Supporting Information

Extremely Amyloidogenic Single-Chain Analogs of Insulin's H-Fragment: On the Structural Adaptability of an Amyloid Stretch.

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1. **Predictions of amyloidogenic tendencies of A-B and B-A based on FoldAmyloid.**
2. **Aggregation of H, A-B, B-A at a close-to-neutral pH: kinetic data and IR spectra of fibrils.**
3. **CD spectra of seeded amyloid fibrils.**

1. Predictions of amyloidogenic tendencies of A-B and B-A based on FoldAmyloid.

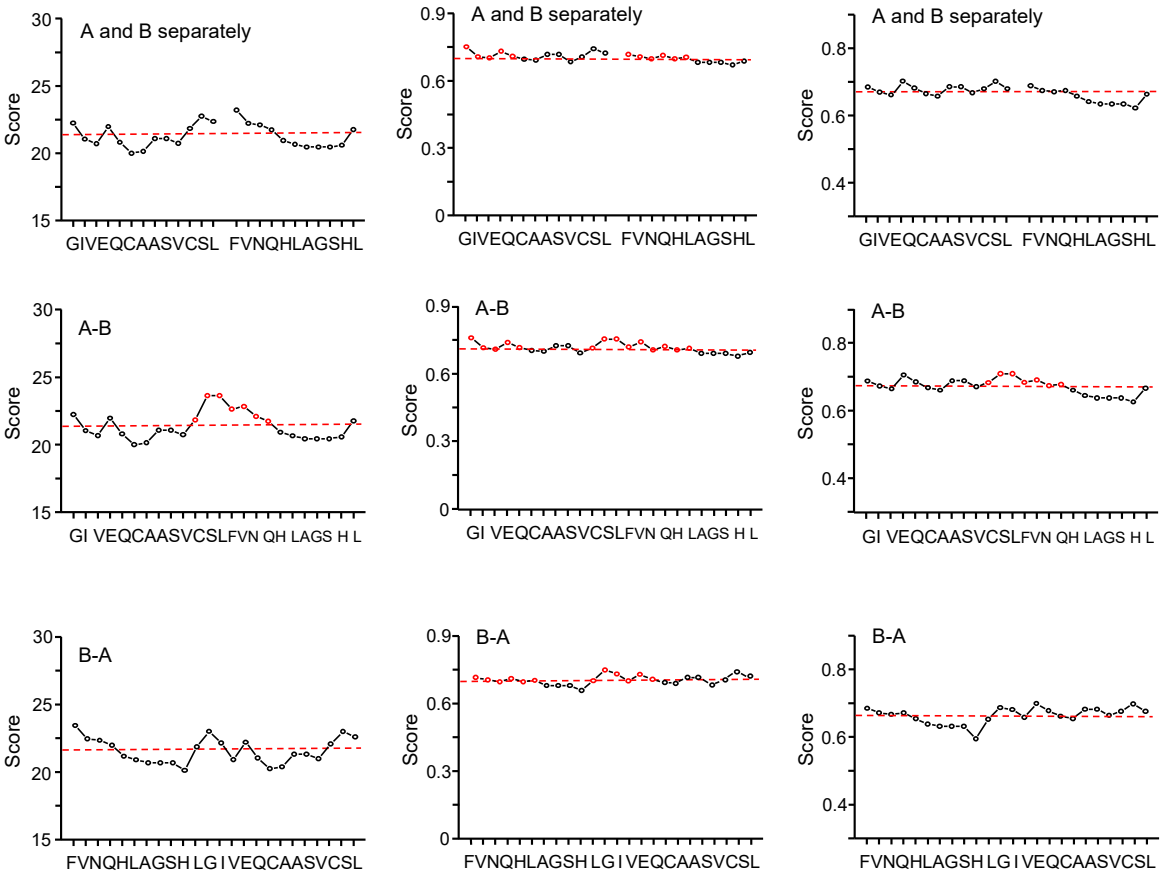

Figure S1. Prediction of amyloidogenic tendencies of separate A- and B-chain parts of H-fragment (top row) and of whole A-B (middle row) and B-A (bottom row) peptides based on FoldAmyloid (S1). Left column: Expected number of contacts 8Å; middle column: bone-bone donors; right column: bone-bone acceptors. Overall, the algorithm does not capture significant differences between separated A and B segments. We note slightly increased scores for the middle section of A-B partly overlapping the TANGO results (**Fig. 1** of the main article).

2. Aggregation of H, A-B, B-A at a close-to-neutral pH: kinetic data and IR spectra of fibrils.

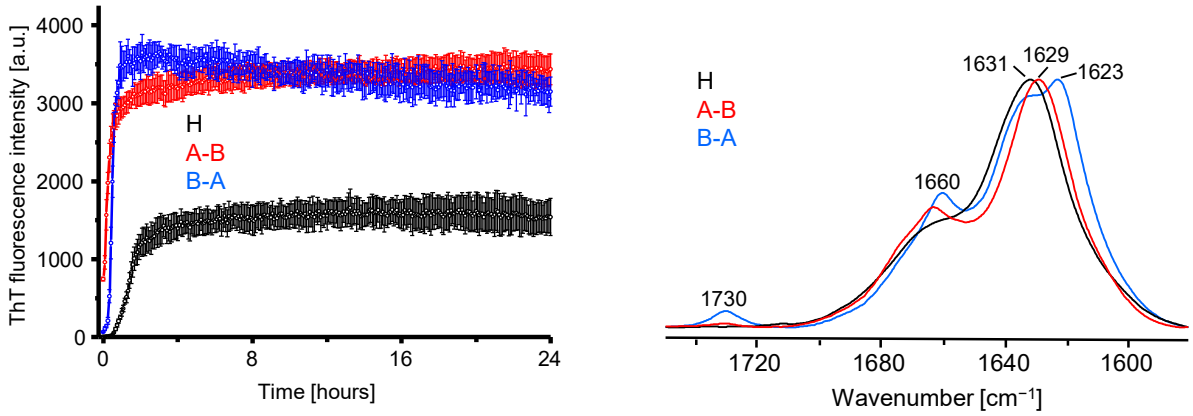

Figure S2. Left panel: kinetic trajectories of fibrillization of H, A-B, and B-A in TRIS buffer, pH 7.5 monitored by ThT fluorescence. Other conditions were the same as in the experiments reported in **Fig. 4A** of the main article except that the dilution buffer contained 50 mM TRIS with pH set to 7.5 (which was maintained within 0.1 pH unit after mixing with the DMSO-dissolved peptides). Right panel: ATR FT-IR spectra of aggregates collected afterward. It is clear that all peptides preserve the highly amyloidogenic characters at this pH and the relative aggregation rate order A-B > B-A >> H is maintained. Also, the fine IR features characteristic for fibrils of B-A and A-B are conserved.

3. CD spectra of seeded amyloid fibrils.

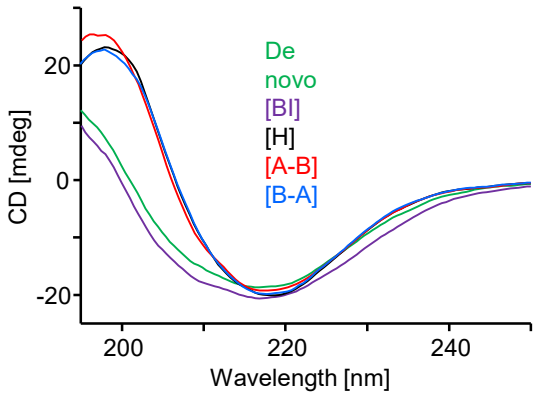

Figure S3. Far-UV CD spectra of insulin fibrils formed spontaneously (green line), seeded homologously (violet line) and cross-seeded with fibrils of H (black line), A-B (red line), and B-A (blue line) fibrils (data corresponding to the IR spectra shown in Fig. 7B of the main article). We note consistent single minima below 220 nm indicative of β-sheet conformation. The decreased spectral intensity in the short wavelength range for fibrils formed in the absence of synthetic peptide seeds may be related to different light scattering properties of these aggregates.

References:

[S1] Garbuzynskiy, S. O., Lobanov, M. Y., & Galzitskaya, O. V. (2010). FoldAmyloid: a method of prediction of amyloidogenic regions from protein sequence. ***Bioinformatics***, 26(3), 326-332.