HDX for Aggregating Proteins

Protein aggregation is the cause of Alzheimer's Disease (AD), Parkinson's Disease, and Huntington's Disease, motivating the development of new approaches to understand this process. Amyloid beta (Aβ) has a propensity to aggregate and affects brain function. In AD, the aggregation of the 40- and 42-aa-long Aβ peptide, generally called $A\beta_{40}$ or $A\beta_{42}$, is proposed to be involved in the onset of the disease, with $A\beta_{42}$ is being the most neurotoxic and amyloidogenic member of the family.

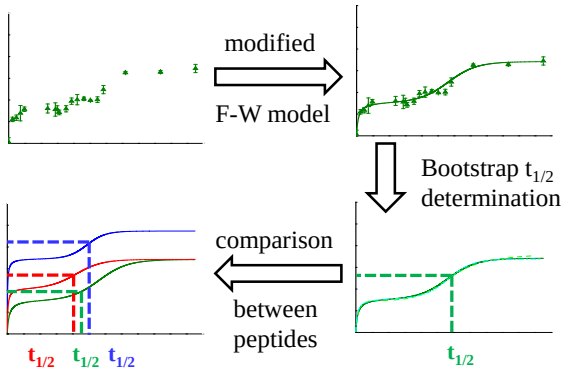

Figure 1. A typical workflow for a pulsed HDX platform for aggregating proteins (e.g., Abeta).

The structures pertinent to the first and last stages in $A\beta_{42}$ aggregation have been studied extensively by NMR (1), X-ray (2) and fluorescence (3). Nonetheless, knowledge of the soluble aggregates of $A\beta_{42}$ is lacking. This is due both to the heterogeneity of the oligomers formed in the early stages of oligomerization and the time course of their formation. Thus, a time-dependent measurement strategy is required. The application of HDX is challenging because both HDX and aggregation occur simultaneously. In order to separate the oligomerization and HDX events, we developed a pulsed HDX strategy. In this experiment, $A\beta_{42}$ is allowed to incubate for various times and then the solvent is exchanged for D_2O for 1 minute (i.e., a pulse of HDX). The reaction is quenched and analysis proceed as in a regular HDX experiment. The proteolytic fragments contain the details of aggregation at various stages and indicate that $A\beta_{42}$ aggregates in an auto-catalytic fashion. The pulsed HDX curves are fitted by an adaptation of Finke-Watzky modeling (4). A single parameter $t_{1/2}$, the time point at which the number of $A\beta_{42}$ molecules in one state equals half of that in beginning state, can be used to characterize the system behavior. A bootstrap strategy in the $t_{1/2}$ analysis is used to evaluate the precision, allowing the use of a simple t-test for establishing differences. By comparing the $t_{1/2}$ values, we obtained the "aggregation order" of different regions (Figure 1). The results show that the middle region (i.e., 20-35) seeds the aggregation process, followed by involvement of the C- and then the N-terminus (i.e., 1-19 and 36-42, which are formed upon pepsin proteolysis). This method also allows a direct examination of other factors that affect oligomerization. Examples include higher temperature and agitation which accelerate aggregation, and the presence of Cu^{2+} which inhibits it. The HDX results corroborate previous work (5). Continued work int his area includes generating site specific data using ETD or ECD. While this approach is straightforward, aggregation conditions and kinetics must be measured beforehand.

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