Supplementary Information

Quantification of the Concentration of Aβ42 Propagons during the Lag Phase by an Amyloid Chain Reaction Assay

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Additional experimental data

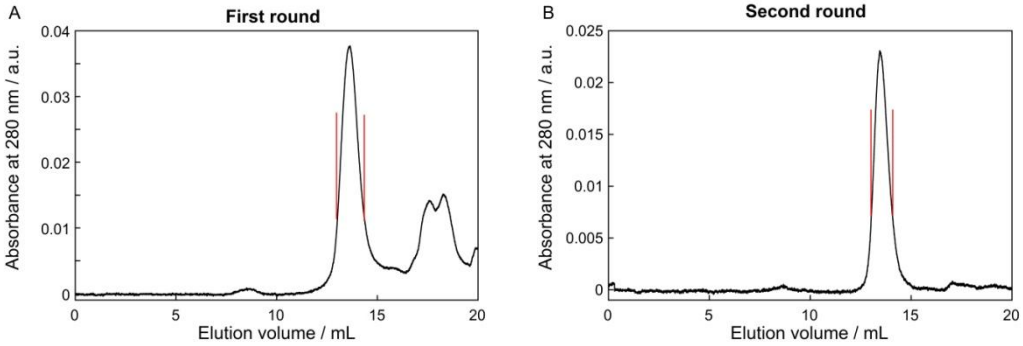

Figure S1. Repeated gel filtration to obtain pure monomer for the kinetic studies. A 1 x 30 cm Superdex 75 column was operated in 20 mM sodium phosphate buffer, pH 8.0 with 0.2 mM EDTA and 0.02% (w/v) sodium azide. The peptide was loaded in 1 mL of 6 M GuHCl and the absorbance at 280 nm was measured in a 5 mm flow-through cuvette (10 mm equivalent is shown). The monomer was collected between the vertical red lines. Between the first (A) and second (B) round, the collected peptide was lyophylized and re-dissolved in 6 M GuHCl.

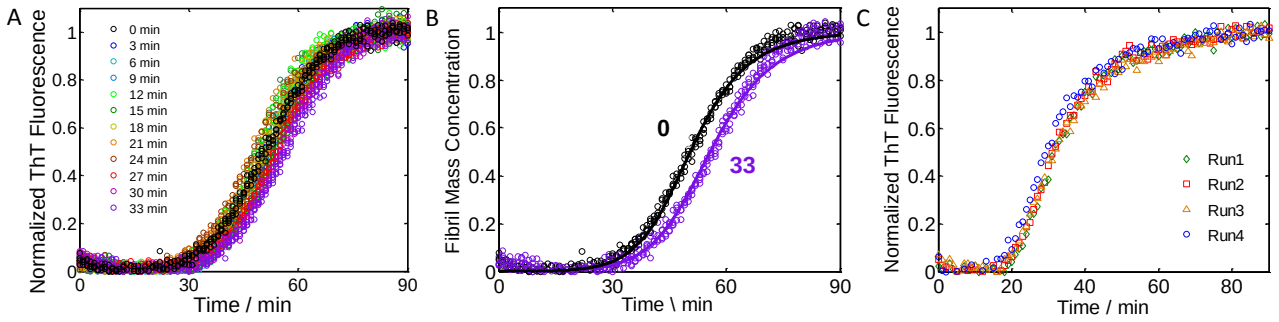

Figure S2. A) Aggregation kinetics of each filtrate of the trap-and-seed experiment supplemented with fresh 4 μM monomer monitored by ThT fluorescence. The average of four replicates is shown for each trapping time. B) Theoretical simulations of the aggregation profiles of the filtrates at time 0 and 33 min (continuous lines) are compared to experimental data. C) Seeded aggregation kinetics for fresh monomer supplemented with retentates. An example of the reproducibility of the four replicates for each trapping time is shown for the reference trapping time of 15 minutes.

The kinetic profiles corresponding to the filtrates at time 0 and 33 minutes can be well described by model simulations considering the microscopic rate constants evaluated in a previous study[1] and an initial monomer concentration of 4 μM and 3.73 μM, respectively (Figure S2B). The difference between the two values, 247±13 nM, is well in agreement with the concentration of fibrils at time 33 min evaluated by the chain polymerization assay (178±8 nM, see Figure 5 in the main text), therefore closing the mass balance.

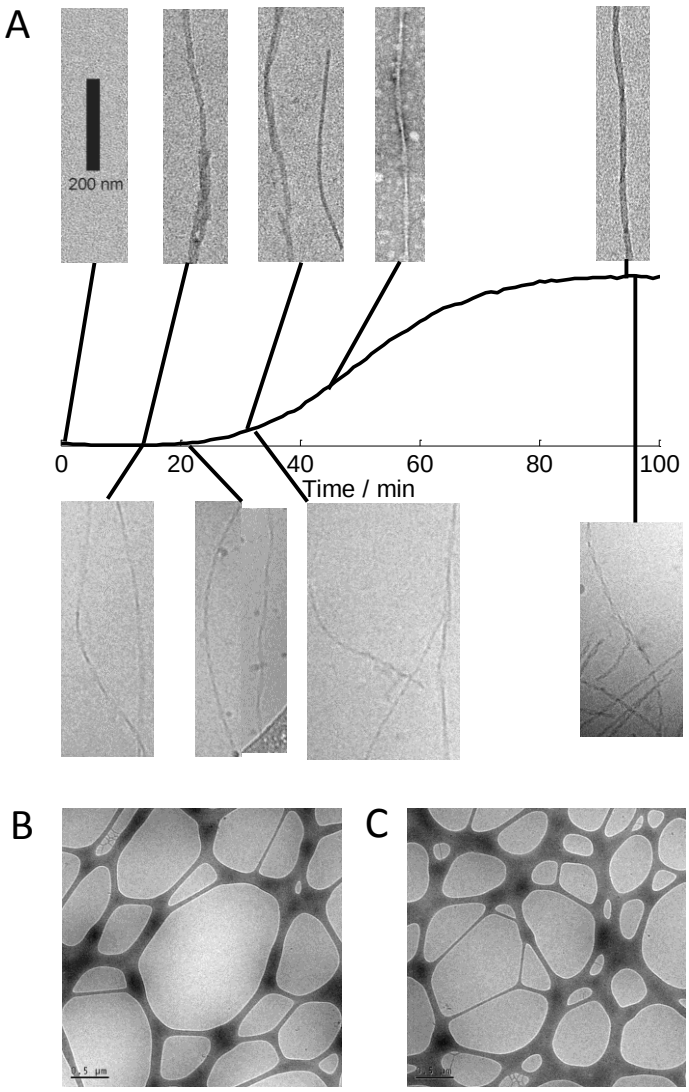

Figure S3 A) TEM with uranyl acetate stain (top) and cryo-TEM (bottom) of Aβ42 as a function of aggregation time. The time points for sample collection are indicated by black lines from the respective panels. B-C) Wide field cryo-TEM panels at 0 time (B) and at 5 minutes (C) confirm the absence of fibrils before the starting of the reaction.

Theoretical calculation of the chain polymerization reaction kinetics

The introduction of the seeds accelerates the soluble monomer conversion into fibrils by elongation, which is characterized by a much faster kinetics compared to nucleation processes. The acceleration of fibril formation is amplified by secondary nucleation processes which are catalysed by the accumulating fibril surface. It is worth noting that the effect of the seeds on the secondary

nucleation rate itself can be neglected, since in the seeded experiments the introduced seed mass (and therefore surface) is a small fraction of the total monomer pool and is negligible after few percentages of conversion. The key parameter governing the seed effect is the fibril number concentration, P_0, and not the fibril mass, M_0, as illustrated by the simulations shown in Figure S4.

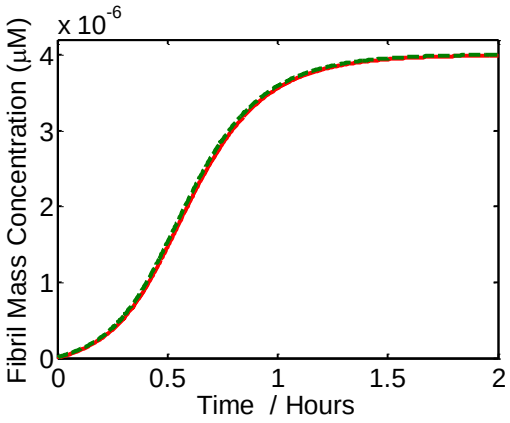

Figure S4: Simulations of the kinetics of fibril formation of a 4 μM monomeric Aβ42 solution supplemented with fibril number concentration P_0 neglecting (—), and considering (--) the seed mass M_0. The seed mass concentration M_0 is 10 nM and average fibril length is 1000 monomeric unit.

The positive feedback induced by secondary nucleation processes is progressively less relevant at increasing seed concentrations, as shown by the simulations in Figure S5. With increasing seed concentration, the monomer is preferentially sequestered into the pre-exiting fibrils by the fast elongation process, thereby reducing the number of new fibrils generated by secondary nucleation processes. This consideration predicts that the addition of a small percentage of mature fibrils will decrease the concentration of soluble oligomers generated during the fibril formation process of a given Aβ42 solution without modifying the final fibril mass load.

The effect can be approximately described as follows. The characteristic time required to consume the soluble monomers by elongation of the introduced pre-existing fibrils, P_0, is $\tau = \log(m_0)/-2k_+P_0$. The number of new fibrils generated during this characteristic time, approximating a constant secondary nucleation rate equal to the maximum rate during the process, can be expressed as:

$$P = k_2 \overline{M}\,\overline{m}^2 \tau = k_2 0.33 m_0 \left(0.66 m_0\right)^2 \frac{\log(m_0)}{-2k_+P_0} \qquad \text{(S1)}$$

The above equation overestimates the concentration of new fibrils generated since the secondary nucleation rate has been approximated as constant and equal to the maximum value during the

process, which corresponds to monomer conversion equal to 66%. Although approximate, Eq. S1 captures semi-quantitatively the effect of the seed concentration on the reduction of the generation of new fibrils, as shown in Figure S4d.

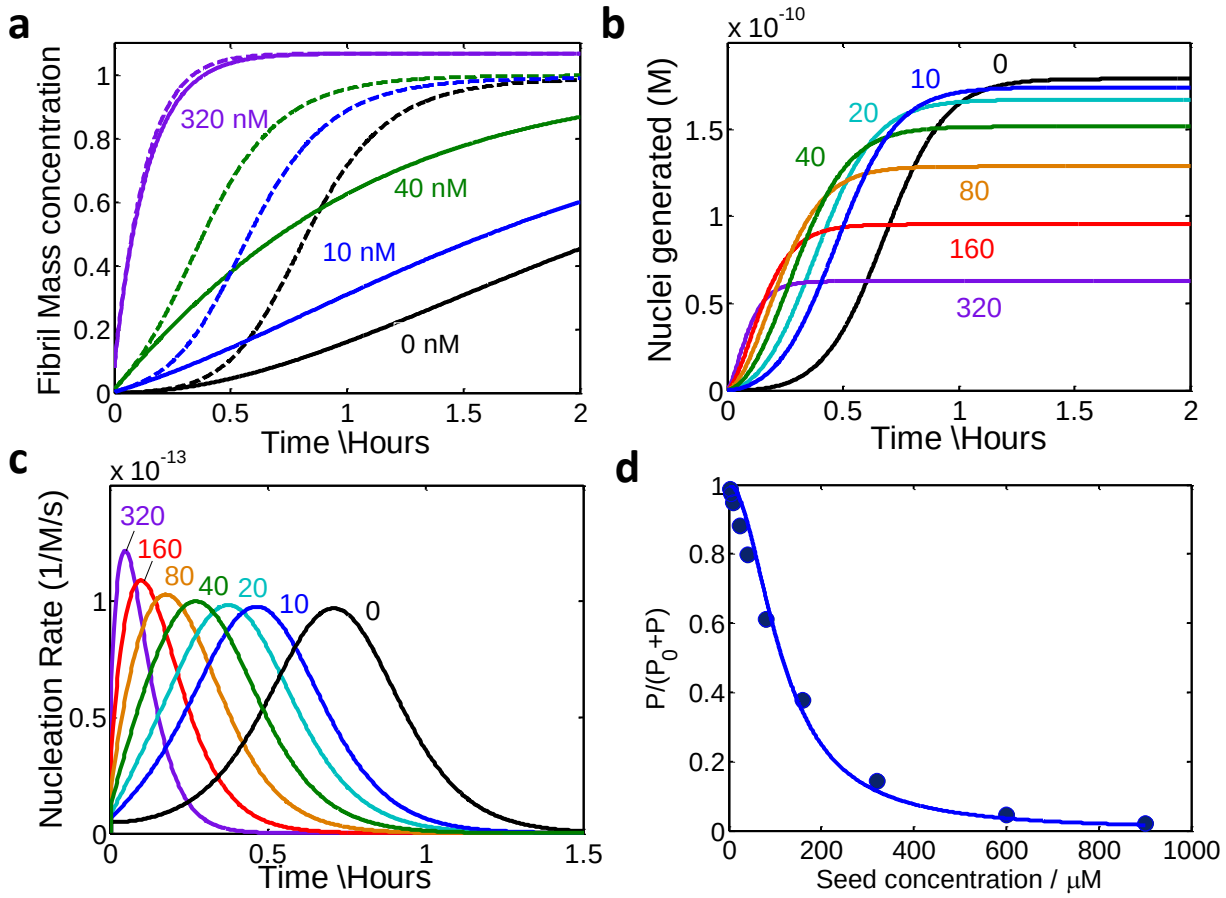

Figure S5. The positive feedback of secondary nucleation processes in un-seeded and seeded reactions. a) Model simulations of fibril formation of 4 μM monomeric Aβ42 solutions in the presence (dot lines) and in the absence (continuous lines) of secondary nucleation processes for an un-seeded reaction (black curves) and a reaction seeded with 10 nM fibrils (blue curves), 40 nM (green curves) and 320 nM (violet curves). b) Simulated time evolution of the concentration of new fibrils generated by secondary nucleation processes at different seed concentrations (indicated by the numbers closed to the lines, in nM). c) Simulated time evolution of the secondary nucleation rates corresponding to the reactions in b) at different seed concentrations. d) Fraction of the fibrils generated by secondary nucleation processes with respect to the total fibril amount at the different seed concentrations corresponding to the simulations in c). Continuous line represents calculations according to Equation S1.

The functional form of the scaling of the half-time with the seed concentration has been previously derived from considerations based on Equation 4 of the main text as:[2]

$$t_{0.5} = \frac{1}{\kappa}\left[\log\left(\frac{1}{C_+}\right) + \log\left(\left(\frac{B_+ + C_+}{\alpha}\right)(0.5)^{-1\Big/\left(\frac{k_\infty^2}{\kappa k_\infty}\right)} - B_+\right)\right], \quad \text{(S2)}$$

At limited amount of initial seeding, eq. S2 can be simplified into:

$$t_{0.5} = \frac{1}{\kappa}\left[\log\left(\frac{1}{C_+}\right) + \log\left(\theta\left[(0.5)^{-1/\theta} - 1\right]\right)\right], \text{ with } \theta = \sqrt{2\Big/\left[n_2\left(n_2+1\right)\right]}. \quad \text{(S3)}$$

The limit value for the half-time of the un-seeded experiment is equal to:

$$t_{0.5} = \kappa\left(\log\frac{2k_2 m_0^{\,n_2+1}}{k_n m_0^{\,n_C}} + \log\left(\theta\left[(0.5)^{-1/\theta} - 1\right]\right)\right) \quad \text{(S4)}$$

With increasing concentrations of seeds, the contribution of nuclei formed by primary nucleation events, represented by the term λ^2 in the rate constant C_+, can be neglected with respect to the introduced seeds. The constant C_+ can be expressed as $C_+ = c \cdot M_0$, with the constant term $c = \frac{k_+}{\kappa L} + \frac{1}{2m_0}$, and the half-time scales linearly with the logarithm of the seed concentration:

$$t_{0.5} = \frac{1}{\kappa}\left(-\log M_0 - \log c + \log\left(\theta\left[(0.5)^{-1/\theta} - 1\right]\right)\right) \quad \text{(S5)}$$

It is worth noting that the slope $1/\kappa$ depends on the single combination of microscopic rate constants k_+k_2, representing the multiplication and growth rate of the fibrillar mass, while the half-time of the un-seeded kinetics depends on both the combination k_+k_2 and the combination k_n/k_2, related to the relative impact of primary over secondary nucleation events.

In Figure S5 we show the half-time as a function of the seed concentration calculated using both the full eq. S2 and the simplified eq. S5, considering the same microscopic reaction rate constants evaluated in a previous kinetic study[1] and keeping as single fitting parameter the average fibril length, evaluated equal to 400 monomeric units. Remarkably, the theoretical calculations provide a slope $1/\kappa$ equal to 19.26 min and an half-time for the un-seeded experiment equal to 50 min, in excellent agreement with the values measured experimentally (equal to 14.6 min and 50.1 min, respectively). The corresponding integrated kinetic laws calculated with Eq. 4 in the main text are shown in Figure 1 in the main text.

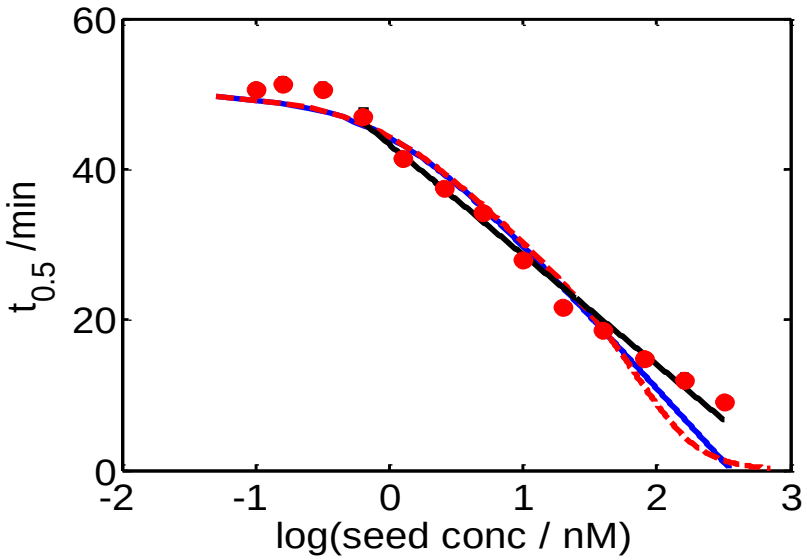

Figure S6: Scaling of the half-time for Aβ42 aggregation experiments versus initial seed concentrations: (●) experimental data; (—) values calculated according to Equation S2; (--) values calculated according to Equation S5 valid at low seed concentrations; (—) fitting to experimental data.

Time evolution of total fibrillar mass for un-seeded experiments at low monomer conversion

For an un-seeded experiment the early time behaviour of Equation 4 in the main text equals:[3]

$$M(t) = m_0\left(C_+ e^{\kappa t} - C_- e^{-\kappa t}\right) = 2m_0 C\cosh(\kappa t) = A\cosh(\kappa t) \qquad \text{(S6)}$$

with $C = C_{\pm} = \frac{\lambda^2}{2\kappa^2} = \frac{k_n m_0^{n_C}}{2k_2 m_0^{n_2+1}} = \frac{k_n}{2k_2 m_0}$, $A = 2m_0 C = \frac{k_n}{k_2}$ and $\kappa = \sqrt{2m_0^{n_2+1}k_+ k_2}$, where the sizes of the nuclei produced by primary (n_C) and secondary (n_2) nucleation events are both equal to 2.[1]

Considering the initial concentration $m_0 = 4$ μM, the values $A = 1$ and $\kappa = 0.003\, s^{-1}$ evaluated from the fitting of the data in Figure 4 correspond to a set of combinations of microscopic kinetic constants equal to $k_+k_2 = 7\cdot10^{10}$ $s^{-2}M^{-3}$ and $k_n/k_2 = 1\cdot10^{-9}$ M, which are in well agreement with the values previously obtained by the analysis of non-seeded kinetics of fibril formation during the overall time course of the process, $k_+k_2 = 3\cdot10^{10}$ $s^{-2}M^{-3}$ and $k_n/k_2 = 3\cdot10^{-8}$ M.[1]

References

(1) Cohen, S.I.A. et al., *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 9758-63.

(2) Cohen, S.I.A.; Vendruscolo, M.; Dobson, C.M.; Knowles, T.P. J. Chem. Phys. **2011**,*135*, 065106.

(3) Cohen, S.I.A.; Vendruscolo, M.; Welland, M.E.; Dobson, C.M.; Terentjev, E.M.; Knowles, T.P. J. Chem. Phys. **2011**, *135*, 065105.