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Kinetic Asymmetry of Subunit Exchange of Homo-Oligomeric Protein as Revealed by Deuteration-assisted Small-Angle Neutron Scattering

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Supporting Materials

Sample preparation

Human proteasome α7 subunit was expressed using *Escherichia coli* [BL21(DE3)]. For the preparation of deuterated subunits, the cells were grown in M9 minimal media containing deuterated glucose (2 g/L) and 99.8% D_2O. After sonication and centrifugation, cell lysates were subjected to anion exchange chromatography (DEAE Sepharose Fast Flow, GE Healthcare). Roughly purified samples were dialyzed and further purified by HPLC system sequentially using an anion exchange column (RESOURCE Q, GE Healthcare) and gel-filtration column (HiLoad 26/60 Superdex 200 pg, GE Healthcare). The purified proteins were concentrated to 5 mg/ml in buffer solutions composed of 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, and 100% H_2O or 98.2% D_2O. In the aqueous solution, the α7 subunits simultaneously form the double heptameric ring, which was confirmed by the SANS experiment.

The protocol for scattering experiments is schematically illustrated in fig. S1. In step 1, four isotopically different α7 solutions were prepared: h-α7 in H_2O (solution #1), h-α7 in D_2O (solution #2), d-α7 in H_2O (solution #3), and d-α7 in D_2O (solution #4). In step 2, the 81% D_2O solution of the h-α7 tetradecamer (solution #5) was prepared by mixing solution #1 with solution #2, whereas the 81% D_2O solution of the d-α7 tetradecamer (solution #6) was prepared by mixing solution #3 with solution #4. After

mixing, time evolution of SANS of solutions #5 and #6 were measured, the results of which are shown in Fig. 2A. Finally, in step 3, the 81% D_2O solution of the 1:1 mixture of the h-α7 and the d-α7 tetradecamers (solution #7) was prepared by mixing equal amounts of solutions #5 and #6. Again, after the mixing, time evolutions of SAXS and SANS of this solution were measured for 12 h, the results of which are shown in Fig. 2B and 2C, respectively.

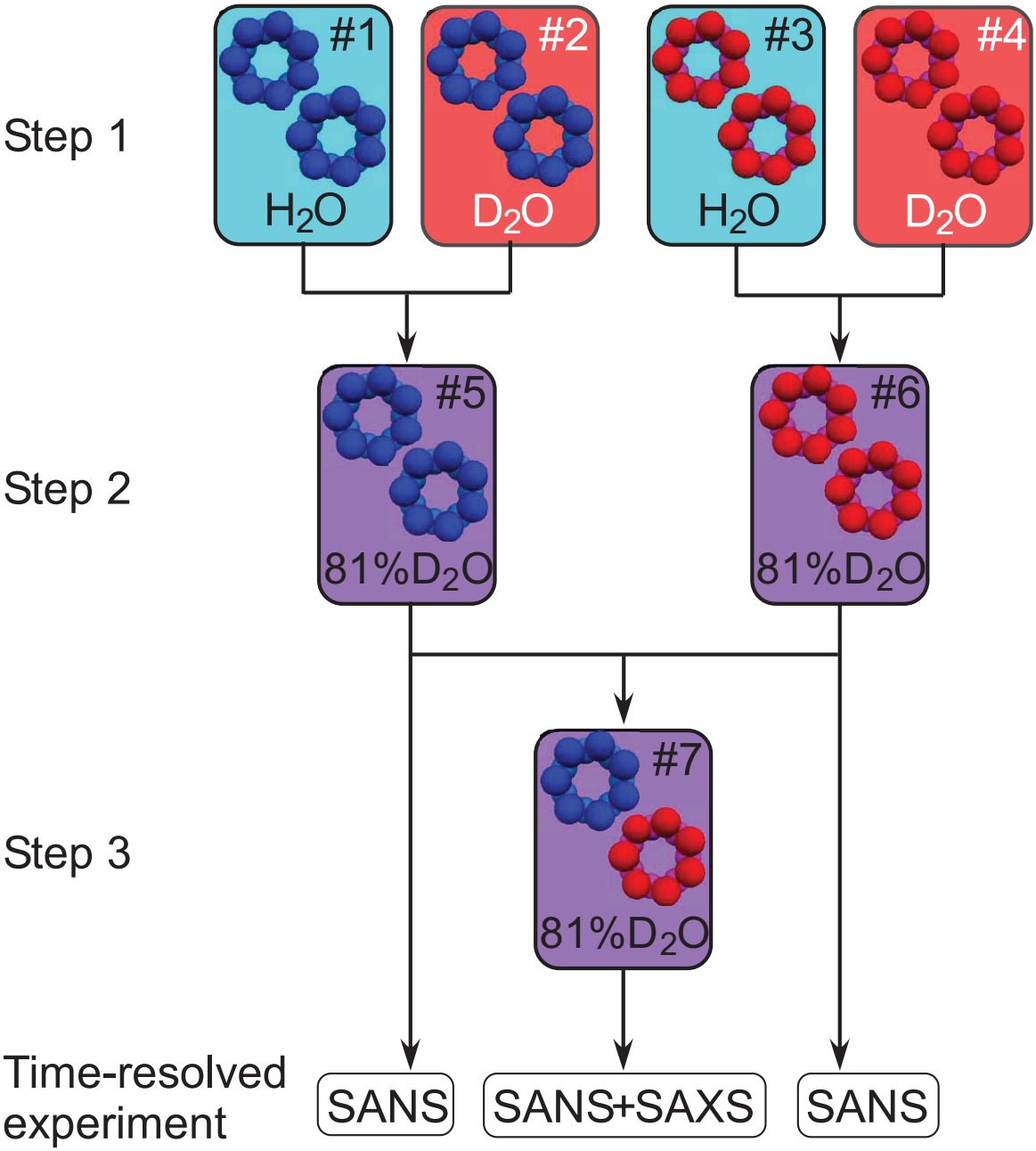

Figure 1: Sample preparation for scattering experiments.