Text S2: Model simulations.

Using the equations of the model described in Text S2 we have simulated the initial rates of nucleation as a function of the initial protein concentrations.

1. **Amyloid nucleation of a stable partially unfolded amyloidogenic intermediate.** In this case the I state is the most stable state. This is equivalent to the linear polymerization model of Oosawa and Kasai. To simulate this case we can assume that $K_I$ is large compared to $K_U$.

The following data were calculated for $K_I = 100$, $K_U = 0.12$, and different values of $K_A$. $k_f = 1 \text{ min}^{-1}$.

![Graph showing the relationship between initial rate and protein concentration with different values of $K_A$.](image)

The slope changes gradually from 2 to 1 as $K_A$ increases. This is logical because for low $K_A$, the monomer governs the pre-equilibrium but it has to self-associate into dimers to form nuclei. For high values of $K_A$ most of the protein is oligomeric from the beginning of the process and the process appears first order.

2. **Amyloid nucleation of a folded protein.**
In this case the pre-oligomerization equilibrium is coupled to the folding-unfolding equilibrium. The relative population of I and Ai will be reduced depending on the values of the equilibrium constants $K_I$ and $K_U$.

a) Effect of $K_I$ and $K_A$. $K_A$ was varied independently of $K_I$ for $K_U = 0.12$ and $k_f = 1 \text{ min}^{-1}$.
The rate of nucleation depends very strongly on $K_A$ and $K_I$:

- For $C_0 \ll (K_A K_I)^{-1}$ the rate of nucleation is very low and scales on the second power of $C_0$.

- If $C_0 \gg (K_A K_I)^{-1}$, the rate tends to a limit imposed by $k_F$ and the nucleation is first order.

- If $C_0 \approx (K_A K_I)^{-1}$, there is a transient increase in the slope of the double logarithmic plot. This is due to an abrupt displacement of the oligomerization equilibrium as $C_0$ increases. The value $(K_A K_I)^{-1}$ acts as a critical concentration.

b) Next we simulated curves for the same product $K_A K_I = 10^3$ but changing their relative values. $K_U$ and $k_F$ are the same as in a)
In the region of the critical concentration $C_0 \approx (K_A K_I)^{-1}$ there is a transient slope increase. The slope is higher for higher $K_A$ and lower $K_I$ values.

c) Effect of the global unfolding equilibrium constant $K_U$:

Simulations were made varying $K_U$ and the resulting values of the unfolding Gibbs energy, $\Delta G_U$. Values of $K_A$ and $K_I$ are as in b)
The unfolding Gibbs energy does not have a strong effect on the initial rates because it has a relatively low effect on the population of the amyloidogenic intermediate.