Turning Affibody Molecules into Efficient Peptide Binders by Dimerization

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Affibody molecules are small (6 kDa) three-helical affinity proteins[1]. Phage display selections of binders from an affibody library against the amyloid beta peptide yielded a variant with 20-pM affinity and with a unique mode of dimeric binding, demonstrating a 2:1 stoichiometry. Biophysical characterization revealed structural rearrangements in both the affibody domains and the amyloid beta peptide upon binding and that the peptide is buried in a tunnel-like cavity, which inhibits the aggregation into senile plaques[2]. Dimeric binders for other peptides have been engineered, demonstrating similar structural rearrangements and mode of binding, indicating that the new dimeric scaffold is well suited for such molecular recognitions. The presentation will include unpublished preclinical data for the amyloid beta binder focused on biodistribution, safety, brain uptake as well as preventive effect in an Alzheimer's disease murine model.

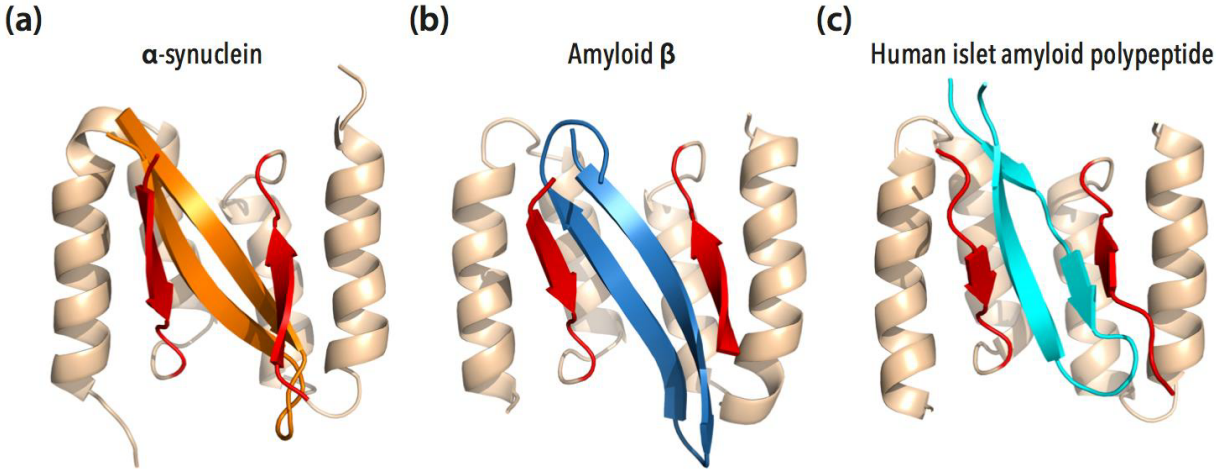

Figure 1. Three-dimensional structures of dimeric affibody molecules in complex with aggregation-prone peptides. *Affibody dimers are shown in gold. The first part of each affibody domain that is typically folded into an alpha helix is instead forming a beta strand (high-lighted in red) and the aggregation prone peptide is folded into a beta hairpin conformation and together they make up a four-stranded beta sheet. (a) Affibody dimer in complex with α-synuclein peptide in orange. PDB ID: 4BXL. (b) Affibody dimer in complex with amyloid β peptide in blue. PDB ID: 2OTK. (c) Affibody dimer in complex with human islet amyloid polypeptide. PDB ID: 5K5G.*

The study highlights first of all the power of directed evolution, where selection from a monomeric affibody library yielded a new type of dimeric binder that is structurally different from the original affibody domain and with a distinct mode of binding, as well as the impact of library design on success in directed evolution.

1. Ståhl S. *et al.* Affibody Molecules in Biotechnological and Medical Applications. *Trends Biotechnol.* **2017** Aug;35(8):691-712.
2. Hoyer W. *et al*. Stabilization of a beta-hairpin in monomeric Alzheimer's amyloid-beta peptide inhibits amyloid formation. *Proc Natl Acad Sci U S A.* **2008** Apr 1;105(13):5099-5104.