

Supporting Information

A universal method for detection of amyloidogenic misfolded proteins

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Biotinylated peptoid and peptides. The ligands were prepared using the submonomer method, essentially as described previously by Zuckermann, et al. (J. Am. Chem. Soc. 1992, *114*, 10646, J. Am. Chem. Soc. 2003, *125*, 8841, and J. Pep. Prot. Res. 1992, *40*, 498). Rink amide resin, Fmoc-aminohexanoic acid, and beta-alanine tbu ester were purchased from EMD Biosciences (San Diego CA) and all other reagents were purchased from Sigma-Aldrich (St Louis Mo). The ligands were purified by HPLC using a 30 x 50 mm SF C18 column, a 30 ml/min flow rate in MeCN/H₂O/TFA with UV detection at 214 nm for 16 min (gradient noted below). The ligands were checked for purity with an analytical HPLC-MS equipped with a 2.1 mm 5 μ m Hypersil ODS column. The retention times using a 0.8 ml/min flow rate, 5-95% MeCN/H₂O/TFA over 3.5 min, and UV detection at 214 nm for 6 min, MS are noted below. The purified ligands were lyophilized and stored at -20C.

Characterization data for peptoids employed. The peptoids are of the format biotin-Ahx-Ahx-peptoid-NH₂ and are shown in shorthand to indicate the peptoid sequence from amine to carboxy terminus: A= Aromatic (the phenylalanine mimic N-benzylglycine), + = positively charged (the lysine mimic N-(4-aminobutyl)glycine), - = negatively charged (the glutamic acid mimic N-(2-carboxyethyl)glycine).

Shorthand	Code	prep gradient	t _R (prep)	t _R (analytical)	exact mass	mass observed
+++A+A	CS-U30329-3a	B-16m-2060	5.53	2.14	1255.67	1256.4
+++++++	CS-U30329-5a	B-16m-0545	2.8	1.16	1365.94	1367.7
++AA++A	CS-U30329-5b	B-16m-1040	4.09	1.67	1422.86	1425.5
+A+A+A+	CS-U30329-5c	B-16m-1040	4.03	1.64	1422.86	1424.5
+++AAA+	CS-U30329-5d	B-16m-1040	4.18	1.72	1422.86	1425.5
-----	CS-U30329-5e	B-16m-0545	4.37	1.41	1372.57	1371.3
--AA--A	CS-U30329-5f	B-16m-1040	6.53	1.97	1426.65	1425.3
-A-A-A-	CS-U30329-5g	B-16m-1040	6.52	1.96	1426.65	1425.3
---AAA-	CS-U30329-5h	B-16m-1040	6.84	2.02	1426.65	1425.3
++-++-	CS-U30329-5i	B-16m-0545	2.97	1.30	1368.78	1369.5
+--+--+	CS-U30329-5j	B-16m-0545	3.18	1.32	1368.78	1371.4
+++---+	CS-U30329-5k	B-16m-0545	3.12	1.31	1368.78	1371.4
--A-A	CS-U30329-5l	B-16m-1040	5.59	1.81	1279.58	1278.3

Preparation of Glutathione and ASR1 beads. To covalently bind ASR1 and glutathione to beads, magnetic beads displaying carboxylic acids (M270 Dynabeads, Dynal Invitrogen, Carlsbad CA) were washed 2 times with 0.1 M MES buffer, pH 5, and then incubated with an activated solution of the maleimidation reagent BMPH (*N*-[β -Maleimidopropionic acid] hydrazide, ThermoScientific) (33 mM BMPH, 130 mM EDC in MES buffer) for 30 min rt. The beads were quenched with Tris buffer (50 mM Tris buffer, pH 7.5), washed in phosphate buffer, and added to 5 mM thiolated ligand in degassed phosphate. The beads were rotated for 21 hours, then washed in phosphate buffer and stored.

Detection of aggregated Ab42 from AD brain homogenates. A β 42 aggregates from AD brain homogenate (ADBH, 0-20 nl of 10% brain homogenate) were captured with 0.09 mg of covalently bound ASR1 or glutathione beads for 1 hour at 37°C in 100 μ l of 80% plasma in capture buffer (50 mM Tris, 150 mM NaCl, 1% Tween-20, 1% Triton X-100 pH 7.5). The beads were then washed with TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween-20 pH 7.5), and bound proteins were eluted and denatured with guanidine thiocyanate HCl (GdnSCN, 6M, rt, 30 min). The eluted A β was subsequently diluted into TBST and detected by an A β 42-specific ELISA, utilizing an A β x-42 specific capture antibody (12F4, Covance, Princeton, NJ) and HRP-conjugated 4G8 antibody (Covance) for detection. Specifically, ELISA plates were coated with 2 μ g/mL 12F4 antibody in 0.1 M NaH₂PO₄ / 1% NaCl / pH 6, washed with TBST, and blocked with 1% BSA, 3% sucrose in TBS for 1 hour at 37°C. 0.2 μ g/mL HRP-conjugated 4G8 antibody in 0.1% BSA, 0.01% casein in PBS was diluted 1:1 with the sample and applied to the 12F4-coated plates for 1 hour at room temperature before detection by a chemiluminescent substrate (Lumiphos Plus, Lumigen Beckman Coulter Southfield MI).

Detection of aggregated prion from CJD brain homogenates. The prion test was performed in a similar manner to the Ab42 AD brain homogenate MPA, using 1 μ l of 10% brain homogenate in 70% plasma and 0.1 mg peptide- or ASR1-coated beads. Prion elution was performed with 3M GdnSCN for 10 min rt, followed by addition of bicarbonate. The plate was incubated at 4C for 48h to coat the plate, followed by blocking in 3% BSA-TBS (1h 37C). Detection was performed using POM2 and POM17 (*PLoS One* **2008**, 3, (12), e3872, provided by Adriano Aguzzi, University of Zurich) antibodies (0.05 μ g/ml) as primary antibodies, G α m-AP (1:2500) as a secondary antibody, and LumiPhosPlus (Lumigen Beckman Coulter Southfield MI) as the substrate.

Detection of aggregated amylin from pancreatic tissue. The amylin test was performed in a similar manner to the A β 42 AD brain homogenate MPA, using 0-200 nl/ml of 10% pancreas homogenate spiked into 80% plasma and 0.3 mg covalently bound ASR1 beads. Amylin elution/denaturation was performed with 6M GdnSCN for 30 min rt. The eluted amylin was detected with a total human amylin ELISA kit (Linco Millipore Billerica MA), according to manufacturer's instructions.

Detection of aggregated α -synuclein. The α -synuclein test was performed in a similar manner to the A β 42 AD brain homogenate MPA, using 0-25 ng/ml of in vitro fibrillized peptide and 0.09 mg of covalently bound ASR1 beads. α -synuclein elution and denaturation was performed with 6M GdnSCN for 30 min rt. The eluted α -synuclein was detected with an α -synuclein ELISA kit (Invitrogen, Carlsbad CA).

Detection of aggregated serpin. The serpin A1 test was performed in a similar manner to the A β 42 AD brain homogenate MPA, using 0-100 ng/ml of in vitro fibrillized protein and 0.45 mg of covalently bound ASR1 beads. Serpin elution and denaturation was performed with 0.1N NaOH rt for 10 min. The eluate was neutralized with 0.12 M NaH₂PO₄, 0.4% Tween-20 and detected with a serpin A1 ELISA kit (Genway, San Diego CA).

Capture efficacy of different peptoids. Biotinylated peptoids (10 μ M) were incubated with paramagnetic streptavidin-coated beads (M280 Streptavidin Dynabeads, Dynal Invitrogen, Carlsbad CA) for 30 min-1h at 37C. After washing, a brain homogenate sample from prion-infected hamsters (300 nL/ml) or AD patient (10 nl/assay) spiked into CSF was added to the beads (1 mg/assay). The A β assay was as described above, with the following modifications 1) 0.1 M NaOH at 80°C for 30 minutes for denaturation/elution, followed by neutralization with 0.12 M NaH₂PO₄, 0.4% Tween-20 and 2) use of the Pierce Supersignal ELISA Femto Substrate (Thermo Fisher Scientific, Rockford, IL). For the prion assay, elution and denaturation were performed as described above, and the eluted prion protein added to a plate coated with the prion-specific antibody 3F4 (Covance, Princeton NJ). After incubation, the eluted prion was detected with POM2-AP conjugate (*PLoS One* **2008**, 3, (12), e3872, provided by Adriano Aguzzi, University of Zurich) and the Lumiphos Plus substrate (Lumigen Beckman Coulter Southfield MI).

Limit of detection study with different oligomers. Covalently bound ASR1 beads (0.9 mg), 5x capture buffer (25 ul, 1x = 50 mM Tris, 150 mM NaCl, 1% Tween-20, 1% Triton X-100 pH 7.5) and serially diluted oligomers spiked into pooled normal CSF (100 ul) were added to the wells of microtiter plates. The plate was incubated for 1 hour at 37°C, the beads were washed with TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween-20 pH 7.5) and 1% Zwittergent 3-14, and the bound proteins eluted and denatured with 0.1 M NaOH at 80°C for 30 minutes. The eluate was neutralized with 0.12 M NaH₂PO₄, 0.4% Tween-20 and applied to an Abeta-triplex MSD immunoassay (Meso Scale Discovery, Gaithersburg, Maryland), which was used according to the manufacturer's instructions. For the curves shown in Figure 2E, a similar protocol was used to capture guanidine-denatured, globulomer, and fibril spiked into 200 ul of 1/200 sera. The Zwittergent wash was omitted for this assay.

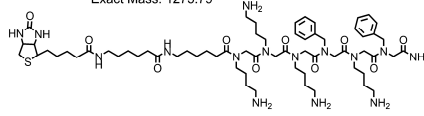
Oligomer specific assay. The oligomer specific ELISA used for epitope mapping used microtiter plates coated with 6E10 antibody (2ug/mL, 200uL/well, Covance, Princeton NJ). Sample and biotin-labeled 6E10 (50 ng/ml, Covance, Princeton NJ) were added to the plate and incubated for 1 hr. After washing away unbound material, SA-HRP (10 ng/ml in TBST with 1% BSA) was added to the plate and incubated for 1h. After washing away unbound material, the chemiluminescent substrate (SuperSignal Femto, Pierce ThermoFisher Scientific, Rockford IL) was added to the plate and the signal detected with a luminometer.

Figure S1. Structures of the peptoids tested as capture reagents.

Figure S2. Characterization of the oligomers. A) Oligomer was added to a sandwich ELISA using an antibody that recognizes A β residues 17-24 for both capture and detection. Signal for the oligomer (but not monomer) was observed, indicating that at least two 4G8 epitopes are available on the oligomer surface. Data shown are the average of replicate samples \pm SD. B) Native gel of E22G, WT, and E22K monomers (M) and oligomers (O).

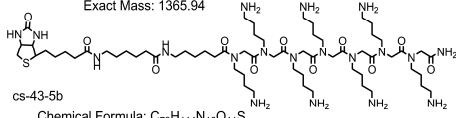
cs-43-3a

Chemical Formula: $C_{64}H_{106}N_{15}O_{10}S$
Exact Mass: 1275.79



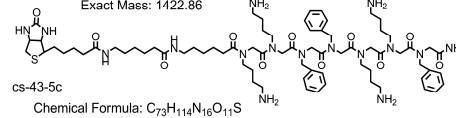
cs-43-5a

Chemical Formula: $C_{64}H_{122}N_{13}O_{11}S$
Exact Mass: 1365.94



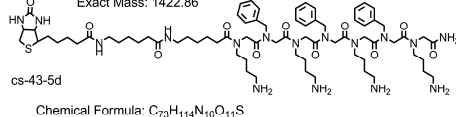
cs-43-5b

Chemical Formula: $C_{73}H_{114}N_{16}O_{11}S$
Exact Mass: 1422.86



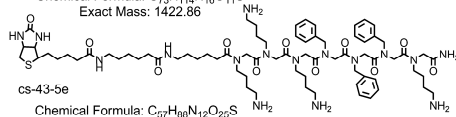
cs-43-5c

Chemical Formula: $C_{73}H_{114}N_{16}O_{11}S$
Exact Mass: 1422.86



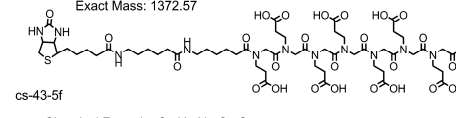
cs-43-5d

Chemical Formula: $C_{73}H_{114}N_{16}O_{11}S$
Exact Mass: 1422.86



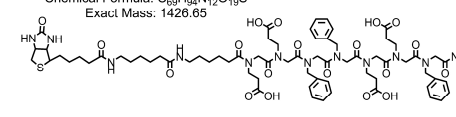
cs-43-5e

Chemical Formula: $C_{67}H_{68}N_{12}O_{20}S$
Exact Mass: 1372.57



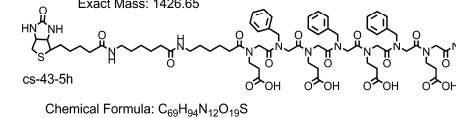
cs-43-5f

Chemical Formula: $C_{65}H_{94}N_{12}O_{19}S$
Exact Mass: 1426.65



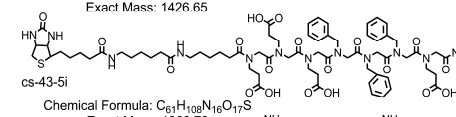
cs-43-5g

Chemical Formula: $C_{69}H_{94}N_{12}O_{19}S$
Exact Mass: 1426.65



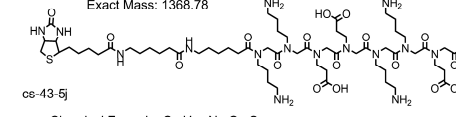
cs-43-5h

Chemical Formula: $C_{69}H_{94}N_{12}O_{19}S$
Exact Mass: 1426.65



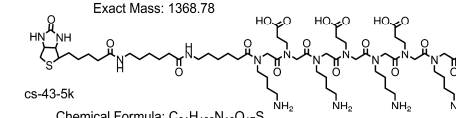
cs-43-5i

Chemical Formula: $C_{61}H_{108}N_{16}O_{17}S$
Exact Mass: 1368.78



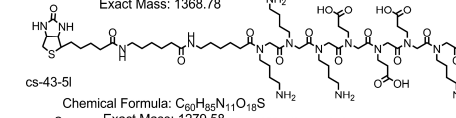
cs-43-5j

Chemical Formula: $C_{61}H_{108}N_{16}O_{17}S$
Exact Mass: 1368.78



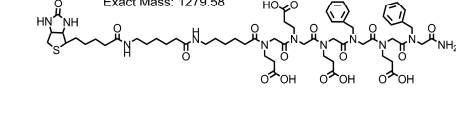
cs-43-5k

Chemical Formula: $C_{61}H_{108}N_{16}O_{17}S$
Exact Mass: 1368.78

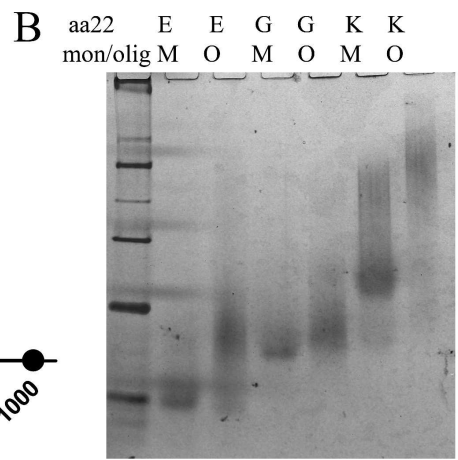
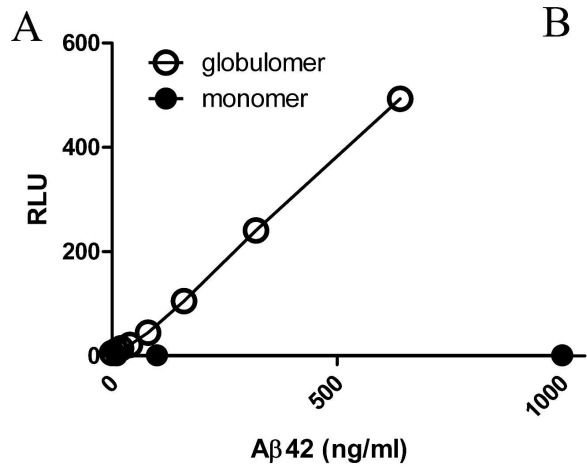


cs-43-5l

Chemical Formula: $C_{60}H_{88}N_{11}O_{18}S$
Exact Mass: 1279.58



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