

Supplementary Information:
**The effect of tensile stress on the conformational free energy
landscape of disulfide bonds**

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I. SIMULATION METHODS AND COMPUTATIONAL DETAILS

A. Equilibrium and Metadynamics Force Field Simulations

All force field molecular dynamics (MD) simulations were carried out with the software suite GROMACS-4.5.3.^{1,2} The OPLS-AA^{3,4} all-atom force field was applied for simulating the polypeptide as well as cystine and diethyl disulfide (DEDS) together with using the SPC water model⁵ for explicit solvation. The simulations were run in the NVT ensemble with periodic boundary conditions. The temperature of the systems was kept constant at 300 K by coupling to Nosé-Hoover chain thermostats⁶, an integration time step of 2 fs was used, trajectory coordinates were recorded every 200 fs for analysis, and all bond lengths were constrained using LINCS.⁷ The electrostatic interactions were incorporated using a reaction field method⁸ that has been shown to perform well for the thermodynamics of protein folding⁹ and thus for computing conformational probability density maps are required here.

In order to converge the sampling of the conformational χ_2/χ'_2 -space, metadynamics acceleration^{10,11} was carried out using the PLUMED plugin¹² together with GROMACS-4.5.5. During metadynamics, the conformational sampling of the χ_2 and χ'_2 torsional angles, being the collective variables for metadynamics, as defined in Fig. 1 of the main text were accelerated for all three systems using Gaussian biasing functions (height: 0.23 kcal/mol, width: 0.1 radians) and deposited every 10 ps for a total simulation length of 300 ns. We assume that by sampling up to a free energy of ≈ 17 kcal/mol via metadynamics almost all possible conformations of the disulfides should be properly sampled as the highest strain energy in a disulfide moiety is found to be of that order or magnitude^{13,14} (corresponding to the Cys37-Cys54 bond of 3-oxoacyl-acyl carrier protein reductase from *Thermotoga maritima*, PDB ID 1O5I). In the case of DEDS and cystine, we assume this value to be even lower as they are free at their termini and hence not that much constrained as disulfide bonds in proteins.

DEDS, cystine and the polypeptide model Ile-Cys-Leu-Ser-Glu-Pro-Asp-Val-His-Cys-Gln (with the disulfide bond between the 2nd and 10th cysteine residue, were generated using PyMOL¹⁵, minimized (1000 steps using the steepest descent algorithm) and subsequently equilibrated. The species were solvated with SPC water in a periodic box of (3.0 x 3.0 x 3.0) nm³ for DEDS and cystine whereas a simulation box of (5.0 x 4.0 x 4.0) nm³ was used

for the polypeptide using 886, 880, and 2633 solvent molecules, respectively. In addition to the solvent, 10 Na^+ and 9 Cl^- ions were also added to the protein model system in order to keep the system neutral. The termini of the polypeptide were capped with hydrogens as to avoid any Coulomb attraction bias on the conformational free energies when using such a rather short peptide.

Force-clamp MD simulations of these species (that have been equilibrated at zero force) were then performed using the same computational approach as described above by explicitly including constant forces¹⁶ thus sampling isotensional force-transformed free energy surfaces^{17,18}; see Ref.¹⁹ for a detailed review on these computational techniques. In the present case, constant stretching forces from zero up to $F_0 = 2.0$ nN were applied to the terminal N and C atoms (of cystine and the polypeptide) and the two methyl C atoms (for DEDS) along the space-fixed x axis, applied in a systematically increasing manner for 2 ns in opposite directions along the x -coordinate to equilibrate the stretched molecule. Further, production runs were carried out for DEDS, Cystine and Polypeptide for 150, 300 and 1300 ns respectively and assessed all the necessary data. VMD²⁰ was used to analyze the dihedrals, the mechanical coordinate q (i.e. the distance between the atoms on which the external force acts), and various other structural parameters. The probability distribution functions and free energy surfaces were obtained from the isotensional metadynamics simulations and were visualized using the commercial software package MATLAB-7.6.0 (The MathWorks Inc., Natick, MA, 2000). The populations of the open conformers in all these models were computed. The "openness" and "closed" nature of the χ torsionals of DEDS were benchmarked by the specific values of their internal dihedrals.¹⁸ In the case of χ_3 angle, any conformation within the dihedral range of $180 \pm 50^\circ$ defines the *trans* conformation and all others defines a *normal* standard conformation of χ_3 . Similarly, χ_2 and χ'_2 conformations with dihedral angle in the range $180 \pm 50^\circ$ refers to a *closed* conformation and all other values for these dihedrals refers to an *open* conformation. The total closed nature in χ_2 and χ'_2 angles were then obtained and the openness was determined by taking the difference to unity.

B. QM/MM Reference Simulations of DEDS

In order to validate the performance of the intramolecular force field when stretching molecules we carried out reference calculations by treating the smallest system, DEDS, us-

ing electronic structure based (“on the fly”) MD, while sticking to a force field representation of the chemically inert explicit solvent. Thus, hybrid QM/MM MD simulations have been carried out upon applying a constant external force F_0 as before using the well-established CPMD/GROMOS coupling scheme.^{21–23} These dynamical calculations are based on ab initio molecular dynamics (AIMD)²³ using the efficient Car–Parrinello²⁴ propagation scheme as implemented in the CPMD program package²⁵. For the QM system, the BLYP density functional^{26,27} and a plane wave basis set with a cutoff of 70 Ry together with normconserving pseudopotentials²⁸ was utilized in a nonperiodic cubic box of 14 Å. The MM system consisted of 70 water molecules and an OH[−] ion represented by the SPC force field.⁵ These simulations were performed in the canonical ensemble at 300 K using Nosé–Hoover chain thermostats⁶ in order to control the kinetic energy of the nuclei as well as the fictitious kinetic energy of the orbitals. An AIMD time step of 6 a.u. (≈ 0.14 fs) was used for the integration of the Car–Parrinello equations of motion using a fictitious mass parameter for the orbitals of $\mu = 700$ a.u. together with the atomic masses of all hydrogens substituted by deuterium masses to allow for larger fictitious orbital mass and thus a larger time step resulting into more efficient AIMD propagation.²³ For each force value, the system was equilibrated initially for 2 ps followed by production runs of 200 ps for analyses.

C. QM Simulations on Disulfide Macrocycle

In previous experiments²⁹, chemically engineered macrocycles, so-called “molecular force probes”,^{30,31} have been used in order to generate internal mechanical stress that strains the disulfide bond that is embedded in the macrocycle.¹⁹ For such systems the tensile force corresponding to the produced strain has been extracted by a validated approach.^{29,31}

In order to compute the corresponding C _{α} –C _{α} distance at room temperature, an AIMD²³ simulation of an isolated disulfide macrocycle (macrocycle 9²⁹, has been performed. Importantly, no external force must be applied to the macrocycle, where strain is generated purely intramolecularly due to the photoswitch, stiff stilbene, being in the cis/trans conformation, see Fig. 1. These AIMD simulations have been conducted as explained in the previous section using a 20 Å cubic box, proper hydrogen masses, and thus a timestep of 3 a.u. with $\mu = 400$ a.u. was set to be a cubic box of 20 Å. Again, the system was initially equilibrated for 2 ps followed by 120 ps for computing the average C _{α} –C _{α} distance.

II. SUPPLEMENTARY FIGURES

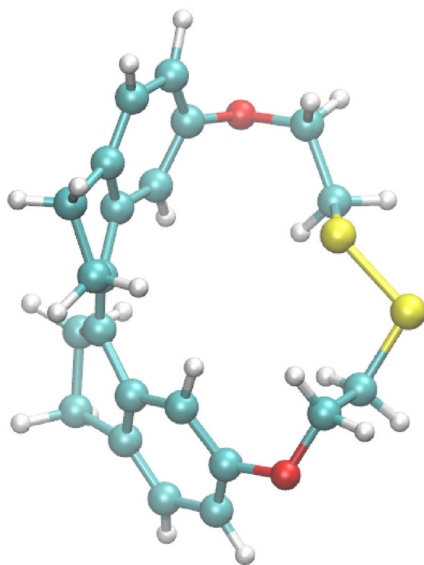


FIG. 1: Macrocycle²⁹ with the photoswitch, stiff stilbene, in its trans conformation leading to a strained disulfide bond.

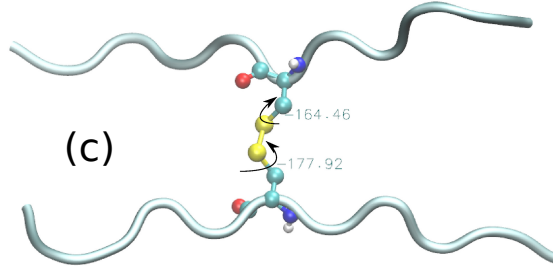
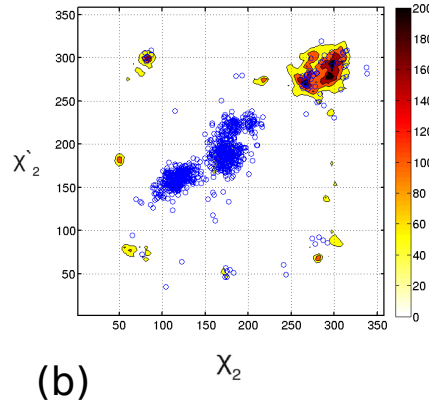
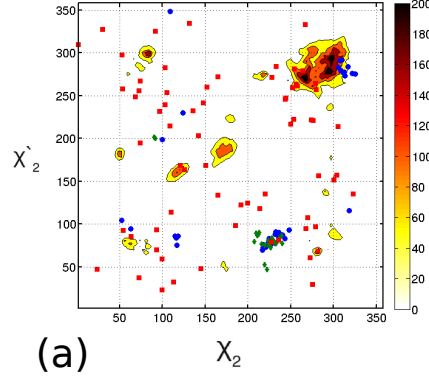


FIG. 2: (a) Probability distribution function $P(\chi_2, \chi'_2)$ obtained from analyzing the X-ray crystal structures of disulfides in the Protein Data Bank (101087 observations) shown by contour lines with superimposed scatter plot of the conformational states of disulfide bonds in TDi proteins (40 observations) in blue circles, DO proteins (27 observations) as green diamonds and interchain Ig proteins (69 observations) as red squares. (b) Same as panel a but superimposing the scatter plot (circles) of the conformational states of disulfide bonds in intrachain Ig proteins (927 observations) showing the preference of closed/closed conformations, i.e. $\chi_2/\chi'_2 \approx 180^\circ/180^\circ$. (c) Configuration of a representative interstrand disulfide bond (CYS A 132 – CYS A 192) in intact IgG1 monoclonal antibody (PDB ID 1IGY), which shows a closed/closed conformation that is typical to the preferred ones in panel b. The bridge is strained as a result of being suspended between the two strands shown in tube representation resulting into $\chi_2/\chi'_2 \approx 165^\circ/178^\circ$.

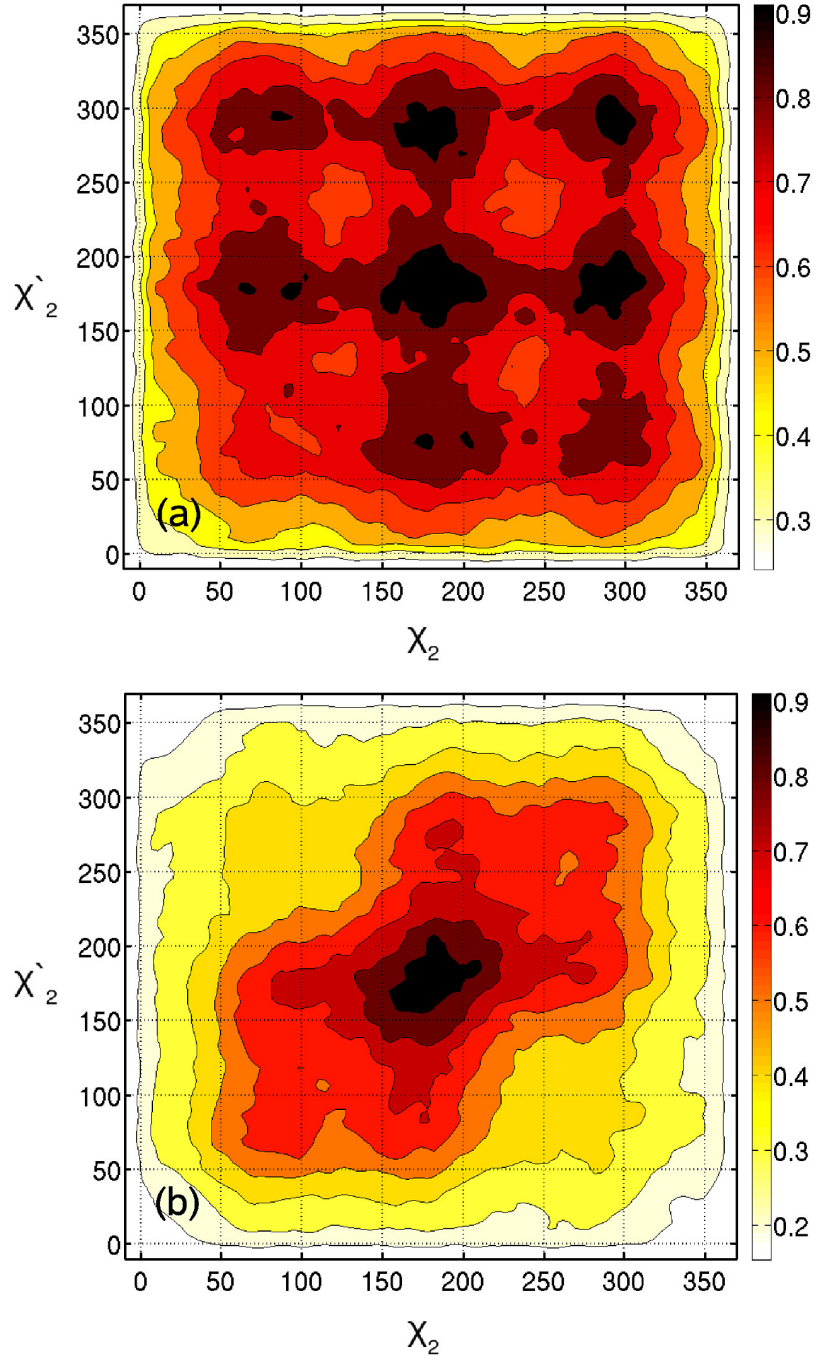


FIG. 3: Probability distribution functions $P(\chi_2, \chi'_2)$ obtained from metadynamics simulations for DEDS at zero force and 0.3 nN in panels a and b, respectively.

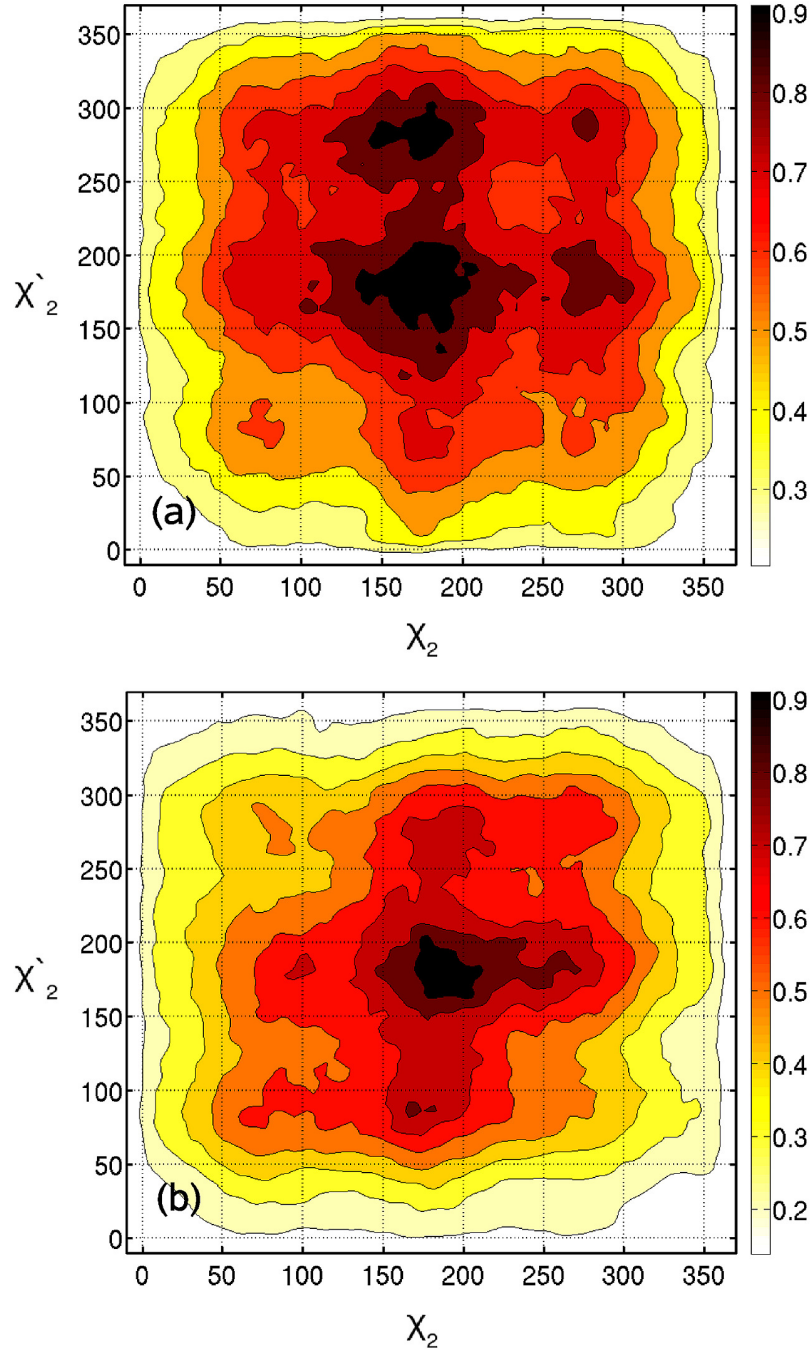


FIG. 4: Probability distribution functions $P(\chi_2, \chi'_2)$ obtained from metadynamics simulations for cystine at zero force and 0.3 nN in panels a and b, respectively.

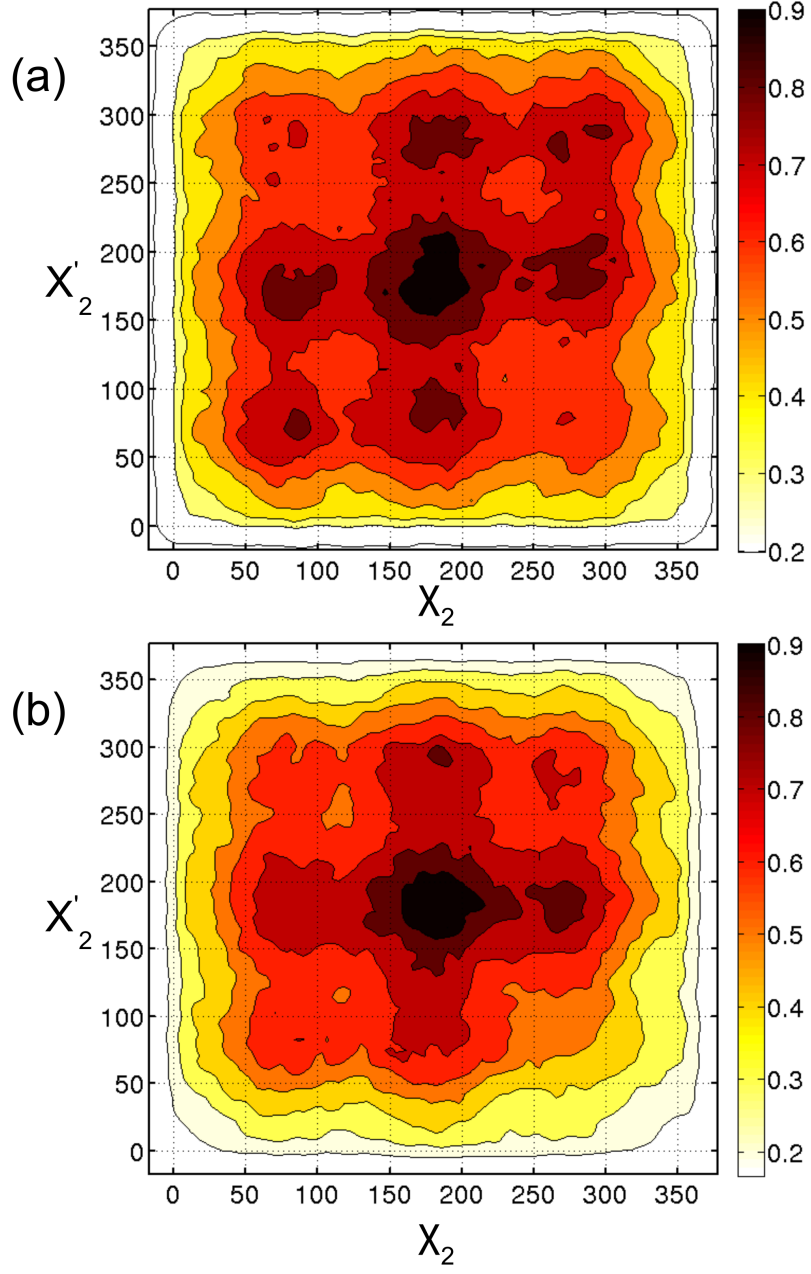


FIG. 5: Probability distribution functions $P(\chi_2, \chi'_2)$ obtained from metadynamics simulations for DEDS and cystine at 0.1 nN force in panels a and b, respectively.

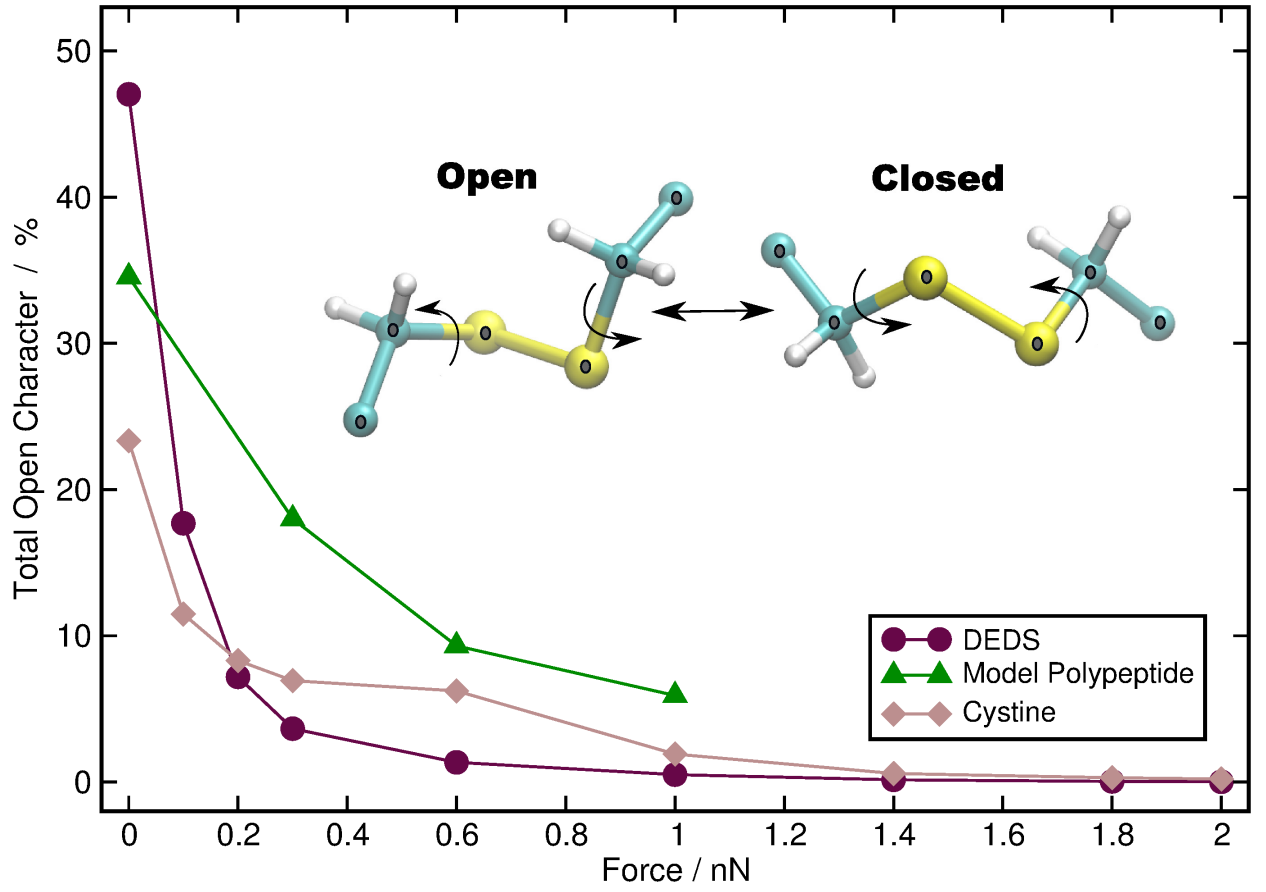


FIG. 6: Total open character according to the χ_2 and χ'_2 dihedral angles (see text) for the model polypeptide, cystine and DEDS as a function of constant external force. The inset shows the closed–closed (the right picture, χ_2 and χ'_2 in the range $180 \pm 50^\circ$) and the open–open conformation (the left picture, χ_2 and χ'_2 all other values for the dihedral apart from $180 \pm 50^\circ$) in these dihedrals. These data have been extracted from 150, 300, 1300 ns of zero force and force–clamp MD simulations performed on DEDS, cystine, and the model polypeptide, respectively.

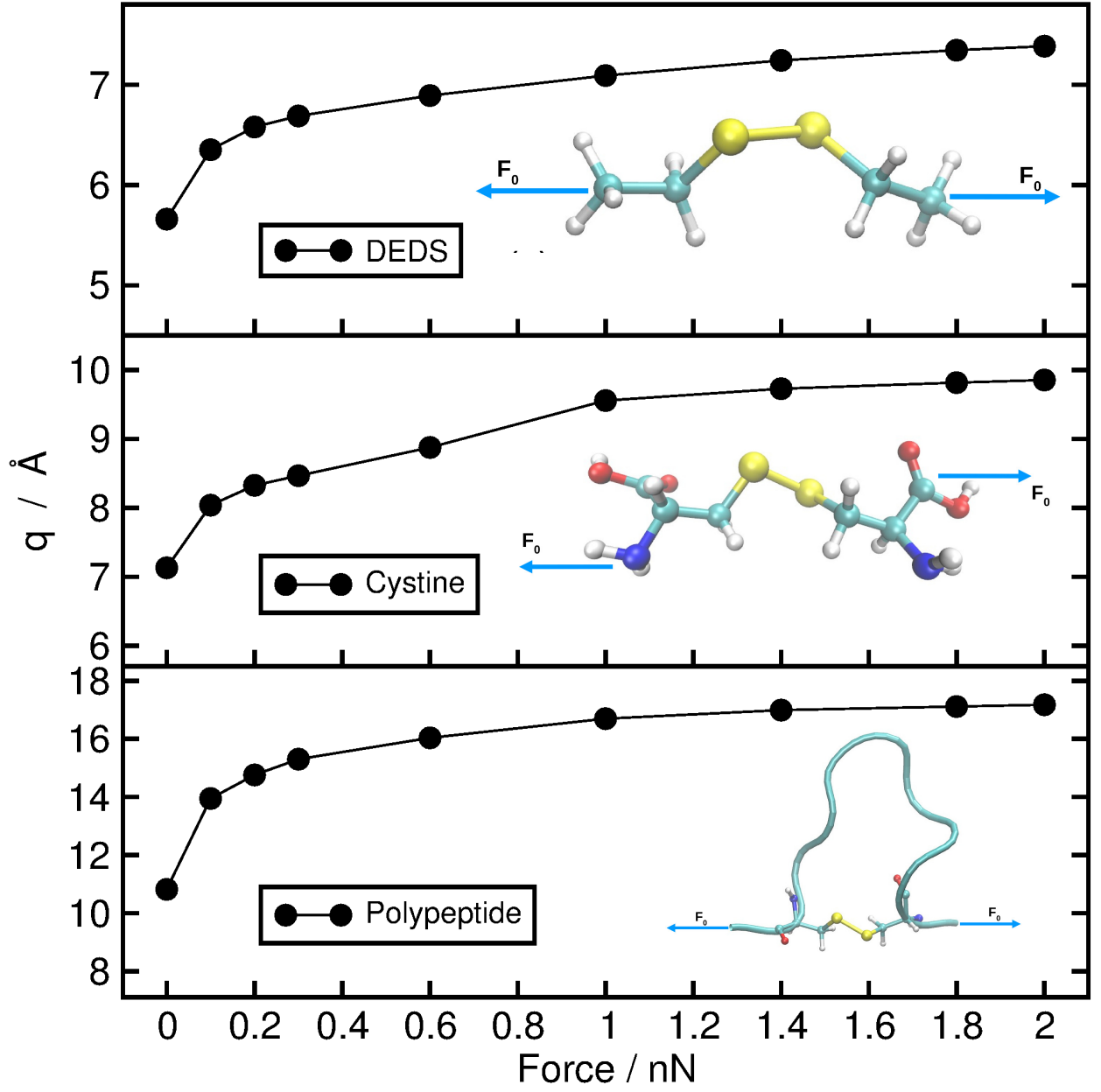


FIG. 7: Mechanical coordinate, q , for the model polypeptide, cystine and DEDS as a function of constant external force.

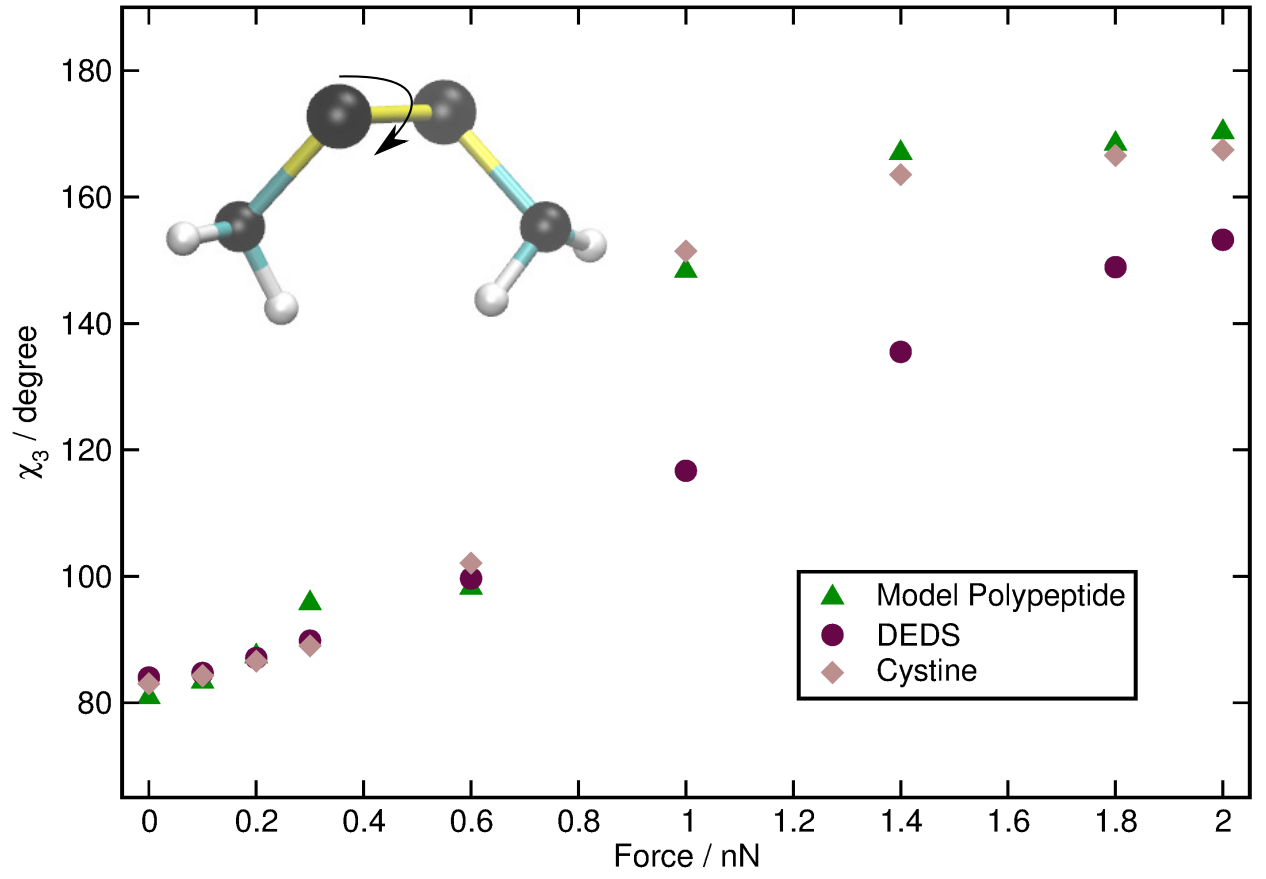


FIG. 8: Central disulfide dihedral angle C-S-S-C, χ_3 , for the model polypeptide, cystine and DEDS as a function of constant external force; the inset visualizes the respective torsional degree of freedom. Note that this behavior, which is very similar for all three systems, is similar to the one found earlier for a cystine molecule.³²

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