

# **Supporting Information for:**

## **Structural Refinement from Restrained-Ensemble**

### **Simulations Based on EPR/DEER Data:**

#### **Application to T4 Lysozyme**

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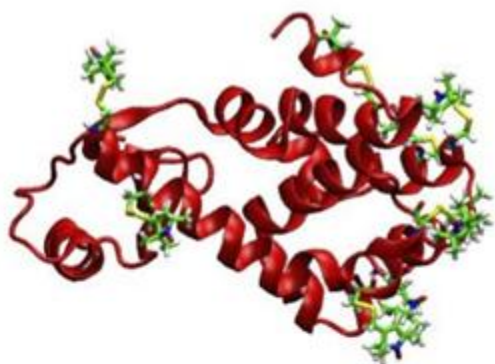
<sup>2</sup>Department of Molecular Physiology and Biophysics,  
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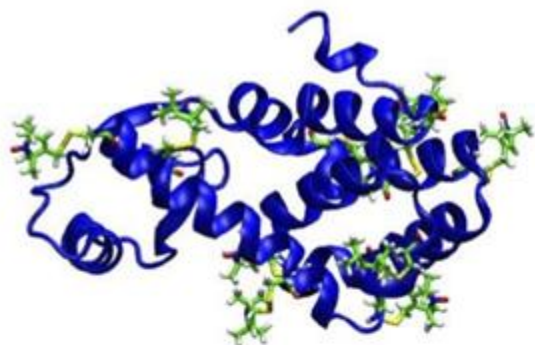
**Table S1.** T4L double mutants

Site1	Site2	Source	Site1	Site2	Source	Site1	Site2	Source	Site1	Site2	Source
59	159	C	65	135	A	82	132	C	94	132	C
60	90	C	72	119	E	82	134	D	108	123	D
60	94	C	72	131	E	82	155	C	108	134	D
60	109	C	72	151	E	83	155	D	108	155	C
60	154	C	75	119	E	85	119	E	109	134	C
61	128	D	75	131	E	85	131	E	115	155	C
61	135	A	75	151	E	85	151	E	116	134	C
62	109	D	76	119	E	93	108	D	119	128	B
62	123	D	76	131	E	93	112	C	119	131	B
62	134	C	76	151	E	93	123	C	123	131	B
62	155	D	79	119	E	93	134	D	128	155	D
64	122	C	79	131	E	93	154	C	140	151	B
65	76	A	82	94	C	94	123	D			
A: Borbat, 2002 <sup>1</sup> B: Alexander, 2008 <sup>2</sup> C: Kazmier, 2011 <sup>3</sup> D: to be described elsewhere E: current mutants											

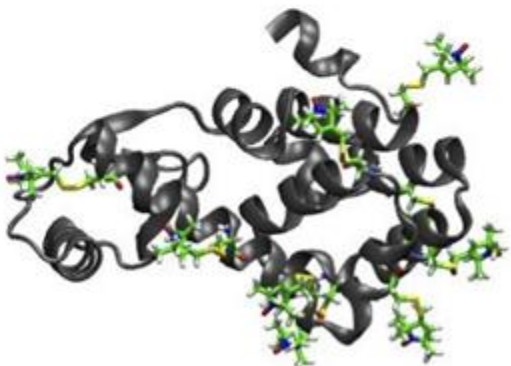
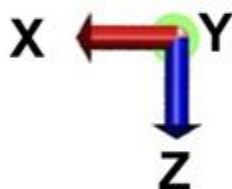
**Figure S1.** Conventional unrestrained MD Simulation set up for 37 spin labeled T4 Lysozymes. Spin-labels are far from each other so that they do not interact with each other. The distance between the center of mass of each T4L is  $80\text{\AA}$  from each other; thereby, they are far enough to cause any interaction. Similarly, the spin-label side chains were also kept at least four residues apart with a distance of about  $10\text{-}15\text{\AA}$  from each other; therefore, the interaction between the spin-labels are also minimum.



**A**



**B**

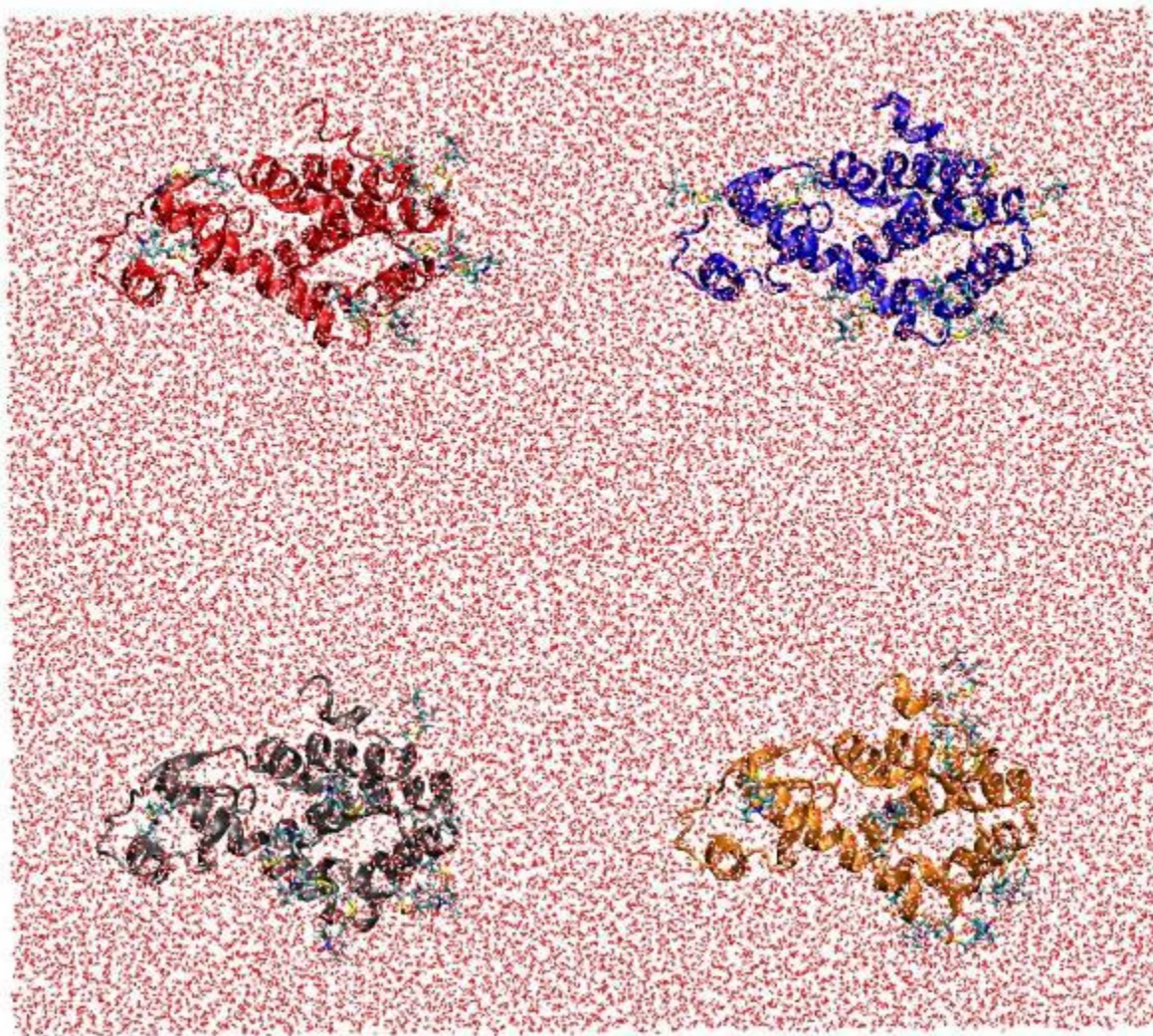


**C**



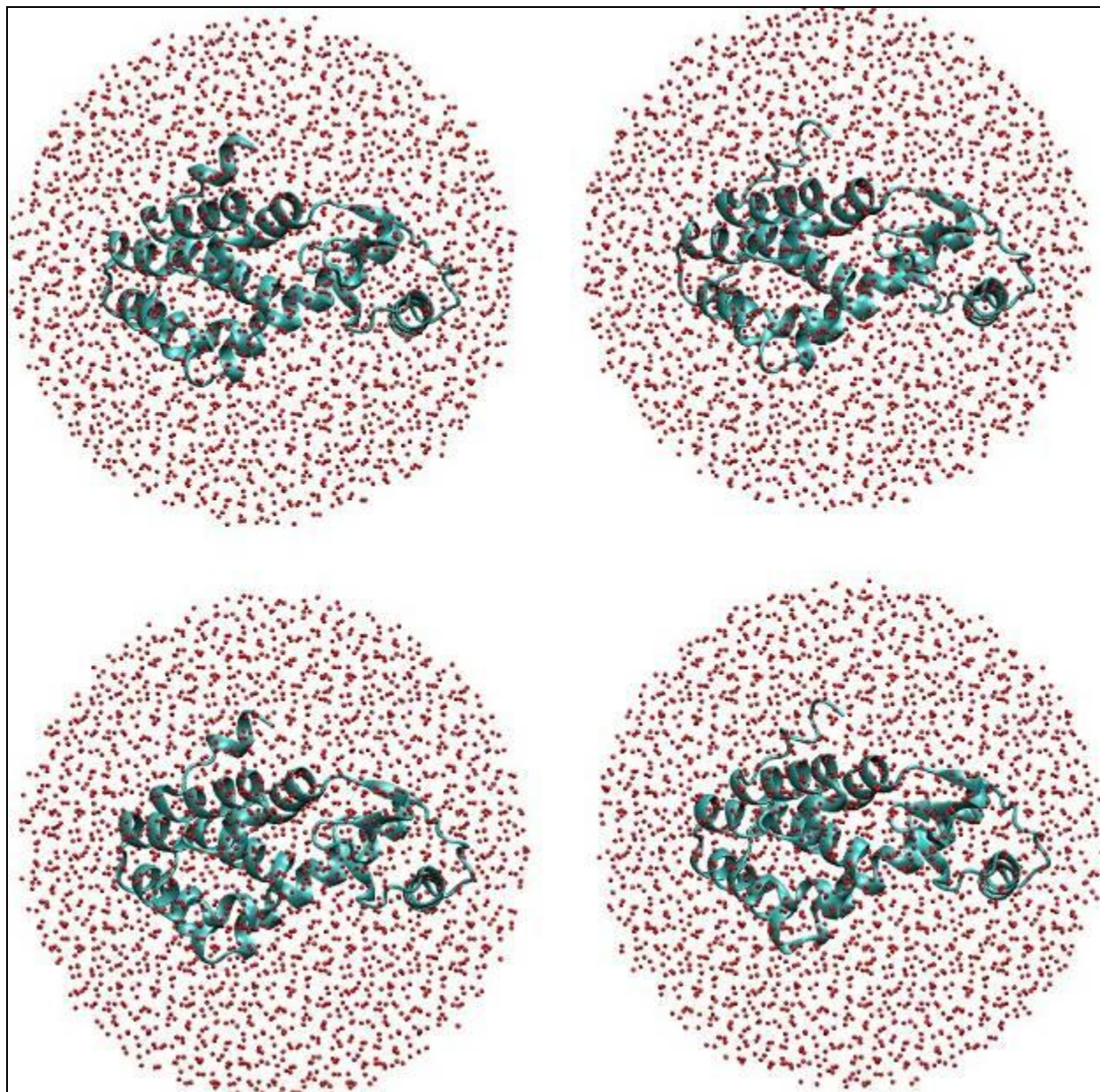
**D**

**Figure S2.** The T4L system is solvated by TIP3P waters in a rectangular box for MD and LES simulation.

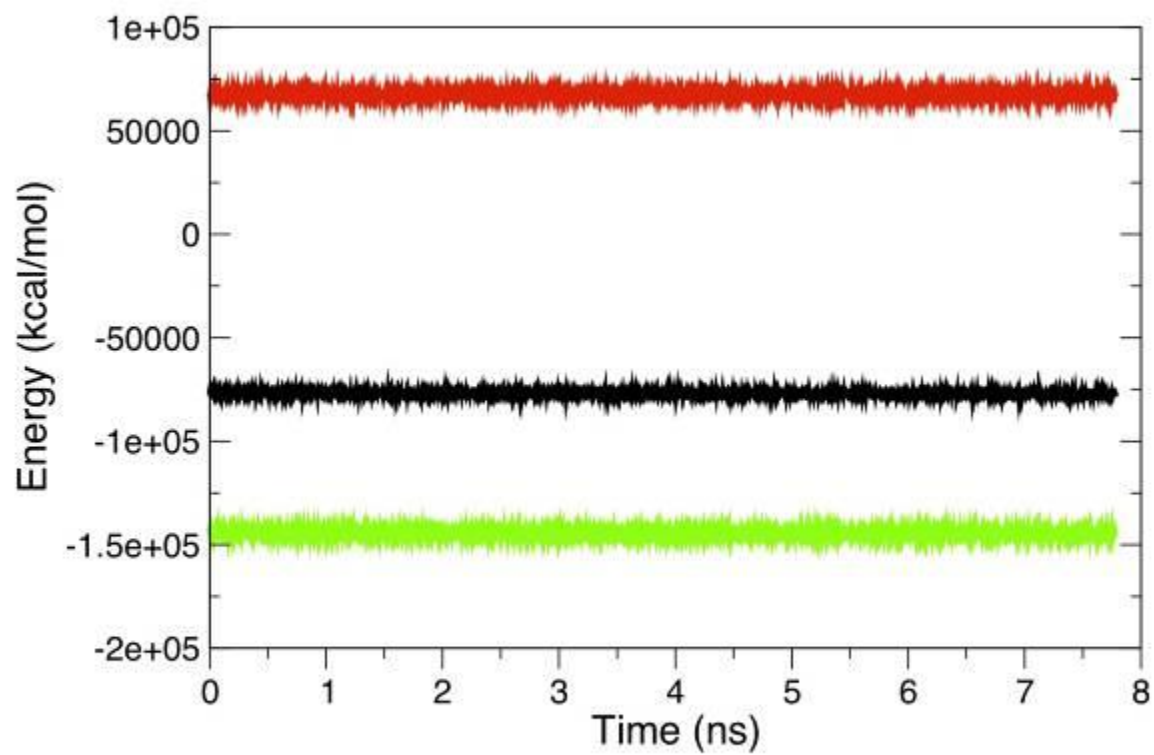




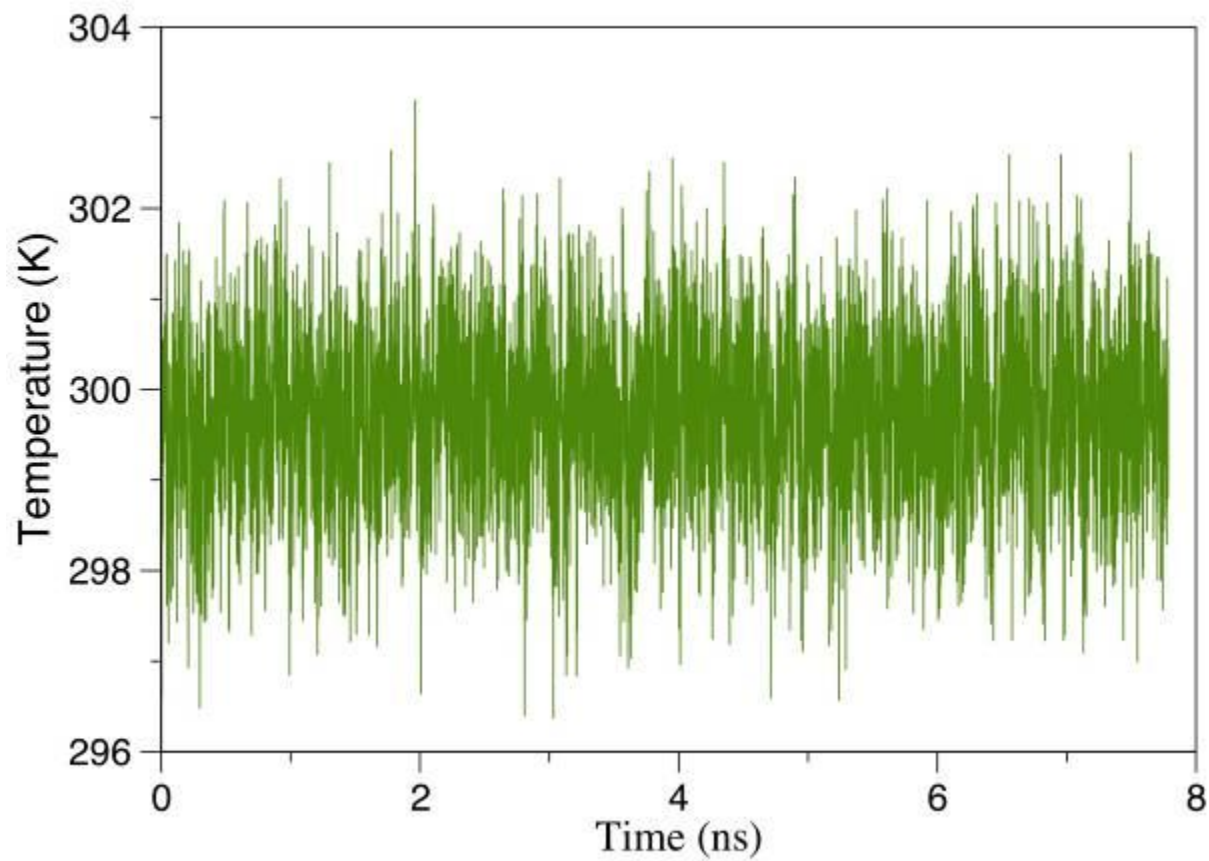
**Figure S3.** Simulation set up in RE simulation. The number of water molecules from the MD and LES simulation box is reduced from 41547 to 19479 by keeping only the waters that are within  $35\text{\AA}$  from the center of mass of each T4L systems.



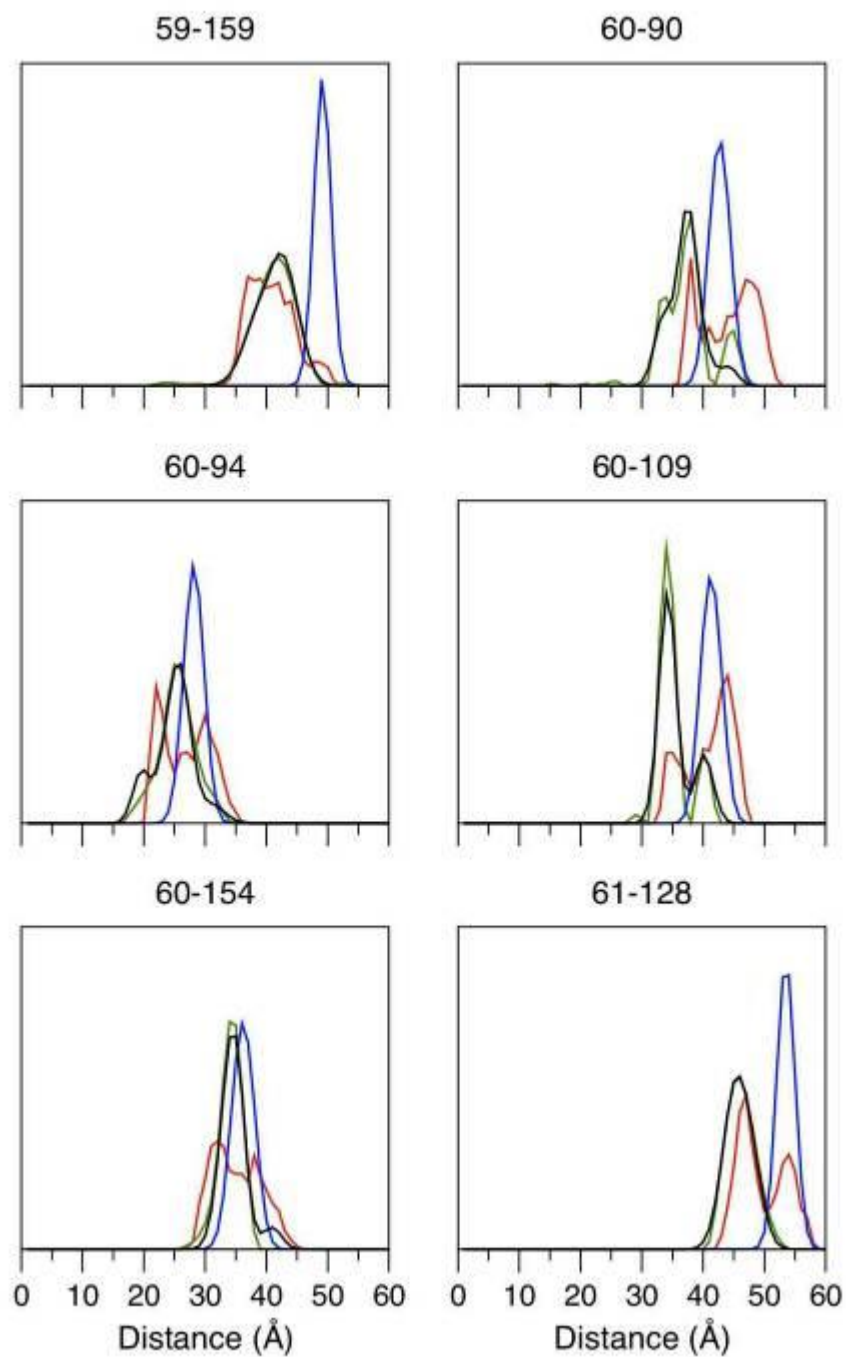
**Figure S4.** Energy plots for the restrained ensemble simulation, divided into total (*black*), kinetic (*red*) and potential (*green*) energies.



**Figure S5.** Temperature vs. time plot in restrained ensemble simulation. Temperatures of the replica atoms were also kept at 300K.

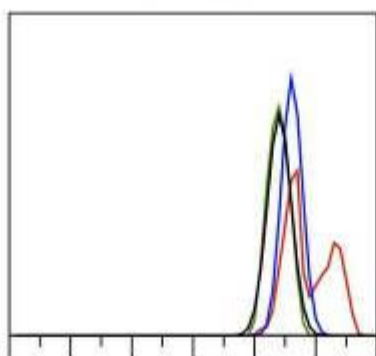


**Figure S6.** Comparison of the spin-spin distance histograms obtained from conventional molecular dynamics (red line), locally enhanced sampling (blue line), restrained ensemble simulation (green line) and experiment (black line).

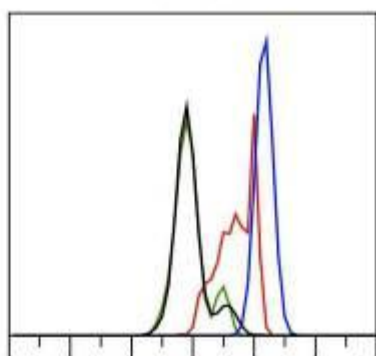




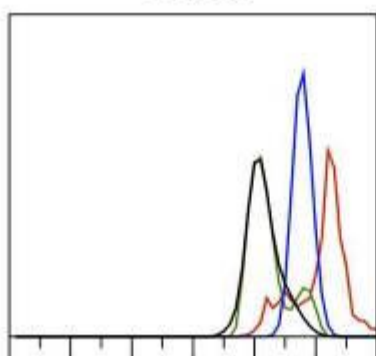
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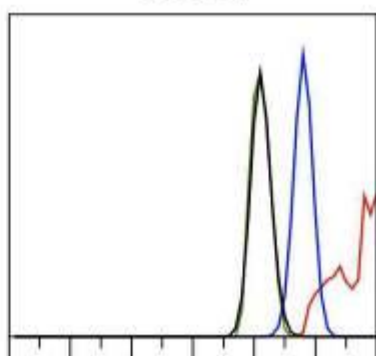
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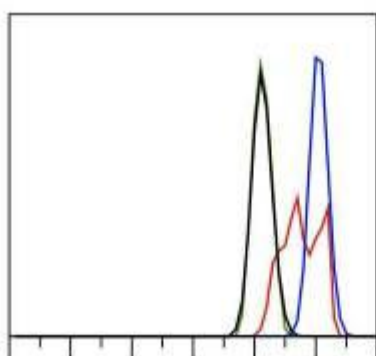
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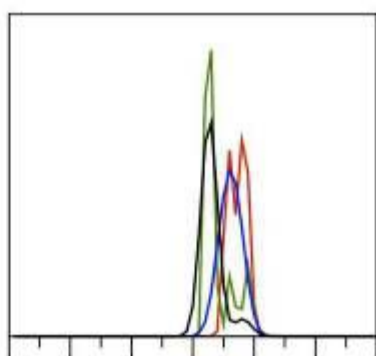
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62-155



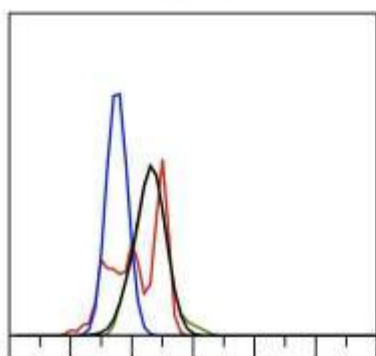
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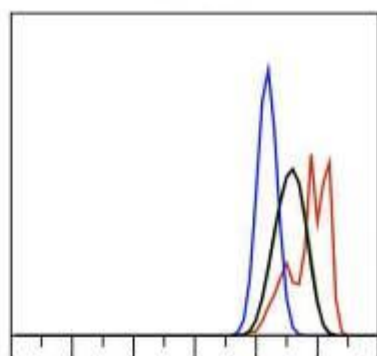
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Distance (Å)

0 10 20 30 40 50 60  
Distance (Å)

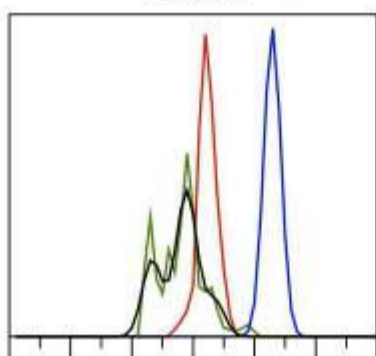
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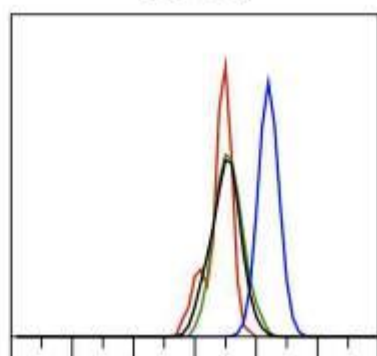
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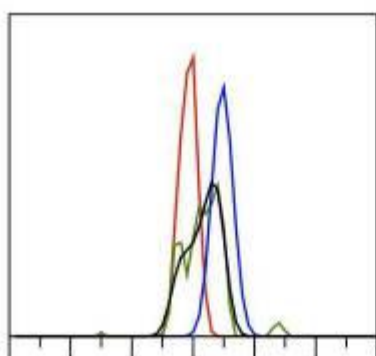
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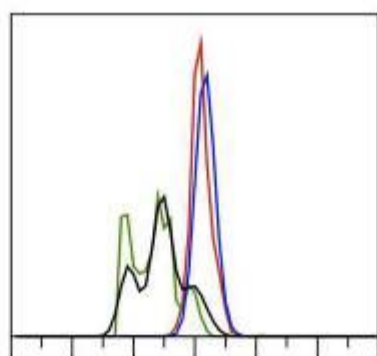
72-131



72-151

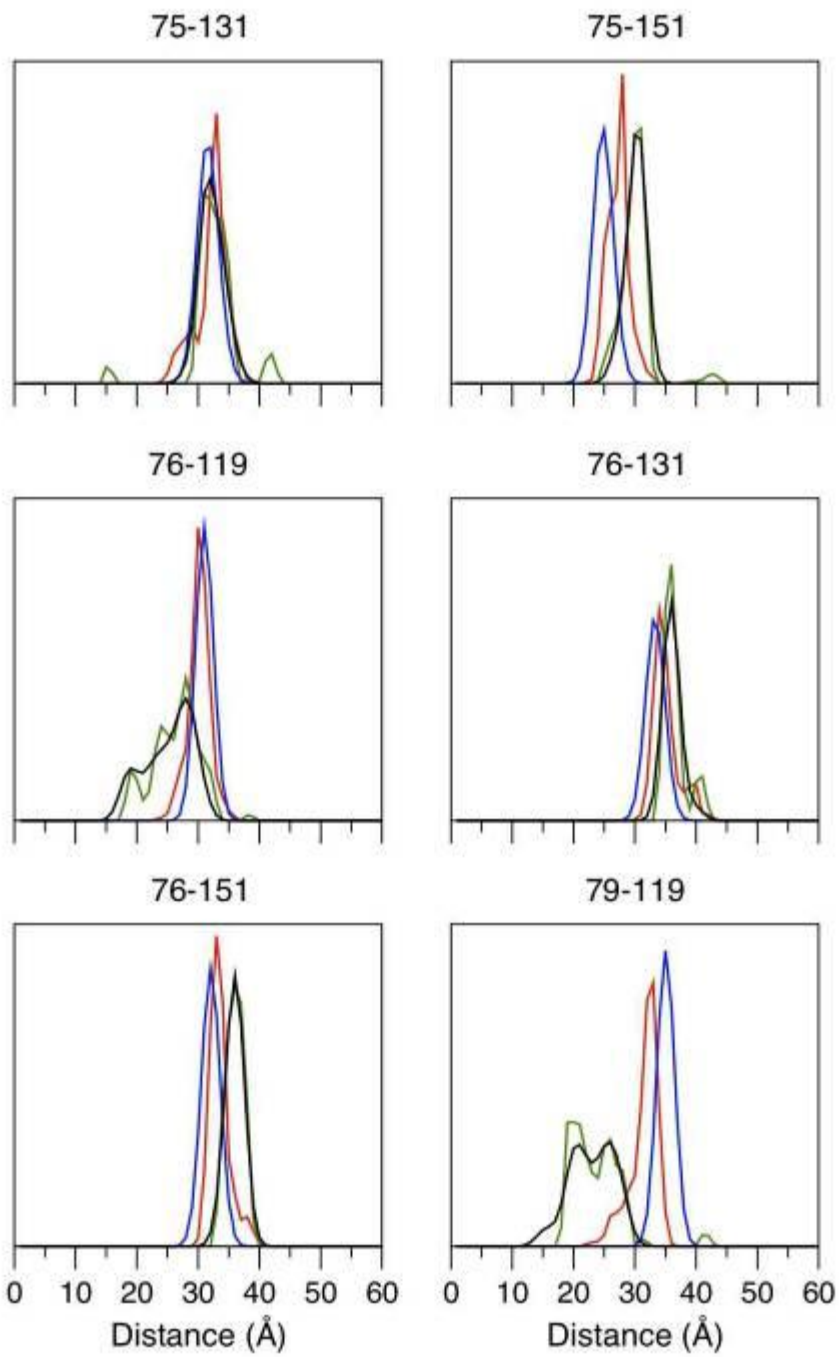


75-119

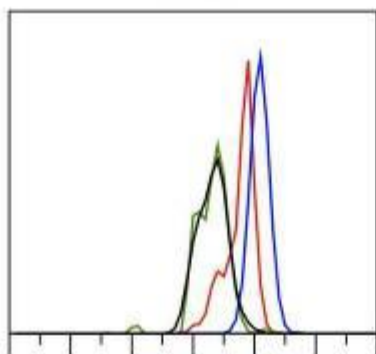


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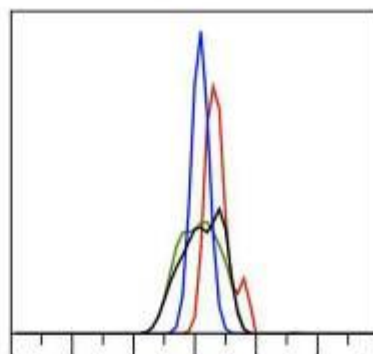
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Distance (Å)



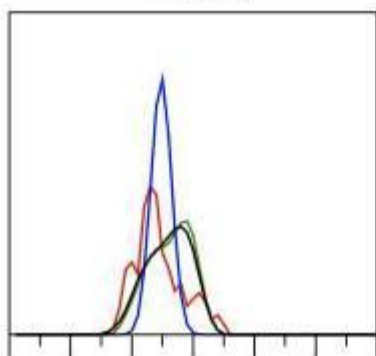
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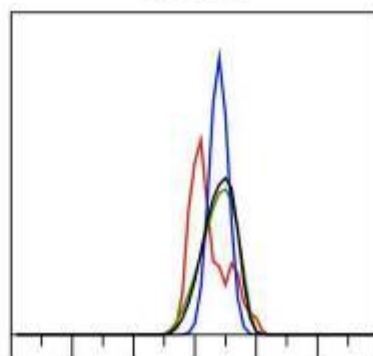
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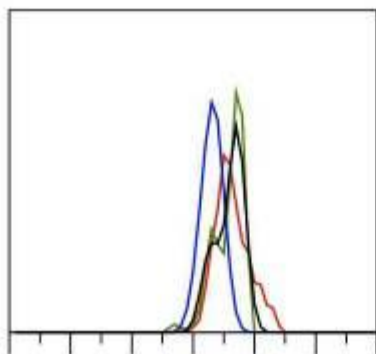
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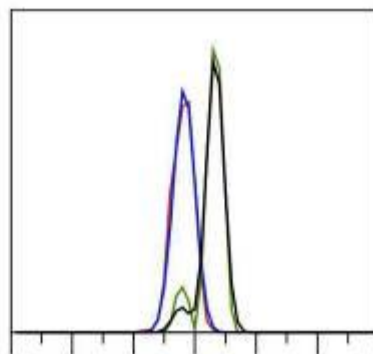
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82-155



83-155

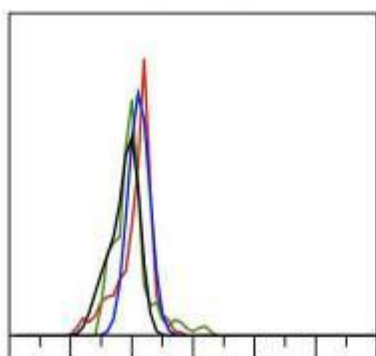


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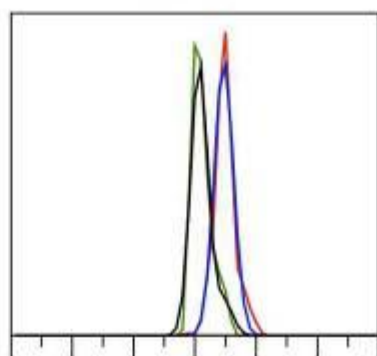
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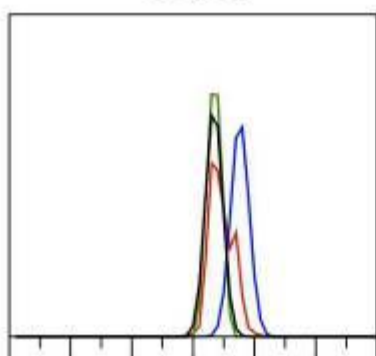
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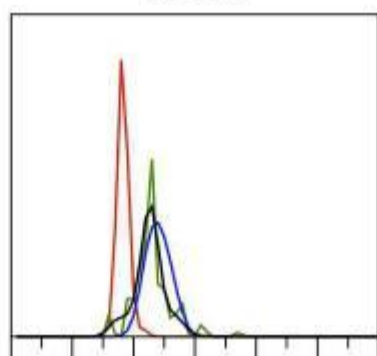
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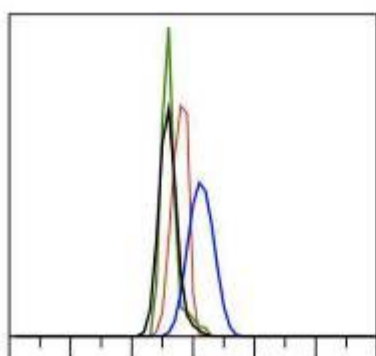
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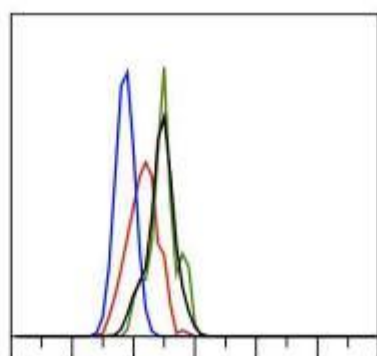
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93-112



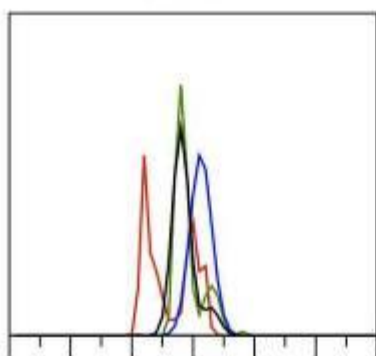
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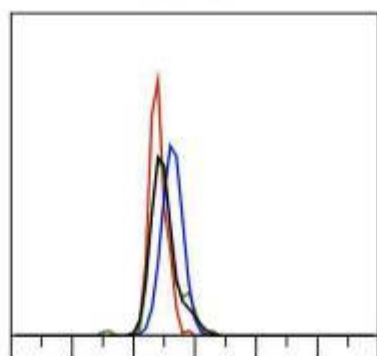
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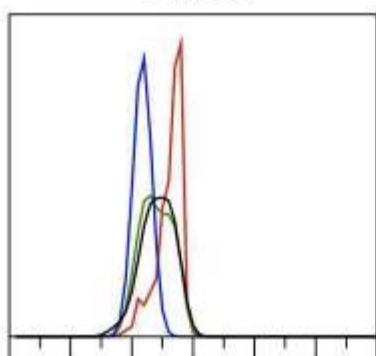
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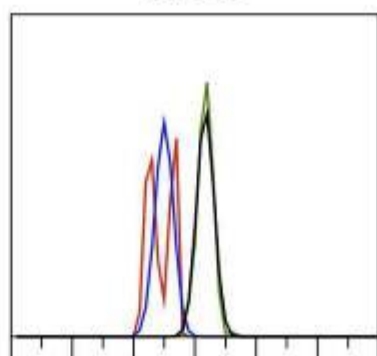
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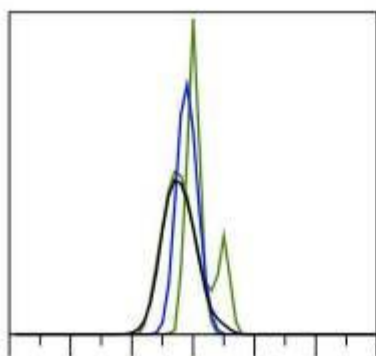
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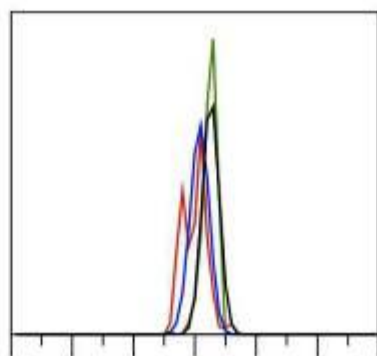
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108-123



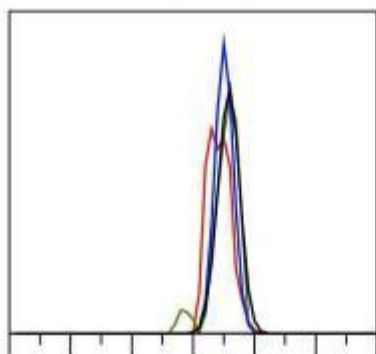
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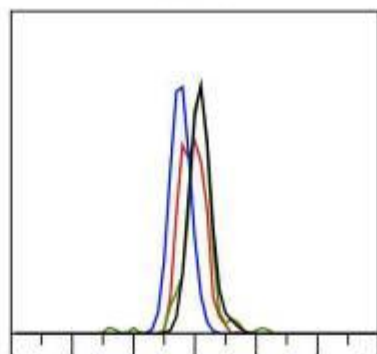
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Distance (Å)

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Distance (Å)

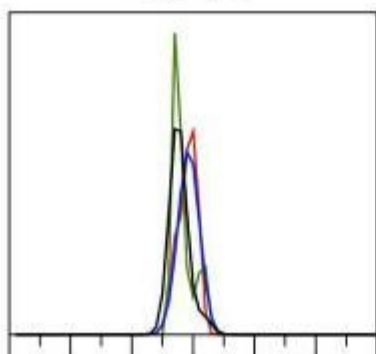
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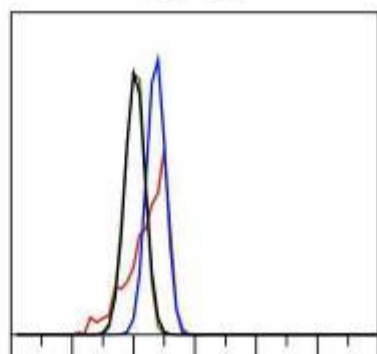
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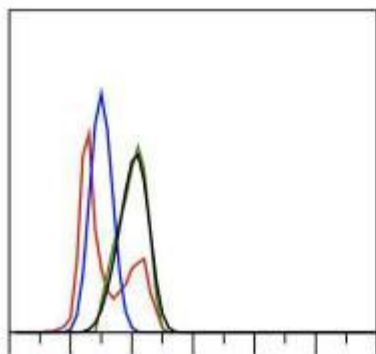
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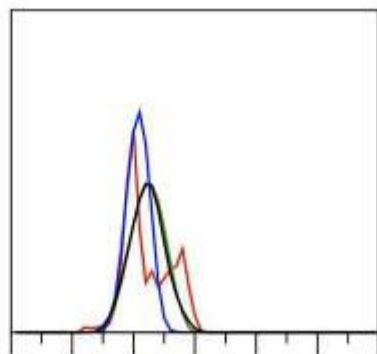
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119-128



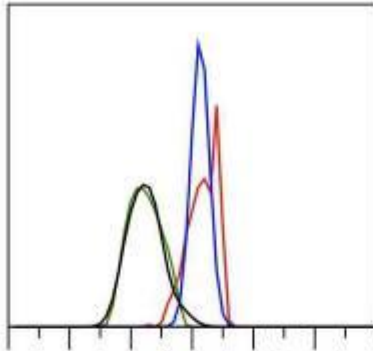
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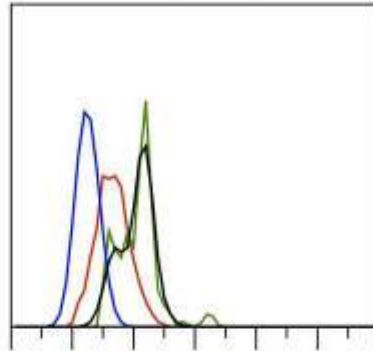
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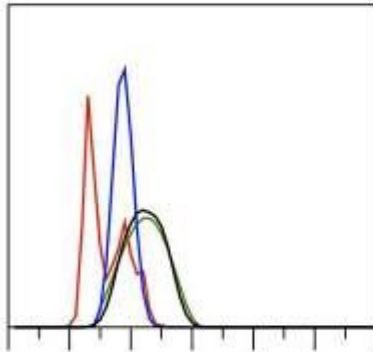
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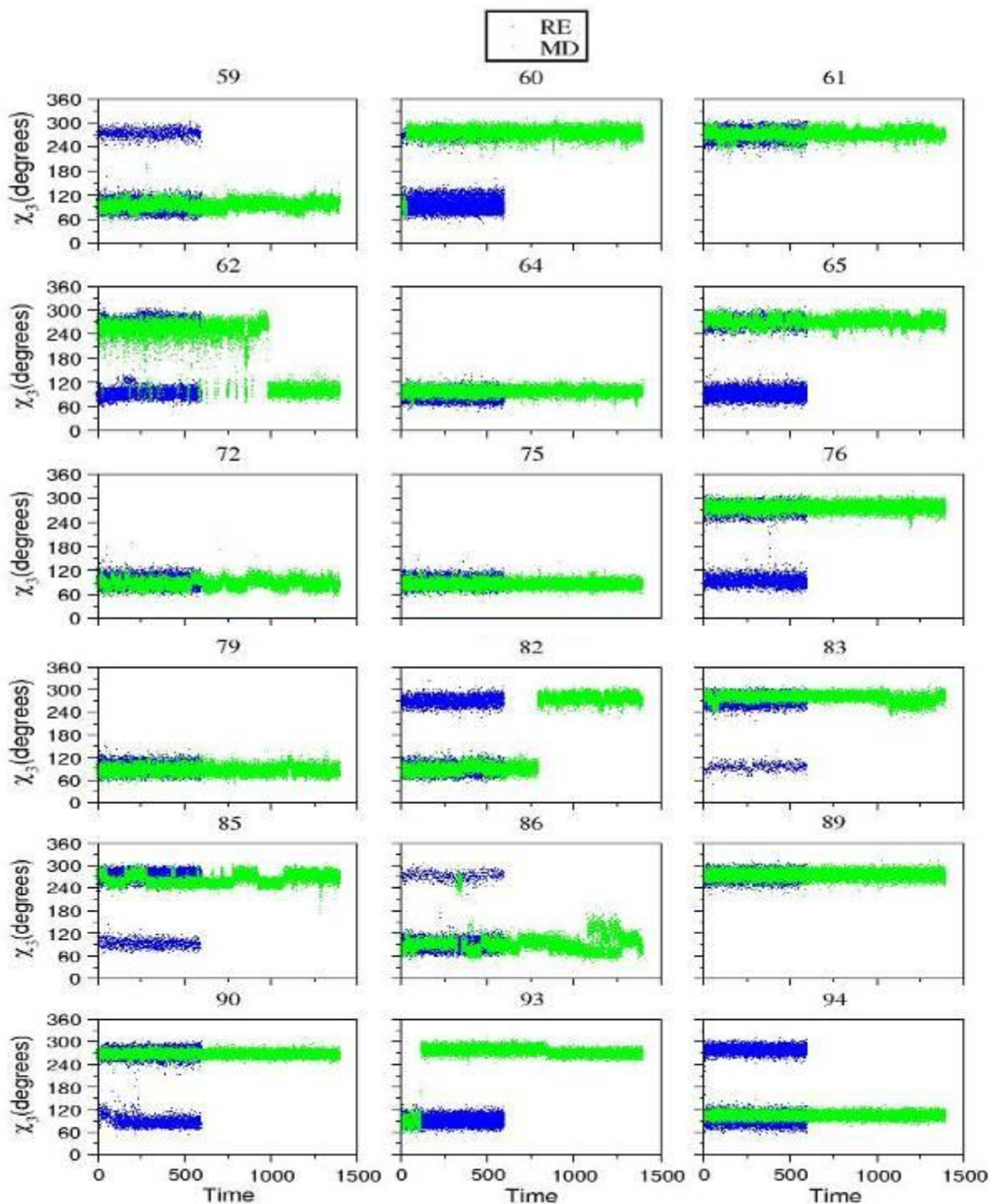


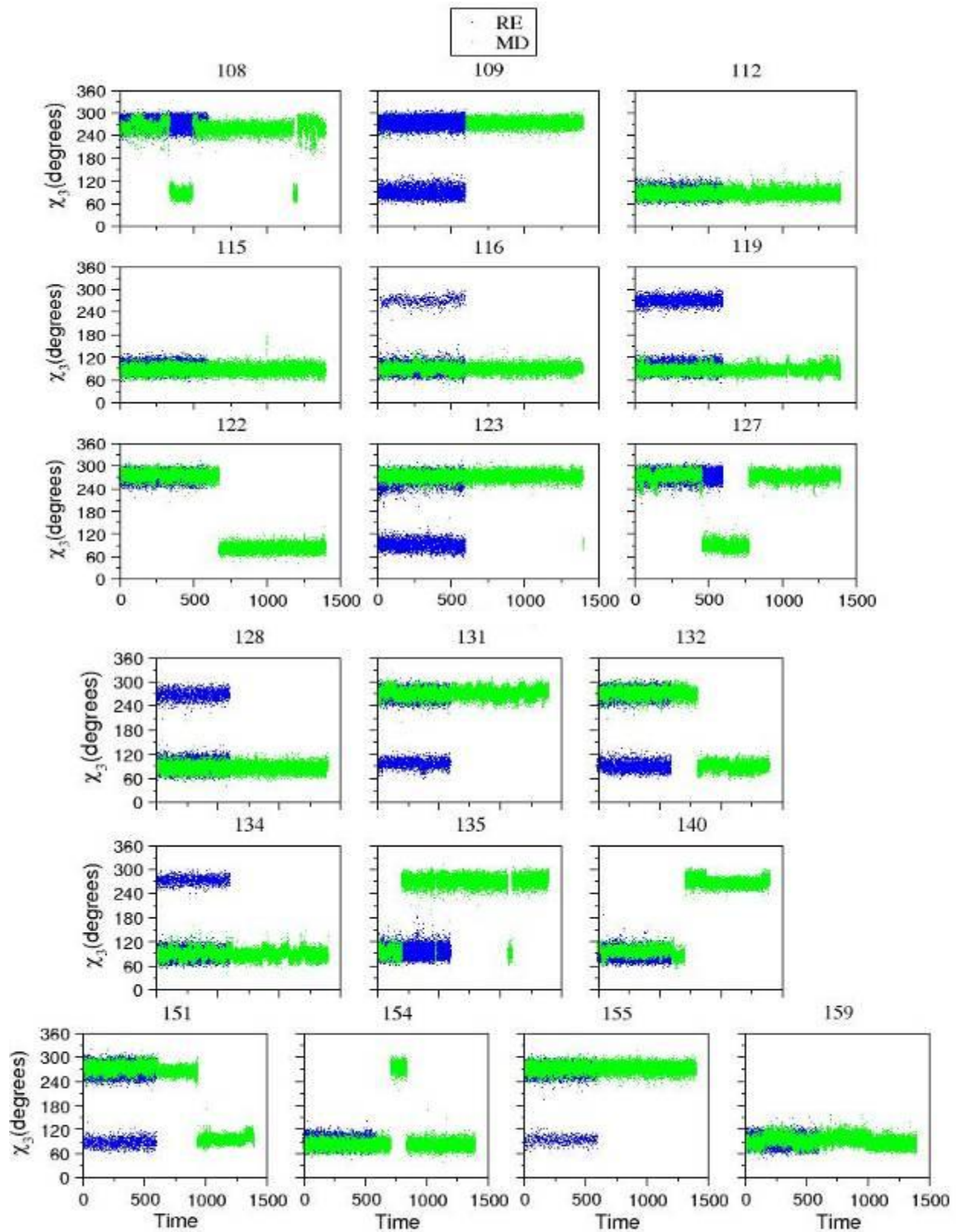
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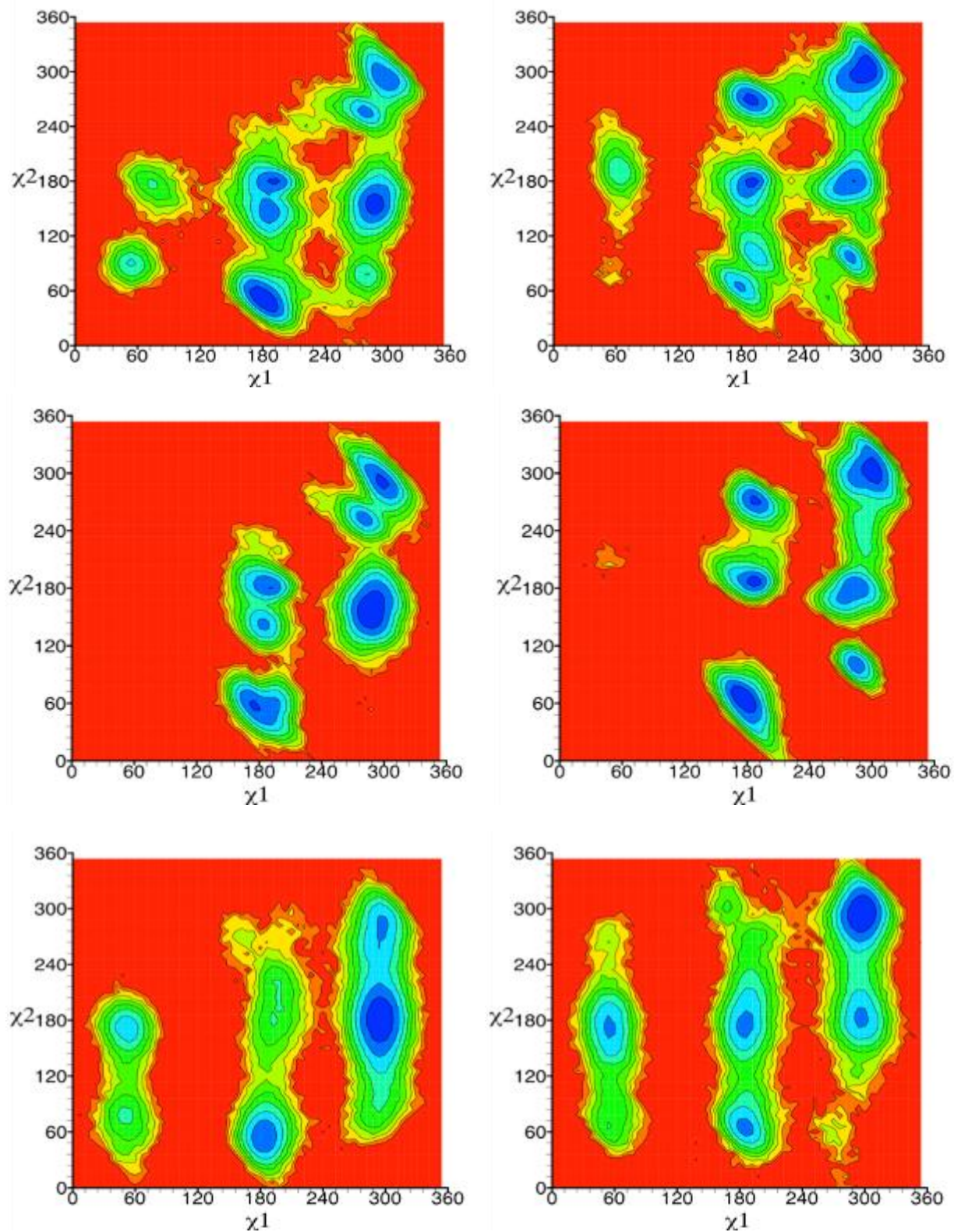


**Figure S7.** Time dependence of the dihedral angle  $\chi_3$  for all the spin-labels obtained from 140 ns conventional MD (blue) and first 600 ps RE (green) simulation. MD time scale is divided by 100 to fit the data from both restrained ensemble and molecular dynamics simulations.



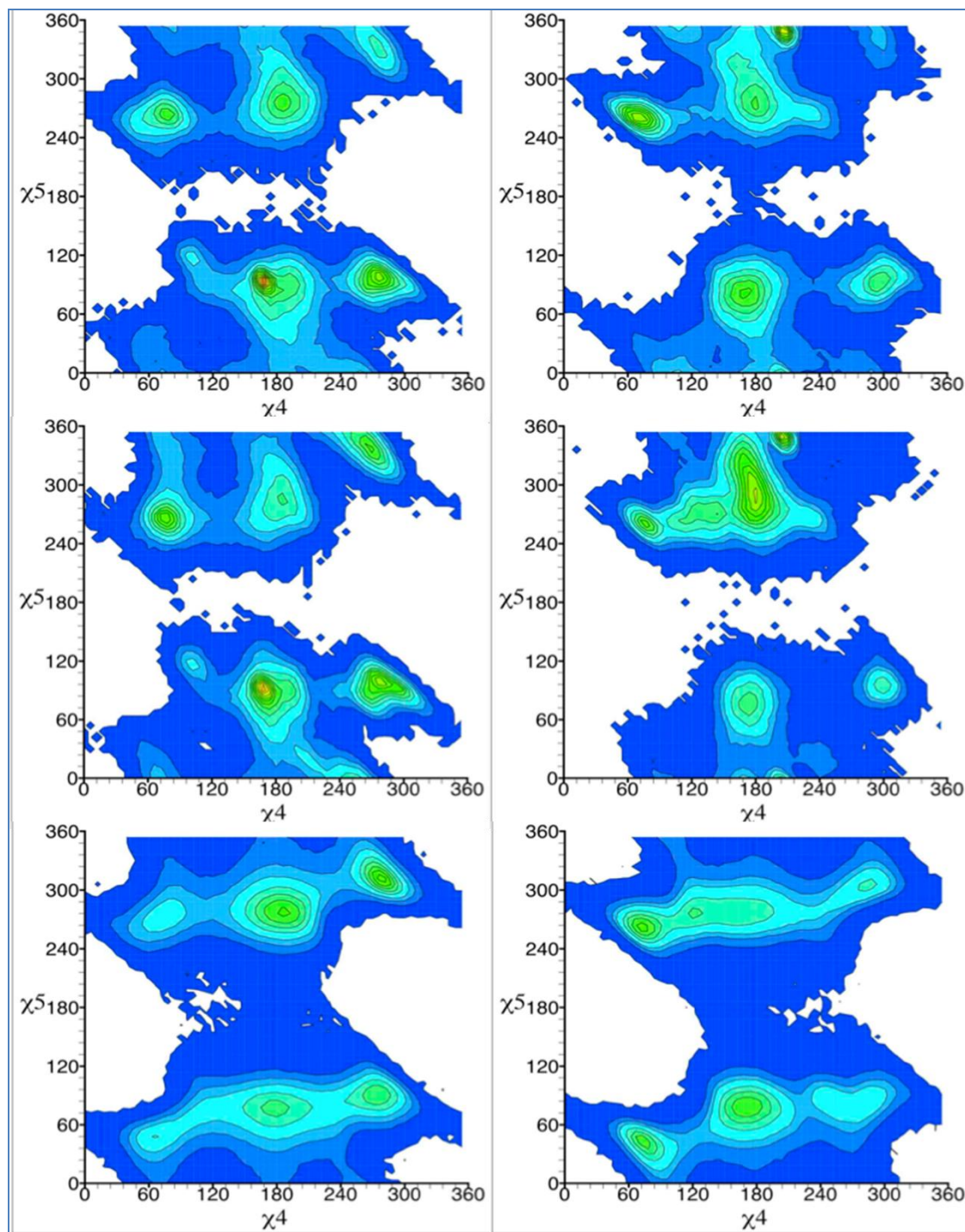


**Figure S8.** The 2D-PMF along  $\chi_1$  and  $\chi_2$  of spin-label attached to T4 lysozyme at  $\chi_3 \sim 90^\circ$  (left panel) and  $270^\circ$  (right panel) obtained from conventional MD (upper panel), locally enhanced sampling (middle panel) and restrained ensemble (lower panel) simulations. The units of the angles  $\chi_1$  and  $\chi_2$  are in degrees and the contour levels are drawn at 1 kcal/mol interval.



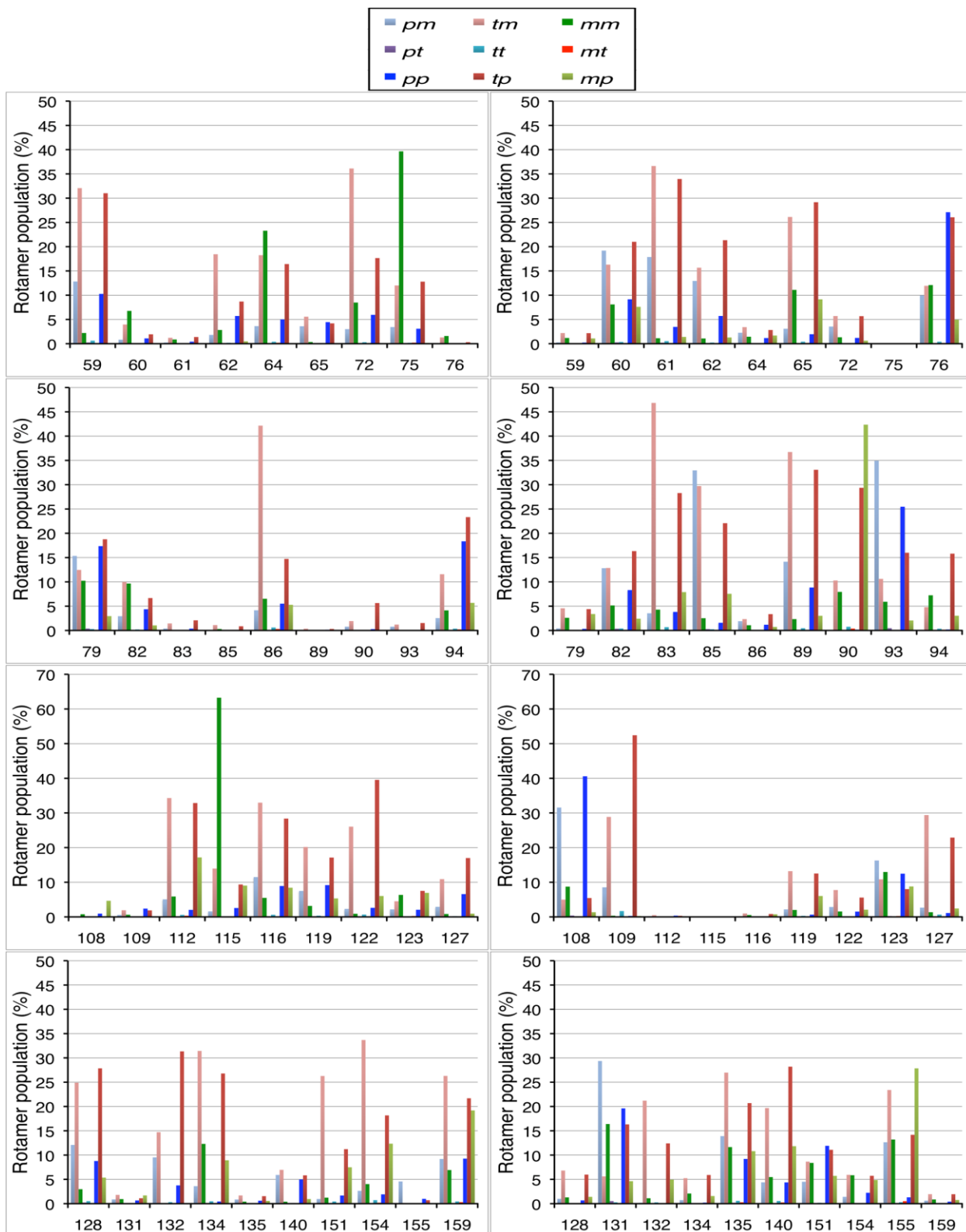


**Figure S9.** Rotamer population distribution along  $\chi_4$  and  $\chi_5$  of spin label attached to T4 Lysozyme at  $\chi_3 \approx 90^\circ$  (left panel) and  $270^\circ$  (right panel) obtained from conventional MD (upper panel), locally enhanced sampling (middle panel) and restraint ensemble (lower panel) simulations. The units of the angles  $\chi_4$  and  $\chi_5$  are in degrees.

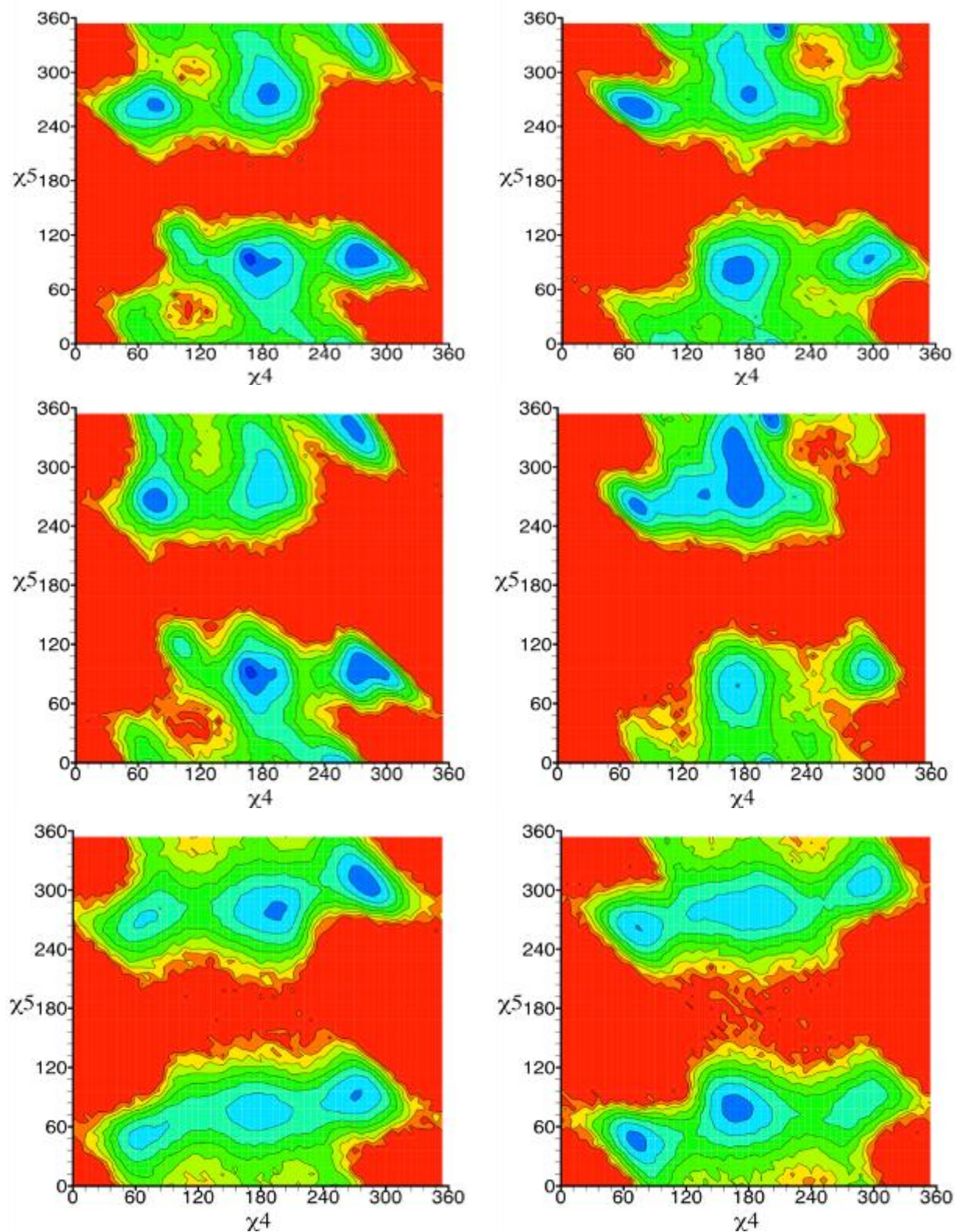




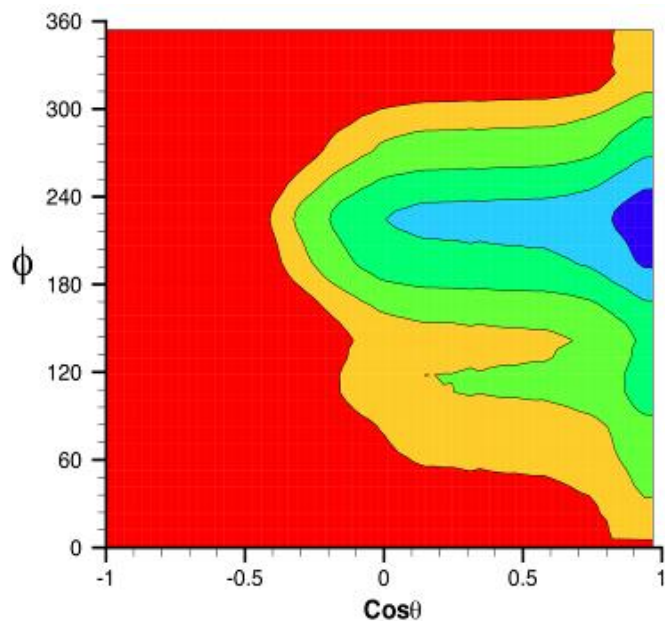
**Figure S10.** Rotamer population (%) along  $\chi_4$  and  $\chi_5$  at  $\chi_3 \approx 90^\circ$  (left panel) and  $270^\circ$  (right panel) of 37 individual spin labels attached to T4 Lysozyme in solution obtained from restraint ensemble simulations.



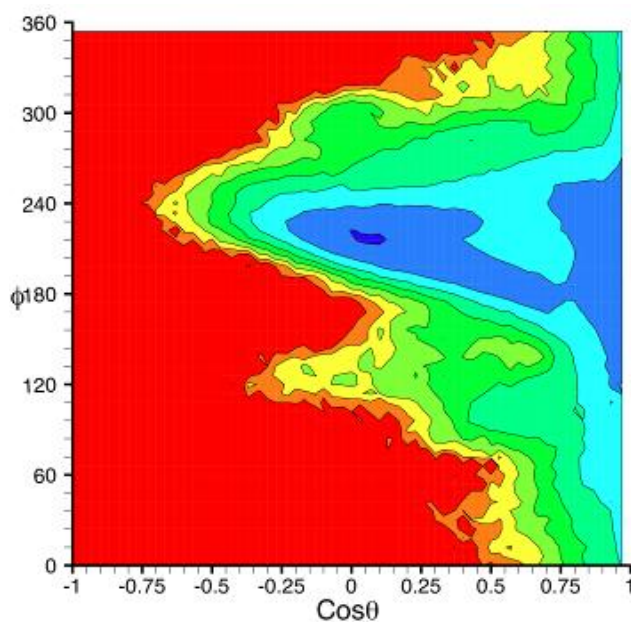
**Figure S11.** The 2D-PMF along  $\chi_4$  and  $\chi_5$  of spin-label attached to T4 Lysozyme at  $\chi_3 \sim 90^\circ$  (left panel) and  $270^\circ$  (right panel) obtained from conventional MD (upper panel), locally enhanced sampling (middle panel) and restrained ensemble (lower panel) simulations. The units of the angles  $\chi_4$  and  $\chi_5$  are in degrees and the contour levels are drawn at 1 kcal/mol interval.



**Figure S12.** The correlation of the  $C_\beta-C_\alpha-ON$  angle and the  $N-C_\alpha-C_\beta-ON$  dihedral angle, where the left panel represents the 2D PMF calculated from the product of the 1D PMFs of the  $C_\beta-C_\alpha-ON$  and the  $N-C_\alpha-C_\beta-ON$  dihedral angles, while the right hand side is the joint 2D PMF calculated from the RE simulation.

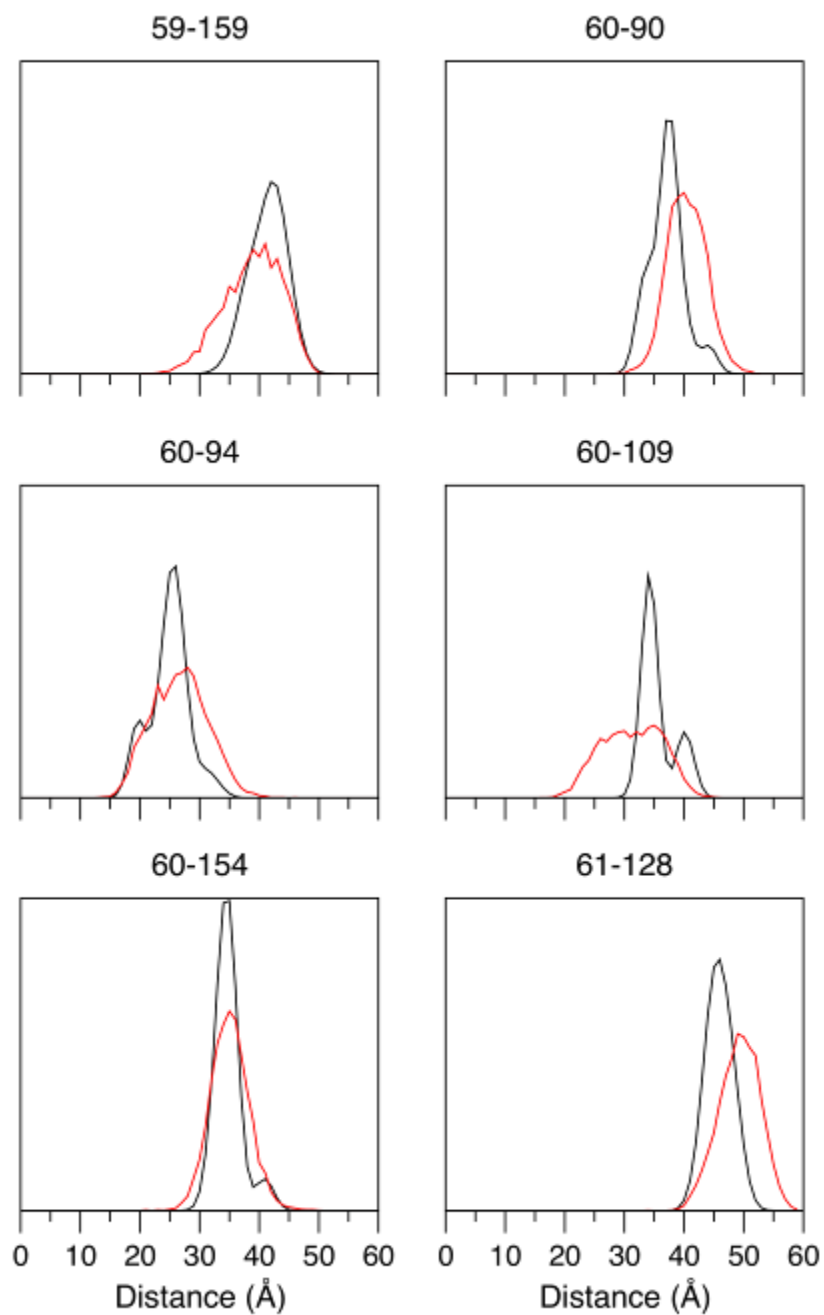


PMF ( $\phi$ ) x PMF ( $\theta$ )

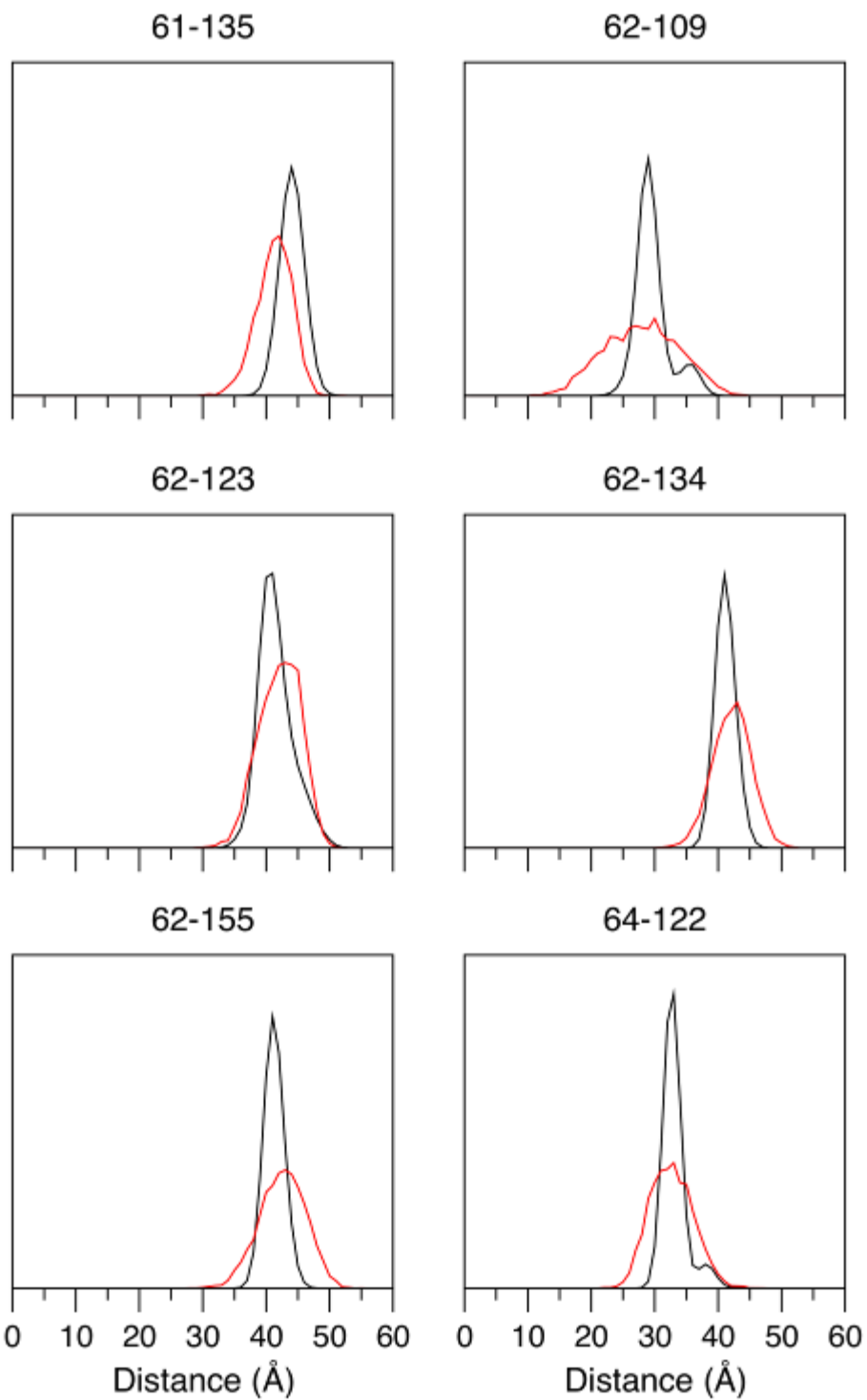


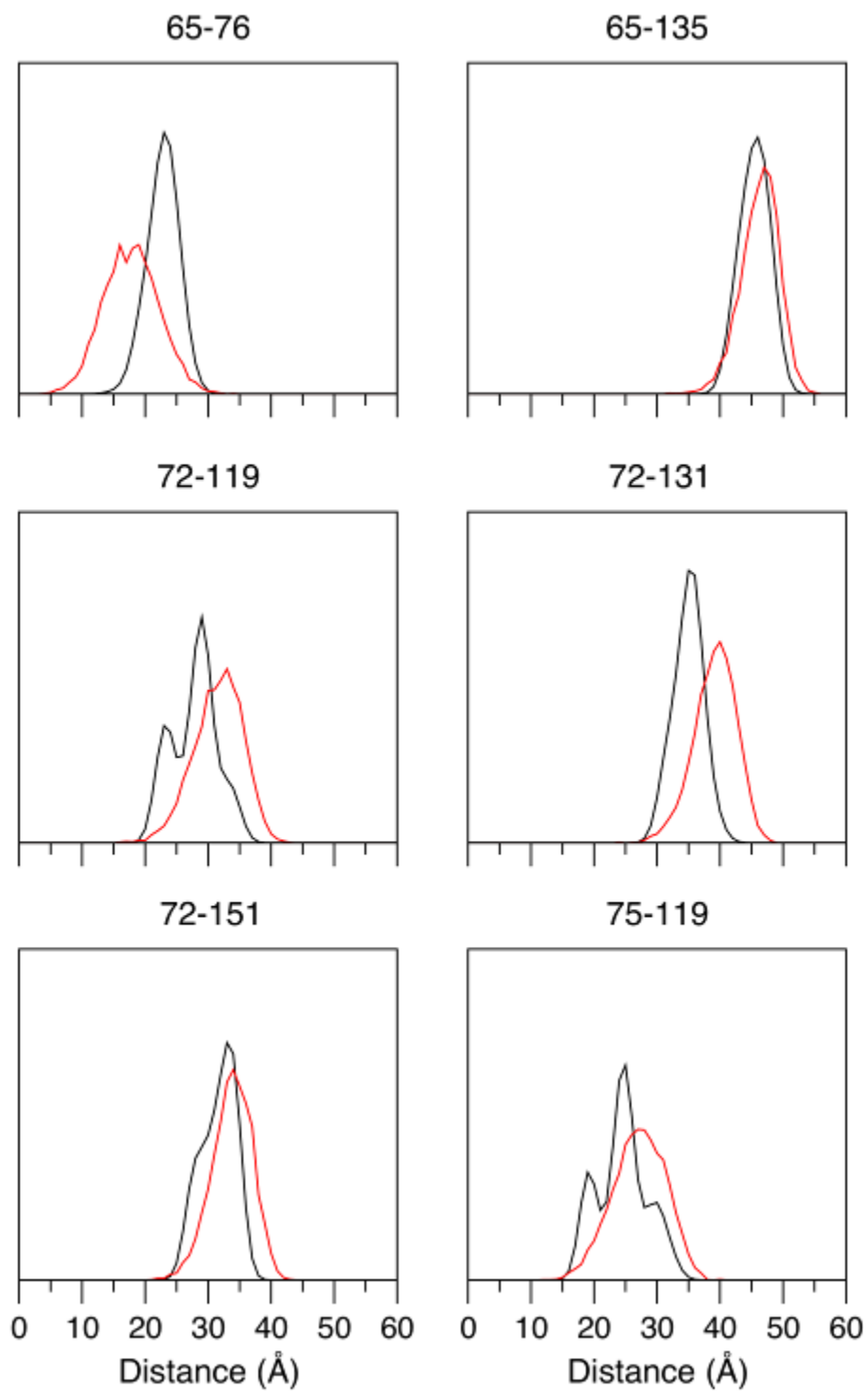
PMF ( $\phi, \theta$ )

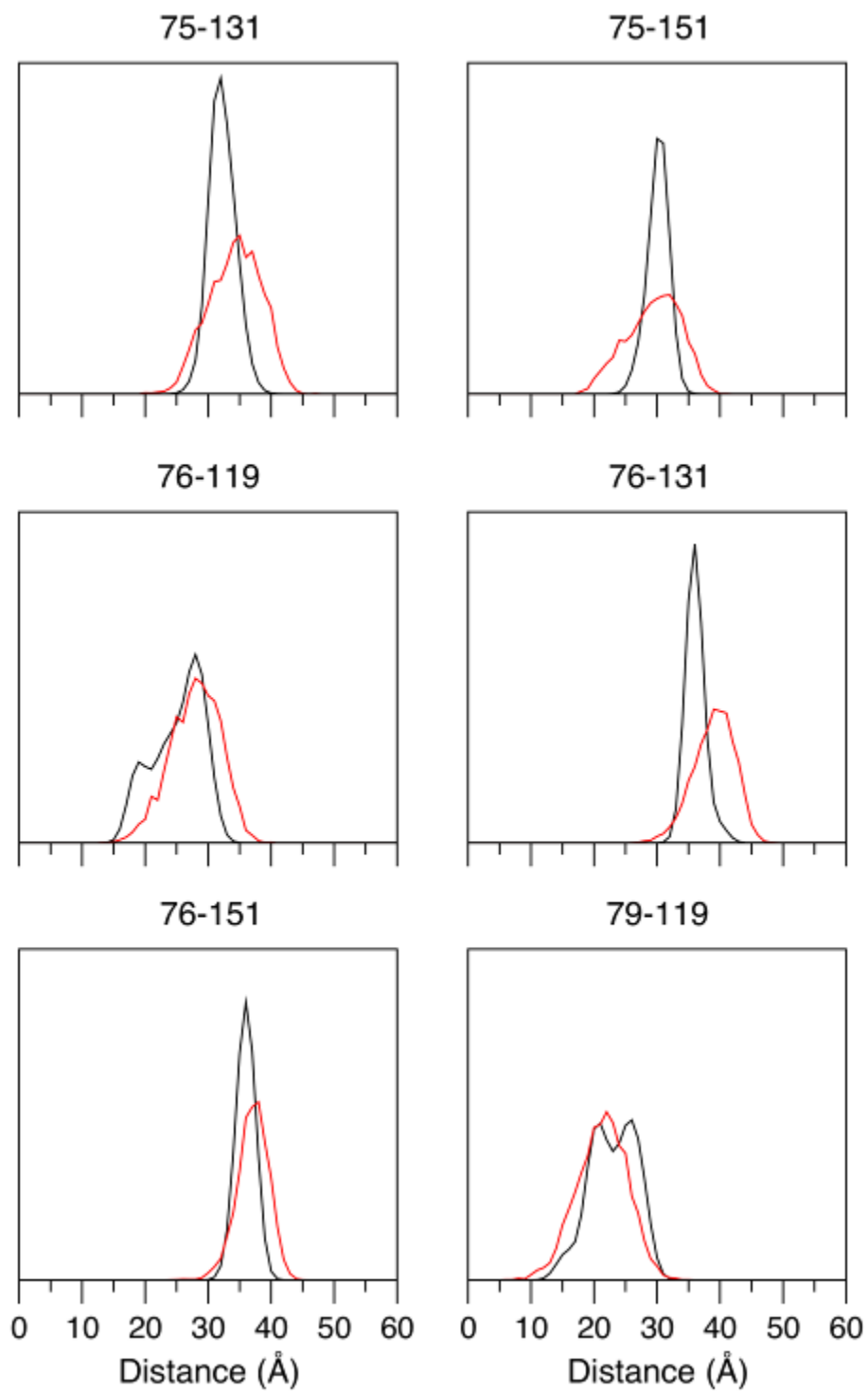
**Figure S13.** Comparison of the spin-spin distance histograms obtained from 5ns molecular dynamics simulation of the ON dummy atom linked T4L (red line) and experiment (black line).

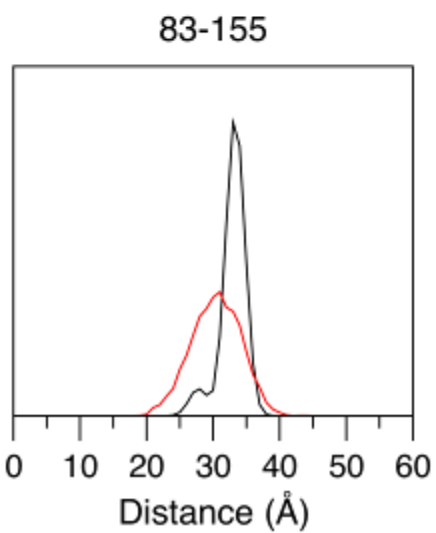
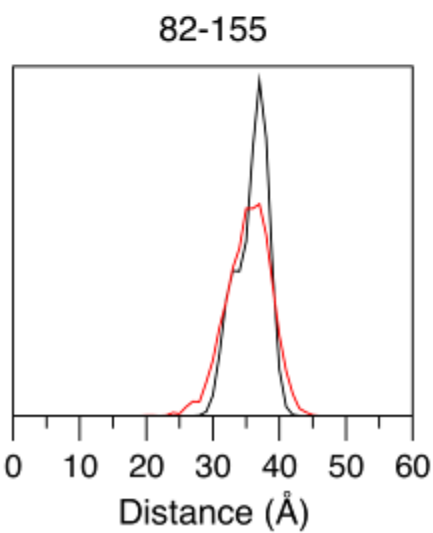
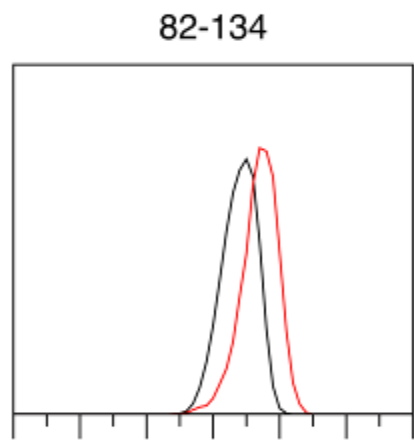
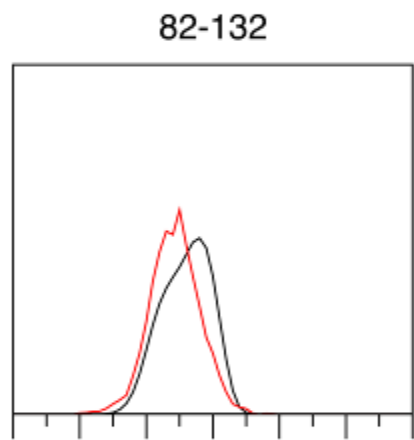
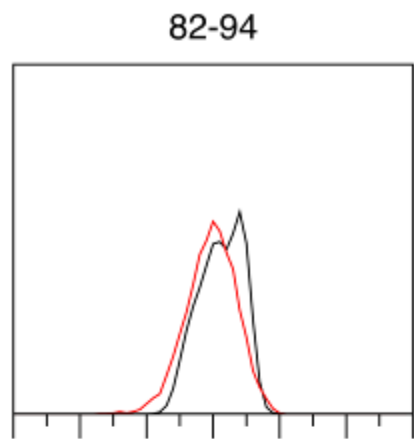
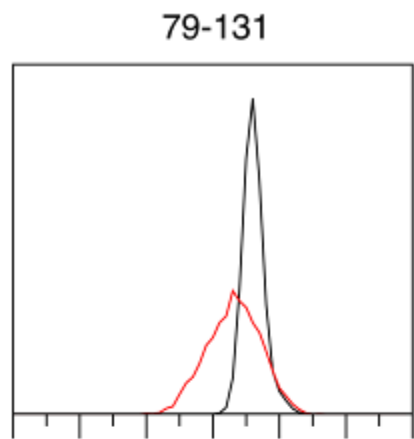




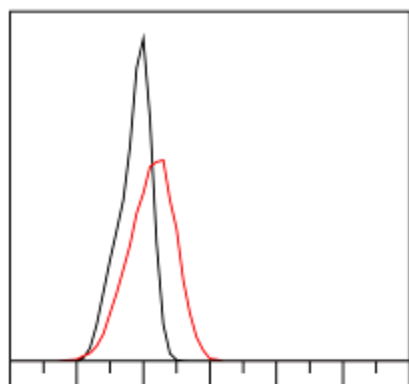




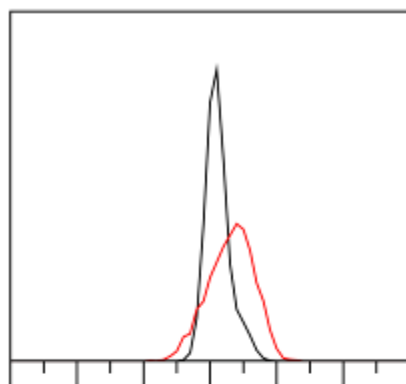




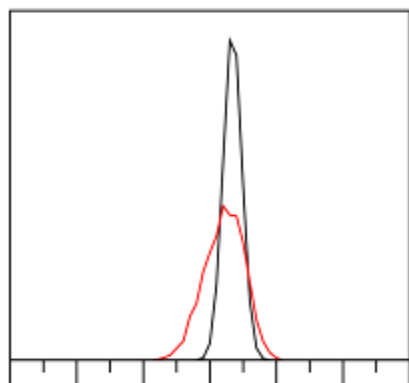
85-119



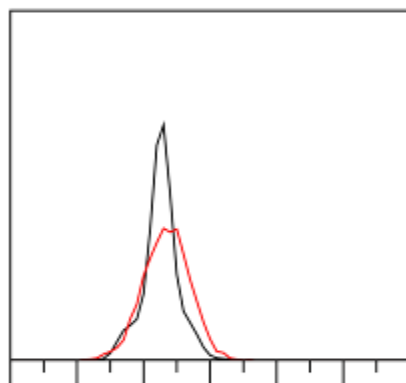
85-131



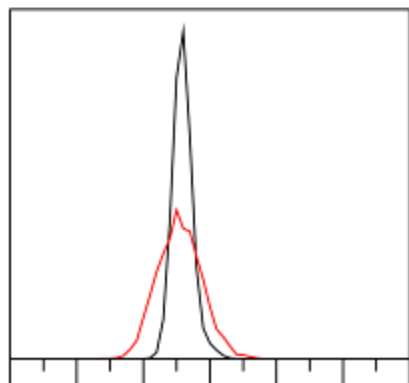
85-151



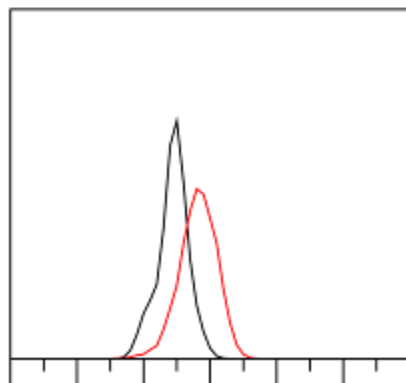
93-108



93-112



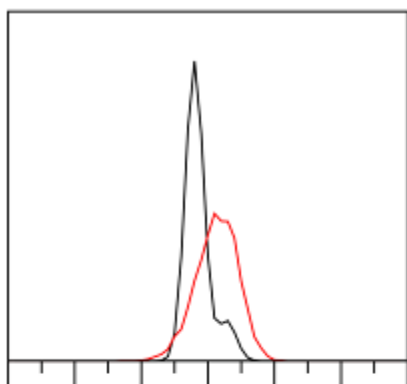
93-123



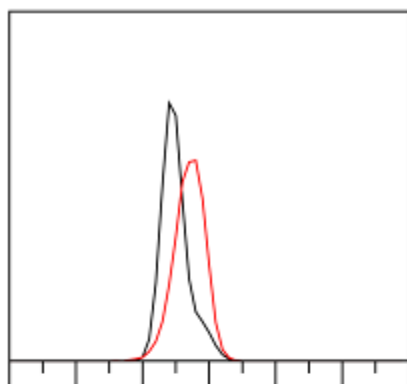
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Distance (Å)

0 10 20 30 40 50 60  
Distance (Å)

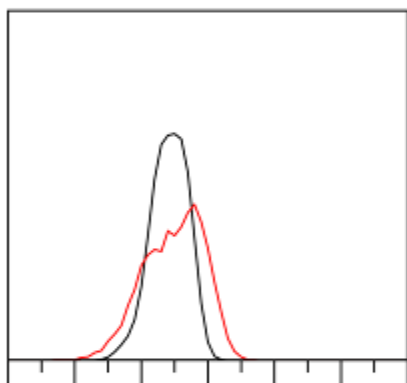
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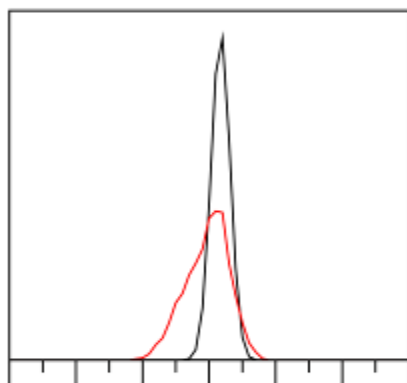
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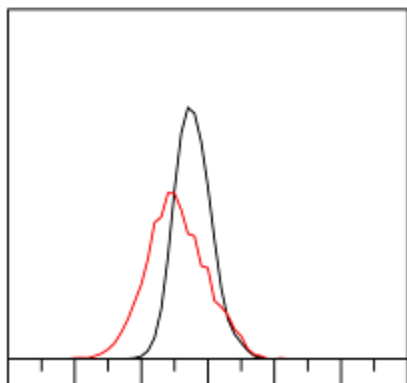
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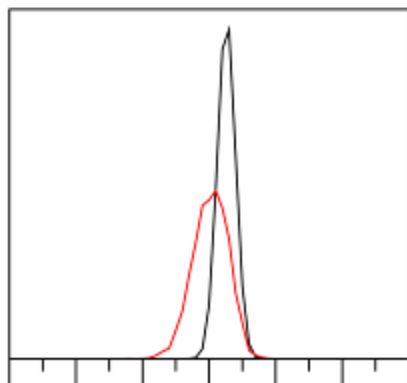
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108-123



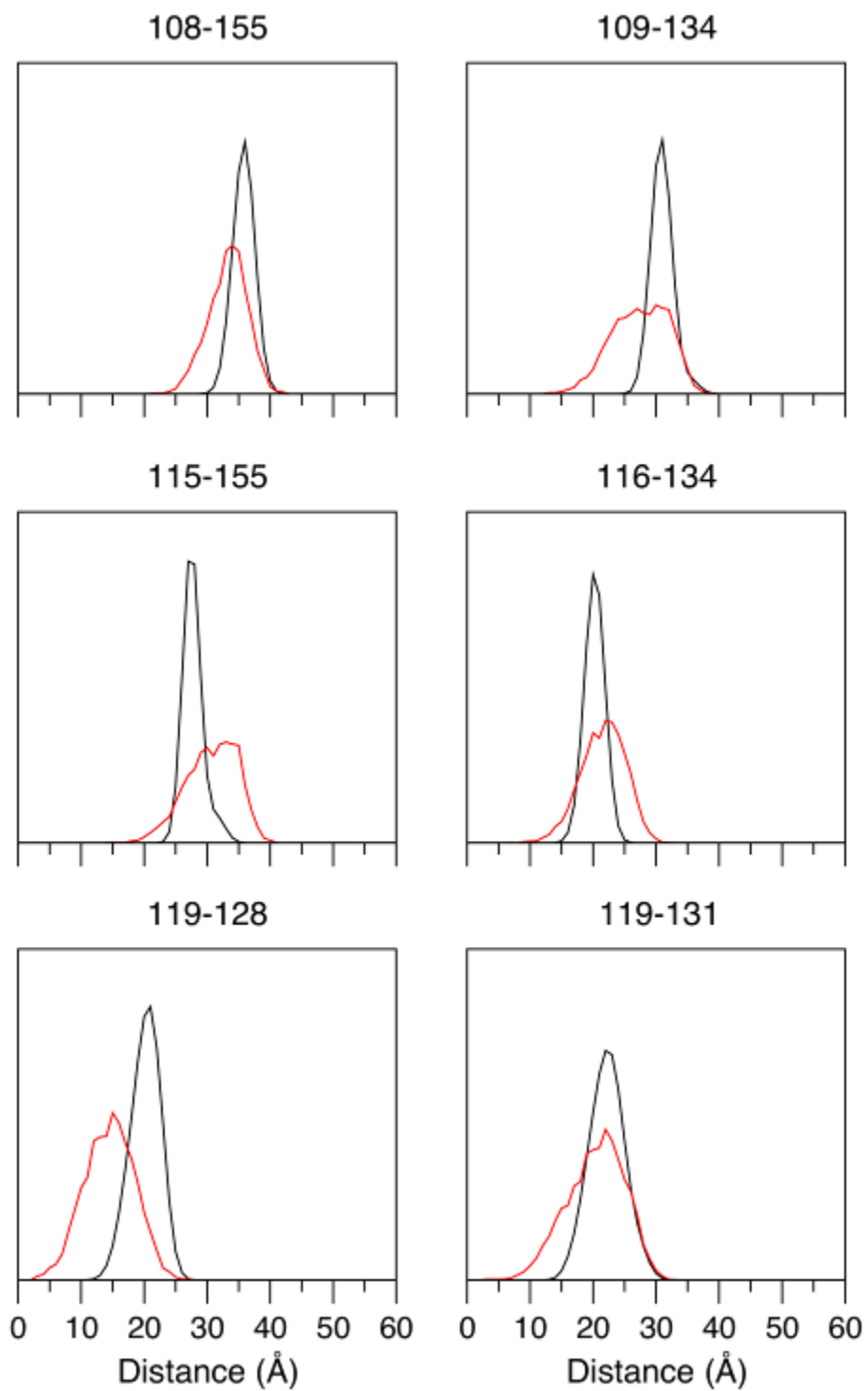
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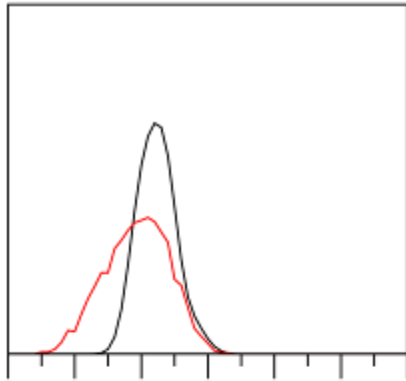
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Distance (Å)

0 10 20 30 40 50 60  
Distance (Å)

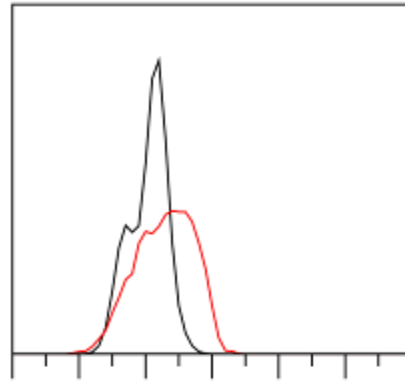




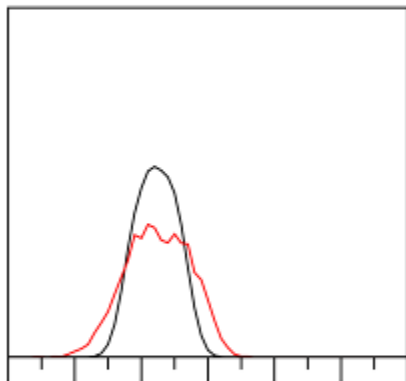
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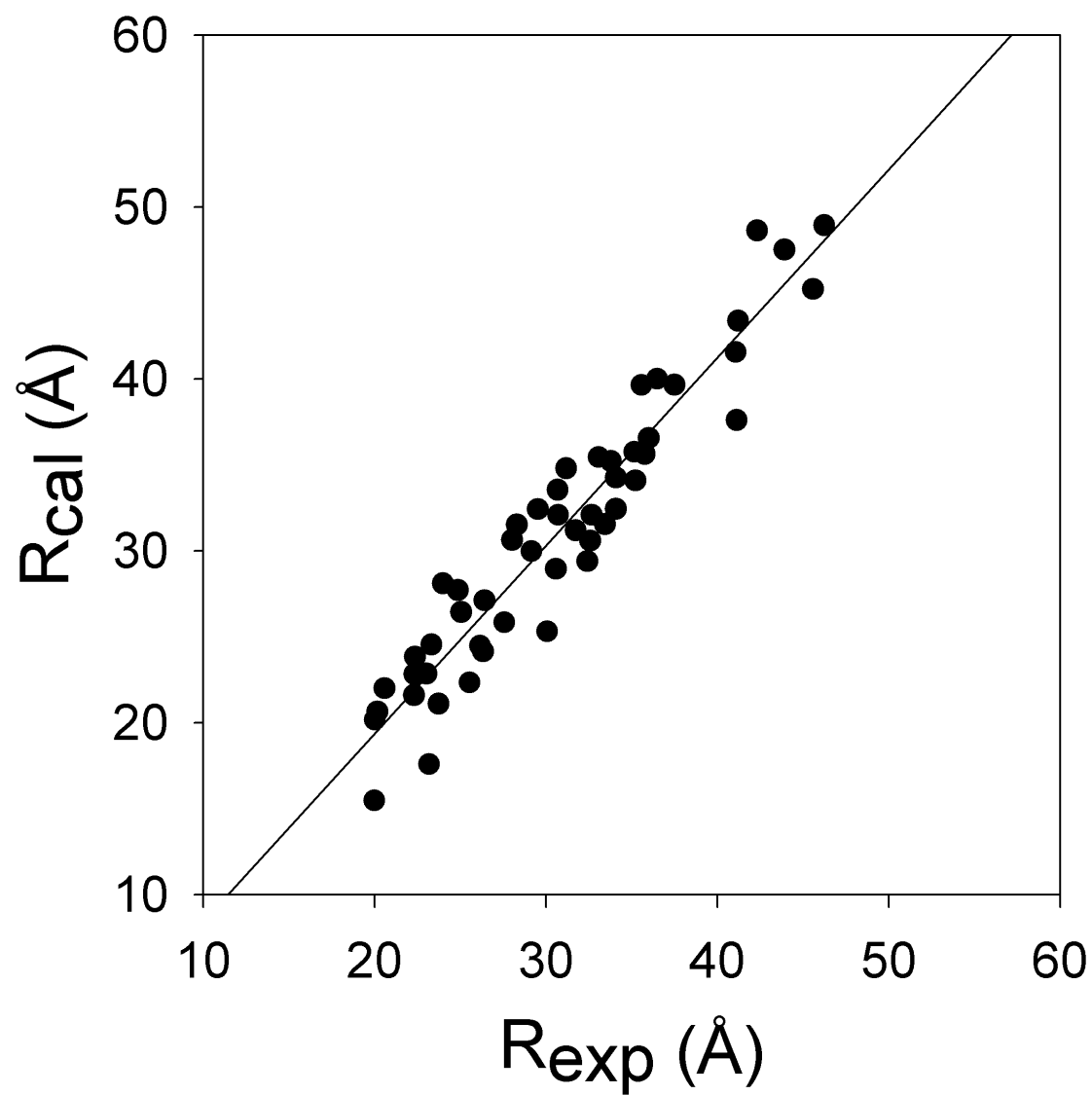
128-155



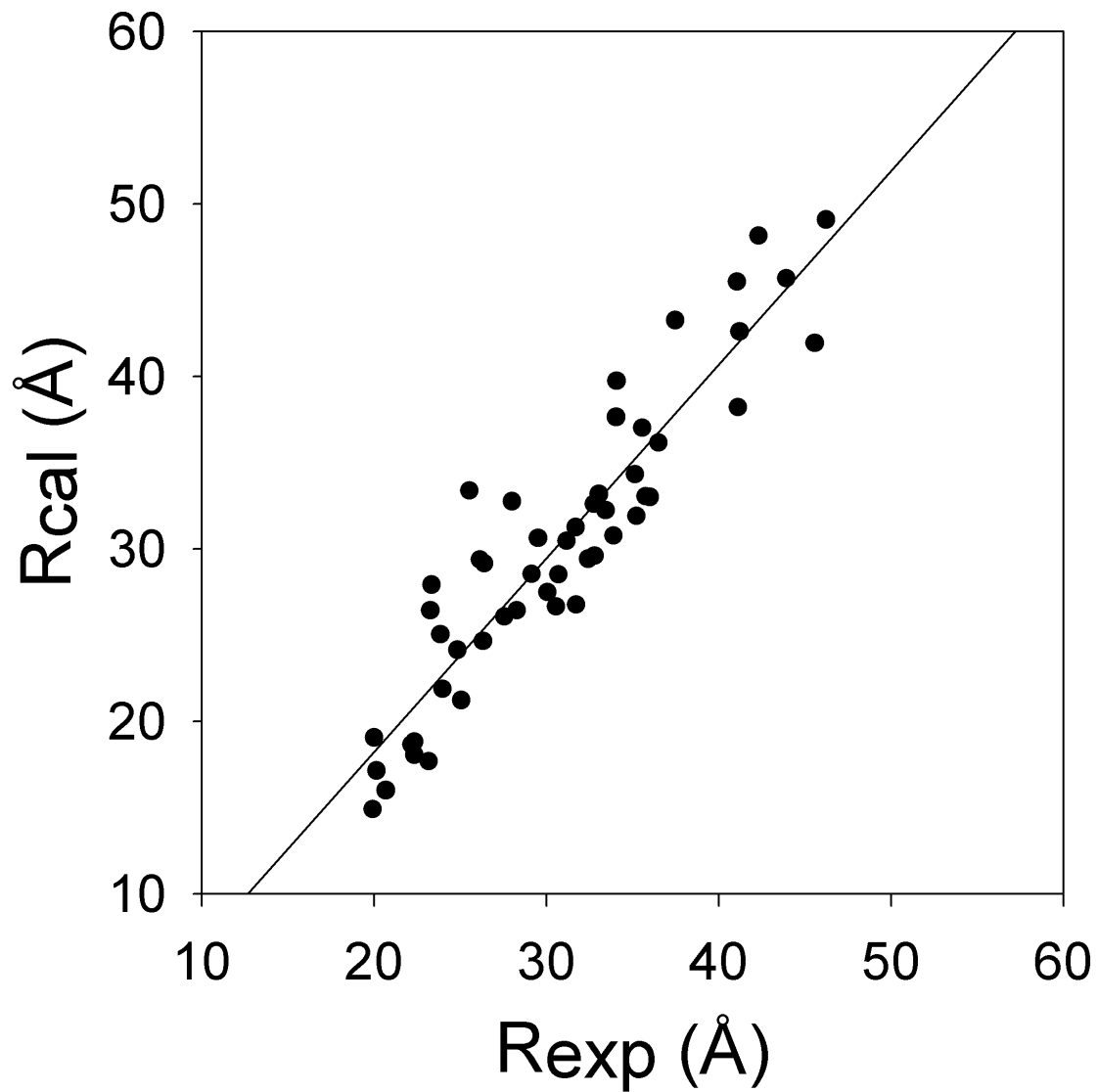
140-151



**Figure S14.** Comparison of 51 inter-label average experimental ( $R_{\text{exp}}$ ) and dummy nitroxide atom MD simulation ( $R_{\text{cal}}$ ) distances in T4L. The correlation coefficient was found to be 0.91.



**Figure S15.** Correlation of 51 inter label experimental ( $R_{\text{exp}}$ ) and MMM ( $R_{\text{cal}}$ ) distances in T4L which gave a correlation coefficient of 0.85.



**Figure S16.** Force field parameter (**A**) and topology (**B**) for pseudo nitroxide dummy atom.

**A.**

\* Parameter for nitroxide dummy atom

\*

BONDS

CT1 OND 0.5 7.9

CP1 OND 0.5 7.9 ! proline

ANGLES

CT2 CT1 OND 1.0 46.0

CT3 CT1 OND 1.0 46.0

CP2 CP1 OND 1.0 46.0 ! proline

CT1 CT1 OND 1.0 46.0 ! Isoleucine

DIHEDRALS

NH1 CT1 CT2 OND 1.9 1 43.0

NH1 CT1 CT3 OND 1.9 1 43.0

NH1 CP1 CP2 OND 1.9 1 43.0 ! Protein

NONBONDED

OND 0.0 -0.05 2.0

NBFIX

OND OT 0.0 0.0

OND HT 0.0 0.0

OND OND 0.0 0.0

END

**B.**

\* Topology for nitroxide dummy atom

\*

31 1

MASS 200 OND 15.999400 O

PRES ONDUM 0.00

GROUP

ATOM ON OND 0.0

BOND CA ON

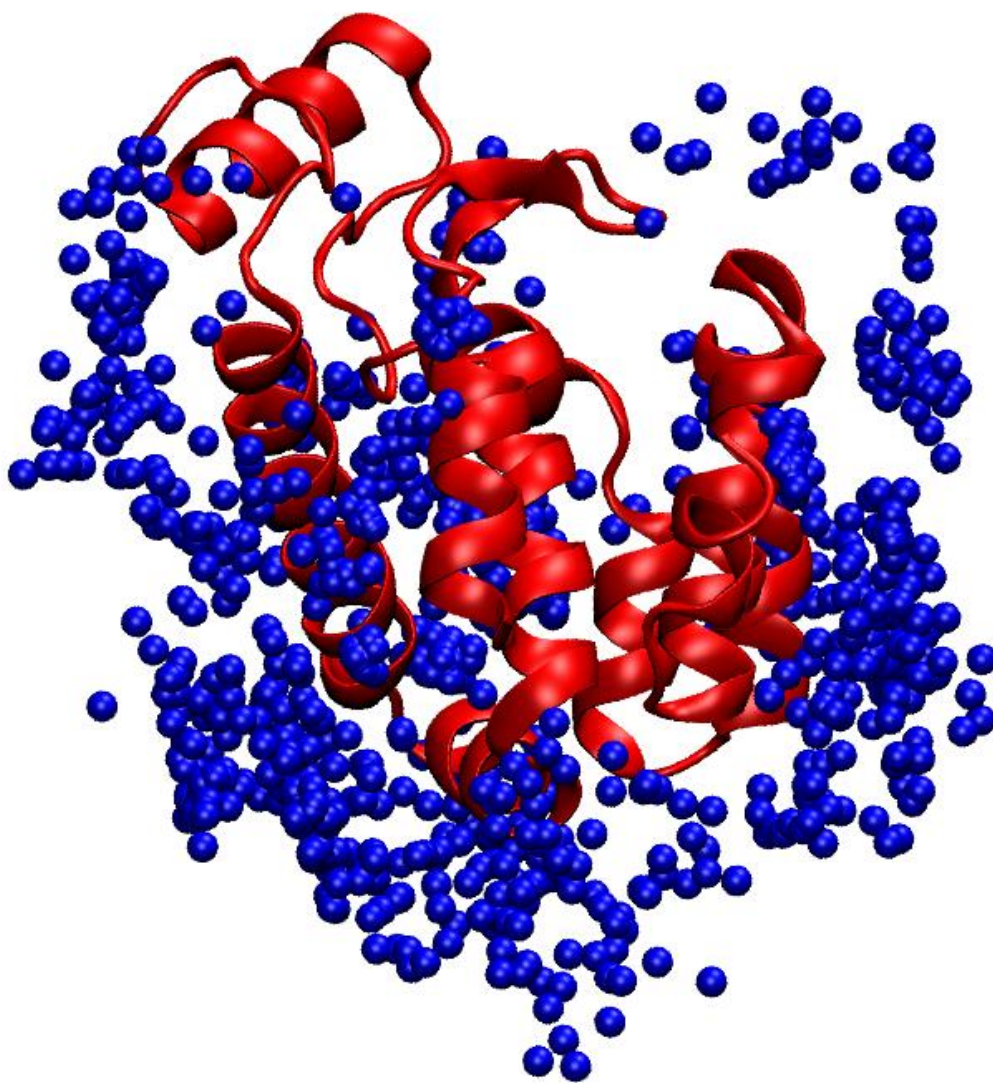
ANGLE CB CA ON

DIHE N CA CB ON

IC N CA CB ON 1.4603 110.28 -137.00 124.91 7.02

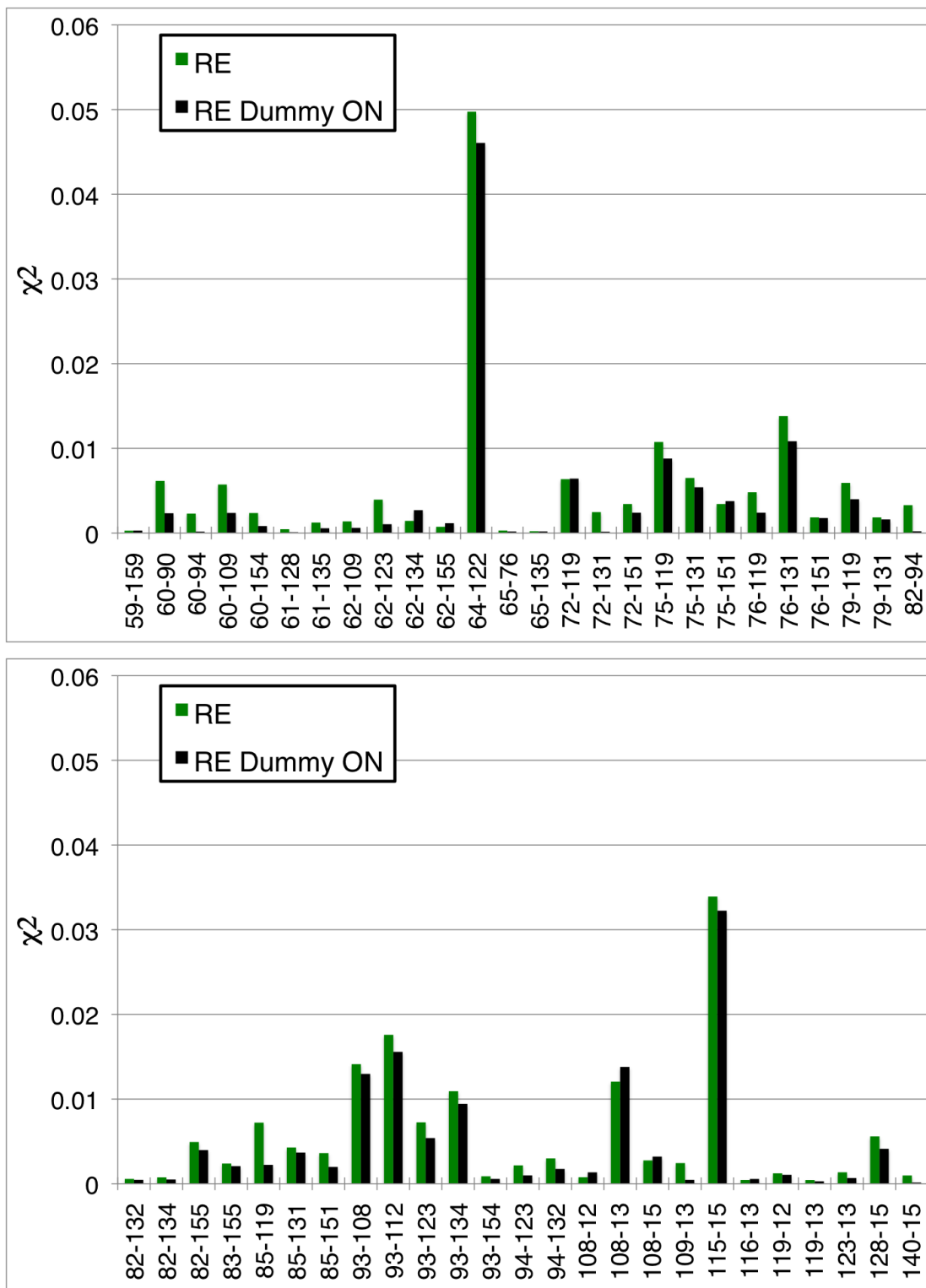
END

**Figure S17.** Cartoon representation of T4L with replicated ON dummy atoms colored in blue used for RE simulation.

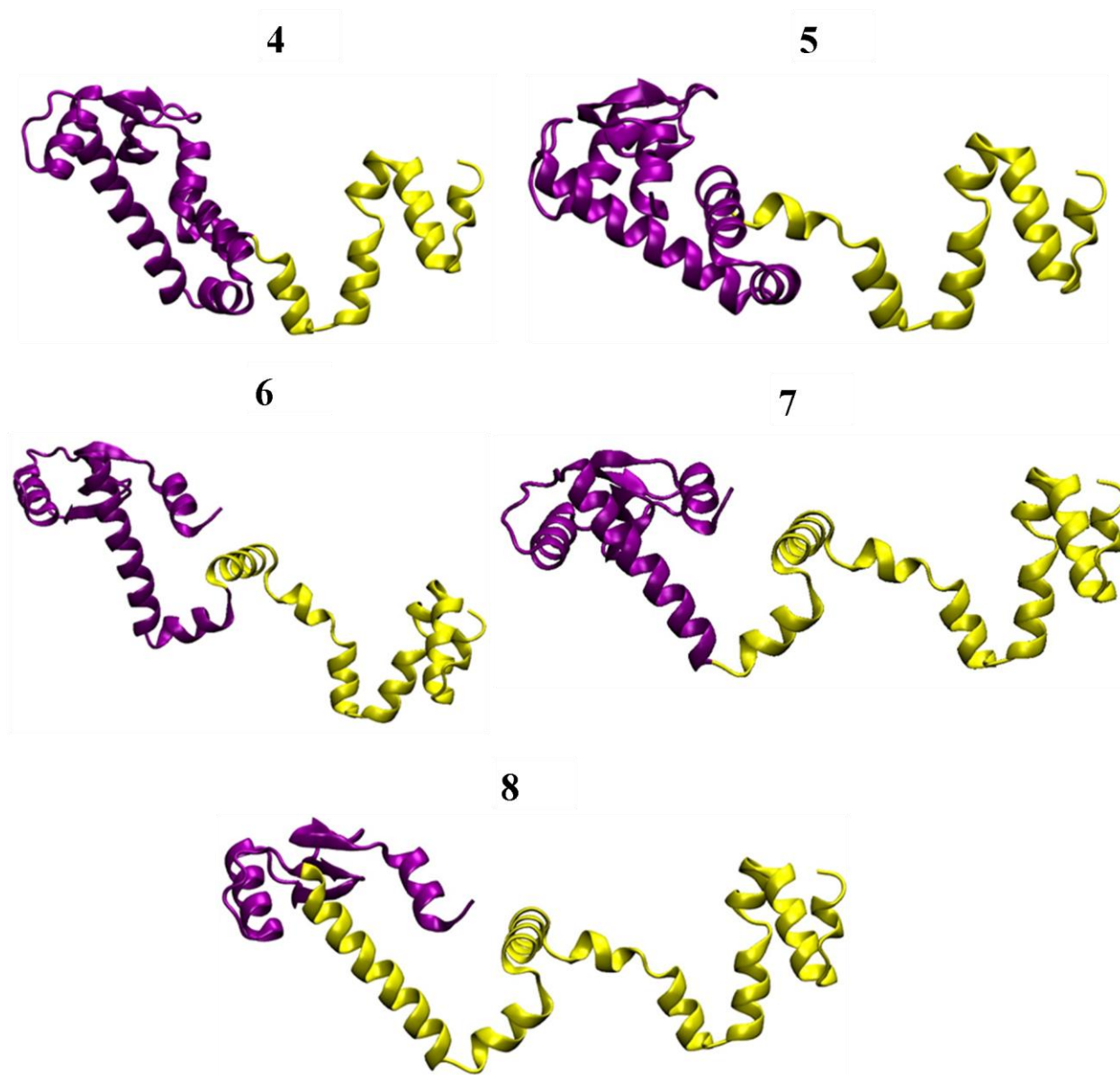




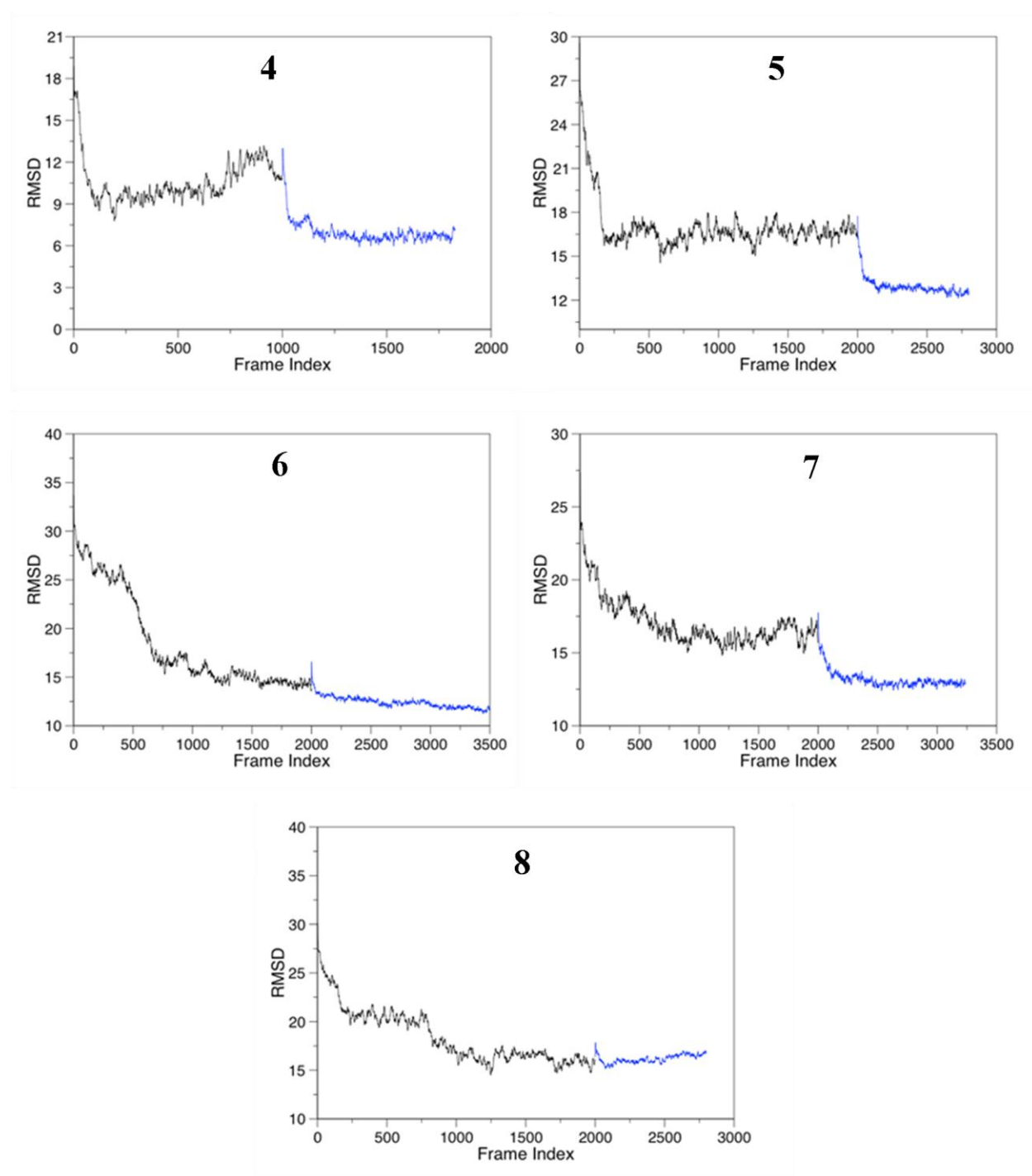
**Figure S18.** Difference in spin-spin distance histograms between experiment and RE simulations conducted with the spin label side chain (green line) and ON dummy atom (black)



**Figure S19.** Cartoon representation of five distorted T4L structures, which were not able to be refined with RE simulation. The distorted area of the structures are colored in yellow and the undistorted area is colored in purple. The numbers in each of these structures represent the number of helices that are distorted.



**Figure S20.** Root mean square deviation of backbone atoms of the distorted residues in T4L, shown in Figure S23, with respect to the X-ray structure obtained from NOE and RE simulations colored in black and blue, respectively.



**Table S2.** Experimental ESP/DEER distance distribution data which are simultaneously used in the RE simulation. These distance distributions are normalized to 1.

108	123	
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2.000000e+000	0.000000e+000	
3.000000e+000	0.000000e+000	
4.000000e+000	0.000000e+000	
5.000000e+000	0.000000e+000	
6.000000e+000	0.000000e+000	
7.000000e+000	0.000000e+000	
8.000000e+000	0.000000e+000	
9.000000e+000	0.000000e+000	
1.000000e+001	0.000000e+000	
1.100000e+001	0.000000e+000	
1.200000e+001	0.000000e+000	
1.300000e+001	0.000000e+000	
1.400000e+001	0.000000e+000	
1.500000e+001	0.000000e+000	
1.600000e+001	0.000000e+000	
1.700000e+001	0.000000e+000	
1.800000e+001	0.000000e+000	
1.900000e+001	0.000000e+000	
2.000000e+001	0.000000e+000	
2.100000e+001	2.622869e-004	
2.200000e+001	8.536026e-003	
2.300000e+001	3.285770e-002	
2.400000e+001	6.590217e-002	
2.500000e+001	1.011810e-001	
2.600000e+001	1.343203e-001	
2.700000e+001	1.517339e-001	
2.800000e+001	1.477354e-001	
2.900000e+001	1.290671e-001	
3.000000e+001	1.007966e-001	
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4.900000e+001	0.000000e+000
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108 134	
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4.100000e+001	0.000000e+000



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1.300000e+001	0.000000e+000

1.400000e+001	0.000000e+000
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1.900000e+001	1.971317e-002
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3.000000e+001	3.773806e-002
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62 109

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93 112

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59 159	
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5.900000e+001	0.0000000e+000
6.000000e+001	0.0000000e+000

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