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Conformational epitopes exposed on misfolded toxic forms of amyloid-beta, tau and alpha-synuclein directly contribute to their seeding activity

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Background: Misfolded, toxic aggregates of proteins capable of prion-like propagation from cell to cell have been implicated in the pathogenesis of neurodegenerative disorders. For example, toxic oligomers of amyloid-beta (Abeta) as well as oligomers and small soluble fibrils of tau and alpha-synuclein (Asyn) have been reported to contribute to neuronal damage and progression of Alzheimer's disease as well as various tauopathies (e.g. FTLD, progressive supranuclear palsy) and synucleinopathies (e.g. Parkinson's, Lewy body dementia, multiple system atrophy). Misfolding of these proteins into toxic forms leads to the exposure of conformational epitopes not normally present on the healthy form of the protein. In these studies, we sought to determine whether these small misfolded regions may not only represent a target for therapeutic antibodies but also directly contribute to the pathogenic seeding activity of misfolded proteins.

Methods: Computational modeling was used to predict conformational epitopes likely to become solvent-exposed on toxic aggregates of Abeta, tau and Asyn but not on non-toxic forms of the proteins. Antibodies raised against cyclic peptide scaffolds replicating these conformational epitopes were tested for binding to various forms of each protein by surface plasmon resonance (SPR) to confirm restricted expression on toxic species. The seeding activity of the conformational peptide epitope scaffolds was tested in a thioflavin T (ThT) propagation assay by measuring the fibrillogenic aggregation of protein monomers with and without the addition of peptide epitope over time.

Results:
Antibodies raised against predicted conformational epitopes of misfolded Abeta, Asyn and tau showed selective recognition of toxic species by SPR indicating that the epitopes are not exposed on normal, healthy forms of these proteins. The conformational cyclic peptide scaffolds, but not the linear versions of the same peptides, showed seeding activity in ThT propagation assays for all 3 proteins.

Conclusions: Small misfolded regions (conformational epitopes) exposed on misfolded toxic proteins can, on their own, replicate the seeding activity of the full-length protein suggesting that they contribute to its prion-like pathogenicity.

These results also indicate that these conformational epitopes represent a biologically relevant target for therapeutic antibodies.