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 of the protein disulfide isomerase (PDI) family, is a multi-domain protein composed of four thioredoxin-like domains arranged in the order **a**-**b**-**b'**-**a'**. The catalytic **a** and **a'** domains and the chaperone-associated **b** and **b'** domains are thought to function cooperatively through domain motions associated with the protein's biological function [1]. Inverse Contrast Matching Small-Angle Neutron Scattering (iCM-SANS) [2], which exploits the large difference in neutron scattering length between hydrogen and deuterium, enables selective observation of specific domains through segmental deuteration. In this study, segmentally deuterated ER-60 was analyzed using iCM-SANS to investigate the motions of the **a**-**b**-**b'** and **b**-**b'**-**a'** regions in solution.

Hydrogenated and 75% deuterated ER-60 fragments were prepared using an *E. coli* expression system. For 75% deuteration, *E. coli* was cultured in 75% deuterated M9 medium [3], containing 75:25 mixtures of D_2O/H_2O and deuterated/non-deuterated glucose.

Hydrogenated and 75% deuterated ER-60 fragments were ligated using *Oa*AEP [4] to generate two segmentally deuterated constructs, *pd*(**a**)-*h*(**bb'a'**) and *h*(**abb'**)-*pd*(**a'**), where *h* and *pd* indicate hydrogenated and 75% deuterated fragments, respectively.

Size-exclusion chromatography coupled with-SANS (SEC-SANS) measurements were performed for wild-type ER-60 (*h*(**abb'a'**)) and the two segmentally deuterated ER-60 constructs, *pd*(**a**)-*h*(**bb'a'**) and *h*(**abb'**)-*pd*(**a'**). The *h*(**abb'a'**), *pd*(**a**)-*h*(**bb'a'**), and *h*(**abb'**)-*pd*(**a'**) samples were concentrated to 11.2, 1.8, and 4.1 mg mL^{-1}, respectively, in a final volume of 500 µL prior to measurement. The measurements were carried out at 25 °C using a Superdex 200 Increase column. The running buffer consisted of 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 1 mM $CaCl_2$ prepared in 100% D_2O. The flow rate was reduced from 0.5 to 0.1 mL min^{-1} immediately before the elution peak entered the flow cell. The flow was then stopped after the peak maximum had passed, and SANS data were collected in the stopping mode.

SEC successfully removed aggregates from all samples before SANS measurements (Fig. 1). SANS scattering profiles free from contributions of aggregates were obtained for *h*(**abb'a'**), *pd*(**a**)-*h*(**bb'a'**), and *h*(**abb'**)-*pd*(**a'**) (Fig. 2). Structural analysis of the dynamic domain motions of ER-60 is currently in progress.

[1] A. Okuda *et al.*, Sci Rep., **11,** 5655 (2021).
[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.

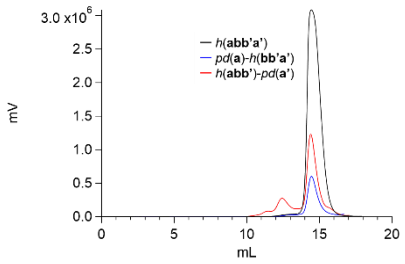

Fig. 1. SEC chromatograms of *h*(**abb'a'**) (black), *pd*(**a**)-*h*(**bb'a'**) (blue), and *h*(**abb'**)-*pd*(**a'**) (red).

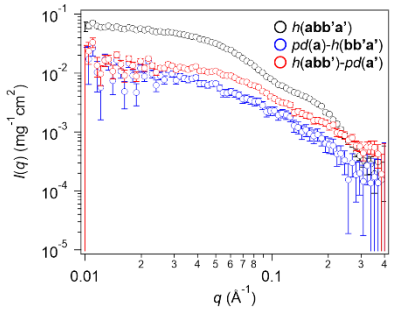

Fig. 2. SEC-SANS scattering profiles of *h*(**abb'a'**) (black), *pd*(**a**)-*h*(**bb'a'**) (blue), and *h*(**abb'**)-*pd*(**a'**) (red).