Structural analysis of amyloid intermediate by small-angle neutron scattering

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A new dementia patient is diagnosed every 3 seconds, and the number of patients is expected to exceed 130 million by 2050. To realize a healthy and long-lived society, it is essential to curb this increasing trend. While abnormal aggregation of proteins (amyloids) in the brain is closely associated with the onsets of the various neurodegenerative diseases that cause dementia, no fundamental treatment has been established. This is because understanding these diseases and developing treatments requires capturing transient changes in proteins, which is difficult with existing techniques. In particular, it is challenging to detect intermediate species such as amyloid nuclei and oligomers that play a critical role in disease onset.

In response to this challenge, we established a rheology-NMR method capable of *in situ* observation of protein aggregation at atomic resolution. Using this method, we observed the aggregation process of the ALS-related protein SOD1 at atomic detail [1]. However, while the rheology-NMR method is powerful for low molecular weight samples, it is not well-suited for structural analysis of *mesoscale* species such as amyloid intermediates, which are crucial for understanding disease mechanisms.

To investigate the molecular shape and morphological changes of intermediates that cannot be captured by rheology-NMR, we performed small-angle neutron scattering (SANS) measurements on SOD1 oligomers captured at different phases of fibril formation. Specifically, in this year's study, we focused on evaluating the structural and thermodynamic stability of these oligomers depending on their formation phase. SANS profiles were acquired immediately after sample preparation and after a 2-hour incubation at room temperature to trace transient structural alterations.

The SANS results revealed that the state and stability of the oligomers dynamically vary depending on the specific phase of fibrillation. The oligomers obtained from the early and middle stages of fibril formation exhibited a pronounced increase in scattering intensity after 2 hours of incubation (Fig. 1, Oligomer A and B). This enhancement indicates that these early-stage intermediate species are structurally unstable and prone to further aggregation or structural rearrangement over time. Conversely, the scattering profile of the oligomers sampled at the late stage of fibril formation remained virtually unchanged even after the 2-hour incubation (Fig. 1, Oligomer C), suggesting that mature and stable oligomeric structures had already been established. These findings successfully capture the phase-dependent evolution of amyloid intermediates, providing crucial mesoscale structural insights that bring us closer to elucidating the underlying mechanisms of amyloid formation.

[1] N. Iwakawa, *et al*. J. Am. Chem. Soc. **143**, 28, 10604–10613 (2021).

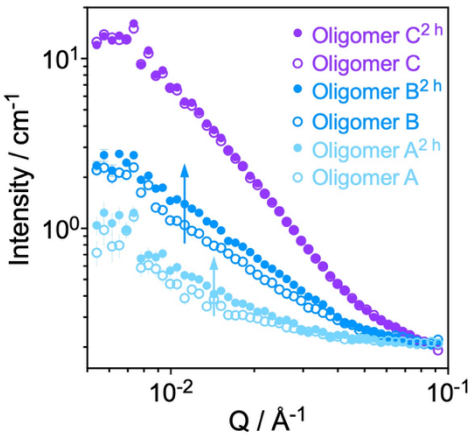

Fig. 1. SANS profiles of SOD1 oligomers at different phases of fibril formation. Light blue, blue, and purple symbols represent oligomers sampled at the early (Oligomer A), middle (Oligomer B), and late (Oligomer C) stages of fibrillation, respectively. Superscription with “2 h” denotes measurements after 2 hours of incubation.