Title:	Site-Specific Fluorescence Depolarization Kinetics Distinguishes the Amyloid Folds Responsible for Distinct Yeast Prion Strains
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Abstract:	The prion determinant of a yeast prion protein, Sup35NM, assembles into β-rich amyloid fibrils that switch the nonprion [psi–] state to the prion [PSI+] state of yeast. Previous studies showed that two distinct forms of amyloids (Sc4 and Sc37), generated in vitro at two different temperatures (4 and 37 °C), recapitulate the strain phenomenon in Saccharomyces cerevisiae. Sc4 demonstrates a strong [PSI+] phenotype, whereas Sc37 shows a weak phenotype. To discern the residue-specific structural and dynamical attributes associated with the amyloids that display strain diversity, we took advantage of the nonoccurrence of tryptophan (Trp) in the NM-domain and created 18 single-Trp variants spanning the entire polypeptide length. The fluorescence readouts from these locations reported the site-specific structural details in Sc4 and Sc37 fibrils. Highly sensitive picosecond fluorescence depolarization measurements at these positions allowed a conformational mobility map to be constructed. Nearly all of the residue positions demonstrated higher local flexibility in Sc4 amyloid, which exhibits a strong phenotype. The differences in the amplitude of local mobility were more pronounced at the two end segments of the N-domain than in the central region. The M-domain is partially exposed and exhibits a higher amplitude of local mobility, indicating a lower degree of chain packing in the amyloid state, as well as a higher mobility in the Sc4 state compared to the Sc37 state. The altered local conformational dynamics in these two distinct amyloid states provide molecular insights into the varied fragility and severing efficiency that govern the inheritance patterns of strong and weak prion strains.
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