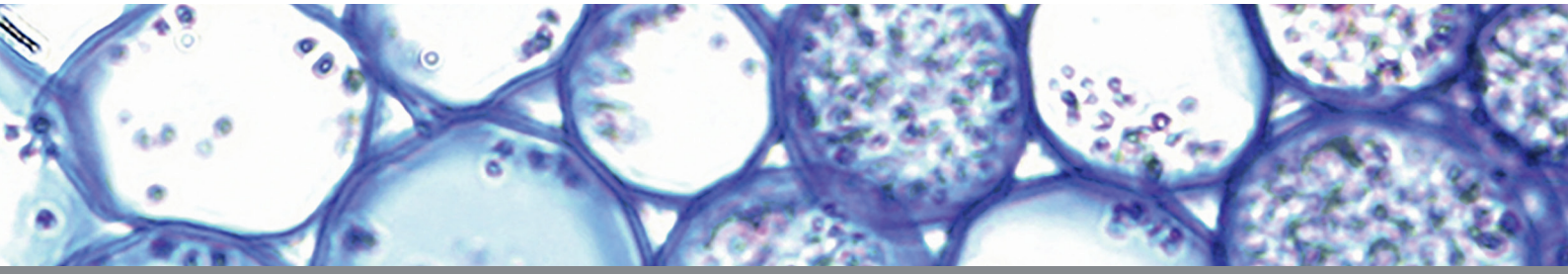

Protein structure resolution

SAXS/WAXS investigation of the protein Sub-unit F of the eukaryotic V1V0 ATPase was performed using the Xeuss 2.0 SAXS/WAXS system, leading to its structure resolution.

Introduction

The Small-angle X-ray Scattering (SAXS) technique is now well known for studying biological systems and more specifically proteins in solution. SAXS measurements allow the determination of the macromolecular shape conformation (by an envelope reconstruction of the investigated protein according to appropriate experimental conditions).
SAXS measurements of protein Sub-unit F were conducted on the Xeuss 2.0 SAXS/WAXS system, which provides a high signal-to noise ratio[1] allowing high data quality collection in the lab with accurate data analysis.

Measurements & results

Measurements were performed on 2, 5, 10 and 17 mg/ml concentration solutions, using the buffer *50 mM HEPES (pH 7.0), 300mM NaCl and 1 mM DTT*, on the Xeuss 2.0 SAXS/WAXS system using a photon counting Pilatus 1M pixel detector.

The figure 1 displays the evolution of I(q=0) as function of the concentration. A clear discrepancy in the intensity scaling away from the linear behavior might indicate either the influence of inter-particle interactions or the presence of aggregates for concentration above 10 mg/ml. Then, the concentration of 10 mg/ml was chosen to provide higher scattering without additional contribution to that of the protein.

The resulting 1D curves are reported in Figure 2. The pair-distance distribution function p(r), calculated using the GNOM[2] software, is shown on Figure 3. The curve displays a principal maximum at 2.8 nm and a protruding shoulder from 5.5 nm up to 7.0 nm. Such results are consistent with synchrotron measurements[3].

Figure 4 shows the low resolution structure of Sub-unit F, restored ab-initio using DAMMIN[2], with a single iteration. The protein appears as a two domain molecule with a large egg-like-shape, connected via a long stalk to a small hook-like region at the end. This looks very close to the obtained external envelope reconstruction from synchrotron data[3].

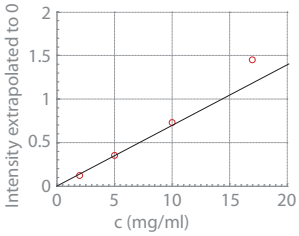

Fig. 1 - Evolution of I(q=0) as function of concentration. The black line is a guide to eye, passing through 0,0.

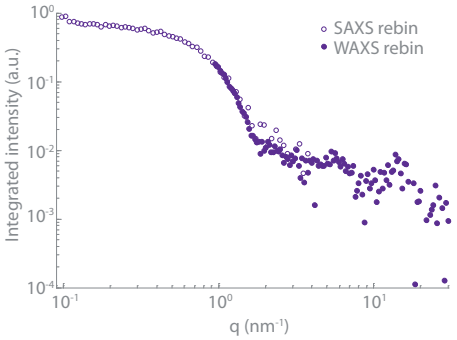

Fig. 2 - 1D curve from Subunit F, c = 10 mg/ml. Exposure time = 6 x 600s. Logarithmic rebin of data.

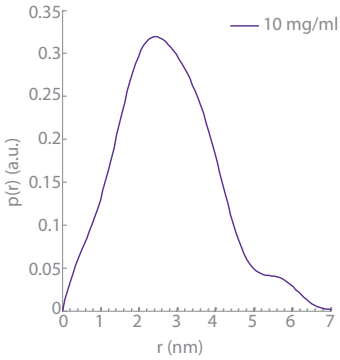

Fig. 3 - Pair-distance distribution function.

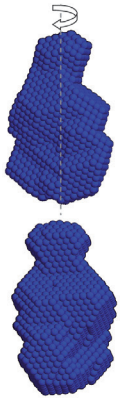

Fig. 4 - 3D structure ab-initio modeling using DAMMIN.

To go further

Data generated with the Xeuss 2.0 was compared by Dr D. Svergun with data collected from the SAXS beamline X33 at the DORIS III storage ring at DESY[4], using the same experimental conditions (buffer, concentration). As reported by Dr D. Svergun, *"The Xeuss data matched well the synchrotron results [...] The accuracy of the lab source ensures a reliable reconstruction of the protein shape."*

[1]TN-XE04-Unique Signal-to-Noise ratio.
[2]Petoukhov et al. J. Appl. Cryst., 2007, 40 s223-s228.
[3]S. Basak et al., Biochimica et Biophysica Acta 1808 (2011) 360-368.
[4]Deutsches Elektronen-Synchrotron, EMBL, Hamburg.

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