Domain-selective structural analysis of the ER-60 using inverse contrast-matching small-angle neutron scattering

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ER-60, an oxidative protein folding enzyme that belongs to the PDI family, is a multi-domain protein consisting of four thioredoxin-like domains, **a** and **a'** domains with catalytically active cysteine pairs and chaperone association **b** and **b'** domains in the order **a-b-b'-a'** [1]. It is thought that each domain works cooperatively to perform its function, and that there is a correlation between the domain motions of different domains depending on the function. To observe the motion and structure of multi-domain proteins in solution, Inverse Contrast Matching Small-Angle Neutron Scattering (iCM-SANS) [2], which takes advantage of the large difference in neutron scattering length between hydrogen and deuterium, is useful.

First, we prepared hydrogenated **a** and **a'** domains of ER-60 and 75% deuterated **bb'** domain of ER-60 with the *E. coli* expression system. To prepare 75% deuterated recombinant protein, *E. coli* was cultured in 75% and 95% deuterated M9 medium [3]. In 75% deuterated M9 medium, the ratio of D_2O and deuterated glucose were 75%. In 95% deuterated M9 medium, D_2O ratio was 95%, and hydrogenated glucose was 100%. If 95% deuterated M9 medium could be utilized without any problems, it would be possible to save the cost of deuterated glucose.

Second, the hydrogenated and deuterated domains of ER-60 were ligated in the order **a-bb'-a'** using the ligating enzyme *Oa*AEP [4]. However, due to aggregation, the ligated sample could not be used for SANS experiments. Therefore, we decided only to perform contrast matching experiments of **bb'** domains.

2 mg/mL 75% deuterated **bb'** domains from 75% and 95% deuterated M9 medium were measured using SANS in 100% and 70% D_2O buffer (20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM $CaCl_2$) at 25 °C. As expected, 75% deuterated **bb'** domains were scatteringly invisible due to contrast matching (Fig. 1A), while in 70% D_2O buffer these were observed (Fig. 1B). Since **bb'** domains prepared from 75% and 95% deuterated M9 medium were both scatteringly invisible, it was shown that using 95% deuterated M9 medium would result in cost savings.

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[2] M. Sugiyama *et al.*, J. Appl. Crystallogr., **47,** 430–435 (2014).
[3] A. Okuda *et al.*, Biophys Physicobiol., **18,** 16-27 (2021).
[4] A. Okuda *et al.*, Angew. Chem. Int. Ed. (2022) **62**, e202214412.

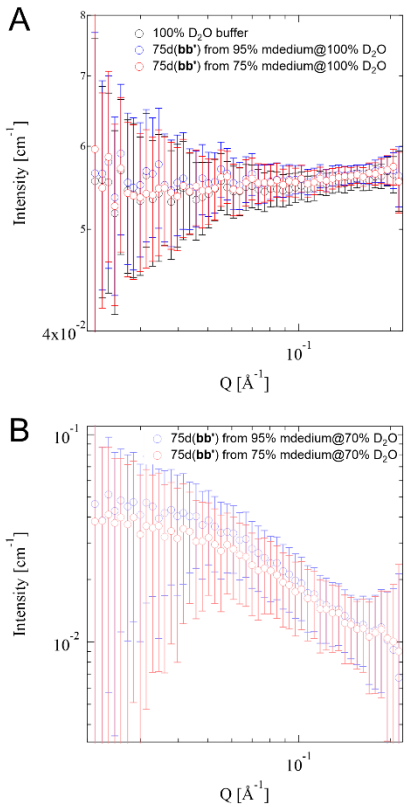

Fig. 1. SANS profiles of (A) 75% deuterated **bb'** domain in 100% D_2O buffer and (B) 75% deuterated **bb'** domain in 70% D_2O buffer.