# Mechanical force can fine-tune redox potentials of disulfide bonds
Supporting Information

Ilona B. Baldus
Molecular Biomechanics
Heidelberg Insitute for Theoretical Studies,
Heidelberg, Germany

Frauke Gräter [1]
Molecular Biomechanics
Heidelberg Insitute for Theoretical Studies,
Heidelberg, Germany
and
MPG-CAS Partner Institute and
Key Laboratory for Computational Biology,
Shanghai, China

[1]Corresponding author. Address: Molecular Biomechanics Heidelberg Insitute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany, Tel.: (+49)6221-533267, Fax: (+49)6221-533298 and MPG-CAS Partner Institute and Key Laboratory for Computational Biology; 320 Yueyang Lu, Shanghai, 200031, China.
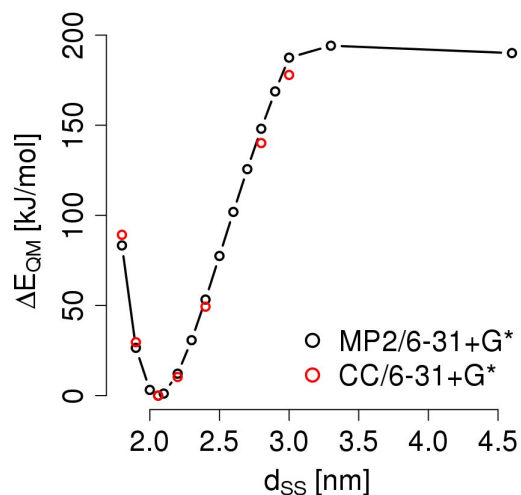
Supporting Figure 1: Restrained optimization of cystine with pure QM, using MP2/6-31+G* *in vacuo*. The bond length was elongated stepwise, then optimization was performed, while freezing the position of both disulfide atoms. As expected, the dissociation of the disulfide is described by a Morse-like potential. Contrary to our pulling simulations, restricting the disulfide bond length allows the ends of the molecule to collapse. QM/MM calculations using the MP2/6-31+G* level of theory is a widely used method, which, however requires validation for the disulfide system used here. To validate that the method is appropriate, we calculated the single point energies for the disulfide bond elongation, using coupled cluster (CC), and conclude that using MP2 is of sufficient accuracy. For better comparison, we set both minimum energy values to zero. See Supporting Table 1 for details.

Supporting Table 1: Absolute electronic energies, E_{elec}, of optimized cystine at a given sulphur-sulphur distance. Energies were optained from restrained optimization: The sulphur-sulphur bond length was elongated stepwise, while freezing the positions of the sulphur atoms. Compare Supporting Figure 1

| d_{SS} [nm]	E_{elec} MP2/6-31+G* [kJ/mol]	CC/6-31+G* [kJ/mol]
1.80	-3779832	-3780049
1.90	-3779889	-3780108
2.00	-3779912	
2.06	-3779916	-3780138
2.10	-3779914	
2.20	-3779903	-3780128
2.30	-3779885	
2.40	-3779862	-3780089
2.50	-3779838	
2.60	-3779814	
2.70	-3779790	
2.80	-3779767	-3779998
2.90	-3779747	
3.00	-3779728	-3779960
3.30	-3779721	
4.60	-3779725	
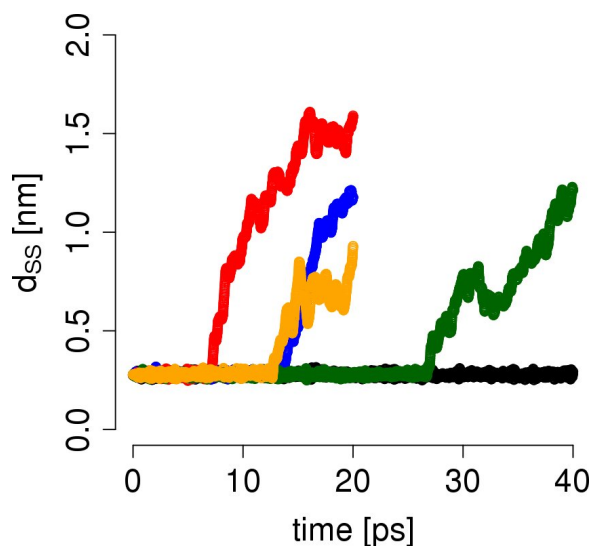
Supporting Figure 2: The radical anion of cystine opens in four out of five independet trajectories at $F = 0$ and at $T = 300\,\mathrm{K}$, resulting in a thiolate anion and a thiyl radical. As we find the open state to be the equilibrium state, it is independent of force. Thus, its energy can be accounted as a constant and the relative changes of the redox potential by force are independent from the energy of the reduced state.

We considered three different products as possible reduced state: The doubly protonated reduced state, as shown in Figure 1 in the main paper, the protonated radical and the radical anion. We found all three possible product states to open at ambient conditions, and thus being independent of force. Supporting Table 2 shows sulphur-sulphur distances optained from energy minimization at different levels of theories of the protonated radical. Any level of theory higher than PM3 shows that the sulphur-sulphur bond opens. Supporting Figure 2 shows the disulfide bond length in the anion radical in dependence of time. The bond opened in four out of five times within 40 ps.

Supporting Table 2: Optimization of the protonated radical, that is now neutral in charge, shows dissociation for any level of theory higher than PM3

method	d_{SS} [Å]
AM1	1.99
PM3	1.95
UB3LYP/6-31+G*	3.19
UMP2/6-31+G*	3.93
QM/MM	3.25
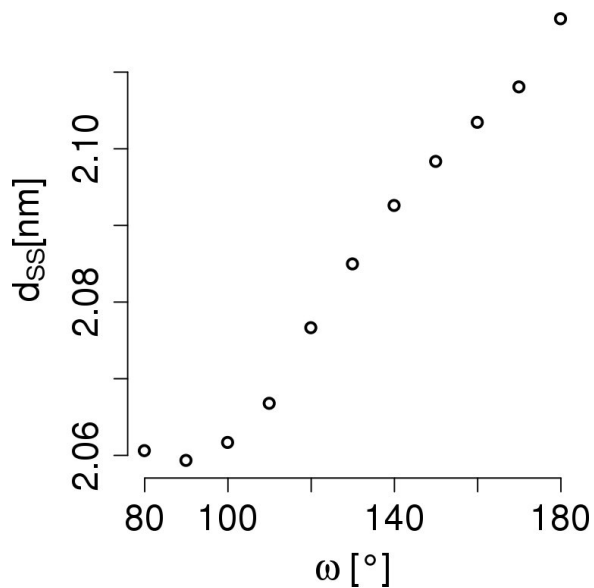
Supporting Figure 3: Restrained optimization of cystine with pure QM, using MP2/6-31+G* *in vacuo*. Here, the dihedral angle enclosing the disulfide bond was enlarged stepwise and restrained while optimization was performed. In accordance with our QM/MM calculations, the disulfide bond length shows a minimum for $\omega > \omega_{min}$.

Supporting Table 3: Absolute electronic energies, E_{elec}, of cystine at different forces, simulated at ambient conditions ($T = 300\,\mathrm{K}$, $p = 1\,\mathrm{bar}$)

Force [pN]	E_{elec} [kJ/mol]
0	-2295386 $\pm$ 3.515647
30	-2295395 $\pm$ 4.617036
50	-2295398 $\pm$ 5.113785
100	-2295404 $\pm$ 4.845556
166	-2295382 $\pm$ 3.387290
332	-2295381 $\pm$ 4.375979
498	-2295372 $\pm$ 4.905127
664	-2295378 $\pm$ 1.929652
830	-2295374 $\pm$ 3.513925
1162	-2295372 $\pm$ 4.914236
1660	-2295359 $\pm$ 3.316494
2490	-2295338 $\pm$ 3.358569
3320	-2295292 $\pm$ 5.296942