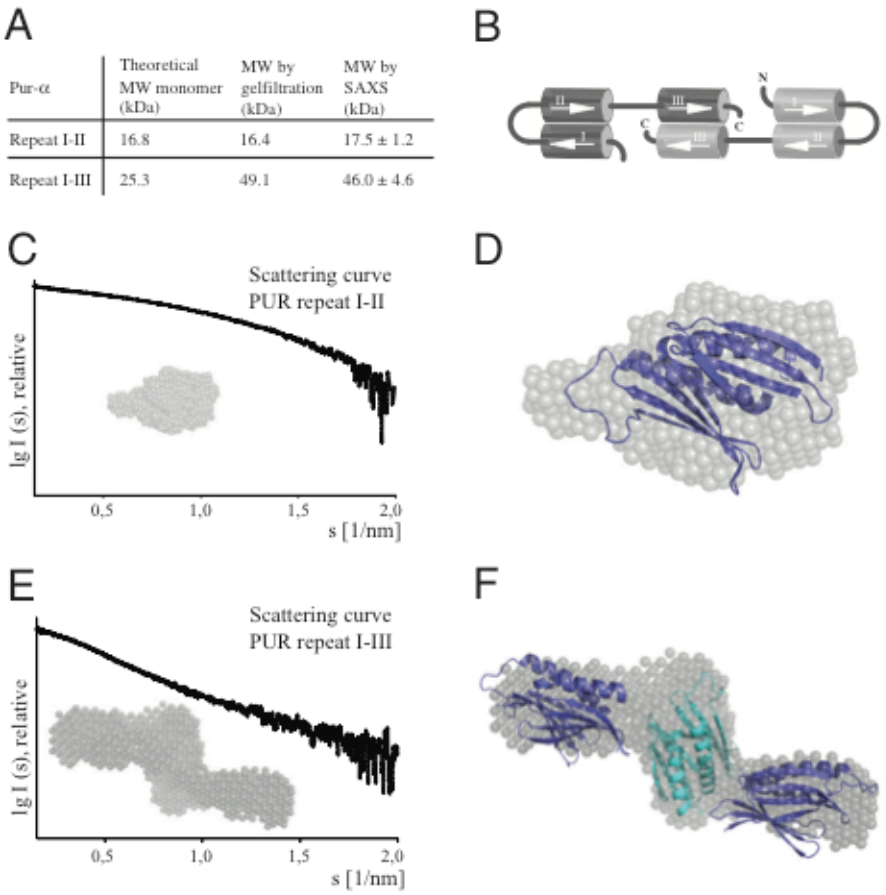
Publication:

Graebsch, A., Roche, S., Niessing, D.: Pur-alpha like proteins are members of the Whirly class of nucleic acid binding proteins.
Proc. Natl. Acad. Sci. USA 106:18521-18526 (2009) – Direct Submission.

Abstract from publication:

The PUR protein family is a distinct and highly conserved class that is characterized by its sequence-specific RNA- and DNA-binding. Its best-studied family member, Pur-α, acts as a transcriptional regulator, as host factor for viral replication, and as cofactor for mRNP localization in dendrites. Pur-α-deficient mice show severe neurologic defects and die after birth. Nucleic-acid binding by Pur-α is mediated by its central core region, for which no structural infor- mation is available. We determined the x-ray structure of residues 40 to 185 from Drosophila melanogaster Pur-α, which constitutes a major part of the core region. We found that this region contains two almost identical structural motifs, termed ‘‘PUR repeats,’’ which interact with each other to form a PUR domain. DNA- and RNA-binding studies confirmed that PUR domains are indeed functional nucleic-acid binding domains. Database analysis show that PUR domains share a fold with the

Whirly class of nucleic-acid binding proteins. Structural analysis combined with mutational studies suggest that a PUR domain binds nucleic acids through two independent surface regions involving concave β-sheets. Structure-based sequence alignment revealed that the core region harbors a third PUR repeat at its C terminus. **Subsequent characterization by small-angle x-ray scattering (SAXS) and size- exclusion chromatography indicated that PUR repeat III mediates dimerization of Pur-α. Surface envelopes calculated from SAXS data show that the Pur-α dimer consisting of repeats I to III is arranged in a Z-like shape. This unexpected domain organization of the entire core domain of Pur-α has direct implications for ssDNA/ ssRNA and dsDNA binding.**



Pur-α	Theoretical MW monomer (kDa)	MW by gelfiltration (kDa)	MW by SAXS (kDa)
Repeat I-II	16.8	16.4	17.5 ± 1.2
Repeat I-III	25.3	49.1	46.0 ± 4.6

Fig. 5. Analysis by SAXS. (*A*) Table summarizing molecular-weight calculations by size-exclusion chromatography and by SAXS. (*B*) Schematic drawing depicting the role of individual PUR repeats in the formation of PUR domains and Pur-α dimerization. (*C*) Scattering curve of Pur-α (I–II) and representative surface envelope calculated from SAXS measurements. (*D*) A fit of the crystal structure into the surface envelope of Pur-α (I–II) confirms the presence of intramolecular PUR domains in solution. (*E*) Scattering curve of Pur-α (I–III) and representative surface envelope calculated from SAXS measurements. These envelopes adopt a Z-like shape. (*F*) A fit of three PUR domains into the Z-like envelope is consistent with the presence of two intramolecular PUR domains and one intermolecular PUR domain (*B*). Structural models of Pur-α (I-II) were positioned manually in the envelopes to demonstrate the compatibility of envelopes with the size of PUR domains.

(Taken from Graeabsch et al. 2009)