Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

Self-inhibition of insulin amyloid-like aggregation

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Methods

Insulin aggregation

Recombinant human insulin was purchased from Sigma Aldrich (91077C). Insulin amyloid-like fibrils were prepared by incubation of fresh insulin solution in 100 mM phosphate buffer pH 2.4 (with and without NaCl) at 60 °C for 24 hours in quiescent conditions. To follow aggregation kinetics, (0.5–5.0 mg/ml) samples were prepared as described above, with addition of 100 µM Thioflavin T (ThT). Aggregation kinetics were recorded at constant 60 °C temperature using QIAGEN Rotor-Gene Q real-time analyzer. Increase of ThT fluorescence intensity upon fibril formation was observed using green channel (excitation 470 nm; emission 510 nm).

Kinetics of aggregation

Initial kinetic parameters of aggregation were obtained as described by Nielsen et al.[1], using the following sigmoidal equation:

$$Y = y_i + m_i t + \frac{y_f + m_f t}{1 + e^{-\left[\frac{t - t_{50}}{\tau}\right]}} \quad (1)$$

Where Y is the fluorescence intensity, t is the time and t_{50} is the time when 50 % of maximal fluorescence intensity is reached. The initial baseline during the lag time is described by $y_i+m_i t$ and the final baseline after the growth phase has ended is described by $y_f+m_f t$ (**Supplementary Fig. S7**).

ThT maximum intensities showed linear increase upon the raise of protein concentration. Fibril concentration was estimated by matching maximum ThT fluorescence intensity to the respective initial insulin concentration for each experimental curve (assuming that aggregation efficiency is 100%) using the following equation:

$$Fibril\ concentration = \frac{\left(y_x - y_{min}\right) \times c}{\left(y_{max} - y_{min}\right)} \quad (2)$$

Where y_x is the ThT fluorescence intensity at time x, y_{min} and y_{max} are the minimal and maximal fluorescence intensities, c – initial insulin concentration.

Data analysis

Insulin aggregation models and experimental data fitting were done using rModeler (Ubicalc Software). Fitting was performed using “Classic”, “Saturated elongation”, “Classic + Tetramers” and “Classic + Capping” models on experimental results from insulin aggregation with and without NaCl.

Steps of amyloid aggregation were described using the following equations:

Primary nucleation, a process in which native protein molecules (M) change their secondary structure and become aggregation centers (A) with rate constant k_n:

$$\frac{d[A]}{dt} = k_n[M]^2 \quad (3)$$

Elongation (equation 4) or **saturated elongation (equation 5),** responsible for fibril growth (F), during which amyloidogenic proteins are added to aggregation centers (A) with rate constant k_+, where K_M determines the monomer concentration at which this process saturates:

$$\frac{d[F]}{dt} = k_+[M][A] \quad (4)$$

$$\frac{d[F]}{dt} = \frac{k_+[M][A]}{1 + \frac{[M]}{K_M}} \quad (5)$$

Secondary nucleation, the process of aggregation center (*A*) formation on the surface of fibrils (*F*) with rate constant k_2, using the surface as a catalyst:

$$\frac{d[A]}{dt} = k_2[M]^2[F] \quad (6)$$

Fibril fragmentation, resulting in the creation of new aggregation centers (*A*) due to breaks in the fibrils (*F*) with rate constant k_-:

$$\frac{d[A]}{dt} = k_-[F] \quad (7)$$

Equilibrium between monomers (*M*) and tetramers (*T*), where k_t *and* k_m are the tetramer and monomer formation rate constants:

$$\frac{d[T]}{dt} = k_t[M]^4 \quad (8)$$

$$\frac{d[M]}{dt} = 4k_m[T] \quad (9)$$

"Capping" of aggregation centers (*A*) by tetramers (*T*) with rate constant k_c:

$$\frac{d[A]}{dt} = -k_c[A][T] \quad (10)$$

Dynamic light scattering (DLS)

For DLS experiments, freshly prepared 0.5-5.0 mg/ml insulin solutions at different NaCl concentrations were filtered through 0.45 µm syringe filters. The size measurements were performed at 60 °C using Malvern Zetasizer µV. For each sample 3 repeats of 10 scans were recorded.

Circular dichroism (CD)

For CD experiments, 5 mg/ml insulin samples, with and without 100 mM NaCl, were filtered through 0.45 µm syringe filters. The samples were incubated at 60 °C and CD spectra were measured every 15 minutes in the 190-280 nm wavelength region using a Jasco J-815 Spectropolarimeter with a 0.1 mm path length cuvette. Each measurement was repeated 3 times.

Atomic force microscopy (AFM)

For AFM experiments 30 µl of the 5.0 mg/ml (diluted 10 times) insulin fibril solutions, containing 0, 25, 50, 75 and 100 mM NaCl, were deposited on freshly cleaved mica and left to adsorb for 1 min, the samples were gently rinsed with water and dried using airflow. AFM images were recorded in the Tapping-in-Air mode at a drive frequency of approximately 300 kHz, using Bruker Dimension Icon scanning probe microscope system and aluminium-coated silicon tips RTESPA-300 as a probe.

Fourier-transform infrared spectrometry (FTIR)

To prepare monomer samples, 5.0 mg of insulin were dissolved in 100 mM phosphate buffer pD 2.4 (in D_2O) with and without 100 mM NaCl.

To prepare fibril samples, insulin fibrils were separated from buffer solution by centrifugation at 20 000 x g for 30 min and later resuspended in D_2O, the procedure was repeated three times. All samples were sonicated for 1 min using Bandelin Sonopuls 3100 ultrasonic homogenizer equipped with MS73 tip (using 50% of the power, total energy applied to the sample - 1.12 kJ).

The FTIR spectra were recorded using Bruker Vertex 80v IR spectrometer equipped with mercury cadmium telluride (MCT) detector. For all measurements, CaF_2 transmission windows and 0.05 mm Teflon spacers were used. Spectra were recorded at room temperature under vacuum conditions (~ 2 mBar). For each spectrum, 256 interferograms of 2

cm^{-1} resolution were co-added. A corresponding buffer spectrum was subtracted from each sample spectrum. All the spectra were normalised to the same area of amide I/I' band (1700-1595 cm^{-1}). All data processing was performed using GRAMS software.

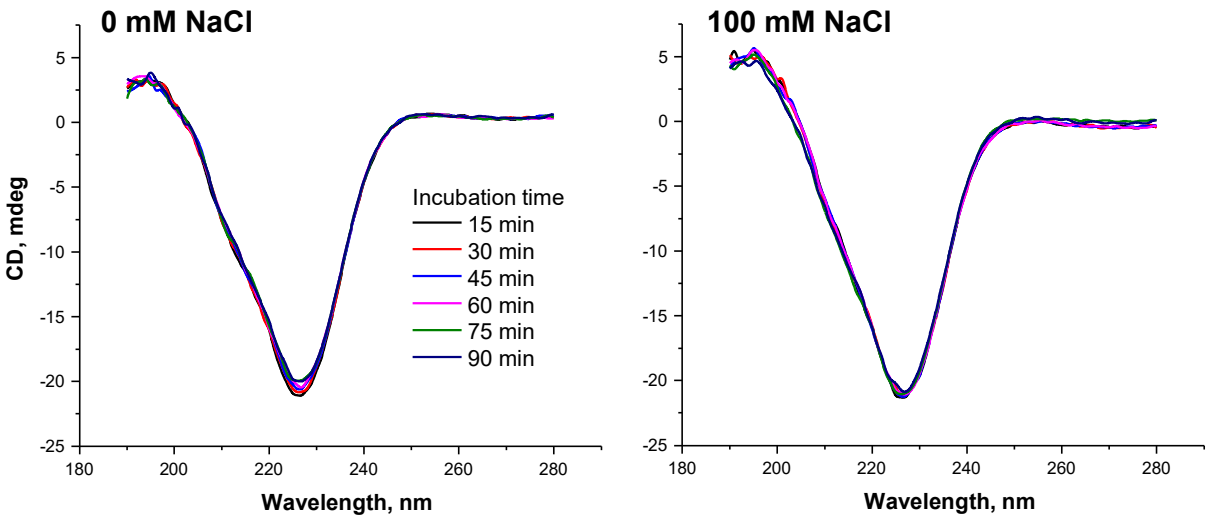

Figure S1. CD spectra of 5 mg/ml insulin samples in the absence and presence of 100 mM NaCl, measured every 15 min at 60°C during the lag phase.

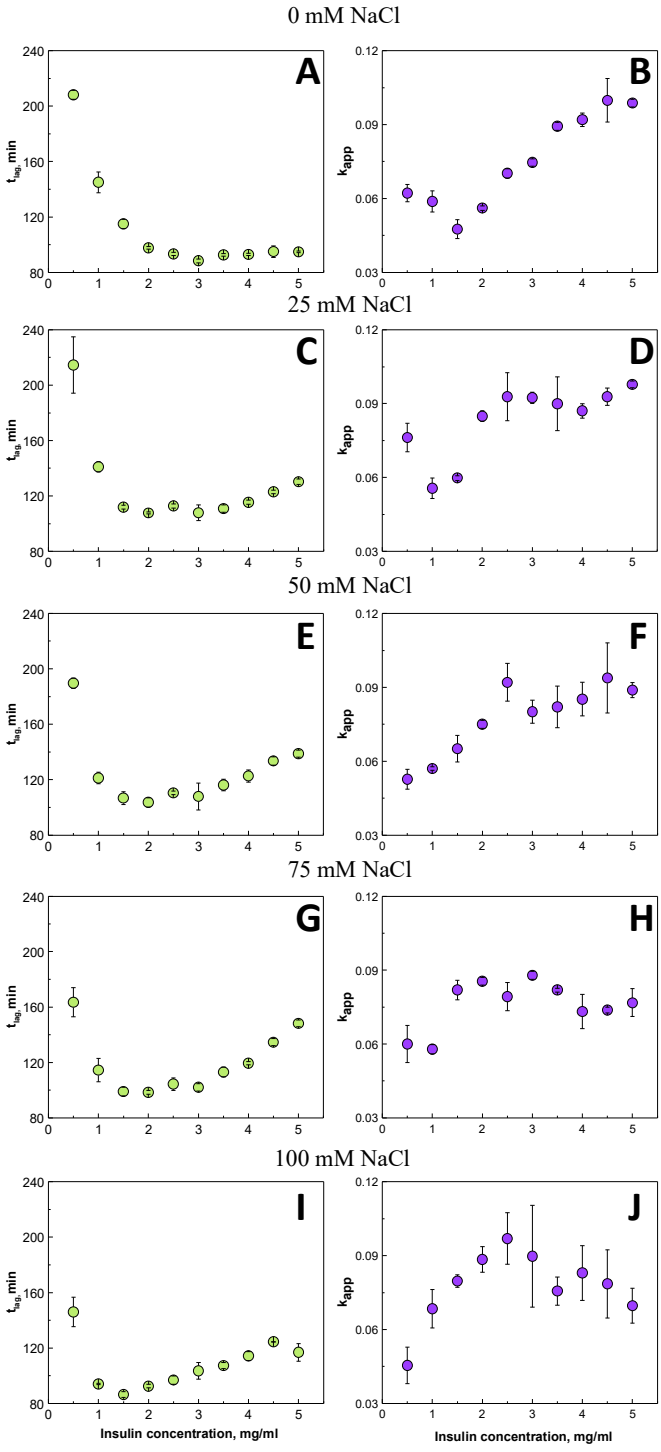

Figure S2. Dependence of insulin aggregation lag time (t_{lag}) (**A, C, E, F, I**) and apparent growth rate constant (k_{app}) (**B, D, F, H, J**) on concentration under a range of NaCl concentrations from 0 to 100 mM. Error bars are standard deviations estimated from three repeats.

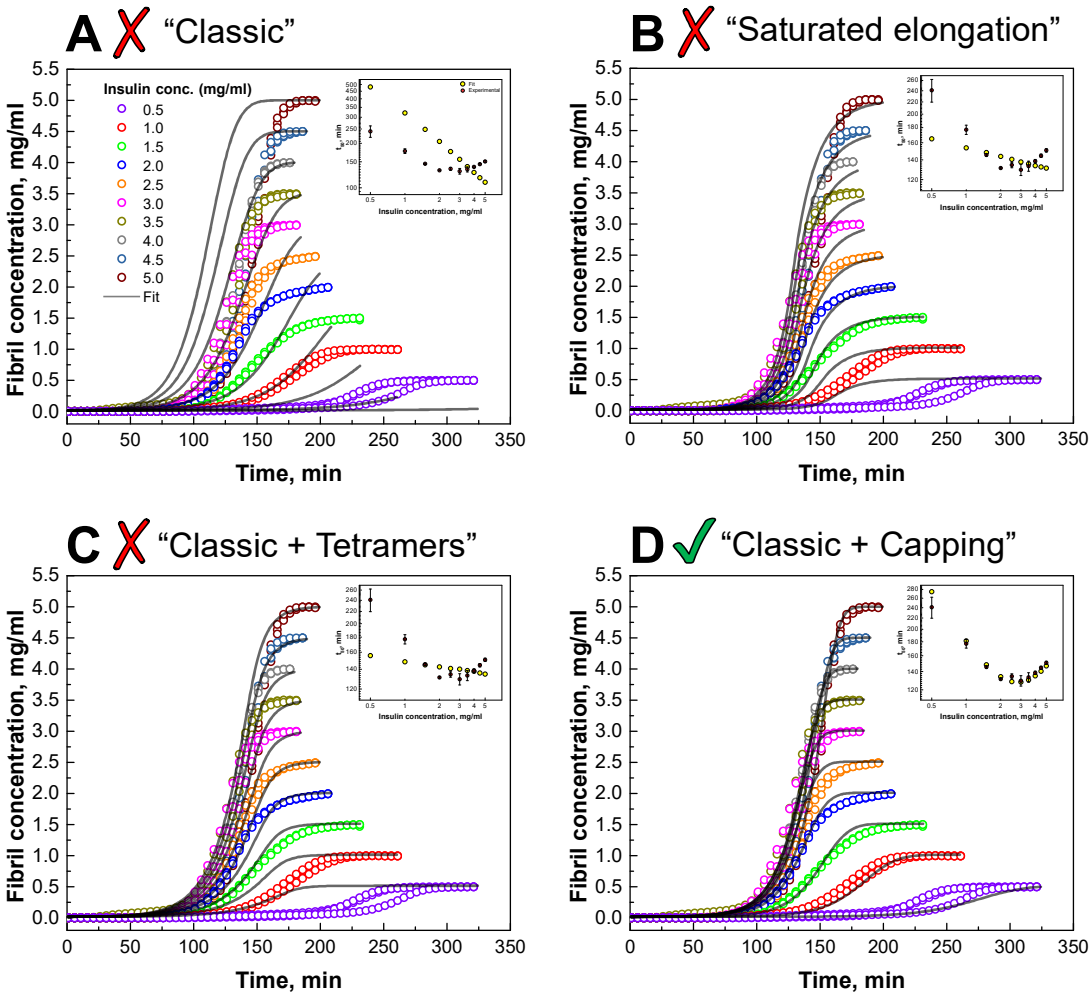

Figure S3. Global fits of experimental data with 25 mM NaCl. The global fit of "Classic" (**A**), "Saturated elongation" (**B**), "Classic + Tetramers" (**C**) and "Classic + Capping" (**D**) model to the experimental data. In each case primary and secondary nucleus size was set to 2. Open circles represent normalised experimental data (3 repeats for each protein concentration).

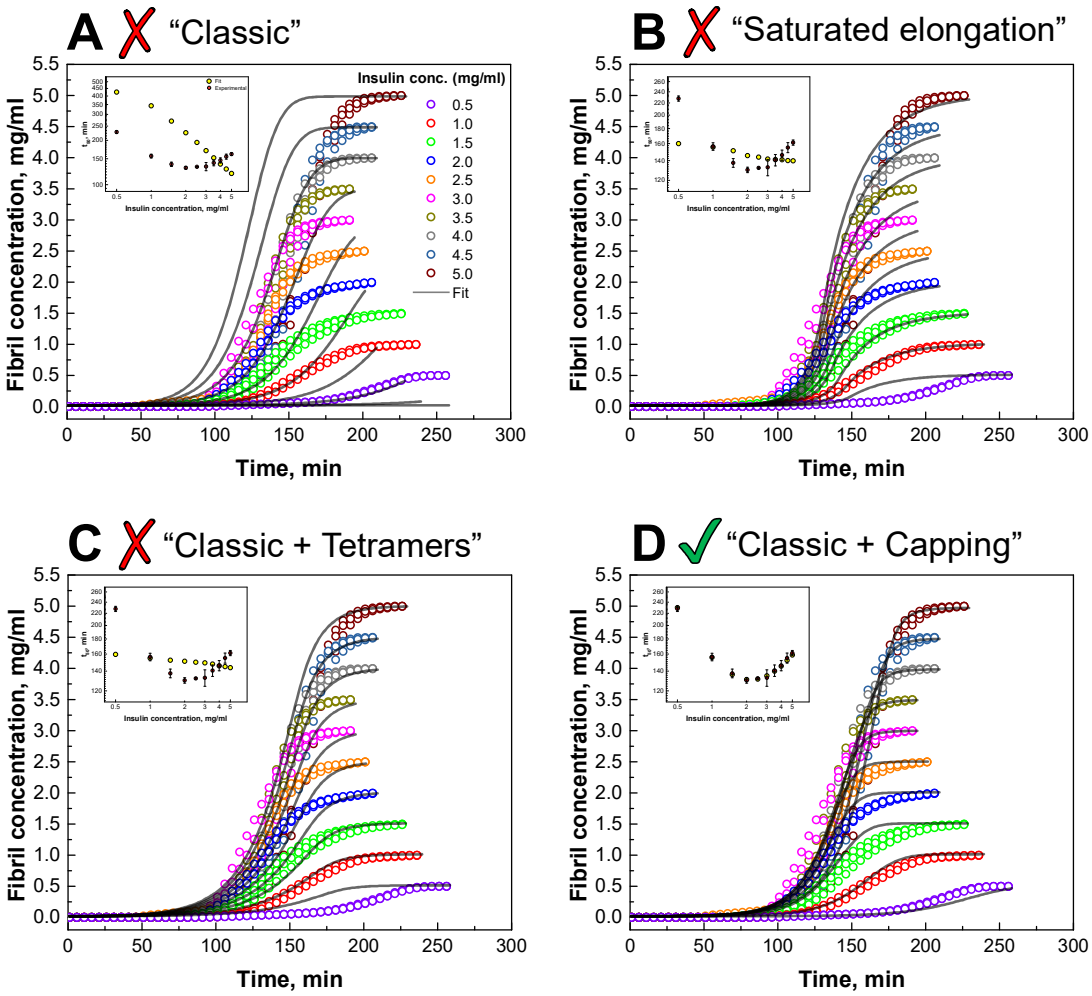

Figure S4. Global fits of experimental data with 50 mM NaCl. The global fit of "Classic" (**A**), "Saturated elongation" (**B**), "Classic + Tetramers" (**C**) and "Classic + Capping" (**D**) model to the experimental data. In each case primary and secondary nucleus size was set to 2. Open circles represent normalised experimental data (3 repeats for each protein concentration).

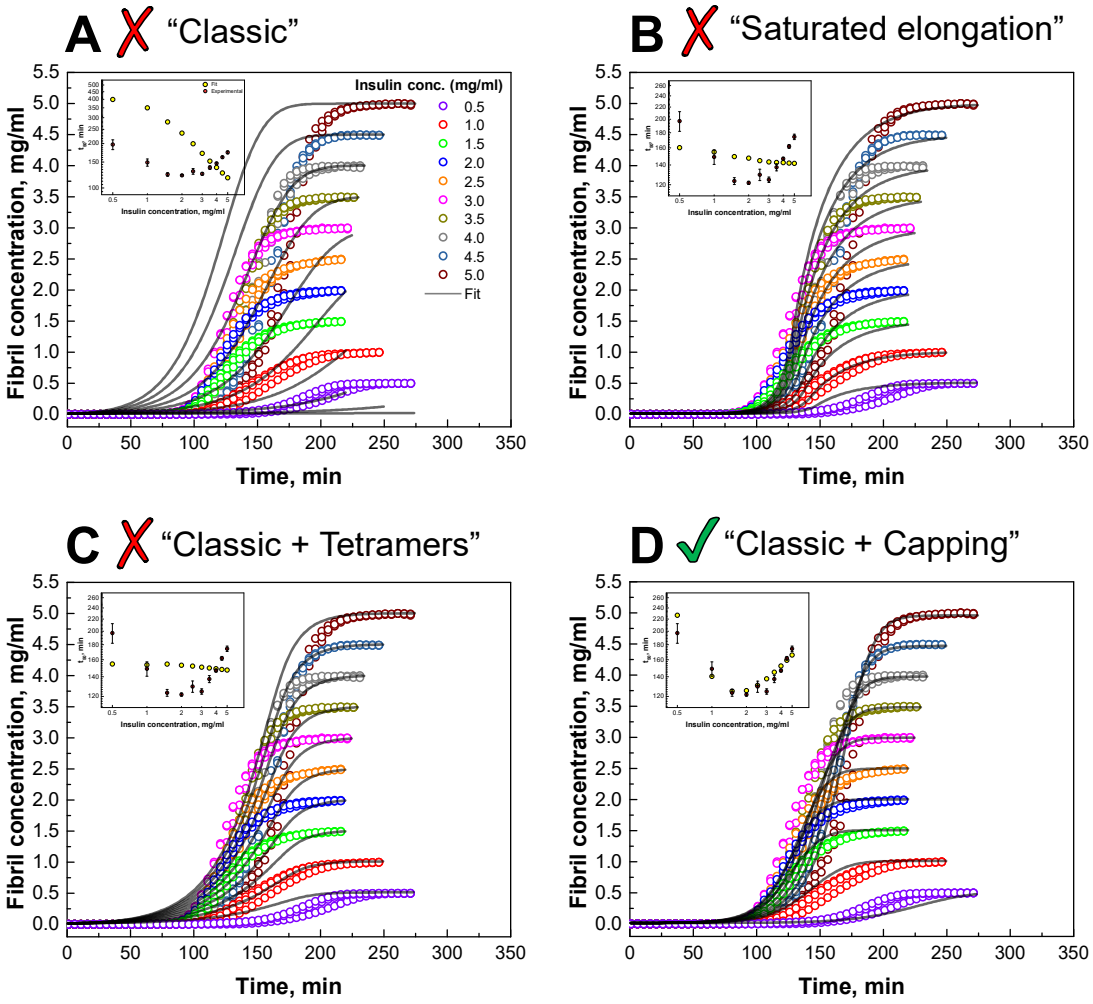

Figure S5. Global fits of experimental data with 75 mM NaCl. The global fit of "Classic" (**A**), "Saturated elongation" (**B**), "Classic + Tetramers" (**C**) and "Classic + Capping" (**D**) model to the experimental data. In each case primary and secondary nucleus size was set to 2. Open circles represent normalised experimental data (3 repeats for each protein concentration).

$$Y = y_i + m_i t + \frac{y_f + m_f t}{1 + e^{-\left[\frac{t - t_{50}}{\tau}\right]}}$$

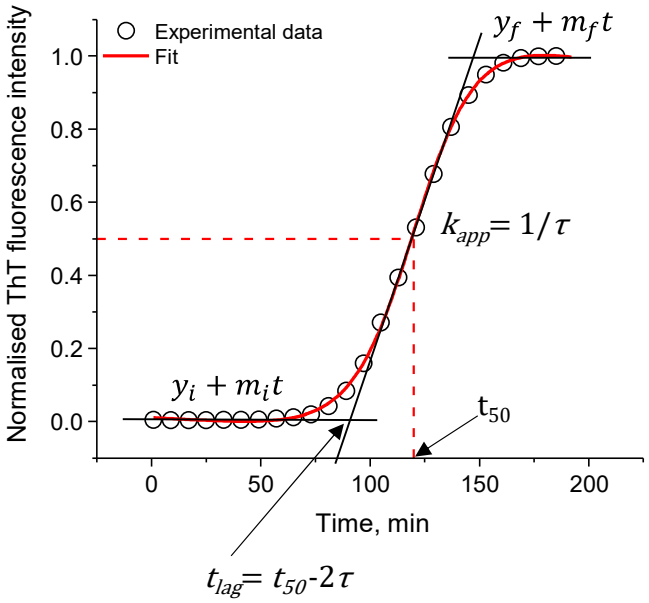

Figure S6. Initial kinetic parameters of aggregation can be found using sigmoidal fit: t_{50} is the time when 50 % of maximal fluorescence intensity is reached; t_{lag} is the time during which no detectable aggregation occurs; k_{app} is the apparent rate constant of fibril growth.

References

1 L. Nielsen, R. Khurana, A. Coats, S. Frokjaer, J. Brange, S. Vyas, V. N. Uversky and a L. Fink, Effect of environmental factors on the kinetics of insulin fibril formation: elucidation of the molecular mechanism., *Biochemistry*, 2001, **40**, 6036–46.