Supplementary Information

Inhibition of Insulin Amyloid Fibrillation by a Novel Amphipathic Heptapeptide: Mechanistic Details Studied by Spectroscopy in Combination With Microscopy

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Tab1e S1: Time parameters of Insulin amyloid fibrillation: Lag time and Half time of the insulin amyloid fibrillation in presence and absence of different concentration of KR7 peptides.

Sample	**Lag Time (minutes)**	**$T_{1/2}$ (minutes)**
Insulin Control	94.1±0.6	113.53±0.8
Insulin + KR7 (1:1)	95.2±2.1	144.5±3.4
Insulin + KR7 (1:0.5)	98.1±1.8	126.1±2.7

Tab1e S2: Qualitative analysis of chemical shift perturbation (CSP) of insulin in presence of equimolar concentration of peptide KR7 (insulin:KR7 =1:1) acquired from NOESY experiment. The symbol + indicates less CSP and ++ indicates higher CSP.

Residue	**CSP**
Insulin Chain A	
Ser12	+
Leu13	+
Leu16	+
Tyr19	+
Cys20	+
Insulin Chain B	
Leu11	+
Val12	++
Glu13	++
Ala14	++
Leu15	++
Tyr16	+
Leu17	++
Val18	++
Gly20	+
Gly23	++
Glu21	++
Phe24	+
Tyr26	+
Thr27	++

Tab1e S3: Details of interacting partner from peptide KR7 and insulin protein conferred from docked structures: The nature of interaction and distance between KR7 and insulin, obtained from HADDOCK.

KR7 residues	Insulin residue		Nature of interaction	Inter-atomic distance (Å)
	Residue	Chain		
Lys1	Glu21	B	Electrostatic Interaction	2.5
Lys1	Leu17	B	Electrostatic Interaction	2.1
Lys1	Tyr16	B	Electrostatic Interaction	2.4
Trp3	Tyr16	B	Hydrophobic Interaction	3.2
Trp4	Gly20	B	Electrostatic Interaction	1.7
Trp4	Phe24	B	Hydrophobic Interaction	4.1
Arg6	Glu13	B	Electrostatic Interaction	1.8
Arg7	Tyr26	B	Electrostatic Interaction	1.8

Figure S1: Mammalian insulin sequence; A) Aligned sequence of mammalian insulin, amino acids represented in conventional single letter code, black letters indicate conserved residues while red indicates non-conserved residues, the green letters indicate conserved residues reported to be important in amyloid fibrillation event. B) Hydrophobicity plot (Protscale server) of bovine insulin showing E13 to L17 of chain B as the most hydrophobic region as indicated by arrows.

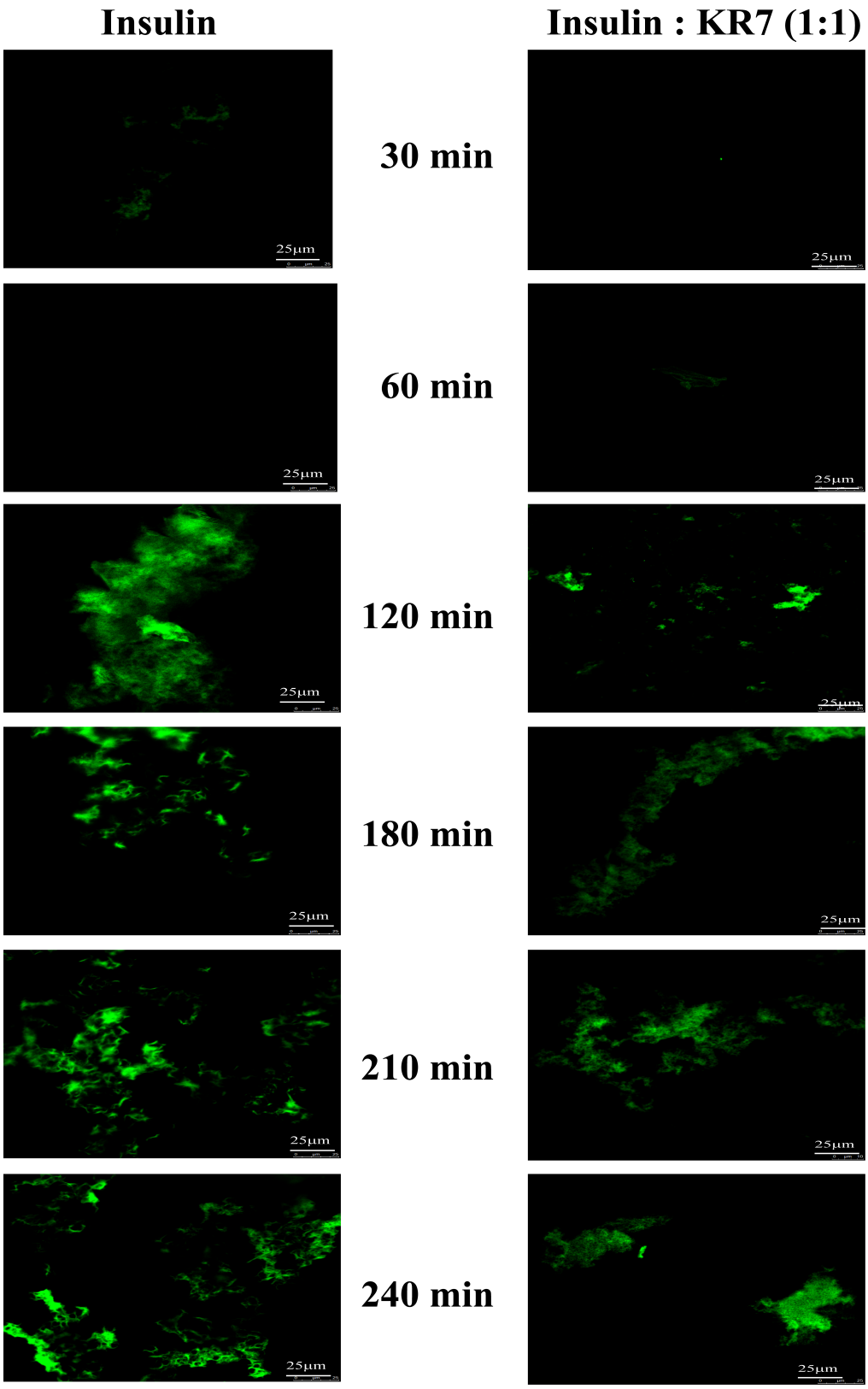

Figure S2: Confocal images of insulin fibrillation by ThT fluorescence: Fluorescence confocal images of insulin amyloids recorded at different time points in the course of insulin fibrillation in the presence (1:1) and absence of KR7 peptide.

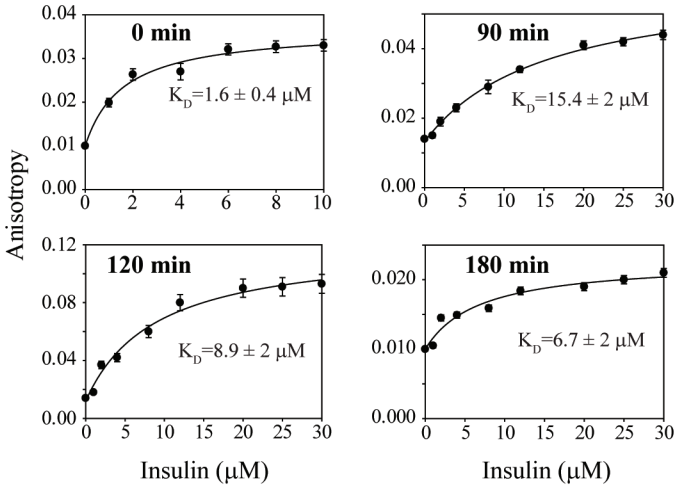

Figure S3: Fluorescence anisotropy measurements of the binding of KR7 to insulin at different time points. Anisotropy of the tryptophan fluorescence of 5 μM KR7 peptide in 10 mM sodium phosphate buffer containing 100 mM NaCl (pH 7.4) with gradual addition of insulin from 1 μM to 20 μM concentration from a 2 mg/mL stock solution in 50 mM citrate phosphate, 100 mM NaCl, pH 2.6 aggregated at 335 K for the indicated time period.

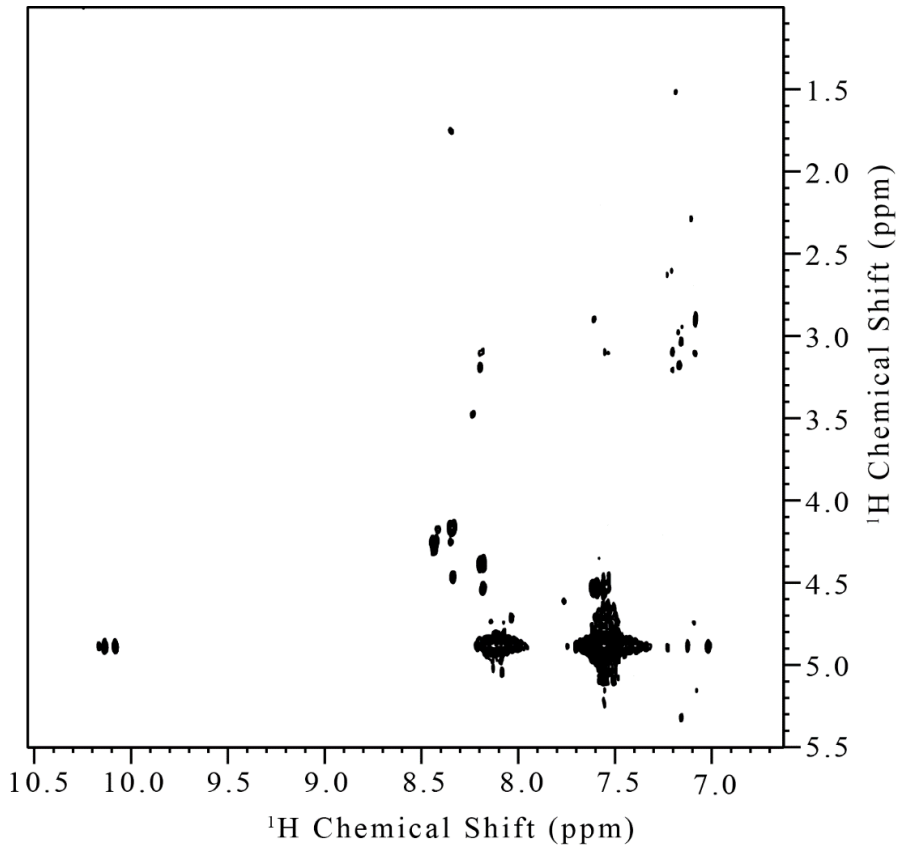

Figure S4: The finger print region of the 2D ^{1}H-^{1}H *tr*NOESY spectra (Bruker Avance III 500 MHz NMR spectrometer, 150ms NOESY mixing time) of KR7 in the context of insulin, recorded at 298 K and at pH 2.6 (adjusted with HCl) with 100 mM NaCl.

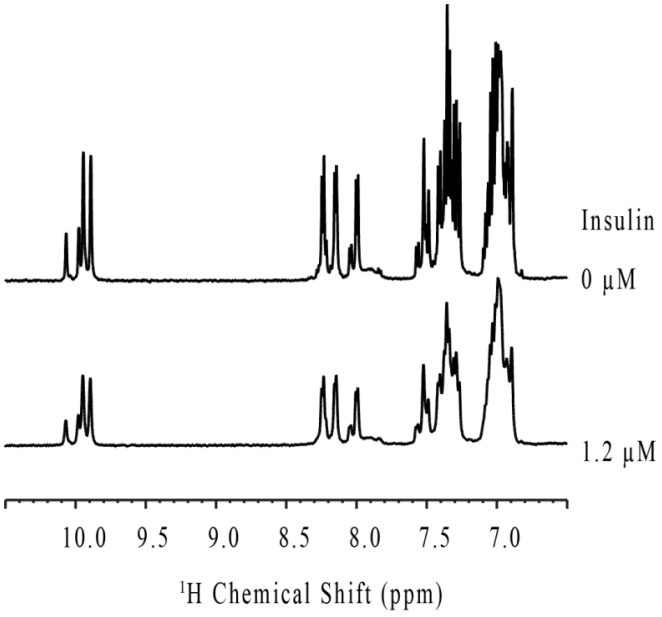

Figure S5: Selected region of one-dimensional ^{1}H NMR spectra of 1mM KR7 recorded at 298 K using Bruker Avance III 500 MHz spectrometer, showing the line width broadening in the aromatic and amide region upon addition of insulin.