

## Insulin Fibrillation and Protein Design: Topological Resistance of Single-Chain Analogs to Thermal Degradation with Application to a Pump Reservoir

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### Abstract

Insulin is susceptible to thermal fibrillation, a misfolding process that leads to nonnative cross- $\beta$  assembly analogous to pathological amyloid deposition. Pharmaceutical formulations are ordinarily protected from such degradation by sequestration of the susceptible monomer within native protein assemblies. With respect to the safety and efficacy of insulin pumps, however, this strategy imposes an intrinsic trade-off between pharmacokinetic goals (rapid absorption and clearance) and the requisite physical properties of a formulation (prolonged shelf life and stability within the reservoir). Available rapid-acting formulations are suboptimal in both respects; susceptibility to fibrillation is exacerbated even as absorption is delayed relative to the ideal specifications of a closed-loop system. To circumvent this molecular trade-off, we exploited structural models of insulin fibrils and amyloidogenic intermediates to define an alternative protective mechanism. Single-chain insulin (SCI) analogs were shown to be refractory to thermal fibrillation with maintenance of biological activity for more than 3 months under conditions that promote the rapid fibrillation and inactivation of insulin. The essential idea exploits an intrinsic incompatibility between SCI topology and the geometry of cross- $\beta$  assembly. A peptide tether was thus interposed between the A- and B-chains whose length was (a) sufficiently long to provide the “play” needed for induced fit of the hormone on receptor binding and yet (b) sufficiently short to impose a topological barrier to fibrillation. Our findings suggest that ultrastable monomeric SCI analogs may be formulated without protective self-assembly and so permit simultaneous optimization of pharmacokinetics and reservoir life.

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**Abbreviations:** (BrdU) bromodeoxyuridine, (CD) circular dichroism, (Gu) guanidine, (HI) human insulin, (HPI) human proinsulin, (HPLC) high-performance liquid chromatography, (IGF-1R) insulin-like growth factor-1 receptor, (IGF-I) insulin-like growth factor-I, (IR-B) insulin receptor isoform B, (MALDI-TOF MS) matrix-assisted laser desorption/ionization-time of flight mass spectrometry, (MS) mass spectrometry, (NaCl) sodium chloride, (NMR) nuclear magnetic resonance, (NPH) neutral protamine Hagedorn, (PBS) phosphate-buffered saline, (PDB) Protein Data Bank, (rp-HPLC) reverse-phase high-performance liquid chromatography, (SCI) single-chain insulin, (ThT) thioflavin T

**Keywords:** amyloid, closed-loop system, insulin pump, intraperitoneal pump, pump reservoir

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