Abundant Aβ fibrils in ultracentrifugal supernatants of aqueous extracts from Alzheimer's disease brains

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Abstract

Soluble aggregates of amyloid-β (Aβ), often called oligomers, are believed to be principal drivers of neurotoxicity, spreading of pathology, and symptoms in Alzheimer's disease (AD), but little is known about their structures in human brain. Aβ oligomers have been defined as aggregates found in supernatants following ultracentrifugation of aqueous extracts. We now report the unexpected presence of abundant Aβ fibrils in high-speed supernatants from AD brains that were extracted by soaking in aqueous buffer. The fibrils did not appear to form during extract preparation, and their numbers by EM correlated with ELISA quantification of aggregated $A\beta_{42}$. Cryo-EM structures of Aβ fibrils from aqueous extracts were identical to those from sarkosyl-insoluble AD brain homogenates. The fibrils in aqueous extracts were immunolabeled by lecanemab, an Aβ aggregate-directed antibody reported to improve cognitive outcomes in AD. We conclude that Aβ fibrils are abundant in aqueous extracts from AD brains and have the same structures as those from amyloid plaques. These findings have implications for understanding the nature of Aβ oligomers and for designing oligomer-preferring therapeutic antibodies.

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Introduction

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47   Amyloid β (Aβ) and tau proteins convert from soluble monomers into insoluble fibrils

48   when they aggregate. Interest in fibril formation derives from their presence in amyloid plaques

49   and tau tangles in Alzheimer's disease (AD) brains, and from the causation of neurodegenerative

50   disease by mutations that accelerate aggregation of Aβ and tau.[1,2] Fibrils can be extracted from

51   *postmortem* brains and pelleted by differential ultracentrifugation in the presence of detergents

52   such as N-lauryl sarcosine (sarkosyl). Detergent-insoluble Aβ has been reported to be less toxic

53   than Aβ from aqueous ultracentrifugal supernatants prepared without detergent.[3] Aqueous

54   supernatants contain putatively soluble Aβ assemblies that disrupt synaptic plasticity[3–5] and

55   neurite morphology,[6,7] promote tau phosphorylation,[6,8,9] decrease memory in rodent models[3] and

56   accelerate Aβ seeding.[10–13] Because such assemblies are not pelleted by ultracentrifugation and

57   are not monomeric, they have been termed "soluble" oligomers, and their neurotoxicity makes

58   them attractive therapeutic targets.

59   The most promising antibodies in advanced trials for AD target aggregated Aβ.[14] They

60   include lecanemab (BAN2401), a humanized mouse monoclonal antibody reported to improve

61   cognitive outcomes in a phase 3 trial[15,16] and to bind preferentially to soluble Aβ aggregates over

62   insoluble fibrils.[15] Here, we use the term "assembly" or "aggregate" instead of "oligomer,"

63   because *oligo* implies a *few* monomers, whereas assemblies in aqueous extracts may contain

64   thousands of non-covalently bound monomers. We use the term "protofilament" to mean a single

65   stack of monomers in a β-pleated sheet; in the case of Aβ fibrils, two protofilaments form one

66   fibril.[16]

67   Synthetic Aβ can aggregate *in vitro* into assemblies of various sizes. However, their

68   relevance for AD is uncertain because of the difficulty in extracting assemblies of known

69   structure and solubility from human brain.[17] The term "soluble" is operational because Aβ

70   aggregates are found in aqueous supernatants after ultracentrifugation, but ultracentrifugation

71   protocols vary and are often poorly described. Most aqueously extracted aggregates of Aβ from

72   AD brains have been reported to be high molecular weight (HMW): they elute in or near the void

73   volume of size exclusion chromatography (SEC) columns (≥500 kDa).[18,19] Low MW aggregates

74   isolated from AD brain, including covalent dimers,[20] are particularly toxic,[3,19] but disassembly of

75   HMW aggregates *ex vivo* is often necessary to observe LMW aggregates and demonstrate their

76   toxicity.[3,6,19] The structure of any of these aggregates is unknown.

77   Dynamic non-covalent interactions render extraction of Aβ assemblies under non-

78   denaturing conditions challenging. A recent method uses soaking of minced bits of AD cerebral

79   cortex in Tris-buffered saline (TBS) rather than tissue homogenization, to avoid shearing plaques

80   and other large Aβ aggregates; these soaking extracts exhibit similar neurotoxicity to whole brain

81   homogenates.[21,22] We previously reported a non-denaturing method to further enrich for Aβ

82   aggregates from soaking extracts of AD cortex by immunoprecipitation with the calcium-

83   sensitive Aβ antibody B24.[23] We were surprised to observe Aβ fibrils in the B24 eluates of

84   putatively soluble aqueous extracts.

85   We now report that both Aβ fibrils and tau paired helical filaments (PHFs) are present in

86   aqueous soaking extracts, even in the absence of immunoprecipitation. With sufficient

87   centrifugal force, virtually all Aβ aggregates could be pelleted. By electron cryo-microscopy

88   (cryo-EM), these fibrils had the same structures as those from sarkosyl-insoluble homogenates of

89   AD brains.[16] Our results suggest that many HMW Aβ aggregates from aqueous extracts of AD

90   brains are amyloid fibrils. These fibrils can be immunolabeled by lecanemab, a putatively

91   oligomer-preferring Aβ antibody that has been reported to improve cognitive function in early

92   AD.

93

Results

Aβ fibrils can be pelleted from aqueous extracts of Alzheimer's disease brain

96   For immunoprecipitation using the calcium-requiring Aβ antibody B24,[23] we started with

97   aqueous extracts prepared by mincing and soaking multiple regions of AD cortex in TBS without

98   homogenization (Table 1).[21] We previously immunoprecipitated Aβ with B24 in the presence of

99   calcium and eluted Aβ using only the chelator EGTA to avoid conditions which might denature

100   Aβ aggregates.[23] The resulting HMW Aβ aggregates from AD brains could then be re-pelleted in

101   a benchtop centrifuge at 20,000 g in a 1.5-ml tube for 1 h (pelleting distance ~1 cm), with no

102   detectable Aβ remaining in the supernatants by ELISA. Examining these pellets by EM revealed

103   the presence short Aβ fibrils.[23]

104   We now asked if Aβ fibrils could be pelleted and observed without immunoprecipitation.

105   We spun 1 ml TBS soaking extracts (Table 1, Step #7), choosing arbitrarily the same 20,000 g

106   protocol for 1 h. Soluble Aβ aggregates would not be expected to pellet. However, a substantial

107   proportion of aggregated Aβ (*i.e.,* that which was detectable by a monomer-preferring ELISA

108   only after denaturation in 5 M GuHCl) was depleted from the supernatants and recovered in the

109   pellets (Fig 1A, center). The natively monomeric Aβ in the original supernatants (*i.e.,* not

110   requiring GuHCl denaturation for detection by the monomer-preferring ELISA) was not depleted

111   by the re-centrifugation, as expected (Fig 1A, right). Examining the re-centrifugation pellets by

112   EM revealed amyloid fibrils that reacted with N-terminal anti-Aβ antibody D54D2 (Fig 1B). In

113   aqueous extracts from AD brains, we also noticed the presence of fibrils resembling PHFs that

114   stained with antibodies directed against the tau C-terminus and against phosphotau (Fig S1). In

115   this report, we focus our analysis on Aβ fibrils. No fibrils were observed in the pellets from

116   aqueous extracts of two control brains (C1 and C2), and only a single fibril was seen on an entire

117   EM grid of a third control brain (C3).

118   Aβ fibrils were not contaminants from the ultracentrifugation pellets because they were

119   also present when only the top ~20% of the supernatants was collected (Fig S2A). To rule out

120   that the sedimentable fibrils we observed had adhered to the surface of the minced tissue (*e.g.,* on

121   the cut surfaces of plaques) rather than diffused out of the tissue into the soaking buffer, we

122   rinsed the minced brain bits with 15 volumes of extraction buffer through a 100 μm filter prior to

123   soaking. We found no reduction in the amount of extracted Aβ aggregates measured

124   biochemically (Fig S2B) or as amyloid fibrils seen by EM. Further arguing in favor of diffusion

125   of fibrils from the minced brain bits into the aqueous buffer rather than carryover from the cut

126   tissue surface was the observation (also documented in Fig S2 of Hong *et al.*[21]) that diffusion of

127   Aβ into the buffer required 15-30 min to plateau, a similar rate as the diffusion of control soluble

128   brain proteins.

129

130   **Sufficient centrifugal force can pellet HMW Aβ aggregates**

131   When accounting for the pelleting distance, our initial and re-centrifugation protocols

132   were approximately equal in pelleting efficiency: the pelleting distance in thin-wall tubes in the

133   SW41Ti (initial soaking extract) is ~9 cm when full (Table 1, Step #5), whereas it is only ~1 cm

134   for 1 ml in a microcentrifuge tube at a 45-degree angle in the benchtop centrifuge (Table 1, Step

135 #7). Thus, the benchtop centrifugation should accomplish the same pelleting efficiency at an ~9-

136 fold lower g-force. It was therefore possible that if higher g-forces had been used initially, the

137 fibrils would not have been recoverable from the supernatant during re-centrifugation. We

138 modified the initial ultracentrifugation step, using the fixed-angle TLA100.3 rotor (pelleting

139 distance ~2 cm) at increasing g-forces, instead of the SW41Ti (pelleting distance ~9 cm). We

140 found that g-forces $\geq$250,000 g across the shorter distance depleted nearly all biochemically

141 detectable aggregated Aβ from the supernatants (Fig 1C) but retained native monomers (Fig 1D).

142 In 475,000 g supernatants, addition of GuHCl denaturation released 1.8-fold more Aβ monomers

143 than without GuHCl, whereas in 20,000 g supernatants, GuHCl addition released 54-fold more

144 Aβ monomers (Fig 1E). This suggests that most Aβ in the 475,000 g supernatants consisted of

145 soluble native monomers and covalent dimers.[3,20] To verify that Aβ aggregates were

146 quantitatively depleted from supernatants obtained at or above 250,000 g, we used high-

147 sensitivity sandwich ELISAs with two different published aggregate-preferring capture

148 antibodies[24,25] that do not require GuHCl denaturation (Fig 1F). Neither assay could detect

149 aggregates in the $\geq$250,000 g supernatants. Moreover, residual Aβ in the 475,000 g supernatants

150 exhibited a LMW SEC profile (Fig 1G).

151 We conclude that most Aβ, and all its HMW aggregates from aqueous brain extracts are

152 made of particles in suspension, consistent with at least some of them being fibrils. We then

153 compared this finding to Aβ in cerebrospinal fluid. Detection of CSF Aβ by our aggregate-

154 specific ELISA was unaltered regardless of the centrifugal force used (Fig 1H), suggesting that

155 the Aβ aggregates detected in human CSF were soluble, in contrast to those in aqueous extracts

156 of AD brain.

157

7

158 Aβ fibrils in aqueous soaking extracts do not appear to form during sample

159 preparation

160   We next sought evidence of *postmortem* artifact: that the fibrils were not present during

161 life but polymerized from smaller non-fibrillar aggregates during the *postmortem* interval or

162 during sample processing. We continued using the standard soaking extract protocol using the

163 SW41Ti rotor at 200,000 *g*,[21] followed by re-centrifugation in the tabletop centrifuge at 20,000 *g*

164 (Table 1).[21]

165   One possibility was that Aβ polymerization occurred along the plastic tube surface during

166 re-centrifugation of soaking extracts. We performed re-centrifugation in which standard soaking

167 extracts were spun and their supernatants moved to new tubes at intervals. The Aβ in each pellet

168 was quantified by ELISA following GuHCl denaturation. If nucleation of new fibrils had

169 occurred, we would have expected a lag in the accumulation of Aβ in the pellets, whereas

170 pelleting of pre-existing particulates would be linearly time-dependent until they had been

171 depleted from the supernatants. We observed no lag phase, suggesting that pelleted Aβ did not

172 arise from nucleation of new fibrils along the plastic tubes (Fig S2C, D). Storage of standard

173 soaking extracts in siliconized or BSA-coated tubes also had no effect compared to standard

174 polypropylene (Fig S2E).

175   The next possibility was that Aβ polymerization occurred during freezing and thawing of

176 the soaking extracts. When proceeding directly from the standard SW41Ti (Table 1, step 5) to a

177 second spin in the benchtop centrifuge (step 7) without freezing, Aβ aggregates as measured by

178 ELISA could still be pelleted (Fig S3A), and fibrils were observed by immuno-EM (Fig S3B).

179 Nevertheless, we also found that freezing a freshly prepared extract at -80°C for storage, as

180 typically performed, resulted in re-pelleting more Aβ from a soaking extract once thawed

181   compared to storage at 4°C (Fig S3C). We also found that the re-pelleting of Aβ was

182   concentration-dependent: when the SW41Ti ultracentrifugation supernatants were diluted at least

183   four-fold before freezing, less aggregated Aβ could be re-pelleted after thawing (Fig S3D). To

184   assess further whether polymerization occurred during the freeze/thaw cycle, we added inhibitors

185   of Aβ aggregation, including aggregate-binding antibodies, 0.45% CHAPS,[26] and *scyllo*-

186   inositol,[27,28] immediately after the initial ultracentrifugation of the soaking extracts and before

187   freezing the supernatants. None of these agents reduced the amount of Aβ that could be re-

188   pelleted after thawing (Fig S3E, F). We also expected that if fibrils had formed *ex vivo* from

189   monomers and/or soluble LMW aggregates during freeze/thaw, then filtering the soaking

190   extracts immediately after the original ultracentrifugation and before freezing should have had no

191   effect on the recovery and pelleting of Aβ. We found, however, that freshly prepared soaking

192   extracts were depleted of Aβ aggregates by a 20-nm pore size alumina filter (Fig S3G) but

193   passed through a 100-nm filter to control for non-size dependent binding to the filter. These

194   results implied that most Aβ assemblies were at least 20 nm in size prior to freezing the original

195   soaking extract. SEC of a freshly prepared TBS extract immediately after the original

196   ultracentrifugation (without freezing) revealed that Aβ still eluted in the void volume of a

197   Superdex 200 Increase column (~$\geq$500 kDa) (Fig S3H). Together, these results suggest that Aβ

198   aggregates were HMW and likely fibrillar prior to freezing and storage of the original soaking

199   extracts. Increased pelleting of Aβ fibrils after freeze/thaw may be due to increased clumping of

200   existing fibrils but probably not to formation of new fibrils.

201   Another possibility was that polymerization occurred after death but before tissue

202   freezing. To exclude this artifact as best as possible, we prepared a soaking extract and applied it

203   to the Superdex 200 Increase column within 8 h of an AD patient's death without freezing either

204   the tissue or the extract. Again, virtually all the ELISA-detectable aggregated Aβ eluted in the

205   void volume of a Superdex 200 Increase column (Fig S3I-K), and fibrils could be pelleted during

206   re-centrifugation (Fig S3L).

207

Fibrils account for a substantial proportion of high-molecular weight Aβ

aggregates in soaking extracts

210   We manually counted the D54D2-immunoreactive fibrils in the re-centrifugation pellets

211   from 13 AD brains, using 30 high-powered EM fields each. Using linear regression, we found a

212   correlation between the average number of fibrils per field and the Aβ levels measured by ELISA

213   after GuHCl denaturation in those same extracts (Fig 1I).

214

Fibrils from soaking extracts of AD brain have the same cryo-EM structures

as those from sarkosyl-insoluble preparations

217   The cryo-EM structures of Aβ fibrils from the sarkosyl-insoluble fractions of AD brains

218   were recently determined.[16] We now asked if the fibrils we identified in aqueous soaking extracts

219   resembled those from sarkosyl-insoluble homogenates. Compared to Aβ fibrils from sarkosyl-

220   insoluble fractions, the soaking extract-derived pellets showed shorter fibrils that were less

221   clumped together, compatible with their relative resistance to pelleting. Cryo-EM structure

222   determination of soaking extract-derived fibrils from two cases of sporadic AD (AD7 and AD11)

223   revealed identical structures to those reported previously for Aβ fibrils from sarkosyl-insoluble

224   preparations.[24] (Fig 2). Case AD7 had only Type I fibrils, whereas case AD11 had both Type I

225   and Type II fibrils, as defined previously.[16]

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226   Because they were present in the same re-centrifuged soaking extracts, we also

227   determined the cryo-EM structures of the tau filaments. They were identical to those reported

228   previously for PHFs from sarkosyl-insoluble fractions of AD brains (Fig S4).[29]

229

230   **Lecanemab labels Aβ fibrils from soaking extracts**

231   Lecanemab was derived from monoclonal antibody 158 that was selected to bind

232   synthetic aggregates of Aβ bearing the AD-causing *APP* "Arctic" mutation E22G.[1,15] Synthetic

233    $A\beta_{40}$[E22G] forms more soluble aggregates than the wild-type $A\beta_{40}$ peptide *in vitro*, and the

234   mechanism of action of lecanemab is thought to relate to its preference for small, soluble Aβ

235   aggregates over amyloid fibrils.[30] Lecanemab has been shown to immunoprecipitate Aβ from

236   aqueous AD brain extracts,[15] so we asked whether lecanemab can bind the fibrils we observed.

237   We found lecanemab could decorate Aβ fibrils (Fig 3A, B) that had been re-pelleted from AD

238   soaking extracts. We also examined antibody h1C22, which we had previously reported to bind

239   better to synthetic Aβ aggregates than to monomers and fibrils,[7,23,24] and we again observed

240   labeling of Aβ fibrils (Fig 3C-E).

241   Since lecanemab labeled Aβ fibrils from aqueous brain extracts, we hypothesized that it

242   would only immunoprecipitate Aβ from extracts prepared at *g*-forces that do not deplete

243   aggregates detectable by other means, such as aggregate-preferring ELISAs that capture Aβ with

244   1C22 or 71A1 (Fig 1F).[25] We found that lecanemab could only immunoprecipitate Aβ from

245   aqueous AD brain supernatants that had been ultracentrifuged at $\leq$100,000 *g* in the TLA100.3

246   rotor (pelleting distance ~2 cm) (Fig 3F), similar to 1C22 (Fig. 1F). In contrast, bapineuzumab,

247   which binds both monomeric and aggregated Aβ, could still immunoprecipitate some Aβ from

248   250,000 and 475,000 *g* supernatants.

249   Lecanemab has been shown to label amyloid plaques by immunohistochemistry at the

250   light microscopic level.[31] To determine if it stains plaque fibrils, we prepared ultrathin

251   cryosections from AD cortex using minimal fixation, so as to preserve antibody epitopes.

252   Although the cytoarchitecture was not well preserved as expected, we observed lecanemab

253   labeling of amyloid fibrils *in situ* (Fig 3G-J) and minimal background from protein A gold alone

254   (Fig 3K).

255

Discussion

257   Characterization of Aβ aggregates in aqueous extracts of AD brain has been difficult, but

258   our results suggest that at least some are identical in tertiary structure to insoluble amyloid

259   fibrils. After gentle soaking extraction (diffusion) from frozen or fresh AD cerebral cortex in

260   aqueous buffer, almost all the Aβ aggregates were retained in the void volume of a Superdex 200

261   Increase column, agreeing with previous studies.[18,19,21] The Aβ aggregates in these soaking

262   extracts could be re-pelleted, and the resultant pellets contained amyloid fibrils that were labeled

263   by several anti-Aβ antibodies, including the putatively soluble aggregate-preferring

264   lecanemab.[15,32] The aqueously extracted fibrils had the same cryo-EM structures as those of Aβ

265   fibrils from the sarkosyl-insoluble pellets of homogenized AD cortex.[16] While we cannot rule out

266   the presence of some non-fibrillar HMW Aβ assemblies, a correlation between total Aβ levels

267   measured by ELISA and the number of Aβ fibrils seen by EM (Fig. 1I) suggests that amyloid

268   fibrils account for a substantial proportion of HMW aggregates that diffuse into aqueous extracts.

269   An unavoidable limitation of this work is the need to examine AD brains *postmortem*.

270   Although we attempted to mitigate the likelihood of artifacts, we cannot completely exclude

271   disassembly of some plaques into free-floating fibrils after death that then entered our soaking

272   extracts. We also cannot completely exclude the *ex vivo* aggregation of monomers or LMW

273   aggregates (*e.g.*, dimers) into fibrils. While we addressed this possibility by testing the effects of

274   small-pore filters and inhibitors of Aβ aggregation, and by using fresh tissue when possible,

275   some *ex vivo* aggregation may have occurred.

276   If small Aβ fibrils diffuse through human brain, they may be in equilibrium with amyloid

277   plaques. By immunohistochemistry, APP transgenic mice show halos surrounding methoxy-

278   XO4-labeled amyloid plaques that are immunoreactive with Aβ aggregate-preferring

279   antibodies.[33] The halos may consist of Aβ fibrils too loosely packed to shift the fluorescence

280   spectra of such dyes and having more access to local synaptic endings than do the fibrils within

281   compacted plaque cores. Supporting this model is our observation that aqueously diffusible

282   fibrils have the same cryo-EM structures as detergent-insoluble Aβ fibrils. Experiments in mice

283   in which the microglial AD risk gene *TREM2* was knocked out, or else microglia were depleted,

284   have shown that compaction of Aβ by microglia may prevent fibril access to dendrites and

285   synapses.[34–36] Soaking brain extracts prepared identically to those studied here (Table 1) have

286   been shown to confer neurotoxicity: impaired synaptic plasticity in mouse hippocampal slices

287   and neuritic dystrophy (reduced neurite length and branching) in cultured human neurons.[21]

288   Moreover, we previously found that HMW aggregates from AD brain had less bioactivity toward

289   both synapses and microglia than did the LMW aggregates (including dimers) into which they

290   disassembled.[19] We now propose that many of these HMW assemblies are short fibrils; thus,

291   partial disassembly of diffusible Aβ fibrils may be required to exert neurotoxicity.

292   Our results have implications for Aβ-targeting therapies. Lecanemab was derived from

293   mouse monoclonal antibody 158, which is reported to preferentially recognize intermediate and

294   lower molecular weight forms of synthetic Aβ aggregates.[15] However, lecanemab also reduces

295   amyloid PET signal, a marker of fibrillar Aβ,[37] and labels plaques by immunohistochemistry. [31]

296   Based on our finding that aqueous AD brain extracts contain Aβ fibrils that are decorated by

297   lecanemab, we speculate that lecanemab's binding target in the human brain may include

298   diffusible fibrils with the same structure as those in insoluble plaques. It remains possible that

299   lecanemab's target may also include non-fibrillar aggregates because we could not exclude their

300   presence.

301   Finally, while we focused on Aβ, we also observed PHF tau present in our soaking

302   extracts. Others have observed that tau seeding activity in aqueous extracts correlates with

303   molecular weight by SEC and inversely with the centrifugal force generating the extracts,

304   implying that larger and heavier tau aggregates are more seed-competent.[12,13] Based on our cryo-

305   EM analysis, we suspect that these preparations contained short tau fibrils with the same fold as

306   PHFs from sarkosyl-insoluble preparations.[38] Supporting this model are the observations that

307   recombinant tau assemblies shortened by sonication were not pelleted by ultracentrifugation,[39]

308   and that tau fibrils were the most seed-competent in brain extracts from transgenic mice

309   overexpressing human mutant P301S tau.[40] Future efforts aimed at the rational design of

310   therapeutic agents that bind and clear tau seeds should consider that they may be PHF.

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319   authors declare no competing interests.

320

Tables

Step #	Steps in standard protocol	Modifications of standard protocol for the specified control experiments, and relevant figure number
1	Frozen tissue thawed, grey matter dissected	**Fig S3I-L:** Fresh tissue
2	Tissue chopped using McIlwain Tissue Chopper set to 0.5 mm width	
3	Tissue bits soaked in extraction buffer 30 min 4°C with nutation in 50-ml tube	**Fig S2B:** Tissue bits first rinsed with ~15 volumes extraction buffer before soaking
4	2,000 *g* fixed angle (pelleting distance ~4 cm) 10 min 4°C, top 90% supernatant collected	
5	200,000 *g* in SW41Ti (pelleting distance ~9 cm) 110 min 4°C, top 90% of supernatant collected	**Fig S2A:** Top 90% of supernatant divided into top 2 ml and bottom ~7 ml **Fig 1C-G:** 20,000 – 475,000 *g* in TLA100.3 (pelleting distance ~2 cm) for 110 min 4°C; top 90% of supernatant collected
6	Aliquot 1 ml into 1.5-ml Eppendorf LoBind tubes; store -80°C	**Fig S2E:** Aliquot into siliconized or BSA-coated tubes **Fig S3C, H:** Store at 4°C **Fig S3D**: Dilute serially before freezing **Fig S3E-F**: Add various Aβ aggregation inhibitors before freezing **Fig S3G**: Filter through aluminum oxide filters before freezing
7 (re-centrifugation)	Thaw aliquot(s), 20,000 *g* fixed angle (pelleting distance ~1 cm) 60 min 4°C. Retain input, supernatant and pellet for ELISA and/or EM	

Table 1. The soaking extract method and its modifications for control experiments in this report

Figure Legends

325   **Fig 1. Aqueous soaking extracts of AD brain contain insoluble and fibrillar Aβ. (A)** Aliquots

326   of aqueous soaking extracts from ultracentrifugal supernatants of AD cortex (see Methods and

327   Table 1) were thawed and spun at 20,000 g in a tabletop centrifuge, and Aβ was quantified by

328   ELISA with (center) and without (right) 5 M GuHCl. Note the different y-axis scales. GuHCl is a

329   chaotropic agent which denatures Aβ aggregates and allows detection by a monomer-preferring

330   ELISA. Aβ detectable without GuHCl denaturation is monomeric. ELISAs revealed that

331   aggregated Aβ, (center) but not Aβ monomers (right), could be re-pelleted. **(B)** EM of fibrils in

332   the pellets of re-centrifuged aqueous soaking extracts of brain AD7 were labeled by anti-Aβ N-

333   terminal antibody D54D2. Scale bar = 100 nm. **(C-F)** Ultracentrifugation pellets high MW Aβ

334   aggregates. A TBS soaking extract of brain AD6 was prepared by mincing cortex, soaking in

335   TBS for 30 min, followed by a 10 min spin at 2,000 g (Table 1, steps 1-4). This supernatant was

336   then divided and ultracentrifuged in a TLA100.3 rotor (pelleting distance ~2 cm) at indicated g

337   forces, and Aβ quantified in the supernatant. Monomer-preferring ELISA performed with (C) or

338   without (D) denaturation with GuHCl showed that Aβ aggregates but not native monomers were

339   depleted as the g-force increased, as did the ratio of Aβ concentration with:without GuHCl (E).

340   **(F)** Two different aggregate-preferring ELISAs showed abolished signal as the g-force increased.

341   **(G)** Superdex 200 Increase size exclusion chromatography of the 475,000 g supernatant revealed

342   a low MW Aβ profile. **(H)** In contrast to brain, CSF Aβ aggregates were not depleted as g-force

343   increased. **(I)** The amount of Aβ measured in soaking extracts after GuHCl denaturation

344   correlated with the number of fibrils present in the pellet after centrifugation and manual blinded

345   counting by immunogold EM. Line represents simple linear regression. Drawings made with

346   Biorender.com.

347

348 **Fig 2. Aβ fibrils from aqueous extracts of AD brains have the same cryo-EM structures as**

349 **amyloid fibrils from sarkosyl-insoluble homogenates.** **(A)** XY-cross-sections of cryo-EM

350 maps of Aβ filaments from soaking extracts of cases AD7 and AD11. For each map, a sum of the

351 reconstructed densities for several XY-slices, approximating one β-rung, is shown. Aβ filament

352 types I and II[16] are indicated at top left; the percentages of Type I and Type II filaments relative

353 to the total of imaged filaments are shown at top right. Resolutions at bottom left. Scale bar = 1

354 nm. **(B, C)** Cryo-EM density maps (in transparent grey) and atomic models for Aβ Type I (in

355 orange in B), Aβ Type II (in blue in C). **(D, E)**, Comparison of the cryo-EM structures of Aβ

356 Type I (B) filaments and Aβ Type II (C) filaments extracted from aqueous soaking extracts vs.

357 sarkosyl-insoluble homogenates (in grey for Type I and Type II filaments) from brains of AD

358 patients.[16] Structures are shown as sticks (above) for one protofilament and as ribbons (below)

359 for the other.

360

361 **Fig 3. Lecanemab decorates Aβ fibrils from Alzheimer's disease brains. (A,B)** Immunogold

362 labeling of pelleted material from the re-centrifugation of AD7 TBS soaking extract reveals

363 immunoreactivity of Aβ fibrils with lecanemab (arrowheads). PHF in the pellet did not react

364 with lecanemab (arrows). **(C, D)** Immunogold labeling with h1C22 Aβ aggregate-preferring

365 antibody shows similar decoration of soaking extract fibrils. **(E)** Omitting primary antibody

366 shows no labeling of fibrils, but infrequent background protein A-gold particles. **(F)** ELISA

367 quantification of $A\beta_{42}$ immunoprecipitated from AD6 aqueous extracts followed by washing,

368 elution and denaturation in 5 M GuHCl. Bapineuzumab, but not lecanemab, still

369 immunoprecipitated Aβ from the 250,000 g and 475,000 g (TLA100.3, pelleting distance ~2 cm)

370 aqueous soaking extract supernatants. **(G-I)** Immunogold labeling of paraformaldehyde-fixed

18

371   ultrathin cryosections from AD11 (G,H) and AD7 (I,J) brains reveals labeling of amyloid fibrils

372   by lecanemab *in situ*. (**K**) Protein A gold alone without lecanemab (brain AD7) shows minimal

373   background labeling. Scale bar = 100 nm.

374

375   **Fig S1. Presence of tau filaments in aqueous AD brain extracts.** Immunogold labeling of

376   pellets from the 20,000-*g* tabletop re-centrifugation of TBS soaking extracts of brains AD12 and

377   AD8 revealed PHFs immunoreactive with antibodies to the tau C-terminus and to tau

378   phosphorylated at T205 or S404. Scale bar = 100 nm.

379

380   **Fig S2. Aβ fibrils in soaking extracts are not contaminants from the ultracentrifugation**

381   **pellet or adhered to the tissue, and do not arise due to plastic-induced polymerization. (A)**

382   Equivalent quantities of Aβ aggregates could be pelleted from the top 2 ml and bottom 7 ml

383   portions of the ultracentrifugal supernatant of brain AD6. (**B**) Rinsing minced brain bits of

384   AD12 in 15 vol of aqueous extraction buffer to remove adherent Aβ aggregates prior to soaking

385   does not decrease Aβ recovery. (**C**) Centrifugation of two thawed AD TBS soaking extracts

386   reveals the absence of a lag phase in the accumulation of Aβ in the pellet. The soaking extracts

387   were centrifuged sequentially for progressively longer times, with each pellet quantified for

388   $A\beta_{42}$, to search for a lag phase that could indicate nucleation. No lag phase was observed. (**D**)

389   Expanded view of the first few timepoints of (C) confirms linear pelleting without a lag phase.

390   (**E**) Storage of frozen AD4 TBS soaking extracts in siliconized or BSA-coated tubes does not

391   alter amount of Aβ recovered in the pellets vs. standard polypropylene Protein LoBind tubes.

392

393   **Fig S3. No evidence that Aβ fibrils from soaking extracts form *postmortem*, but the amount**

394   **recovered is enhanced by freeze-thaw. (A)** The SW41Ti supernatant from three different AD

395   brain soaking extracts was immediately aliquoted and spun in a tabletop centrifuge at 20,000 *g*

396   *without freezing*. Aβ$_{42}$ ELISA reveals the ability to pellet Aβ (red). **(B)** Immunogold EM of

397   pellet from the AD7 soaking extract in (A) (no freezing) reveals Aβ-immunoreactive (D54D2)

398   fibrils. **(C)** More Aβ (by ELISA post-GuHCl) is re-pelleted from soaking extracts frozen at -

399   80°C than kept at 4°C (N=7 AD brains). Left, total Aβ pelleted; right, Aβ pelleted as % of Aβ

400   input. *P<0.05, **P<0.01 by two-tailed paired student's t-test. **(D)** Serial dilution of original

401   TBS soaking extract (AD7) immediately after removal from the ultracentrifuge and before

402   freezing diminishes recovery of Aβ$_{42}$ upon re-pelleting. **(E)** Addition of 7 different Aβ

403   monoclonal antibodies (10 µg/ml) or CHAPS at 0.45% to TBS soaking extract (AD4)

404   immediately after the ultracentrifugation step did not decrease subsequent re-pelleting after

405   freeze-thaw storage. **(F)** Addition (2 mM) of anti-Aβ *agg*regation agent *scyllo*-inositol (or its

406   inactive stereoisomer *chiro*-inositol) to TBS soaking extract (AD9) immediately after the

407   ultracentrifugation did not decrease subsequent re-pelleting after freeze-thaw storage. **(G)** A 20-

408   nm alumina (Whatman Anotop) filter removed more Aβ aggregates than a 100-nm filter

409   (control) before freezing a freshly prepared TBS soaking extract of brain AD8, suggesting that

410   most Aβ particles were larger than 20 nm before freezing. **(H)** Aβ mostly elutes in the void

411   volume of a Superdex 200 Increase column both before and after freezing (-80°C) freshly

412   prepared TBS soaking extract of brain AD4. **(I-K)** A soaking extract from brain AD10 was made

413   and analyzed by size exclusion chromatography (Superdex 200 Increase) by <8 h after death.

414   Monomer-preferring ELISA performed with (I) and without (J) denaturation with GuHCl shows

415   Aβ$_{42}$ aggregates mostly in the void volume, as confirmed with an Aβ aggregate-preferring

20

416   ELISA (K). **(L)** After freezing soaking extracts and later thawing and re-pelleting, D54D2-

417   immunoreactive fibrils were present in the pellets. Scale bar = 100 nm.

418

419   **Fig S4. Tau fibrils from soaking extracts of AD cortex have the same structures as those of**

420   **PHFs in sarkosyl-insoluble homogenates. (A)** Cryo-EM maps of PHFs from soaking extracts

421   of AD7 and AD11. For each map, a sum of the reconstructed densities for several XY-slices,

422   approximating one β-rung, is shown. Resolutions at bottom left. Scale bar = 1 nm. **(B)** Cryo-EM

423   density map (in grey) and atomic model for PHF (in purple). **(C)** Comparison of the cryo-EM

424   structures of PHFs extracted from soaking extracts (in magenta) and from sarkosyl-insoluble

425   homogenates (in grey) of AD brains.[29] Structures are shown as sticks for one protofilament

426   (above) and as ribbons for the other protofilament (below).

427

428

429

References

430

431   1.   Nilsberth, C. *et al.* The 'Arctic' APP mutation (E693G) causes Alzheimer's disease

432   by enhanced Aβ protofibril formation. *Nat Neurosci* **4**, 887–893 (2001).

433   2.   Selkoe, D. J. & Hardy, J. The amyloid hypothesis of Alzheimer's disease at

434   25 years. *EMBO Mol Med* **8**, 595–608 (2016).

435   3.   Shankar, G. M. *et al.* Amyloid-β protein dimers isolated directly from Alzheimer's

436   brains impair synaptic plasticity and memory. *Nat Med* **14**, 837–842 (2008).

437   4.   Hughes, C. *et al.* Beta amyloid aggregates induce sensitised TLR4 signalling

438   causing long-term potentiation deficit and rat neuronal cell death. *Commun Biol* **3**,

439   79 (2020).

440   5.   Li, S. *et al.* Soluble oligomers of amyloid Beta protein facilitate hippocampal long-

441   term depression by disrupting neuronal glutamate uptake. *Neuron* **62**, 788–801

442   (2009).

443   6.   Jin, M. *et al.* Soluble amyloid beta-protein dimers isolated from Alzheimer cortex

444   directly induce Tau hyperphosphorylation and neuritic degeneration. *Proc Natl*

445   *Acad Sci U S A* **108**, 5819–24 (2011).

446   7.   Jin, M. *et al.* An in vitro paradigm to assess potential anti-Aβ antibodies for

447   Alzheimer's disease. *Nat Commun* **9**, 2676 (2018).

448   8.   De Felice, F. G. *et al.* Alzheimer's disease-type neuronal tau hyperphosphorylation

449   induced by A beta oligomers. *Neurobiol Aging* **29**, 1334–1347 (2008).

450   9.   Forny-Germano, L. *et al.* Alzheimer's disease-like pathology induced by amyloid-β

451   oligomers in nonhuman primates. *J Neurosci* **34**, 13629–13643 (2014).

452   10.   Langer, F. *et al.* Soluble Aβ Seeds Are Potent Inducers of Cerebral β-Amyloid

453   Deposition. *Journal of Neuroscience* **31**, 14488–14495 (2011).

454   11.   Katzmarski, N. *et al.* Aβ oligomers trigger and accelerate Aβ seeding. *Brain*

455   *Pathology* **30**, 36–45 (2020).

456   12.   Takeda, S. *et al.* Neuronal uptake and propagation of a rare phosphorylated high-

457   molecular-weight tau derived from Alzheimer's disease brain. *Nat Commun* **6**, 8490

458   (2015).

459   13.   Dujardin, S. *et al.* Tau molecular diversity contributes to clinical heterogeneity in

460   Alzheimer's disease. *Nat Med* **26**, 1256–1263 (2020).

461   14.   Karran, E. & De Strooper, B. The amyloid hypothesis in Alzheimer disease: new

462   insights from new therapeutics. *Nat Rev Drug Discov* **21**, 306–318 (2022).

463   15.   Tucker, S. *et al.* The Murine Version of BAN2401 (mAb158) Selectively Reduces

464   Amyloid-β Protofibrils in Brain and Cerebrospinal Fluid of tg-ArcSwe Mice.

465   *Journal of Alzheimer's Disease* **43**, 575–588 (2015).

466   16.   Yang, Y. *et al.* Cryo-EM structures of amyloid-β 42 filaments from human brains.

467   *Science (1979)* **375**, 167–172 (2022).

468   17.   Benilova, I., Karran, E. & De Strooper, B. The toxic Aβ oligomer and Alzheimer's

469   disease: an emperor in need of clothes. *Nat Neurosci* **15**, 349–357 (2012).

470   18.   Mc Donald, J. M. *et al.* The aqueous phase of Alzheimer's disease brain contains

471   assemblies built from ∼4 and ∼7 kDa Aβ species. *Alzheimer's & Dementia* **11**,

472   1286–1305 (2015).

473   19.   Yang, T., Li, S., Xu, H., Walsh, D. M. & Selkoe, D. J. Large Soluble Oligomers of

474   Amyloid β-Protein from Alzheimer Brain Are Far Less Neuroactive Than the

475   Smaller Oligomers to Which They Dissociate. *J Neurosci* **37**, 152–163 (2017).

476   20.   Brinkmalm, G. *et al.* Identification of neurotoxic cross-linked amyloid-β dimers in

477   the Alzheimer's brain. *Brain* **142**, 1441–1457 (2019).

478   21.   Hong, W. *et al.* Diffusible, highly bioactive oligomers represent a critical minority

479   of soluble Aβ in Alzheimer's disease brain. *Acta Neuropathol* **136**, 19–40 (2018).

480   22.   Sideris, D. I. *et al.* Soluble amyloid beta-containing aggregates are present

481   throughout the brain at early stages of Alzheimer's disease. *Brain Commun* **3**,

482   fcab147–fcab147 (2021).

483   23.   Stern, A. M. *et al.* A calcium-sensitive antibody isolates soluble amyloid-β

484   aggregates and fibrils from Alzheimer's disease brain. *Brain* **147**, 2528–2540

485   (2022).

486   24.   Yang, T. *et al.* A highly sensitive novel immunoassay specifically detects low

487   levels of soluble Aβ oligomers in human cerebrospinal fluid. *Alzheimers Res Ther*

488   **7**, 14 (2015).

489   25.   Liu, L. *et al.* An ultra-sensitive immunoassay detects and quantifies soluble Aβ

490   oligomers in human plasma. *Alzheimer's & Dementia* **18**, 1186–1202 (2022).

491   26.   Esparza, T. J. *et al.* Soluble Amyloid-beta Aggregates from Human Alzheimer's

492   Disease Brains. *Sci Rep* **6**, 38187 (2016).

493   27.   Jin, M. & Selkoe, D. J. Systematic analysis of time-dependent neural effects of

494   soluble amyloid β oligomers in culture and in vivo: Prevention by scyllo-inositol.

495   *Neurobiol Dis* **82**, 152–163 (2015).

496   28.   McLaurin, J., Golomb, R., Jurewicz, A., Antel, J. P. & Fraser, P. E. Inositol

497   Stereoisomers Stabilize an Oligomeric Aggregate of Alzheimer Amyloid β Peptide

498   and Inhibit Aβ-induced Toxicity*. *Journal of Biological Chemistry* **275**, 18495–

499   18502 (2000).

500   29.   Fitzpatrick, A. W. P. *et al.* Cryo-EM structures of tau filaments from Alzheimer's

501   disease. *Nature* **547**, 185–190 (2017).

502   30.   Lannfelt, L. *et al.* Perspectives on future Alzheimer therapies: amyloid-β

503   protofibrils - a new target for immunotherapy with BAN2401 in Alzheimer's

504   disease. *Alzheimers Res Ther* **6**, 16 (2014).

505   31.   Johannesson, M. *et al.* Elevated soluble amyloid beta protofibrils in Down

506   syndrome and Alzheimer's disease. *Molecular and Cellular Neuroscience* **114**,

507   103641 (2021).

508   32.   Lannfelt, L. *et al.* Perspectives on future Alzheimer therapies: amyloid-β

509   protofibrils - a new target for immunotherapy with BAN2401 in Alzheimer's

510   disease. *Alzheimers Res Ther* **6**, 16 (2014).

511   33.   Koffie, R. M. *et al.* Oligomeric amyloid beta associates with postsynaptic densities

512   and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci*

513   *U S A* **106**, 4012–4017 (2009).

514   34.   Meilandt, W. J. *et al.* Trem2 deletion reduces late-stage amyloid plaque

515   accumulation, elevates the Aβ42:Aβ40 ratio, and exacerbates axonal dystrophy and

516   dendritic spine loss in the PS2APP Alzheimer's mouse model. *The Journal*

517   *of Neuroscience* 1819–1871 (2020) doi:10.1523/JNEUROSCI.1871-19.2019.

25

518 35. Casali, B. T., MacPherson, K. P., Reed-Geaghan, E. G. & Landreth, G. E.

519   Microglia depletion rapidly and reversibly alters amyloid pathology by

520   modification of plaque compaction and morphologies. *Neurobiol Dis* **142**, 104956

521   (2020).

522 36. Parhizkar, S. *et al.* Loss of TREM2 function increases amyloid seeding but reduces

523   plaque-associated ApoE. *Nat Neurosci* **22**, 191–204 (2019).

524 37. Swanson, C. J. *et al.* A randomized, double-blind, phase 2b proof-of-concept

525   clinical trial in early Alzheimer's disease with lecanemab, an anti-Aβ protofibril

526   antibody. *Alzheimers Res Ther* **13**, 80 (2021).

527 38. Falcon, B. *et al.* Tau filaments from multiple cases of sporadic and inherited

528   Alzheimer's disease adopt a common fold. *Acta Neuropathol* **136**, 699–708 (2018).

529 39. Weismiller, H. A. *et al.* Structural disorder in four-repeat Tau fibrils reveals a new

530   mechanism for barriers to cross-seeding of Tau isoforms. *Journal of Biological*

531   *Chemistry* **293**, 17336–17348 (2018).

532 40. Jackson, S. J. *et al.* Short Fibrils Constitute the Major Species of Seed-Competent

533   Tau in the Brains of Mice Transgenic for Human P301S Tau. *Journal of*

534   *Neuroscience* **36**, 762–772 (2016).

535 41. Zivanov, J., Nakane, T. & Scheres, S. H. A Bayesian approach to beam-induced

536   motion correction in cryo-EM single-particle analysis. *IUCrJ* **6**, 5–17 (2019).

537 42. Rohou, A. & Grigorieff, N. CTFFIND4: Fast and accurate defocus estimation from

538   electron micrographs. *J Struct Biol* **192**, 216–221 (2015).

539   43.   Zivanov, J., Nakane, T. & Scheres, S. H. W. Estimation of high-order aberrations

540   and anisotropic magnification from cryo-EM data sets in RELION-3.1. *IUCrJ2* **7**,

541   253–267 (2020).

542   44.   He, S. & Scheres, S. H. W. Helical reconstruction in RELION. *J Struct Biol* **198**,

543   163–176 (2017).

544   45.   Chen, S. *et al.* High-resolution noise substitution to measure overfitting and

545   validate resolution in 3D structure determination by single particle electron

546   cryomicroscopy. *Ultramicroscopy* **135**, 24–35 (2013).

547   46.   Casañal, A., Lohkamp, B. & Emsley, P. Current developments in Coot for

548   macromolecular model building of Electron Cryo-microscopy and Crystallographic

549   Data. *Protein Science* **29**, 1055–1064 (2020).

550   47.   Yamashita, K., Palmer, C. M., Burnley, T. & Murshudov, G. N. Cryo-EM single-

551   particle structure refinement and map calculation using Servalcat. *Acta*

552   *Crystallographica Section D* **77**, 1282–1291 (2021).

553

554

Materials and Methods

Aqueous soaking extraction of Aβ and tau fibrils from Alzheimer's disease brains and their re-centrifugation

The soaking method for aqueous extraction of diffusible Aβ aggregates from AD brain has been described previously[21,22] and is depicted in Table 1. Brain donor demographic and pathologic information is described in Table S1. Grey matter was dissected from fresh or frozen cortical regions of individuals with AD and minced using a McIlwain tissue chopper (razor blade) set at 0.5 mm. The resultant brain bits were soaked for 30 min in a 1:5 ratio (w:v) of TBS extraction buffer (25 mM Tris supplemented with 150 mM NaCl, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 2 μg/ml pepstatin, 120 μg/ml 4-benzenesulfonyl fluoride hydrochloride, 5 mM NaF, pH 7.2) in 50-ml Eppendorf Protein LoBind tubes, and then spun at 2,000 g in a Fiberlite F14-14 x 50cy rotor in a Sorvall Lynx 6000 centrifuge for 10 min at 4°C (pelleting distance ~4 cm depending on volume). The top ~90% of the supernatants were transferred to thin-wall polypropylene tubes (Beckman catalog #331372) and spun using an SW41Ti rotor at 40,000 rpm for 110 minutes in an Optima L90K ultracentrifuge at 4°C (pelleting distance ~9 cm). The top ~90% supernatants were retained and frozen at -80°C in 1 ml aliquots. For re-centrifugation (see Results), the frozen aliquots were thawed and spun in an Eppendorf 5417R centrifuge equipped with an F-45-30-11 rotor or else a TOMY MX-307 centrifuge equipped with an AR015-24 rotor at 20,000 g for 2 h at 4°C (pelleting distance ~1 cm). Prior to this re-centrifugation, a 50 μl portion of each aliquot was retained for Aβ ELISA. The final supernatants, as well as the associated pellets, were collected and retained for ELISAs and electron microscopy (EM). For Aβ42 monomer ELISAs, pellets were resuspended and denatured in 50 μl TBS containing 5 M GuHCl and 5 mM EDTA. Similarly, for Aβ42 monomer ELISAs on the original soaking extracts

28

578   (inputs) and their recentrifuged supernatants, each was denatured by mixing them at a 2:5 ratio

579   (v:v) with TBS containing 7 M GuHCl and 5 mM EDTA, to reach a final GuHCl concentration

580   of 5 M. For analysis by negative-staining EM, final pellets were resuspended in 15 µl cold water.

581

582   **ELISAs**

583   Aβ monomer-preferring ELISAs were home-brew immunoassays run on the Meso Scale

584   Discovery (MSD) platform, as described.[23] Samples containing GuHCl were diluted before

585   ELISA, such that the GuHCl concentration was <0.25 M, to avoid interference with the antibody

586   reaction. All steps were at room temperature. Plates were coated with capture antibody in PBS

587   overnight and blocked in 5% MSD Blocker A in TBS with 0.05% Tween-20 (TBS-T) for 1 h.

588   Samples were then applied for 1.5 h after being diluted in 1% Blocker A in TBS-T. Plates were

589   washed 3 times in TBS-T, then biotinylated detector antibody and MSD Strepavidin-Sulfotag

590   (1:5000) were applied for 1.5 h. After three more washes in TBS-T, the plates were detected with

591   2x MSD read buffer. The lower limit of quantification (LLoQ) was defined as the lowest

592   standard with a luminescence value at least twice the blank average, and the lower limit of

593   detection (LLoD) was defined as the lowest standard with values greater than the blank average

594   plus twice the blank standard deviation. Aβ aggregate-preferring ELISAs were home-brew

595   assays run on the Millipore SMCxPRO platform, as described previously.[25] Antibodies, sources,

596   and concentrations are listed in Table S2.

597

598   **Negative staining and immunoelectron microscopy**

599   Carbon-coated grids (EMS) were glow-discharged at 25 mA for 20 s. For liquid samples

600   (resuspended final pellets from soaking extracts), 5 µl were adsorbed to the grid for 1-5 min.

601   Excess liquid was blotted off with filter paper (Whatman #1). Grids were then floated on a drop

602   of water, blotted again, and stained with 1% uranyl acetate for 15 s. Excess stain was blotted off

603   with filter paper. For immunostaining after their adsorption to the grids, samples were blocked in

604   1% BSA for 10 min. Antibodies diluted in 1% BSA were then added for 20 min and washed 3

605   times in PBS. Protein A-gold (Cell Biology UMC Utrecht) in 1% BSA was applied for 30 min,

606   followed by two washes in PBS and four washes in water. All grids were examined on a JEOL

607   1200EX transmission electron microscope equipped with an AMT 2k CCD camera. Except for

608   lecanemab, primary antibodies were purchased from Cell Signaling Technologies and diluted as

609   follows: Anti-Aβ N-terminus (D54D2) 1:10; anti-tau C-terminus (D1M9X) 1:20; anti-tau pS404

610   (D2Z4G) 1:50; anti-tau pT205 (E7D3E) 1:50. Lecanemab was obtained from Sanofi and used at

611   a final concentration of 13.3 µg/ml. To quantify fibrils, thirteen 0.9-ml soaking extracts were

612   centrifuged for 2h at 20,000 g in an Eppendorf FA-45-30-11 rotor, and the pellets were

613   resuspended in 20 µl water. Samples were immunolabeled with D54D2 as above, and 30

614   micrographs were taken at random by one experimenter (AMS) from well-stained portions of

615   each grid at 25,000x magnification. The image order was randomized by a second experimenter

616   (ALM) and given to a third blinded experimenter (ME) who counted the number of

617   immunolabeled fibrils.

618   For ultrathin cryosections, AD brain tissues were fixed (fresh or after freezing) in 4%

619   paraformaldehyde and stored at 4°C. Prior to freezing in liquid nitrogen, the fixed tissue samples

620   were infiltrated with 2.3 M sucrose in PBS for 15 min. Frozen samples were sectioned at a

621   thickness of 60 nm at -120°C and the sections transferred to formvar/carbon coated copper grids.

622   Grids were floated on PBS, and downstream immunolabeling was carried out as above.

623   Contrasting/embedding of the labeled grids was carried out on ice in 0.3% uranyl acetate in 2%

624   methyl cellulose for 10 min.

625

Size exclusion chromatography

627   Size exclusion chromatography of human brain soaking extracts was performed using

628   ÄKTA FPLC and a Superdex 200 Increase 10/300 column (Cytiva). Extracts (350 µl) were

629   injected into the column using a 0.5-ml loop. Running buffer was TBS (25 mM Tris, 150 mM

630   NaCl, pH 7.4) at 0.5 ml/min. 0.5-ml fractions were collected and used directly for ELISA

631   without lyophilization.

632

Electron cryo-microscopy

634   Four to five 1-ml aliquots of soaking extracts were thawed on ice and spun in 1.5 ml

635   Eppendorf tubes in an Eppendorf FA-45-24-11 rotor at 20,000 g for 1 h. All the pellets were

636   resuspended and combined into 1 ml cold water. Following a 30 min centrifugation at 20,000 g

637   at 4°C, pellets were resuspended in 10 µl of 20 mM Tris-HCl, pH 7.4. Samples were centrifuged

638   at 3,000 g for 3 min and applied to glow-discharged holey carbon gold grids (Quantifoil Au

639   R1.2/1.3, 300 mesh), which were glow-discharged with an Edwards (S150B) sputter coater at 30

640   mA for 30 s. Aliquots (3 µl) of samples were applied to the grids and blotted for approximately

641   3-5 s with filter paper (Whatman) at 100% humidity and 4 °C, using a Vitrobot Mark IV

642   (Thermo Fisher). Datasets were acquired on a Titan Krios G3 microscope (Thermo Fisher

643   Scientific) operated at 300 kV. Images for case AD7 were acquired using a Falcon-4 detector

644   without energy filter. Images for case AD11 were acquired on a Gatan K3 detector in super-

645   resolution counting mode, using a Bio-quantum energy filter (Gatan) with a slit width of 20 eV.

646   Images were recorded with a total dose of 40 electrons per $Å^2$.

647   Movie frames were gain-corrected, aligned, dose-weighted and then summed into a

648   single micrograph using RELION's own motion correction program.[41] Contrast transfer function

649   (CTF) parameters were estimated using CTFFIND-4.1.[42] All subsequent image-processing steps

650   were performed using helical reconstruction methods in RELION.[43,44] Filaments were picked

651   manually. Reference-free 2D classification was performed to select suitable segments for further

652   processing. Deposited maps of Type I Aβ42 filaments (EMD-13800), Type II Aβ42 filaments

653   (EMD-13809),[16] and tau PHFs (EMD-3741 and EMD-0259)[16] low-pass filtered to 10 Å were

654   used as initial models. Combinations of 3D auto-refinement and 3D classifications were used to

655   select the best segments for each structure. For all structures, Bayesian polishing[41] and CTF

656   refinement[43] were performed to further increase the resolution of the reconstructions. Final

657   reconstructions were sharpened using standard post-processing procedures in RELION, and

658   overall resolutions were estimated from Fourier shell correlations at 0.143 between the

659   independently refined half-maps, using phase randomisation to correct for convolution effects of

660   a generous, soft-edged solvent mask.[45]

661   Atomic models comprising β-sheet rung were built in Coot,[46] starting from available

662   models for Type I Aβ42 filaments (PDB:7Q4B), Type II Aβ42 filaments (PDB:7Q4M), and

663   PHFs (PDB:6HRE). Coordinate refinements were performed using *Servalcat*.[47] Final models

664   were obtained using refinement of only the asymmetric unit against the half-maps in *Servalcat*.

665

666   **Data availability**

667   Cryo-EM maps have been deposited in the Electron Microscopy Data Bank (EMDB) under

668   accession numbers EMD-15770, EMD-15771 and EMD-15772. Corresponding refined atomic

669   models have been deposited in the Protein Data Bank (PDB) under accession numbers 8AZS,
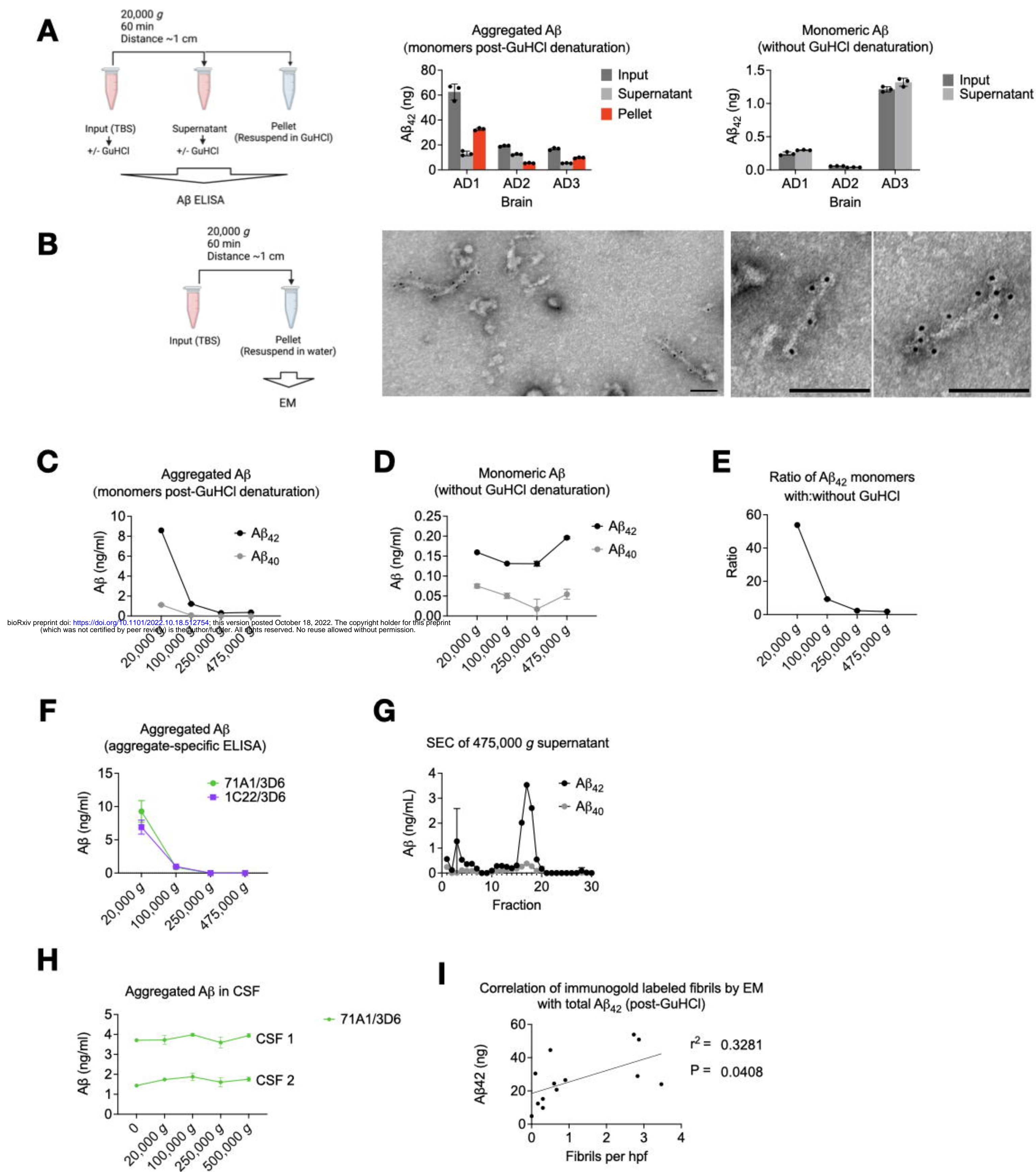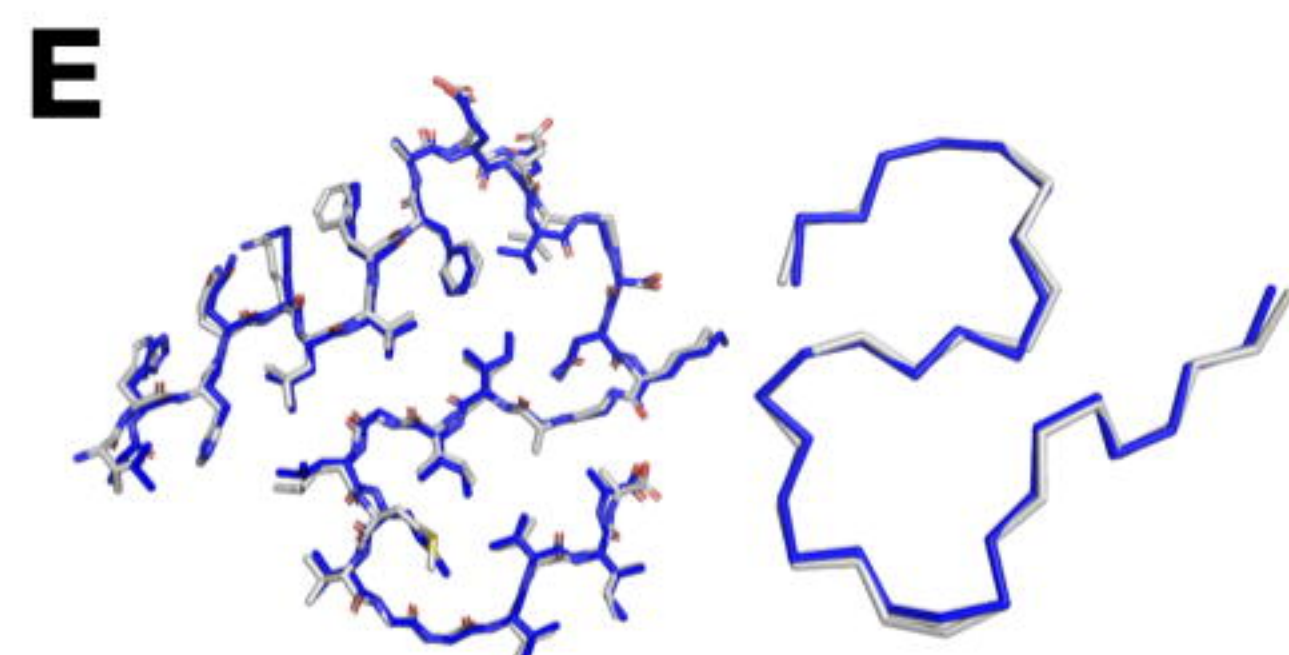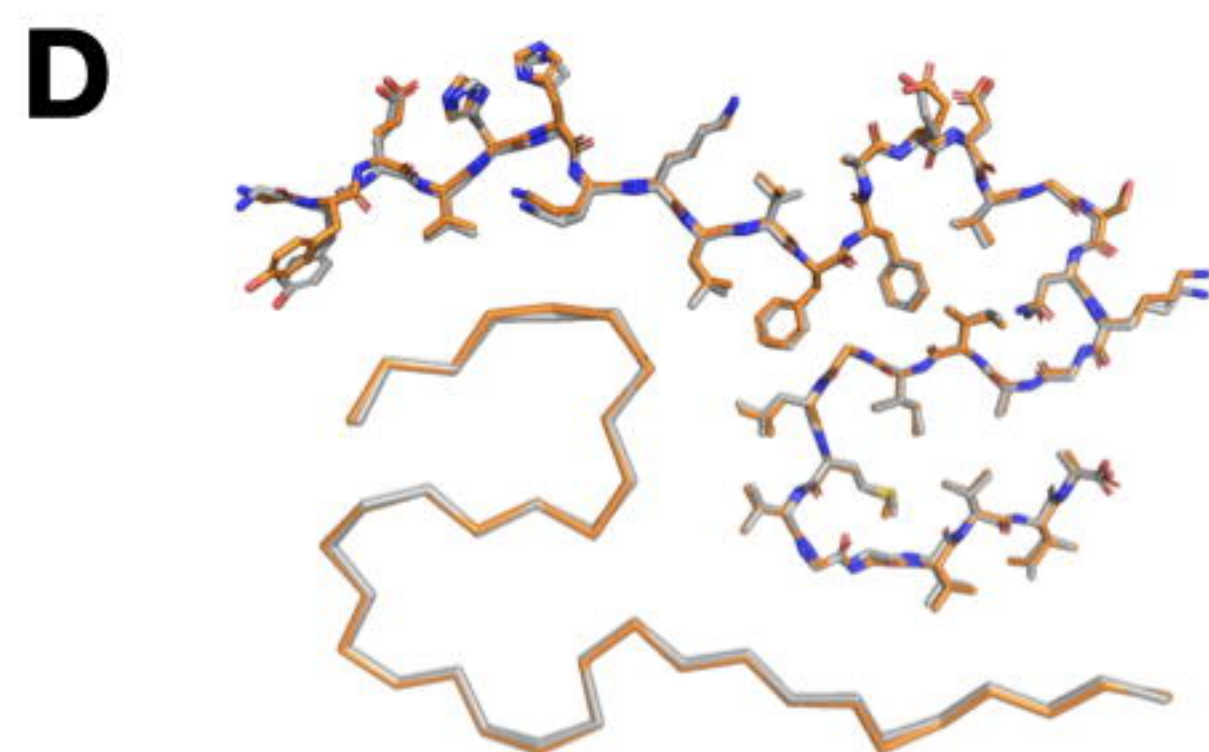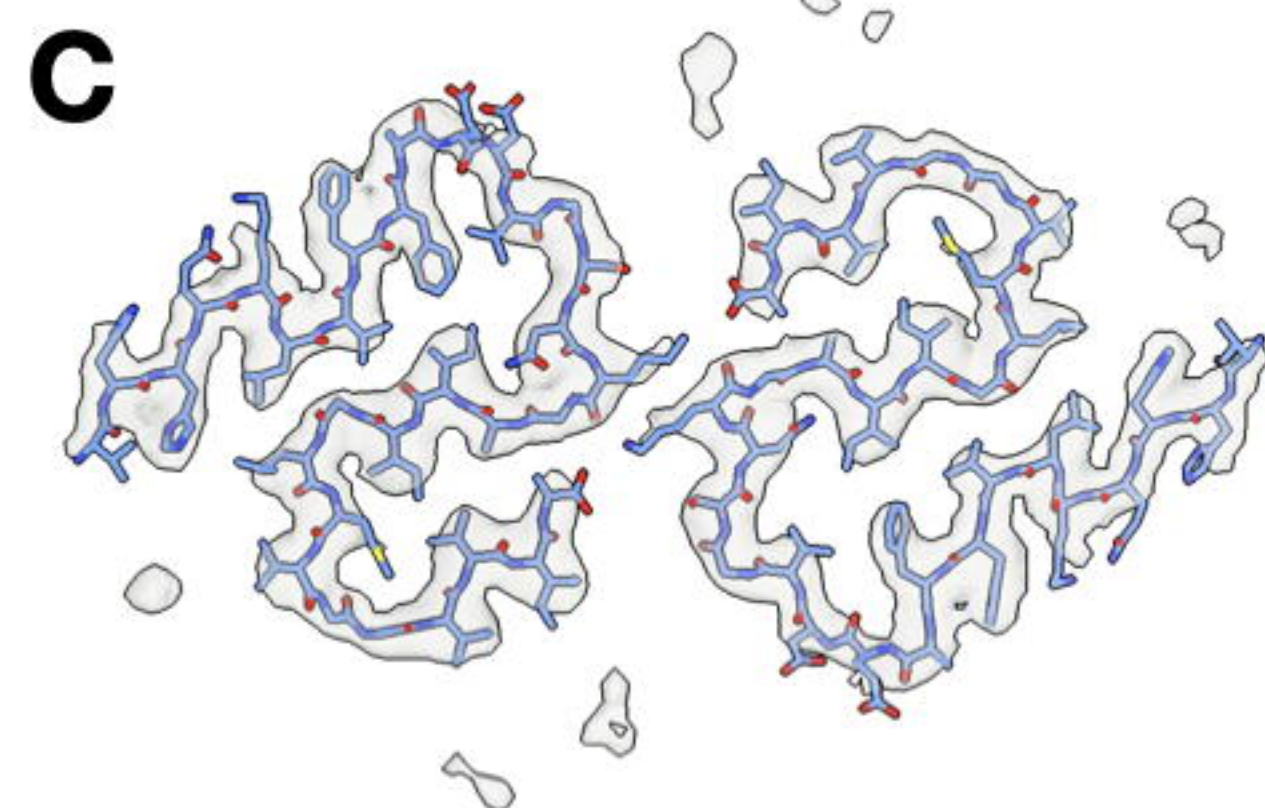
670   8AZT and 8AZU.
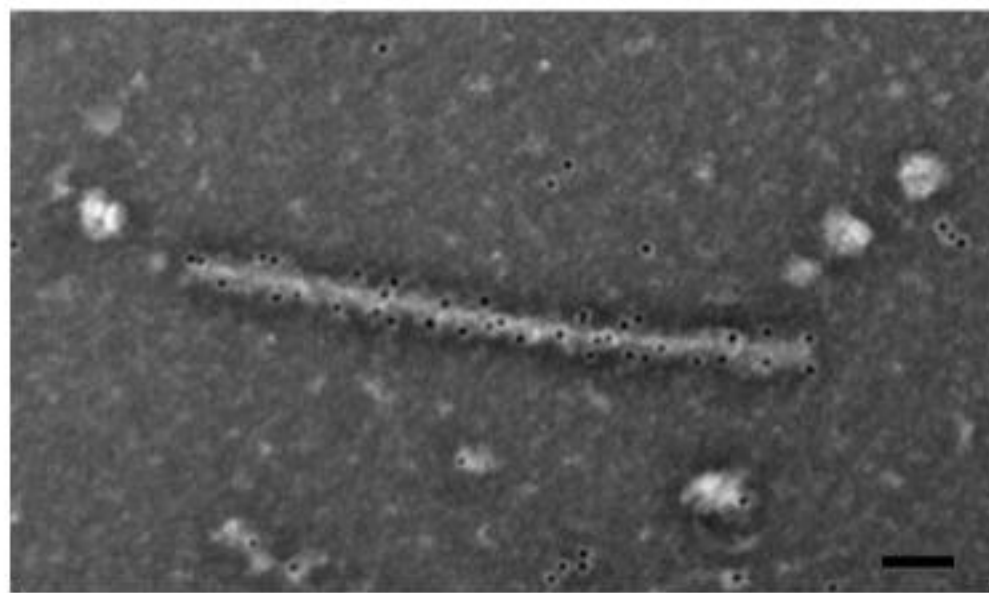
A

Aggregated Aβ
(monomers post-GuHCl denaturation)

Monomeric Aβ
(without GuHCl denaturation)

B

C

Aggregated Aβ
(monomers post-GuHCl denaturation)

D

Monomeric Aβ
(without GuHCl denaturation)

E

Ratio of Aβ$_{42}$ monomers
with:without GuHCl

F

Aggregated Aβ
(aggregate-specific ELISA)

G

SEC of 475,000 g supernatant

H

Aggregated Aβ in CSF

I

Correlation of immunogold labeled fibrils by EM
with total Aβ$_{42}$ (post-GuHCl)

r^2 = 0.3281

P = 0.0408

Fig 1

Fig 2

A Lecanemab
B Lecanemab
C h1C22
D h1C22

E No antibody

F
Immunoprecipitation:
■ Lecanemab
■ Bapineuzumab

$A\beta_{42}$ (ng)

3
1
0.15
0.10
0.05
0.00

20k   100k   250k   475k
RCF_{av} (g)

G Lecanemab
H

I Lecanemab
J
K No antibody

Fig 3

Tau C-terminus

Tau-pT205
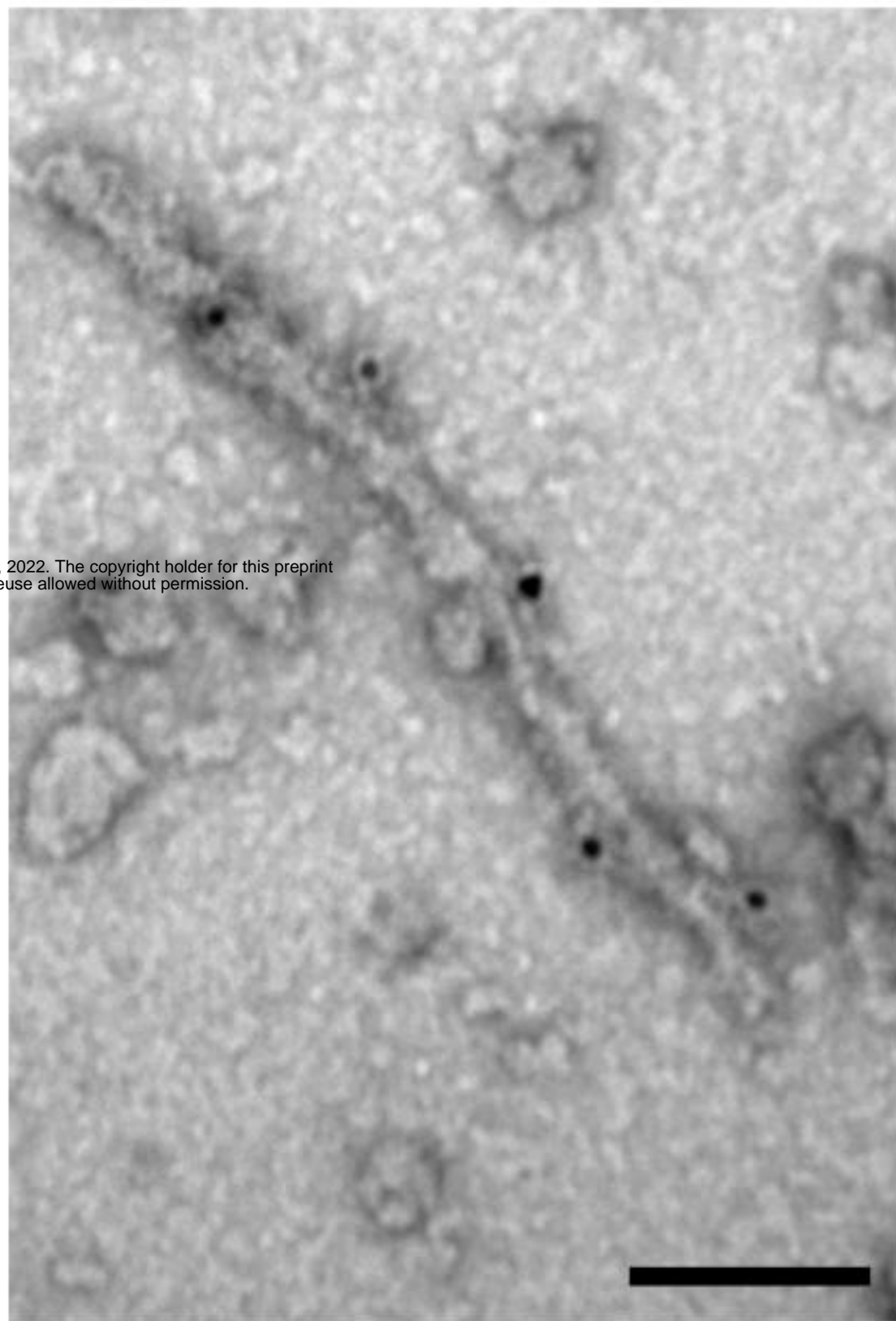
Tau-pS404
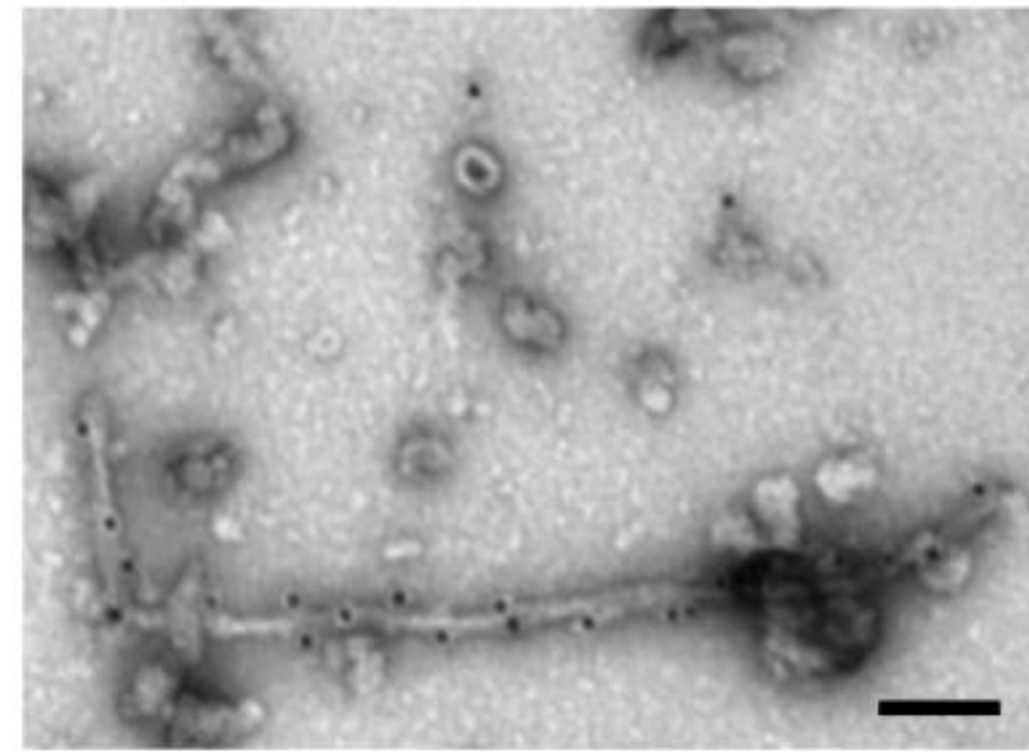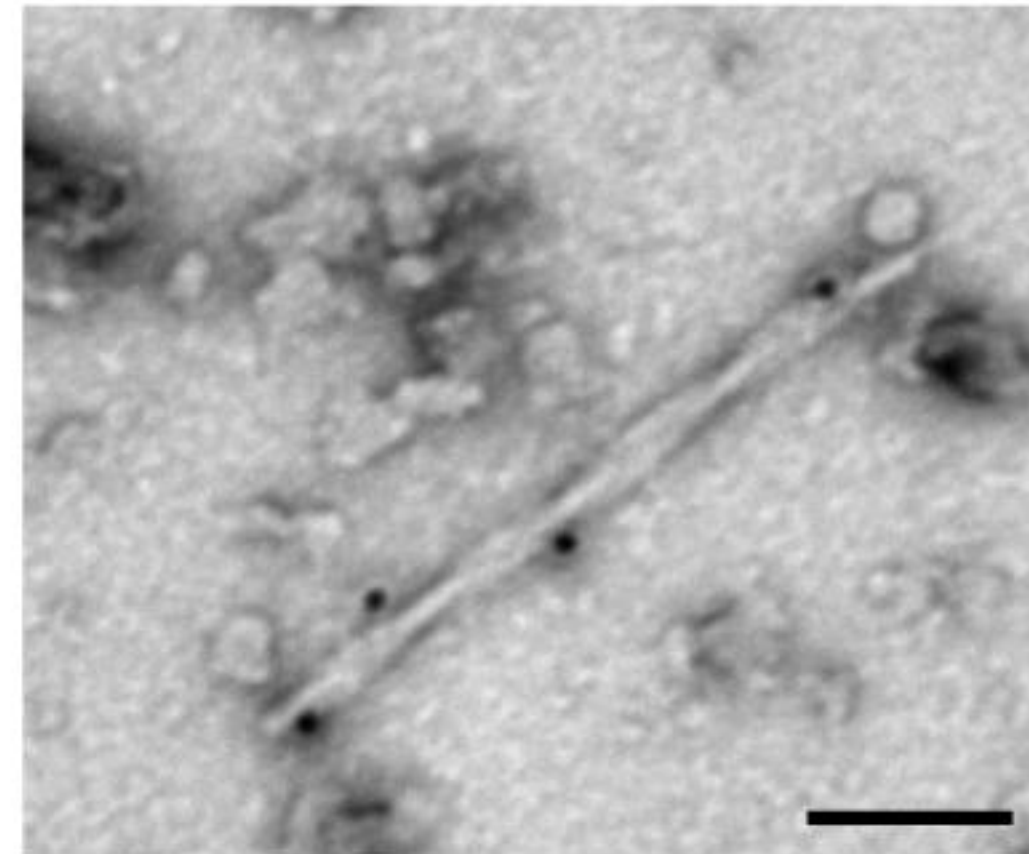
Fig S1

A

Aggregated Aβ
(monomers post-GuHCl denaturation)

■ Input
■ Supernatant
■ Pellet

y-axis: $A\beta_{42}$ (ng), 0–20
x-axis: Bottom ~7 ml, Top 2 ml
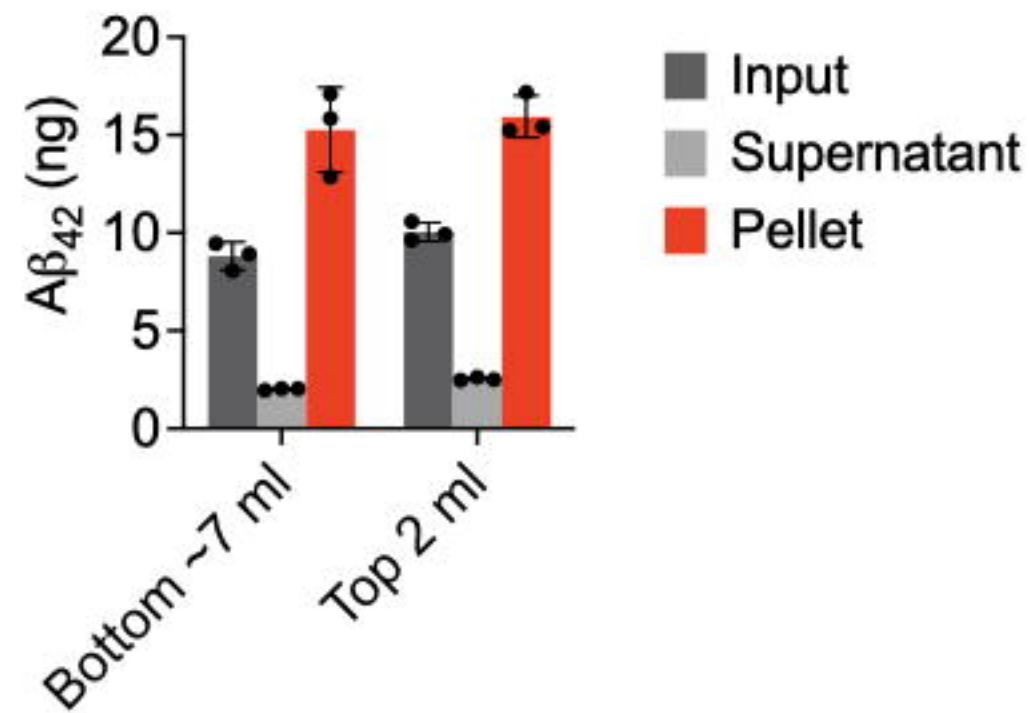
B

Rinsing tissue in 15 volumes
extraction buffer prior to soaking
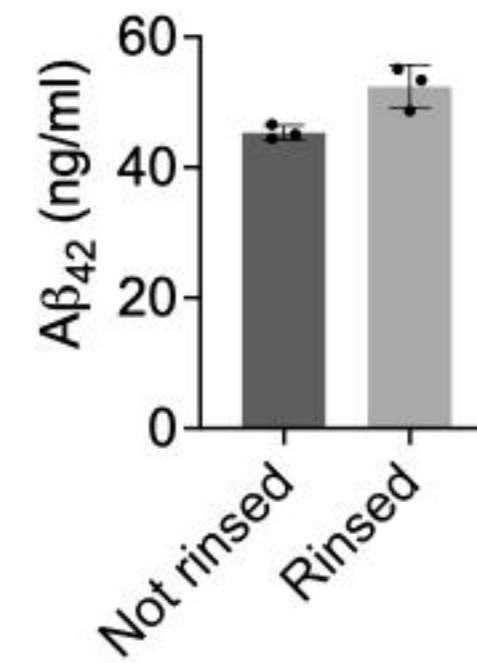
y-axis: $A\beta_{42}$ (ng/ml), 0–60
x-axis: Not rinsed, Rinsed

C

Timecourse of accumulation of aggregated
Aβ in pellet during re-centrifugation

y-axis: $A\beta_{42}$ (ng), 0–8
x-axis: Time (min), 0–250
● AD5
● AD4

D

Timecourse of accumulation of aggregated
Aβ in pellet during re-centrifugation

y-axis: $A\beta_{42}$ (ng), 0–4
x-axis: Time (min), 0–40
● AD5
● AD4

E
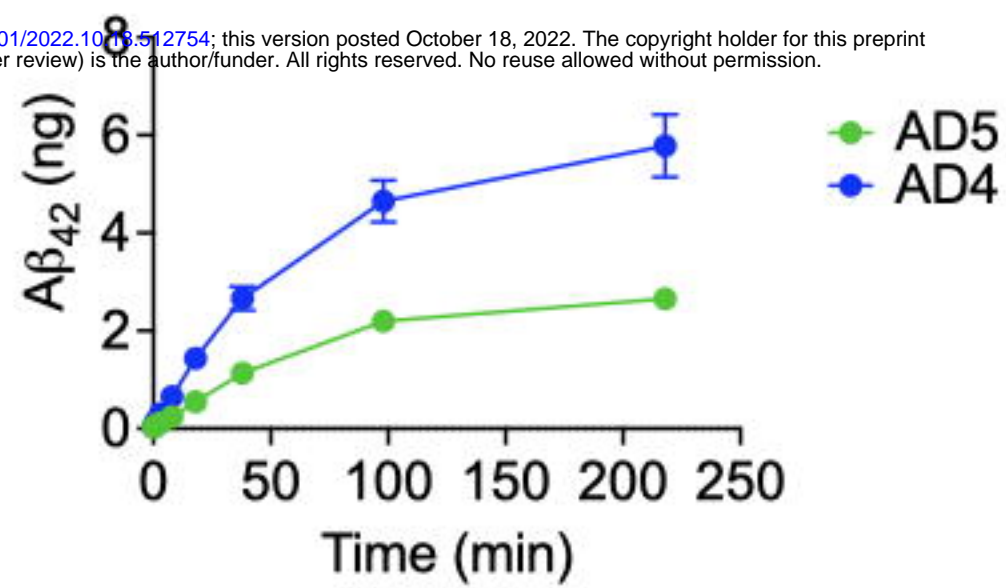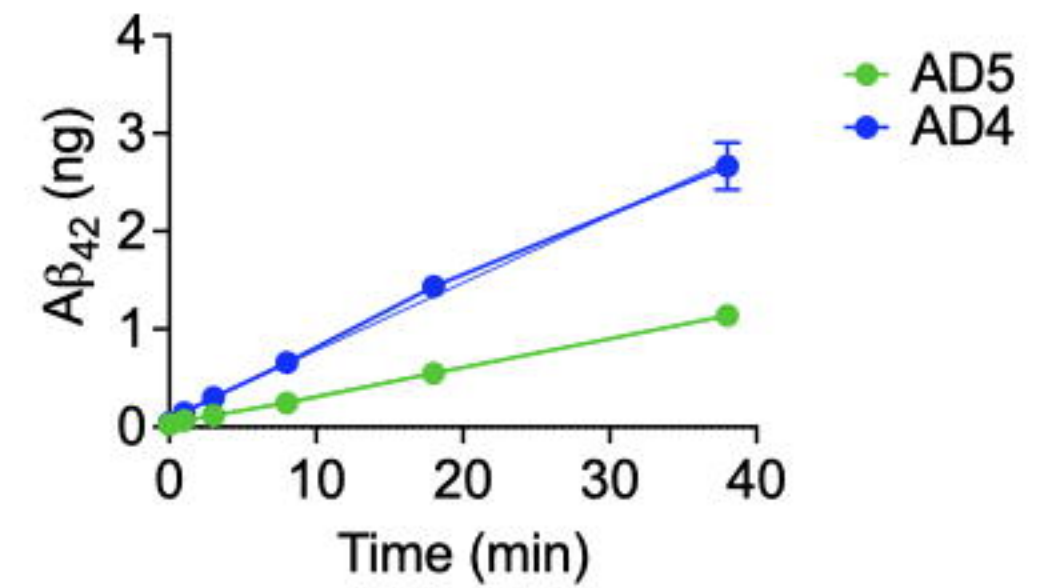
Re-centrifugation of Aβ aggregates by
plastic tube type

y-axis: $A\beta_{42}$ (ng), 0.0–2.0
x-axis: LoBind, Siliconized, LoBind + BSA

■ Input
■ Supernatant
■ Pellet

Fig S2

A Re-centrifugation of aggregated Aβ in soaking extracts stored at 4°C

B Fibrils obtained from soaking extracts stored at 4°C

C Storage temperature effect on re-centrifugation of aggregated Aβ

D Effect of dilution prior to freezing on re-centrifugation of aggregated Aβ

E Effect of aggregation inhibitors prior to freezing on subsequent re-centrifugation of aggregated Aβ

F Effect of inositol prior to freezing on subsequent re-centrifugation of aggregated Aβ

G Effect of filtration prior to freezing on subsequent re-centrifugation of aggregated Aβ

H SEC of Aβ aggregates

I Aggregated Aβ (monomers post-GuHCl denaturation)

J Monomeric Aβ (without GuHCl denaturation)

K Aggregated Aβ (aggregate-specific ELISA)

L

Fig S3

A

AD7   AD11

PHF   PHF

3.5Å   1nm   3.1Å

B

C

Fig S4