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**Supporting Material**

# Branching in amyloid fibril growth

Christian Beyschau Andersen, Hisashi Yagi, Mauro Manno, Vincenzo Martorana, Tadato Ban, Gunna Christiansen, Daniel Erik Otzen, Yuji Goto, Christian Rischel

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Andersen C. B., Yagi H., Manno M., Martorana V., Ban T.,

Christiansen G., Otzen D. E., Goto Y. Rischel C.

**Supplementary Text and Figure**

## Theory of seeded fibril growth

In the following, we calculate the effect of changing seed concentration in a very simple model of fibril growth with or without secondary nucleation. We consider only the initial part of the growth kinetics, where the monomer concentration is approximately constant. Similar models have been used by previous workers in the field (1-4). Fibrils grow by monomer addition at ends, so the concentration of peptide monomers incorporated in fibrils [$A_f$] grows proportionally to the concentration of ends [$E$]:

$$\frac{d[A_f]}{dt} = k_e[E] \qquad \text{(Eq. 1)}$$

where $k_e$ is a rate constant, possibly dependent on the monomer concentration. If the formation of new ends is insignificant, the concentration of ends remains constant, and the increase in fibril mass will be linear in time, with a slope proportional to the number of seed ends:

$$[A_f]_{\text{no sec}}(t) = k[E]t$$

where the subscript *no sec* indicates the absence of a secondary nucleation mechanism. An increase in seed concentration results in a higher slope. This scenario is plotted in Figure 7a in the main text.

A secondary nucleation mechanism, *e.g.*, branching, generates new ends with a rate proportional to the amount of fibril (1):

$$\frac{d[E]_{\text{sec}}}{dt} = k_s[A_f]_{\text{sec}} \qquad \text{(Eq. 2)}$$

where the rate constant $k_s$ again possibly depends on the concentration of free monomers. By solving the coupled differential equations in Eq. 1 and Eq. 2, we find that both the number of ends and the concentration of monomers incorporated into fibrils grow exponentially

$$[E]_{\text{sec}}(t) = [E]_0 \exp(\kappa t)$$

$$\left[A_f\right]_{\text{sec}}(t) = \frac{\kappa}{k_s}[E]_0 \exp(\kappa t)$$

where $[E]_0$ is the initial concentration of ends (from the seeds) and $\kappa = \sqrt{k_e k_s}$ . We can again compare the growth kinetics in the cases of different seed concentrations giving different initial concentration of ends $[E]_0^1$ and $[E]_0^2$ :

$$\left[A_f\right]_{\text{sec}}^1(t) = \frac{\kappa}{k_s}[E]_0^1 \exp(\kappa t)$$

$$\left[A_f\right]_{\text{sec}}^2(t) = \frac{\kappa}{k_s}[E]_0^2 \exp(\kappa t) = \frac{\kappa}{k_s}[E]_0^1 \exp(\kappa(t+\tau))$$

with

$$\tau = \ln\left([E]_0^2 / [E]_0^1\right)/\kappa$$

It is seen that an increase in the seed concentration will not affect the exponential nature of the growth, but only give a shift of the time axis which reduces the lag time. This scenario is plotted in Figure 7b in the main text. It is also seen that both the form of the seeded fibrillation kinetics and the response to a change in seed concentration are changed dramatically by the presence of a secondary nucleation mechanism.

## References


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**Supplementary Figure 1**

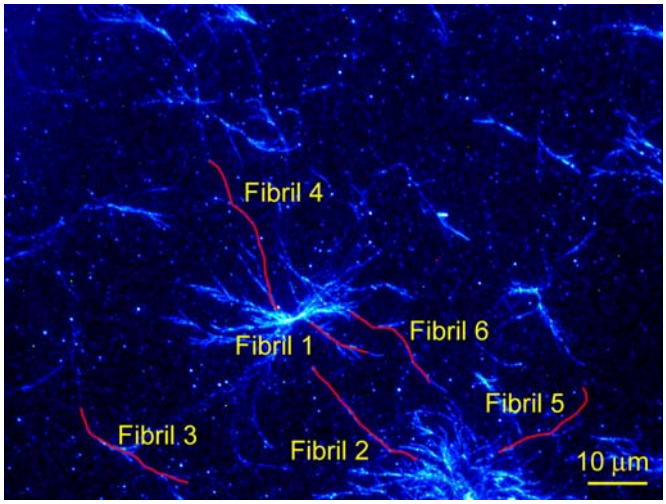


Illustration of the fibrils measured in Figure 3b of the main text. The bar represents 10 µm.

Supplementary Video 1. Real-time TIRFM QuickTime movie showing fibril growth from a seeded solution of glucagon. Fibrils grow initially by end-to-end addition of monomers to the seeds, but as time progresses, an increasing number of fibrils are formed by branching from already formed fibrils.

Supplementary Video 2. A single fibril is protruding from a cluster of seeds in the left part of the image. As the fibril grows, it continually generates new fibrils by branching from the side. This process repeats itself on the newly formed fibrils and ultimately a cascade of new fibrils are formed. Bar represents 10 μm.