Stop-and-go kinetics in amyloid fibrillation

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Many human diseases are associated with protein aggregation and fibrillation. Using glucagon as a model system for protein fibrillation we show that fibrils grow in an intermittent fashion, with periods of growth followed by long pauses. Remarkably, even if the intrinsic transition rates vary considerably in each experiment, the probability of being in the growing (stopping) state is very close to 1/4 (3/4), suggesting the presence of 4 independent conformations of the fibril tip. We discuss this possibility in terms of existing structural knowledge.

Protein fibrillation is the process by which misfolded proteins tend to form large linear aggregates [1]. Its importance is related to the role played in many degenerative diseases, such as Parkinson's, Alzheimer's, Huntington and prion diseases [2]. While our knowledge of the structural properties of these fibrils improves at great pace [3, 4], the dynamics of their growth process is still poorly understood. The formation of amyloid fibrils involves at least two steps: the formation of growth centers by primary nucleation, which is often a slow process, followed by elongation through addition of monomers [5]. In many cases, a so-called secondary nucleation mechanism is also involved, whereby new growth centers are formed from existing fibrils [6, 8, 9]. Whereas the process of secondary nucleation is known to entail a number of different mechanisms [9, 10], the primary elongation process has not been elucidated to the same level of detail.

In this communication, we present an experimental and theoretical study of the elongation process of glucacon fibrils. Glucagon is a small peptide hormone consisting of only 29 amino acids produced in the pancreas. It has the opposite effect to that of insulin and therefore increases blood glucose levels when released. As a model system for protein fibrillation, glucagon kinetics has provided insights into the early oligomerization stages of the process [11–13], the interplay between growth and fibril morphology [14, 15] and amyloid branching [9]. Here, we focus on the properties of the late-stage elongation process.

Experiments were performed on samples of glucagon monomers in solution. In order to detect the growth, a specialized fluorescence microscopy technique was applied, the so-called Total Internal Reflection Fluorescence Microscopy (TIRFM) (Supplementary method). The TIRFM images of the fibrillation process were obtained at initial glucagon concentration of $\rho = 0.25$ mg/ml in aqueous buffer (50 mM glycine HCl pH 2.5) with preformed seeds. Images of the growth are shown in Fig. 1 at three consecutive times $t = 0,\ 216\ ,\ 407$ min.

Because the fibrils grow along the glass slide we are able to track each fibril length as function of time. We monitor 16 independent fibrils for each image frame in the experiment. The time interval, Δt, between frames varies from a minimum value of $\Delta t_{min} = 1$ min. to a maximum value $\Delta t_{max} = 35$ min., with a typical value of $\Delta t = 10$ min.. The total duration of the experiment is $t = 525$ min. The combined results for the 16 fibrils are shown in Fig. 2.

A striking feature of the fibril dynamics is its discrete nature, where long periods of growth are interrupted by extended periods of stasis (stop state). This prompted us to collect the statistics of time spent in the growth (g) and the stop (s) state, $f_g(t)$ and $f_s(t)$ respectively, for all 16 fibrils. In Fig. 3, these distributions are shown on semi-logarithmic plots. Note that the finite sampling rate implies that actual time spent in given state can only be estimated in terms of upper and lower bounds (Supplementary Methods). The upper estimates are shown by the dashed blue curves and the lower estimates are shown by the full blue curves. As seen in the figure, the difference between the distributions for the upper and lower estimates of both f_g and f_s are marginal. All distributions are very well fitted by exponential functions, $f_g(t) \sim \exp(-k_- t)$ and $f_s(t) \sim \exp(-k_+ t)$, as shown by the yellow curves (here, the dashed yellow curve is the fit to the upper estimates and the full yellow curve

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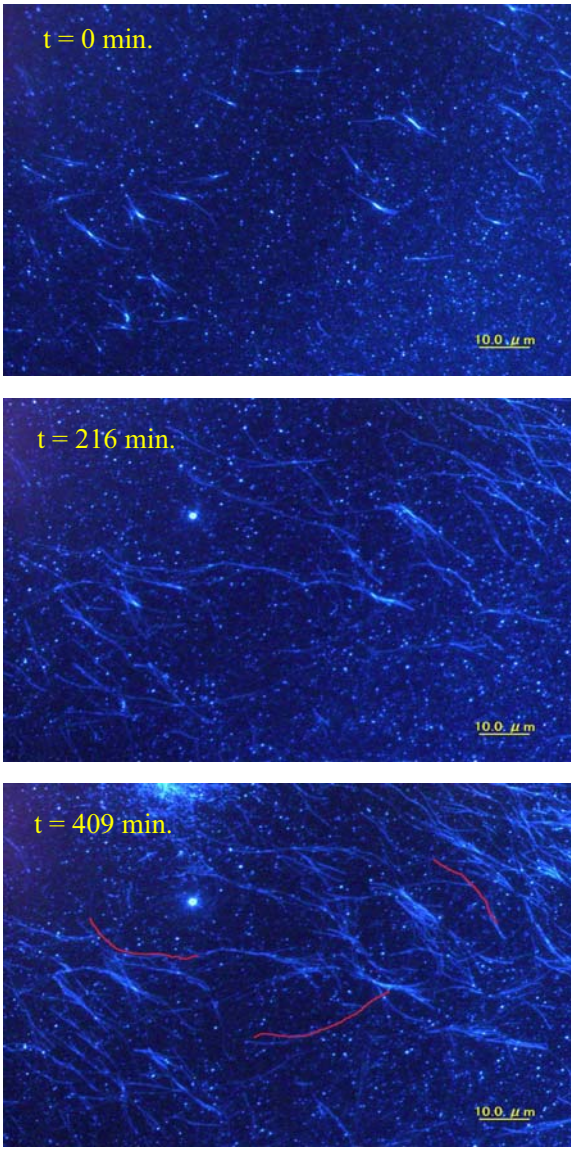

FIG. 1: TIRFM images of glucacon fibril growth with initial glucagon concentration of $\rho = 0.25$ mg/ml in aqueous buffer (50 mM glycine HCl pH 2.5) at three consecutive times after the initiation of the aggregation. Red lines in the last picture mark examples of fibrils which are tracked during the growth process.

is the fit of the lower estimates). The fits are of excellent quality over almost two decades, as signified by high R values ($R^2 > 0.98$). The values obtained are $k_+ = 9.0 \cdot 10^{-3}$ min^{-1} and $k_- = 2.8 \cdot 10^{-2}$ min^{-1}. A series of four independent experiments have been performed with the same glucagon monomer concentration (0.25 mg/ml) and pH (2.5) with differences in the seed concentrations (∼20% variation) and data acquisition only (Supplementary Table 1). In all four cases the same analysis and procedure was performed resulting in exponential distributions of similar high quality.

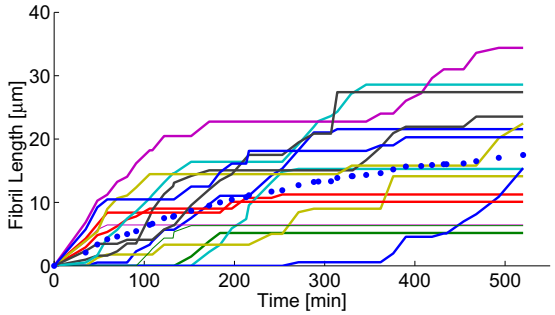

FIG. 2: Length as a function of time for 16 fibrils tracked from the images shown in Fig 1. Note the long plateaus, corresponding to the stop states, followed by shorter (on average) growing periods. The average growth is indicated with a dotted blue line. The sampling time between each image can be seen from the time separation between each point.

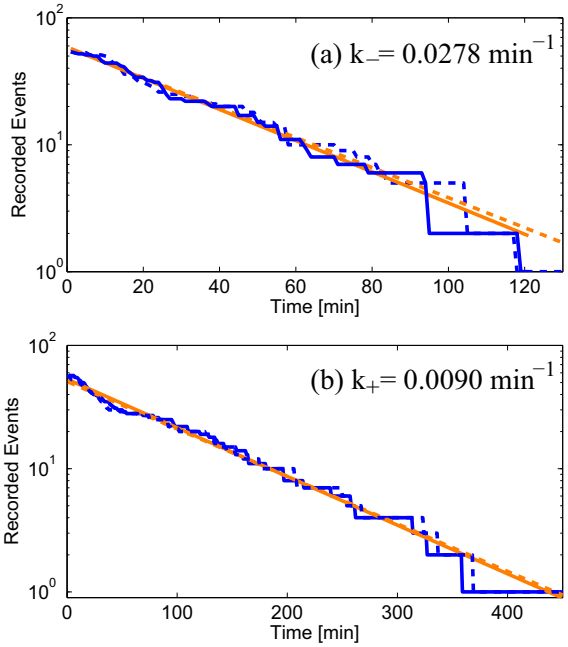

FIG. 3: Growth $f_g(t)$ (a) and stop $f_s(t)$ (b) times distributions on semi-logarithmic scales. Blue lines are data and yellow straight lines are the exponential fit. Both for data and fits, continuous lines are the lower estimates and dashed lines are the upper estimates (see text). All fits are of extremely good quality as indicated by the large R-value, $R^2 > 0.98$.

The observed stop and go behaviour of fibril dynamics is clearly not associated with the discrete nature of monomer attachment (Supplementary Methods). The simplest model of the process is to assume that the fibril exhibits two internal states: one in which it is allowed to grow, with a rate g and one in which it cannot

grow (Supplementary Figure 1). Consistent with data, the time spent in each state would be exponentially distributed and the transition rates between the two states can therefore be identified with the observed rates k_+ (stop$\rightarrow$ growth) and k_- (growth $\rightarrow$ stop). Denoting the total rate $k = k_+ + k_-$, one can calculate the probability of being in the growing state, $p_+ = k_+/k$ and in the stopped state, $p_- = k_-/k$. By comparing the results from 4 independent experiments we observe that the rates k_+ and k_- vary quite significantly, up to a factor 3 (Supplementary Table 1). A possible explanation for this could be the variation in sampling frequency, due to heating of the sample by the laser. Indeed, the dependency of the fibril growth on both the laser intensity as well as the illumination time has been observed under similar experimental conditions for β_2-microglobulin kinetics [16].

The striking result is that, even though both transition rates vary among experiments, they combine in such a way that the probabilities of growing and stopping, p_+ and p_-, do not change appreciably in different experiments (Supplementary Table 1). In particular, p_+ is always very close to 1/4 and consequently p_- is very close to 3/4. Notice that, if the difference between the growing and the stopped state would have been due to an energy gap, one would have expected the population ratio of the two states to be much more sensitive to variations in the individual transition rates, $k_\pm$. Conversely, the constancy of this ratio suggests that the energy difference between the growing and the stopped states to be irrelevant. This ratio could then reflect the presence of three stopped configurations for each growing one, all of them being iso-energetic.

Recently, similar stop-and-go behavior of the fibril elongation of $A\beta$ -peptides [17, 18] and α-synuclein [19] have been reported although the timescales involved are 1-2 orders of magnitude faster. This suggests that the observed kinetics reflects the presence of some kind of structural change at the fibril ends, which is not necessarily specific to glucagon. In this picture, the protein properties would affect the barrier height, and thus the timescale of the process, only.

If our hypothesis about the existence of approximately isoenergetic states holds up to scrutiny, it suggests that there may be an additional dimension in the fibrillation energy landscape that cannot easily be identified by conventional techniques. Glucagon is known to adopt a number of different conformations depending on the fibrillation conditions, but these conformations differ considerably in energy and are unlikely to co-exist to an equal extent [20] . Rather, it is possible that we have a number of closely related states with different propagation properties which are separated by high local activation barriers within a relatively flat ground state level. Further experimental studies are required to establish the validity of our scenario.

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Author Contribution

CBA, DO, HY and YG designed the experiment. JFB, JF, SK, SP and MHJ analyzed the results. JFB, SK, SP, DO and MHJ wrote the manuscript.

Competing interests statement

The authors declare no competing financial interests.

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