Proliferation of amyloid-β42 aggregates occurs through a secondary nucleation mechanism


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The generation of toxic oligomers during the aggregation of the amyloid-β (Aβ) peptide Aβ42 into amyloid fibrils and plaques has emerged as a central feature of the onset and progression of Alzheimer’s disease, but the molecular pathways that control pathological aggregation have proved challenging to identify. Here, we use a combination of kinetic studies, selective radiolabeling experiments, and chemical microscopy to track the rates of formation of both fibrils and oligomers and the resulting cytotoxic effects. Our results show that once a small but critical concentration of amyloid fibrils has accumulated, the toxic oligomeric species are predominantly formed from monomeric peptide molecules through a fibril-catalyzed secondary nucleation reaction, rather than through a classical mechanism of homogenous primary nucleation. This catalytic mechanism couples together the growth of insoluble amyloid fibrils and the generation of diffusible oligomeric aggregates that are implicated as neurotoxic agents in Alzheimer’s disease. These results reveal that the aggregation of Aβ42 is promoted by a positive feedback loop that originates from the interactions between the monomeric and fibrillar forms of this peptide. Our findings bring together the main molecular species implicated in the Aβ aggregation cascade and suggest that perturbation of the secondary nucleation pathway identified in this study could be an effective strategy to control the proliferation of neurotoxic Aβ42 oligomers.

Results and Discussion

Microscopic Mechanisms. The general method underlying the kinetic analysis builds on earlier work (10, 14–17, 18, 19, 21, 22) and considers all of the possible sources of new aggregates, which consist of two or more monomers, from the species present in the system, as shown in Table 1, from both primary (10, 19, 23–25) and secondary (11, 12, 14, 26–28) pathways. Primary pathways, such as homogenous nucleation (10, 19), generate new aggregates at a rate dependent on the concentration of monomers alone and independent of the concentration of existing fibrils. Secondary pathways are the complementary class of mechanisms that generate new aggregates at a rate dependent on the concentration of existing fibrils. The latter class can be subdivided into monomer-independent processes, such as fragmentation (11, 12, 18, 26), with a rate depending only upon the concentration of existing fibrils, and monomer-dependent processes, such as secondary nucleation (14, 22, 27, 28), where the surfaces of existing fibrils catalyze the nucleation of new aggregates from the monomeric state, with a rate dependent on both the concentration of monomers and that of existing fibrils. Together, these three classes of mechanism, shown in Table 1, form the basis of a general description of protein aggregation (15, 17), because they account for the generation of new aggregates from mechanisms that involve monomers alone, existing aggregates alone, or both monomers and existing aggregates. These pathways initially populate oligomeric intermediates (29), which lead to fibrillar forms that elongate at a rate that is independent of their length (10, 15, 17) and represent the bulk of the aggregate mass.

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Table 1. Schema of the general processes that create new aggregates (14, 15)

<table>
<thead>
<tr>
<th>Mechanism involves</th>
<th>Monomers only</th>
<th>Fibris only</th>
<th>Both monomers and fibrils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>Homogeneous nucleation</td>
<td>Fragmentation</td>
<td>Secondary nucleation</td>
</tr>
<tr>
<td>Early time dependence</td>
<td>Polynomial</td>
<td>Exponential</td>
<td>Exponential</td>
</tr>
<tr>
<td>$M(t)/M(\infty) \sim (\lambda t)^b$</td>
<td>$\exp(\gamma t)$</td>
<td>$\exp(\gamma t)$</td>
<td>$\exp(\gamma t)$</td>
</tr>
<tr>
<td>Overall reaction parameter</td>
<td>Strong</td>
<td>Weak</td>
<td>Strong</td>
</tr>
<tr>
<td>$t_{lag} \propto \kappa^{-1} \propto m(0)^{-\frac{1}{2}}$</td>
<td>$t_{lag} \propto \kappa^{-1} \propto m(0)^{-\frac{1}{2}}$</td>
<td>$t_{lag} \propto \kappa^{-1} \propto m(0)^{-\frac{1}{2}}$</td>
<td></td>
</tr>
<tr>
<td>Agreement with data</td>
<td>Fig 1B</td>
<td>Fig 1C</td>
<td>Fig 1D</td>
</tr>
</tbody>
</table>

Check marks and crosses denote whether or not the characteristics of each mechanism match the data in Fig. 1. The fibril structure is adapted from ref. 20.

Linear Theory. These three classes of mechanism, summarized in Table 1, exhibit qualitatively different features, both as a function of time and as a function of the initial monomer concentration (14, 15, 18, 30). These differences are readily observed by considering the behavior of the system for early times before appreciable amounts of monomer have been sequestered into aggregates (15) when the rate equations can be linearized (15, 30). For cases where the mechanism that creates aggregates involves the preexisting fibrils, such as fragmentation or secondary nucleation, positive feedback for the increase of the fibril mass concentration, $M(t)$, results in the evolution of the form (14, 18) $d^2M(t)/dt^2 = \kappa^2M$, and hence exponential growth $M(t) \sim \exp(\gamma t)$ is observed, leading to a strong lag phase (14, 15, 17). The duration of the lag phase is commonly described by a lag time $t_{lag}$ defined as the time at which the aggregate concentration reaches a small fixed percentage of the total peptide concentration. Here, $\kappa = \sqrt{2k_m}k_m$ is the combined parameter that controls proliferation through secondary pathways, $k_m$ is the rate constant for the secondary process, $m$ is the monomer concentration, $k_m$ is the fibril elongation rate constant, and $n_2$ is the reaction order of the secondary pathway with respect to the monomer (15, 30). By contrast, for cases where nucleation is independent of the fibril concentration, such as for classical homogeneous nucleation (10), no feedback is generated, $d^2M(t)/dt^2 = \lambda^2$, and hence slow polynomial growth results, $M(t) \sim \lambda t^2$, with a weak lag phase (10, 15, 30), where $\lambda = \sqrt{2k_m}k_m$ is the combined parameter controlling proliferation through primary nucleation, $k_m$ denotes the primary nucleation rate constant, and $n_2$ is the reaction order of the primary process (10, 15, 30). The reaction orders $n_1$ and $n_2$ need not correspond to structural sizes of nuclei (30). The distinction between polynomial growth for primary processes and exponential-type growth for secondary processes is in general maintained for more complicated pathways, such as cascades through multiple intermediates (15, 30, 31).

A second distinction between the basic mechanisms in Table 1 is whether or not the monomer concentration affects the process. If it does, a high concentration dependence of the lag time is possible, otherwise only a weak dependence emerges because a change in the monomer concentration has no direct effect on the nucleation pathway. It is convenient to describe the monomer dependence of the overall assembly reaction, including elongation-related processes, with a power-law relationship, $t_{lag} \sim m(0)^{\gamma}$, which relates the lag time $t_{lag}$ for the reaction to the initial peptide concentration $m(0)$ (15, 30). The exponent $\gamma$ in the power law for the lag time (14, 15) is to a good approximation defined by the monomer dependence of the combined parameter $\lambda$ in the case where primary pathways are dominant and from $\kappa$ in the case of secondary pathways. In this manner, the exponent is given from $\lambda$ as $\gamma = -n_1/2$ for processes where a classical homogeneous nucleation step is the major source of aggregates (10) and from $\kappa$ as $\gamma = -(n_2 + 1)/2$ for phenomena where secondary nucleation processes dominate (14, 17). A strong monomer dependence, $|\gamma| \geq 1$, can, therefore, always be captured by either primary or secondary nucleation through an appropriate value of $n_1$ or $n_2$. By contrast, monomer-independent secondary processes, such as fragmentation, are associated with a weaker overall monomer scaling (16, 18) corresponding to a monomer reaction order $n_2 = 0$, $\gamma = -1/2$, with this remaining weak monomer

Fig. 1. Experimental kinetics of Aβ42 aggregation under quiescent conditions for 10 initial monomer concentrations. (A) Power-law scaling of the time to half-completion with the initial monomer concentration. The slope gives the scaling exponent $\gamma$ discussed in the text. (B–D) Global fits to the normalized experimental data, using the analytical solutions for systems where (B) the dominant nucleation mechanism is primary nucleation, and there are no secondary pathways (10, 17); (C) (a dominant) fragmentation process is active in addition to primary nucleation (16); and (D) secondary nucleation, in addition to primary nucleation, creates new aggregates, Eq. 1 (see SI Text for further discussion of these fits). The rate constants are (B) $\sqrt{k_m} = 8 \times 10^4 M^{-1}s^{-1}$, with $k_m = 0$, $n_1 = 3$; $\sqrt{k_m} = 10M^{-1}s^{-1}$, with $n_1 = 2$; and (D) $\sqrt{k_m} = 30M^{-1}s^{-1}$, $\sqrt{k_m} = 2 \times 10^5 M^{-1}s^{-1}$, with $n_1 = n_2 = 2$.}

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dependence originating only from the fibril elongation step in the overall self-assembly pathway.

**Nonlinear Theory.** Due to the complexity inherent in amyloid aggregation, it is challenging to acquire data in the regime where the linear solutions are fully valid, because in this early region the signals from bulk assays are low. To extend the applicability of kinetic analysis (14, 15) to amyloid systems, it is therefore desirable to consider the full reaction time course to maximize the constraints on the molecular mechanisms determined from the experimental data (22, 30). At later times in the reaction, as the monomer is consumed, the equations describing the overall assembly process become highly nonlinear and are challenging to integrate (15–17, 18, 22, 30). We have, however, recently derived self-consistent rate laws for the assembly process that are valid for the entire time course of the reaction (22). The full rate law reveals that the same two principal parameters $\kappa$ and $\lambda$, which were identified in the early time behavior (Table 1), define much of the macroscopic behavior in the nonlinear regime also, although the rate laws themselves have a different form, Eq. 1. An analysis of the full time course, therefore, introduces additional constraints without introducing any additional freedom, resulting in a stringent test of the theory and robust mechanistic conclusions.

**Secondary Nucleation Controls Aβ42 Fibril Formation.** To obtain a clear picture of the molecular mechanisms that give rise to Aβ42 fibrils, it is essential to generate highly reproducible experimental data reporting on this process. We have been able to collect these types of data at pH values and concentrations of the peptide that relate to physiological conditions (32) by controlling carefully the inertness of surfaces within which solutions of the peptide are contained and by purifying the recombinant monomeric peptide, using repeated applications of size-exclusion chromatography to ensure well-defined initial conditions before initiating kinetic analysis (14, 15) to amyloid systems, it is therefore desirable to consider the full reaction time course to maximize the constraints on the molecular mechanisms determined from the experimental data (22, 30). At later times in the reaction, as the monomer is consumed, the equations describing the overall assembly process become highly nonlinear and are challenging to integrate (15–17, 18, 22, 30). We have, however, recently derived self-consistent rate laws for the assembly process that are valid for the entire time course of the reaction (22). The full rate law reveals that the same two principal parameters $\kappa$ and $\lambda$, which were identified in the early time behavior (Table 1), define much of the macroscopic behavior in the nonlinear regime also, although the rate laws themselves have a different form, Eq. 1. An analysis of the full time course, therefore, introduces additional constraints without introducing any additional freedom, resulting in a stringent test of the theory and robust mechanistic conclusions.

The value for the scaling exponent, which describes how the lag time or half-time of the reaction scales with the initial concentration of monomer, measured in Fig. 1A (and for preseeded experiments that report on this change). It is interesting to note from Fig. 2 that increasing levels of shear force microscopy studies have captured the formation of such nuclei (34).

**Rational Alteration of the Aggregation Pathway.** A factor that has contributed greatly to previous difficulties in developing a clear picture of Aβ42 aggregation is the high sensitivity of the kinetics of aggregation to even small changes in the reaction conditions. Here, we can use such differences in aggregation behavior in a systematic manner to reveal the underlying microscopic mechanisms. Thus, having shown that Aβ42 aggregation under quiescent conditions is controlled by a fibril-catalyzed secondary nucleation process, we sought to modify the dominant factors determining the aggregation pathway by introducing shear forces through shaking and thereby identify the signals in the kinetic data that report on this change.

The rate equations (16, 22) lead to the intriguing prediction for Aβ42 that, if such shear gradually introduces fibril fragmentation as a molecular mechanism (Fig. 2), the scaling exponent (16, 22, 30) — relating the lag time or half-time to the monomer concentration — will change monotonically from the quiescent value of $-1.33$ and approach the theoretical limit of $-0.5$ (Table 1) associated with fragmentation (16, 22, 30) at very high agitation rates (Fig. 2 A–E, Lower). Remarkably, the overall effect of fragmentation is incorporated in the rate equations through the introduction of a single additional parameter relative to the quiescent case (SI Text), $\sqrt{k_b/\kappa}$, by use of which we are able to fit very closely the kinetic traces at each agitation rate (Fig. 2 B–E, Upper). Furthermore, we verified using electron microscopy and seeding experiments that the morphology of the fibrils remained unchanged (Figs. S4 and S5). The global nature of the fit is equivalent to the ability to predict quantitatively the behavior of the system with changes in experimental conditions; such a situation is likely to be found only when the model captures correctly the molecular events taking place in the reaction.

It is interesting to note from Fig. 2 that increasing levels of shear change not only the power law for the half-time, but also the characteristic form of the kinetic profiles at the late stages of the reaction (Fig. S4) (30). A change occurs because fragmentation, unlike secondary nucleation, is not directly affected by the depletion...
show that no radioactivity is detectable in the oligomer and for late times (22) predicts a sharper, double
Experimental kinetics for A
The analysis of the kinetic data indicates that a major
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Shear alters the symmetry of the reaction pro
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The data in Fig. 4A show that the rate of generation of oligomers is dramatically enhanced in the solution that contains preformed fibrils even though the initial concentration of soluble peptide is kept constant. Crucially, because the added fibrils in these experiments are unlabeled, these results establish that oligomers are formed from the monomeric peptide, but in a reaction that is catalyzed very strongly by the presence of fibrils (Fig. S6). We also carried out the complementary experiments where unlabeled monomers were incubated with radiolabeled fibrils; the data in Fig. 4A show that no radioactivity is detectable in the oligomer fraction, confirming that the oligomers do not originate from the preformed fibrils themselves (e.g., through fragmentation or dissociation), but rather are formed from monomers through secondary nucleation. We verified these results using immunochrom-}

Conferring the Source of Oligomer Populations with Radioactive Peptides. The analysis of the kinetic data indicates that a major and continuous source of new fibrils under quiescent conditions is a secondary nucleation mechanism that involves both the monomeric peptide and mature amyloid fibrils. Because a large number of peptides are required to form ordered fibrillar forms that are detected in ThT measurements, aggregates generated through the secondary pathway must initially be in prefibrillar, oligomeric states that can escape detection by this method (24, 35). To observe directly these ThT-invisible oligomer populations, which can ultimately convert to fibrils, and pinpoint their molecular origin, we studied a pair of samples with the same concentration of 35S-radiolabeled peptide, but one containing in addition to the soluble radioactive peptide a small concentration of unlabeled preformed fibrils. We measured the concentration of oligomers in both aggregating samples by quantifying through liquid scintillation assays the radioactivity in the oligomer fractions obtained from size-exclusion chromatography. This highly sensitive method of detecting oligomers has the advantage of not requiring any chemical labels and, therefore, leaves all of the chemical characteristics of the peptide intact.
Direct measurement of oligomer populations, using radioactive AJ42 peptides. (A) Samples of monomer (light blue bar) or monomer mixed with 1% preformed fibrils (dark blue bar and right bar) with selective radiolabeling of monomer or fibrils, as indicated in red, were incubated followed by size-exclusion chromatography and liquid scintillation counting. The counts for the oligomer fractions are shown below the respective samples. The monomer counts are shown in Fig. S7. (B) Probing the chromatography fractions with the 6E10 antibody confirms the dramatically enhanced production of small oligomers in the presence of fibrils. Time $\Delta t_1 = 24$ min. (C) Reduction in cell viability (MTS) for reactions without (light blue bars) and with (dark blue bars) a small concentration of added fibrils under the same conditions as in A and after filtration through a 200-nm filter. Values are averages over nine measurements at $\Delta t_2 = 5$; 6.7 min. Gray bars are the initial (monomer) and end (fibril) reaction time points. (D) Normalized kinetic time courses without (light blue) and with (dark blue) added preformed fibrils that correspond to those in A–C. The rapid increase in the slope of the assay with preformed fibrils (dark blue) after ca. 10 min, before the matched reaction without preformed fibrils (light blue) has generated significant aggregate mass, indicates rapid creation of new aggregates through secondary nucleation (30) (SI Text). The concentration of monomeric AJ42 was 4 μM and the mass concentration of added fibrils was 40 nM.

Fig. 4. Direct measurement of oligomer populations, using radioactive AJ42 peptides. (A) Samples of monomer (light blue bar) or monomer mixed with 1% preformed fibrils (dark blue bar and right bar) with selective radiolabeling of monomer or fibrils, as indicated in red, were incubated followed by size-exclusion chromatography and liquid scintillation counting. The counts for the oligomer fractions are shown below the respective samples. The monomer counts are shown in Fig. S7. (B) Probing the chromatography fractions with the 6E10 antibody confirms the dramatically enhanced production of small oligomers in the presence of fibrils. Time $\Delta t_1 = 24$ min. (C) Reduction in cell viability (MTS) for reactions without (light blue bars) and with (dark blue bars) a small concentration of added fibrils under the same conditions as in A and after filtration through a 200-nm filter. Values are averages over nine measurements at $\Delta t_2 = 5$; 6.7 min. Gray bars are the initial (monomer) and end (fibril) reaction time points. (D) Normalized kinetic time courses without (light blue) and with (dark blue) added preformed fibrils that correspond to those in A–C. The rapid increase in the slope of the assay with preformed fibrils (dark blue) after ca. 10 min, before the matched reaction without preformed fibrils (light blue) has generated significant aggregate mass, indicates rapid creation of new aggregates through secondary nucleation (30) (SI Text). The concentration of monomeric AJ42 was 4 μM and the mass concentration of added fibrils was 40 nM.

confirming the formation of oligomers through secondary nucleation in a fibril-dependent manner.

The combination of the kinetic experiments and the detailed analysis of the chromatography fractions reveals that low molecular weight oligomers are formed in a pathway that involves both the monomer and fibrils. A key question, however, is whether the toxicity known to be associated with AJ42 aggregation can originate from this same pathway. To address this issue, we measured the reduction in viability (Fig. 4C) and the increase in cytotoxicity (Fig. S8) of SH-SY5Y human neuroblastoma cells when exposed to oligomers formed as a result of secondary nucleation. We studied two solutions with an identical monomer concentration and, therefore, an identical population of oligomers generated by primary nucleation; marked differences in the resulting toxicity are evident, however, when a small concentration of preformed fibrils was added to one of these solutions to trigger the production of secondary oligomers as shown above. As the fibrils themselves are observed not to give rise to a high level of toxicity, these observations identify specifically that the major source of cytotoxic oligomers results from a process that involves both the monomeric peptide and the fibrils, i.e., secondary nucleation.

Significance and Conclusions

These results establish a general picture for the self-assembly of AJ42 that brings together all of the species in the aggregation cascade (Fig. 5). Initially, in the absence of fibrils, all oligomers have to be generated through primary pathways because secondary nucleation requires the presence of fibrils. Once a critical concentration of amyloid fibrils has formed, however, secondary nucleation will overtake primary nucleation as the major source of new oligomers and further proliferation becomes exponential in nature (14, 16, 22) due to positive feedback (Fig. 5). The identification of secondary nucleation underlines the importance of elucidating the detailed structures of amyloid fibrils and their surfaces, information that will motivate molecular simulations to determine the origins of their surface-catalytic activity. The critical concentration of fibrils, above which secondary nucleation becomes the dominant mechanism generating new aggregates, is given from the ratio of the primary to secondary nucleation rate constants, $k_n = k_n/k_2$; the parameters obtained in Fig. 1D define this concentration to be of the order of 10 nM. A survey of literature values (Tables S1 and S2) shows that the aggregate loads in the brains of patients suffering from AD are much greater than this critical concentration, and hence the results suggest that secondary nucleation is likely to be active under these conditions. It is therefore interesting to speculate that the secondary nucleation process identified in this in vitro study as the origin of the toxicity of AJ42 aggregation could also play a major role in vivo, even accounting for the fact that differences in the morphological character and accessible surface area of the amyloid fibrils may cause variations in the rate of oligomer formation through secondary nucleation for different plaque loads.

In agreement with this idea, clear signatures of secondary nucleation are apparent in studies of living systems, as a halo of brils, i.e., secondary nucleation. A key question, however, is whether secondary nucleation requires the presence of fibrils themselves are unaffected, whereas the generation of oligomers through the secondary nucleation pathway is by definition very significantly enhanced. Furthermore, in the vicinity of plaques, dendritic spines have been found to be disrupted in a manner that depends on their distance from the plaques (39), an observation that suggests that the latter structures are not toxic by themselves in vivo but instead facilitate the generation of toxic oligomers by surface catalysis. The molecular picture that emerges from the present study, therefore, provides a mechanism by which the accumulation of amyloid fibrils is coupled to the generation of low molecular weight diffusive aggregates from monomeric peptide, thereby connecting together all of the main components in the AJ cascade. This conclusion suggests that an important approach for suppressing the production of neurotoxic AJ42 oligomers could be to focus on altering the secondary, rather than (or in addition to) the primary, nucleation pathway. Indeed, once the critical concentration of fibrils is exceeded, further perturbation of the primary nucleation pathway ceases to be effective in reducing the overall proliferation of oligomers, as most new aggregates are not created via this mechanism.

Materials and Methods

Additional information can be found in SI Text.

Fig. 5. Schematic showing the overall reaction pathway and the corresponding rate constants identified in this paper. The approximate rates of the elongation-related processes have been identified in previous work (33, 35, 36),
**Integrated Rate Law.** When both primary and secondary pathways are active, the integrated rate law describing the generation of total fibril mass, \( M(t) \), over time as a function only of the initial conditions and the rate constants of the system is given as (16, 22).

\[
M(t) = \frac{1}{\beta_+ + \beta_-} \frac{\beta_+ + \beta_-}{(\beta_+ + \beta_- + \beta_0)c_0} \left( \frac{\beta_+}{\beta_-} \right)^{\frac{t}{\tau}} e^{-k_0 t} \tag{1}
\]

Although many distinct parameters, including microscopic rate constants for primary nucleation \( k_o \), elongation \( k_i \), depolymerization \( k_{\text{dep}} \), and fibril-catalyzed secondary nucleation \( k_{\text{fi}} \), are required to capture the complete assembly process (16, 22), only two particular combinations of the rate constants define much of the macroscopic behavior; these parameters are related to the rate of formation of new aggregates through primary pathways \( \beta = 2k_0k_i/m(0)^{1/2} \) and through secondary pathways \( k = k_m/k_0 \), where \( k_m = k_i \) when \( n_i = 0 \). Indeed, Eq. 1 depends on the rate constants through these two parameters, \( \beta \) and \( k \), alone because \( \beta = (k_0 + k_m)/(2k_i) \), \( k_0 = \sqrt{2k_i/n_0 + 1} \), and \( k_m = \sqrt{2k_i/n_0} \). The initial concentration of soluble monomers is \( m(0) \) and the reaction orders describing the dependencies of the primary and secondary pathways on the monomer concentration are \( n_i \) and \( n_0 \).

**Materials.** We expressed in Escherichia coli and purified, as described previously (40), the Aβ(1-42) peptide (MDAEFRHDSGYEVHHKLVFFAADGVS-NKGAIILGMVGVGVI). Radiolabeled Aβ42 was expressed and purified in the same way, except that cells were grown in minimal medium supplemented with [35S]methionine 2 min before induction. Aliquots of purified Aβ42 were thawed and subjected to gel filtration on a Superdex 75 column in 20 mM sodium phosphate buffer, pH 8, 80 μM EDTA 0.02% NaN3.

**Radiolabeling and Immunochemistry.** The peptide samples were taken from an ongoing seeded or unseeded aggregation reaction (Fig. 4) and immediately loaded into a 1 x 30-cm Superdex 75 column. Eluted fractions (2 μL) were diluted 1:4 in scintillation solution (Ready Safe liquid Scintillation Mixture; Beckman Coulter) and placed in a scintillator (Beckman LS6500IC) for counting for a total of 120 min per sample. The counts for fractions with average elution volumes of 6, 8, and 10 mL were binned as oligomer counts (sum of 3- to 20-mer, because the dominant monomer peak makes dimer quantification inaccurate) and counts for fractions eluting at 12, 14, and 16 mL were binned as monomer counts. The experiments were repeated with unlabeled species, and 1-mL eluted fractions were concentrated by lyophilization, dissolved in 8 M urea, and applied to a PDVF membrane for semi-quantitative analysis using E610 primary antibody (Signet) and alkaline phosphatase-conjugated rabbit anti-mouse secondary antibody (Dako).

**Cytotoxicity and Cell Viability Assays.** Assays were performed on SH-SYSY human neuroblastoma cells cultured under standard conditions. The peptide samples were taken from ongoing seeded or unseeded reactions and subjected to filtration through a 200-nm filter (Anapou). Control peptide, monomer, and fibril were not filtered. Buffer controls were both filtered and unfiltered. The cells were then cultured in the presence of the peptides, buffer, or media for a further 24 h before the cytotoxicity and viability assays were performed. Caspase-3/7 activity was measured using the Apo-ONE Homogeneous Caspase-3/7 assay (Promega). Cell viability was measured using the Cell Titer 96 Aqueous One MTS reagent (Promega).

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Supporting Information

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SI Materials and Methods

All chemicals were of analytical grade. The amyloid-β Aβ(M1-42) peptide (MDAEFRHDSEYEVHHQKLVELFAEDVGSNKGA-IIGLMVGGVVIA) was expressed in Escherichia coli and purified as described previously (1). In short, the purification procedure involved sonication of *E. coli* cells, dissolution of inclusion bodies in 8 M urea, ion exchange in batch mode on DEAE cellulose resin, centrifugation through a molecular weight cutoff (MWCO) of 30,000 filter and, finally, concentration using a MWCO of 3,000 filter. The purified peptide was frozen as identical 1-mL aliquots. Radiolabeled Aβ(M1-42) was expressed and purified in the same way, except that cells were grown in minimal medium that was supplemented with [35S] methionine 2 min before induction.

Preparation of Samples for Kinetic Experiments. For kinetic experiments, aliquots of purified Aβ42 were thawed and subjected to two rounds of gel filtration on a Superdex 75 column in 20 mM sodium phosphate buffer, pH 8, with 200 μM EDTA and 0.02% NaN₃. The latter part of the monomer peak, Fig. S1, was collected on ice and was typically found to have a concentration (determined by quantitative amino acid analysis purchased from BMC Uppsala) of 5–12 μM. The gel filtration step removes traces of preexistent aggregates and exchanges the buffer for the one used in the fibril formation experiments. The monomer generated in this way was supplemented with 6 μM thioflavin T (ThT) from a 1.2-mM stock and was used to prepare by dilution a series of samples of concentrations between 0.5 and 6 μM Aβ(M1-42) in 20 mM sodium phosphate buffer, pH 8, with 200 μM EDTA and 0.02% NaN₃; before dilution, the solutions were also supplemented with 6 μM ThT so that all samples contain the same ThT concentration. The dilutions were made in low-spin Eppendorf tubes (Axygen) on ice, using careful pipetting to avoid introduction of air bubbles. Each sample was then pipetted into multiple wells of a 96-well half-area plate of black polystyrene with a clear bottom and PEG coating (Corning 3881), 100 μL per well. The samples were added to the plate from lower to higher concentration, after which the plate was sealed with a plastic film (Corning 3095).

Kinetic Assays. Assays were initiated by placing the 96-well plate at 37 °C and shaking at the designated orbital speed in a plate reader (Fluostar Omega or Fluostar Optima; BMGLabtech). The ThT fluorescence was measured through the bottom of the plate every 343 s (with an excitation filter of 440 nm and an emission filter of 480 nm) with continuous shaking (when relevant) between measurements. Each reading lasted for 43 s and the intervening shaking (or quiescent) period lasted for 300 s. The ThT fluorescence was followed for 3–5 repeats of each monomer concentration. The formation of fibrils was verified using transmission electron microscopy (1).

Preseeded Kinetic Assays. Kinetic experiments were set up as above for multiple samples of Aβ42 in 20 mM sodium phosphate buffer, pH 8, with 200 μM EDTA, 6 μM ThT, and 0.02% NaN₃. The ThT fluorescence was monitored for 1.5 h to verify the formation of fibrils. The samples were then collected from the wells into low-speed and quiescent conditions. The latter part of the monomer peak, Fig. S1, was collected on ice, using careful pipetting to avoid introduction of air bubbles. ThT so that all samples contain the same ThT concentration. The ThT fluorescence was measured in the plate reader every 60 s for multiple samples of Aβ42 aggregate mass concentration under our carefully controlled conditions by carrying out several control experiments (Fig. S2). In particular, we observed that the fluorescence intensity from ThT at the end of aggregation reactions scales linearly with the total Aβ42 peptide concentration in the system (Fig. S2A). Because these fluorescence values correspond to a system where almost all of the peptide is in aggregated form (2), this control demonstrates that the fluorescence from ThT is linearly related to the Aβ42 aggregate mass concentration.

Furthermore, in a second control, we used radiolabeled peptide coupled to liquid scintillation experiments to determine directly the initial monomer concentration in an aggregating reaction and also the free monomer concentration at the half-time of the same aggregation reaction as indicated by the ThT reaction profile. This measurement, shown in Fig. S2B, confirmed that at the half-time indicated by the ThT assay, precisely half of the total monomer in the system did indeed remain as free monomer. These control experiments verify explicitly the faithfulness of ThT as a reporter of Aβ42 aggregate mass concentration in our kinetic studies.

It is important to reconcile the robust linear relationship between ThT fluorescence and the total aggregate mass and free monomer concentrations with the fact that ThT reports on fibrillar species. As discussed in the main text, a component of the total aggregate mass that corresponds to low molecular weight aggregates not yet of fibrillar structure may, therefore, not be detected in bulk measurements made using ThT. Importantly, the direct contribution to the aggregate mass concentration due to these ThT-invisible oligomers during the aggregation reaction is observed by radiolabeling experiments, through comparing the radiocounts in Figs. 4A and Fig. S7, to be no more than around 1% of the total protein mass in the system. Oligomers are therefore not a significant component of the total aggregate concentration when weighted by mass. The combination of our ThT and radiolabeling measurements shows directly that, at the half-time of the reaction, approximately half of the protein mass remains in the monomeric state, approximately half is in fibrillar form, and only a small fraction is present as oligomers. Measurements of the mass concentration of fibrillar species via ThT fluorescence are hence equal to those of the total aggregate mass concentration, which includes also oligomeric species, to within around 1%, as shown in Fig. S2. This conclusion is equivalent to the statement that $M(t) + m(t) = m_{tot}$, where $M(t)$ is the fibrillar mass concentration, $m(t)$ is the free monomer concentration, and $m_{tot}$ is the total concentration of peptide in the closed system.

Kinetic Data Presentation. The fractional fibrillar mass concentration was calculated from the ThT fluorescence measurements, using the result from our calibration experiments that the two are linearly related. The kinetic traces in Figs. 1 and 2 display the results of three to five replicates overlaid to present transparently the high reproducibility and low spread in the data. Where error bars are shown elsewhere, they are SEs determined from the results of three to five replicates at each concentration. At least two identical plates of 96 solutions were examined at each shaking speed and at quiescent conditions.
Transmission Electron Microscopy. Transmission electron microscopy (TEM) images were acquired using a Philips CM120 BioTWIN electron microscope equipped with a postcolumn energy filter (Gatan GIF100) and a CCD camera. The acceleration voltage was 120 kV. A carbon-coated formvar grid was placed upside down on a droplet of each sample, followed by a quick rinse and then placing the grid upside down on a droplet of 1.5% (wt/vol) uranyl acetate (3). TEM images were taken for samples at quiescent condition at zero time; at 15, 30, and 45 min into the lag phase; and after reaching the equilibrium plateau; and for samples shaken at 100, 200, 300, and 600 rpm after reaching the equilibrium plateau.

Analysis of Monomer and Oligomer Populations Using Radiolabel and Monoclonal Antibodies. Aggregation was monitored by ThT fluorescence for samples of 5 μM Ap42 with and without 50 nM of preformed seeds in 20 mM sodium phosphate buffer, pH 8, with 200 μM EDTA and 0.02% NaN3 with 6 μM ThT. Samples with 3S Ap42 were supplemented with seeds formed from unlabeled peptide, and samples of unlabeled Ap42 monomer were supplemented with seeds formed from [35S]Ap42. The aggregation process was monitored until the time point marked \( t = \Delta t \) in Fig. 4D, i.e., the time when the ThT fluorescence intensity of seeded samples has reached 50% of the maximum value and unseeded samples are still in the lag phase. The samples were collected from the wells and immediately injected into a 1 × 30-cm Superdex 75 column. Eluted fractions (2 mL per fraction) were diluted 1:4 in scintillation solution (Ready Safe Liquid Scintillation Mixture; Beckman Coulter) and placed in a scintillator (Beckman LS6000IC) for counting for a total of 120 min per sample. All samples were counted in sequence, 10 min per sample, and 12 such sequences were performed. The counts for fractions eluting at 6, 8, and 10 mL were binned as oligomer counts and counts for fractions eluting at 12, 14, and 16 mL were binned as monomer counts (average elution volume of fraction given). The experiments were repeated with unlabeled monomer and no seeds or unlabeled seeds, and 1-mL fractions collected during elution from the Superdex 75 column were concentrated by lyophilization, dissolved in 8 M urea, and applied to a PVDF membrane for semiquantitative analysis using 6E10 primary antibody (Signet) and alkaline phosphatase-conjugated rabbit anti-mouse secondary antibody (Dako). The centers of the fractions shown in the eight dots in Fig. 4B correspond to elution volumes of 5.2, 6.6, 8.0, 9.5, 10.8, 12.2, 13.6, and 15.2 mL.

Cytoxicity and Cell Viability Assays. Assays were performed on SH-SY5Y human neuroblastoma cells cultured under standard conditions at 37 °C in a humidified incubator with 5% CO2. Cells were seeded at a density of 25,000 per well in a white-walled, clear-bottomed 96-well plate and cultured for 24 h in DMEM/10% FBS. The culture media were then replaced with prewarmed phenol red free DMEM without serum into which the peptide samples or NaPO4 buffer were diluted 1:4. The peptide samples were taken directly from an ongoing seeded or unseeded aggregation reaction and subjected to filtration through a 200-nm filter (Anapour). Control peptide, monomer, and fibril were not filtrated. Buffer controls were both filtrated and unfiltrated. The cells were then cultured in the presence of the peptides, buffer, or media for a further 24 h before the cytotoxicity and viability assays were performed. Caspase-3/7 activity was measured using the Apo-ONE Homogeneous Caspase-3/7 assay (Promega). The fluorogenic caspase-3/7 substrate was diluted 1:100 in the lysis buffer provided and added to the cell medium at a 1:1 ratio. The reagent/cell mix was then incubated for 1 h before measuring the fluorescence at excitation 480 nm/emission 520 nm in an Optima Fluostar plate reader. Cell viability was measured using the Cell Titer 96 Aqueous One MTS reagent from Promega. The MTS reagent was added to the cell culture medium and incubated with the cells at 37 °C in a humidified incubator with 5% CO2 before the absorbance at 495 nm was measured in an Optima Fluostar plate reader. All values given for both assays are buffer subtracted. The reduction in viability was calculated as the percentage reduction in MTS signal (absorbance at 495 nm) for each sample compared with that of the buffer-treated cells.

Theoretical Analysis

Global Analysis of Experimental Kinetic Data. The time to half-completion of each curve was determined by extracting from the experimental profiles the data points corresponding to a fractional fibrillar mass concentration value of between 0.4 and 0.6 and then performing a linear regression of these data points. The resulting straight-line fit was used to determine the time to half-completion for every individual curve, and the mean and SE were then found for each concentration (Fig. 2 A–E, Lower). The scaling exponent was found by a nonlinear fit of a power-law relationship, \( \tau_{50\%} = \beta m(t) \gamma \), for \( \beta, \gamma \) to these half-time data, weighted inversely by the square of the SEs of the data at each concentration. The scaling relationship observed at quiescent conditions is shown in Fig. S3A over an extended range of concentrations in comparison with Fig. L4.

The two-parameter quiescent global fit in Fig. 1D was performed using the analytical rate law presented in our previous analysis (4). For \( \lambda \), the time evolution of a polymerization reaction in which the dominant mechanism is primary nucleation with secondary nucleation. The single-parameter global fits in Fig. 2 were performed using an analytical solution for the case where both fragmentation and monomer-dependent secondary nucleation mechanisms are considered, given as Eq. 1 with redefinitions of \( \kappa = \sqrt{2k,m(0)k_3m(0)^2 + k_-} \) and \( k_\infty = k_0/2\left((-2\gamma - 1)(-2\gamma - 1 - 1)+2\gamma/|n_e|^2\right)^{1/2} \), where \( \gamma \) is the experimental scaling exponent observed in each case (−0.94, −0.76, −0.66, and −0.62 from Fig. 2). In these fits the rate constants found in the analytical global fit in Fig. 1D were fixed to these predetermined values, leaving only one free kinetic parameter, \( k_0, k_- \). The final single-parameter fit in Fig. 2E was also performed using the analytical result in ref. 4 for a system proliferating solely through aggregate fragmentation, yielding an identical result. All global analytical fits were carried out using a Levenberg–Marquardt algorithm.

Relating Scaling Exponents to Mechanisms. Information regarding the dominant mechanisms was inferred from the scaling exponents based on our previous analysis (4–6). In particular, a powerful example of the dominance of the two principal parameters \( \lambda \) and \( \kappa \) is given by the power-law behavior \( \tau_{50\%} \sim m(t)^\gamma \) that relates the half-time, \( \tau_{50\%} \), at which half of the total peptide is present in the aggregated form, to the initial peptide concentration \( m(0) \); the exponent \( \gamma \) in this power law is a good approximation given by \( \gamma = -n_e/2 \) for processes where the primary nucleation step is the major source of oligomers and by \( \gamma = -(m + 1)/2 \) for phenomena where secondary nucleation processes dominate (4–7). The lag time before the observation of aggregates follows the same scaling behavior; we use here the half-time because it is available accurately from experimental data. A strong overall monomer dependence in Table 1 refers to the possibility, but not a guarantee (8), of a scaling exponent \( \gamma \geq 1 \), whereas a weak dependence refers to a scaling exponent \( \gamma < 1 \).

The value for the scaling exponent measured in Fig. L4 and Fig. S3A for Ap42 under quiescent conditions is \( \gamma < 1.3 \pm 0.03 \). In addition, the data in Fig. S3B show that the same value for the scaling exponent is observed even when a small amount of preformed fibrils is added at the beginning of the reaction to bypass primary nucleation (7), indicating that primary nucleation does not make a large contribution to the observed monomer scaling. It is interesting to note that these observations exclude aggregate
fragmentation, believed to be vital in the propagation of prions, as the dominant mechanism driving Aβ42 aggregation, because this process would result in an exponent $\gamma \leq -0.5$ as it corresponds to a monomer-independent secondary pathway, $n_2 = 0$. The value of the scaling exponent observed is, however, consistent with a dominant secondary nucleation pathway characterized by a monomer dependence of $n_2 = 2$ and a minor contribution from primary nucleation, the effect of which is to lower the magnitude of the scaling exponent (4–7) from the value $\gamma = -(n_2 + 1)/2 = -1.5$ toward the value given for proliferation through primary nucleation only, $\gamma = -1$ for $n_2 = 2$; the addition of preformed fibrils at the beginning of the reaction results in a similar minor reduction in the magnitude of the scaling exponent below $\gamma = -1.5$ (5, 9). We directly confirm this conclusion by checking explicitly in Fig. 1D the high degree to which the experimental data determined for the full time course of the reaction are matched by the predictions from the rate law, Eq. 1, when all 10 initial peptide concentrations are used and the only two free parameters, $\sqrt{k_c k_p}$ and $\sqrt{k_c k_s}$ that enter $x$ and $\lambda$, are fixed globally to the same values for all 10 measured peptide concentrations to provide the best fit for the entire dataset.

Having established the mechanism of aggregation for Aβ42 at quiescent conditions, the rate equations (4) predict that, if fibril fragmentation is gradually introduced as a molecular mechanism for Aβ42 in addition to the processes already found under quiescent conditions, the scaling exponent will move from the quiescent case toward $\gamma = -1$. In particular, the rapid increase in the slope of the time courses observed.

Fits to Alternative Mechanisms at Quiescent Conditions. Primary nucleation. The global fit in Fig. 1B is to the classical Oosawa theory of nucleation polymerization (10, 11), which is recovered to leading order by Eq. 1 when the rates of the secondary pathways are set equal to zero (5, 6). In this case, the scaling behavior that is observed for the half-time, Fig. 1A, can be best accounted for by a primary nucleation exponent of around $n_1 = 3$, leaving the single combined rate parameter $k_c k_p$ that controls proliferation through primary nucleation and growth to be fitted globally, resulting in the fit shown in Fig. 1B. It is clear that although the scaling behavior of the half-time is approximately recovered, the model cannot even qualitatively describe the kinetic reaction time courses observed.

This result is in agreement with the kinetic data in Fig. 4D, which point in a model-independent manner to the dominant role of secondary pathways, rather than primary nucleation, in generating most new aggregates (7). When preformed fibrils are added at the beginning of the reaction in Fig. 4D, the kinetic profile (dark blue) goes toward completion before the corresponding reaction without preformed fibrils (light blue) has generated significant aggregate mass; in particular, the rapid increase in the slope of the assay with preformed fibrils (dark blue) after ca. 15 min indicates rapid creation of new aggregates (7). The matched profile without preformed fibrils (light blue) shows that primary nucleation is not rapidly creating new aggregates at this time, and by definition the addition of fibrils cannot affect primary nucleation, pinpointing the origin of the new aggregates as the effect of secondary pathways (7). The radiocounting experiments shown in Fig. 4A provide independent evidence for this conclusion.

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Fragmentation. If the fragmentation rate is allowed to vary in addition to the primary nucleation rate, but with the secondary nucleation rate set equal to zero, the best fit of the model (4) to the kinetic data corresponds to a system where most new aggregates are still created through primary nucleation, but where fragmentation is active as a minor mechanism. This result is inconsistent with the data in Fig. S3B, where it is observed that primary nucleation does not make a large contribution to the monomer scaling. Moreover, the resulting fit does not accurately capture the characteristic shapes of the reaction profiles at early and late times, which are hallmarks of a secondary nucleation process (7). Furthermore, a model with only a minor contribution from a secondary pathway is inconsistent with both the kinetic data in Fig. 4D (as explained in Fits to Alternative Mechanisms at Quiescent Conditions, Primary nucleation) and with the radiocounting experiments in Fig. 4A, which both demonstrate that a secondary pathway is active as the dominant, rather than a minor, mechanism.

Because a global fit to a model where both primary nucleation and fragmentation are active results in a situation where fragmentation is a minor, rather than a dominant, mechanism, an additional constraint was added to generate a representative fit to the data in Fig. 1 for the model where fragmentation is the dominant mechanism that is generating new aggregates. Specifically, we enforced $k_c k_p = (10^7 \text{M}^{-1} \text{s}^{-1}) k_s k_c$, which corresponds to fragmentation becoming the dominant mechanism once the aggregate mass concentration reaches around 10 nM. The resulting fit is shown in Fig. 1C, where it is clear that neither the shape of the reaction profiles nor the scaling exponent, which in a system dominated by fragmentation is $\gamma = -0.5$, can be recovered by a model that describes the situation where filament fragmentation is the dominant mechanism responsible for generating new aggregates. This is in agreement with the radiolabeling experiments shown in Fig. 4A, where it is observed that the oligomers formed through the secondary process are generated from monomeric peptide (i.e., through secondary nucleation) and not from existing aggregates (i.e., through fragmentation), hence providing independent verification that fragmentation is not the dominant mechanism generating oligomers.

Predicted Change in Scaling Exponent Observed at High Monomer Concentration. The reduction in the half-time scaling exponent with increasing shear, identified in Fig. 2 A–E, is summarized in Fig. S3B, and summarized in Fig. 3B, stems qualitatively from the fact that growth in a system driven by a monomer-dependent nucleation process has a higher dependence on the monomer concentration relative to growth in a system fragmenting under shear. A further prediction of the theory (4–6), therefore, is that at high monomer concentrations, fragmentation will become relatively less important in determining the growth kinetics if monomer-dependent nucleation processes are active. This feature emerges experimentally in the form of a deviation from the power-law relationship between the half-time and the peptide concentration at the highest concentrations used in this study. These data points are shown as open circles in Fig. 2 A–E, and Fig. S3B and fall markedly below the best-fit lines that describe the rest of the data at lower concentrations. Indeed, if the four best-fit lines in Fig. S3B that correspond to aggregation under external shear (red, orange, green, and blue) were to be extended to higher concentration, they would cross the best-fit line given from measurements of aggregation under quiescent conditions (extended purple line). This would correspond to a physically impossible situation because shear cannot slow down the polymerization reaction. Instead of following this nonphysical trend, the data indicate a change in exponent as a function of concentration that has the effect of reducing the half-times for the reactions under shear to values below the best-fit lines, such that the datasets thereby avoid, as the initial monomer concentration is increased, crossing each other or the best-fit line obtained under quiescent conditions. More specifically, the theoretical analysis shows that at high monomer concentrations all of the power-law relationships in Fig. S3B must become parallel to the
scaling law corresponding to that for the quiescent system. Very
generally, these results highlight the close connection between
the scaling exponent and the molecular mechanism of filaments
growth, with a change of exponent being a hallmark of a change
in molecular mechanism.

Scaling Exponent Does Not Originate from Primary Nucleation. To
verify that the monomer scaling exponent that we measured at
quiescent conditions does not contain a large contribution from
primary nucleation, we performed experiments where primary
nucleation was bypassed (7) through the addition of a small
amount of preformed fibrils. The monomer scaling exponent
observed under these conditions (Fig. S3B) is the same as that
observed in the absence of seed material (Fig. S3A). These ex-
periments show that primary nucleation does not contribute
a significant part of the monomer scaling. This experimental
result eliminates the possibility that the monomer scaling exponent
at quiescent conditions emerges from proliferation involving fil-
ament fragmentation and a significant contribution from primary
nucleation with a large nucleation exponent $n_c$, which could in-
crease the magnitude of the scaling exponent from the frag-
mentation-dominated limit, $\gamma = -1/2$, significantly toward the
value given for primary nucleation alone, $\gamma = -n_c/2$. For this type
of mechanism, the scaling exponent would revert to the value for
a fragmentation-dominated system, $\gamma = -1/2$, when primary nu-
cleation is bypassed, in contradiction to the experimental data for
the case where preformed fibrils are added at the beginning of the
reaction (Fig. S3B). By contrast, the monomer-dependent sec-
ondary nucleation mechanism is fully consistent with the data in
Fig. S3. Note that a minor reduction in the magnitude of the
scaling exponent below $\gamma = -1.5$ is also observed in the presence
of preformed fibrils (Fig. S3B), in agreement with the predictions
of the kinetic theory (5,9).

Fibril Morphology Is Similar at All Shear Rates. We observed experi-
mentally that the fibril morphology is similar at the different shear
rates under which the reactions were carried out in Fig. 2. Using
electron microscopy (Fig. S4), no fibrils were found at zero time
(consistent with the isolation of monomer just before starting the
experiment). Fibrils were found in all other samples, i.e., taken at
15, 30, and 45 min into the lag phase and after reaching the
equilibrium plateau. The fibrils found under all conditions are
remarkably similar (Fig. S4): typically, fibrils with a diameter of
the order of 10 nm are seen. A greater prevalence of short fibrils is
observed at higher shaking speed, but some very long fibrils are
seen at all levels of shear. The average length at quiescent
conditions is expected to be of the order (4,12) $\delta k_v m(0)/\kappa \approx 1 \mu m$,
consistent with observations here, and calculated using the rate
constants determined in the main text with an extension $\delta \approx 1 A$
per monomer (13,14).

Furthermore, we carried out experiments to determine whether
the kinetics of reactions seeded with fibrils formed at varying
levels of shear differed. When preformed fibrils formed at dif-
ferent shaking speeds are added to monomeric peptide, the ac-
celeration of the reaction is very similar in all cases (Fig. S5).
In particular, through adding high concentrations of preformed
fibrils formed at different shaking speeds to monomeric peptide
(Fig. S5A), it is observed that the rates of elongation of fibrils
formed at different levels of shear, which are proportional to the
initial slopes of the reaction profiles (7), are very similar. Simi-
larly, adding low concentrations of seed material to monomeric
peptide (Fig. S5B) reveals that fibrils generated at different
levels of shear promote secondary nucleation very similarly.

Acceleration Through Seeding Is Due to Fibrils and Not Oligomers. To
determine whether the acceleration of the reaction that we
observed upon addition of preformed aggregate material to
monomeric peptide in Fig. 4D is due to preformed fibrils or
oligomers, we performed control experiments (Fig. S6) where
the preformed aggregate material is filtered before being added
to monomeric peptide. When the filtrate (<200 nm) is added to
monomeric peptide, significant acceleration is not observed (Fig.
S6C); when the retentate (>200 nm) is added to monomer
peptide, the reaction is accelerated in the same manner as when
the unfiltered preformed aggregate material is used (Fig. S6B).
These experiments verify that it is fibrils (larger than 200 nm,
consistent with the TEM images in Fig. S4), rather than oligomers,
that are primarily acting to accelerate the reaction.

Experimental Measurements of Aß42 Aggregate Concentrations from
Patients with Alzheimer’s Disease and Control Subjects. Experimental
measurements of Aß loads in patients with Alzheimer’s disease
(AD) and age-matched control subjects were collated from the
literature and are shown together in Table S1. Table S2 shows
the median and the upper and lower quartiles for the patients
with AD and the healthy controls from this collated dataset.

Six of these studies (15–20) reported values for insoluble Aß1–
42, whereas two reported values for insoluble Aß−42 (21,22),
and two reported values for only total insoluble Aß (23,24). The
studies (15–20) where concentrations for soluble and insoluble
Aß40 and Aß42 were reported individually suggest that a mea-
surement of total insoluble Aß would be equal to within an order
of magnitude of the concentration of insoluble Aß42, and so
these values are included in the analysis (Table S1). Three of
the studies (16,21,22) reported values for multiple brain regions;
the individual concentrations reported generally differ by less
than one order of magnitude, and so an average is reported in
Table S1. In cases where some of the control subjects displayed
aggregate loads below the stated detection threshold (18,19),
the threshold value itself was used as the Aß42 concentration,
resulting in an overestimate for the aggregate loads in these
control subjects in this analysis.

The measurements in each study were made using varying
eXtraction and immunoassay techniques. Despite the spread
in reported values across the different measurements, the collated
dataset described in Tables S1 and S2 establishes a broad view
of the concentration of aggregated Aß42 in control subjects and
patients with AD. It is interesting to note in particular that the
concentration of Aß42 aggregates observed in the brains of
patients with AD is on average two orders of magnitude above
the critical concentration of aggregates (ca. 10 nm) where, in
our study, secondary nucleation became more important than pri-
mary nucleation in creating new oligomers.

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measurements to microscopic mechanisms of protein aggregation. J Mol Biol 421(2–3):
160–171.
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Fig. S1. Size exclusion chromatograms obtained during the multistep Aβ42 purification process used in these studies. (A) First round of purification where the monomer peak is initially selected (red dashed lines) and the second peak is mainly Tris and EDTA from the initial purification protocol (1). (B) This is followed by at least one further round of purification, wherein the center of the remaining monomer peak is selected (red dashed lines) to give the monomeric peptide to be used in studies.

Fig. S2. Calibration of the relationship between fluorescence intensity from ThT and Aβ42 aggregate mass concentration. (A) Linear relationship between fluorescence intensity from ThT and that from total Aβ42 at reaction completion (here taken as the average fluorescence value between 10 and 12 h) where all of the monomeric peptide has been sequestered into aggregates (2). (B) Fraction of remaining free monomer measured by radiocounting at a time corresponding to the half-time as indicated by a ThT assay. The count of radiolabeled monomer shows that at the time reported by ThT as the half-time there is indeed precisely half of the monomeric peptide remaining in solution.
Fig. S3. (A) Power-law scaling relationship between the half-time, $\tau_{50\%}$, and the initial monomer concentration, $m(0)$, at quiescent conditions. The scaling exponent, $\tau_{50\%} \propto m(0)^\gamma$, over the extended range of concentrations shown here is $\gamma = -1.34 \pm 0.02$, consistent with the data shown in Fig. 1A. (B) Power-law scaling exponent between the half-time, $\tau_{50\%}$, and the initial monomer concentration, $m(0)$, for the case where 60 $\mu$M preformed fibrils is also added at the beginning of the reaction to bypass primary nucleation. The slope is the same as in A, indicating the monomer scaling exponent does not have a large contribution from primary nucleation.

Fig. S4. TEM images of fibrils formed at different levels of shear (from ca. 3.4 $\mu$M Aj42). The fibrils are all of similar morphology, with a shortening of the average length at higher shaking speeds. The average length at quiescent conditions is expected to be approximately (4, 11) $\delta + m(0)/x = 1 \mu$m, consistent with observations here and calculated using the rate constants determined in the main text with an extension $\delta = 1$ A per monomer (12, 13). Fibrils are observed during the lag phase for all conditions, as expected (7). The end time refers to the time at which the plateau is reached in the reaction profile. No aggregates are observed at zero time.
Fig. S5. Fibrils formed at different levels of shear accelerate the reaction very similarly. (A) Thirty percent (monomer equivalent) preformed fibrils formed at different shaking speeds (as annotated) are added to monomeric peptide (ca. 5 μM) at quiescent conditions. The initial slope, which is proportional to the elongation rate of the preformed fibrils (7), is very similar in all cases. (B) One percent (monomer equivalent) preformed fibrils formed at different shaking speeds (as annotated) are added to monomeric peptide (ca. 5 μM) at quiescent conditions. The resulting reaction profiles are very similar, indicating that preformed fibrils formed at each shaking speed are associated with very similar rates of secondary nucleation.

Fig. S6. Verification that the acceleration of the reaction observed upon the addition of preformed aggregate material is due to the addition of preformed fibrillar species rather than oligomers. An aggregation reaction (ca. 10 μM) is followed until completion, upon which the solution, as shown in A, is filtered through a 200-nm filter. (B and C) The retentate on the filter (B) and the filtrate (C) are added to monomeric peptide (ca. 5 μM). Only the retentate is observed to significantly accelerate the reaction. The concentrations of preformed aggregates in B and C are given as a percentage of the concentration of monomer peptide.
Fig. S7. Measurements of the monomer fractions for the same samples for which the oligomer fractions are shown in Fig. 4A, quantified by size-exclusion chromatography and selective radiolabeling. The ratio of the monomer concentration without (light blue bar) to that with (dark blue bar) preformed fibrils added is 2 at the measurement time, consistent with the kinetic data in Fig. 4D, because the sample without added fibrils (light blue bar) is still in the lag phase, whereas the sample with added fibrils (dark blue bar) has reached approximately the reaction half-time. The mass concentrations of oligomers in Fig. 4A are two orders of magnitude lower than the corresponding mass concentrations of monomers measured here. The combination of our ThT and radiolabeling measurements shows directly that, at the half-time of the reaction, approximately half of the protein mass remains in the monomeric state, approximately half is in fibrillar form, and only a small fraction is present as oligomers.
Fig. S8. Increase in cytotoxicity (caspase) was measured for reactions without (light blue bar) and with (dark blue bar) a small concentration of added preformed fibrils under the same conditions as the corresponding measurements of the decrease in cell viability (MTS) in Fig. 4C. In both assays, the highest toxicity is observed under conditions where the oligomer population generated through secondary nucleation is high.

<table>
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<tr>
<th>Reference</th>
<th>Estimated Aβ42 aggregate load/nM</th>
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<td>Control subjects</td>
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<td>Bao et al. (15) [table 3]</td>
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<tr>
<td>Hellstroem-Lindahl et al. (16) [figures 1 and 4]</td>
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<td>Gravina et al. (19) [table 1 and text]</td>
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<td>Tamaoka et al. (20) [table 1]</td>
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The specific source of the data within each reference is indicated in brackets. Values for the median and quartiles from this overall dataset are given in Table S2.
Table S2. Values for the median and the quartiles for Aβ42 aggregate loads calculated from the dataset shown in Table S1

<table>
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<tr>
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<td>Upper quartile</td>
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