# De-Novo designed 13 mer hairpin-peptide arrests Insulin and inhibits its aggregation:Role of OH-π interactions between water and hydrophobic amino acids.

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## SUPPORTING MATERIAL

**Table S1.a** calculation of secondary structure percentage from K2D software of intial stage/0min.

| SAMPLES | % of α-HELIX | % of β-STRAND | RANDOM COIL |
|---|---|---|---|
| **Insulin 0min** | 66 | 18 | 21 |
| **Insulin-B2T_YY 0min** | 52 | 16 | 22 |
| **Insulin-B2T_TT \0min** | 53 | 15 | 22 |
| **Insulin-B2T_FF 0min** | 59 | 17 | 24 |
| **Insulin-B2T_KV 0min** | 49 | 28 | 33 |

**Table S1.b** calculation of secondary structure percentage from K2D software of final stage/800min.

| SAMPLES | % of α-HELIX | % of β-STRAND | RANDOM COIL |
|---|---|---|---|
| **Insulin 800min** | 21 | 45 | 45 |
| **Insulin-B2T_YY 800min** | 12 | 30 | 48 |
| **Insulin-B2T_TT 800min** | 62 | 16 | 22 |
| **Insulin-B2T_FF 800min** | 11.5 | 32 | 37 |
| **Insulin-B2T_KV 800min** | 6.93 | 47 | 45 |

**Table S1.c** calculation of secondary structure percentage from K2D software of four peptides.

| SAMPLES | % of α-HELIX | % of β-STRAND | RANDOM COIL |
|---|---|---|---|
| | | | |
| **B2T_YY** | 6.71 | 38.21 | 50.5 |
| **B2T_TT** | 3.95 | 50.1 | 45.7 |
| **B2T_FF** | 6.45 | 40.32 | 52.5 |
| **B2T_KV** | 11.27 | 35.21 | 44.2 |

***Table S2.a*** ITC parameters of B2T_YY and insulin.

| **THERMODYNAMIC PARAMETERS** | **VALUE** |
|---|---|
| **ENTHALPY CHANGE ΔH** | $1.647*10^4$ cal $M^{-1}$ |
| **ENTROPY CHANGE ΔS** | 72.1 cal/mol/deg |
| **FREE ENERGY CHANGE ΔG** | $-9.5*10^3$ kcal $M^{-1}$ |
| **DISSOCIATION CONSTANT $K_a$** | $4.96*10^3$ $M^{-1}$ |

Table S2.b ITC parameters of B2T_FF and insulin.

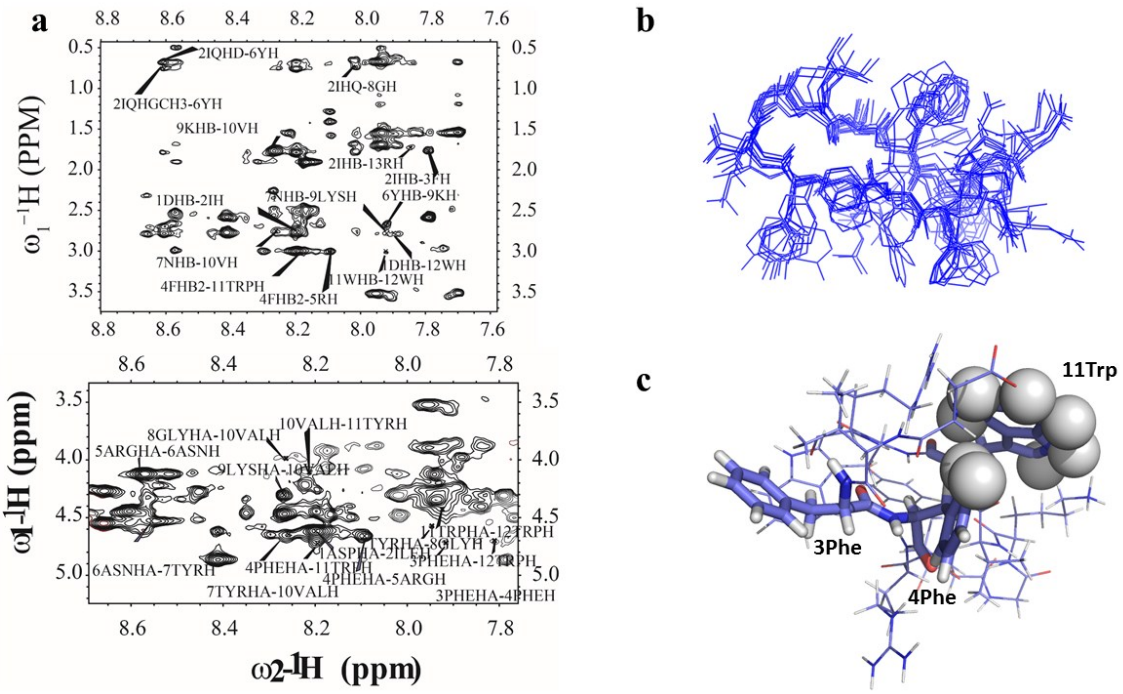
| **THERMODYNAMIC PARAMETERS** | **VALUE** |
|---|---|
| **ENTHALPY CHANGE ΔH** | $-3.095*10^4$ cal $M^{-1}$ |
| **ENTROPY CHANGE ΔS** | -93.9 cal/mol/deg |
| **FREE ENERGY CHANGE ΔG** | $11.7*10^3$ kcal $M^{-1}$ |
| **DISSOCIATION CONSTANT $K_a$** | $1.42*10^3$ $M^{-1}$ |

Table S2.c ITC parameters of B2T_TT and insulin.

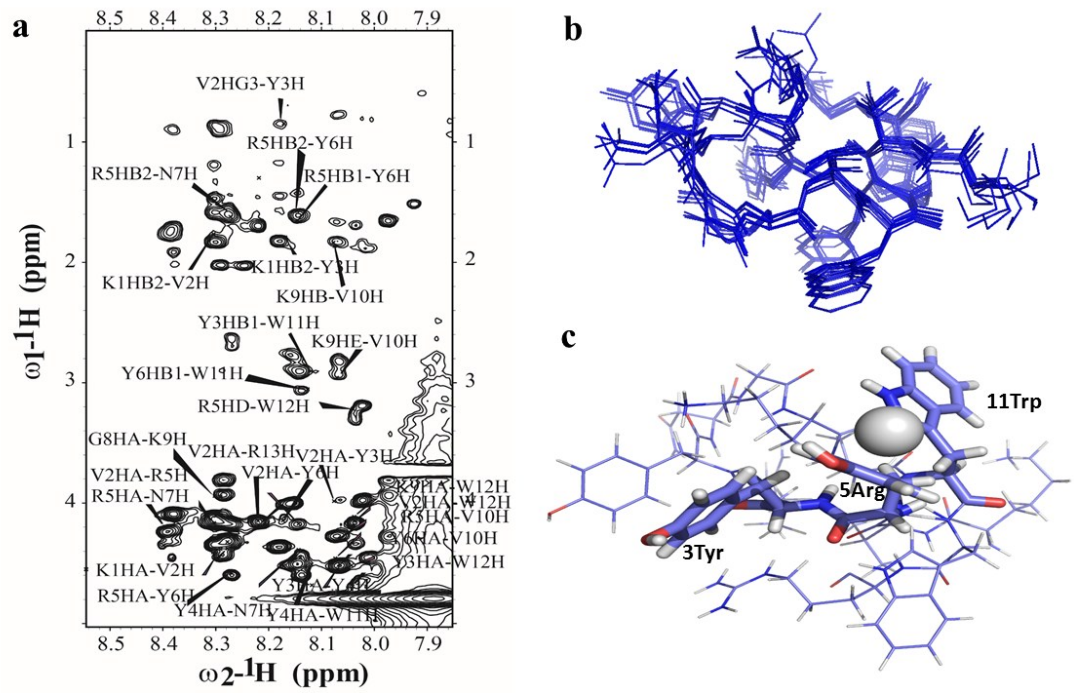
| **THERMODYNAMIC PARAMETERS** | **VALUE** |
|---|---|
| **ENTHALPY CHANGE ΔH** | $2.996*10^3$ cal $M^{-1}$, $-4.623*10^3$ cal $M^{-1}$, $2.706*10^3$ cal $M^{-1}$, $-2.190*10^3$ cal $M^{-1}$ |
| **ENTROPY CHANGE ΔS** | 30.1 cal/mol/deg, 6.37 cal/mol/deg, 30.2 cal/mol/deg and 12.5 cal/mol/deg |
| **FREE ENERGY CHANGE ΔG** | $-24.3*10^6$ kcal $M^{-1}$, $4.7*10^3$ kcal $M^{-1}$, $5.7*10^3$ kcal $M^{-1}$ , $-5.3*10^3$ kcal $M^{-1}$ |
| **DISSOCIATION CONSTANT $K_a$** | $2.3*10^4$ $M^{-1}$, $6.01*10^4$ , $4.16*10^4$ and $2.16*10^4$ $M^{-1}$ |

Table S2.d- ITC parameters of B2T_KV and insulin.

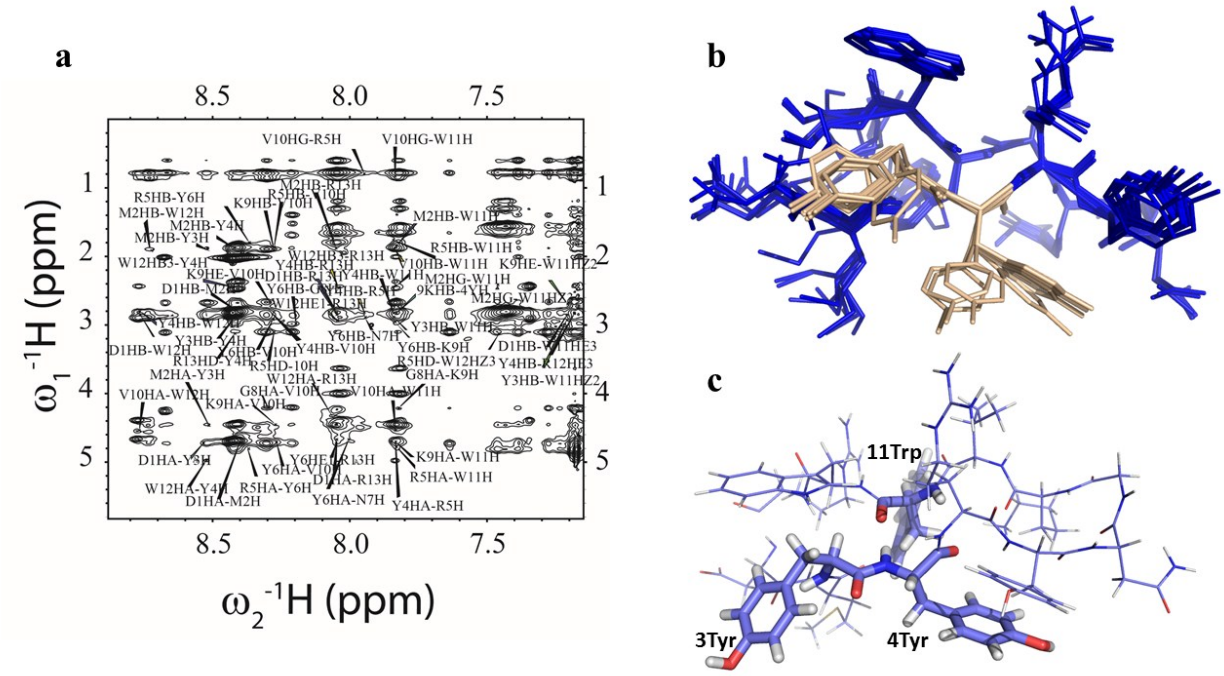
| THERMODYNAMIC PARAMETERS | VALUE |
|---|---|
| ENTHALPY CHANGE ΔH | $1.493*10^{3}$ cal $M^{-1}$ |
| ENTROPY CHANGE ΔS | 28.0 cal/mol/deg |
| FREE ENERGY CHANGE ΔG | $-28*10^{6}$ kcal $M^{-1}$ |
| DISSOCIATION CONSTANT $K_a$ | $1.04*10^{-5}$ $M^{-1}$ |



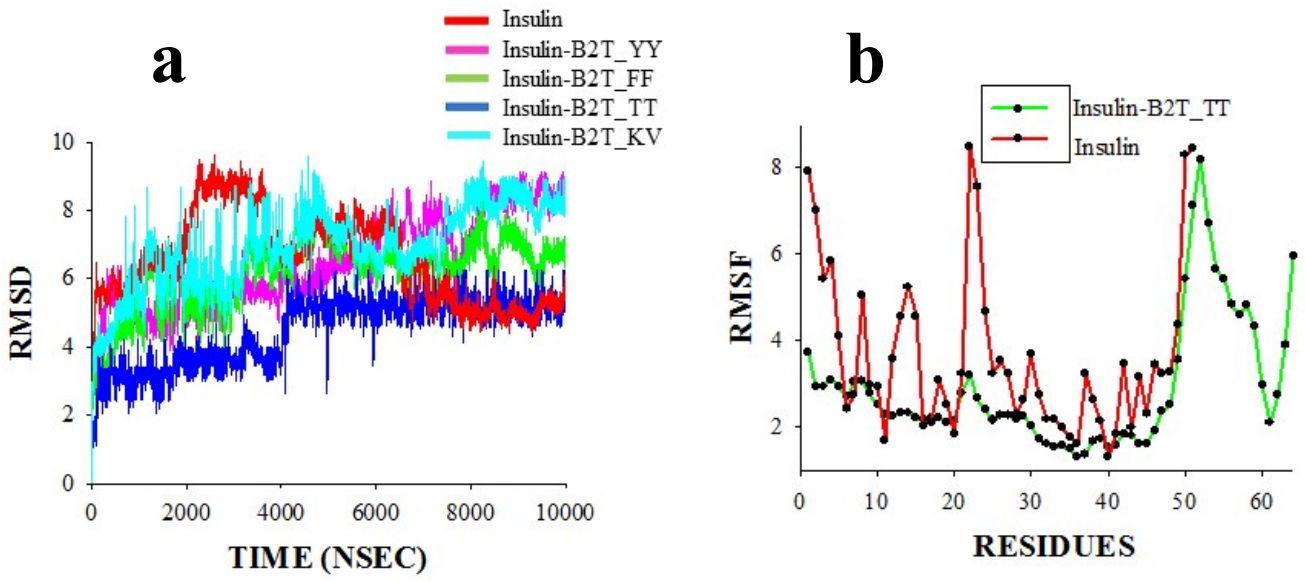

*SI figure 1* a) Noesy spectrum of B2T_FF, b) ensemble structure of B2T_FF and c) showing the orientation of the residues during hairpin structure formation.



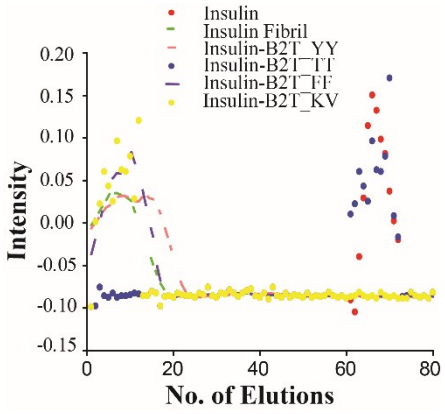

*SI figure 2* a) Noesy spectrum of B2T_KV, b) ensemble structure of B2T_KV and c) showing the orientation of the residues during hairpin structure formation.



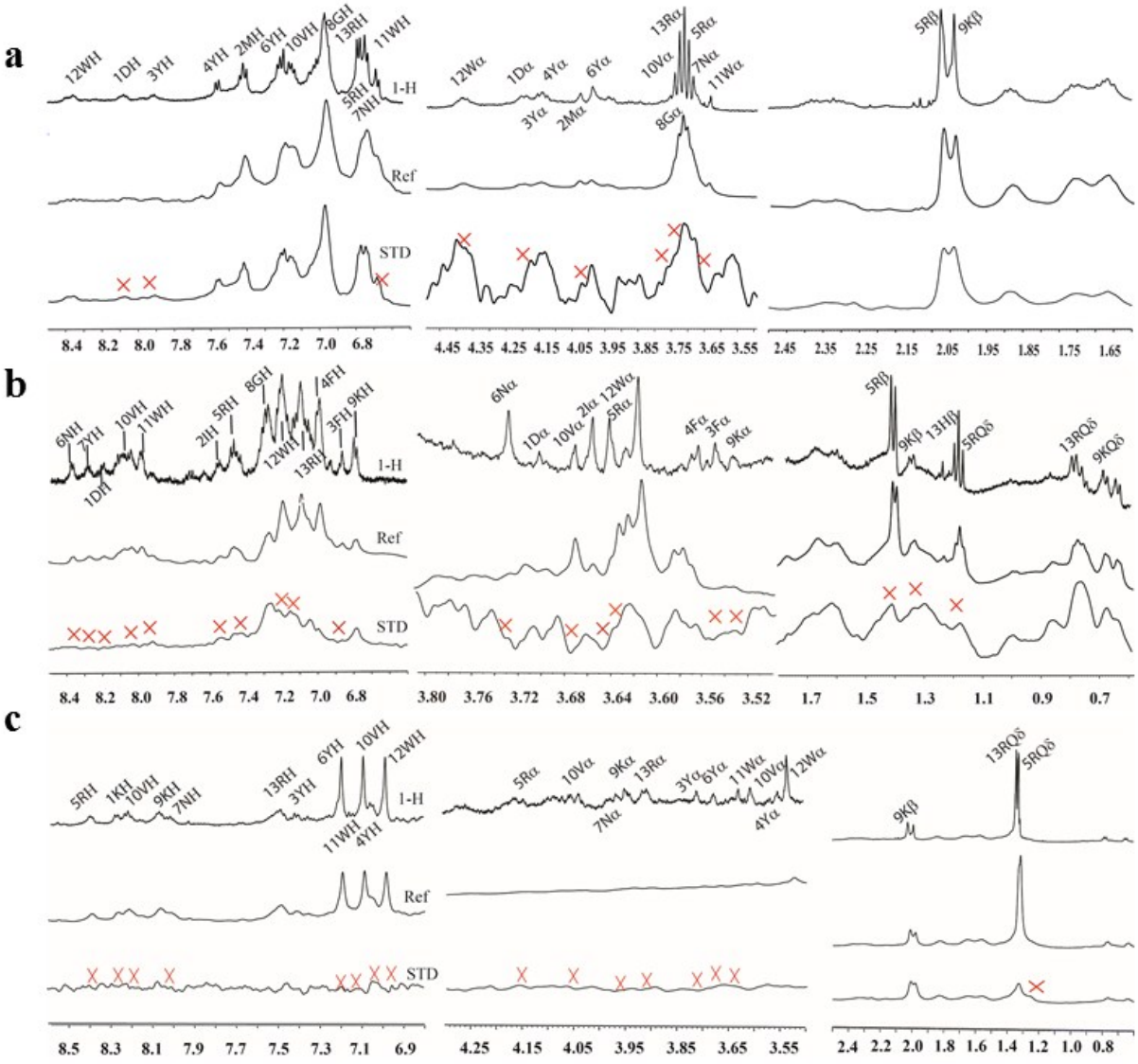

*SI figure 3* a) Noesy spectrum of B2T_YY, b) ensemble structure of B2T_YY and c) showing the orientation of the residues during hairpin structure formation.




*SI figure 4* a) RMSD graph calculated from 100ns of molecular simulation run of insulin, insulin-B2T complex peptides. b) RMSF graph of insulin and insulin-B2T_TT complex.




*SI Figure 5* Size-exclusion chromatography of insulin pre-heating, insulin-fibrils and insulin-B2T peptides.




*SI figure 6* a) STD NMR of B2T_YY, b) STD NMR of B2T_FF and c) STD NMR of B2T_KV.