Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Combining EPR spectroscopy and X-ray crystallography to elucidate the structure and dynamics of conformationally constrained spin labels in T4 lysozyme single crystals

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Supporting Information

Table S1. Crystallographic data collection and refinement statistics for T4 lysozyme 115/119RX determined at 298 K.

PDB entry	5NXO
Wavelength (Å)	1.542
Resolution range (Å)	23.22 - 1.803 (1.868 - 1.803)
Space group	$P3_221$
Unit cell (*a*=*b*, *c*; Å)	61.07, 97.07
Total reflections	35824 (2882)
Unique reflections	19432 (1809)
Multiplicity	1.8 (1.6)
Completeness (%)	0.98 (0.92)
Mean I/sigma(I)	13.92 (1.12)
Wilson B-factor (Å^2)	25.52
R-merge	0.03501 (0.5192)
R-meas	0.04951 (0.7342)
$CC_{1/2}$	0.999 (0.625)
CC*	1 (0.877)
Reflections used in refinement	19430 (1812)
Reflections used for R-free	956 (80)
R-work	0.1696 (0.2710)

R-free	0.2147 (0.3086)
CC(work)	0.970 (0.722)
CC(free)	0.941 (0.707)
Number of non-hydrogen atoms	1434
Protein residues	164
RMS(bonds) (Å)	0.019
RMS(angles) (°)	1.88
Ramachandran favored (%)	98
Ramachandran allowed (%)	1.8
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.71
Clashscore	1.48
Average B-factor (Å^2)	29.59
macromolecules	28.82
solvent	39.78

Statistics for the highest-resolution shell are shown in parentheses.

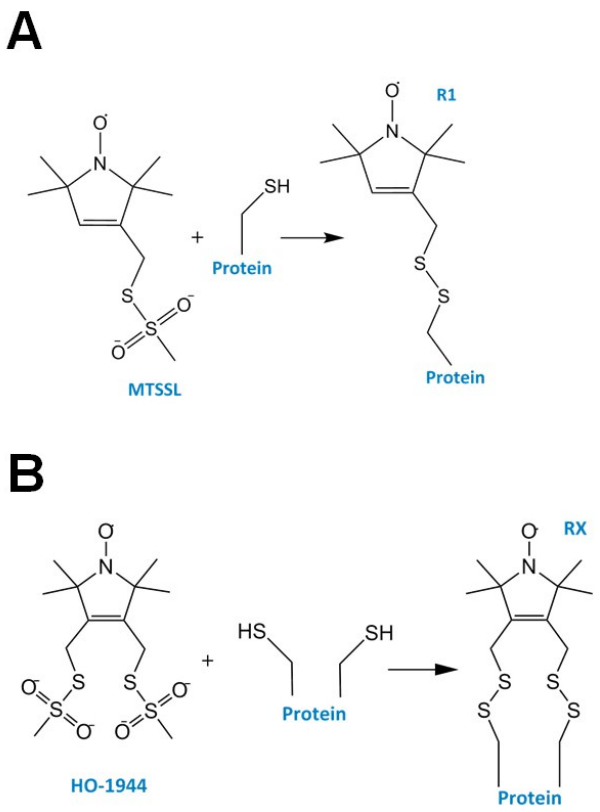

Figure S1. Schematic drawing of the labeling strategy. **(A)** Site-directed spin labeling of a single cysteine residue using the spin label 1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl (methanethiosulfonate, MTSSL) resulting in a covalent attachment via a disulfide bond. **(B)** Site-directed spin labeling of two adjacent cysteine residues using 2,2,5,5-tetramethyl-3,4-bis(sulfanylmethyl)-2,5-dihydro-1H-pyrrol-1-ol resulting in two covalent disulfide bonds.

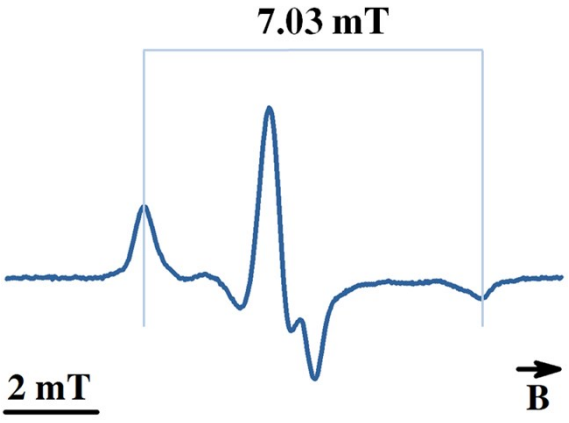

Figure S2. X-band EPR spectrum of a homogenous suspension of crushed single crystals of T4L variant 118R1 performed at room temperature. The spectral width was determined to 7.03 mT.

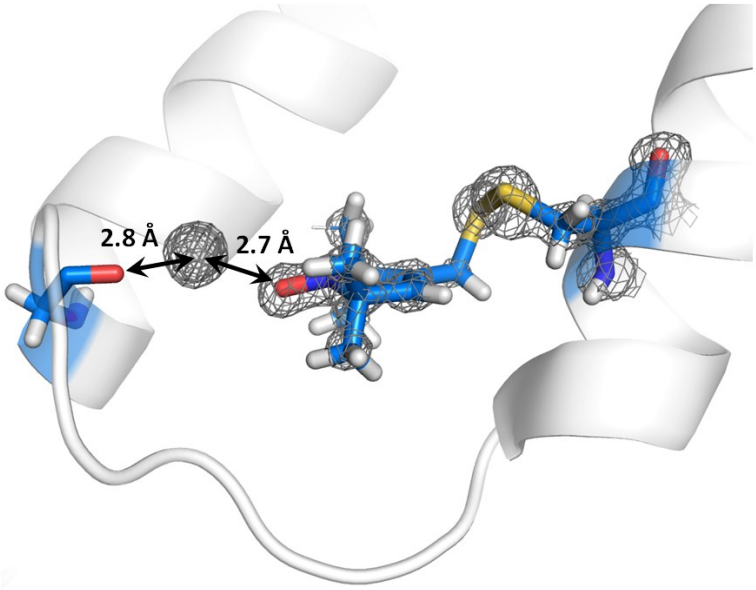

Figure S3. Stick model of the side-chain modification L118R1 (PDB entry 5JDT) superimposed by a $2F_o$-F_c difference electron density map contoured at 1.8 σ (gray mesh).[4] Additional electron density corresponds to a water molecule, which facilitates hydrogen bonds between the oxygen atom of the nitroxide and the carbonyl moiety of residue G107.

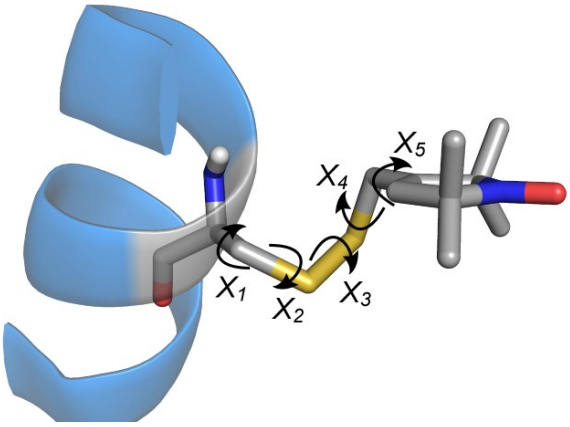

Figure S4. Stick model of the chemical structure of the R1 side chain exemplarily attached to position 118C of T4 lysozyme. The internal dynamics of the R1 side chain commonly considered to be characterized by rotations around the five dihedral angles X_1- X_5.

Table S2. Static and dynamic parameters for EPR line-shape simulation based on the SLE.

A_{xx}	A_{yy}	A_{zz}	g_{xx}	g_{yy}	g_{zz}	log R_{xx}	log R_{yy}	log R_{zz}	β_D	S	lw
0.62 mT	0.59 mT	3.7 mT	2.0086	2.0068	2.0023	7.8-8.2	7.8-8.2	7.8-8.2	0	0.91	0.17 mT

Table S3. EPR fit parameters obtained using the effective Hamiltonian approach by fitting an X-band EPR spectrum of a homogenous suspension of crushed single crystals at room temperature. Best fit parameters are shown for T4L variant 118R1.

Variant	A_{xx} [mT]	A_{yy} [mT]	A_{zz} [mT]	g_{xx}	g_{yy}	g_{zz}	Lw (FWHM) [mT]
118R1	0.74	0.68	3.55	2.0084	2.0062	2.0023	0.25

Table S4. EPR fit parameters obtained using the effective Hamiltonian approach by fitting an X-band EPR spectrum of a homogenous suspension of crushed single crystals at room temperature. Best fit parameters are shown for T4L variant 115/119RX.

Variant	A_{xx} [mT]	A_{yy} [mT]	A_{zz} [mT]	g_{xx}	g_{yy}	g_{zz}	Lw (FWHM) [mT]
115/119RX	0.69	0.58	3.65	2.0084	2.0063	2.0023	0.20

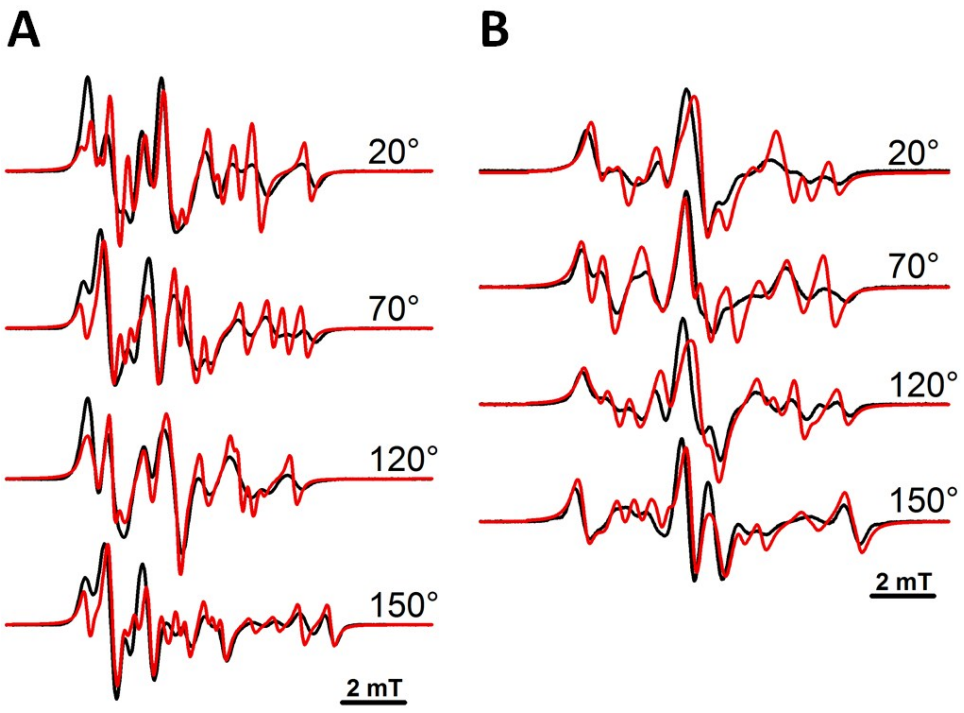

Figure S5. Four EPR Q-band (**A**) and X-band (**B**) spectra out of an angle dependent series taken for a single crystal of the T4L variant 118R1 (black traces). Angles are given with respect to a reference orientation (0°), for which the orientation of the unit cell was determined by X-ray crystallography. Red traces are simulations to the measured line shape using the effective Hamiltonian approach with principal components of the Zeeman- and hyperfine interactions as shown in **Table S3**.

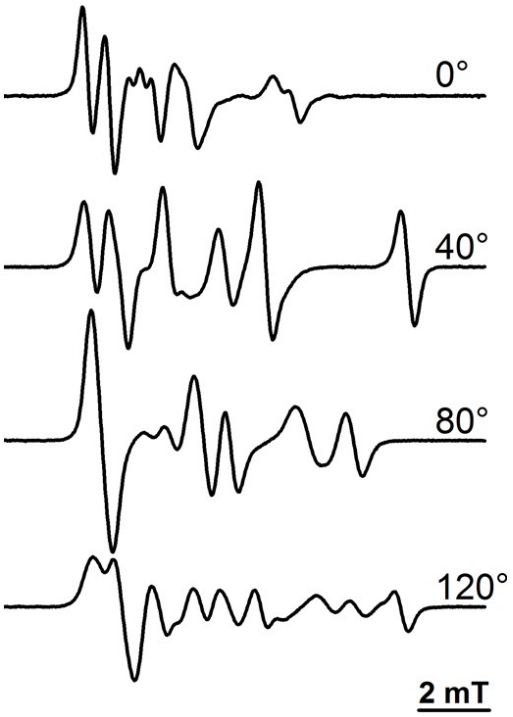

Figure S6. Four EPR Q-band spectra out of an angle dependent series taken from a single crystal of the T4L variant 115/119RX. Angles are given with respect to a reference orientation (0°), for which the orientation of the unit cell was determined by X-ray crystallography.