Supplementary Information

Isolating toxic insulin amyloid reactive species that lack β-sheets and have wide pH stability

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Guinier Analysis

The Guinier analysis is a model-independent method (it does not require an a priori knowledge about the sample) which includes a linearized fitting of the SANS data to extract structural information of the sample. Using the Guinier approximation (1) *I(Q)*, the scattered intensity, can be expanded as:

$$\boldsymbol{I(Q)} = \boldsymbol{I}_0 \exp\left(-\frac{\boldsymbol{Q}^2 \boldsymbol{R}_g^2}{3}\right) \quad (1)$$

where I_0 is the forward scattered intensity, Q the scattering variable and R_g the radius of gyration. The Guinier analysis involves plotting ln($I(Q)$) vs Q^2: a linear fit gives R_g and I_0 from the slope and intercept, respectively. The R_g represents the effective size of the scattering "particle", while I_0 can be used to estimate the molecular weight of the "particle" (2). The usefulness of this plot stems from the fact that the obtained particle "size" R_g is independent of the absolute intensity I_0 and of any model. Because the Guinier approximation is valid only in the region where $Q*Rg \cong 1$ (see **Supplementary Table 1**), only the low Q range was considered. The scattering data were reduced and analyzed using a macro written by Oakridge National Laboratory using Igor Pro (Wavemetrics Inc., Portland, OR).
The I_0 is used to determine the molecular weight (MW) of the molecule in solution using the following equation:

$$I(0) = \overline{cM_w(\Delta\rho)^2 v^2 / N_A} \quad (2)$$

where c is the concentration of protein (g/ml), M_w is the molecular weight, $\Delta\rho$ is the contrast of the scattering length density between the protein and the solvent and is 3.4 x 10^{10} cm^{-2} for D_2O, v is the partial specific volume of protein and is 0.73 cm^3/g and N_A is Avogadro's number. Two additional scale factors were applied to *I(0),* including the absolute scale factor for the detector distance used, which was 1.4462 x 10^{-5} and multiplied the value of *I(0),* and the sample thickness of 0.2 cm was used to divide *I(0).*

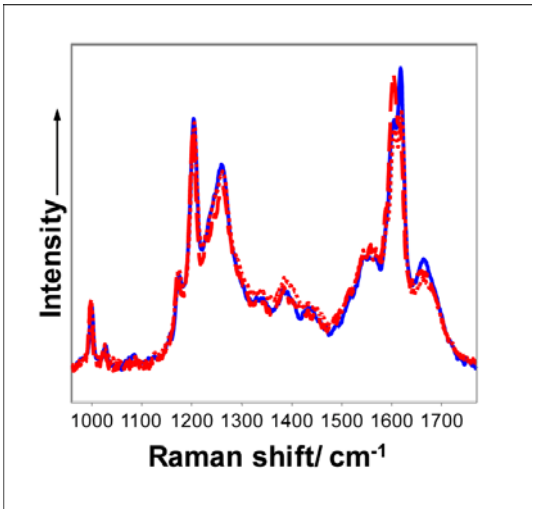
References

1. Guinier, A. and G. Fournet, *Small-angle scattering of x-rays*. 1955, New York: Wiley.
2. Jacrot, B. and G. Zaccai, *Determination of molecular weigh by neutron scattering.* Biopolymers, 1981. **20**: p. 2413-2426.

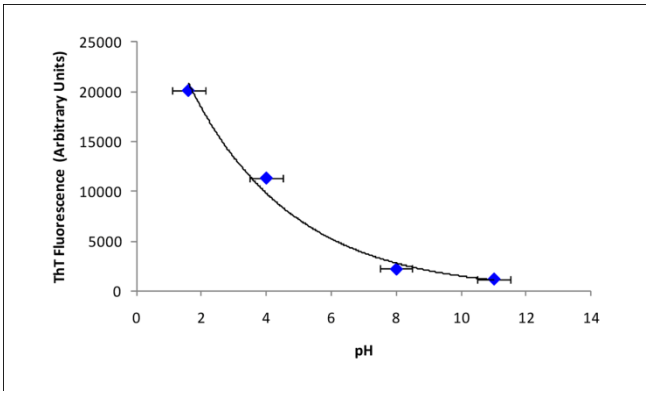
Supplementary Table 1: Parameters of the Guinier analysis

Sample	$R_g \times Q$ min	$R_g \times Q$ max
Native pH 1.6	0.4	1.5
$I_{F,}$	1.2	1.4
$I_{F,}$	0.9	1.2
I_F Filtered	0.3	1.0

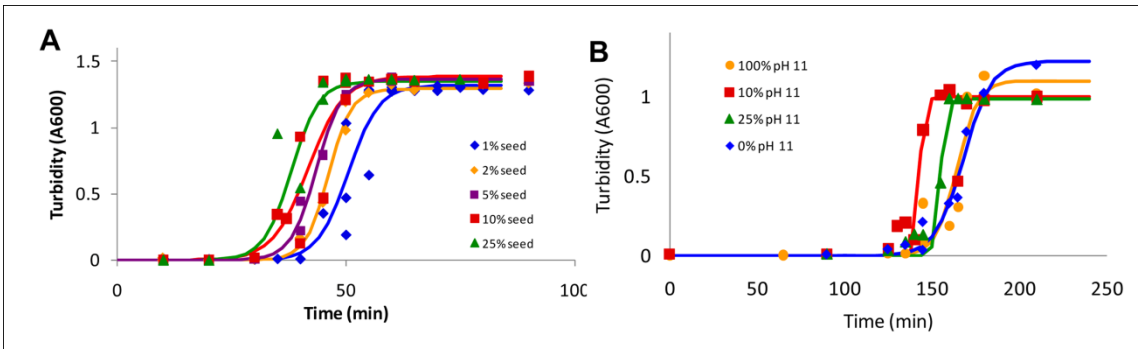
Supplementary Figures



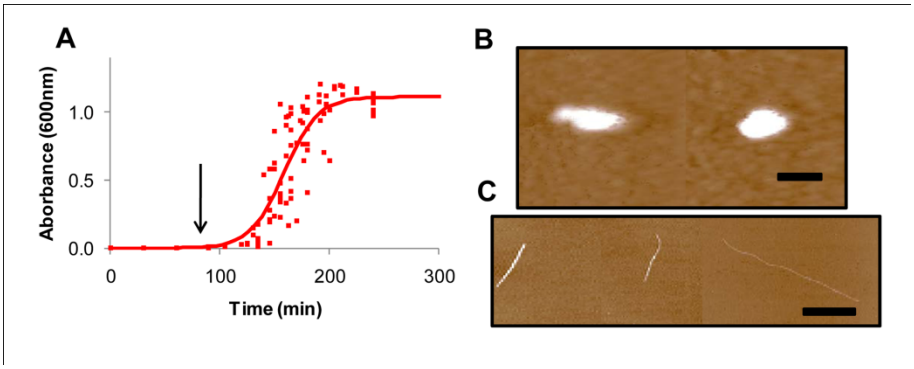
Supplementary Figure 1: DUVRR of insulin at pH 11. The secondary structure of insulin that had been in fibrils (I_F – dotted red line) was compared with native insulin (solid blue line) that had not previously been in fibrils (I_N – dashed red line) and no difference was found.



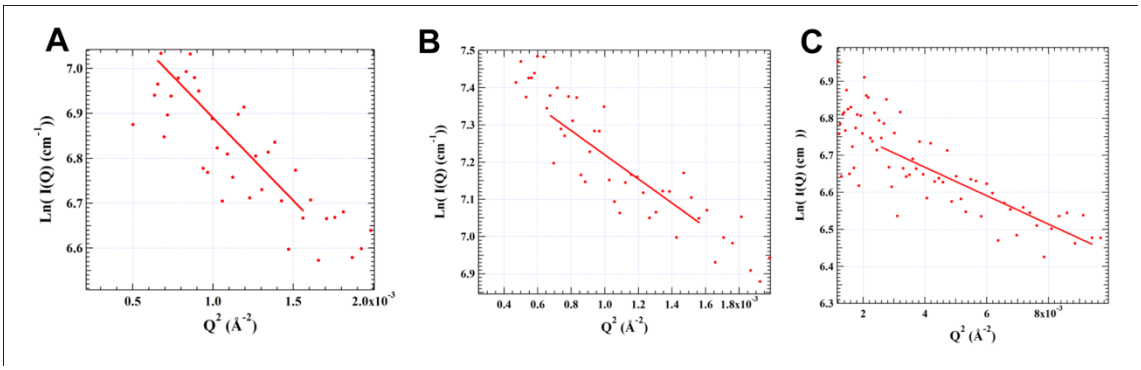
Supplementary Figure 2: Change in ThT fluorescence with pH. ThT is pH sensitive: It has a high signal at pH 2 and is greatly reduced at pH 11.



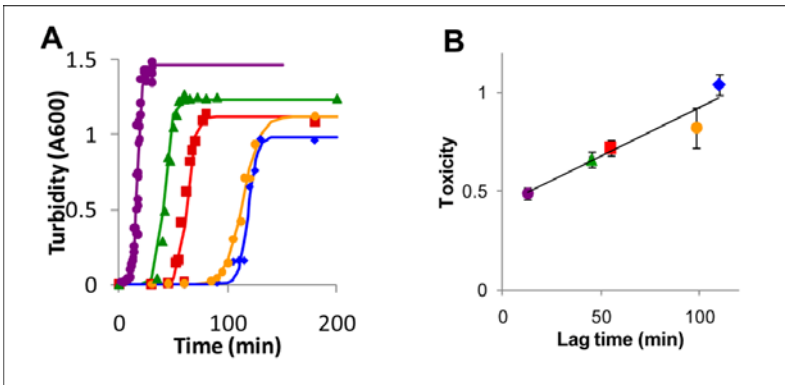
Supplementary Figure 3: The seeding effect of disaggregated and pH cycled insulin. (A) Disaggregated insulin (I_F') seeds a kinetic run in a dose dependent manner, whereas (B) insulin that was pH cycled (I_N') does not seed the reaction. Note that the time axes are on a different scale.



Supplementary Figure 4: Images of aggregates and fibrillar aggregates in lag time samples from native insulin. (A) Samples were taken at 75 minutes into the fibrillation run and imaged with AFM. (B) Aggregates similar in size to those found in the disaggregated fibril samples (**Fig. 4C**) and (C) fibrils were found in the samples. Scale bars are 100 nm in B and 500 nm in C.



Supplementary Figure 5: The Guiner analysis of SANS data. (A) pH 11 disaggreated insulin (I_F), (B) pH 1.6 disaggregated insulin (I_F'), and (C) pH 1.6 disaggregated insulin that was filtered with a 0.22 µm filter.



Supplementary Figure 6: Dose response of retentate seeding. (A) Seeding of a 2 mg/ml insulin run at 65°C and pH 1.6 with 1% of fibrils (purple squares), retentate (green triangles), retentate diluted 1:10 (red squares), retentate diluted 1:80 (orange circles) and no seeding (blue diamonds). (B) When the MTT reduction on PC-12 cells of each sample was compared to the lag time of that sample, there was a linear correlation with an R^2 of 0.91.