Supporting Information for

The Ability of Insulin to Inhibit Amyloid Formation by ProIAPP Processing Intermediate Is Significantly Reduced in the Presence of Sulfated Glycosaminoglycans.

Authors: Hui Wang, Daniel P. Raleigh

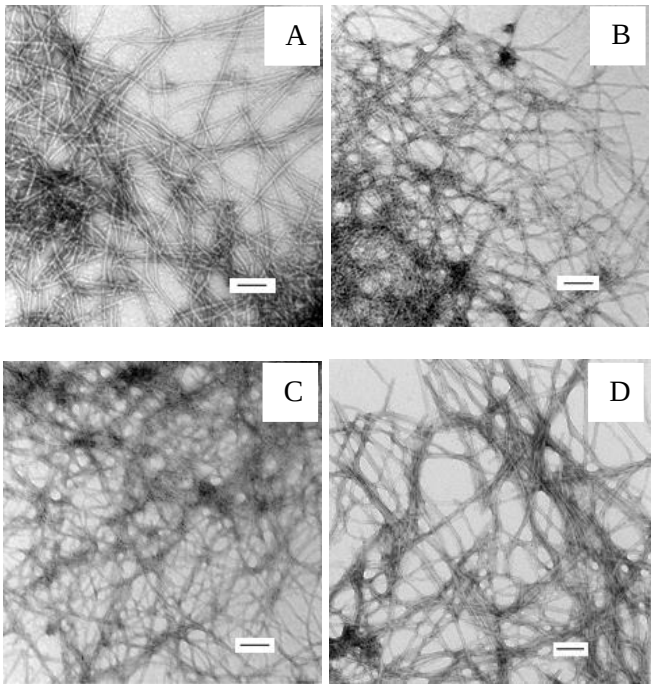

Figure S1. TEM images of mixtures of IAPP and insulin at different ratios of IAPP to insulin in the absence of HS. The images correspond to time points at the end of the reactions displayed in figure 2 of the article. (A) TEM image of a 100:1 mixture of IAPP and insulin, IAPP was in 100 fold excess. (B) TEM image of a 80:1 mixture of IAPP and insulin, IAPP was in 80 fold excess. (C) TEM image of a 60:1 mixture of IAPP and insulin, IAPP was in 60 fold excess. (D) TEM image of a 40:1 mixture of IAPP and insulin, IAPP was in 40 fold excess. Scale bars represent 100 nm. Aliquots were removed at the end of each experiment for TEM analysis.

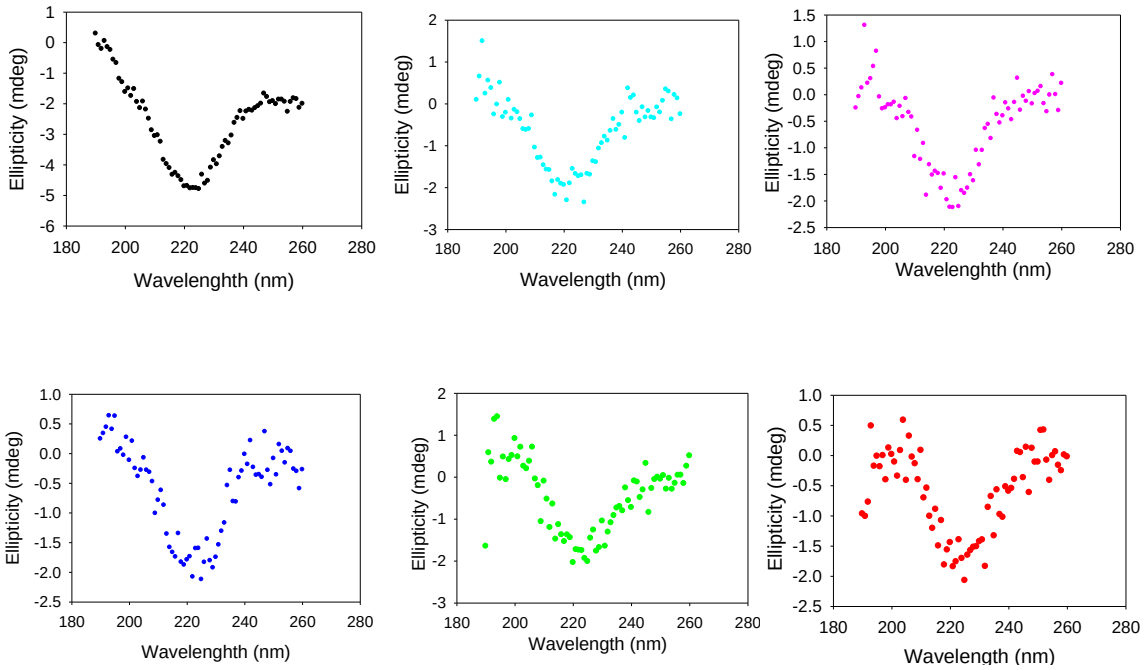

Figure S2. CD spectra of mixtures of IAPP and insulin at different ratios of IAPP to insulin in the absence of HS. The spectra correspond to time points at the end of the reactions displayed in figure 2 of the article. The color coding used here is the same as in figure 2 of the article. Aliquots were removed at the end of each kinetic experiment.

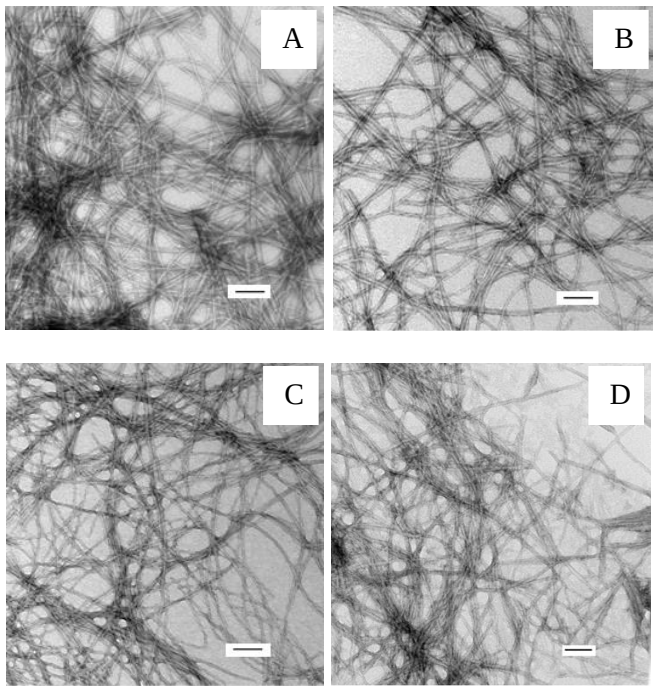

Figure S3. TEM images of mixtures of proIAPP$_{1-48}$ and insulin mixtures at different ratios of proIAPP$_{1-48}$ to insulin in the absence of HS. The images correspond to time points at the end of the reactions displayed in figure 3 of the article. (A) TEM image of a 100:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 100 fold excess. (B) TEM image of an 80:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 80 fold excess. (C) TEM image of a 60:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 60 fold excess. (D) TEM image of a 40:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 40 fold excess. Scale bars represent 100 nm. Aliquots were removed at the end of each experiment for TEM analysis.

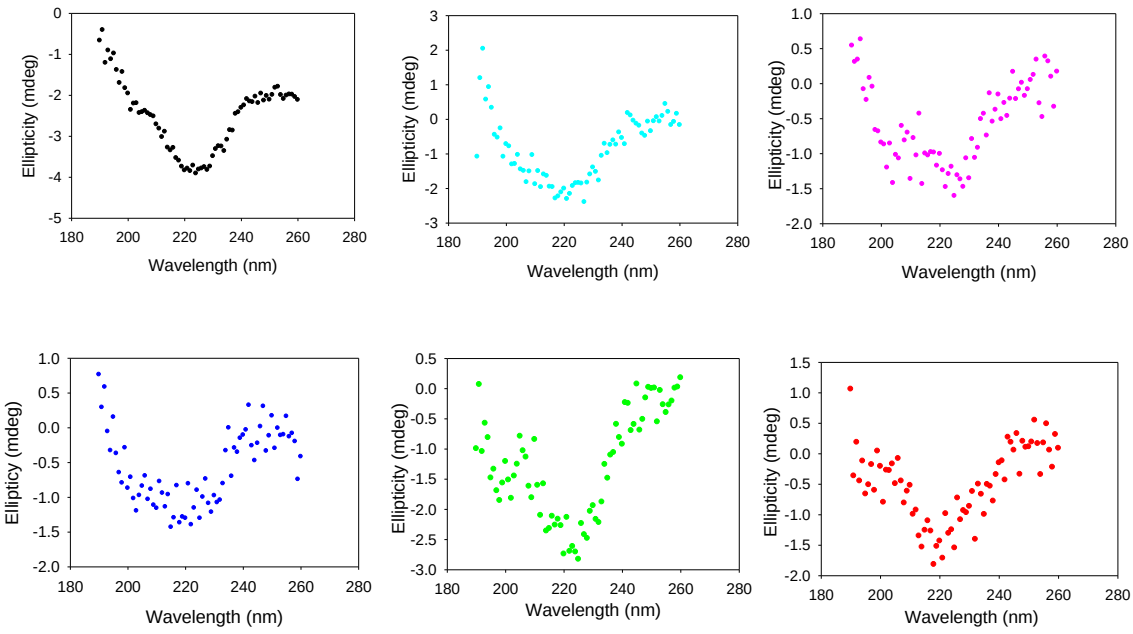

Figure S4. CD spectra of mixtures of proIAPP$_{1\text{-}48}$ and insulin at different ratios of proIAPP$_{1\text{-}48}$ to insulin in the absence of HS. The spectra correspond to the time points at the end of the reactions displayed in figure 3 of the article. The color coding used here is the same as in figure 3 of the article. Aliquots were removed at the end of each kinetic experiment.

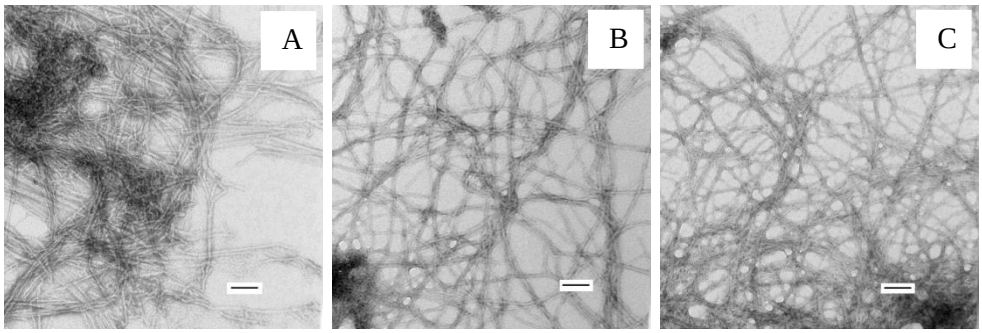

Figure S5. TEM images of mixtures of IAPP and insulin at different ratios of IAPP to insulin in the presence of HS. The images correspond to the time points at the end of the reactions displayed in figure 4 of the article. (A) TEM image of a 20:1 mixture of IAPP and insulin, IAPP was in 20 fold excess. (B) TEM image of a 5:1 mixture of IAPP and insulin, IAPP was in 5 fold excess. (C) TEM image of a 1:1 mixture of IAPP and insulin. Scale bars represent 100 nm. Aliquots were removed at the end of each experiment for TEM analysis.

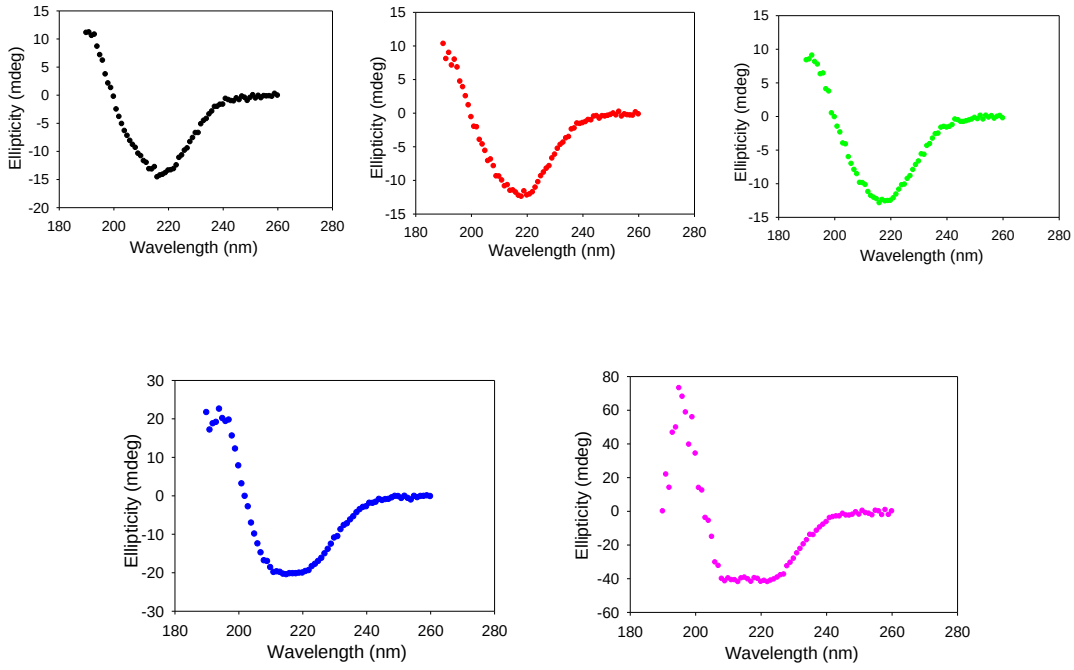

Figure S6. CD spectra of the mixture of IAPP and insulin at different ratios of IAPP to insulin in the presence of HS. The spectra correspond to the time points at the end of the reactions displayed in figure 4 of the article. The color coding used here is the same as in figure 4 of the article. Aliquots were removed at the end of each kinetic experiment.

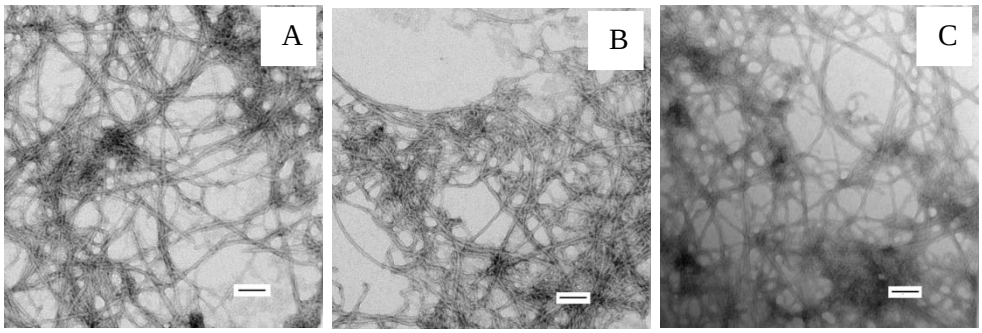

Figure S7. TEM images of mixtures of proIAPP$_{1-48}$ and insulin at different ratios of proIAPP$_{1-48}$ to insulin in the presence of HS. The images correspond to the time points at the end of the reactions displayed in figure 5 of the article. (A) TEM image of a 20:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 20 fold excess. (B) TEM image of a 5:1 mixture of proIAPP$_{1-48}$ and insulin, proIAPP$_{1-48}$ was in 5 fold excess. (C) TEM image of a 1:1 mixture of proIAPP$_{1-48}$ and insulin. Scale bars represent 100 nm. Aliquots were removed at the end of each experiment for TEM analysis.

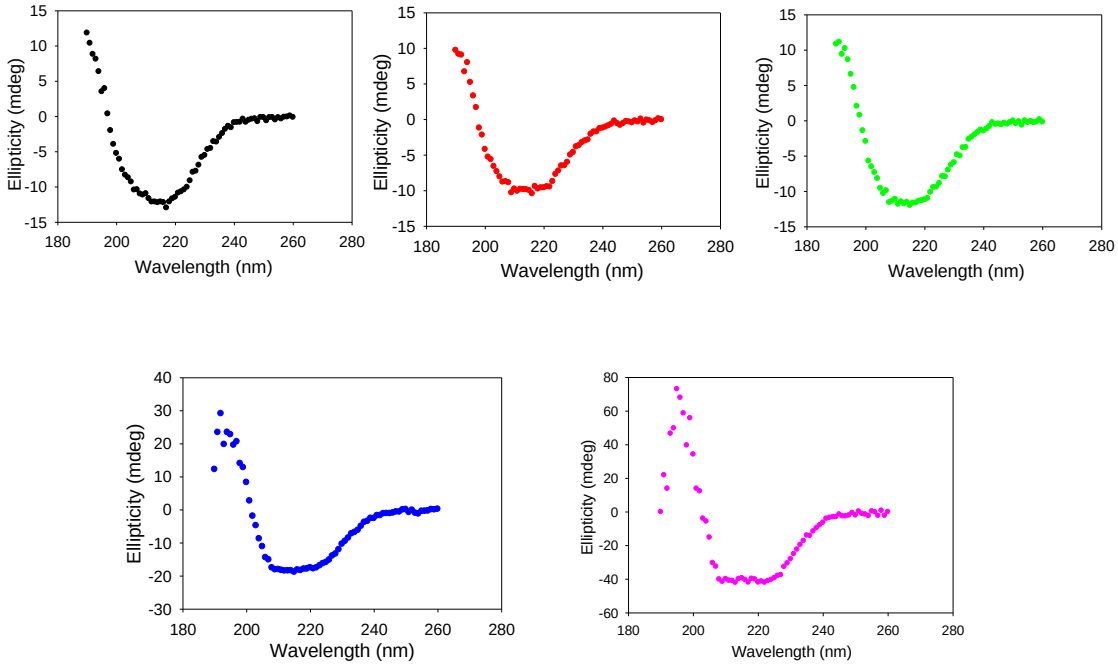

Figure S8. CD spectra of the mixture of proIAPP$_{1\text{-}48}$ and insulin at different ratios of proIAPP$_{1\text{-}48}$ to insulin in the presence of HS. The spectra correspond to the time points at the end of the reactions displayed in figure 5 of the article. The color coding used here is the same as in figure 5 of the article. Aliquots were removed at the end of each kinetic experiment.

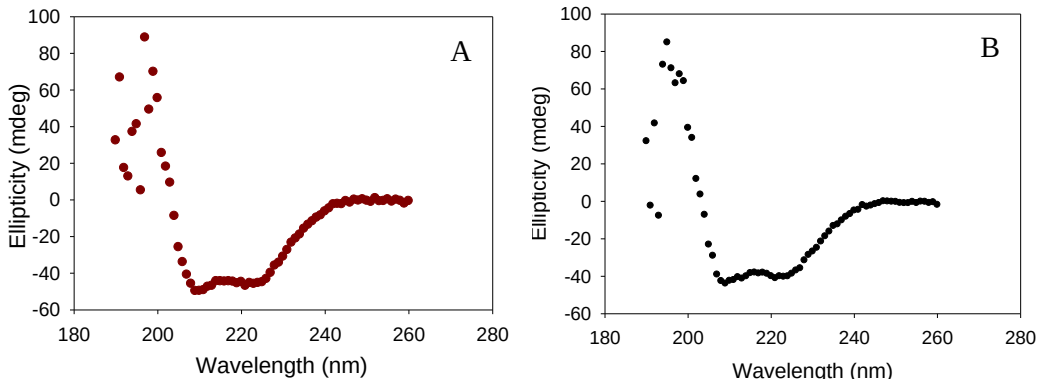

Figure S9. CD spectra of insulin in the absence of HS and of a mixture of insulin and HS. (A) The spectrum corresponds to the time point at the end of the reaction displayed as the brown curve in figure 4 of the article. An aliquot was removed at the end of the kinetic experiment shown in figure 4 of the article. The kinetic experiment was conducted in 20 mM Tris-HCl (pH 7.4) and 2% HFIP (v/v) without stirring at 25 ℃. (B) The spectrum of insulin without HS and without any incubation. Insulin was at 80 μM and HS was at 1.3 μM, when present.

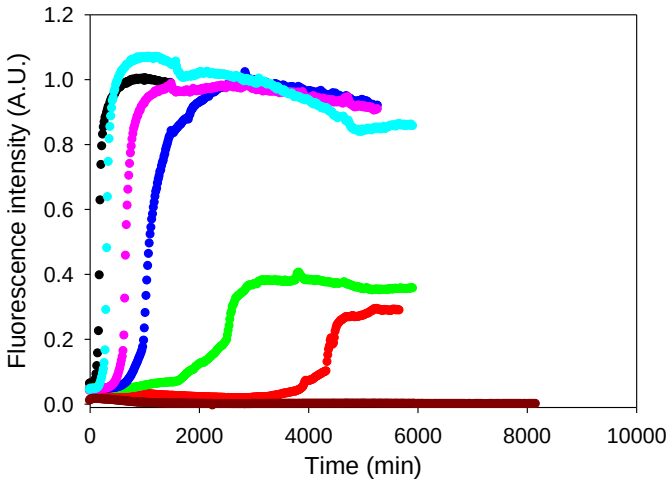

Figure S10. Plot of the unnormalized data displayed in figure 2A of the article.

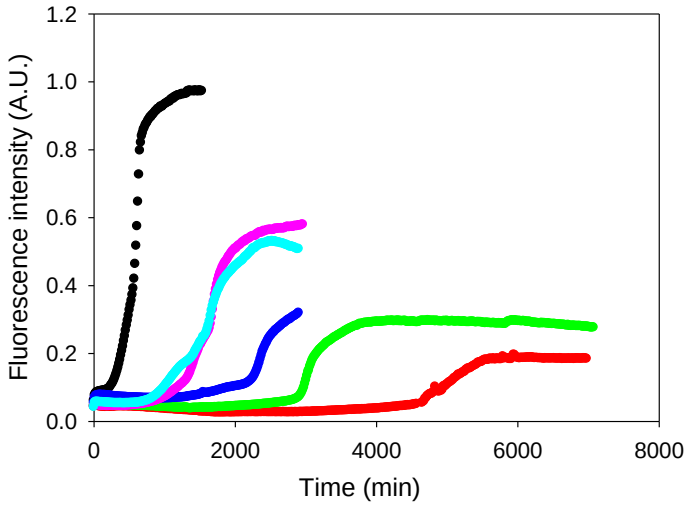

Figure S11. Plot of the unnormalized data displayed in figure 3A of the article.

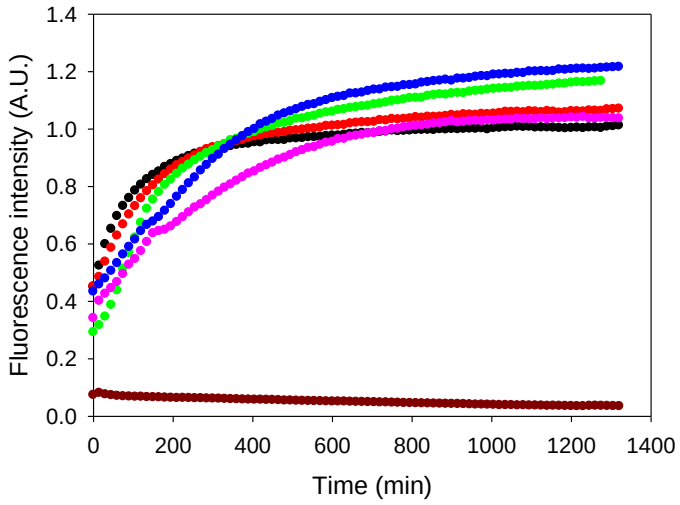

Figure S12. Plot of the unnormalized data displayed in figure 4A of the article.

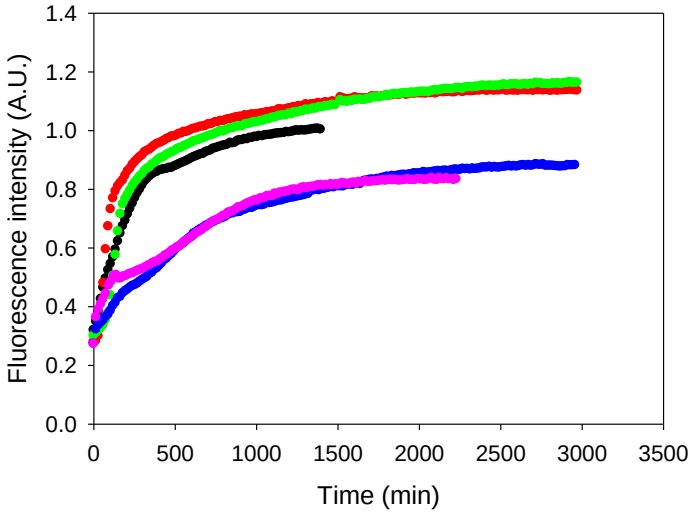

Figure S13. Plot of the unnormalized data displayed in figure 5A of the article.

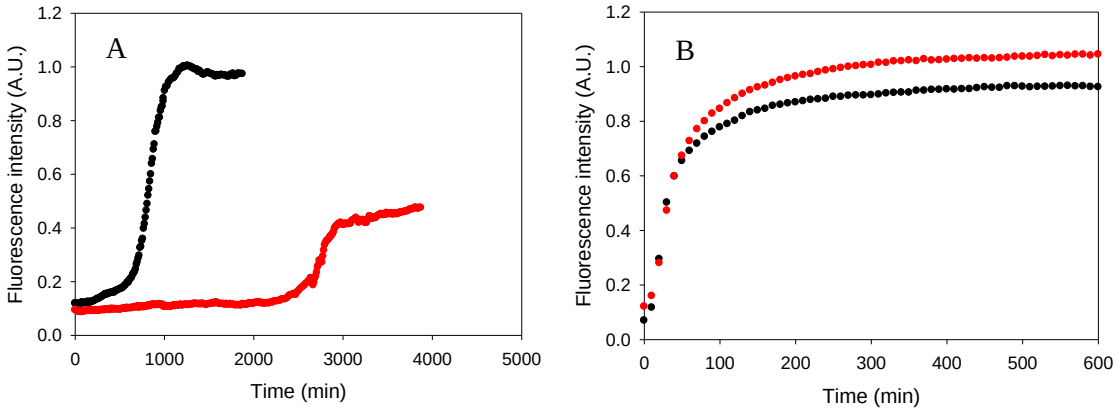

Figure S14. Plot of the unnormalized data displayed in figure 6A (A) and figure 6B (B) of the article.

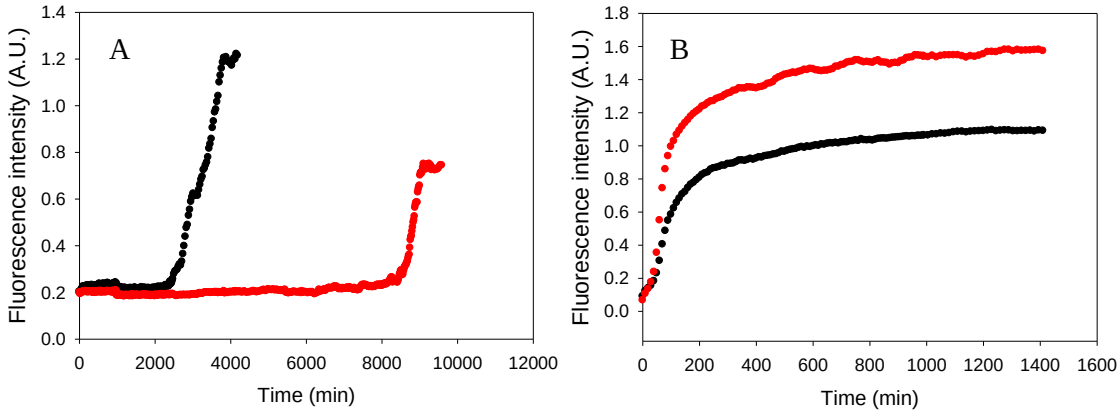

Figure S15. Plot of the unnormalized data displayed in figure 7A (A) and figure 7B (B) of the article.

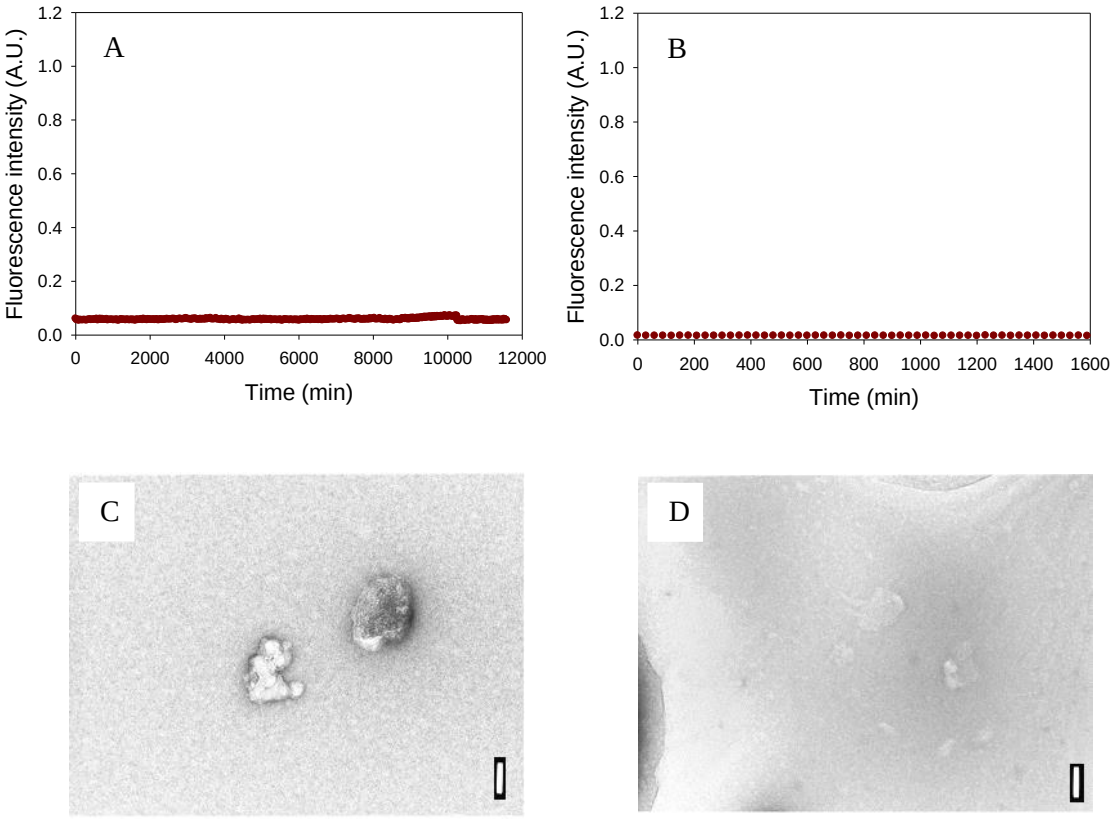

Figure S16. Insulin does not form amyloid during the time course of the experiments conducted in the absence of HFIP. (A) Thioflavin-T curve recorded for a sample of insulin in the absence of IAPP, HS and HFIP. (B) Thioflavin-T curve recorded for a sample of insulin in the presence of HS, but without IAPP or HFIP. Kinetic experiments were conducted in 20 mM Tis-HCl (pH 7.4) without stirring at 25 ℃. The concentration of insulin was 0.8 μM. HS, when present, was at 1.3 μM. No HFIP was present. (C) TEM image of a sample removed at the end of the curve shown in panel A. (D) TEM image of a sample removed at the end of the curve shown in panel B. Scale bars represent 50 nm.