–Supporting Information–

Investigation of Stability and Disulfide Bond Shuffling of Lipid Transfer Proteins by Molecular Dynamics Simulation[†]

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RUNNING HEADER: LTP Stability and Disulfide Bond Shuffling

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Table S1: Key results of the analysis of the different structures of LTP2 as labelled using VADAR (*1*). ASA is the surface area accessible to a water molecule and VOL is the sum of volumes for all residues in the protein.

	% of residues in specified region								
	Ramachandran plot (ϕ/ψ)				ω	ASA (nm^2)		VOL (nm^3)	
Model	core	allowed	generous	outside	core	obs*	exp*	obs*	exp*
R-LTP2-N	73	18	4	0	76	3900	4103	7415	4955
W-LTP2-X	98	1	0	0	92	4100	4090	7981	7917
W-LTP2-N	75	22	2	0	100	4333	4090	8247	7918
R-LTP2-H	88	7	2	0	86	4618	4103	8219	7955

* obs and exp refer to the observed and expected values, respectively.

Table S2: Rmsd between LTP2 structures (nm). The structures were first aligned by minimising the rmsd between the backbone atoms (C, Cα, N) of the structured portion of W-LTP2-X (residues 4-60); the rmsd was then computed for the same atoms.

Model	W-LTP2-X	W-LTP2-N	R-LTP2-H
R-LTP2-N	0.43	0.47	0.35
W-LTP2-X		0.24	0.29
W-LTP2-N			0.35

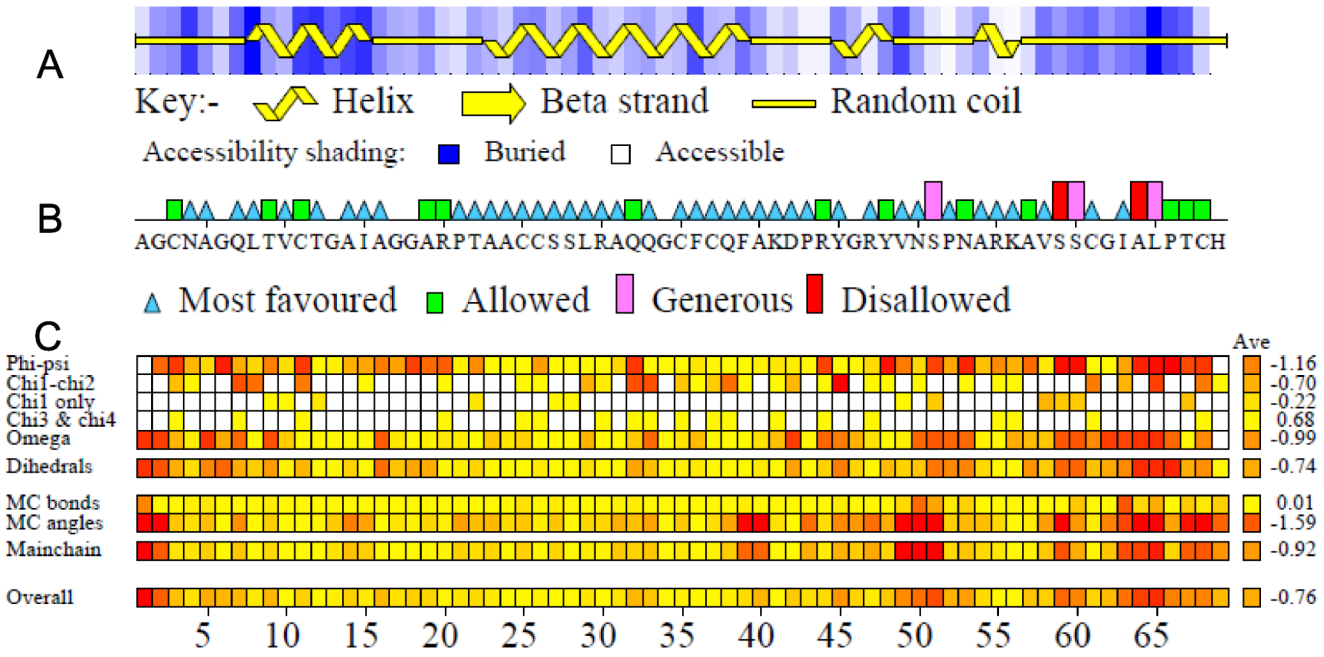

Fig. S1: Key output of the analysis of R-LTP2-N with PROCHECK (*2*). A) Secondary structure and solvent accessibility, B) location of the ϕ/ψ angles on the ramachandran plot and C) G-factors for each class of structural properties for each residue. Ideally, G-factors should be above 0.5; dark squares and values below -1.0 indicate potential problems. Note that χ_1 G-factors are only shown for residues without χ_2 angles.

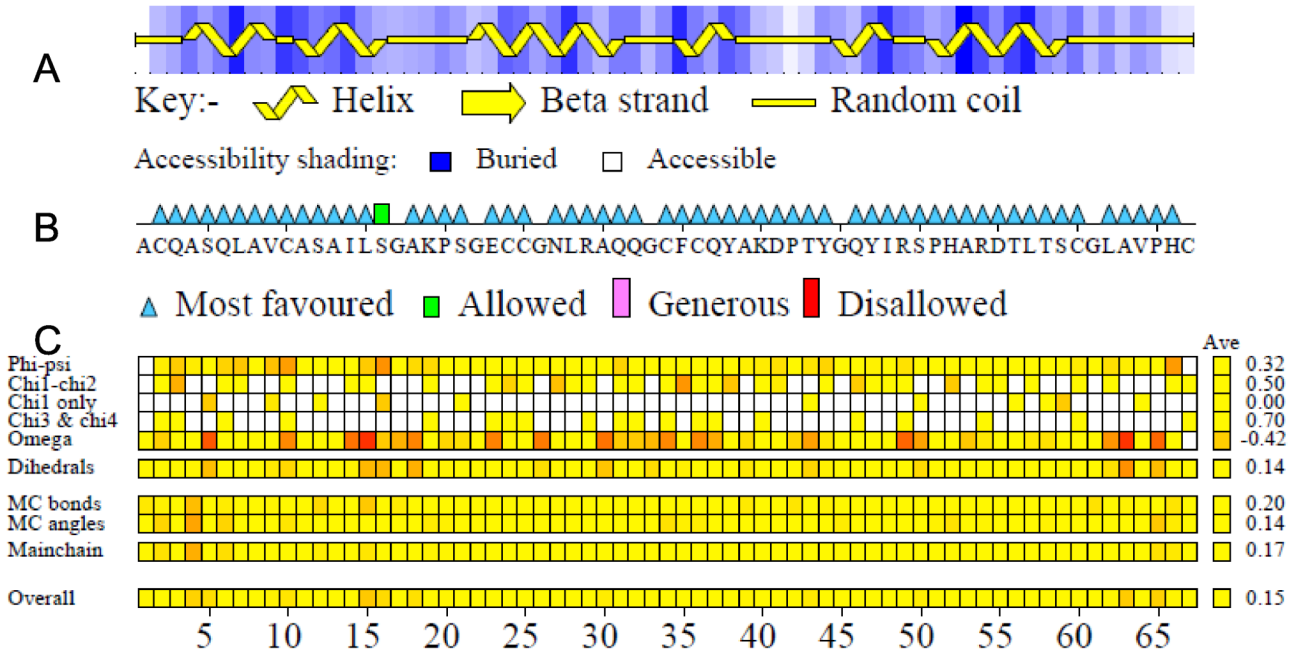

Fig. S2: Key output of the analysis of W-LTP2-X with PROCHECK (*2*). A) Secondary structure and solvent accessibility, B) location of the ϕ/ψ angles on the ramachandran plot and C) G-factors for each class of structural properties for each residue. Ideally, G-factors should be above 0.5; dark squares and values below -1.0 indicate potential problems. Note that χ_1 G-factors are only shown for residues without χ_2 angles.

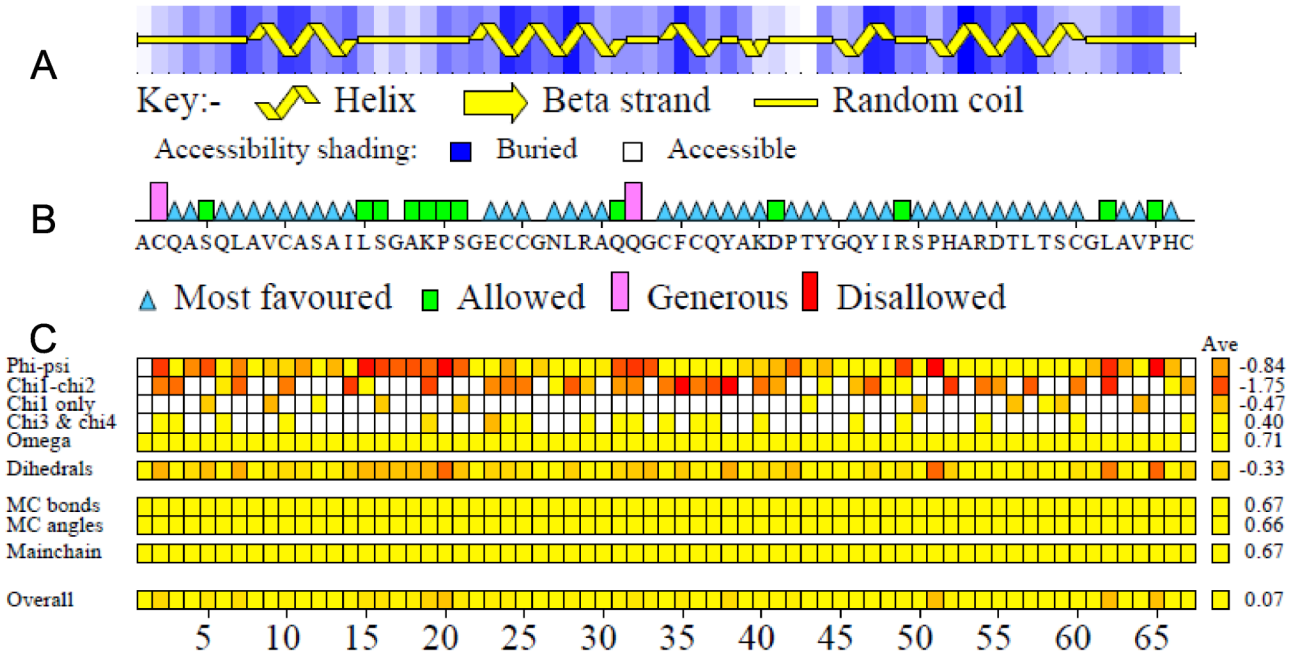

Fig. S3: Key output of the analysis of W-LTP2-N with PROCHECK (*2*). A) Secondary structure and solvent accessibility, B) location of the ϕ/ψ angles on the ramachandran plot and C) G-factors for each class of structural properties for each residue. Ideally, G-factors should be above 0.5; dark squares and values below -1.0 indicate potential problems. Note that χ_1 G-factors are only shown for residues without χ_2 angles.

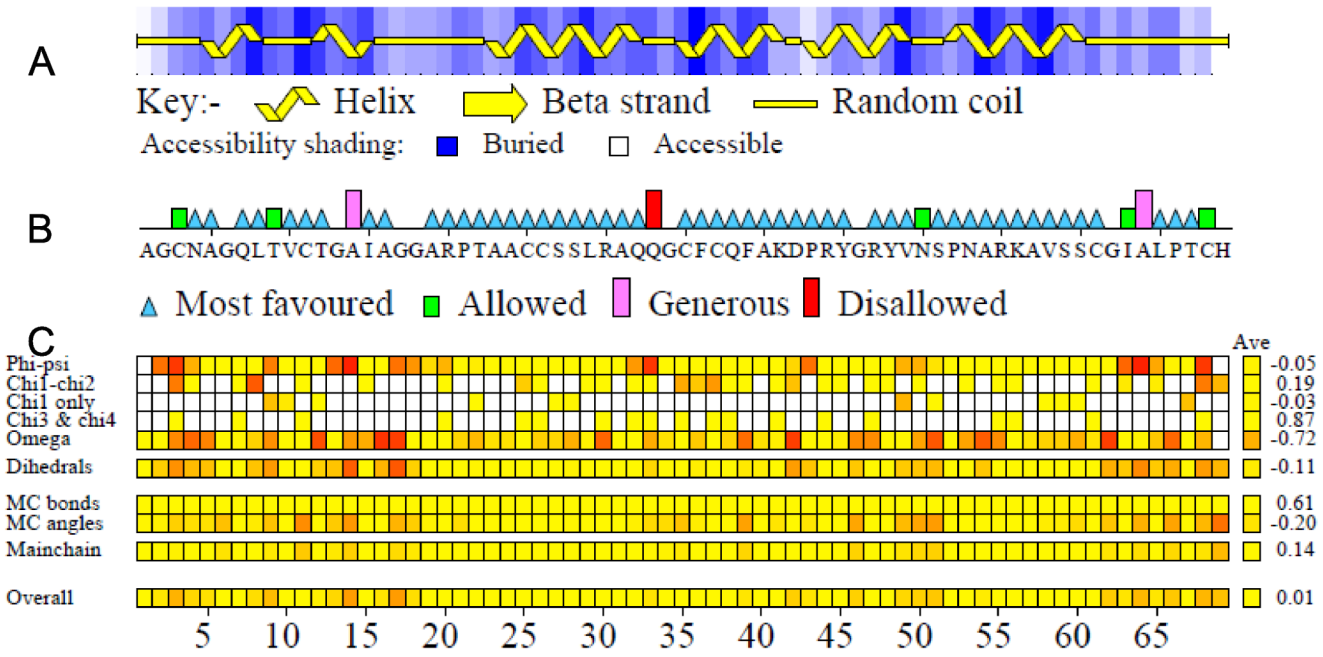

Fig. S4: Key output of the analysis of R-LTP2-H with PROCHECK (*2*). A) Secondary structure and solvent accessibility, B) location of the ϕ/ψ angles on the ramachandran plot and C) G-factors for each class of structural properties for each residue. Ideally, G-factors should be above 0.5; dark squares and values below -1.0 indicate potential problems. Note that χ_1 G-factors are only shown for residues without χ_2 angles.

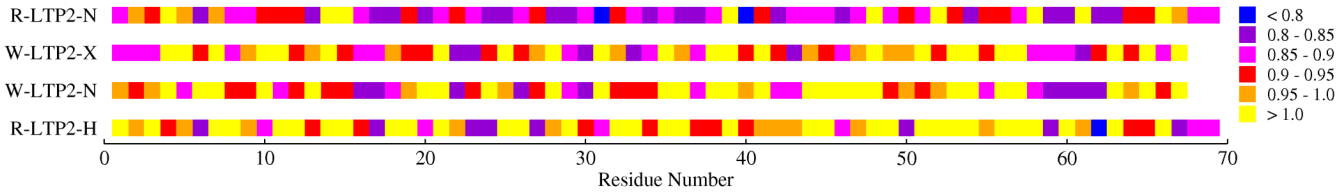

Fig. S5: Fractional volume of each residue of each LTP2 structure as labelled, computed with VADAR (*1*). Fractional volumes less than 0.80 indicate compression or atomic overlaps.

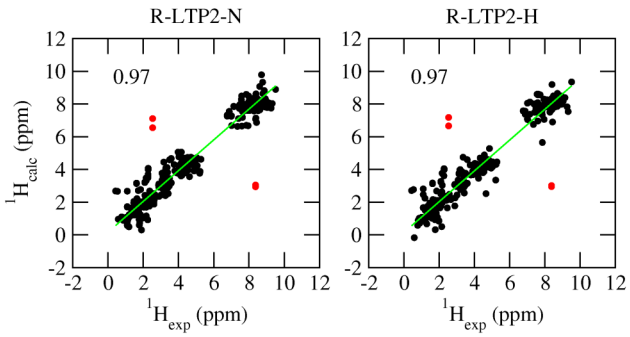

Fig. S6: Comparisons of the experimental ^{1}H chemical shifts measured for R-LTP2 with those back-calculated using SHIFTX (*3*) from R-LTP2-N and R-LTP2-H as labelled. The green line shows a linear regression fit to the data. The red dots show outliers which were not included in the fitting procedure, as they are most likely due to incorrect assignment of the experimental data. The correlation coefficient is shown on each plot.

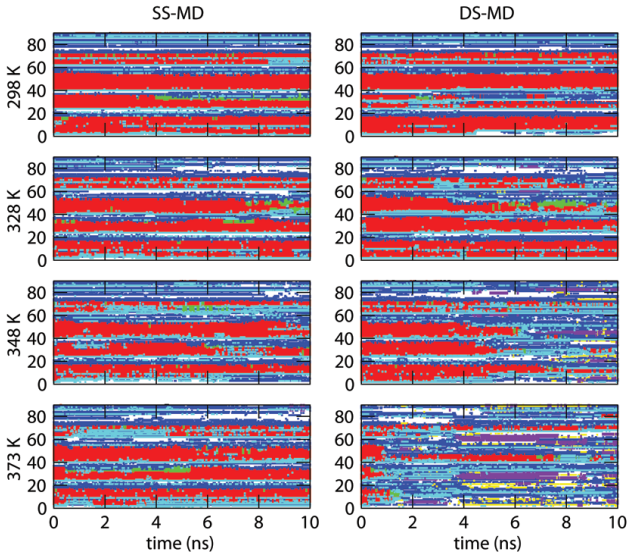

Fig. S7: Time series of the secondary structure ((black) 3_{10}-helix, (red) α-helix, (green) π-helix, (blue) bend, (yellow) β-bridge, (violet) β-strand and (cyan) turn) of R-LTP1-X during the SS-MD and DS-MD simulations at different temperatures as labelled.

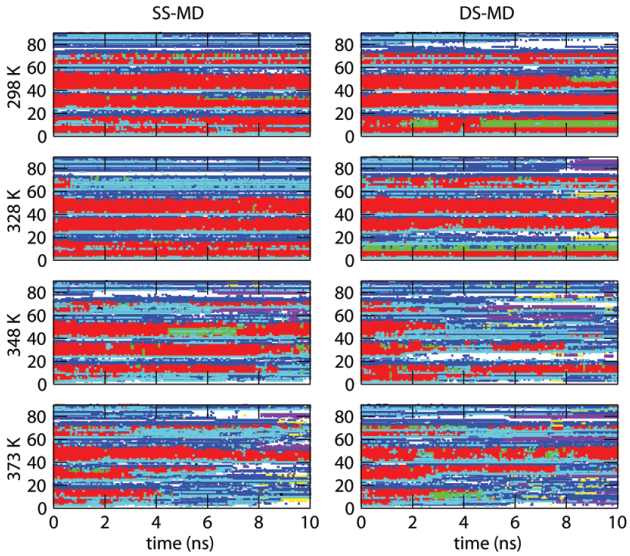

Fig. S8: Time series of the secondary structure ((black) 3_{10}-helix, (red) α-helix, (green) π-helix, (blue) bend, (yellow) β-bridge, (violet) β-strand and (cyan) turn) of R-LTP1-N during the SS-MD and DS-MD simulations at different temperatures as labelled.

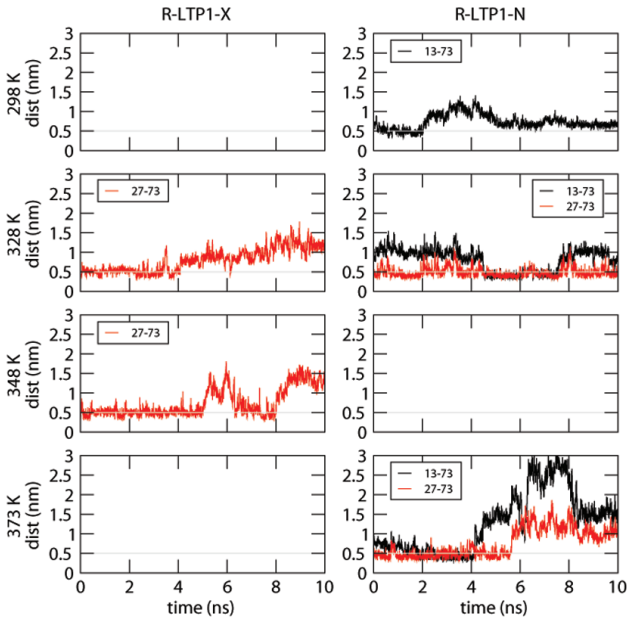

Fig. S9: Time series of the sulfur-sulfur distances of the non-native cysteine residue pairs that are less than 0.5 nm apart for more than 10% (1 ns) of the DS-MD simulations of R-LTP1-X and R-LTP1-N at different temperatures as labelled. The grey line indicates 0.5 nm.

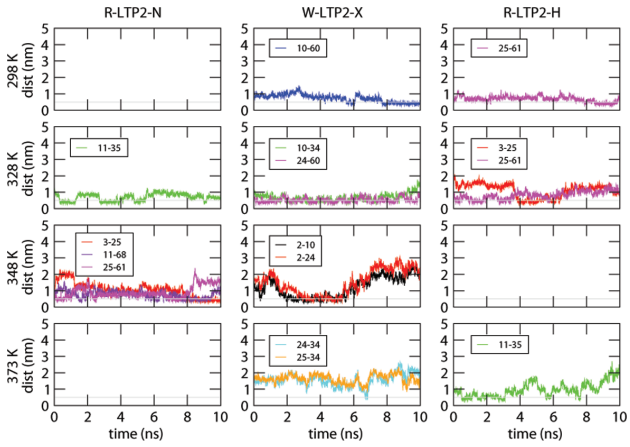

Fig. S10: Time series of the sulfur-sulfur distances of the non-native cysteine residue pairs that are less than 0.5 nm apart for more than 10% (1 ns) of the DS-MD simulations of R-LTP2-N, W-LTP2-X and R-LTP2-H at different temperatures as labelled. The grey line indicates 0.5 nm.

References

1. Willard, L., Ranjan, A., Zhang, H., Monzavi, H., Boyko, R. F., Sykes, B. D., and Wishart, D. S. (2003) VADAR: a web server for quantitative evaluation of protein structure quality. *Nucl. Acids Res. 31*, 3316–3319.

2. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) PROCHECK - a program to check the stereochemical quality of protein structures. *J. App. Cryst. 26*, 283–291.

3. Neal, S., Nip, A. M., Zhang, H., and Wishart, D. S. (2003) Rapid and accurate calculation of protein ^{1}H, ^{13}C and ^{15}N chemical shifts. *J. Biomol. N.M.R. 26*, 215–240.