Electronic Supplementary Material (ESI) for Molecular BioSystems.


# SUPPORTING INFORMATION

# Conformational thermodynamics guided structural reconstruction of bio-molecular fragments

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**Table S1.** The solvent accessible surface area (SASA) of the ensemble averaged conformations of model A, model B, $2Ca^{2+}$-TnC and $4Ca^{2+}$-TnC.

| **TnC** | **SASA (Å$^2$)** |
|---|---|
| Model A | 9374 |
| Model B | 9375 |
| $2Ca^{2+}$-TnC | 9842 |
| $4Ca^{2+}$-TnC | 9838 |

**Table S2**. The backbone conformational entropy change of central linker residues in model B compared to model A.

| **Residues** | $T\Delta S_i^{conf}$ **(kJ/mol)** |
|---|---|
| M81 | 0.84 |
| V82 | 0.89 |
| R83 | 2.23 |
| Q84 | 3.89 |
| M85 | 2.05 |
| K86 | 0.26 |
| E87 | 0.69 |
| D88 | ~0 |
| A89 | -0.94 |
| K90 | -3.71 |
| G91 | ~0 |
| K92 | -4.39 |
| S93 | -4.68 |
| E94 | -3.32 |
| E95 | 1.28 |
| E96 | 1.91 |

**Table S3.** Homologous sequences with variable similarity from PDB compared to the query sequence of "QMKEDAKGKSEEE" corresponding to the missing fragment of TnC. The secondary structures, like helix (H, G), turn (T), bend (S) and beta strand (B, E) are assigned through DSSP.

| PDB id-chain id | Sequence | Secondary structure |
|---|---|---|
| **2I0Z-A** | QMKED-------- | HHTTS-------- |
| **1AYZ-A** | -MKED-------- | -HHHS-------- |
| **4HD5-A** | -MKED-------- | -EETT-------- |
| **3BAT-A** | -MKED-------- | -HHHH-------- |
| **1Y6Z-A** | -MKED-------- | ---TT-------- |
| **1M1S-A** | ----DAKGKS--- | -----TT-EE--- |
| **3HQ2-A** | -----AKGKS--- | -----HHHTT--- |
| **3BDU-A** | -----AKGK---- | -----EE------ |
| **3DDL-A** | --------KSEEE | --------HHHHT |
| **2I6X-A** | --------KSEEE | --------S-HHH |
| **3LMZ-A** | --------KSEEE | ---------SHHH |
| **3ZN3-A** | --------KSEEE | --------HH--- |
| **2Z4T-A** | --------KSEEE | --------HHHHH |
| **4H0P-A** | ---------SEEE | ---------EEEE |
| **4QAN-A** | ---------SEEE | ---------SSHH |
| **1Y0U-A** | ---------SEEE | ----------HHH |
| **1CFD-A** | ---------SEEE | ---------SHHH |
| **3GTD-A** | ---------SEEE | ----------HHH |
| **3RFU-A** | ---------SEEE | ---------EEEE |
| **2A5Y-A** | QMKED-------- | HHHHH-------- |
| **1BJ8-A** | -MKEDGKG----- | -EESSS------- |
| **2BFH-A** | -MKEDG------- | -E-TTS------- |
| **1K3B-B** | -MKED-------- | ---SS-------- |
| **1ITF-A** | -MKED-------- | -HHHH-------- |
| **4ACJ-A** | -MKED-------- | -SSSE-------- |
| **3UNF-K** | -MKED-------- | -EETT-------- |
| **3LFL-A** | -MKED-------- | -HHHH-------- |
| **4XKK-A** | -MKED-------- | -E-GG-------- |
| **1KCM-A** | -MKED-------- | -TGGG-------- |
| **1AQU-A** | -MKED-------- | -HHH--------- |
| **4OA6-A** | -MKED-------- | -S---------- |
| **1VK1-A** | --KEDAK------ | --HHHHH------ |
| **2JI5-A** | --KEDAK------ | --HHHHT------ |

| | | |
|---|---|---|
| **1WZN-A** | --KEDAK------ | --HHT-S------ |
| **3RP9-A** | --KEDAK------ | ---HHHH------ |
| **2EQU-A** | ---EDAKG----- | ---GGGT------ |
| **1NI4-B** | ---EDAKG----- | ---HHHHH----- |
| **1E8X-A** | ---EDAK------ | ---HHHH------ |
| **3R3J-A** | ------KGKSE-- | ------TT--H-- |
| **2ODR-A** | ------KGKSE-- | ------HHHHH-- |
| **4BFR-A** | -------GKSEEE | -------TB-HHH |
| **1N1B-A** | ---------SEEE | ----------HHH |
| **1EF1-C** | --------RSEEE | --------TGGGG |
| **1AD6-A** | --------KSEEE | --------HHHHH |
| **4BC6-A** | --------KSEEE | ---------SHHH |
| **1LFH-A** | --------KSEEE | --------S-HHH |
| **1LFI-A** | --------KSEEE | --------S-TTH |
| **2CRK-A** | --------KSEEE | ---------HHHH |
| **3BJU-A** | --------KSEEE | --------S-HHH |
| **1M1E-B** | ---------SEEE | ---------HHHH |
| **1Y8Q-A** | ---------SEEE | ----------HHH |
| **3EVR-A** | ---------SEEE | ---------HHHH |
| **4OY4-A** | ---------SEEE | ----------HHH |
| **3S9V-A** | ---------SEEE | ---------BHHH |
| **2F05-A** | ---------SEEE | ----------HHH |
| | | |

**Table S4.** The destabilized and disordered residues of $2Ca^{2+}$-TnC with respect to model A (set A).

| **Residues** | $\Delta G_i^{conf}$ **(kJ/mol)** | $T\Delta S_i^{conf}$ **(kJ/mol)** |
|---|---|---|
| F28 | 0.91 | 1.80 |
| Q50 | 1.39 | 8.47 |
| F77 | 0.74 | 1.01 |
| M80 | 0.42 | 3.30 |
| M81 | 0.17 | 2.36 |
| D88 | 0.39 | 3.22 |
| R102 | 1.35 | 1.19 |
| T124 | 0.67 | 2.03 |
| E126 | 1.57 | 6.04 |
| H127 | 0.52 | 3.62 |
| V128 | 1.87 | 1.32 |
| D132 | 0.52 | 0.28 |
| M157 | 2.20 | 7.57 |

| | | |
|---|---|---|
| V160 | 1.11 | 5.14 |

**Table S5.** Comparison of inter-helical angles, $\theta_{i,j}$ between helices $i$ and $j$ of the ensemble averaged conformations of model A, model B and the constructed models from Rosetta, RaptorX, PHYRE2 and ModLoop.

| **ANGLE** | **Model A** | **Model B** | **Rosetta model** | **RaptorX model** | **PHYRE2 model** | **ModLoop model** |
|---|---|---|---|---|---|---|
| $\theta_{a,b}$ | 144° | 138° | 137° | 129° | 137° | 129° |
| $\theta_{c,d}$ | 114° | 134° | 148° | 133° | 148° | 128° |
| $\theta_{e,f}$ | 160° | 146° | 102° | 108° | 102° | 94° |
| $\theta_{g,h}$ | 141° | 128° | 106° | 102° | 106° | 100° |

**Figure Captions**

**Fig S1.** Initial apo-TnC models (a) model A and (b) model B. (c) RMSD and (d) RMSF of model A (black), model B (red), $2Ca^{2+}$-TnC (green) and $4Ca^{2+}$-TnC (blue).

**Fig S2.** The equilibrium probability distributions of backbone dihedral angles, $P_i^A(\phi)$ (black solid line), $P_i^A(\psi)$ (black dashed line) of (a) K86, (b) E87, (c) D88, (d) A89 in model A and similarly $P_i^B(\phi)$ (black solid line), $P_i^B(\psi)$ (black dashed line) of (e) K86, (f) E87, (g) D88, (h) A89 in model B. The insets represent the corresponding $\phi$-$\psi$ scatter-plots, where helix conformation is shown in black dots.

**Fig S3.** The equilibrium probability distributions of backbone dihedral angles, $P_i^A(\phi)$ (black solid line), $P_i^A(\psi)$ (black dashed line) of (a) K92, (b) S93, (c) E94 and (d) E95 in model A and similarly $P_i^B(\phi)$ (black solid line), $P_i^B(\psi)$ (black dashed line) of (e) K92, (f) S93, (g) E94 and (h) E95 in model B. The insets represent the corresponding $\phi$-$\psi$ scatter-plots, where helix conformation is shown in black dots.

**Fig S4.** The distribution of (a) inter-domain distance $P(d_{N-C})$, (b) inter-domain angle $P(\theta_{N-C})$ and (c) radius of gyration $P(R_g)$ calculated from the ensemble of model A (black solid line) and that of model B (gray dashed line). (d) The distribution of $M85_{C\alpha} - E95_{C\alpha}$ distances, $P(d^{C\alpha}_{M85-E95})$ in the NMR, PDB id 1TNW (black solid line) and the simulated (gray dashed line) ensemble of $4Ca^{2+}$-TnC.

**Fig S5.** Homology based models of apo-TnC from (a) Rosetta using PDB template 1TOP, (b) RaptorX using PDB template 1NCX, (c) ModLoop using PDB template 1YV0. The central linker residues M85-E95 are coloured in black.

**Fig S6.** (a) RMSD plot of ModLoop model. (b) The distribution of radius of gyration $P(R_g)$ calculated from the ensemble of ModLoop model. Conformational thermodynamic changes in ModLoop model with respect to model B, illustrating (c) stabilized (green) and destabilized (yellow) residues (d) ordered (green) and disordered (yellow) residues. (e) The energy minimized $2Ca^{2+}$-TnC-TnI docked complex where the destabilized and disordered interfacial residues of $2Ca^{2+}$-TnC with respect to the ModLoop model are shown in red.

**Fig S7.** The energy minimized $2Ca^{2+}$-TnC-TnI docked complex where the destabilized and disordered interfacial residues of $2Ca^{2+}$-TnC with respect to the model A are shown in red.

**Fig S8.** The ordered (green) and disordered (yellow) residues (a) in the C-terminal domain of $2Ca^{2+}$-TnC, (b) in the N-terminal domain of $4Ca^{2+}$-TnC, (c) in the C-terminal domain of $4Ca^{2+}$-TnC with respect to model B.

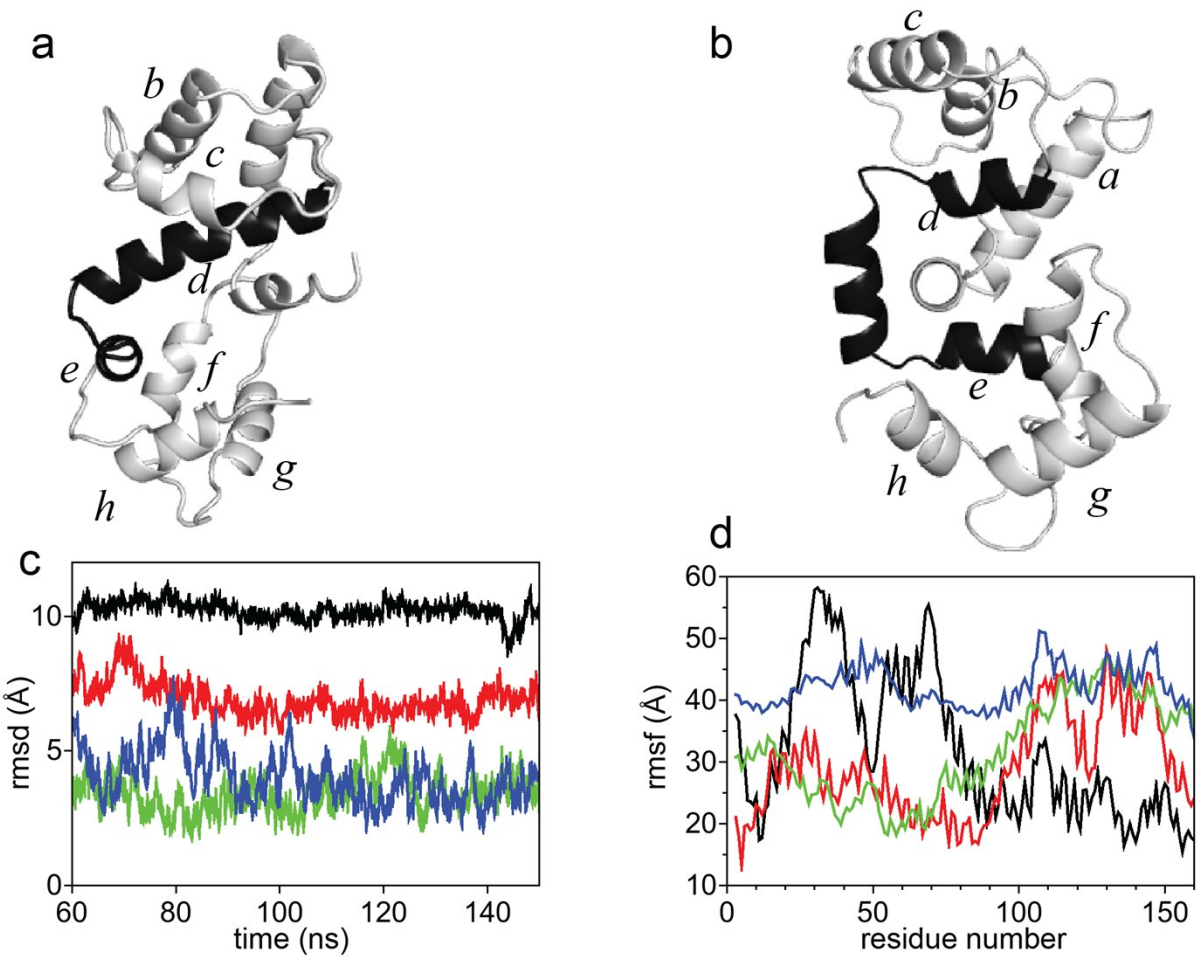


**Fig S1.**

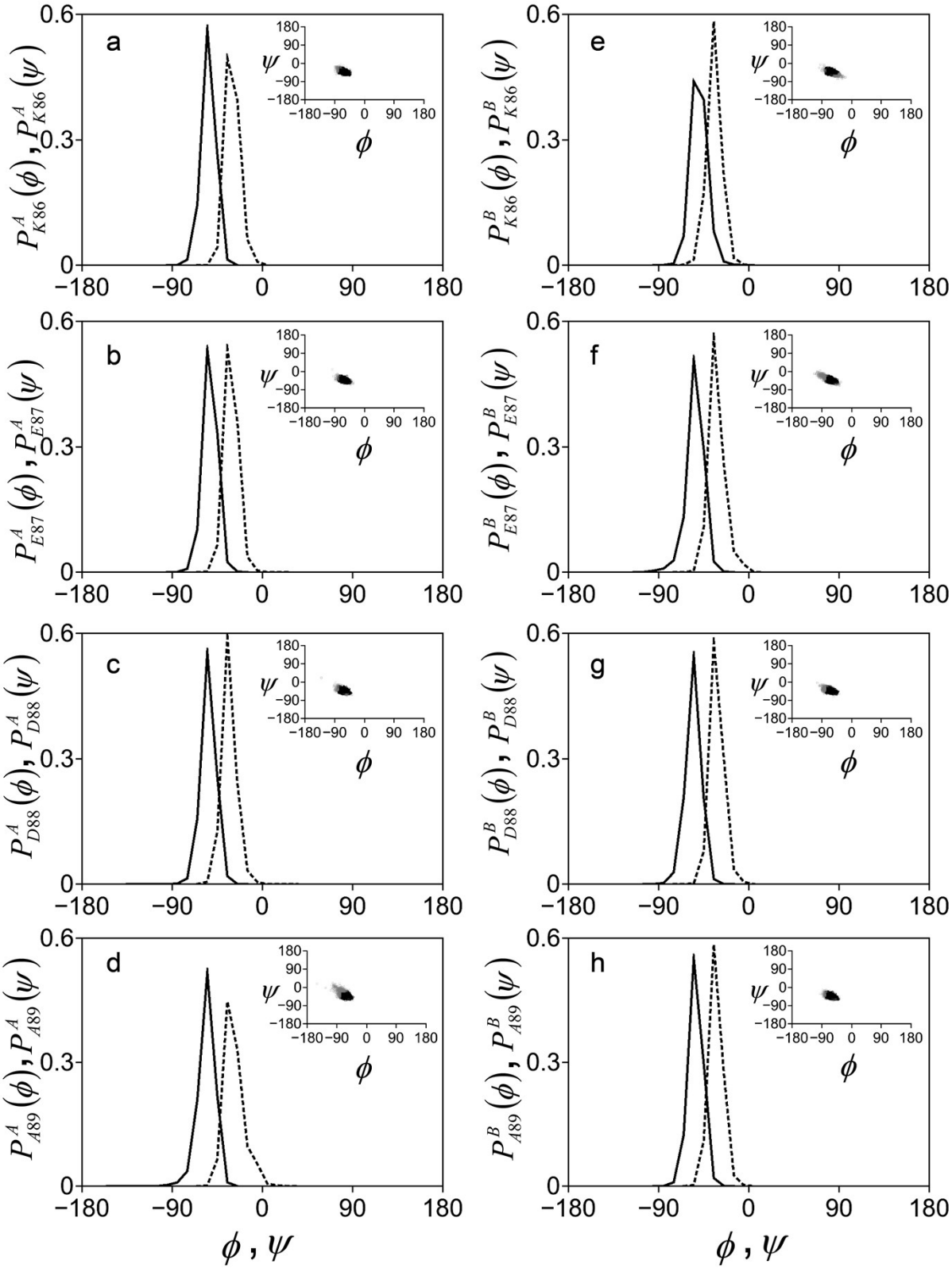
a
b
c
d
e
f
g
h
$P^A_{K86}(\phi), P^A_{K86}(\psi)$
$P^A_{E87}(\phi), P^A_{E87}(\psi)$
$P^A_{D88}(\phi), P^A_{D88}(\psi)$
$P^A_{A89}(\phi), P^A_{A89}(\psi)$
$P^B_{K86}(\phi), P^B_{K86}(\psi)$
$P^B_{E87}(\phi), P^B_{E87}(\psi)$
$P^B_{D88}(\phi), P^B_{D88}(\psi)$
$P^B_{A89}(\phi), P^B_{A89}(\psi)$
0.6
0.3
0
−180 −90 0 90 180
$\phi$
$\psi$
$\phi, \psi$

**Fig S2.**

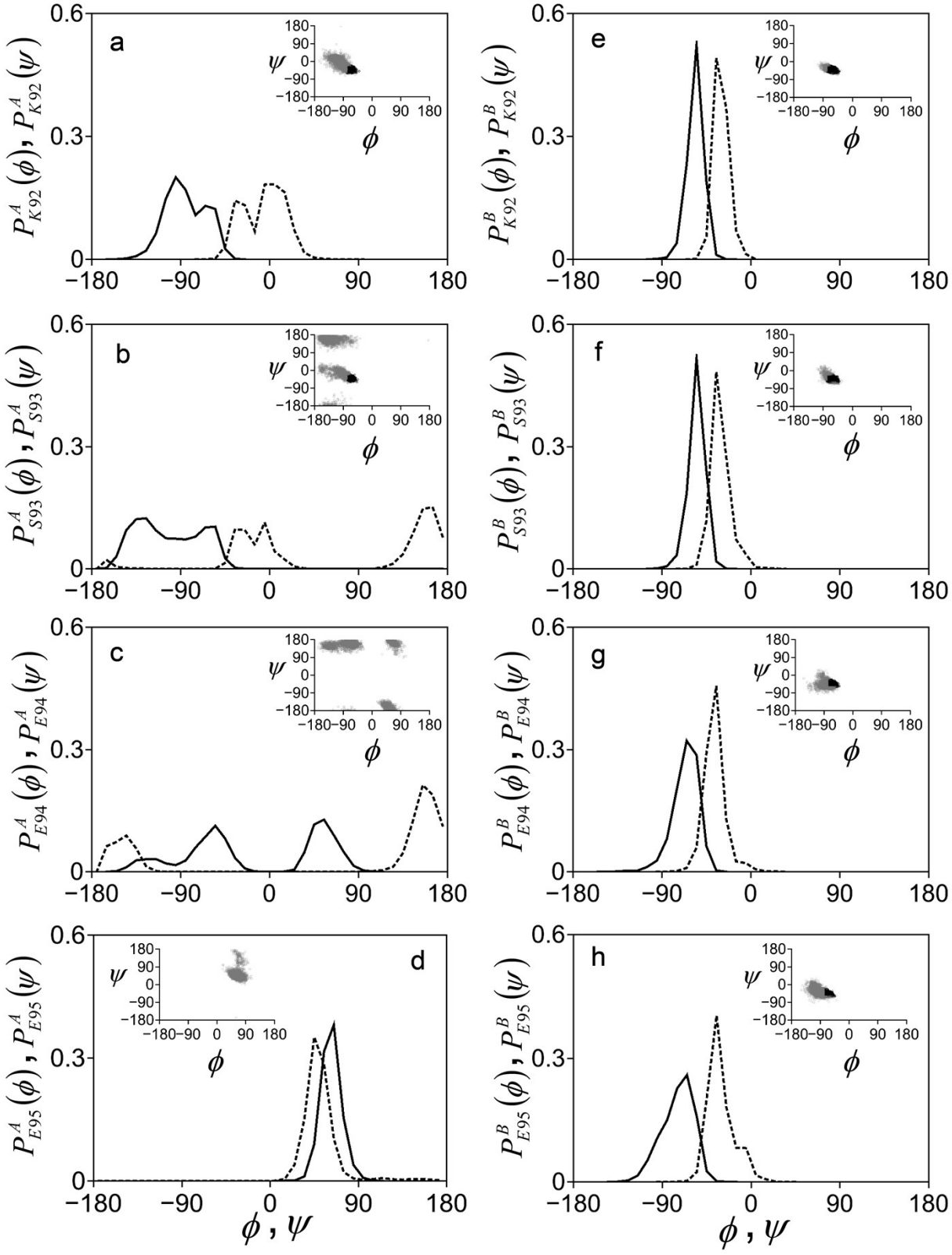
a
b
c
d
e
f
g
h
$P^A_{K92}(\phi), P^A_{K92}(\psi)$
$P^A_{S93}(\phi), P^A_{S93}(\psi)$
$P^A_{E94}(\phi), P^A_{E94}(\psi)$
$P^A_{E95}(\phi), P^A_{E95}(\psi)$
$P^B_{K92}(\phi), P^B_{K92}(\psi)$
$P^B_{S93}(\phi), P^B_{S93}(\psi)$
$P^B_{E94}(\phi), P^B_{E94}(\psi)$
$P^B_{E95}(\phi), P^B_{E95}(\psi)$
0.6
0.3
0
−180 −90 0 90 180
$\phi$ , $\psi$
$\phi$
$\psi$
180 90 0 −90 −180
−180 −90 0 90 180

**Fig S3.**

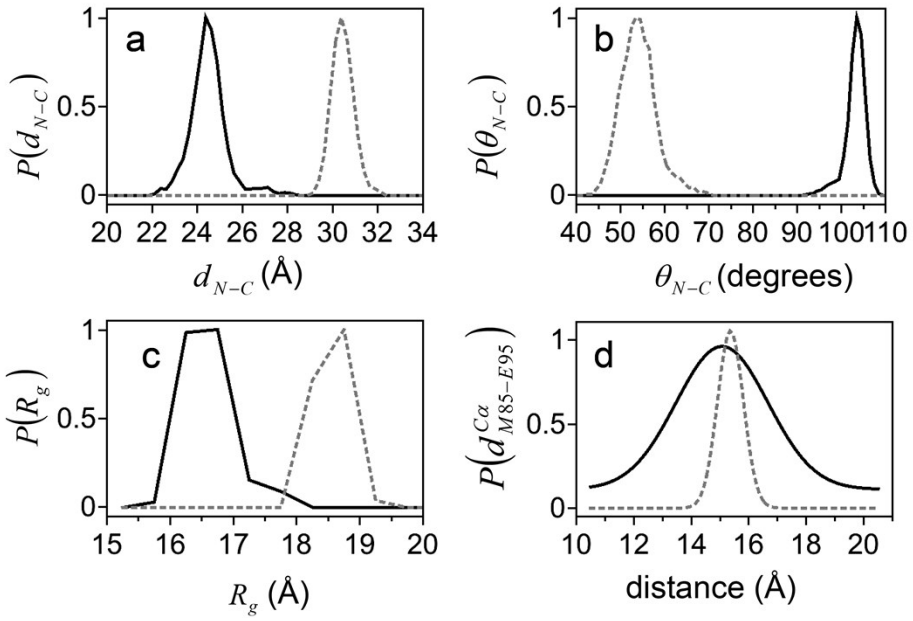
a
b
c
d
$P(d_{N-C})$
$P(\theta_{N-C})$
$P(R_g)$
$P(d^{C\alpha}_{M85-E95})$
$d_{N-C}$ (Å)
$\theta_{N-C}$ (degrees)
$R_g$ (Å)
distance (Å)

**Fig S4.**

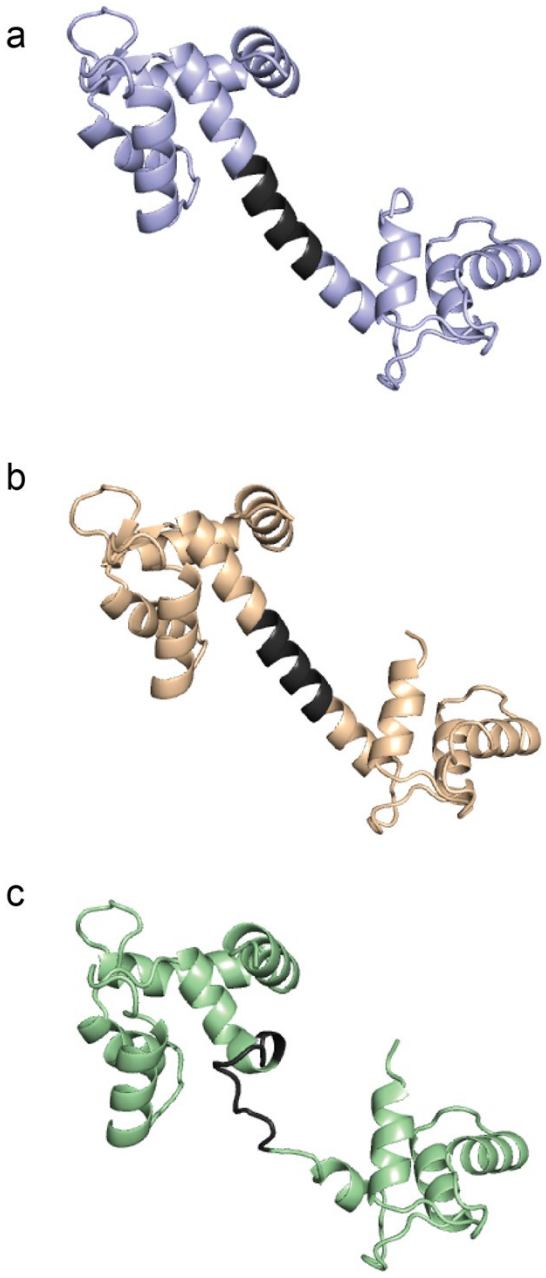


**Fig S5.**

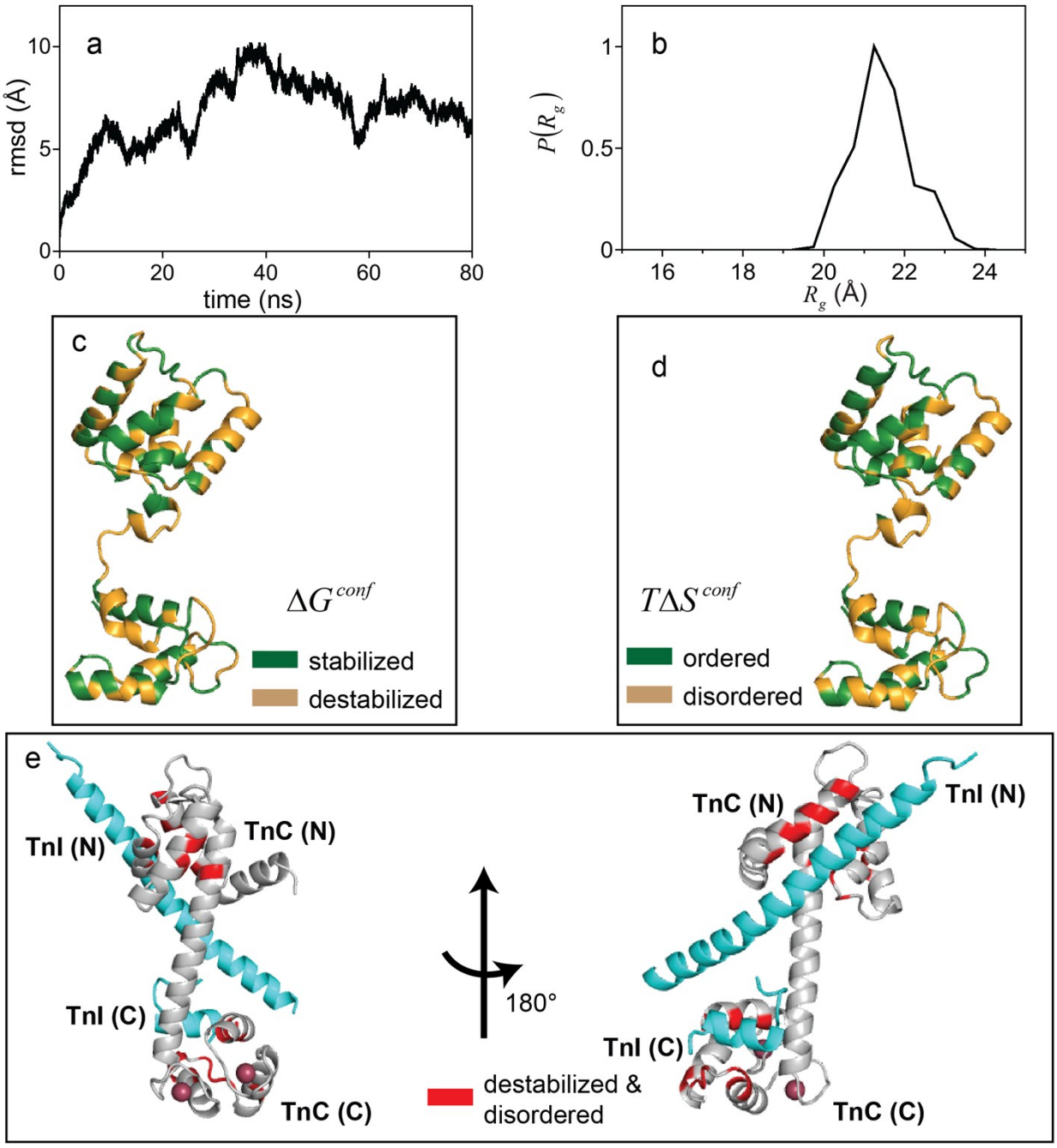
a
rmsd (Å)
time (ns)
b
$P(R_g)$
$R_g$ (Å)
c
$\Delta G^{conf}$
stabilized
destabilized
d
$T\Delta S^{conf}$
ordered
disordered
e
TnI (N)
TnC (N)
TnI (C)
TnC (C)
180°
destabilized &
disordered

**Fig S6.**

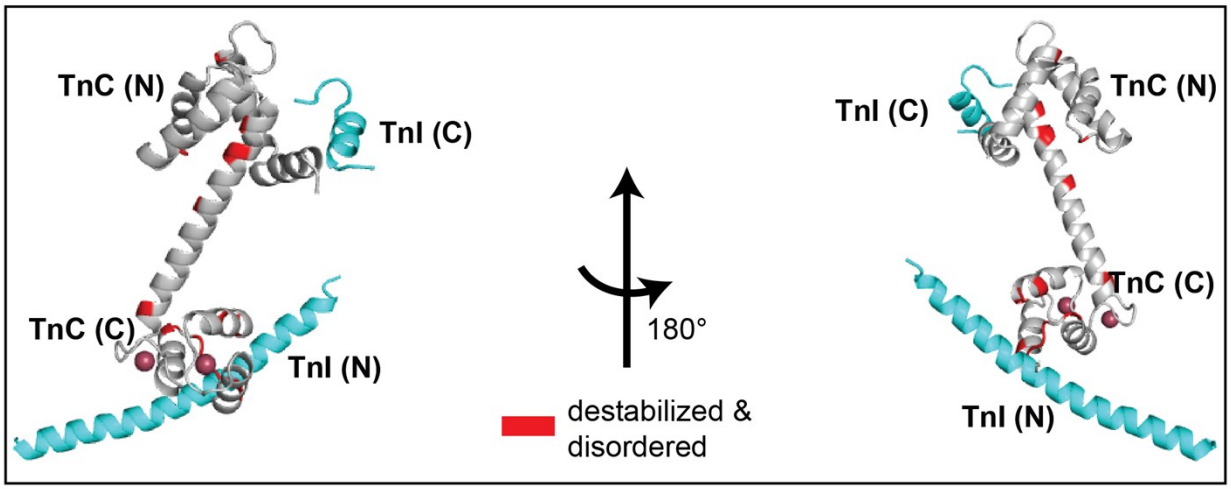
TnC (N)
TnI (C)
TnC (C)
TnI (N)
180°
destabilized &
disordered
TnI (C)
TnC (N)
TnC (C)
TnI (N)

**Fig S7.**

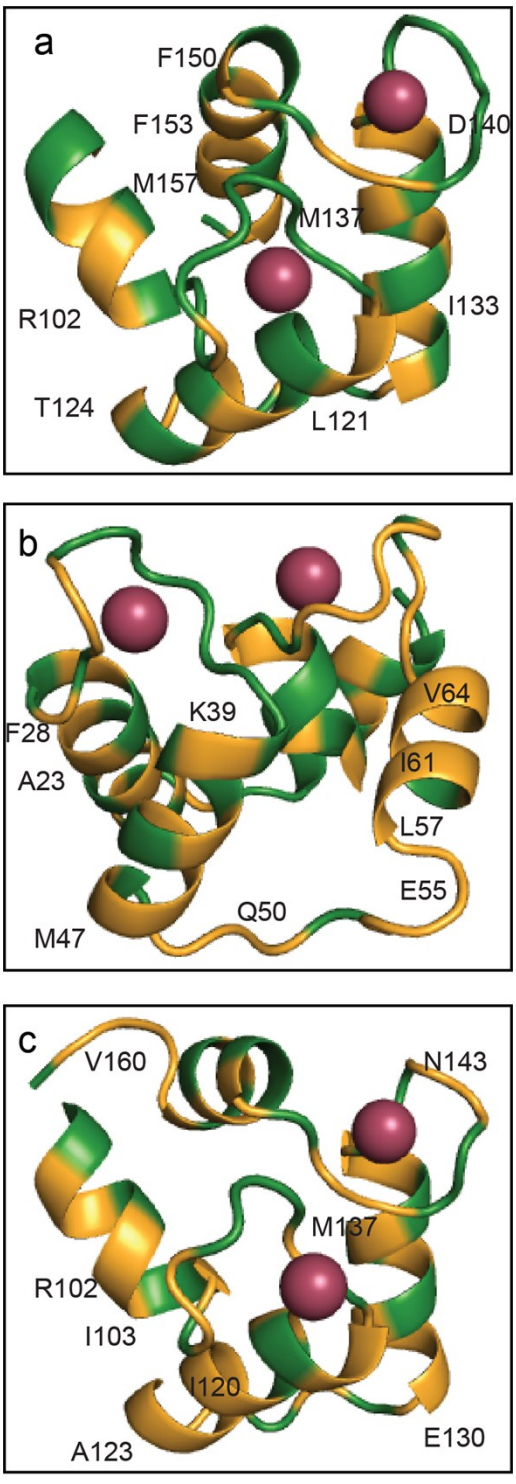
a
F150
F153
D140
M157
M137
R102
I133
T124
L121
b
K39
V64
F28
I61
A23
L57
E55
Q50
M47
c
V160
N143
M137
R102
I103
I120
A123
E130

**Fig S8.**