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Supplemental Information

Solution Structure of an Intramembrane Aspartyl Protease via Small Angle Neutron Scattering

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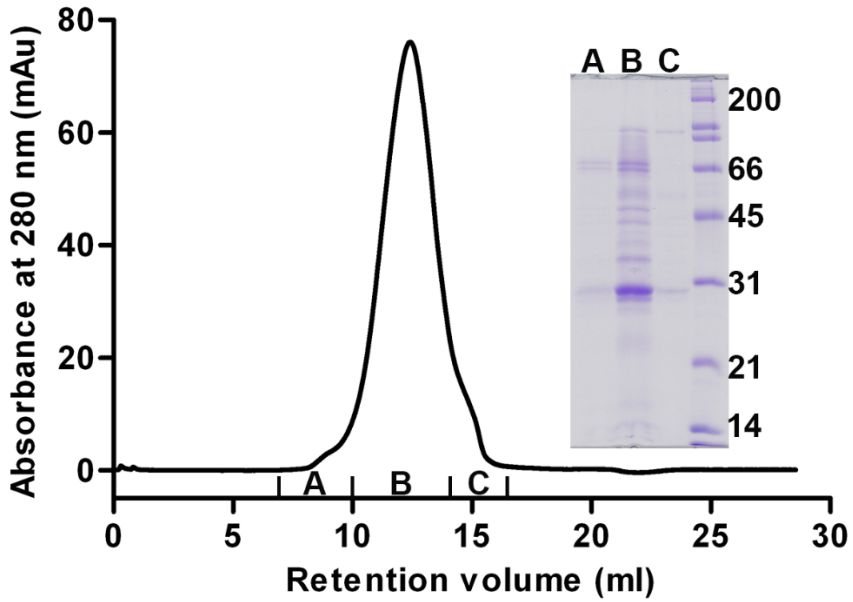

Figure S1. *d*-MmIAP purification. Final size exclusion chromatogram (Superose-12 10/300 GL column) for *d*-MmIAP equilibrated with 20 mM Hepes (pH 7.5), 250 mM NaCl, 48.5% D_2O and 0.05% total DDM, of which 44% (w/v) is tail-deuterated d_{25}-DDM (Anatrace). Inset: SDS-PAGE analysis of pooled fractions labeled A, B, C. Region labelled "B" was used in the SANS experiment.

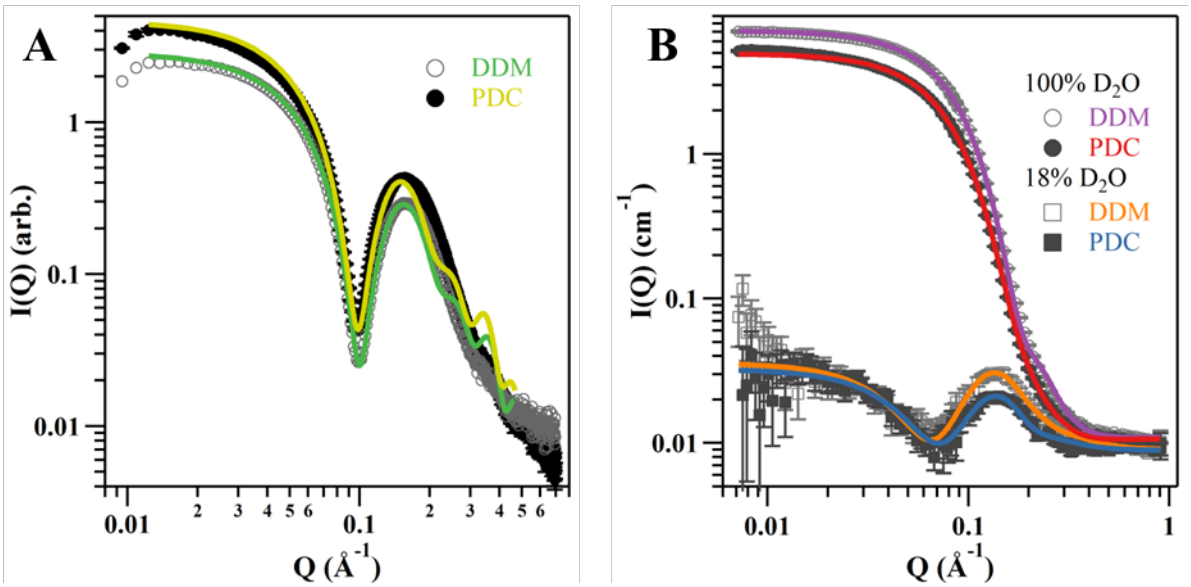

Figure S2. Preliminary (A) SAXS and (B) SANS data collected for solutions of micelles (DDM – unfilled markers) and the protein-detergent complex (PDC – filled markers). Strong similarity was observed in scattering profile shapes between these two conditions at all measured contrasts. Core-shell ellipsoid model fits are shown by the solid lines, colored according to the legend.

Particle molecular volume estimates

The SANS data were placed on absolute intensity scale, and an independent estimation of particle size from the forward scattering intensity (I_0) is therefore in principle possible. The sample molecular weight (MW) can be determined using the calibrated I_0 value, sample concentration (c), and particle contrast ($\Delta\rho$) according to the following equation:

$$MW = \frac{I_0 \cdot N_A}{c(\Delta\rho \cdot v)^2} \quad (1)$$

where N_A is Avagadro's number, and v is the partial specific volume of the protein (1). However, as Tainer et al. (2) have pointed out, this approach is difficult, given the limits in accuracy of known particle concentration and other factors. SANS experiments also have the added challenge of accurately assessing the particle's neutron contrast, particularly when the particle is a protein expressed in deuterated growth media.

An estimate of the protein contrast was made by inferring the level of deuterium incorporation at non-exchangeable positions in the protein from published empirical evidence. Previous studies suggest that a protein expressed in growth media containing ~90% D_2O yields a protein with 70-75% incorporation of deuterium at the non-exchangeable positions of the protein (3). Particle concentration was estimated to be 2.52 ±0.38 mg·mL^{-1} by NanoDrop spectrophotometer (ThermoScientific) measurements of the absorbance at 280 nm using a calculated molar extinction coefficient of 33,920 M^{-1} cm^{-1} from the IAP primary sequence. Although no absorbance in this region was observed for protein-free detergent micelle solutions, we cannot completely exclude the possibility that the suspension of membrane protein in detergent micelles interferes with this concentration estimate. From the apparent intensity of our gel electrophoresis data, we place an upper boundary of the concentration at 5 mg·mL^{-1}. This limit, in conjunction with the uncertainties in particle contrast, yields a total range of uncertainty

in molecular weight that is too broad to identify the oligomeric state of MmIAP from I_0 with any useful accuracy.

Conversely, we obtained estimates for the particle volume from two intensity scale independent methods: The DAMMIN model yields a total excluded particle volume of 20,590 Å^3 in fair agreement with the calculated volume of monomeric MmIAP (31,258 Å^3 from PDB 4HYC, chain A, using VADAR v1.8, Univ. of Alberta, or 40,778 Å^3 from primary sequence using MULCh, University of Sydney). The concentration-independent approach described by Rambo and Tainer to determine molecular weight was also employed (2), using the SAS invariant Q_R and the following relationship for proteins:

$$Mass = \left(\frac{Q_R}{0.1231}\right)^{1.0} \qquad (2)$$

where $Q_R = V_c^2 \cdot R_g^{-1}$, and V_c is the volume-of-correlation. Additional details and plots used to determine the volume-of-correlation from the integrated area of $Q^*I(Q)$ are provided in Figure S3 below. This approach yields a molecular weight of 16.4 kDa, smaller than a single molecule of MmIAP. Both estimates support the conclusion that MmIAP in solution is a rather small scattering object, a result that is consistent with the hypothesis of MmIAP being monomeric rather than oligomeric.

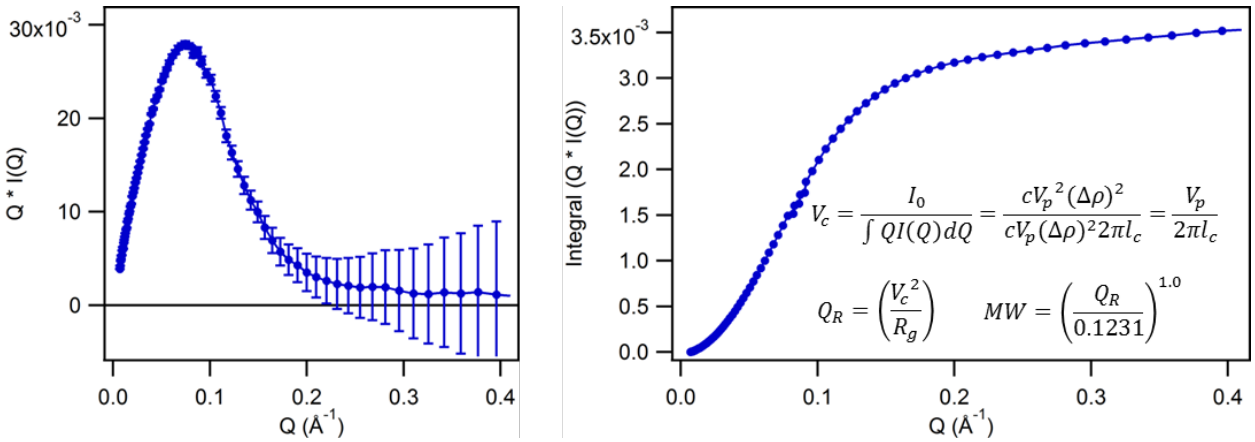

Figure S3. Determination of protein molecular weight from the SAS invariant Q_R. Data are replotted first as a modified Kratky plot (Q*I(Q) vs Q), where flexible and non-flexible proteins, and even unfolded proteins, should converge with zero intensity at high Q. This plot is then integrated to an upper limit defined by the scattering signal decay or plateau of integration (Q_{max} ≈ 0.25 was used here). The ratio of I_0 to this integrated value determines the volume-of-correlation for the particle to be used in estimating the molecular weight of the particle.

Supporting References

1. Zaccai, N. R., I. N. Serdyuk, and J. Zaccai. 2017. Methods in Molecular Biophysics. Cambridge University Press.
2. Rambo, R. P., and J. A. Tainer. 2013. Accurate assessment of mass, models and resolution by small-angle scattering. Nature 496:477-+.
3. Leiting, B., F. Marsilio, and J. F. O'Connell. 1998. Predictable Deuteration of Recombinant Proteins Expressed inEscherichia coli. Analytical biochemistry 265:351-355.