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1 **Direct measurement of lipid membrane disruption connects**
2 **kinetics and toxicity of A β 42 aggregation**

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21
22 **Abstract**

23
24 The formation of amyloid deposits within human tissues is a defining feature of more than
25 fifty medical disorders, including Alzheimer's disease. Strong genetic and histological
26 evidence links these conditions to the process of protein aggregation, yet it has remained
27 challenging to identify a definitive connection between aggregation and pathogenicity. Using
28 time-resolved fluorescence microscopy of individual synthetic vesicles, we show for the
29 A β 42 peptide implicated in Alzheimer's disease that the disruption of lipid bilayers correlates
30 linearly with the time course of the levels of transient oligomers generated through secondary
31 nucleation. These findings suggest a specific role of oligomers generated through the
32 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.

35 **Main text**

36
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 A β 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 Ca²⁺ 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 A β 42, the 42-residue A β 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 A β 42 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 A β 42 oligomers generated by
66 secondary nucleation [27] in driving membrane permeation.

67

68 Results

69 **Link between aggregation of A β 42 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 A β 42 was
72 incubated at concentrations between 2 and 4 μ M 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 A β 42 fibrils (seeds) to a solution of 2 μ M monomeric A β 42 (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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺
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 $\text{A}\beta_{42}$ 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 $2\ \mu\text{M}$
127 $\text{A}\beta_{42}$ (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 A β 42 aggregation**

159 We then carried out a series of additional experiments in which the rates of key microscopic
160 steps in the A β 42 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 A β 42), 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 A β 42), 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 $\text{A}\beta_{42}$ 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 $\text{A}\beta_{42}$ 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 $\text{A}\beta_{42}$ 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 A β 42 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 A β 42 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 A β 42 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 A β 42 associated with neurotoxicity within the brain
223 [33]. In addition, the concentration-dependent induction of A β 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 A β 42, to the disruption of lipid membranes. These
229 results, therefore, show directly that aggregation of the A β 42 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

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243

244 **Author contributions.** P.F. and S.D. performed the experiments; T.C.T.M. developed the
245 theoretical model and performed the kinetic analysis; all authors participated in designing the
246 study, interpreting the results and writing the paper. P.F., S.D. and T.C.T.M. contributed
247 equally to this work.

248

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

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251

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253

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333

334

335

336

337

338

Figure captions

339

340 **Figure 1. Link between aggregation of A β 42 and lipid bilayer permeability: secondary**
341 **nucleation generates the oligomers that are associated with the lipid bilayer permeation**
342 **induced by A β 42 aggregation. (a)** Schematic illustration of the network of molecular steps
343 involved in A β 42 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 Ca²⁺ influx during A β 42 aggregation when monomeric
346 A β 42 was incubated under quiescent conditions at concentrations of 2 μ M (first row), 3 μ M
347 (second row), 4 μ M (third row), and 2 μ M in the presence of 2.5% preformed seed fibrils
348 (fourth row). **(b)** Best fit of experimental Ca²⁺-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 Ca²⁺ influx
353 (Supplementary Note 1, Eq. 8, solid lines). The various measurements of Ca²⁺ influx at
354 different concentrations of A β 42 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 μ M monomeric A β 42 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 Ca²⁺ 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 Ca²⁺-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 A β 42. (a)-(b)** The effects of two different types of molecular chaperone on
381 lipid bilayer permeability were measured as monomeric A β 42 was incubated at a
382 concentration of 2 μ M under quiescent conditions in the presence of **(a)** DNAJB6 (0.01%
383 molar equivalents to monomeric A β 42) or **(b)** Brichos (10% molar equivalents to monomeric
384 A β 42). The plots show both the time evolution of amyloid fibril formation and of Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ influx induced by the
399 aggregation of 2 μ M A β 42 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 A β 42 oligomers in generating aggregation-**
403 **associated damage in different systems.** Common role of secondary A β 42 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 A β 42 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 A β 42**

417 The recombinant A β 42 (M1-42) peptide (MDAEFRHDSGYEVHHQKLVFF
418 AEDVGSNKGAIIGLMVGGVVIA), here called A β 42, 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 A β 42 for kinetic experiments**

423 Solutions of monomeric recombinant A β 42 were prepared as previously described by
424 dissolving the lyophilised A β 42 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 $\epsilon_{280} = 1490 \text{ L mol}^{-1} \text{ cm}^{-1}$.

428

429 **Measurement of aggregation kinetics of A β 42**

430 For kinetic experiments the A β 42 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 A β 42.

440

441 **A β 42 aggregation**

442 A β 42 aggregation reactions were performed in 20 mM sodium phosphate buffer, pH 8,
443 supplemented with 20 μ M ThT and 200 μ M 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 Ca²⁺ influx were then taken into low-binding tubes at the desired times after the plate was
449 placed in the incubator.

450

451 **Seeded aggregation**

452 Pre-formed fibrils of A β 42, which are used as seed, were prepared just before the experiment.
453 Fibrils were prepared by aggregating 4 μ M A β 42 for overnight in 20 mM sodium phosphate,
454 200 μ M EDTA, pH 8.0 and 20 μ M 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 μ M A β 42) 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 A β 42 fibrils (2.5%) were subsequently added to the 2 μ M freshly
460 prepared monomer solution to perform seeded aggregation. This aggregation reaction was
461 also performed in 20 mM sodium phosphate, 20 μ M ThT, 200 μ M EDTA at pH 8.0 at 37°C
462 under quiescent conditions.

463

464 **Preparation and purification of the BRICHOS domain**

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

466

467 **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 μ M 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 μ m. 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 (9x9mm²,
497 Biorad, Hercules, CA, product number SLF-0601) were affixed to the glass slides. 50 μ L 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 μ L 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 ($10 \text{ W}/\text{cm}^2$) 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 ($T=295\text{K}$). 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 $\text{A}\beta 42$ 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

540 **Data analysis to quantify the extent of Ca²⁺ 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 ($F_{\text{background}}$), in
544 the presence of an aggregation mixture ($F_{\text{aggregate}}$), and after the addition of ionomycin
545 ($F_{\text{ionomycin}}$). The relative influx of Ca²⁺ into an individual vesicle due to aggregates of A β 42
546 peptide was then determined using the following equation:

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

547 The average degree of Ca²⁺ influx was calculated by averaging the Ca²⁺ influx into individual
548 vesicles.

549

550 **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 A β 42 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 \sim 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

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