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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

deleterious biological function, and establish a direct causative connection between amyloid formation and its pathological effects.

Main text

Neurodegenerative conditions including Alzheimer's and Parkinson's diseases [1-5] have emerged as major challenges to the health and social systems of the modern world as a result of their increasing prevalence in our ageing populations. A substantial body of data indicates that protein aggregation is a key factor underlying these disorders [6-14]. Recent progress in understanding the molecular mechanisms of aggregation has revealed that these processes typically involve a primary nucleation step, followed by the growth of the initial aggregates through an elongation process (Fig. 1a) [15-18]. Once a critical quantity of fibrils is formed, however, the aggregation reaction can be accelerated dramatically by secondary processes in which fibrils formed during the aggregation reaction promote the formation of further aggregates (Fig. 1a) [17,18]. A prominent example of such secondary processes is surface-catalysed secondary nucleation, which is particularly significant for the Aβ peptides associated with Alzheimer's disease, and has been linked to a variety of mechanisms of neuronal damage [4,5,17,18]. Indeed, the disruption of the lipid bilayer within cellular membranes by oligomeric protein aggregates, and the consequent loss of Ca^{2+} homeostasis, has been proposed as a general mechanism of neurotoxicity [19-21].

In the context of Alzheimer's disease, a key objective is to connect the time dependence of lipid bilayer permeability to that of the aggregation reaction and to the resulting distribution of aggregated forms of Aβ42, the 42-residue Aβ peptide that has been most strongly linked to neurological damage. Such data would serve to establish the species and processes associated with an ongoing Aβ42 aggregation reaction that are primarily responsible for causing lipid bilayer permeability. This information would be particularly useful for elucidating the mechanisms of pathological protein aggregation, which still remain poorly understood, a possible reason for the heterogeneous outcome of clinical trials aimed at targeting amyloid formation [22-24].

To address this question, we have combined recent advances in the mechanistic analysis of experimental measurements of protein aggregation kinetics in vitro [15,25] with the development of a single molecule optochemical approach able to quantify the degree of membrane permeability resulting from the aggregation reaction at any point in time [26]. Using this platform, we reveal the dominant role of transient Aβ42 oligomers generated by secondary nucleation [27] in driving membrane permeation.

Results

Link between aggregation of Aβ42 and lipid bilayer permeability

We first monitored the extent of membrane disruption by measuring the time dependence of the permeability of the lipid bilayers of synthetic vesicles when monomeric Aβ42 was incubated at concentrations between 2 and 4 μM under solution conditions where the kinetics of amyloid fibril formation have been shown to be highly reproducible [28] (Extended Data Figure 1 and Online Methods). We also added 2.5% molar monomer equivalents of pre-formed Aβ42 fibrils (seeds) to a solution of 2μM monomeric Aβ42 (Online Methods). Addition of such seed fibrils accelerates the aggregation reaction by bypassing the primary nucleation step, providing a convenient and robust way to disentangle secondary nucleation from primary nucleation [17-18]. In each case, at specific time points, aliquots were removed from the aggregating solutions, added to lipid vesicles, and the extent of Ca^{2+} influx measured using the optochemical method [26]. We found that the ability of the aggregation reaction mixture to induce bilayer permeability increased with time, reaching a maximum value in the growth phase that is clearly observable in bulk measurements, and then decreased during the remainder of the aggregation reaction (Figs. 1b,c).

Since membrane permeation is directly linked in our assay to the aggregation process, we utilised a chemical kinetics approach to describe the time evolution of Ca^{2+} influx, $\Phi(t)$, in terms of the concentrations $c(t)$ at time t of different types of aggregate species that could give rise to membrane permeability [16] (Fig. 1a and Supplementary Note 1). In particular, due to the transient nature of Ca^{2+} influx, these aggregate species include intermediate oligomers generated either by primary nucleation (primary oligomers) or by secondary nucleation (secondary oligomers) [27]. We used the law of mass action to represent Ca^{2+} influx very generally as a power-law of the concentration of oligomers, $\Phi(t) \propto c(t)^{\gamma}$, where $\gamma \geq 1$ is the reaction order of membrane permeation with respect to the concentration of oligomers (Supplementary Note 1). γ is a measure of the cooperativity between oligomers in causing membrane permeation. We then described the time evolution of the concentrations of primary and secondary oligomers during aggregation using a master equation (Supplementary Note 1, Eq. 1). Using approaches drawing on self-consistent field theory [16,27], we derived explicit mathematical expressions for the time course of fibril formation

and for the concentrations of oligomers in these two specific mechanistic scenarios (Supplementary Note 1), and then compared each of them to the experimental data describing the extent of Ca^{2+} influx over time (Fig. 1b,c). Specifically, we first fitted globally the different kinetic traces of amyloid fibril formation to our kinetic model (Supplementary Note 1, Eq. 2) to determine combined rate parameters associated with primary and secondary nucleation (Supplementary Table 1). These parameters were then implemented in the theoretical oligomer concentration curves (Supplementary Note 1, Eqs. 5 and 6), leaving the combined rates of oligomer dissociation and conversion, the proportionality constant between oligomer concentrations and the extent of Ca^{2+} influx, and the reaction order γ as fitting parameters (Supplementary Table 2 and Supplementary Note 1).

This global analysis reveals that the membrane permeation data are well described by a scenario in which the large majority of the species causing lipid bilayer permeability is generated by secondary nucleation (Fig. 1c) but not by primary nucleation (Fig. 1b). Note that the former scenario (Fig. 1c) explicitly considers contributions from both primary and secondary oligomers (Supplementary Note 1, Eq. 8), even though the primary oligomers are a significant proportion of the oligomer population only during the initial stages but are otherwise outnumbered by secondary oligomers for the rest of the reaction (Extended Data Figure 2). We also note that in comparing the different mechanistic scenarios, we consider fit quality globally across a variety of conditions (concentration, seeds), since fits to individual kinetic traces are insufficient to accept or reject a particular mechanism [16]. In particular, the inability of the primary oligomer model to describe the membrane permeation data stems from the fact that the primary nucleation pathway for Aβ42 is negligible in the presence of pre-formed fibrils (Extended Data Figure 3) [17,18]. To provide further support to the hypothesis that secondary nucleation generates the majority of species responsible for membrane permeation, we performed a set of additional experiments, where the rate of secondary nucleation was modulated by removing fibrils during aggregation through centrifugation (Online Methods). Specifically, an aggregation reaction starting with 2 μM Aβ42 (lag time close to 1 hour) was stopped after 40 minutes. The aggregation mixture was then centrifuged for 15 minutes to remove fibrils and reduce secondary nucleation. We also performed a control experiment where the aggregation was stopped at 40 minutes and restarted at 60 minutes without any centrifugation. In both cases, membrane permeation was measured at 2 hours. We found that membrane permeation is significantly reduced when the mixture is centrifuged, but there is no significant change when aggregation is only interrupted (Fig. 1d). This result is in line with the prediction that this protocol would significantly

diminish the observed membrane permeation from secondary oligomers, which are now present at lower concentrations, but leave unaffected the toxicity from primary oligomers. The best global fit of the permeation data to the secondary oligomer model yields an exponent $\gamma \sim 1.0 \pm 0.1$ (Fig. 2a). This finding implies that the time course of Ca^{2+} influx correlates with the concentration of secondary oligomers, suggesting that membrane permeation is a first order reaction with respect to the population of secondary oligomers and is thus independent of interactions between oligomers (Fig. 2b and Extended Data Figure 4a).

We next investigated the question of whether the secondary oligomers are able to exert their damaging effects directly on their formation or if they have first to convert into species that are distinct from those that propagate the aggregation reaction and which possess more specific deleterious activity ('lethal oligomers') analogous to events described for prion diseases [29]. To this end we considered a scenario in which oligomers generated initially through secondary nucleation were assumed not to be toxic but to induce bilayer permeation only after a conversion step to such lethal oligomers over timescales comparable to, or longer than, that of the overall aggregation process (Supplementary Note 1, Eq. 9). The comparison between the model predictions and the experimental time course of membrane permeation shows, however, that this model is not able to capture the kinetics of the observed behaviour (Fig. 2c,d and Extended Data Figure 4b), implying that the oligomers generated on the surfaces of fibrils drive membrane permeation without the need for a structural reorganization that is slow relative to the overall aggregation process. As such, this finding establishes a direct connection between the process of amyloid fibril formation and the toxic effects associated with protein oligomers.

Molecular chaperones modulate lipid bilayer permeation by Aβ42 aggregation

We then carried out a series of additional experiments in which the rates of key microscopic steps in the Aβ42 aggregation mechanism were modulated selectively by molecular chaperones [30,31] (Fig. 3) in order to test further the conclusion that secondary oligomers are correlated with lipid bilayer permeation. We performed experiments in the presence of the chaperone DNAJB6 (0.01% molar equivalents to monomeric Aβ42), which at this concentration has been shown to inhibit mainly primary nucleation [31] (Extended Data Figure 5), and the Brichos domain (10% molar equivalents to monomeric Aβ42), that has been shown to suppress secondary nucleation [30] (Extended Data Figure 6). The experiments were performed at low concentrations of the chaperones in order still to be able

to detect a measurable degree of Ca^{2+} influx, as these chaperones are able to inhibit very effectively the levels of oligomers generated by nucleation processes. In each case, we recorded the effect of each chaperone on the total aggregate mass concentration, as well as on the extent of Ca^{2+} influx (Fig. 3).

The alterations of the rate constants for primary and secondary nucleation in the presence of the chaperones were determined by fitting the aggregate mass measurements to the analytical expression for the time course of amyloid fibril formation (Supplementary Note 1, Eq. 2 and Supplementary Table 3). These rate parameters were then used to simulate the effects of the chaperones on the population of secondary oligomers over time (Supplementary Note 1, Eq. 4, solid lines), without the introduction of any additional fitting parameters. In the presence of DNAJB6 (Fig. 3a), we observed a retardation of membrane permeation that is in agreement with the reduction of the rate of primary nucleation as shown by the theoretical prediction (solid line). In the presence of Brichos, the rate and total extent of Ca^{2+} influx were observed to be reduced, in agreement with the theoretical prediction for the inhibition of secondary nucleation (Fig. 3b). Moreover, turning off secondary nucleation increases the relative importance of oligomers generated by primary nucleation shifting the predicted peak of Ca^{2+} influx to earlier times, in agreement with the experiments. We also studied the effect on membrane permeation of a mutational variant of DNAJB6, M3 (Online Methods). M3 has been found to leave the overall aggregation reaction of Aβ42 unchanged (Extended Data Figure 7a) [31], and indeed, we find here that M3 has no detectable effect on the extent of Ca^{2+} influx (Extended Data Figure 7b). Overall, therefore, the results of this study demonstrate that the oligomers generated by secondary nucleation during the aggregation of Aβ42 are the major species responsible, at least in vitro, for disrupting lipid bilayers and permitting an influx of Ca^{2+} ions into vesicles.

Secondary Aβ42 oligomers in different systems

Finally, we sought to investigate whether or not the predictions from our model would also allow us to rationalise the role of secondary oligomers in driving toxicity in cellular and animal models (Fig. 4). To this end, we compared our lipid bilayer permeation data with the reduction of viability of cells in culture and with the reduction of gamma oscillations in mouse brain slices, each representing a different readout of the toxic effects of aggregation in vivo [30]. In particular, we measured the maximal extent of lipid bilayer permeation in the presence of increasing concentrations of Brichos to reduce the rate of secondary nucleation

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

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

DISCUSSION

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

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

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

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

Competing interests. The authors declare no competing interests.

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Figure captions

Figure 1. **Link between aggregation of Aβ42 and lipid bilayer permeability: secondary nucleation generates the oligomers that are associated with the lipid bilayer permeation induced by Aβ42 aggregation. (a)** Schematic illustration of the network of molecular steps involved in Aβ42 aggregation and the oligomeric species with the potential to cause lipid bilayer permeability (see Supplementary Note 1 for details) [17,27]. **(b)-(c)** Kinetic analysis of the time evolution of the extent of Ca^{2+} influx during Aβ42 aggregation when monomeric Aβ42 was incubated under quiescent conditions at concentrations of 2 μM (first row), 3 μM (second row), 4 μM (third row), and 2 μM in the presence of 2.5% preformed seed fibrils (fourth row). **(b)** Best fit of experimental Ca^{2+}-influx data to a kinetic model that assumes only oligomers generated by primary nucleation to be responsible for lipid bilayer permeation (Supplementary Note 1, Eq. 7, solid lines). Coefficient of determination for global fits $R^2 = 0.91$. **(c)** Best fit of the same experimental data to a kinetic model that assumes oligomers generated through secondary nucleation to be responsible for Ca^{2+} influx (Supplementary Note 1, Eq. 8, solid lines). The various measurements of Ca^{2+} influx at different concentrations of Aβ42 and in the presence of pre-formed fibrils are consistent with a kinetic model where secondary nucleation generates the majority of oligomers that cause lipid bilayer permeability. The fitting parameters, the derivation of the equations and a detailed description of the analysis can be found in the Supplementary Note 1 and Supplementary Tables 1 and 2. **(d)** Fibril spin down experiment. An aggregation reaction starting with 2 μM monomeric Aβ42 was taken out at 40 min and put back at 60 min following centrifugation to remove fibrils (purple data) or without centrifugation (green data). The bar charts show the measured for the extent of Ca^{2+} influx at 2 hours. Error bars indicate the standard error of the mean (SEM) over triplicates.

Figure 2. Role of oligomer cooperativity and structural reorganization. (a)-(b) Best fit of experimental Ca^{2+}-influx data to a secondary oligomer kinetic model with reaction order $\gamma = 1$ (low oligomer cooperativity, **(a)**) and $\gamma > 1$ (high oligomer cooperativity, **(b)**; fit shown here for $\gamma = 3$, see Extended Data Figure 4a). **(c)-(d)** Analysis of membrane

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

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

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

Online Methods

Preparation and purification of recombinant Aβ42

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

Preparation of recombinant Aβ42 for kinetic experiments

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

Measurement of aggregation kinetics of Aβ42

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

and incubated at 37°C under quiescent conditions using the bottom reading mode (440-nm excitation filter, 480-nm emission filter). For each new preparation of protein, the aggregation kinetics were checked by performing reactions at different concentrations of Aβ42.

Aβ42 aggregation

Aβ42 aggregation reactions were performed in 20 mM sodium phosphate buffer, pH 8, supplemented with 20 μM ThT and 200 μM EDTA. Samples were prepared in low-binding Eppendorf tubes on ice by avoiding any introduction of air bubbles. All aggregation reactions were performed in a 96-well half area, low-binding, clear-bottom PEG coated plate (Corning 3881, Kennebuck ME, USA). Plates were sealed to prevent any evaporation. Aggregation assays were performed at 37°C under quiescent conditions. Aliquots for measurements of Ca^{2+} influx were then taken into low-binding tubes at the desired times after the plate was placed in the incubator.

Seeded aggregation

Pre-formed fibrils of Aβ42, which are used as seed, were prepared just before the experiment. Fibrils were prepared by aggregating 4 μM Aβ42 for overnight in 20 mM sodium phosphate, 200 μM EDTA, pH 8.0 and 20 μM ThT. ThT fluorescence was monitored over time to ensure that the fibrils were formed. Then samples were collected from the wells into low-binding Eppendorf tubes and sonicated for 2 min in a sonicator bath at room temperature. Under this condition, the final concentration of fibrils (4 μM Aβ42) was considered to be equal to the initial concentration of the monomer, as there was negligible presence of free monomer left in solution. Preformed Aβ42 fibrils (2.5%) were subsequently added to the 2 μM freshly prepared monomer solution to perform seeded aggregation. This aggregation reaction was also performed in 20 mM sodium phosphate, 20 μM ThT, 200 μM EDTA at pH 8.0at 37°C under quiescent conditions.

Preparation and purification of the BRICHOS domain

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

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

Human DNAJB6b (isoform b, UniProt ID O75190-2) with a hexa-His tag was expressed recombinantly in E. coli ER2566 and purified as described previously [31] but with an

additional washing step using 8 M urea during the affinity chromatography in order to remove bound bacterial proteins [37]. Just prior to its use, DNAJB6 was dialysed into the assay buffer (20 mm sodium phosphate buffer pH 8, 0.2 mm EDTA, 0.02% sodium azide) using Slide-A-Lyser MINI (Thermo Scientific, Rockford, IL).

Preparation and purification of dye filled vesicles

The dye filled vesicles were prepared as previously described [26]. Phospholipids 16:0-18:1 PC (catalogue no - 850457) and biotinylated lipids 18:1-12:0 Biotin PC (catalogue no - 860563) were purchased from Avanti Polar Lipids (Alabama, USA) in the form of powder and chloroform solutions respectively. Chloroform stock solutions were mixed such that the ratio between 16:0-18:1 PC and 18:1-12:0 biotin PC was 100:1, and the chloroform was then removed under vacuum in a desiccator overnight. The samples were then dissolved in HEPES buffer (pH 6.5) with 100 μM Cal-520 and five freeze-and-thaw cycles were performed using dry ice and a water bath. The solution was passed at least 10 times through an extruder (Avanti Polar Lipids, Alabama, USA) with a membrane of an appropriate size cut off of 200 μm. The size of the vesicles was determined using a Zetasizer (Zetasizer Nano ZSP, Malvern Instruments, Malvern, UK). To separate non-incorporated dye molecules from the solution surrounding the vesicles, size-exclusion chromatography was performed [26].

Preparation of PEGylated slides and immobilization of single vesicles

We followed the previously described protocol for slide preparation to perform the membrane permeabilisation assay [26]. Borosilicate glass coverslides (VWR International, 22x22 mm, product number 63 1-0122) were cleaned by subsequent sonication in 2% (v/v) Hellmanex III (Hellma GmbH & Co. KG, Muellheim, Germany) in milliQ water, twice in milliQ water, methanol and again in water for 10 min each. The glass slides were dried under a nitrogen stream, and plasma-etched using an argon plasma cleaner (PDC-002, Harrick Plasma, Ithaca, NY) for 20 minutes before Frame-Seal incubation chambers (9x9mm^2, Biorad, Hercules, CA, product number SLF-0601) were affixed to the glass slides. 50 μL of a mixture of 100:1 PLL-g-PEG (SuSoS AG, Duebendorf, Switzerland) and PLL-g-PEG biotin (SuSoS AG, Duebendorf, Switzerland) (1 g/L) in reaction buffer (50 mM Hepes, pH 6.5) was added to the coverslide inside of the chamber and incubated for 30 min. Then the coverslides were washed 3 times with filtered reaction buffer. 50 μL of a solution of 0.1 mg/mL Neutravidin (ThermoScientific, Rockford, IL 61105, USA) in reaction buffer was added to the coverslide and incubated for 15 min, and washed 3 times with reaction buffer. Then, 50

μL of the solution of purified vesicles was added to the coverslide and incubated for 30 min before washing carefully at least 5 times with reaction buffer.

Imaging using Total Internal Reflection Fluorescence Microscope

Imaging was performed using a homebuilt Total Internal Reflection Fluorescence Microscope (TIRFM) based on an inverted Olympus IX-71 microscope as previously described in detail [26]. A 488 nm laser (Toptica, iBeam smart, 200 mW, Munich, Germany) was used to excite the sample. The expanded and collimated laser beam was focused using two Plano-convex lens onto the back-focal plane of the 60X, 1.49NA oil immersion objective lens (APON60XO TIRF, Olympus, product number N2709400) to a spot of adjustable diameter. The fluorescence signal was collected by the same objective and was separated from the excitation beam by a dichroic (Di01-R405/488/561/635, Semrock). The emitted light was passed through an appropriate set of filters (BLP01-488R, Semrock and FF01-520/44-25, Semrock). The fluorescence signal was then passed through a 2.5x beam expander and imaged onto a 512 x 512 pixel EMCCD camera (Photometrics Evolve, E VO-512-M-FW-16-AC-110). Images were acquired with a 488nm laser (10 W/cm^2) for 50 frames with a scan speed of 20 Hz and bit depth of 16 bits. Each pixel corresponds to 100 nm. All the measurements were carried out under ambient conditions (T=295K). The open source microscopy manager software Micro Manager 1.4 was used to control the microscope hardware and image acquisition.

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

The imaging of the induced membrane permeability was performed as previously described [26]. Single vesicles tethered to PLL-PEG coated borosilicate glass coverslides (VWR International, 22x22 mm, product number 63 1-0122) were placed on an oil immersion objective mounted on an inverted Olympus IX-71 microscope. Each coverslide was affixed to Frame-Seal incubation chambers and was incubated with 50 μL of HEPES buffer of pH 6.5. Just before the imaging, the HEPES buffer was replaced with 50 μL Ca^{2+} containing buffer solution L-15. 16 (4x4) images of the coverslide were recorded under three different conditions (background, in the presence of Aβ42 and after addition of ionomycin (Cambridge Bioscience Ltd, Cambridge, UK), respectively). The distance between each field of view was set to 100 μm, and was automated (bean-shell script, Micromanager) to avoid any user bias. After each measurement the script allowed the stage (Prior H117, Rockland, MA, USA) to move the field of view back to the start position such that identical fields of view could be

acquired for the three different conditions.

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

The fluorescence intensity of individual vesicles was determined as previously described [26]. The recorded images were analysed using ImageJ to determine the fluorescence intensity of each spot under the three different conditions, namely background ($F_{background}$), in the presence of an aggregation mixture ($F_{aggregate}$), and after the addition of ionomycin ($F_{ionomycin}$). The relative influx of Ca^{2+} into an individual vesicle due to aggregates of Aβ42 peptide was then determined using the following equation:

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

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

Fibril spin down experiment

To check if the oligomers formed during secondary nucleation are the major source of species causing membrane permeabilisation, we performed an aggregation where the rate of secondary nucleation was substantially reduced by removing insoluble fibrils from the aggregation reaction. We aggregated 2 μM Aβ42 in 20 mM sodium phosphate buffer at pH 8, mixed with 20 μM ThT, 200 μM EDTA for 40 minutes in clear bottom 96 well plates (lag phase ~ 1 hour) and then arrested the aggregation by putting it to the ice bath. Immediately we then transferred the aggregation mixture into a low-binding Eppendorf and centrifuged for 15 minutes at 4^{o}C to remove as many fibrils as possible. Then we transferred the supernatant back into clear bottom 96 well plates for 60 minutes at 37^{o}C and followed the aggregation. We also performed a control experiment where the aggregation was stopped at 40 minutes using ice bath and the aggregation restarted at 60 minutes at 37^{o}C without any centrifugation.

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability All simulation and data analysis codes are included in this article and its Supplementary Information. Codes are available from the corresponding authors on request.

Data availability The authors confirm that all data generated and analysed during this study are included in this published article and its Supplementary Information. Source data for Figures 1,3 and 4 are available with the paper online.

methods-only references

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