# Hybrid bioinorganic insulin amyloid fibrils

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## Supplementary information

### Materials and methods

**Materials -** all the chemicals including bovine insulin were purchased from Sigma-Aldrich, and used without further purification

**Transmission Electron Microscopy – A** 20 µl aliquot of mineralized insulin fibril was placed on a copper grid with carbon treatment. Samples were viewed by a Tecnai G2 20 UT microscope working at 200 kV, Energy-dispersive X-ray (EDX) analysis was performed with an EDAX detector with calibration.

**Atomic Force Microscope – A** 20 µl aliquot of mineralized insulin fibril was placed on a silicon wafer with the size of 1 cm * 1cm by nitrogen blowing. Samples were viewed by a Veeco Dimension 3000 microscope.

**Insulin fibril formation** Bovine insulin was dissolved in 2M guanidine hydrochloride and was dialyzed against three rounds of 25 mM HCl at 4 ºC. A stock solution containing 0.1mM bovine insulin in 25 mM HCl was prepared by diluting the dialyzed sample with 25 mM HCl. The reaction mixture was then placed in a hot plate and was kept at 65 ºC for 20 h without agitation, which resulted in the formation of amyloid fibrils as determined by AFM.

**Potassium hexachloroplatinate functionalized insulin fibril** The as prepared solution of amyloid fibrils (0.1 mM) was incubated at room temperature with 0.1 M water soluble hexachloroplatinic acid for 10 minutes. The pH value was kept at about 1. Excess hexachloroplatinic acid was removed by dialysis at 4 °C against three rounds of water, followed by addition of an aqueous solution of potassium chloride. This resulted in the formation of a weakly yellow precipitate. The formation of hexachloroplatinic acid coated fibril was demonstrated by TEM analysis. ***Be cautious, hexachloroplatinic acid is toxic.***

**Calcium apatite mineralized insulin fibril.** Similar to the protocol described above, a 0.1 mM amyloid fibrils solution was incubated with 0.5 M potassium phosphate for 10 minutes. The pH value of the solution was monitored and kept within 9.5-10. Excess phosphate salt was removed by dialysis. During the addition of phosphate, a

precipitation gradually occurs, resulting in the formation of a separated layer. The resulting solution was then incubated for 10 minutes at room temperature with a 0.5 mM calcium chloride aqueous solution, which resulted in the calcification of the amyloid fibrils, which was demonstrated by TEM analysis.

## Figure legends

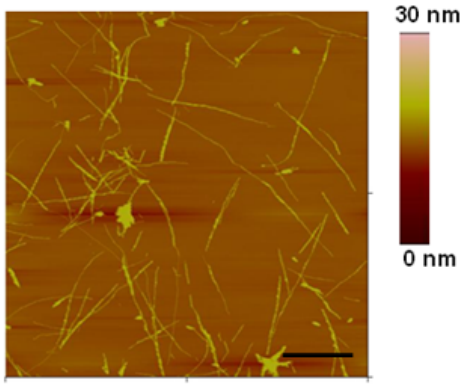


***Figure S1*** AFM image of dilute insulin fibril (Scale bar 1μm). The length is ranged from 1μm to 4 μm.

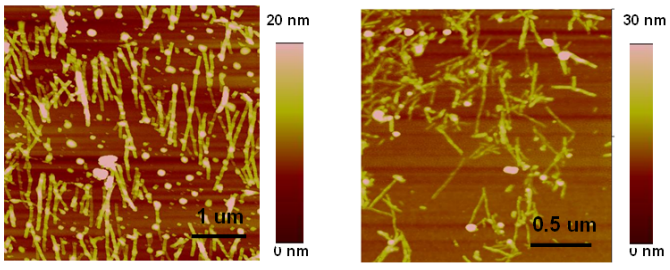


***Figure S2*** AFM image of intermediate product from anionic phosphate decorated (left) and fully functionalized insulin fibril (right). Compared with the pristine amyloid fibril, these fibrils have a larger diameter (about 8 nm and 15 nm respectively).