Electronic supplementary information

To accompany

Rotamer libraries of spin labelled cysteines for protein studies

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Customization of the CHARMM27 force-field for IA-PROXYL

The bending potential for the angle between S-CT2-C atom types, as well as potential for the torsion specified by CT2-S-CT2-C atom types (χ_3 dihedral for IA-PROXYL) are missing in the standard CHARMM27 force field. In the case of IA-PROXYL, the former is needed to account for bending of the S_γ-C_δ-C_ε bond, whereas the latter specifies torsion about the χ_3 dihedral angle, see Fig. S1.

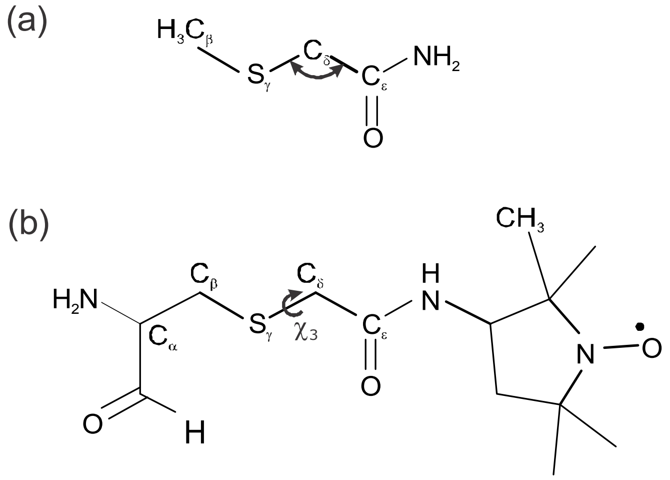

Fig. S1 (**a**) Molecular fragment used for determination of the bending potential for the angle between S-CT2-C atom types. (**b**) IA-PROXYL spin label.

The S-CT2-C bending potential was obtained by fitting vibrational frequencies of the molecular fragment (Fig. S1a) calculated by Tinker to the corresponding frequencies calculated by density functional theory (as implemented in the ORCA package[1]). The potential for the χ_3 torsion corresponding to the CT2-S-CT2-C atom types was not explicitly parameterized but rather replaced with that for CT3-S-CT2-CT2 atoms types. Inspection of other CHARMM27 torsion parameters suggests that similar replacements are commonplace.

Summary for MD simulations and projection of the full length MD trajectory on the set of rotamers for MTSSL and IA-PROXYL

Distributions of dihedral angles χ_1-χ_5 for MTSSL and χ_1-χ_6 for IA-PROXYL were calculated from the corresponding full-length MD trajectories acquired at 175 and 298 K. In the form of histograms or dihedral angle profiles, such distributions are shown as blue lines in Fig. S2 and Fig. S3 for MTTSL and IA-PROXYL respectively. Additionally, canonical dihedral angle values (shown green) as well as dihedral angles of the rotamers forming final rotamer libraries (shown red) are displayed superimposed on the corresponding dihedral angle profiles.

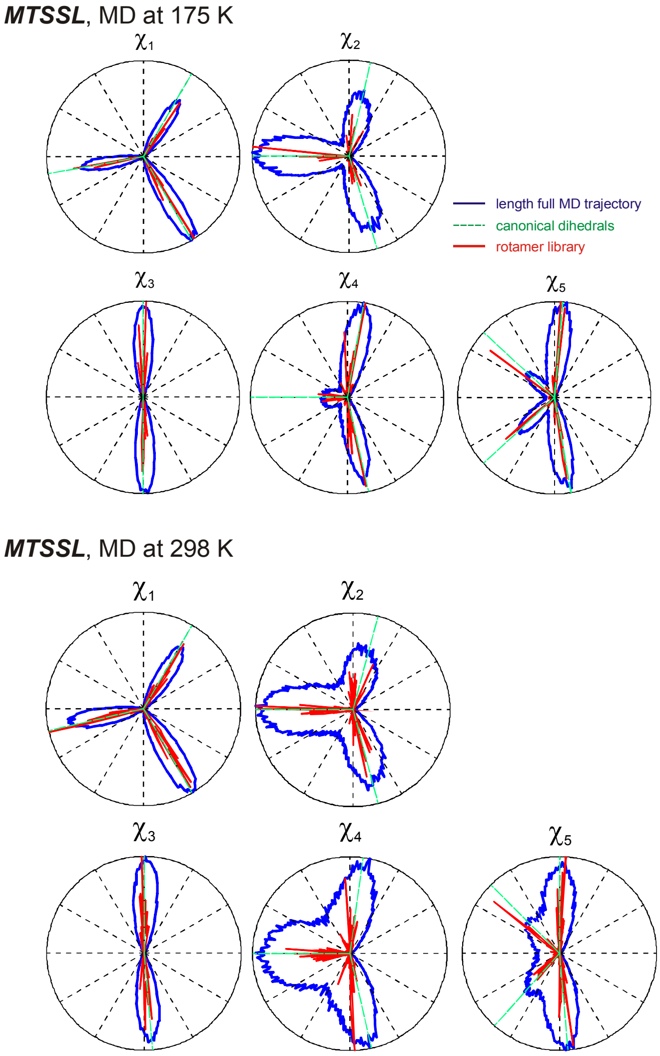

Fig. S2 Distributions of dihedral angles χ_1-χ_5 (*dihedral profiles*) for MTSSL calculated from the full length MD trajectories acquired at 175 and 298 K. Canonical dihedral angles values as well as dihedral angles distributions of the rotamers comprising corresponding final rotamer libraries are shown superimposed.

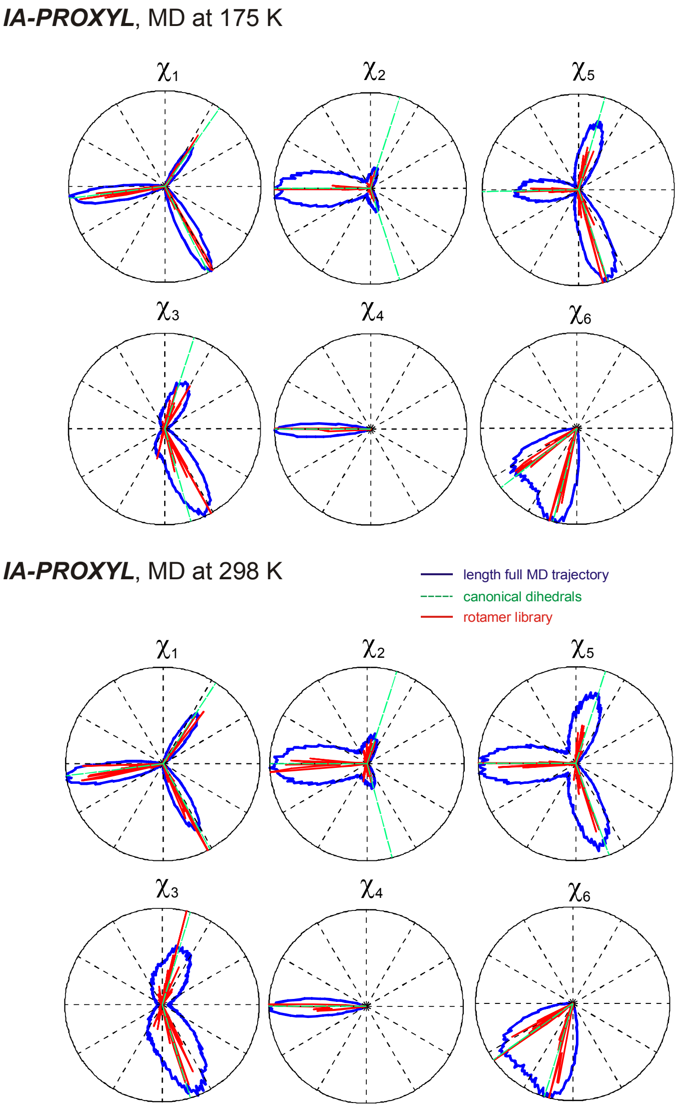

Fig. S3 Distributions of dihedral angles χ_1-χ_6 (dihedral profiles) for IA-PROXYL calculated from the full length MD trajectories acquired at 175 and 298 K. Canonical dihedral angles values as well as dihedral angles distributions of the rotamers comprising corresponding final rotamer libraries are shown superimposed.

References.

1. F. Neese, *Coord. Chem. Rev.*, 2009**, 253**, 526-563.