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Chemical Probes That Selectively Recognize the Earliest Aβ Oligomers in Complex Mixtures

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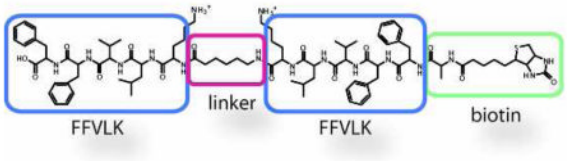
Abstract



Alzheimer's disease (AD) is characterized by the self-assembly of amyloid beta (Aβ) peptides. Recent models implicate some of the earliest Aβ oligomers, such as trimers and tetramers, in disease. However, the roles of these structures remain uncertain, in part, because selective probes of their formation are not available. Towards that goal, we generated bivalent versions of the known Aβ ligand, the pentapeptide KLVFF. We found that compounds containing sufficiently long linkers (~19 to 24 Å) recognized primarily Aβ trimers and tetramers, with little binding to either monomer or higher order structures. These compounds might be useful probes for early Aβ oligomers.

Both *in vitro* and *in vivo*, the Aβ monomer will self-assemble into higher order structures, including dimers, trimers, tetramers and larger oligomers. Eventually, this peptide will form the elongated fibrils that are observed in late-stage AD patients (Figure 1a). Recent studies have suggested that the accumulation of smaller aggregates, not the large fibrils, might better correlate with neurotoxicity.1 For example, patient-derived Aβ dimers and trimers have been shown to inhibit long-term potentiation and damage synaptic plasticity.2 Similarly, synthetic Aβ trimers and tetramers are two-fold more toxic to cultured neurons

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Supporting Information Available: Detailed protocols for ligand synthesis and characterization, Aβ preparation and crosslinking, Western blots and MD simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

than either Aβ monomers, dimers or fibrils.3 Together, these studies suggest that some of the earliest structures in the aggregation pathway might be the most diagnostic of disease.

The Aβ peptide is a 40 or 42 amino acid fragment of the amyloid precursor protein (APP) (Figure 1b). Portions of this peptide are thought to form β-sheets upon release from APP and subsequent stacking of these region appears to nucleate Aβ self-association.4 Within these sites, residues 16 through 20, KLVFF, are especially important.5 Specifically, this motif is thought to interact with itself in adjacent β-strands, with the phenylalanine residues forming key, repetitive inter-molecular contacts.6 The interactions of KLVFF with itself has been studied extensively and these studies have suggested that many Aβ structures have an exposed KLVFF motif at each "end".7 Consistent with this model, KLVFF-based peptides will inhibit Aβ aggregation at high concentrations, presumably by blocking these sites.6e However, KLVFF binds only weakly (K_d > 1 mM)8 and multivalent displays have been found to be required for potent inhibition.9 Multivalent binding is known to significantly enhance avidity and selectivity in many systems, by elevating local ligand concentration, favoring multi-site binding and other mechanisms. Based on these observations, we envisioned that bridging two KLVFF peptides with a linker of the appropriate length might provide a probe for the earliest Aβ oligomers (Figure 1c). This strategy was designed to address a central challenge in building probes that are specific for a subset of Aβ structures. Namely, these oligomers are assembled from identical monomer units and; therefore, they contain many degenerate molecular features, such as high beta-sheet content. By exploiting one of their few distinguishing properties (*e.g.* end-to-end distance between KLVFF motifs), we hoped to circumvent these issues.

To estimate the minimal distance needed to span the ends of an early Aβ aggregate, we assembled a representative KLVFF-based probe *in silico* and then employed molecular dynamics (MD) simulations to examine its binding to a model Aβ repeating unit.7f These studies roughly estimated the distance between KLVFF sites as 13–15 Å in a dimer, 19–20 Å in a trimer, and approximately 24–25 Å in a tetramer (Figure 1d and Supplemental Figure 1). Using microwave-assisted, solid-phase peptide coupling, we then constructed a control compound in which the KLVFF peptide was linked to biotin (Figure 2a). Similarly, we generated four molecules (**d7**, **d13**, **d19**, and **d24**), each containing two KLVFF motifs separated by a variable number of aminohexanoic acid (Ahx) units and a biotin at the N-terminus (Figure 2b). These compounds were named according to the approximate length of the extended linker (*e.g.* **d7** has an estimated linker length of ~ 7 Å).11 We found that these probes were soluble and non-aggregating in aqueous solution at low concentrations (below 10 μM).

To test binding of these probes, we employed an established, UV cross-linking approach to produce Aβ samples containing a mixture of small oligomers.12 Briefly, 25 μM Aβ (1–40) was cross-linked with a Ru(II) catalyst and excess catalyst was removed.13 The resulting "ladder" was separated by native-gel electrophoresis. By silver staining, we observed approximately equal levels of monomer, dimer, and trimer, along with lesser quantities of tetramer and pentamer (Figure 2b). We then transferred these samples to nitrocellulose, incubated with the KLVFF probes (2 μM) and washed extensively. The bound material was localized using streptavidin coupled to horseradish peroxidase (HRP).

Under these conditions, we observed no binding by the KLVFF-biotin control (Figure 2b), a result consistent with its weak affinity.8 Similarly, the KLVFF-based probes with relatively short linkers, **d7** and **d13**, also had weak binding, with a faint band at the molecular weight of an Aβ trimer (Figure 2b and 2c). However, the compounds with longer linkers, **d19** and **d24**, interacted strongly with the trimer and tetramer regions, with some binding to the dimers and pentamers (Figure 2b and 2c). The relatively poor binding to the Aβ dimers

might suggest that it is not as ordered as the other structures, a concept that is consistent with recent MD and NMR studies.6g,7d Importantly, Aβ monomer was not recognized by any of the ligands at these concentrations, supporting an important role for multivalent interactions.

Based upon the results using crosslinked Aβ samples, we wanted to further test binding in more dynamic mixtures. Towards that goal, we used ligand **d24** to probe aged samples of non-crosslinked Aβ (1–42). Samples prepared by this method are known to contain a mixture of monomer (4.5 kDa), poorly resolved trimers and tetramers (~ 12 to 18 kDa) and higher order oligomers (~40 to 200 kDa).14 Consistent with those patterns, we observed Aβ structures of these sizes by either silver staining or Western blots with the anti-Aβ antibody 6E10 (Figure 3a). The KLVFF-biotin control did not recognize any of the bands under these conditions. However, **d24** remained bound to the region corresponding to trimer and tetramer, suggesting that selectivity is maintained (Figure 3a). Minimal binding to monomer was seen and, importantly, we did not observe any interactions with the higher molecular weight oligomers, further emphasizing the selectivity of these probes for early structures in the Aβ aggregation pathway.

Finally, we wanted to evaluate binding in human cerebrospinal fluid (CSF), which provides a more challenging environment than aqueous buffers in which to retain binding. To determine if **d24** could still bind Aβ in this milieu, we added 1 μg of cross-linked Aβ (1–40) to CSF samples from non-AD patients (6 μg of total protein) and characterized the resulting mixture by silver stain, 6E10 antibody and **d24**. From these studies, we concluded that **d24** recognized several unrelated bands within the CSF sample; however, this off-target reactivity was largely restricted to proteins > 50 kDa. Thus, we were still able to visualize binding to Aβ trimers and tetramers (Figure 3b). Similar findings were obtained using Aβ (1–42) (Supplemental Figure 2). Together, these results suggest that the multivalent probes can exploit unique inter-KLVFF distances to distinguish between otherwise closely related Aβ structures. Based on these findings, we anticipate that derivatives of **d24**, with further improvements in affinity and selectivity, may be promising probes for detecting the appearance of the earliest Aβ aggregates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. (a) Caughey B, Lansbury PT. Annu Rev Neurosci. 2003; 26:267–98. [PubMed: 12704221] (b) Hung LW, Ciccotosto GD, Giannakis E, Tew DJ, Perez K, Masters CL, Cappai R, Wade JD, Barnham KJ. J Neurosci. 2008; 28:11950–8. [PubMed: 19005060]
2. (a) Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL. J Neurosci. 2007; 27:2866–75. [PubMed: 17360908] (b) Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Nat Med. 2008; 14:837–42. [PubMed: 18568035] (c) Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ. J Physiol. 2006; 572:477–92. [PubMed: 16469784] (d) O'Nuallain B, Freir DB, Nicoll AJ, Risse E, Ferguson N, Herron CE, Collinge J, Walsh DM. J Neurosci. 2010; 30:14411–19. [PubMed: 20980598]

3. Ono K, Condron MM, Teplow DB. Proc Natl Acad Sci U S A. 2009; 106:14745–50. [PubMed: 19706468]
4. (a) Aguzzi A, O'Connor T. Nat Rev Drug Discov. 2010; 9:237–48. [PubMed: 20190788] (b) Roychaudhuri R, Yang M, Hoshi MM, Teplow DB. J Biol Chem. 2009; 284:4749–53. [PubMed: 18845536]
5. Esler WP, Stimson ER, Ghilardi JR, Lu YA, Felix AM, Vinters HV, Mantyh PW, Lee JP, Maggio JE. Biochemistry. 1996; 35:13914–21. [PubMed: 8909288]
6. (a) Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JI, Van Nostrand WE, Smith SO. Nat Struct Mol Biol. 2010; 17:561–7. [PubMed: 20383142] (b) Bieschke J, Siegel SJ, Fu Y, Kelly JW. Biochemistry. 2008; 47:50–9. [PubMed: 18078350] (b) Bowerman CJ, Ryan DM, Nissan DA, Nilsson BL. Mol Biosyst. 2009; 5:1058–69. [PubMed: 19668872] (c) Chini MG, Scrima M, D'Ursi AM, Bifulco G. J Pept Sci. 2009; 15:229–34. [PubMed: 19090016] (d) Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K. J Mol Biol. 1992; 228:460–73. [PubMed: 1453457] (e) Tjernberg LO, Naslund J, Lindqvist F, Johansson J, Karlstrom AR, Thyberg J, Terenius L, Nordstedt C. J Biol Chem. 1996; 271:8545–8. [PubMed: 8621479] (f) Wood SJ, Wetzel R, Martin JD, Hurle MR. Biochemistry. 1995; 34:724–30. [PubMed: 7827029] (g) Yu L, Edalji R, Harlan JE, Holzman TF, Lopez AP, Labkovsky B, Hillen H, Barghorn S, Ebert U, Richardson PL, Miesbauer L, Solomon L, Bartley D, Walter K, Johnson RW, Hajduk PJ, Olejniczak ET. Biochemistry. 2009; 48:1870–7. [PubMed: 19216516]
7. (a) Antzutkin ON, Balbach JJ, Leapman RD, Rizzo NW, Reed J, Tycko R. Proc Natl Acad Sci U S A. 2000; 97:13045–50. [PubMed: 11069287] (b) Costa PR, Kocisko DA, Sun BQ, Lansbury PT, Griffin RG. J Am Chem Soc. 1997; 119:10487–10493. (c) Hetenyi C, Kortvelyesi T, Penke B. Bioorg Med Chem. 2002; 10:1587–93. [PubMed: 11886820] (d) Horn AH, Sticht H. J Phys Chem B. 2010; 114:2219–26. [PubMed: 20104925] (e) Hwang W, Zhang S, Kamm RD, Karplus M. Proc Natl Acad Sci U S A. 2004; 101:12916–21. [PubMed: 15326301] (f) Luhrs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H, Schubert D, Riek R. Proc Natl Acad Sci U S A. 2005; 102:17342–7. [PubMed: 16293696] (g) Wallace JA, Shen JK. Biochemistry. 2010; 49:5290–8. [PubMed: 20491446] (h) Hu Y, Su B, Kim C-S, Hernandez M, Rostagno A, Ghiso J, Kim JR. Chembiochem. 2010 in press.
8. Cairo CW, Strzelec A, Murphy RM, Kiessling LL. Biochemistry. 2002; 41:8620–9. [PubMed: 12093279]
9. (a) Chafekar SM, Malda H, Merkx M, Meijer EW, Viertl D, Lashuel HA, Baas F, Scheper W. Chembiochem. 2007; 8:1857–64. [PubMed: 17763487] (b) Lowe TL, Strzelec A, Kiessling LL, Murphy RM. Biochemistry. 2001; 40:7882–9. [PubMed: 11425316] (c) Watanabe K, Nakamura K, Akikusa S, Okada T, Kodaka M, Konakahara T, Okuno H. Biochem Biophys Res Commun. 2002; 290:121–4. [PubMed: 11779142] (d) Zhang G, Leibowitz MJ, Sinko PJ, Stein S. Bioconjug Chem. 2003; 14:86–92. [PubMed: 12526697]
10. Kiessling LL, Gestwicki JE, Strong LE. Angew Chem Int Ed Engl. 2006; 45:2348–68. [PubMed: 16557636]
11. Thomas LL, Christakis TJ, Jorgensen WL. J Phys Chem B. 2006; 110:21198–204. [PubMed: 17048945]
12. Bitan G, Teplow DB. Acc Chem Res. 2004; 37:357–64. [PubMed: 15196045]
13. Marinec PS, Evans CG, Gibbons GS, Tarnowski MA, Overbeek DL, Gestwicki JE. Bioorg Med Chem. 2009; 17:5763–8. [PubMed: 19643614]
14. Cerf E, Sarroukh R, Tamamizu-Kato S, Breydo L, Derclaye S, Dufrene YF, Narayanaswami V, Goormaghtigh E, Ruysschaert JM, Raussens V. Biochem J. 2009; 421:415–23. [PubMed: 19435461]

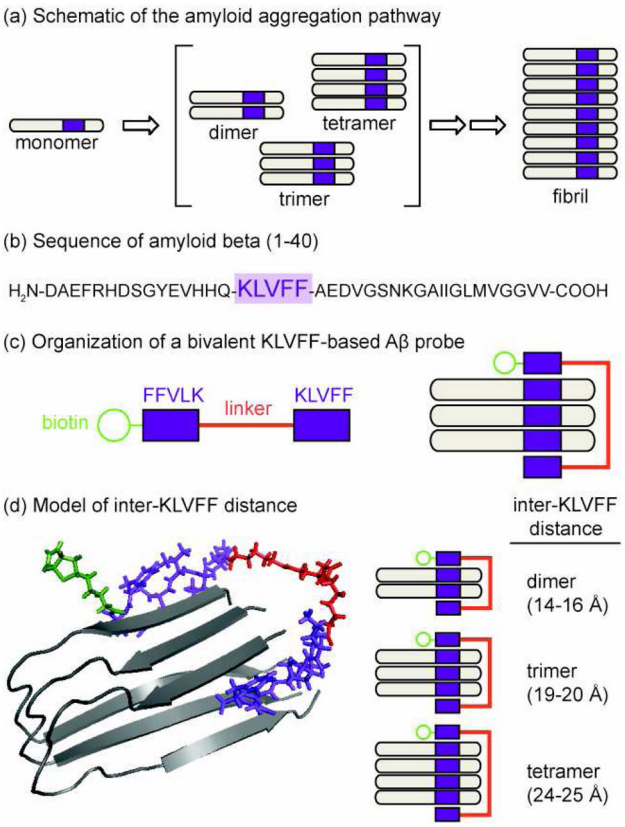

Figure 1.

Design of KLVFF-based probes. (a) Schematic of the Aβ aggregation pathway, highlighting the earliest structures. (b) The sequence of Aβ (1–40), including the core KLVFF motif. (c) Proposed features of a bivalent, KLVFF-based probe. The Aβ is shown in grey, the KLVFF in purple, the linker in red and the biotin tag in green. (d) Snapshot from a molecular dynamics simulation of a KLVFF-based probe bound to a model Aβ trimer. Based on these simulations, an approximate distance between KLVFF sites was estimated.

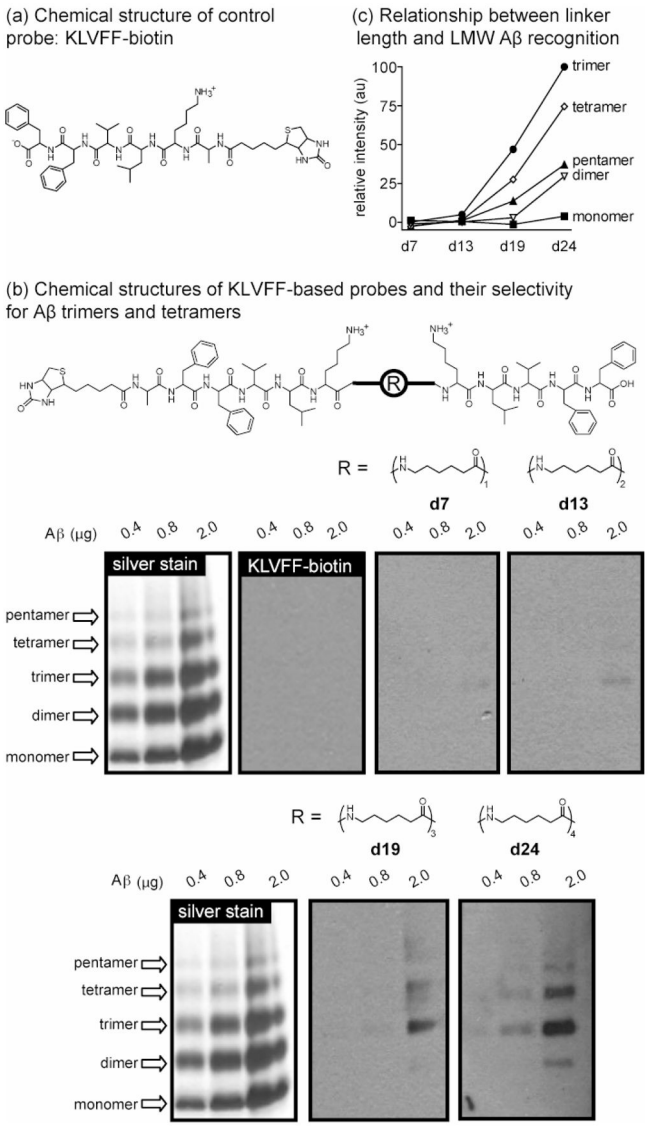

Figure 2.

KLVFF-based probes selectively bind Aβ trimers and tetramers. (a) Chemical structure of the monovalent, KLVFF-biotin probe. (b) Chemical structures and binding properties of bivalent probes. Aβ (1–40) was cross-linked as described, separated using electrophoresis, and transferred to nitrocellulose. Compounds (2 μM) were incubated with the membranes, which were then washed and imaged with streptavidin-HRP. (c) The Aβ band intensities are plotted against the maximum linker length. Results are representative of four independent experiments.

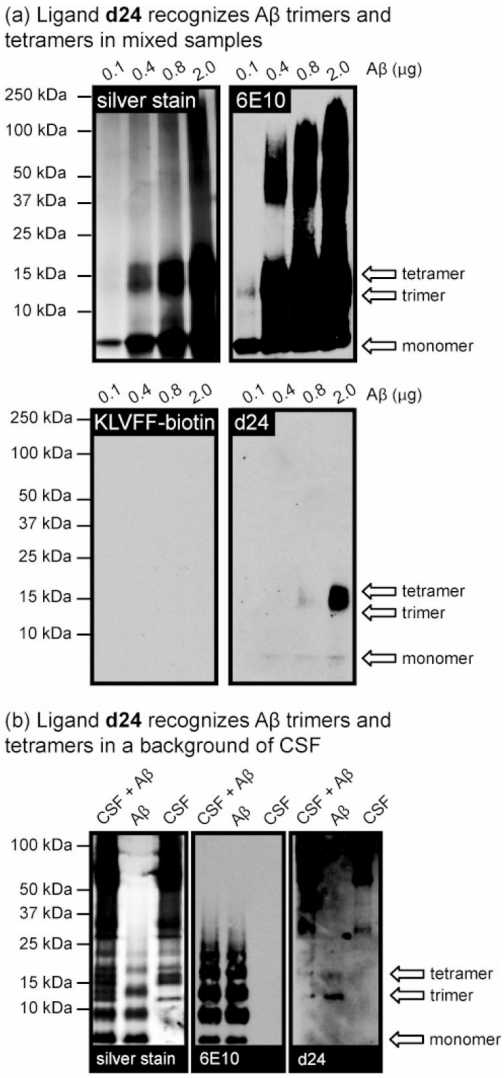

Figure 3.

Ligand **d24** selectively recognize trimers and tetramers in mixed Aβ samples and human cerebrospinal fluid (CSF). (a) Aβ (1–42) was separated and probed as described above. Silver staining or probing with the anti-Aβ antibody 6E10 showed a mixture of monomers, trimers, tetramers, and high-molecular weight oligomers, but **d24** bound predominantly to trimers and tetramers. (b) Cross-linked Aβ (1–40) was added to human CSF and probed with **d24**. Results are representative of at least two independent replicates.