# *Supporting Information*

## Assembly of Glucagon (Proto)fibrils by Longitudinal Addition of Oligomers

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## Experimental Section

**Sample Preparation.** The frozen glucagon powder (purchased from Sigma) was dissolved in Milli-Q water (18.2 MΩ.cm) to a concentration of 1.0 mg/ml (pH 6-7) and then incubated under 37 $^{o}$C for different periods of time. The process of sample preparation for *ex situ* AFM measurement was as following: a drop of 10 µl glucagon solution was deposited onto freshly cleaved mica surface and then rinsed gently by Milli-Q water, finally dried under compressed nitrogen stream. For *in-situ* AFM observation, freshly made glucagon solution with a concentration of 0.5–1 mg/ml was used and injected into the fluid cell. Then the fibrillation of the protein on the mica surface was monitored with tapping mode AFM.

**Instrument.** *Ex-situ* and *in-situ* AFM images were collected in tapping mode on a NanoScope IIIa system (Veeco Instruments). *Ex-situ* AFM measurements were performed in air with controlled humidity (about 35%) at room temperature. Silicon cantilevers were used for AFM imaging in air with a nominal spring constant of about 4.5 N/m and resonant frequency of 150 KHz (NSC12, MikroMasch), while silicon nitride cantilevers (NPS, Veeco) with a nominal spring constant of 0.32 N/m and frequency of 6-10 KHz were used in liquid for *in situ* observation of protein aggregation process. The scan rate for both *ex-situ* and *in-situ* AFM images was 1.0 Hz.

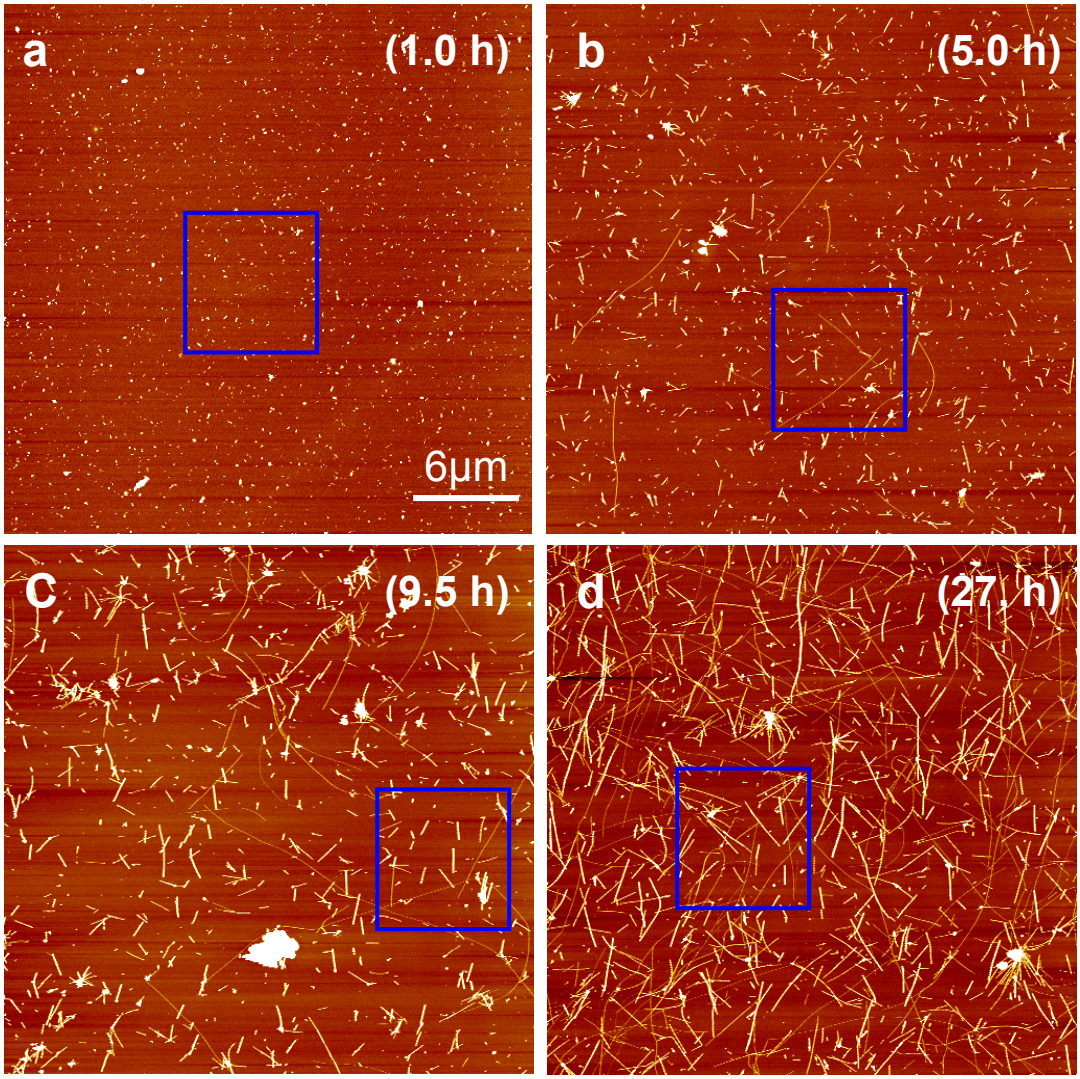


Figure S1. *Ex situ* AFM images of glucagon after incubating for four different time periods. a) 1.0 h, b) 5.0 h, c) 9.5 h, d) 27 h, respectively. The magnified AFM images of indicated areas marked with blue squares are shown in the main text (Fig. 1). Scale size: 30 × 30 $\mu m^2$.

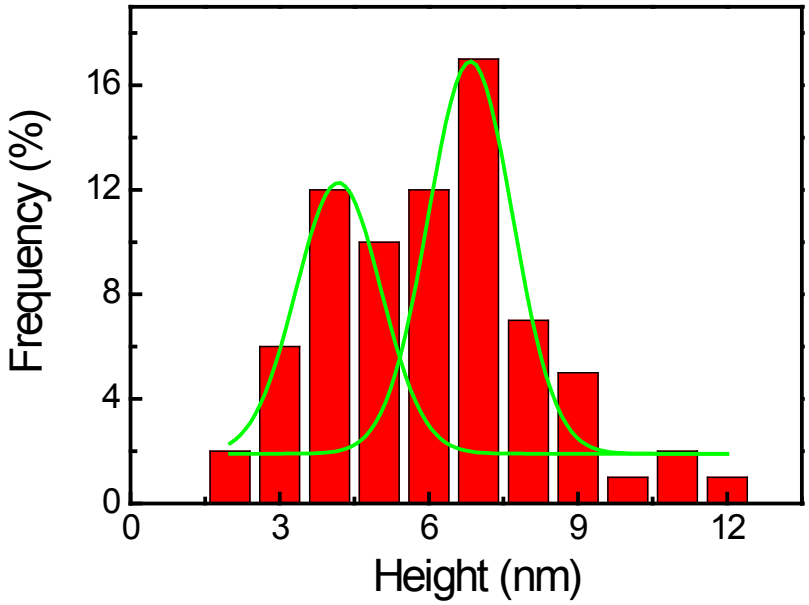


Figure S2. Height distribution of aggregates after incubating for 1 hour. Two green lines are the Gauss fit of two peaks, which are used only for eye guiding.

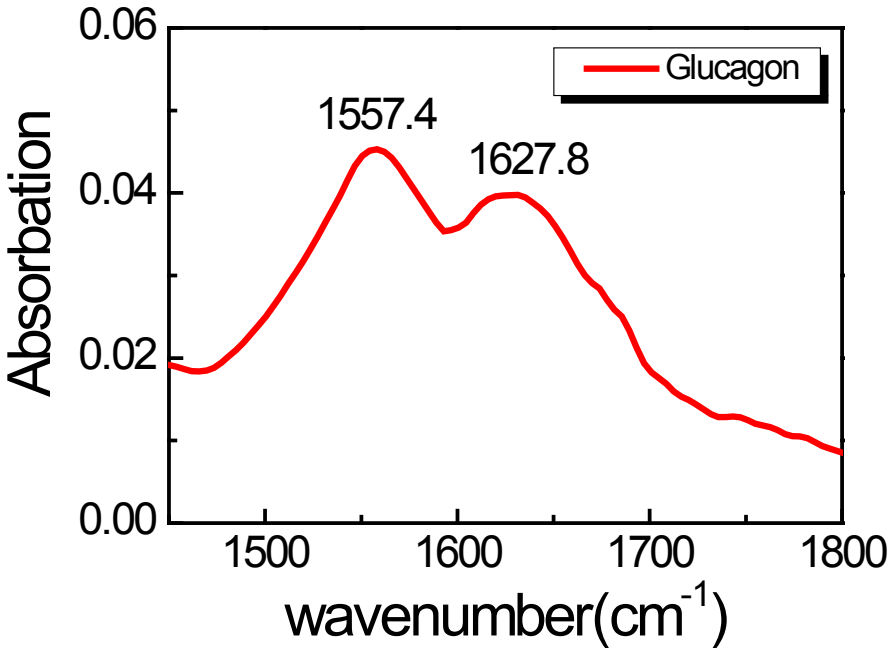


**Figure S3.** The Micro-FTIR spectra of glucagon, showing that these fibrils are clearly rich in β-sheet structures with a maximum absorbance of around 1628 $cm^{-1}$ at amide-I band.

Experiments: Micro-FTIR spectra were recorded using a Thermo Scientific Nicolet 6700 spectrophotometer. This apparatus was equipped with a Nicolet continuum XL microscope and micro-FTIR measurements. A drop of protein solution (3-5 μl) was put on substrate and waited for drying. The absorbance spectra of the samples were collected at a resolution of 4.0 $cm^{-1}$ by subtracting the background.

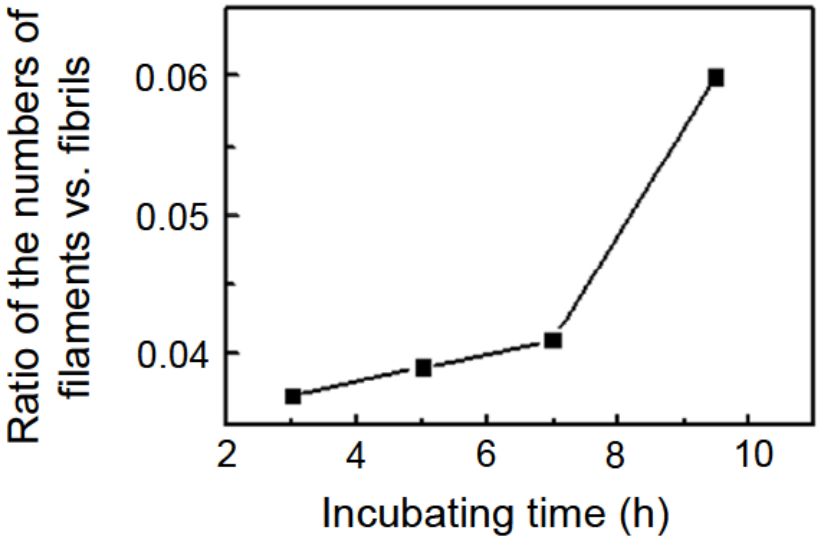


**Figure S4**. Ratio of the numbers of filaments vs. protofibrils as a function of incubating time, showing that protofibrils pre-dominated over filaments at early stage (<7h) and a rapid increase with time.