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Investigation of multi-subunit protein complexes by SAXS (MX-1224)

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Small-angle X-ray scattering has emerged as a powerful method to restore shapes of complex, dynamic, often large macromolecular complexes. We have taken advantage of the unique opportunities offered by the BioSAXS beamline at ESRF (ID14eh3) to investigate the solution properties of an array of proteins and protein complexes that have proved hard to crystallize thus far, and we report hereby on our SAXS studies of a histone methylation reader protein, Yng2p.

Yng2p is part of large multi-subunit complexes involved in histone acetylation, particularly in H4 acetylation via the NuA4 complex. Despite extensive crystallization trials were conducted we were unable to grow Yng2p crystals of sufficient size and quality for detailed crystallographic analysis. Given that we had further experimental evidences that suggested that the Yng2p structure might be much more dynamic than anticipated and adopt a variety of quaternary structures, we set out to investigate its behaviour in solution by SAXS.

We measured SAXS data using the standard setting of the ID14eh3 beamline using 35 uL of concentrated protein sample at different concentrations and using dialysis buffer as reference. The concentrations measured spanned a wide range between 2.4 and 11.3 mg/mL. Under these experimental conditions no radiation damage could be appreciated. Model independent parameters were calculated for each concentration directly from SAXS data after buffer subtraction, resulting in radii of gyration (R_G) of 5.5–7.5 nm and maximum diameter (D_{max}) of 25 nm. The molecular mass estimate derived from Porod volume analysis was roughly in agreement with the theoretical molecular mass for a tetrameric Yng2p complex (124 kDa) but was larger (140–160 kDa). Even though errors of 20% are typical in molecular weight estimations through Porod volume calculations, the apparent excess mass, together with the R_G and D_{max} values, strongly indicate that the shape of Yng2p in solution departs very significantly from a spherical volume.

Furthermore, shape restoration with DAMMIF from merged SAXS data provided an ensemble of shapes that were predominantly elongated in shape. This shape, which can be described as a prolate ellipsoid, could easily explain the larger apparent size of Yng2p in solution with respect to naïve expectations based on a spherical shape.

We hope to pursue further structural analyses on this particle in the context of its physiologic binding partners (sub-complexes of NuA4) with x-ray crystallography (whenever possible) and SAXS to gain a more solid understanding of the factors governing Yng2p oligomerization and how its quaternary structure influences its engagement in NuA4 and in binding specifically methylated tails of histone H4.

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