Supporting Information: Molecular Dynamics of the Proline Switch and its Role in Crk Signaling

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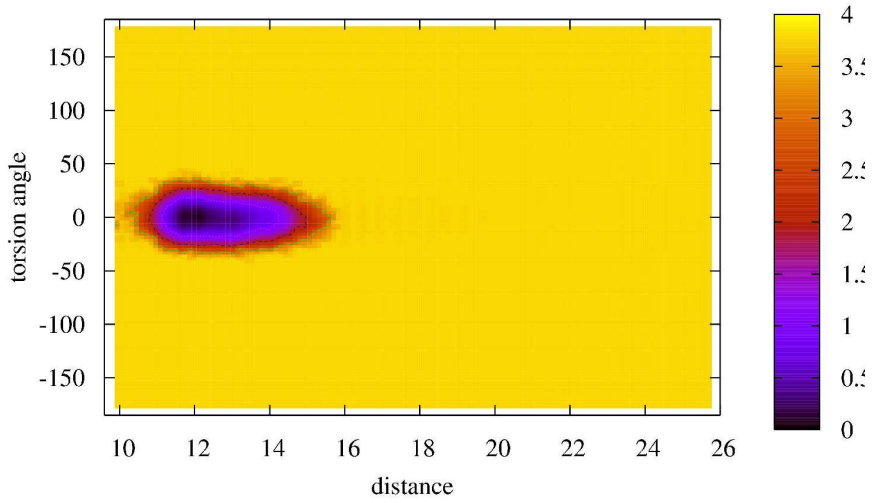
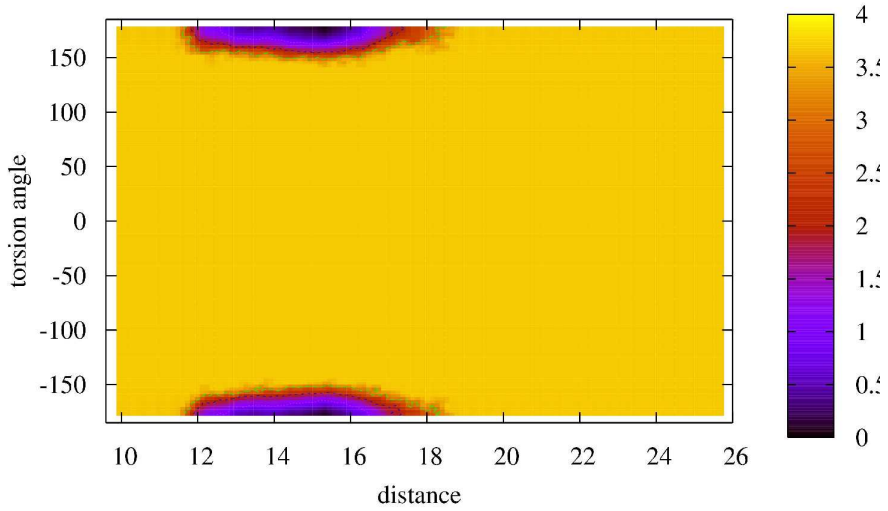


(a)



(b)

Figure S1: Free energy of Crk l-SH3^{C} in 2D (the center of mass distance between the second half of the linker (residue 220 to 238) and the SH3^{C} domain (residue 239 to 297) and the ω dihedral angle of Pro238) from the normal MD simulations. (a) 200 ns normal MD simulation started from the cis state (first model of PDB: 2L3P). (b) 200 ns normal MD simulation started from the trans state (first model of PDB: 2L3Q).

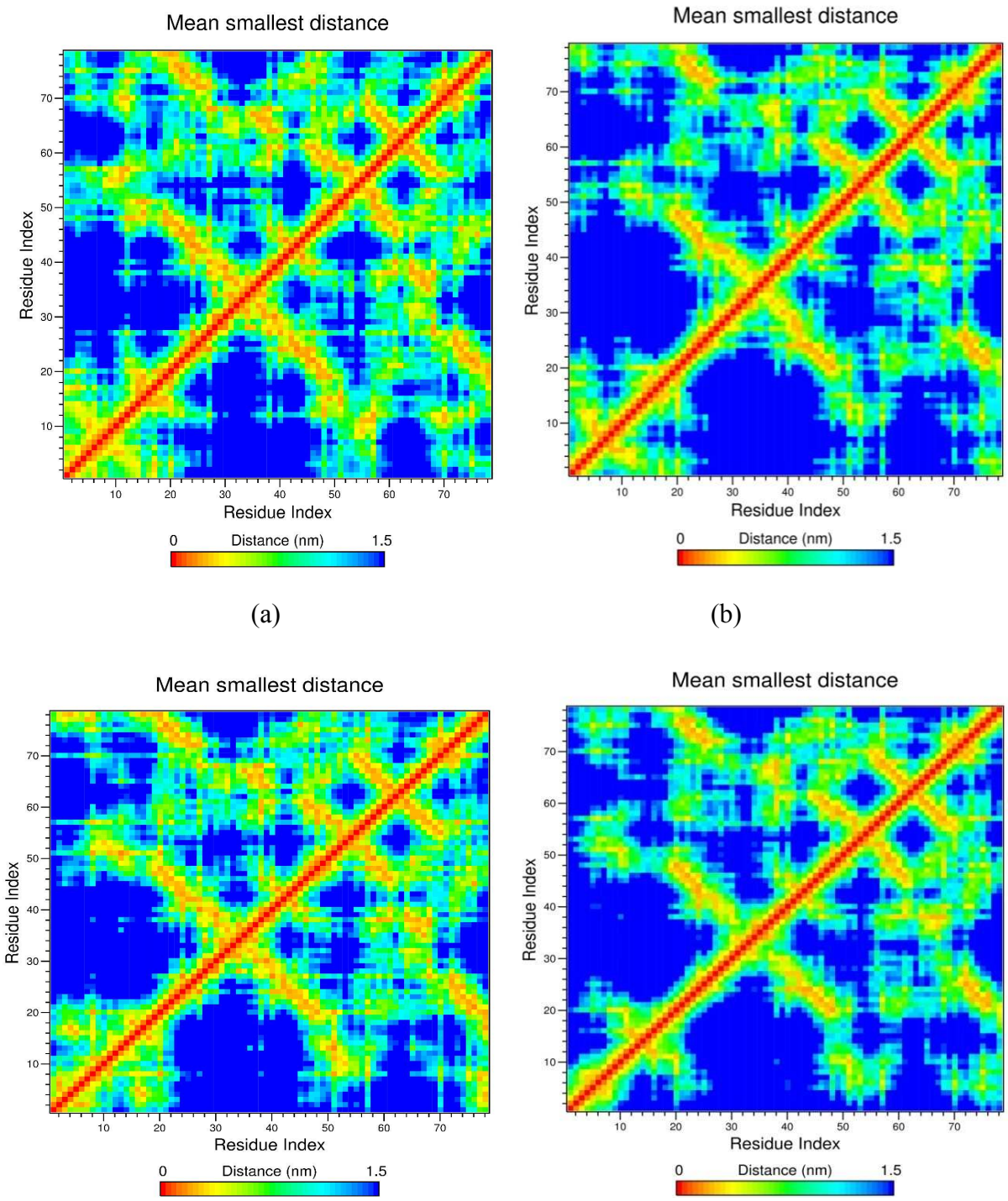
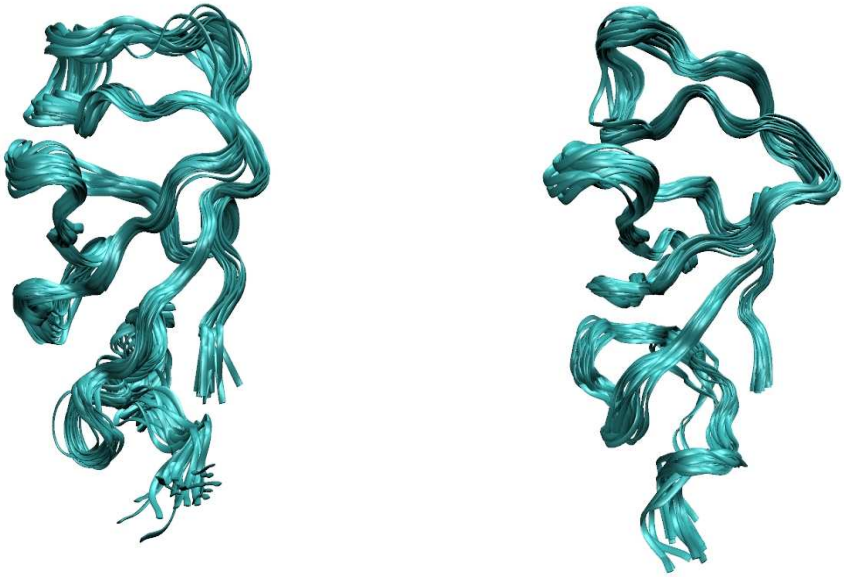


Figure S2: Residue contact maps of Crk l-SH3^{C} from NMR structures and MD simulations. (a) from 20 NMR models of PDB: 2L3P (cis state); (b) from 100,000 snapshorts of the 200ns MD simulation started from the first model of PDB: 2L3P; (c) from 20 NMR models of PDB: 2L3Q (trans state); (d) from 100,000 snapshorts of the 200ns MD simulation started from the first model of PDB: 2L3Q. The differences between the cis and trans states can be found by checking the contact regions between the linker (residues 1 to 20) and the domain (residues 21 to 79). See the main text for more details.



(a) **(b)**

Figure S3: Low-energy conformations (20 each) corresponding to the two minima (two macrostates) on the PMF of l-SH3^{C} constructed from the metadynamics simulation. (a) cis state, (b) trans state.

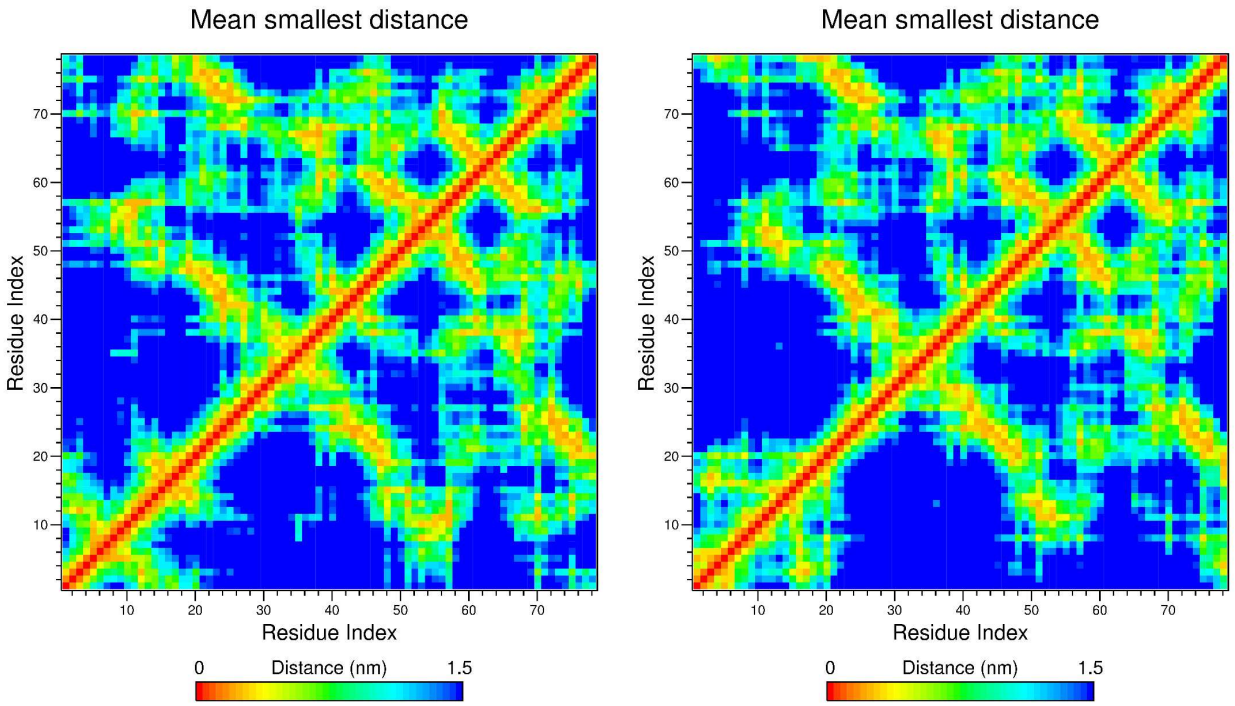
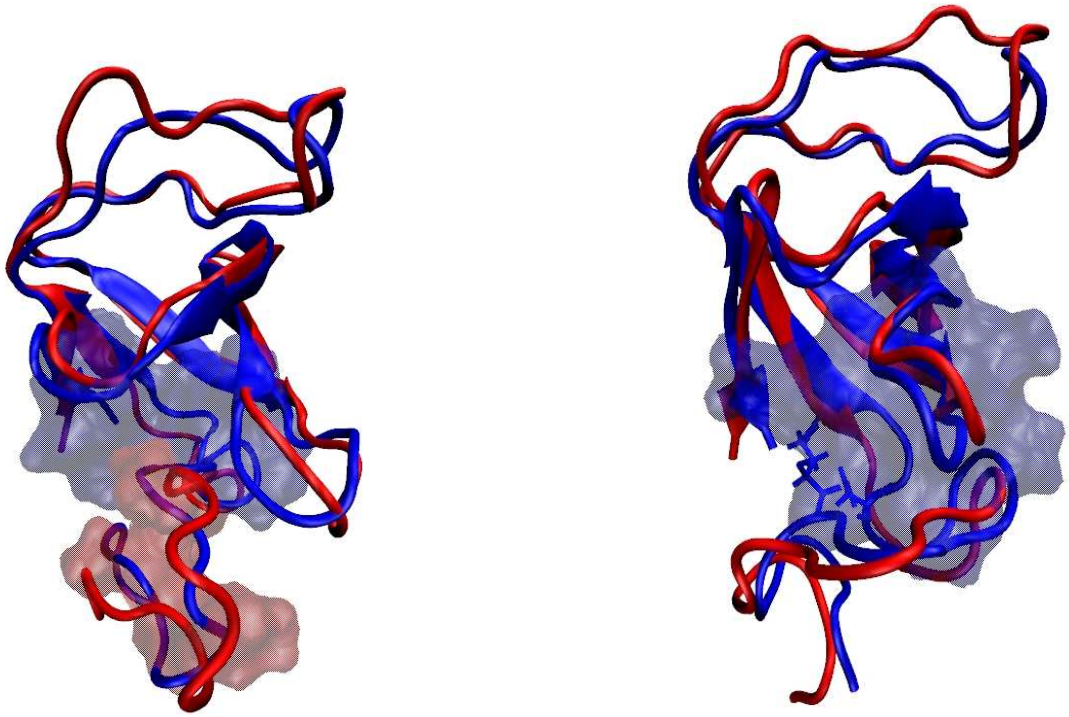


Figure S4: Residue contact maps calculated from the 20 low-energy conformations (Figure 3S) for the two macrostates of Crk l-SH3^{C} (corresponding to the two minima on the PMF constructed from the metadynamics simulation). (a) cis state, (b) trans state.



(a) **(b)**

Figure S5: Comparison of Crk l-SH3^{C} structures from the NMR with that from the metadynamics simulation. (a) Blue: the first NMR model from PDB: 2L3P (cis state). Red: a representative structure of the cis state from the metadynamics simulation. The transparent blue surface area shows the hydrophobic interaction region between the linker (Leu231 and Leu234) and the SH3^{C} domain (Ala241, Val267, Trp276, Phe289, and Val292). In contrast, the red surface area represents the hydrophobic interaction region between the first half (Pro225 and Ile227) and the second half linker (Pro230 and Pro232). (b) Blue: the first NMR model from PDB: 2L3Q (trans state). Red: a representative structure of the trans state from the metadynamics simulation. The transparent blue surface denotes the the hydrophobic contacts between the linker (ala223, Ile227, Pro230 and Pro 232) and the SH3^{C} domain (Phe239, Lys269, Ile270, Asn271, Trp276, and Leu 294). The blue ball and stick residues represent Asn236 and Gln297 forming a hydrogen bond between the linker and the SH3^{C} domain.

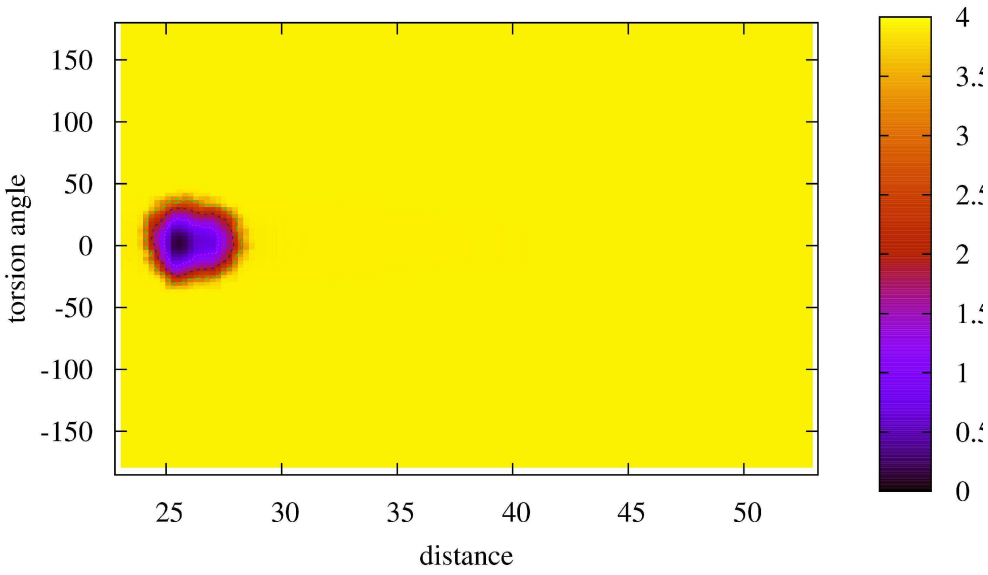


Figure S6: Free energy of Crk $SH3^{N}$-l-$SH3^{C}$ in 2D (the center of mass distance between the $SH3^{N}$ and $SH3^{C}$ domains and the ω dihedral angle of P238) from the 200ns standard MD simulation started from the cis state (first model of PDB: 2L3S).

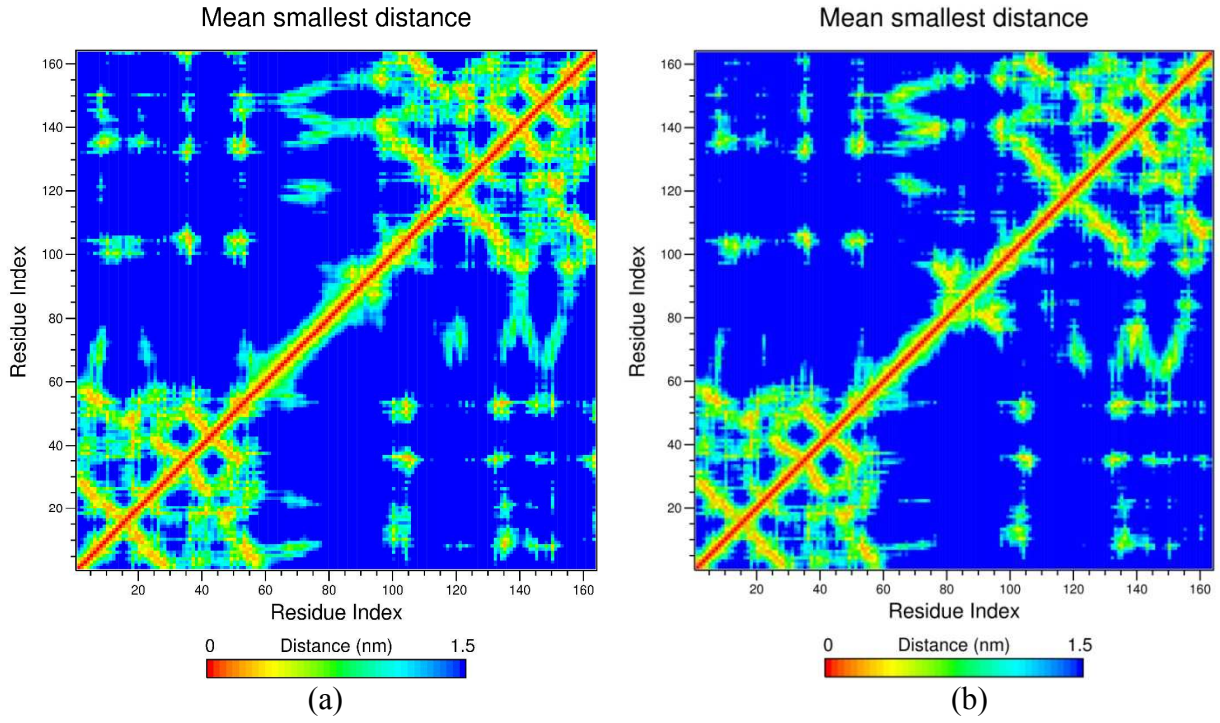
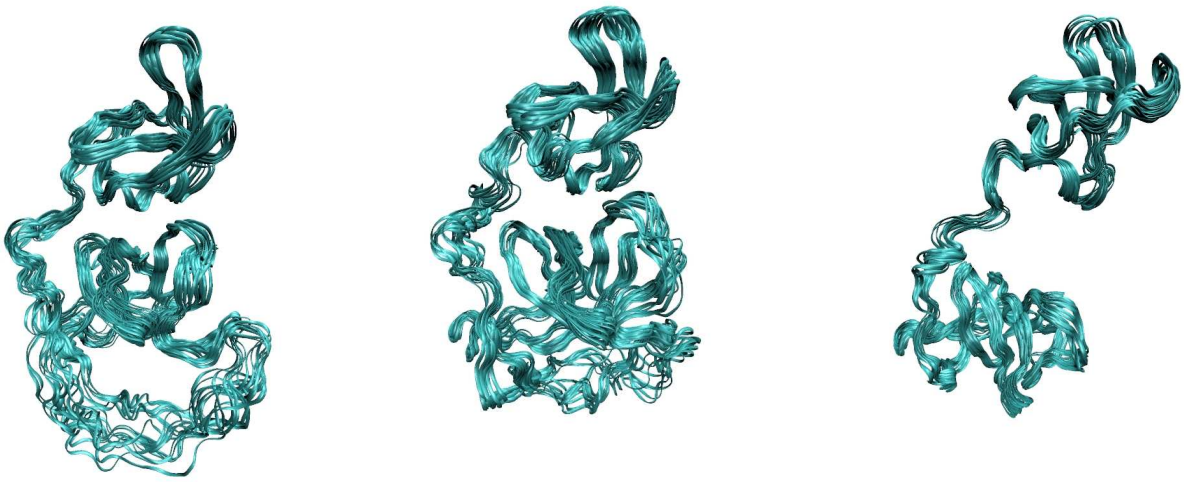


Figure S7: Residue contact maps of Crk $SH3^{N}$-l-$SH3^{C}$ calculated from (a) 20 NMR models of PDB: 2L3S (cis state) and (b) 100,000 snapshots of the 200ns MD simulation started from the first model of PDB: 2L3S.



(a) **(b)** **(c)**

Figure S8: Low-energy conformations (20 each) corresponding to the three minima (three macrostates) on the PMF of Crk $SH3^{N}$-l-$SH3^{C}$ constructed from the metadynamics simulation. (a) cis closed state, (b) trans closed state, and (c) trans open state.

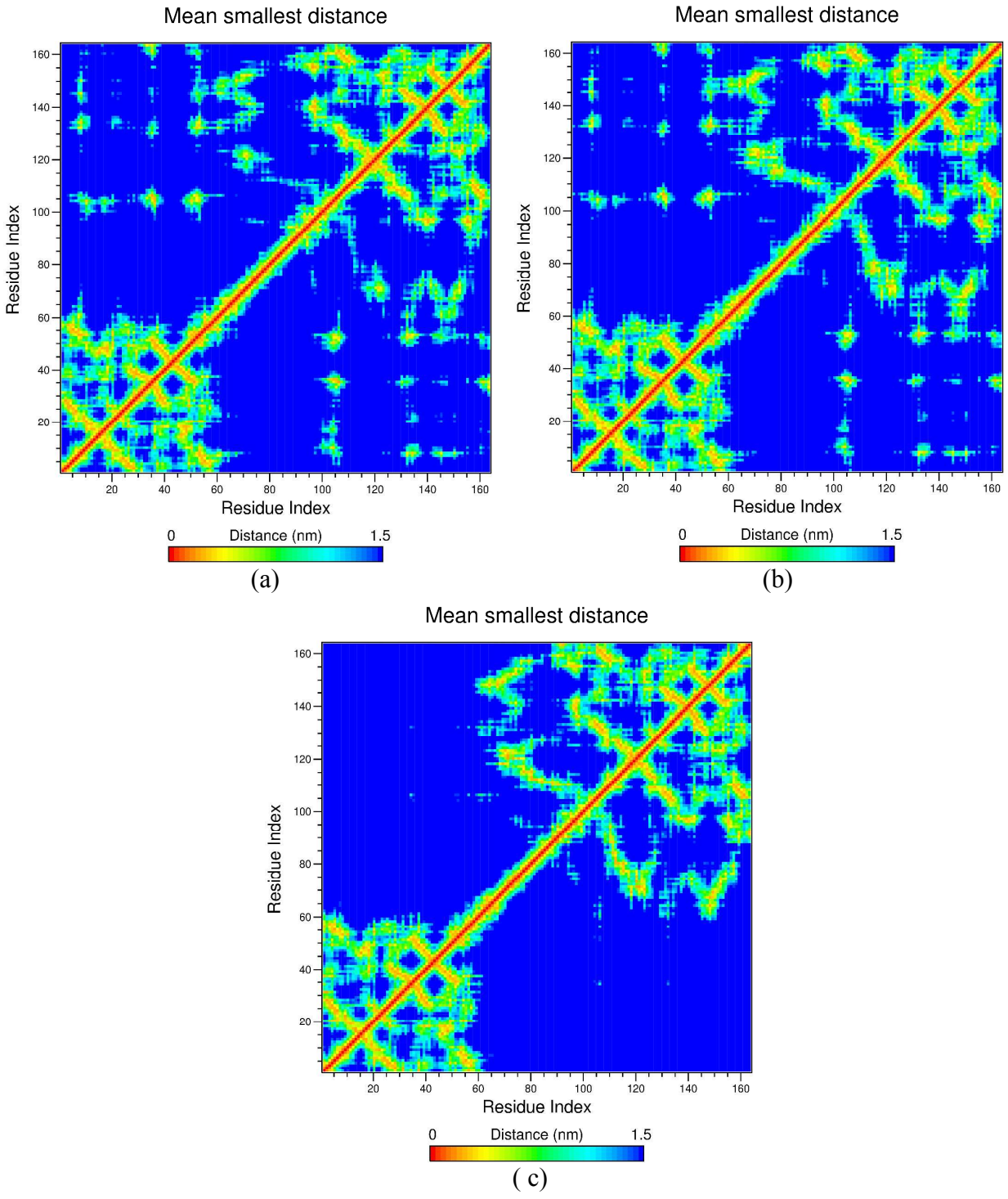


Figure S9: Residue contact maps calculated from 20 low-energy conformations (Figure 8S) representing three macrostates of Crk $SH3^{N}$-l-$SH3^{C}$ (corresponding to the three minima on the PMFs constructed from the metadaynamics simulation). (a) cis closed state, (b) trans closed state, and (c) trans open state.

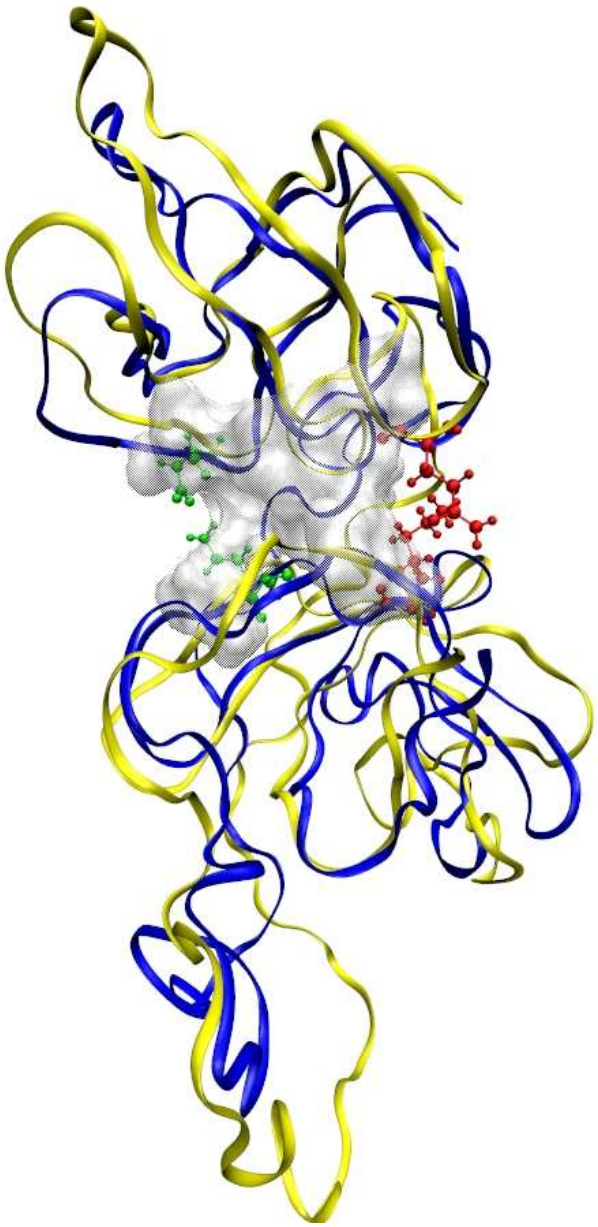


Figure S10: Structure comparison of Crk $SH3^N$-l-$SH3^C$ cis closed state between the NMR and metadynamics simulation. Blue: first model of NMR derived PDB: 2L3S. Yellow: the structure corresponding to the cis closed minimum from the metadynamics simulation. The transparent surface area shows the hydrophobic interaction regions includes the canonical binding site of $SH3^N$ (aromatic resides Phe142, Phe144, Trp170, and Tyr187 along with Pro184 and Pro186) and other three hydrophobic residues from $SH3^C$ (Pro238, Phe239, and Ile270). The Gln 169 and Lys 266 forming a hydrogen bond were displayed as sticks and balls in red. Instead, the Asp143 and Lys269 consisting of a salt bridge in the cis close state were denoted in green. Note that the structures were only aligned to the $SH3^N$ domain.