The slow folding of cross-linked α-helical peptides is due to steric hindrance

SUPPLEMENTARY MATERIAL

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I. R- AND L-CONFORMATIONS OF ARGININE 10

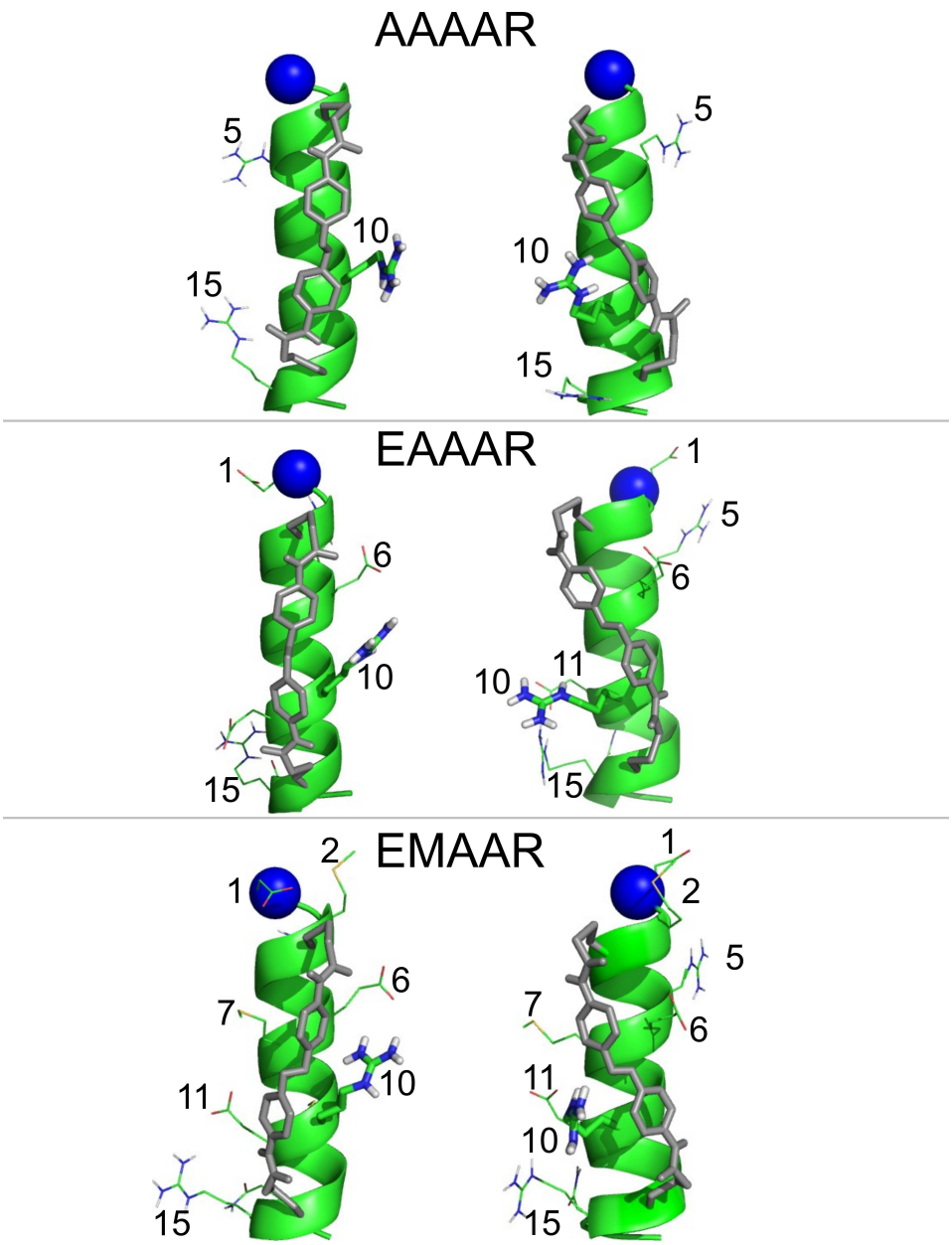

FIG. S1: The two orientations of the Arg10 side chain in the helical state of AAAAR, EAAAR, and EMAAR. Transitions between r-orientation (structures on the left column) and l-orientation (right column) are hindered by the presence of the cross-linker. Atoms in non-Ala side chains are shown by sticks. All structures are aligned with the N-terminus (blue sphere) on top and cross-linker atoms (grey) in the front for optimal comparison of the orientation of the side chains.

II. MFPT-BASED CFEP

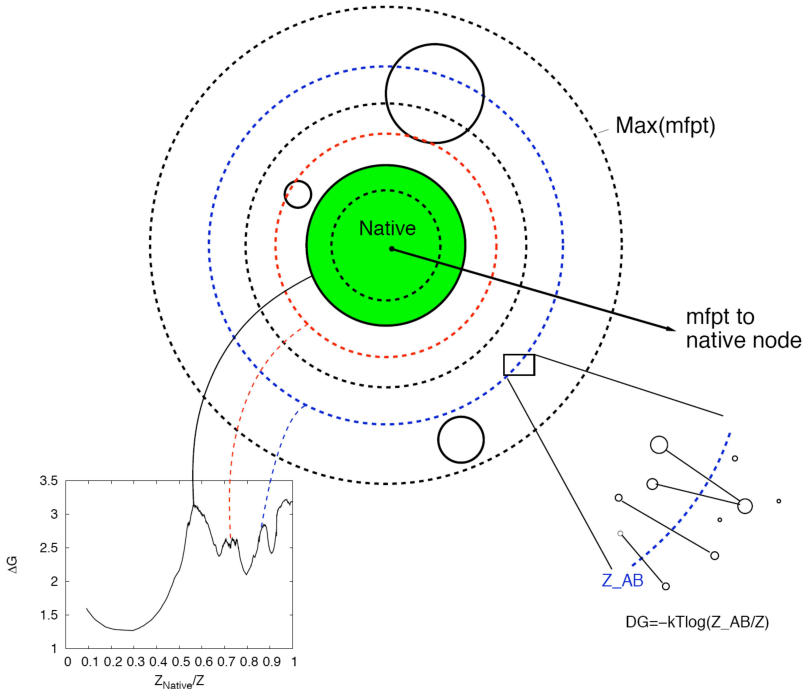

FIG. S2: Schematic illustration of the one-dimensional FEP procedure using mfpt as progress variable. Each of the four solid circles represents a free-energy basin while concentric dashed circles represent values of mfpt. For each value of mfpt_c between 0 (native node) and mfpt_{MAX} a point in the profile is obtained. Bottom right: ΔG of the fraction of links crossing the cutting surface at mfpt=mfpt_c. Bottom left: Relative partition function Z_A/Z, where the set A contains nodes with mfpt<mfpt_c. The figure is adapted from Ref.[1]

III. CFEP OF HELICAL ENSEMBLE USING RMSD COARSE GRAINING

The combination of the cFEP with side chain atoms RMSD clustering is particularly effective to determine individual states differing in side chain orientation and packing. On the other hand, the secondary structure coarse-graining used in previous works[2,3] does not take side chain information into account. Therefore, it can group together conformations that are far from each other in configuration space (i.e., distant in terms of side chain orientations) simply because they have the same secondary structure string. Here, RMSD clusters were determined upon optimal fitting of the C_α atoms. Side chain atoms were used for RMSD clustering using a threshold of 2.0 Å for AAAAR and 2.5 Å for EAAAR and EMAAR. Structural fitting according to the C_α-atoms is justified because the analysis focus on the helical ensemble (i.e., composed of similar structures without bending in the chain).

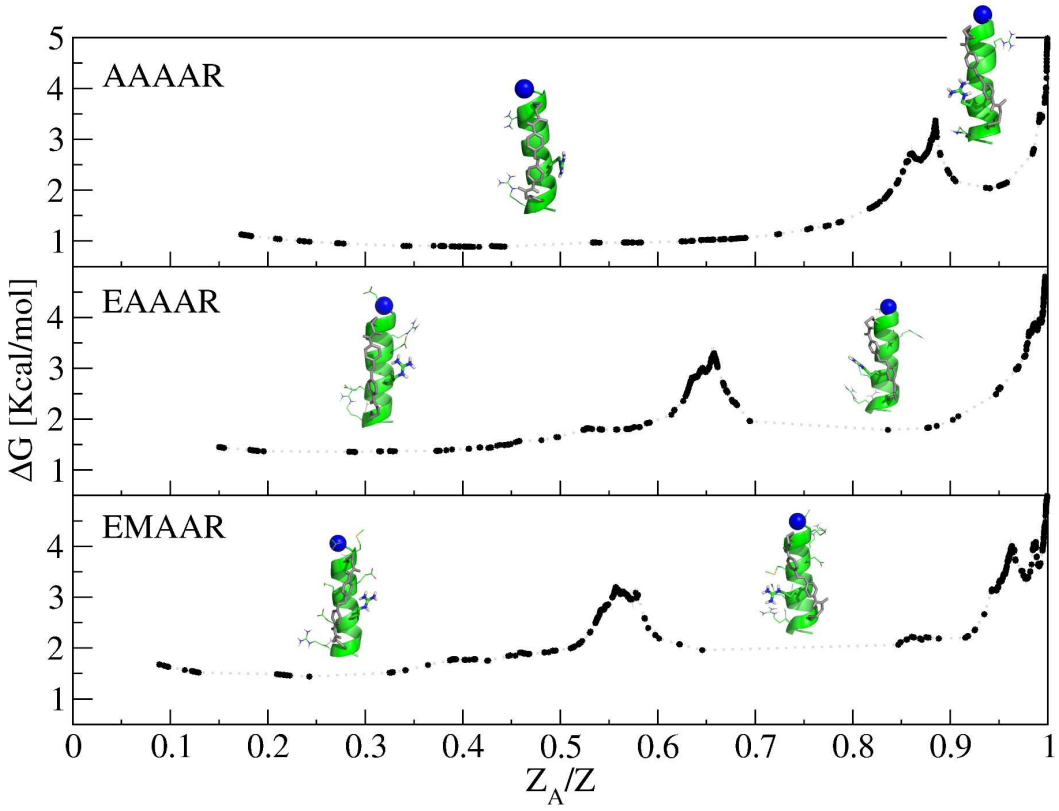

FIG. S3: The cFEP of the trans state shows a free-energy barrier separating RMSD clusters with r-orientation of Arg10 from clusters with l-orientation. The height of the barrier is about 2 kcal/mol. Representative structures are prepared with PyMOL (http://www.pymol.org/). The structures on the left and right side of the barrier have the Arg10 side chain in the r-orientation and l-orientation, respectively. The reaction coordinate is the relative partition function Z_A/Z (see Methods and Ref.[4]) and the mfpt was used to calculate the kinetic distance from the reference node[1] which is the most populated RMSD cluster.

IV. CFEP OF HELICAL ENSEMBLE USING COSθ COARSE GRAINING

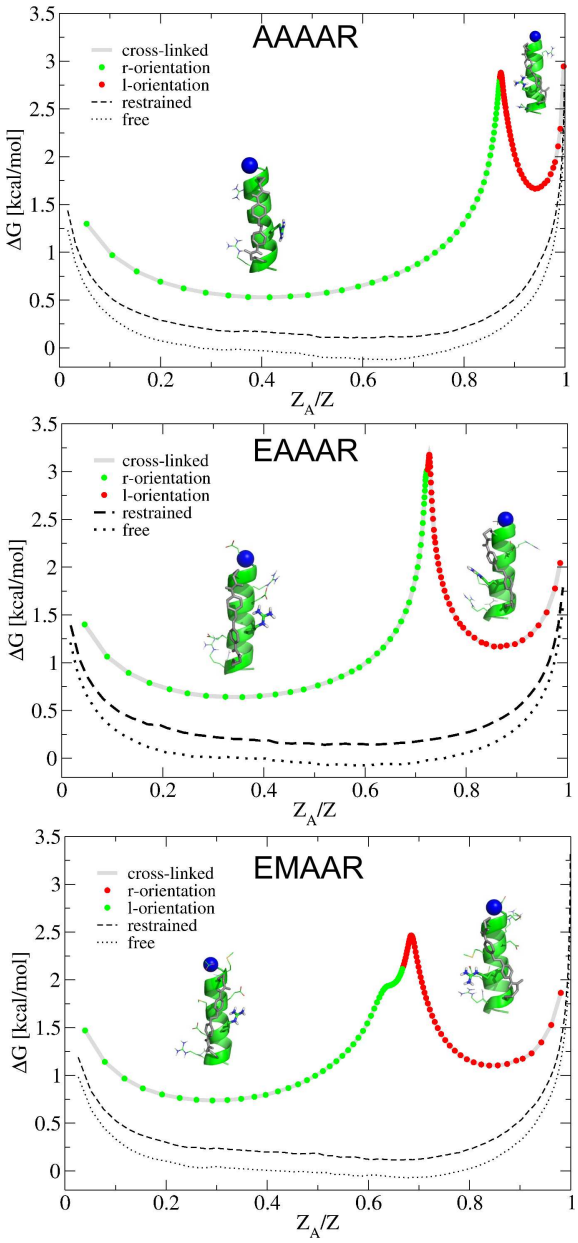

FIG. S4: The cFEP of the helical ensemble of AAAAR (top), EAAAR (middle), and EMAAR (bottom) using cosθ for clustering. The lines with colored circles correspond to the peptide with cross-linker, while the black dashed and dotted lines are the cFEP of the non-bulky cross-linker and free system, respectively. The latter cFEPs were translated along the y-axis for clarity. See also the legend of Fig. 4,top in main text.

V. FIT OF HELICAL TRACES

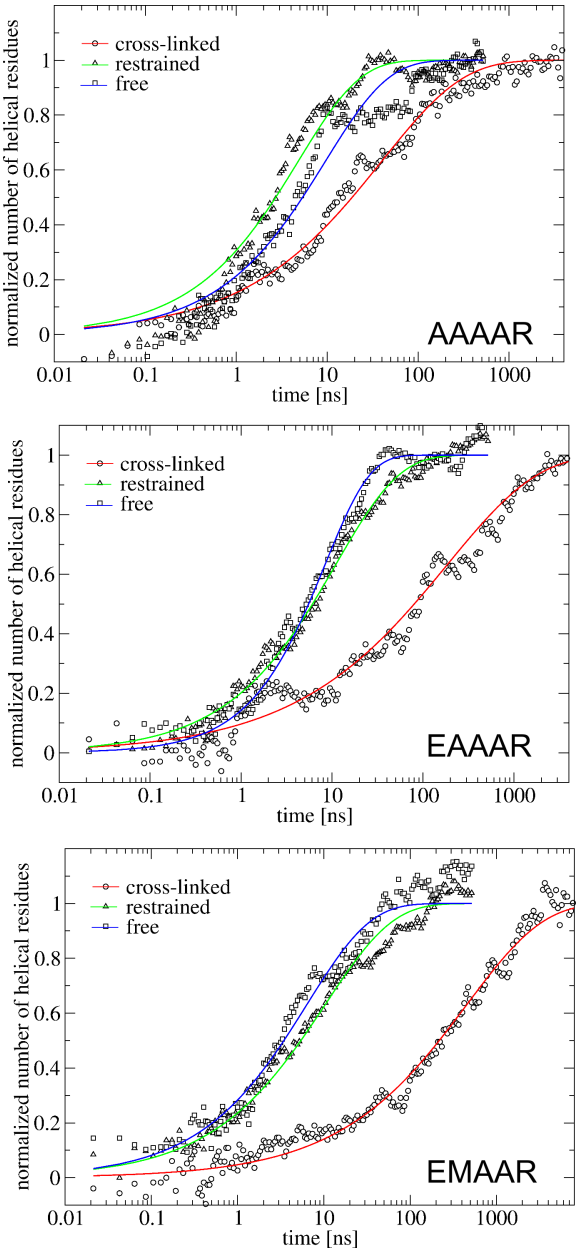

FIG. S5: Kinetic traces of helical content along MD simulations of AAAAR (top), EAAAR (middle), and EMAAR (bottom). The plots show the normalized difference in the number of helical residues (α-, π-, and 3_{10}-helix conformation[5]) between initial and final states. Data points (symbols) are fitted with stretched-exponential functions $(1 - exp(-x/\tau)^{\beta}$, solid lines). Folding times and stretching factors are listed in Table S-I. The folding time increases with increasing number of long side chains and the increase is more pronounced for cross-linked peptides.

	cross-linked		restrained		free	
	τ [ns]	β	τ [ns]	β	τ [ns]	β
AAAAR	40	0.49	5	0.64	9	0.83
EAAAR	185	0.45	11	0.70	8	0.88
EMAAR	456	0.49	10	0.56	6	0.59

TABLE S-I: Results of the stretched exponential fits $(1 - exp(-x/\tau)^{\beta})$.

VI. SUBSTITUTION OF THE CROSS-LINKER WITH A RESTRAINT

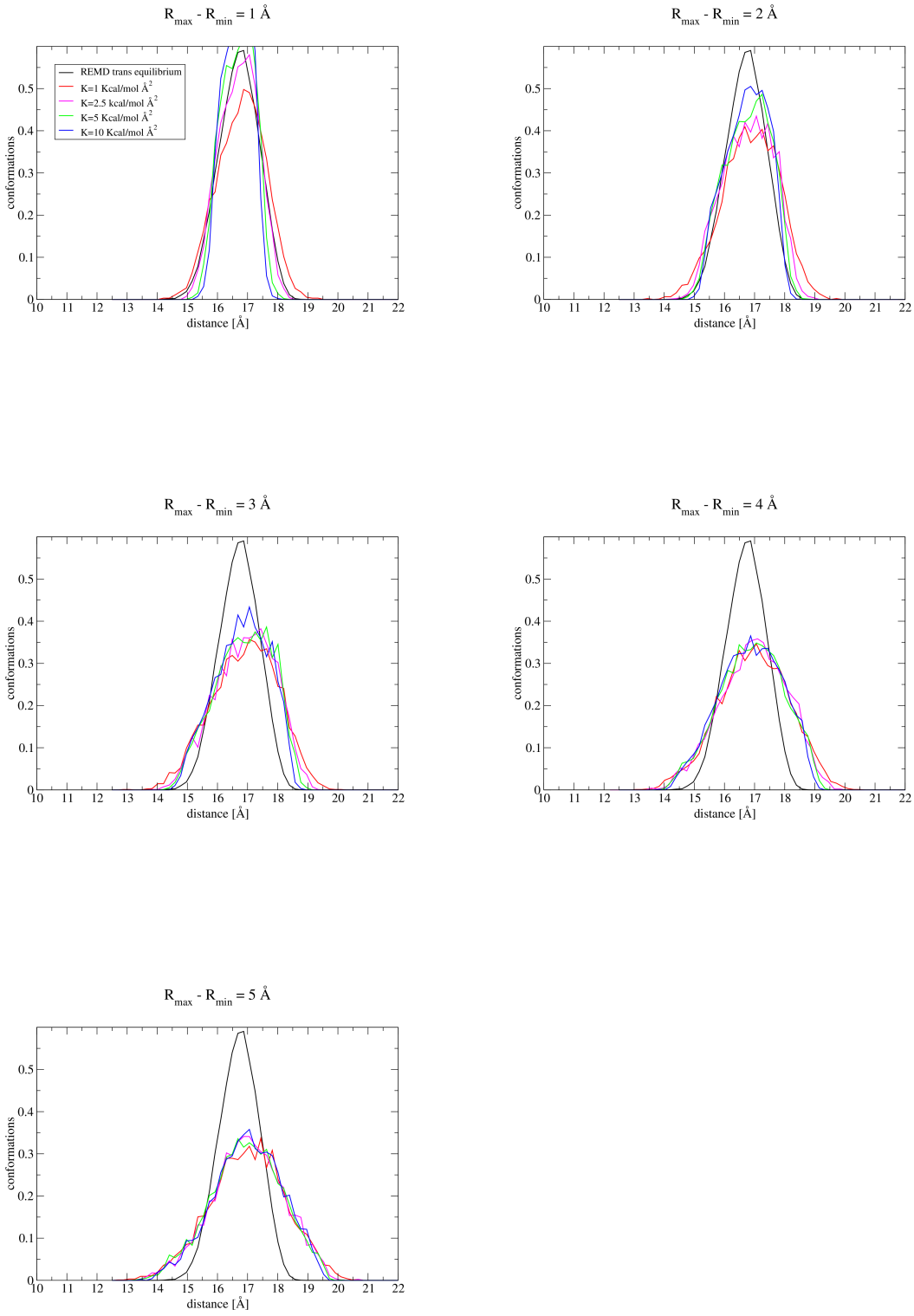

FIG. S6: Cysteine sulfur atoms distance distribution in a 100 ns MD test-run on EAAAR with R_{MAX}-R_{MIN} ranging from 1 to 5 Å and K ranging from 1 to 10 Kcal/mol Å^2, see also Tab. S-II.

R_{min} [Å]	R_{max} [Å]	(R_{min}-R_{max}) [Å]
K=1 [Kcal/mol Å^2]		
14.0	19.0	5
14.5	18.5	4
15.0	18.0	3
15.5	17.5	2
16.0	17.0	1
K=2.5 [Kcal/mol Å^2]		
14.0	19.0	5
14.5	18.5	4
15.0	18.0	3
15.5	17.5	2
16.0	17.0	1
K=5 [Kcal/mol Å^2]		
14.0	19.0	5
14.5	18.5	4
15.0	18.0	3
15.5	17.5	2
16.0	17.0	1
K=10 [Kcal/mol Å^2]		
14.0	19.0	5
14.5	18.5	4
15.0	18.0	3
15.5	17.5	2
16.0	17.0	1

TABLE S-II: Summary of tests performed. R_{MAX}-R_{MIN} varies between 1 Å and 5 Å and K between 1 Kcal/mol Å^2 and 10 Kcal/mol Å^2.

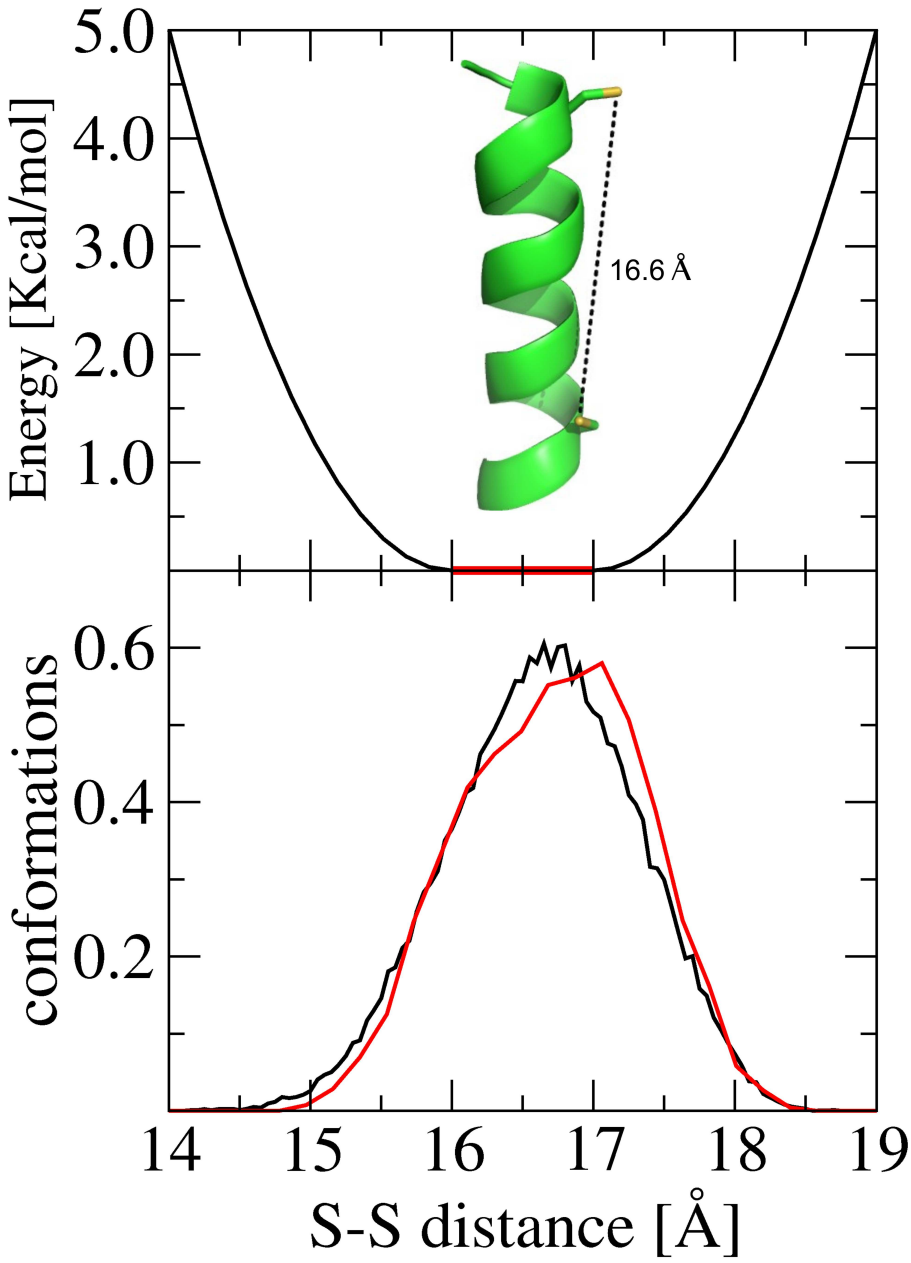

FIG. S7: Distance restraint used to emulate the cross-linker. (Top) The restraining function is harmonic at the borders and zero in the 16.0-17.0 Å interval. The average separation of the sulfur atoms of Cys3 and Cys14 in the trans conformation of the cross-linker is 16.6 Å (inset). (Bottom) Distance distribution when the linker is in trans conformation (black curve). The red curve is the results obtained in a 100 ns kinetic run using the restraining function shown in the top.

[1] S. V. Krivov, S. Muff, A. Caflisch, and M. Karplus, J. Phys. Chem. B **112**, 8701 (2008).

[2] J. A. Ihalainen, B. Paoli, S. Muff, E. H. G. Backus, J. Bredenbeck, G. A. Woolley, A. Caflisch, and P. Hamm., Proc. Natl. Acad. Sci. USA **105**, 9588 (2008).

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