Deciphering the structural basis for amyloid secondary nucleation

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Amyloids are highly ordered protein fibrillar assemblies (nanofibrils) intimately associated with a range of incurable disorders, including Alzheimer's disease (AD). A critical characteristic of amyloid diseases is the surface-catalyzed accumulation of cytotoxic amyloid intermediates through a process known as secondary nucleation, although the mechanisms underlying this process remain unclear. The primary goal of this study is to elucidate the structural determinants that govern surface-catalyzed nucleation, for which we have developed a strategy cantered around the creation of single-chain recombinant amyloid-like proteins (SCRAPs). These SCRAPs, designed to mimic amyloid fibril hotspots in a soluble form, aim to facilitate the seeding of amyloid formation, offering a valuable tool for investigating sequence dependency within amyloid hotspots through site-directed mutagenesis and systematic variation of amino acid residues.

To effectively mimic the entire surface of AD-associated amyloid-β (Aβ) fibrils, we have designed various SCRAPs to display different segments of the Aβ fibril surface. This approach, known as "motif scanning", ensures comprehensive coverage of the amyloid surface, enabling a detailed study of its properties. We employed the Thioflavin T (ThT) fluorescence assay to assess the seeding efficacy of AβSCRAPs. By introducing varying concentrations of different SCRAPs into solutions containing 3 μM of Aβ42 and measuring the ThT fluorescence at sequential time points, we observed that at higher concentrations, AβSCRAPv2_002 could seed amyloid formation, reducing the half-time of aggregation by approximately 25%. This effect is similar to the cross-seeding phenomenon observed with Aβ and the yeast prion Sup35, suggesting that AβSCRAPv2_002 successfully mimics certain critical features necessary for secondary nucleation. These results not only underscore the potential of SCRAPs in exploring amyloid formation but also suggest a new avenue for therapeutic intervention in amyloidogenic diseases.