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Direct measurement of lipid membrane disruption connects kinetics and toxicity of Aβ42 aggregation

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Abstract

The formation of amyloid deposits within human tissues is a defining feature of more than fifty medical disorders, including Alzheimer's disease. Strong genetic and histological evidence links these conditions to the process of protein aggregation, yet it has remained challenging to identify a definitive connection between aggregation and pathogenicity. Using time-resolved fluorescence microscopy of individual synthetic vesicles, we show for the Aβ42 peptide implicated in Alzheimer's disease that the disruption of lipid bilayers correlates linearly with the time course of the levels of transient oligomers generated through secondary nucleation. These findings suggest a specific role of oligomers generated through the catalytic action of fibrillar species during the protein aggregation process, in driving

33   deleterious biological function, and establish a direct causative connection between amyloid

34   formation and its pathological effects.

Main text

37   Neurodegenerative conditions including Alzheimer's and Parkinson's diseases [1-5] have

38   emerged as major challenges to the health and social systems of the modern world as a result

39   of their increasing prevalence in our ageing populations. A substantial body of data indicates

40   that protein aggregation is a key factor underlying these disorders [6-14]. Recent progress in

41   understanding the molecular mechanisms of aggregation has revealed that these processes

42   typically involve a primary nucleation step, followed by the growth of the initial aggregates

43   through an elongation process (Fig. 1a) [15-18]. Once a critical quantity of fibrils is formed,

44   however, the aggregation reaction can be accelerated dramatically by secondary processes in

45   which fibrils formed during the aggregation reaction promote the formation of further

46   aggregates (Fig. 1a) [17,18]. A prominent example of such secondary processes is surface-

47   catalysed secondary nucleation, which is particularly significant for the Aβ peptides

48   associated with Alzheimer's disease, and has been linked to a variety of mechanisms of

49   neuronal damage [4,5,17,18]. Indeed, the disruption of the lipid bilayer within cellular

50   membranes by oligomeric protein aggregates, and the consequent loss of Ca^{2+} homeostasis,

51   has been proposed as a general mechanism of neurotoxicity [19-21].

52   In the context of Alzheimer's disease, a key objective is to connect the time

53   dependence of lipid bilayer permeability to that of the aggregation reaction and to the

54   resulting distribution of aggregated forms of Aβ42, the 42-residue Aβ peptide that has been

55   most strongly linked to neurological damage. Such data would serve to establish the species

56   and processes associated with an ongoing Aβ42 aggregation reaction that are primarily

57   responsible for causing lipid bilayer permeability. This information would be particularly

58   useful for elucidating the mechanisms of pathological protein aggregation, which still remain

59   poorly understood, a possible reason for the heterogeneous outcome of clinical trials aimed at

60   targeting amyloid formation [22-24].

61   To address this question, we have combined recent advances in the mechanistic

62   analysis of experimental measurements of protein aggregation kinetics in vitro [15,25] with

63   the development of a single molecule optochemical approach able to quantify the degree of

64   membrane permeability resulting from the aggregation reaction at any point in time [26].

65   Using this platform, we reveal the dominant role of transient Aβ42 oligomers generated by

66   secondary nucleation [27] in driving membrane permeation.

67

68 Results

69 **Link between aggregation of Aβ42 and lipid bilayer permeability**

70 We first monitored the extent of membrane disruption by measuring the time dependence of

71 the permeability of the lipid bilayers of synthetic vesicles when monomeric Aβ42 was

72 incubated at concentrations between 2 and 4 μM under solution conditions where the kinetics

73 of amyloid fibril formation have been shown to be highly reproducible [28] (Extended Data

74 Figure 1 and Online Methods). We also added 2.5% molar monomer equivalents of pre-

75 formed Aβ42 fibrils (seeds) to a solution of 2μM monomeric Aβ42 (Online Methods).

76 Addition of such seed fibrils accelerates the aggregation reaction by bypassing the primary

77 nucleation step, providing a convenient and robust way to disentangle secondary nucleation

78 from primary nucleation [17-18]. In each case, at specific time points, aliquots were removed

79 from the aggregating solutions, added to lipid vesicles, and the extent of Ca^{2+} influx measured

80 using the optochemical method [26]. We found that the ability of the aggregation reaction

81 mixture to induce bilayer permeability increased with time, reaching a maximum value in the

82 growth phase that is clearly observable in bulk measurements, and then decreased during the

83 remainder of the aggregation reaction (Figs. 1b,c).

84

85

86 Since membrane permeation is directly linked in our assay to the aggregation process, we

87 utilised a chemical kinetics approach to describe the time evolution of Ca^{2+} influx, $\Phi(t)$, in

88 terms of the concentrations $c(t)$ at time t of different types of aggregate species that could

89 give rise to membrane permeability [16] (Fig. 1a and Supplementary Note 1). In particular,

90 due to the transient nature of Ca^{2+} influx, these aggregate species include intermediate

91 oligomers generated either by primary nucleation (primary oligomers) or by secondary

92 nucleation (secondary oligomers) [27]. We used the law of mass action to represent Ca^{2+}

93 influx very generally as a power-law of the concentration of oligomers, $\Phi(t) \propto c(t)^{\gamma}$, where

94 $\gamma \geq 1$ is the reaction order of membrane permeation with respect to the concentration of

95 oligomers (Supplementary Note 1). γ is a measure of the cooperativity between oligomers in

96 causing membrane permeation. We then described the time evolution of the concentrations of

97 primary and secondary oligomers during aggregation using a master equation

98 (Supplementary Note 1, Eq. 1). Using approaches drawing on self-consistent field theory

99 [16,27], we derived explicit mathematical expressions for the time course of fibril formation

100   and for the concentrations of oligomers in these two specific mechanistic scenarios

101   (Supplementary Note 1), and then compared each of them to the experimental data describing

102   the extent of Ca^{2+} influx over time (Fig. 1b,c). Specifically, we first fitted globally the

103   different kinetic traces of amyloid fibril formation to our kinetic model (Supplementary Note

104   1, Eq. 2) to determine combined rate parameters associated with primary and secondary

105   nucleation (Supplementary Table 1). These parameters were then implemented in the

106   theoretical oligomer concentration curves (Supplementary Note 1, Eqs. 5 and 6), leaving the

107   combined rates of oligomer dissociation and conversion, the proportionality constant between

108   oligomer concentrations and the extent of Ca^{2+} influx, and the reaction order γ as fitting

109   parameters (Supplementary Table 2 and Supplementary Note 1).

110   This global analysis reveals that the membrane permeation data are well described by

111   a scenario in which the large majority of the species causing lipid bilayer permeability is

112   generated by secondary nucleation (Fig. 1c) but not by primary nucleation (Fig. 1b). Note

113   that the former scenario (Fig. 1c) explicitly considers contributions from both primary and

114   secondary oligomers (Supplementary Note 1, Eq. 8), even though the primary oligomers are a

115   significant proportion of the oligomer population only during the initial stages but are

116   otherwise outnumbered by secondary oligomers for the rest of the reaction (Extended Data

117   Figure 2). We also note that in comparing the different mechanistic scenarios, we consider fit

118   quality globally across a variety of conditions (concentration, seeds), since fits to individual

119   kinetic traces are insufficient to accept or reject a particular mechanism [16]. In particular,

120   the inability of the primary oligomer model to describe the membrane permeation data stems

121   from the fact that the primary nucleation pathway for Aβ42 is negligible in the presence of

122   pre-formed fibrils (Extended Data Figure 3) [17,18]. To provide further support to the

123   hypothesis that secondary nucleation generates the majority of species responsible for

124   membrane permeation, we performed a set of additional experiments, where the rate of

125   secondary nucleation was modulated by removing fibrils during aggregation through

126   centrifugation (Online Methods). Specifically, an aggregation reaction starting with 2 μM

127   Aβ42 (lag time close to 1 hour) was stopped after 40 minutes. The aggregation mixture was

128   then centrifuged for 15 minutes to remove fibrils and reduce secondary nucleation. We also

129   performed a control experiment where the aggregation was stopped at 40 minutes and

130   restarted at 60 minutes without any centrifugation. In both cases, membrane permeation was

131   measured at 2 hours. We found that membrane permeation is significantly reduced when the

132   mixture is centrifuged, but there is no significant change when aggregation is only interrupted

133   (Fig. 1d). This result is in line with the prediction that this protocol would significantly

134   diminish the observed membrane permeation from secondary oligomers, which are now

135   present at lower concentrations, but leave unaffected the toxicity from primary oligomers.

136   The best global fit of the permeation data to the secondary oligomer model yields an

137   exponent $\gamma \sim 1.0 \pm 0.1$ (Fig. 2a). This finding implies that the time course of Ca^{2+} influx

138   correlates with the concentration of secondary oligomers, suggesting that membrane

139   permeation is a first order reaction with respect to the population of secondary oligomers and

140   is thus independent of interactions between oligomers (Fig. 2b and Extended Data Figure 4a).

141

142   We next investigated the question of whether the secondary oligomers are able to exert their

143   damaging effects directly on their formation or if they have first to convert into species that

144   are distinct from those that propagate the aggregation reaction and which possess more

145   specific deleterious activity ('lethal oligomers') analogous to events described for prion

146   diseases [29]. To this end we considered a scenario in which oligomers generated initially

147   through secondary nucleation were assumed not to be toxic but to induce bilayer permeation

148   only after a conversion step to such lethal oligomers over timescales comparable to, or longer

149   than, that of the overall aggregation process (Supplementary Note 1, Eq. 9). The comparison

150   between the model predictions and the experimental time course of membrane permeation

151   shows, however, that this model is not able to capture the kinetics of the observed behaviour

152   (Fig. 2c,d and Extended Data Figure 4b), implying that the oligomers generated on the

153   surfaces of fibrils drive membrane permeation   without the need for a structural

154   reorganization that is slow relative to the overall aggregation process. As such, this finding

155   establishes a direct connection between the process of amyloid fibril formation and the toxic

156   effects associated with protein oligomers.

157

158   **Molecular chaperones modulate lipid bilayer permeation by Aβ42 aggregation**

159   We then carried out a series of additional experiments in which the rates of key microscopic

160   steps in the Aβ42 aggregation mechanism were modulated selectively by molecular

161   chaperones [30,31] (Fig. 3) in order to test further the conclusion that secondary oligomers

162   are correlated with lipid bilayer permeation. We performed experiments in the presence of

163   the chaperone DNAJB6 (0.01% molar equivalents to monomeric Aβ42), which at this

164   concentration has been shown to inhibit mainly primary nucleation [31] (Extended Data

165   Figure 5), and the Brichos domain (10% molar equivalents to monomeric Aβ42), that has

166   been shown to suppress secondary nucleation [30] (Extended Data Figure 6). The

167   experiments were performed at low concentrations of the chaperones in order still to be able

168   to detect a measurable degree of Ca^{2+} influx, as these chaperones are able to inhibit very

169   effectively the levels of oligomers generated by nucleation processes. In each case, we

170   recorded the effect of each chaperone on the total aggregate mass concentration, as well as on

171   the extent of Ca^{2+} influx (Fig. 3).

172

173   The alterations of the rate constants for primary and secondary nucleation in the presence of

174   the chaperones were determined by fitting the aggregate mass measurements to the analytical

175   expression for the time course of amyloid fibril formation (Supplementary Note 1, Eq. 2 and

176   Supplementary Table 3). These rate parameters were then used to simulate the effects of the

177   chaperones on the population of secondary oligomers over time (Supplementary Note 1, Eq.

178   4, solid lines), without the introduction of any additional fitting parameters. In the presence of

179   DNAJB6 (Fig. 3a), we observed a retardation of membrane permeation that is in agreement

180   with the reduction of the rate of primary nucleation as shown by the theoretical prediction

181   (solid line). In the presence of Brichos, the rate and total extent of Ca^{2+} influx were observed

182   to be reduced, in agreement with the theoretical prediction for the inhibition of secondary

183   nucleation (Fig. 3b). Moreover, turning off secondary nucleation increases the relative

184   importance of oligomers generated by primary nucleation shifting the predicted peak of Ca^{2+}

185   influx to earlier times, in agreement with the experiments. We also studied the effect on

186   membrane permeation of a mutational variant of DNAJB6, M3 (Online Methods). M3 has

187   been found to leave the overall aggregation reaction of Aβ42 unchanged (Extended Data

188   Figure 7a) [31], and indeed, we find here that M3 has no detectable effect on the extent of

189   Ca^{2+} influx (Extended Data Figure 7b). Overall, therefore, the results of this study

190   demonstrate that the oligomers generated by secondary nucleation during the aggregation of

191   Aβ42 are the major species responsible, at least in vitro, for disrupting lipid bilayers and

192   permitting an influx of Ca^{2+} ions into vesicles.

193

194   **Secondary Aβ42 oligomers in different systems**

195   Finally, we sought to investigate whether or not the predictions from our model would also

196   allow us to rationalise the role of secondary oligomers in driving toxicity in cellular and

197   animal models (Fig. 4). To this end, we compared our lipid bilayer permeation data with the

198   reduction of viability of cells in culture and with the reduction of gamma oscillations in

199   mouse brain slices, each representing a different readout of the toxic effects of aggregation in

200   vivo [30]. In particular, we measured the maximal extent of lipid bilayer permeation in the

201   presence of increasing concentrations of Brichos to reduce the rate of secondary nucleation

202 and hence to reduce the production of secondary Aβ42 oligomers. We then used our
203 theoretical model (Supplementary Note 1, Eq. 11 and Extended Data Figure 8) to predict
204 quantitatively the concentrations of secondary oligomers in the presence of different
205 concentrations of Brichos.

206 The resulting solid line in Fig. 4 is able to capture the experimentally measured extent of
207 bilayer permeation in vitro. In addition, however, the reduction in the degree of membrane
208 disruption in the presence of Brichos correlates qualitatively with our previous in vivo
209 experimental measurements of cell death and the impairment of gamma oscillations in mouse
210 brain slices [30] (Fig. 4).

211

212 DISCUSSION

213 Overall, our results suggest a dominant deleterious role of secondary oligomers resulting
214 from the aggregation of Aβ42 in vitro and in vivo. While it is likely that there are additional
215 and more specific mechanisms of toxicity in vivo, such as interactions with receptors and
216 other cellular components [1-5,32], our study indicates that the Aβ42 oligomers are able to
217 cause lipid membrane disruption, which is directly linked to cellular damage [19,20]. In this
218 context, a variety of results obtained previously can be rationalised in terms of secondary
219 oligomers populated during the aggregation process, since their generation requires the
220 presence of both monomeric and fibrillar forms of the protein. Thus, for example, the
221 presence of seed fibrils in addition to monomeric protein in animal models has been found to
222 induce the formation of aggregates of Aβ42 associated with neurotoxicity within the brain
223 [33]. In addition, the concentration-dependent induction of Aβ aggregation, and its associated
224 detrimental effects in model organisms upon administration of pathological brain extracts
225 [34], correlate with the observations from in vitro studies.

226

227 In conclusion, our study links directly the generation of secondary oligomers in the presence
228 of monomeric and fibrillar forms of Aβ42, to the disruption of lipid membranes. These
229 results, therefore, show directly that aggregation of the Aβ42 peptide is specifically linked to
230 the evolution of membrane disruption that gives rise to the type of cellular damage that has
231 been linked to Alzheimer's disease.

232

233 **Methods.** Details of the experimental materials and methods, mathematical modelling, and
234 data fitting are available in the online version of the paper.

235

243

248

249 **Competing interests.** The authors declare no competing interests.

250 **Correspondence and requests for material** should be addressed to DK and TPJK.

251

252 **References**

253

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335

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337

338   **Figure captions**

339

340   **Figure 1**. **Link between aggregation of Aβ42 and lipid bilayer permeability: secondary**

341   **nucleation generates the oligomers that are associated with the lipid bilayer permeation**

342   **induced by Aβ42 aggregation. (a)** Schematic illustration of the network of molecular steps

343   involved in Aβ42 aggregation and the oligomeric species with the potential to cause lipid

344   bilayer permeability (see Supplementary Note 1 for details) [17,27]. **(b)-(c)** Kinetic analysis

345   of the time evolution of the extent of Ca^{2+} influx during Aβ42 aggregation when monomeric

346   Aβ42 was incubated under quiescent conditions at concentrations of 2 μM (first row), 3 μM

347   (second row), 4 μM (third row), and 2 μM in the presence of 2.5% preformed seed fibrils

348   (fourth row). **(b)** Best fit of experimental Ca^{2+}-influx data to a kinetic model that assumes

349   only oligomers generated by primary nucleation to be responsible for lipid bilayer permeation

350   (Supplementary Note 1, Eq. 7, solid lines). Coefficient of determination for global fits

351   $R^2 = 0.91$. **(c)** Best fit of the same experimental data to a kinetic model that assumes

352   oligomers generated through secondary nucleation to be responsible for Ca^{2+} influx

353   (Supplementary Note 1, Eq. 8, solid lines). The various measurements of Ca^{2+} influx at

354   different concentrations of Aβ42 and in the presence of pre-formed fibrils are consistent with

355   a kinetic model where secondary nucleation generates the majority of oligomers that cause

356   lipid bilayer permeability. The fitting parameters, the derivation of the equations and a

357   detailed description of the analysis can be found in the Supplementary Note 1 and

358   Supplementary Tables 1 and 2. **(d)** Fibril spin down experiment. An aggregation reaction

359   starting with 2 μM monomeric Aβ42 was taken out at 40 min and put back at 60 min

360   following centrifugation to remove fibrils (purple data) or without centrifugation (green

361   data). The bar charts show the measured for the extent of Ca^{2+} influx at 2 hours. Error bars

362   indicate the standard error of the mean (SEM) over triplicates.

363

364   **Figure 2. Role of oligomer cooperativity and structural reorganization. (a)-(b)** Best fit of

365   experimental Ca^{2+}-influx data to a secondary oligomer kinetic model with reaction order

366   $\gamma = 1$ (low oligomer cooperativity, **(a)**) and $\gamma > 1$ (high oligomer cooperativity, **(b)**; fit

367   shown here for $\gamma = 3$, see Extended Data Figure 4a). **(c)-(d)** Analysis of membrane

368   permeation measurements using a kinetic model that assumes that oligomers generated
369   initially through secondary nucleation are not damaging upon their formation, but are
370   required to convert into species that are able to induce bilayer permeation. The theoretical
371   predictions were generated assuming either fast ($1/\tau \ll \kappa$, **(c)**) or slow $1/\tau \gtrsim \kappa$, **(d)**; see
372   Extended Data Figure 4b) rate of toxic conversion compared to the characteristic
373   proliferation rate of the aggregation reaction (κ) [16]. This analysis shows that, in order to
374   describe the membrane permeation data, it is not necessary to invoke a slow conversion step
375   of oligomers into species that are able to induce bilayer permeation. If such a conversion step
376   exists, it must occur on a timescale that is faster than that of the overall aggregation process.
377   Error bars indicate the standard error of the mean (SEM) over triplicates.

378

379   **Figure 3**. **Molecular chaperones modulate lipid bilayer permeation induced by the**
380   **aggregation of Aβ42. (a)-(b)** The effects of two different types of molecular chaperone on
381   lipid bilayer permeability were measured as monomeric Aβ42 was incubated at a
382   concentration of 2 μM under quiescent conditions in the presence of **(a)** DNAJB6 (0.01%
383   molar equivalents to monomeric Aβ42) or **(b)** Brichos (10% molar equivalents to monomeric
384   Aβ42). The plots show both the time evolution of amyloid fibril formation and of Ca^{2+} influx
385   in the absence and presence of the respective chaperone. We first fitted our measurements of
386   fibril mass formation to the analytical expression for the aggregation time course
387   (Supplementary Note 1, Eq. 2) to determine how the effective rates of primary and secondary
388   nucleation are affected by the chaperones (solid lines). The rate parameters extracted from
389   the analysis (Supplementary Table 3) were then implemented in the analytical expression for
390   the concentration of secondary oligomers (Supplementary Note 1, Eq. 4) to predict the
391   modulation of lipid bilayer permeability (solid lines). Except for the reduction of the rates of
392   primary and secondary nucleation, the parameters used for calculating the theoretical curves
393   for Ca^{2+} influx were the same as in Fig. 1c. The data in **(a)** and **(b)** agree with a model in
394   which membrane permeation correlates with the levels of oligomers generated by secondary
395   nucleation. Indeed, in such a model, inhibiting primary nucleation using DNAJB6 delays the
396   build-up, but does not reduce significantly the total concentration of oligomers. Inhibiting
397   secondary nucleation using Brichos, however, reduces significantly the total level of
398   oligomers. The bar charts show the relative maximal extent of Ca^{2+} influx induced by the
399   aggregation of 2 μM Aβ42 in the absence and presence of DNAJB6 and Brichos,
400   respectively. Error bars indicate the standard error of the mean (SEM) over triplicates.

401

402 **Figure 4. Common role of secondary Aβ42 oligomers in generating aggregation-**
403 **associated damage in different systems.** Common role of secondary Aβ42 oligomers in
404 generating aggregation-associated damage in different systems. The presence of increasing
405 concentrations of Brichos reduces the experimentally measured maximal levels of lipid
406 bilayer permeability (green triangles). These measurements are consistent with the theoretical
407 prediction of the reduction of Aβ42 oligomers generated by secondary nucleation (solid line,
408 see Supplementary Note 1, Eq. 11). Error bars indicate the standard error of the mean (SEM)
409 over triplicates. Moreover, the reduction in lipid bilayer permeability in the presence of 50%
410 molar equivalents of Brichos correlates broadly with the observed reduction of the viability
411 of cells in culture (blue circles) and the reduced loss in gamma oscillation in mouse brain
412 slices (pink squares). The cell and mouse brain slice data are taken from Ref. [30].

413

414 Online Methods

415

416 **Preparation and purification of recombinant Aβ42**
417 The recombinant Aβ42 (M1-42) peptide (MDAEFRHDSGYEVHHQKLVFF
418 AEDVGSNKGAIIGLMVGGVVIA), here called Aβ42, was expressed in the Escherichia coli
419 BL21 Gold (DE3) strain and purified as described previously with slight modifications
420 [25,35].

421

422 **Preparation of recombinant Aβ42 for kinetic experiments**
423 Solutions of monomeric recombinant Aβ42 were prepared as previously described by
424 dissolving the lyophilised Aβ42 peptide in 6 M GuHCl then purifying the protein using a
425 Superdex 75 10/300 GL column (GE Healthcare Bio-Sciences AB SE-751 84 Uppsala,
426 Sweden). The centre of the elution peak was collected, and the peptide concentration was
427 determined from the absorbance of the integrated peak area using $\varepsilon_{280} = 1490$ L mol^{-1}cm^{-1}.

428

429 **Measurement of aggregation kinetics of Aβ42**
430 For kinetic experiments the Aβ42 monomer was diluted with buffer and supplemented with
431 20 μM ThT. All samples were prepared in low-binding Eppendorf tubes (Eppendorf AG,
432 Hamburg, Germany) on ice. Each sample was then pipetted into multiple wells of a 96-well
433 half-area, low-binding polyethylene glycol coating plate (Corning 3881, Kennebuck ME,
434 USA) with a clear bottom, at 80 μL per well. The 96-well plate was placed in a plate reader
435 (Fluostar Omega, Fluostar Optima, or Fluostar Galaxy; BMG Labtech, Ortenberg, Germany)

436 and incubated at 37°C under quiescent conditions using the bottom reading mode (440-nm

437 excitation filter, 480-nm emission filter). For each new preparation of protein, the

438 aggregation kinetics were checked by performing reactions at different concentrations of

439 Aβ42.

440

Aβ42 aggregation

442 Aβ42 aggregation reactions were performed in 20 mM sodium phosphate buffer, pH 8,

443 supplemented with 20 μM ThT and 200 μM EDTA. Samples were prepared in low-binding

444 Eppendorf tubes on ice by avoiding any introduction of air bubbles. All aggregation reactions

445 were performed in a 96-well half area, low-binding, clear-bottom PEG coated plate (Corning

446 3881, Kennebuck ME, USA). Plates were sealed to prevent any evaporation. Aggregation

447 assays were performed at 37°C under quiescent conditions. Aliquots for measurements of

448 Ca^{2+} influx were then taken into low-binding tubes at the desired times after the plate was

449 placed in the incubator.

450

Seeded aggregation

452 Pre-formed fibrils of Aβ42, which are used as seed, were prepared just before the experiment.

453 Fibrils were prepared by aggregating 4 μM Aβ42 for overnight in 20 mM sodium phosphate,

454 200 μM EDTA, pH 8.0 and 20 μM ThT. ThT fluorescence was monitored over time to ensure

455 that the fibrils were formed. Then samples were collected from the wells into low-binding

456 Eppendorf tubes and sonicated for 2 min in a sonicator bath at room temperature. Under this

457 condition, the final concentration of fibrils (4 μM Aβ42) was considered to be equal to the

458 initial concentration of the monomer, as there was negligible presence of free monomer left

459 in solution. Preformed Aβ42 fibrils (2.5%) were subsequently added to the 2 μM freshly

460 prepared monomer solution to perform seeded aggregation. This aggregation reaction was

461 also performed in 20 mM sodium phosphate, 20 μM ThT, 200 μM EDTA at pH 8.0at 37°C

462 under quiescent conditions.

463

Preparation and purification of the BRICHOS domain

465 proSP-C Brichos was expressed in E. coli and purified as described previously [36].

466

Preparation and purification of the chaperone DNAJB6 and its mutational variant M3

468 Human DNAJB6b (isoform b, UniProt ID O75190-2) with a hexa-His tag was expressed

469 recombinantly in E. coli ER2566 and purified as described previously [31] but with an

470   additional washing step using 8 M urea during the affinity chromatography in order to

471   remove bound bacterial proteins [37]. Just prior to its use, DNAJB6 was dialysed into the

472   assay buffer (20 mm sodium phosphate buffer pH 8, 0.2 mm EDTA, 0.02% sodium azide)

473   using Slide-A-Lyser MINI (Thermo Scientific, Rockford, IL).

474

475   **Preparation and purification of dye filled vesicles**

476   The dye filled vesicles were prepared as previously described [26]. Phospholipids 16:0-18:1

477   PC (catalogue no - 850457) and biotinylated lipids 18:1-12:0 Biotin PC (catalogue no -

478   860563) were purchased from Avanti Polar Lipids (Alabama, USA) in the form of powder

479   and chloroform solutions respectively. Chloroform stock solutions were mixed such that the

480   ratio between 16:0-18:1 PC and 18:1-12:0 biotin PC was 100:1, and the chloroform was then

481   removed under vacuum in a desiccator overnight. The samples were then dissolved in

482   HEPES buffer (pH 6.5) with 100 µM Cal-520 and five freeze-and-thaw cycles were

483   performed using dry ice and a water bath. The solution was passed at least 10 times through

484   an extruder (Avanti Polar Lipids, Alabama, USA) with a membrane of an appropriate size cut

485   off of 200 µm. The size of the vesicles was determined using a Zetasizer (Zetasizer Nano

486   ZSP, Malvern Instruments, Malvern, UK). To separate non-incorporated dye molecules from

487   the solution surrounding the vesicles, size-exclusion chromatography was performed [26].

488

489   **Preparation of PEGylated slides and immobilization of single vesicles**

490   We followed the previously described protocol for slide preparation to perform the

491   membrane permeabilisation assay [26]. Borosilicate glass coverslides (VWR International,

492   22x22 mm, product number 63 1-0122) were cleaned by subsequent sonication in 2% (v/v)

493   Hellmanex III (Hellma GmbH & Co. KG, Muellheim, Germany) in milliQ water, twice in

494   milliQ water, methanol and again in water for 10 min each. The glass slides were dried under

495   a nitrogen stream, and plasma-etched using an argon plasma cleaner (PDC-002, Harrick

496   Plasma, Ithaca, NY) for 20 minutes before Frame-Seal incubation chambers (9x9mm^2,

497   Biorad, Hercules, CA, product number SLF-0601) were affixed to the glass slides. 50 µL of a

498   mixture of 100:1 PLL-g-PEG (SuSoS AG, Duebendorf, Switzerland) and PLL-g-PEG biotin

499   (SuSoS AG, Duebendorf, Switzerland) (1 g/L) in reaction buffer (50 mM Hepes, pH 6.5) was

500   added to the coverslide inside of the chamber and incubated for 30 min. Then the coverslides

501   were washed 3 times with filtered reaction buffer. 50 µL of a solution of 0.1 mg/mL

502   Neutravidin (ThermoScientific, Rockford, IL 61105, USA) in reaction buffer was added to

503   the coverslide and incubated for 15 min, and washed 3 times with reaction buffer. Then, 50

504 μL of the solution of purified vesicles was added to the coverslide and incubated for 30 min

505 before washing carefully at least 5 times with reaction buffer.

506

507 **Imaging using Total Internal Reflection Fluorescence Microscope**

508 Imaging was performed using a homebuilt Total Internal Reflection Fluorescence

509 Microscope (TIRFM) based on an inverted Olympus IX-71 microscope as previously

510 described in detail [26]. A 488 nm laser (Toptica, iBeam smart, 200 mW, Munich, Germany)

511 was used to excite the sample. The expanded and collimated laser beam was focused using

512 two Plano-convex lens onto the back-focal plane of the 60X, 1.49NA oil immersion objective

513 lens (APON60XO TIRF, Olympus, product number N2709400) to a spot of adjustable

514 diameter. The fluorescence signal was collected by the same objective and was separated

515 from the excitation beam by a dichroic (Di01-R405/488/561/635, Semrock). The emitted

516 light was passed through an appropriate set of filters (BLP01-488R, Semrock and FF01-

517 520/44-25, Semrock). The fluorescence signal was then passed through a 2.5x beam expander

518 and imaged onto a 512 x 512 pixel EMCCD camera (Photometrics Evolve, E VO-512-M-

519 FW-16-AC-110). Images were acquired with a 488nm laser ($10~W/cm^2$) for 50 frames with a

520 scan speed of 20 Hz and bit depth of 16 bits. Each pixel corresponds to 100 nm. All the

521 measurements were carried out under ambient conditions (T=295K). The open source

522 microscopy manager software Micro Manager 1.4 was used to control the microscope

523 hardware and image acquisition.

524

525 **Performing the Ca^{2+} influx assay using TIRFM**

526 The imaging of the induced membrane permeability was performed as previously described

527 [26]. Single vesicles tethered to PLL-PEG coated borosilicate glass coverslides (VWR

528 International, 22x22 mm, product number 63 1-0122) were placed on an oil immersion

529 objective mounted on an inverted Olympus IX-71 microscope. Each coverslide was affixed to

530 Frame-Seal incubation chambers and was incubated with 50 μL of HEPES buffer of pH 6.5.

531 Just before the imaging, the HEPES buffer was replaced with 50 μL Ca^{2+} containing buffer

532 solution L-15. 16 (4x4) images of the coverslide were recorded under three different

533 conditions (background, in the presence of Aβ42 and after addition of ionomycin (Cambridge

534 Bioscience Ltd, Cambridge, UK), respectively). The distance between each field of view was

535 set to 100 μm, and was automated (bean-shell script, Micromanager) to avoid any user bias.

536 After each measurement the script allowed the stage (Prior H117, Rockland, MA, USA) to

537 move the field of view back to the start position such that identical fields of view could be

538   acquired for the three different conditions.

539

Data analysis to quantify the extent of Ca^{2+} influx

541   The fluorescence intensity of individual vesicles was determined as previously described

542   [26]. The recorded images were analysed using ImageJ to determine the fluorescence

543   intensity of each spot under the three different conditions, namely background ($F_{background}$), in

544   the presence of an aggregation mixture ($F_{aggregate}$), and after the addition of ionomycin

545   ($F_{ionomycin}$). The relative influx of Ca^{2+} into an individual vesicle due to aggregates of Aβ42

546   peptide was then determined using the following equation:

$$Ca^{2+} \text{ influx} = \Phi = \frac{F_{aggregate} - F_{background}}{F_{ionomycin} - F_{background}}$$

547   The average degree of Ca^{2+} influx was calculated by averaging the Ca^{2+} influx into individual

548   vesicles.

549

Fibril spin down experiment

551   To check if the oligomers formed during secondary nucleation are the major source of

552   species causing membrane permeabilisation, we performed an aggregation where the rate of

553   secondary nucleation was substantially reduced by removing insoluble fibrils from the

554   aggregation reaction. We aggregated 2 μM Aβ42 in 20 mM sodium phosphate buffer at pH 8,

555   mixed with 20 μM ThT, 200 μM EDTA for 40 minutes in clear bottom 96 well plates (lag

556   phase ~ 1 hour) and then arrested the aggregation by putting it to the ice bath. Immediately

557   we then transferred the aggregation mixture into a low-binding Eppendorf and centrifuged for

558   15 minutes at 4°C to remove as many fibrils as possible. Then we transferred the supernatant

559   back into clear bottom 96 well plates for 60 minutes at 37°C and followed the aggregation.

560   We also performed a control experiment where the aggregation was stopped at 40 minutes

561   using ice bath and the aggregation restarted at 60 minutes at 37°C without any centrifugation.

562

563   Further information on experimental design is available in the Nature Research Reporting

564   Summary linked to this article.

565

566   **Code availability** All simulation and data analysis codes are included in this article and its

567   Supplementary Information. Codes are available from the corresponding authors on request.

568

569

570   **Data availability** The authors confirm that all data generated and analysed during this study

571   are included in this published article and its Supplementary Information. Source data for

572   Figures 1,3 and 4 are available with the paper online.

573

574   methods-only references

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