# Supplementary Material

to *"Heparin assisted assembly of somatostatin amyloid nanofibrils results in disordered precipitates by hindrance of protofilaments interactions"*
*by Dharmadana et al.*

## Supplementary results mentioned in the manuscript

### 1. Thioflavin T (ThT) binding assays on pure somatostatin: evidence of hours-long lag phase.

Thioflavin T assays were perfomed on 1%w/w somatostatin acetate in 150 mM NaCl (Figure S1), using the slow kinetics protocol mentioned in the experimental section of the manuscript.

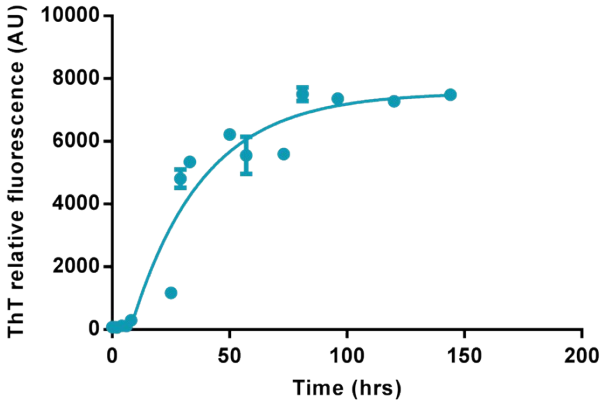


**Figure S1**. Aggregation kinetics of 1%w/w somatostatin acetate in 150 mM NaCl, as followed by ThT fluorescence binding assay.

### 2. Monomer release assay.

Monomer release assays were performed to compare the reversibility of the somatostatin aggregates formed in the presence and absence of heparin (Figure S2).

**Experimental procedure**. Somatostatin samples were prepared both in the presence and absence of heparin. 10 mg/mL (5.8 mM) pure Somatostatin acetate sample was prepared in 150 mM NaCl containing 0.01% sodium azide and stored at 4°C for 3 days before the experiment to allow mature fibril formation. Somatostatin with heparin samples were prepared at the same peptide concentration with a equimolar heparin concentration in 150 mm NaCl containing 0.01% sodium azide, immediately before the experiment. Phosphate buffer saline (PBS, pH 7.4) containing 0.01% sodium azide was used as a release medium. Slide-a-lyzer® dialysis devices (Thermo Fisher Scientific, USA) comprising a dialysis regenerated cellulose and polypropylene membrane with a 3.5kDa cut-off mounted on 50 mL Eppendorf® tubes were used for the study. The samples were transferred onto the dialysis membrane immediately before the experiment. The assembled dialysis devices were filled with 45 mL PBS. The assembled dialysis units were then placed in a shaking water bath at room temperature (25°C). 20 μL aliquots were taken from the release medium at regular time intervals and analysed for peptide concentration using a NanoDrop® (Thermo Fisher Scientific, USA) UV spectrophotometer (280 nm absorbance) after calibration with Somatostatin samples of known concentrations. An equal amount of release medium was replaced immediately after the withdrawing. Experiments were performed in triplicates.

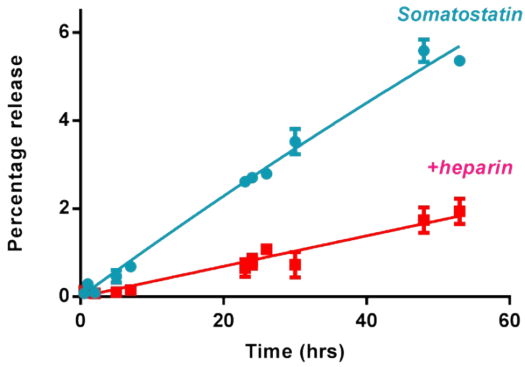


**Figure S2**. Monomer release assay of 1% w/w somatostatin fibrils (blue) and somatostatin 1% w/w with heparin aggregates (red).

### 3. Additional small angle X-ray scattering intensity profiles (SAXS).

Small angle X-ray scattering intensity profiles (Figure S3) used for slope calculations in Table 2 of the manuscript, obtained using the experimental procedure described in the experimental section of the manuscript.

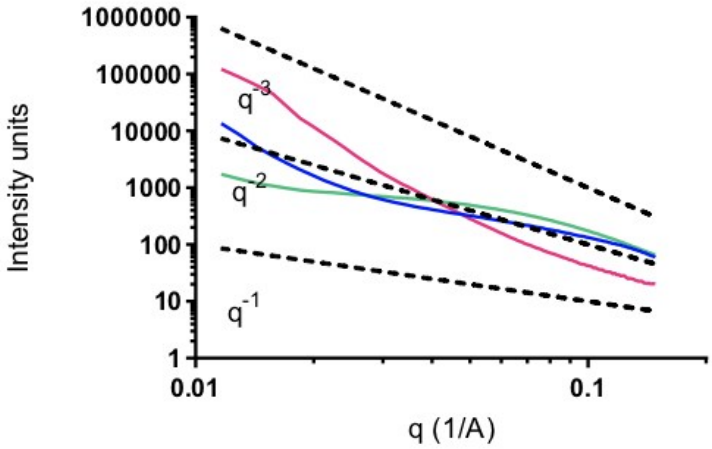


**Figure S3**. Representative SAXS profiles of 1% w/w somatostatin samples: somatostatin alone, 1 week old (green), with heparin (equimolar, 2 days old, red), and chondroitin (equimolar, 2 days old, blue).