Protocol for the preparation of stable monomeric amyloid β

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Introduction

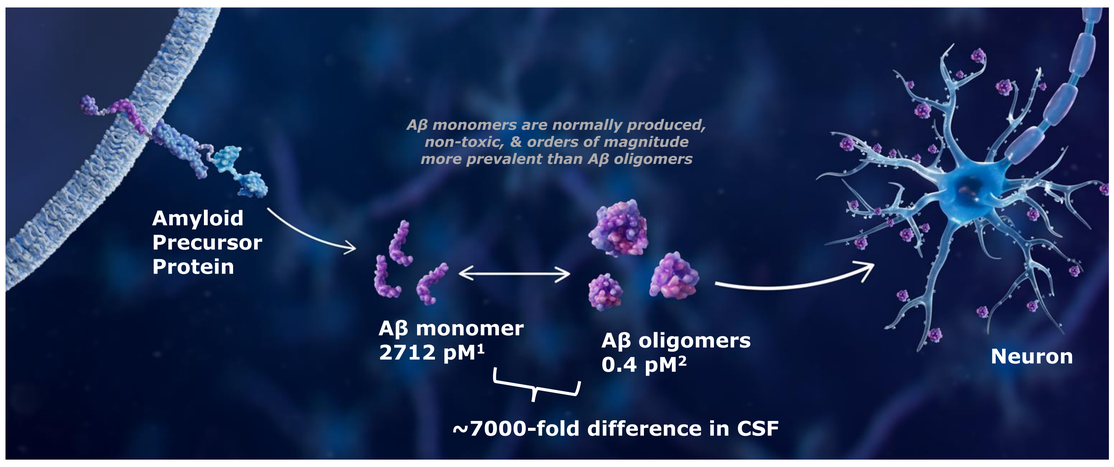
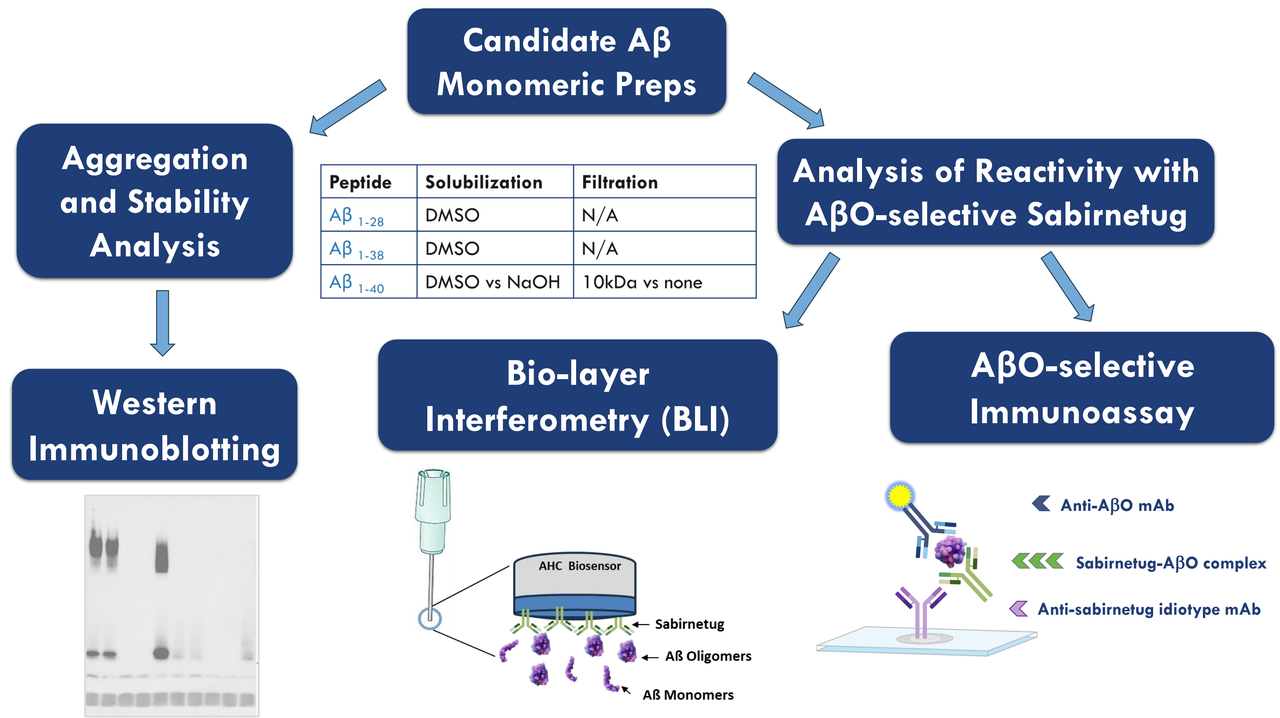


Figure 1. Effective targeting of soluble AβOs requires high selectivity due to significantly greater abundance of Aβ monomers. In the human brain, Aβ monomers are cleaved from the membrane-bound amyloid precursor protein (APP) and then aggregate into soluble Aβ oligomers (AβOs) as well as soluble protofibrils and insoluble fibrils; note the latter two conformers are not pictured here. In Alzheimer's disease (AD), soluble aggregates such as AβOs can bind neuronal synapses and induce synaptic toxicity leading to cognitive decline, which makes them attractive therapeutic targets. Average concentrations of Aβ monomer ($Aβ_{1-40}$ reported here) and AβOs measured in CSF are shown in the schematic.[1,2]

- Soluble amyloid beta oligomers (AβOs) are early, persistent drivers of AD pathogenesis.[3]
- Since Aβ monomers are orders-of-magnitude more abundant than AβOs in AD CSF, understanding selectivity of AβO-targeting drugs & immunoassays is crucial.
- Rapid *in vitro* aggregation of canonical Aβ peptides, including $Aβ_{1-42}$ and $Aβ_{1-40}$, makes selectivity assessment challenging. Thus, methods development preventing Aβ aggregation during analysis is essential.
- We compared published methods for production of pure, stable monomers and applied resultant preparations to assays utilizing the AβO-selective antibody sabirnetug (ACU193), which is currently being tested in the Phase 2 study ALTITUDE–AD (NCT06335173).

Methods



Peptide	Solubilization	Filtration
$Aβ_{1-28}$	DMSO	N/A
$Aβ_{1-38}$	DMSO	N/A
$Aβ_{1-40}$	DMSO vs NaOH	10kDa vs none

Figure 2. Experiment flowchart. Synthetic Aβ peptides (1-28, 1-38, 1-40) were HFIP (hexafluoro-2-propanol) treated, solubilized in NaOH[4] or DMSO,[5] diluted in Ham's F12 media to 100 µM, and in some cases filtered using 10 kDa filters.[6] $Aβ_{1-42}$ was not evaluated as a monomeric standard since it aggregates within 5 minutes in aqueous soutions.[3] The monomeric state and stability of the preparations were characterized by western immunoblotting. Binding of the preparations to an antibody with established AβO selectivity (sabirnetug) was tested by the kinetic measurement technique of biolayer interferometry (BLI) using an anti-human IgG Fc capture (AHC) to immobilize sabirnetug,[8] and by an AβO-selective electrochemiluminescence immunoassay, which is the target engagement assay used in INTERCEPT-AD[9] (Phase 1 study of sabirnetug in early AD), modified to run on the MSD S-PLEX platform for R&D purposes.

Results

$Aβ_{1-28}$ & $Aβ_{1-38}$ oligomerize in < 3 h at 4°C

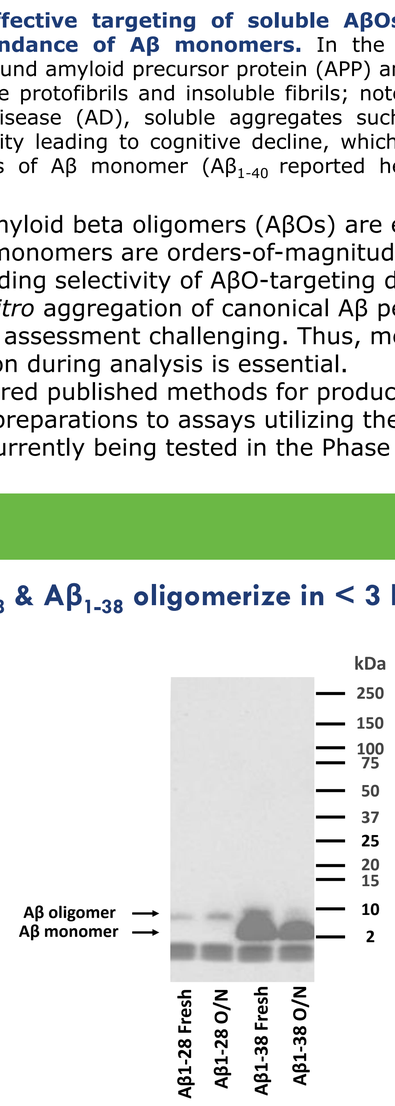


Figure 3. Aβ peptides were solubilized in DMSO/F12 and incubated at 4°C for < 3 h (fresh) or 18-24 h (overnight, O/N), resolved by SDS-PAGE, transferred to a nitrocellulose membrane, and labelled with 82E1 monoclonal antibody against Aβ 1-x. Both preparations contain monomers and oligomeric proteoforms, with $Aβ_{1-38}$ forming a dimer and $Aβ_{1-28}$ a trimer. *Note: molecular weights of the tested peptides: $Aβ_{1-28}$: 3262.7 Da, $Aβ_{1-38}$: 4131.9 Da.*

$Aβ_{1-40}$ oligomerizes but monomer can be purified from oligomers by filtration

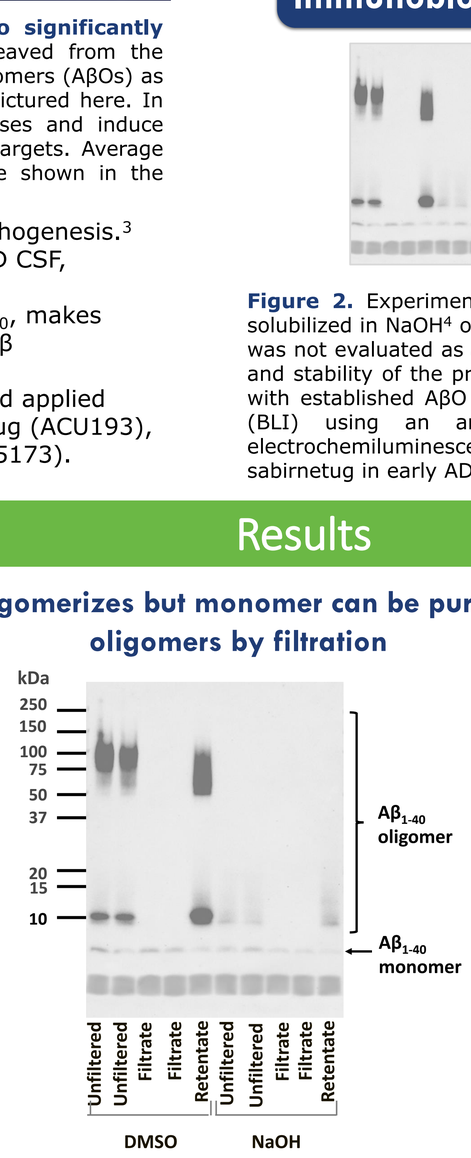


Figure 4. $Aβ_{1-40}$ peptide was HFIP treated, solubilized with DMSO or NaOH, diluted to 100 µM in Ham's F12, and incubated for 18-24 h at 4°C (on ice). After incubation, each preparation was filtered by 10 kDa ultrafiltration (Pierce PES). 90 pmol of each fraction (retentate > 10 kDa; filtrate < 10 kDa) or the unfiltered preparations were analyzed by Western immunoblotting using the anti-AβO antibody ACU988 (Acumen). Fewer, and smaller, oligomers formed with NaOH used as the solvent as compared to DMSO. Filtration removed oligomers.

Filtered $Aβ_{1-40}$ preparation remains stable (purely monomeric) for up to 18-24 h (O/N) at 4°C and for at least 3 h at RT

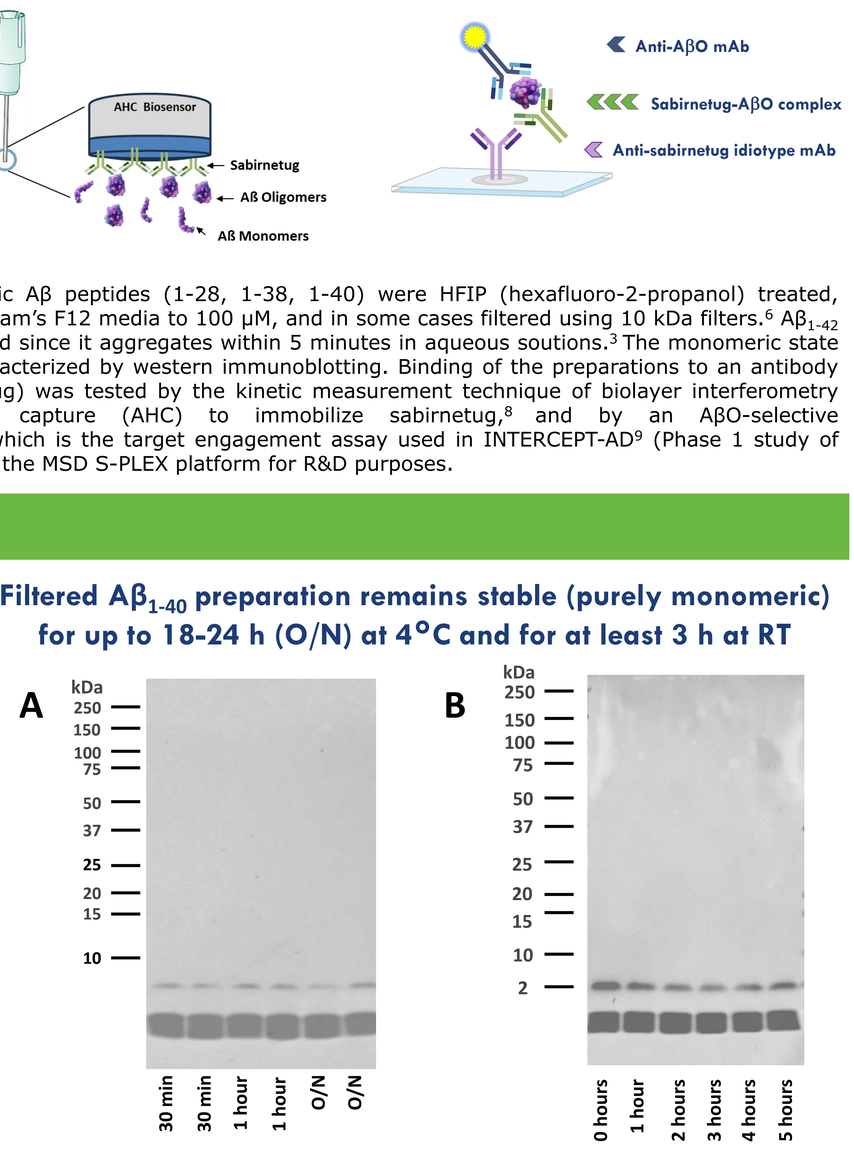


Figure 5. Western blot analysis of 10 kDa filtered $Aβ_{1-40}$ following an incubation (A) at 4°C (on ice) for 0.5-24 h (ACU988) or at 18-22°C (RT) for 0-5 h (82E1) (B). It is unclear if the gray smudge at 75-100 kDa between the 4-5 h lanes in B is a real signal or not; further experiments are required. The removal of aggregates in the initial preparation stabilizes the $Aβ_{1-40}$ peptide over time.

Filtered $Aβ_{1-40}$ monomer is not recognized by AβO-selective sabirnetug in a kinetic assay

Analyte	Max Response (nm)		Affinity (HLB K_D, nM)		Affinity (SS K_D, nM)		Association rate (K_a, 1/M*s)		Dissociation rate, (K_d, 1/s)	
$Aβ_{1-42}$ Oligomers	1	12	6.8	71	174	98	1.28E+04	29	9.20E-05	63
$Aβ_{1-40}$ Unfiltered	0	41	0.15	39	491	117	1.51E+03	23	2.10E-07	9
$Aβ_{1-40}$ 10k Retentate	0	9	2.4	238	444	106	2.95E+03	59	1.39E-05	241
$Aβ_{1-40}$ 10k Filtrate	0	112	NA	NA	NA	NA	NA	NA	NA	NA
$Aβ_{1-28}$	0	61	NA	NA	NA	NA	NA	NA	NA	NA
$Aβ_{1-38}$	0	12	58	173	1.07E+18	141	1.44E+03	72	2.91E-05	172

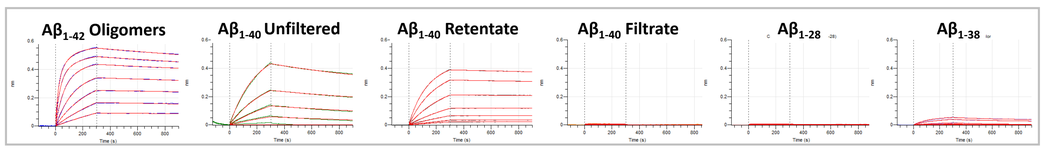


Figure 6. BLI kinetic analysis with AβO-selective antibody sabirnetug. Data tabulated as mean, % coefficient of variation; n = 3-9 replicates from 1-2 independent experiments. Sabirnetug = 5 µg/mL, Aβ preparations titrated 3000-62.5 nM. $Aβ_{1-42}$ oligomer standard = ADDLs.[1] Data was fit by both a 1:2 heterogeneous ligand binding (HLB) model and a steady state (SS) model. K_D = affinity constant; K_a = association rate; K_d = dissociation rate. Association & dissociation rates presented are from the HLB model fit. Below the table are representative BLI traces from each preparation.

- K_a is the least variable, most reliable metric for this dataset.
- AβO-selective sabirnetug shows high affinity binding with fast association rates to all preparations containing high molecular weight AβOs as detected by Western immunoblotting: $Aβ_{1-42}$ oligomers,[7] $Aβ_{1-40}$ unfiltered, & $Aβ_{1-40}$ 10 kDa retentate.
- Sabirnetug did not bind 10 kDa filtered $Aβ_{1-40}$ or $Aβ_{1-28}$. Limited binding was seen for $Aβ_{1-38}$.

Filtered $Aβ_{1-40}$ monomer is not recognized by an AβO-selective immunoassay utilizing sabirnetug

Figure 7. (A) The oligomeric state of each preparation was further evaluated in an AβO-selective electrochemiluminescence immunoassay using MSD platform based on detecting sabirnetug binding to Aβ proteoforms.[8] Stabilized $Aβ_{1-42}$ oligomers (GBS Leiden) were used as a calibrator control. Data points represent mean +/- SD from 1-4 independent experiments. (B) A sandwich immunoassay shows that filtered $Aβ_{1-40}$ at 20 pM interacts with 2G3 & 6E10 antibodies, indicating that the filtered $Aβ_{1-40}$ preparation remains immunoreactive.

- Filtered $Aβ_{1-40}$ monomer yielded no signal in assay utilizing the AβO-selective sabirnetug (panel A).
  - As expected, AβOs of $Aβ_{1-42}$ & $Aβ_{1-40}$ (retentate, unfiltered) did give signal.
  - $Aβ_{1-28}$ (unfiltered) gives no signal & $Aβ_{1-38}$ (unfiltered) gives minimal, suggesting two peptides are monomeric in these assay conditions or that this assay does not recognize dimers/trimers of these peptides.
- However, filtered $Aβ_{1-40}$ is immunoactive when using antibodies not selective for AβOs, e.g., using 2G3 (against $Aβ_{x-40}$, ADx) & 6E10 (against $Aβ_{1-16}$, BioLegend) as the capture and detection antibodies, respectively.

RESEARCH HIGHLIGHTS

- C-terminally truncated Aβ proteoforms including $Aβ_{1-40}$ oligomerize under standard experimental conditions.
- 10 kDa filtration of $Aβ_{1-40}$ enables the production of pure monomeric Aβ solutions, which remain much more stable for short time periods.
- Such preparations are essential to assess selectivity of AβO targeting drugs and assays.
- These preparations may also become useful as a reference calibrator in commercially available in vitro diagnostic (IVD) assays for different Aβ proteoforms or as external quality control for IVD assays.
- Here, we use 10 kDa filtered $Aβ_{1-40}$ to confirm AβO selectivity of sabirnetug, currently in Phase 2 studies (ALTITUDE-AD; NCT06335173).

1. Willemse, et al., 2021. Alzheimers Dement. 13(1): e12182. 2. Ostrowitzki, et al., JAMA Neurol, 2022; 79(11): 1113-1121. 3. Cline et al., J Alzheimers Dis, 2018; 61(s1): S567-S610. 4. Teplow, 2006, Methods Enzymol, 413: 20-33. 5. Stine, et al., 2003, J Biol Chem, 278(13): 11612-22. 6. Velasco et al., 2012, ACS Chem Neurosci, 3(11): 972-81. 7. Chromy et al. Biochemistry. 2003 Nov 11;42(44):12749-60. 8. Abdiche et al. 2008, Analytical Biochem, 377(2):209-17. 9. Cline E. MJ, Zhang H., Sethuraman G., Siemers, E., Dean R.A., Jerecic, J. ACU193-sAβO Complex Measurement in CSF: Additional Analyses Using a Sensitive Assay of Target Engagement for the sAβO-Selective Antibody ACU193 in INTERCEPT-AD. Clinical Trials Alzheimer's Disease; 2023 2023/11/01; Boston, MA.