Supplemental Material

Crystal Structure of the Amyloid-β p3 Fragment Provides a Model for Oligomer Formation in Alzheimer's Disease

Victor A. Streltsov[1], Joseph N. Varghese[1], Colin L. Masters[2], Stewart D. Nuttall[1]
[1]CSIRO Materials Science and Engineering, and Preventative Health Flagship, 343 Royal Parade, Parkville, VIC 3052, Australia. [2]Mental Health Research Institute, The University of Melbourne, Parkville, VIC 3010, Australia.

Supplementary Figures

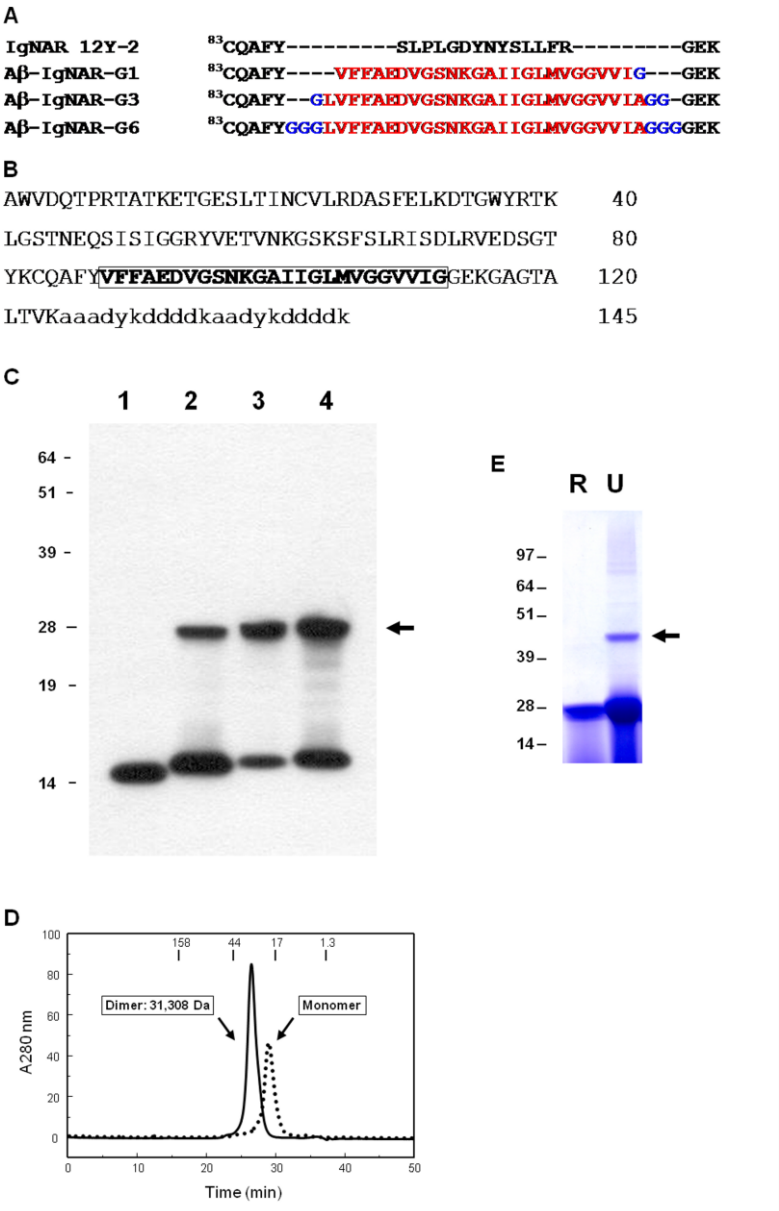

Fig. S1. Construction and crystallization of Aβ-IgNAR chimeric proteins. (*A*) Aβ residues Leu17-Ala42 or Val18–Ile41 were engineered into the IgNAR CDR3 loop region, connected by variable length glycine linkers to promote rotational freedom. For Aβ-IgNAR-G1, the Ala42Gly substitution was required for re-alignment with the IgNAR G-strand. (*B*) Sequence of Aβ-IgNAR-G1 including dual C-terminal octapeptide FLAG purification tags and alanine linker regions (shown in lower case). The Aβ component is boxed and bolded. (*C*) Western blot analysis (reducing conditions) reveals the presence of dimeric species (arrowed at ~30 kDa) for Aβ-IgNAR-G1 (lane 2), Aβ-IgNAR-G3 (lane 3), and Aβ-IgNAR-G6 (lane 4), in contrast to the parental 12Y-2 IgNAR (lane 1). Molecular weight markers (kDa) are indicated on the left. (*D*) Affinity purified Aβ-IgNAR-G1 elutes as a dimer by gel filtration (solid line) in comparison to 12Y-2 IgNAR monomer (dotted line). (*E*) SDS-PAGE of affinity-purified Aβ-IgNAR-G1 under reducing (R) and non-reducing (U) conditions, illustrating formation of tetrameric (arrowed) species in the absence of heating/reducing agent.

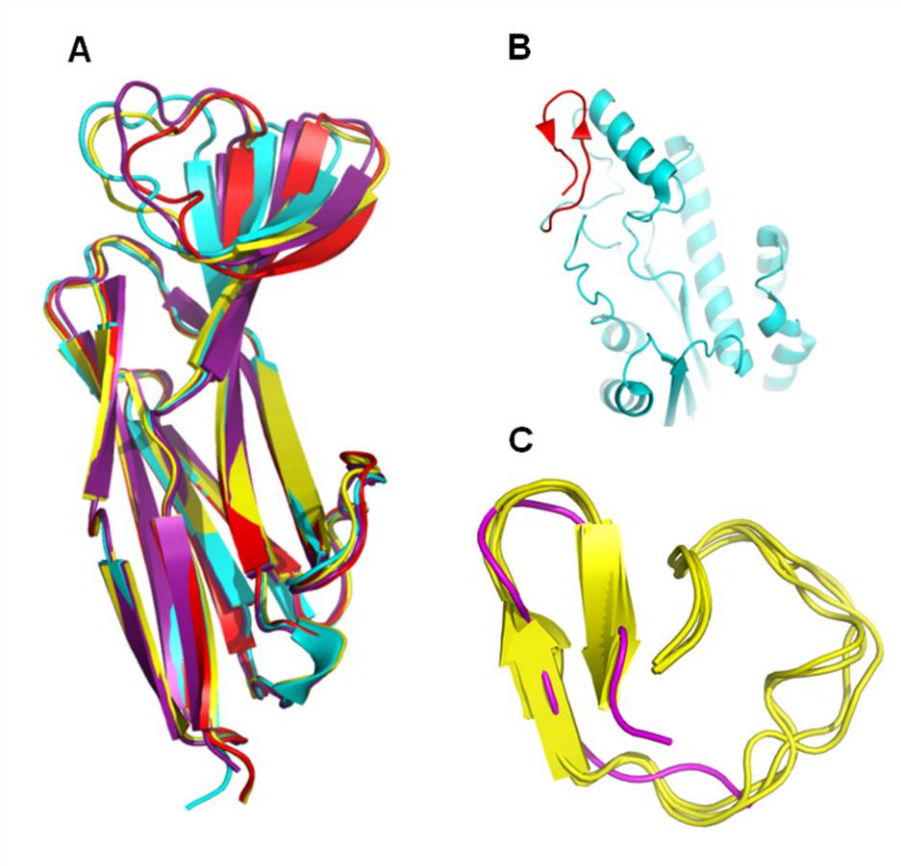

Fig. S2. (*A*) Aβ-IgNAR overlay of chains A-D shows minimal perturbation of the underlying scaffold. Chain are coloured as in Fig. 1*B*. (*B*) Chimeric protein consisting of *Tk*-RNase HII(1-197) (in blue) with C-terminally fused $A\beta_{28\text{-}42}$ (in red) (PDB:1X1P) (Takano et al., 2006). (*C*) $A\beta_{28\text{-}42}$ fragment from (*B*) (in pink) is overlaid with the corresponding residues for Aβ-IgNAR-G1 (in yellow) with a r.m.s.d. = 1.9 Å for 13 atoms.

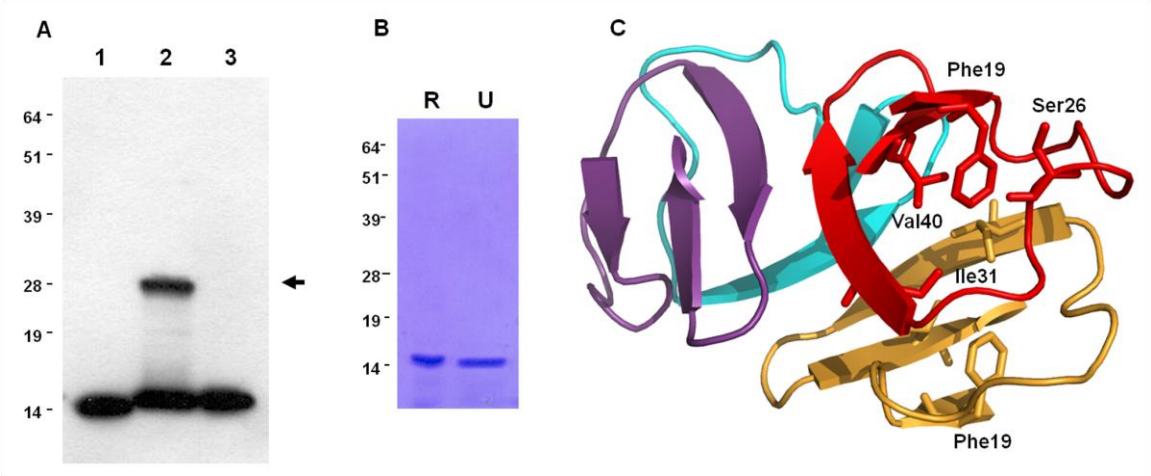

Fig. S3. Mutations that reduce aggregation of the Alzheimer's $A\beta_{1-42}$ peptide (Wurth et al., 2002). (*A*) Mutant Leu34Pro results in a decrease in Aβ-IgNAR-G1 dimerization (arrowed) and reversion to monomeric form. Western blot analysis (reducing conditions) for 12Y-2 IgNAR (lane 1), Aβ-IgNAR-G1 (lane 2), Aβ-IgNAR-G1-Leu34Pro (lane 3). Molecular weight markers (kDa) are indicated on the left. (*B*) SDS-PAGE of affinity-purified Aβ-IgNAR-G1 Leu34Pro under reducing R and non-reducing U conditions, illustrating dissociation to monomeric form. Molecular weight markers (kDa) are indicated on the left. (*C*) Phe19 is important for stabilizing the dimer and it is recognized as affecting the folding and assembly of Aβ by mutations Phe19Ser, Phe19Thr, or Phe19Val (Wurth et al., 2002).

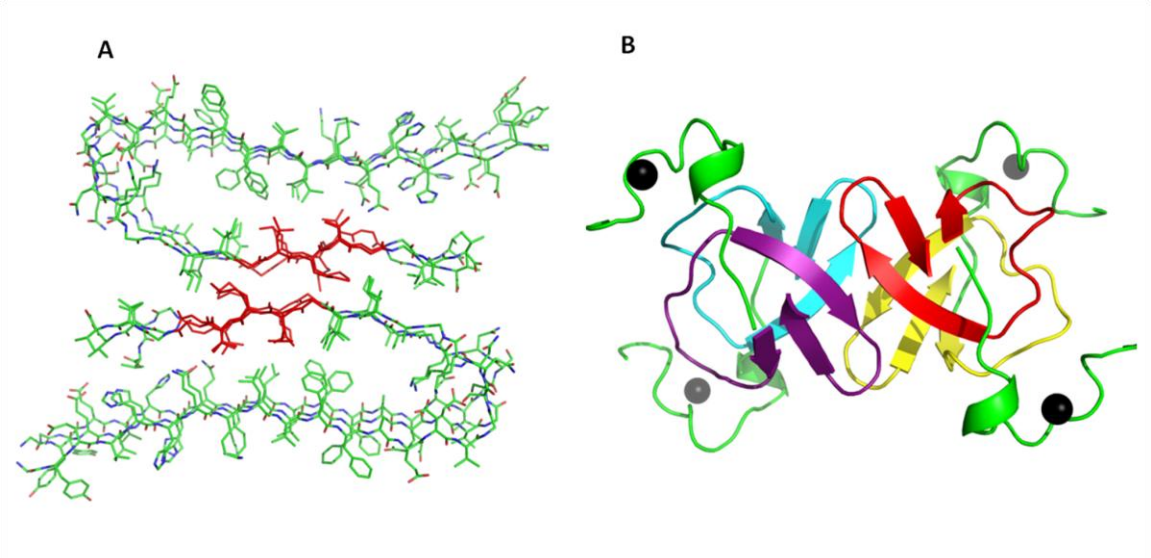

Fig. S4. Construction of the Aβ oligomer model based on the tetramer structure from Aβ-IgNAR with the C-teminal β-sheets aligned to the NMR fibril model (Petkova et al., 2006). (*A*) NMR structure with fragments (AA 33-36) coloured in red are used to align Aβ tetramers. (*B*) Combination of EXAFS structure of $A\beta_{1-16}$-Cu(II) (Streltsov et al., 2008) with crystal structure of $A\beta_{18-41}$ tetramer. The $A\beta_{18-41}$ tetramer chains are coloured as in Fig. 1*B*. The N-terminal fragments $A\beta_{1-17}$ are in green. Black spheres represent transitional metals (Cu, Zn, Fe).

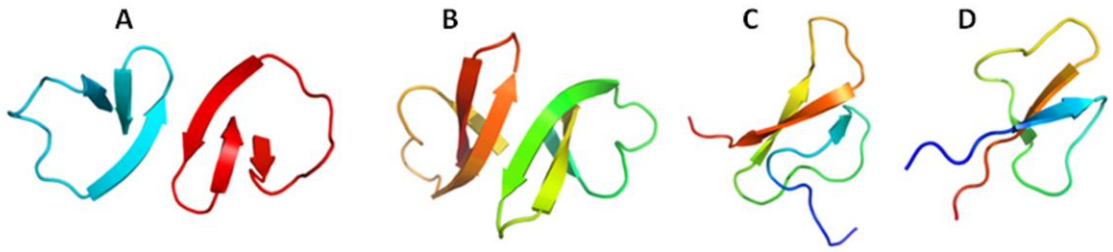

Fig. S5. Similarities between (*A*) $A\beta_{18-41}$ dimer formed by the chains A and D and structures of anti-microbial peptides: (*B*) Crystal structure of human neutrophil alpha-defensin 2 (HNP2) dimer (PDB ID: 1ZMI); (*C*) Solution structure of horseshoe crab antimicrobial peptide tachystatin B (PDB ID :2DCV); (*D*) Solution structure of cryptdin-4, the most potent α-defensin from mouse (PDB ID:1TV0).

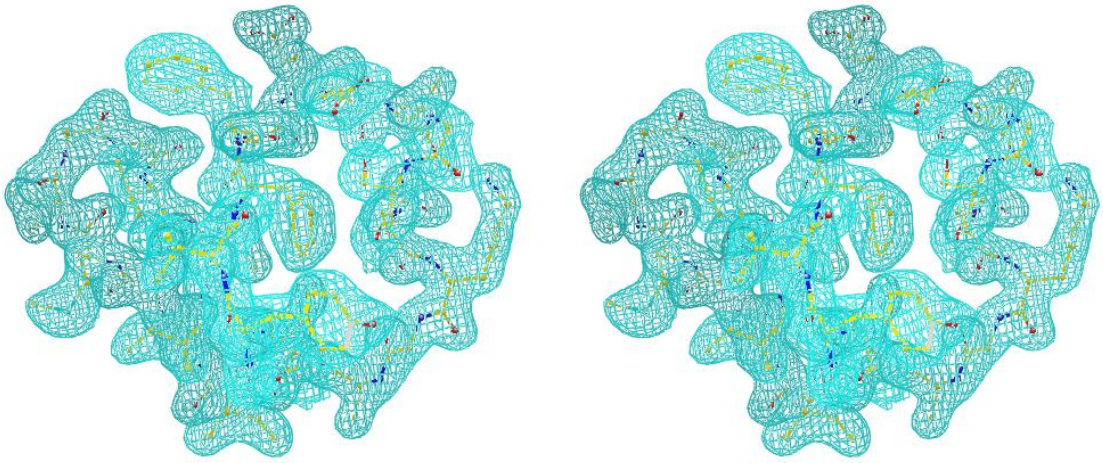

Fig. S6. Stereo image of the $2F_0$-F_c electron density for $A\beta_{18-41}$ fragment (chain A) in Aβ-IgNAR. The map is contoured at 1.0 σ.

Supplementary Tables

Table S1. Oligonucleotides primers used to generate Aβ-IgNAR constructs.

Designation	Designed Use	Orientation	Oligonucleotide Sequence (5'- 3')
8408	5' Amplification; *Sfi*I site	→	GTCTCGCGGCCCAGCCGGCCATGGCCGCATGGGTAGACCAAACACC
8404	3' Amplification; *Not*I site	←	CACGTTATCTGCGGCCGCTTTCACGGTTAATGCGGTGCCAGCTCC
IgNAR/Aβ_1_1	Internal build Aβ into CDR3 loop	←	CCATCAGGCCAATGATCGCACCTTTGTTGCTGCCAACATCTTCCGCAAAGAACACATAGAATGCTTGACACTTATACGTGC
IgNAR/Aβ_1_2	Internal build Aβ into CDR3 loop	←	TTTCACGGTTAATGCGGTGCCAGCTCCTTTCTCACCGCCAATCACAACGCCACCCACCATCAGGCCAATGATCGCACC
IgNAR/Aβ_2_1	Internal build Aβ into CDR3 loop	←	CCATCAGGCCAATGATCGCACCTTTGTTGCTGCCAACATCTTCCGCAAAGAACACCAGACCGCCACCATAGAATGCTTGACACTTATACGTGC
IgNAR/Aβ_2_2	Internal build Aβ into CDR3 loop	←	AATCTGCGGCCGCTTTCACGGTTAATGCGGTGCCAGCTCCTTTCTCACCGCCTCCACCCGCAATCACAACGCCACCCACCATCAGGCCAATGATCGCACC
IgNAR/Aβ_3_1	Internal build Aβ into CDR3 loop	←	CCATCAGGCCAATGATCGCACCTTTGTTGCTGCCAACATCTTCCGCAAAGAACACCAGGCCATAGAATGCTTGACACTTATACGTGC
IgNAR/Aβ_3_2	Internal build Aβ into CDR3 loop	←	AATCTGCGGCCGCTTTCACGGTTAATGCGGTGCCAGCTCCTTTCTCACCGCCACCCGCAATCACAACGCCACCCACCATCAGGCCAATGATCGCACC
IgNAR/Aβ-Wth1	$Leu^{34}Pro$ mutation in Aβ-IgNAR-G1	←	AATCTGCGGCCGCTTTCACGGTTAATGCGGTGCCAGCTCCTTTCTCACCGCCAATCACAACGCCACCCACCATCGGGCCAATGATCGCACC

Table S2. Aβ-IgNAR-G1 buried surface areas and shape complementarity statistics[a].

Chain	A	B	C	D	**Total**[b]
A	-	215.7 *0.590*[c]	589.0 *0.709*	290.8 *0.786*	1095.5
B		-	254.9 *0.742*	563.8 *0.567*	1034.4
C			-	215.8 *0.717*	1044.2
D				-	1087.6
Average					1065.4

[a]For Aβ regions only. [b]Area excluded on first molecule due to interaction with second (in $Å^2$) calculated using point density of 10 points/$Å^2$. [c]Complementarity statistics (in itialics) calculated using the Sc program(Lawrence and Colman, 1993). Probe sphere radius = 1.7 Å.

Supplementary References

Lawrence MC, Colman PM (1993) Shape complementarity at protein/protein interfaces. J Mol Biol 234:946-950.

Petkova AT, Yau WM, Tycko R (2006) Experimental constraints on quaternary structure in Alzheimer's β-amyloid fibrils. Biochemistry 45:498-512.

Streltsov V, Titmuss S, Epa V, Barnham K, Masters C, Varghese J (2008) The structure of the amyloid-β peptide high-affinity copper II binding site in Alzheimer disease. Biophys J 95:3447-3456.

Takano K, Endo S, Mukaiyama A, Chon H, Matsumura H, Koga Y, Kanaya S (2006) Structure of amyloid-β fragments in aqueous environments. FEBS J 273:150-158.

Wurth C, Guimard NK, Hecht MH (2002) Mutations that reduce aggregation of the Alzheimer's Aβ42 peptide: an unbiased search for the sequence determinants of Aβ amyloidogenesis. J Mol Biol 319:1279-1290.