

Supporting Information:

Single-molecule force spectroscopy measurements of bond elongation during a bimolecular reaction

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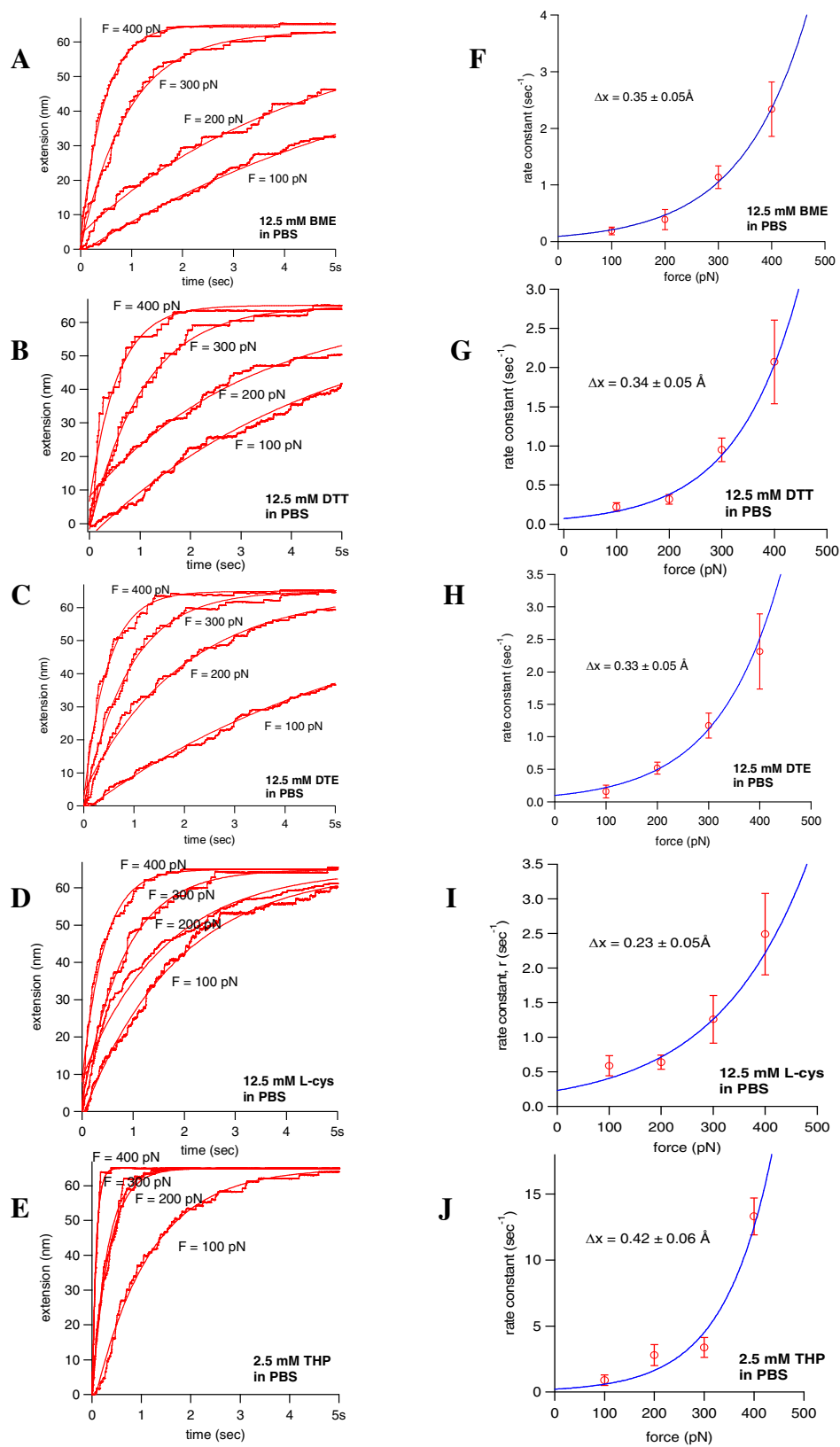


Figure S1: Force-dependent disulfide bond reduction rate constants are obtained by fitting the averaged extension versus time traces with a single-exponential function (A to E) and the distance to the TS (Δx) is obtained using an Arrhenius model (F to J).

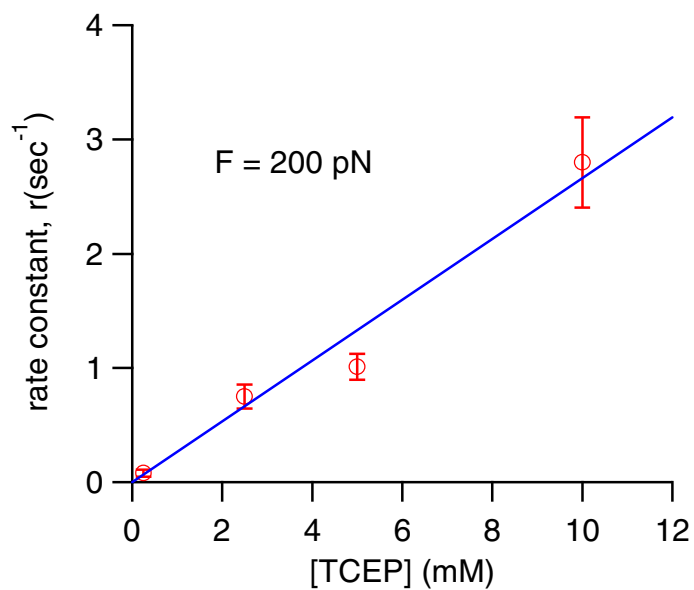


Figure S2: The rate of the disulfide bond reduction is first-order dependent on [TCEP]. Plot of the rate constant, $r(F)$ as a function of [TCEP] at $F = 200$ pN. The solid line is a linear fit to the data. The linear (first-order) dependence on [TCEP] demonstrates that the disulfide reduction by TCEP is a bimolecular reaction.

Results of the quantum chemical calculations

Table S1. Energies (Hartrees)

	B3LYP/ 6-31G(d)	zpv ^a B3LYP/ 6-31G(d)
<i>Reaction 1</i>		
Reactant state (1)	-1620.614764	0.226254
TS (2)	-1620.568993	0.223524
Product state (3)	-1620.614764	0.226254
<i>Reaction 2</i>		
Reactant state (4)	-1564.375741	0.234035
TS (5)	-1564.305902	0.230551
Product state (6)	-1564.346445	0.235443
<i>SCRF-calculations</i>		
Reactant. reaction 1	-1620.639003	
TS reaction 1	-1620.596305	
Reactant. reaction 2	-1564.399646	
TS reaction 2	-1564.340777	

a) Zero point vibrational energy

Table S2. Cartesian coordinates for structure 1.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	-4.381877	0.353680	0.952039
2	6	0	-1.465489	-2.029988	0.887006
3	1	0	-3.852047	1.295355	0.791232
4	1	0	-0.378354	-2.106743	0.798428
5	16	0	-4.027431	-0.832373	-0.406650
6	16	0	-1.938880	-0.892991	-0.471213
7	1	0	-1.935517	-3.003613	0.735346
8	1	0	-5.461981	0.529672	0.922760
9	16	0	4.739716	-0.738239	-0.614607
10	6	0	4.829527	-0.761752	1.222788
11	1	0	5.795158	-0.330967	1.497566
12	1	0	4.027659	-0.156329	1.650174
13	1	0	4.776835	-1.784383	1.602542
14	8	0	1.298066	-1.130294	-0.524034
15	1	0	1.042228	-0.989061	-1.448038
16	1	0	3.474541	-1.222605	-0.705245
17	8	0	-1.726339	2.431225	-0.151796

18	1	0	-2.115565	2.575693	-1.028933
19	8	0	2.217443	1.266603	0.378269
20	1	0	1.765696	2.122563	0.167732
21	1	0	3.048734	1.265135	-0.127302
22	8	0	0.805278	3.557211	-0.126384
23	1	0	-0.118006	3.207425	-0.130822
24	1	0	0.855561	4.094493	0.678515
25	1	0	1.502770	-0.221706	-0.178936
26	1	0	-1.593353	1.460563	-0.110269
27	1	0	-1.740576	-1.619291	1.860850
28	1	0	-4.116796	-0.068158	1.924490

Table S3. Cartesian coordinates for structure 2.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	-3.690187	0.731125	0.449564
2	6	0	-1.739219	-2.204199	-0.617486
3	1	0	-3.286892	0.677102	1.465844
4	1	0	-1.150585	-2.903924	-1.213668
5	16	0	-2.357228	0.988988	-0.790284
6	16	0	-0.686242	-0.771260	-0.140734
7	1	0	-2.568445	-1.820696	-1.213318
8	1	0	-4.387893	1.572562	0.394197
9	16	0	1.143460	-2.313971	0.581620
10	6	0	1.745836	-1.195581	1.901205
11	1	0	1.025131	-1.141674	2.723693
12	1	0	2.690145	-1.585659	2.292447
13	1	0	1.930340	-0.181347	1.522363
14	8	0	2.029057	-0.621647	-1.912276
15	1	0	1.060543	-0.478073	-1.936251
16	1	0	2.075977	-1.307329	-1.188437
17	8	0	-0.430331	2.059878	1.325805
18	1	0	-1.094000	1.963061	0.559390
19	8	0	3.053298	1.544950	-1.094057
20	1	0	2.351671	2.179577	-0.243366
21	1	0	3.195050	2.078492	-1.892236
22	8	0	1.796933	2.802743	0.551203
23	1	0	0.812974	2.452418	0.857554
24	1	0	2.351633	2.883746	1.343910
25	1	0	2.641098	0.635304	-1.411960
26	1	0	-0.387711	1.139948	1.650364
27	1	0	-2.108144	-2.709361	0.276797
28	1	0	-4.240954	-0.189707	0.237940

Table S4. Cartesian coordinates for structure **3**.
Identical to structure 1.

Table S5. Cartesian coordinates for structure **4**.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	4.434971	-0.974534	0.548354
2	6	0	0.756753	-1.854172	0.722004
3	1	0	4.885499	-1.841841	0.061482
4	1	0	0.265622	-0.891158	0.877435
5	16	0	3.114939	-0.265485	-0.512519
6	16	0	1.792232	-1.850708	-0.794186
7	1	0	1.343926	-2.137727	1.599320
8	1	0	5.188550	-0.188948	0.665034
9	6	0	-5.127050	-1.757226	-0.550727
10	1	0	-5.555183	-2.612813	-0.018745
11	1	0	-5.836117	-0.926055	-0.511213
12	1	0	-4.991584	-2.053137	-1.595854
13	8	0	-2.297902	2.480276	-0.440197
14	1	0	-0.014579	4.595015	-0.945437
15	8	0	0.080408	3.650501	-1.137654
16	1	0	-0.828073	3.272896	-1.010613
17	1	0	0.973573	2.871586	0.144476
18	8	0	1.298277	2.352844	0.927707
19	1	0	1.892604	1.677541	0.552349
20	1	0	-0.089932	1.558125	1.362174
21	1	0	-2.967289	3.055144	-0.041024
22	1	0	-0.013251	-2.612120	0.544928
23	1	0	4.049957	-1.251274	1.532460
24	15	0	-3.447370	-1.356278	0.184208
25	1	0	-3.249171	-0.101970	-0.455010
26	1	0	-3.912829	-0.767589	1.397056
27	1	0	-1.931830	1.948315	0.314691
28	1	0	-1.173421	1.251504	2.453973
29	8	0	-0.984371	1.125547	1.512504

Table S6. Cartesian coordinates for structure **5**.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z

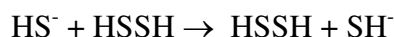
1	6	0	4.088942	-0.719393	0.297354
2	6	0	0.453565	-1.798736	1.331650
3	1	0	4.469893	-1.730756	0.124898
4	1	0	0.123176	-1.003808	2.001063
5	16	0	2.703362	-0.363701	-0.863651
6	16	0	-0.045589	-1.363108	-0.386884
7	1	0	1.541690	-1.839267	1.287876
8	1	0	4.909896	-0.013511	0.132898
9	6	0	-3.088568	-2.112911	-1.052325
10	1	0	-2.916610	-3.157154	-0.777751
11	1	0	-4.159695	-1.890887	-0.994158
12	1	0	-2.755867	-1.971322	-2.085436
13	8	0	-2.272212	2.546257	-0.058863
14	1	0	0.232204	4.377617	-0.538035
15	8	0	0.118092	3.498656	-0.928876
16	1	0	-0.822130	3.245317	-0.724927
17	1	0	0.958139	2.350970	0.016360
18	8	0	1.232480	1.635520	0.664265
19	1	0	1.835437	0.835686	0.052161
20	1	0	0.322141	1.251131	1.025857
21	1	0	-2.848916	3.156745	0.423927
22	1	0	0.055573	-2.773649	1.622205
23	1	0	3.778425	-0.638087	1.345358
24	15	0	-2.118301	-0.996777	0.046745
25	1	0	-2.552591	0.280132	-0.329545
26	1	0	-2.625938	-1.275630	1.325144
27	1	0	-1.824152	1.951642	0.650743
28	1	0	-1.203763	0.970507	2.389831
29	8	0	-1.077161	0.849713	1.435829

Table S7. Cartesian coordinates for structure **6**.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	3.147803	-2.059593	-0.390769
2	6	0	-2.108301	0.176017	2.222452
3	1	0	3.326291	-2.603168	-1.321715
4	1	0	-2.453790	1.207852	2.310972
5	16	0	1.374180	-2.289005	0.043105
6	16	0	-2.572987	-0.575294	0.614500
7	1	0	-1.035882	0.105647	2.401273
8	1	0	3.374364	-1.001231	-0.534956

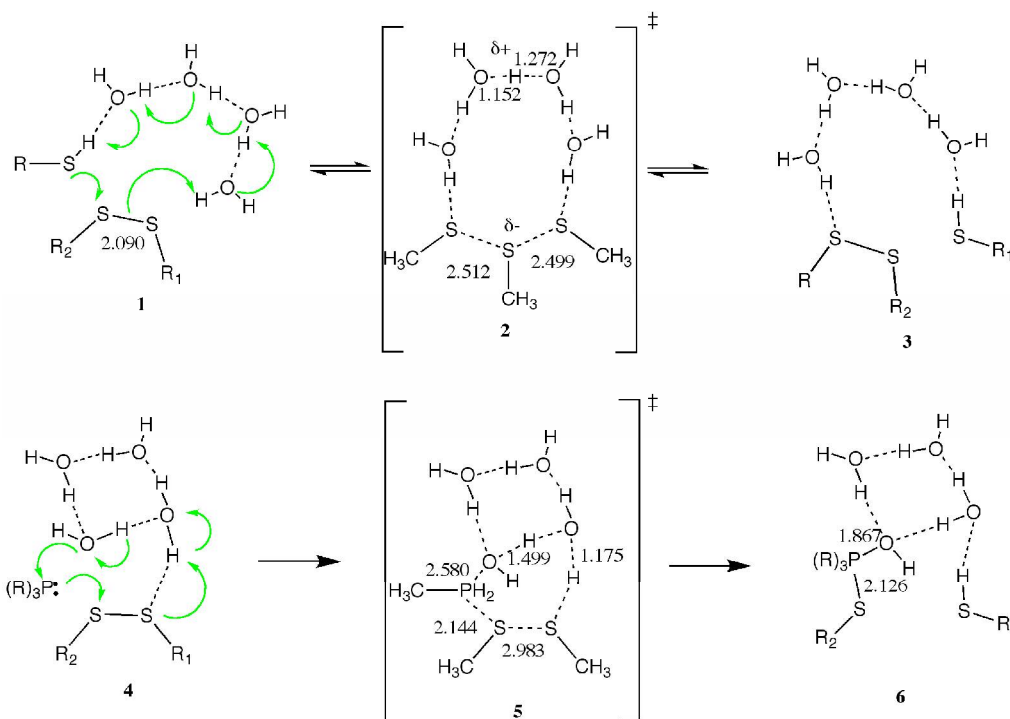
9	6	0	-2.120192	-0.967517	-2.165867
10	1	0	-3.213581	-1.021598	-2.200185
11	1	0	-1.757171	-0.689654	-3.164054
12	1	0	-1.717202	-1.952906	-1.913753
13	8	0	1.364007	2.854517	-1.169844
14	1	0	3.991366	2.017345	0.458112
15	8	0	3.316177	1.404930	0.131778
16	1	0	2.709933	1.955404	-0.422853
17	1	0	2.092203	0.823725	1.413831
18	8	0	1.249194	0.577666	1.855021
19	1	0	1.296255	-1.263964	0.938028
20	1	0	0.575501	1.031982	1.311959
21	1	0	1.366319	3.806656	-0.992408
22	1	0	-2.639014	-0.432169	2.961406
23	1	0	3.793851	-2.472125	0.388544
24	15	0	-1.491960	0.343104	-0.969288
25	1	0	-0.137744	0.067543	-1.220798
26	1	0	-2.072490	1.404733	-1.700802
27	1	0	0.570335	2.503930	-0.694040
28	1	0	-1.423256	2.266943	0.493406
29	8	0	-0.754606	1.667118	0.121891

The simplest conceivable model for the reduction of a protein disulfide bond is the gas phase reaction



It is known that the substitution reaction in the gas phase does not go via a high energy TS, but results via the highly stable symmetrical trisulfide anion, $[\text{HSS}(\text{H})\text{SH}]^-$ as an intermediate.¹ Mainly for this reason, a gas phase model is incomplete, and a realistic TS model must take the effect of solvation explicitly into consideration. We follow Fernandes and Ramos² by adding four water molecules into the supermolecule. We also consider including methyl groups to represent a more realistic representation of the first reaction in question. However, we did not make any *a priori* assumption about whether the attacking thiol is in the protonated or deprotonated form. In the reaction model for the disulfide bond reduction by thiols (reaction 1, main text), the explicit incorporation of water allows for effective deprotonation of the sulfur in the TS, thereby enhancing the inherent nucleophilicity. It must be noted that the largest amplitude of all atom of the reaction coordinate is the hydrogen in the 12 o'clock position (Figure S3 and upper center of Scheme S1). The computed barrier height (including zero point vibrational energy) is 113 kJ/mol. When we impose a dielectric continuum to the supermolecular system, in the form of a SCRF calculation in water, this is reduced to 105 kJ/mol. Neither of these quantities should be equivalent in an absolute sense to the empirical Arrhenius activation energy obtained from the treatment of the experimental data. The reaction mechanism for the phosphine-based disulfide reduction is shown in reaction 2 (main text) and Scheme 1 (lower part). Also for this reaction proton transfer to the departing sulfur is a necessary

requirement for reaction. However, this proton does not stem from the phosphorous which formally neutral during the whole reaction sequence. Instead, it is a by-product of the oxidation which takes place in concert with the S_N2 displacement. The lower part of Scheme S1 displays the mechanism, as calculated with B3LYP/6-31G(d). The barrier height is computed to be 174 kJ/mol. The SCRf calculations reduce this value to 153 kJ/mol. We assume that the product of this reaction (**6**) is easily hydrolyzed to the phosphine oxide $(CH_3)_2H_2PO$, although no attempt was made to model this end of the reaction.



Scheme S1. Reaction mechanisms. $R = R_1 = R_2 = CH_3$. Bond distances are in Å units.

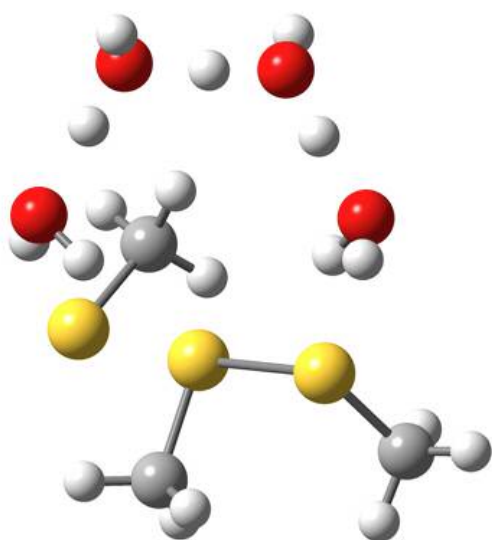


Figure S3. TS for reduction by thiol

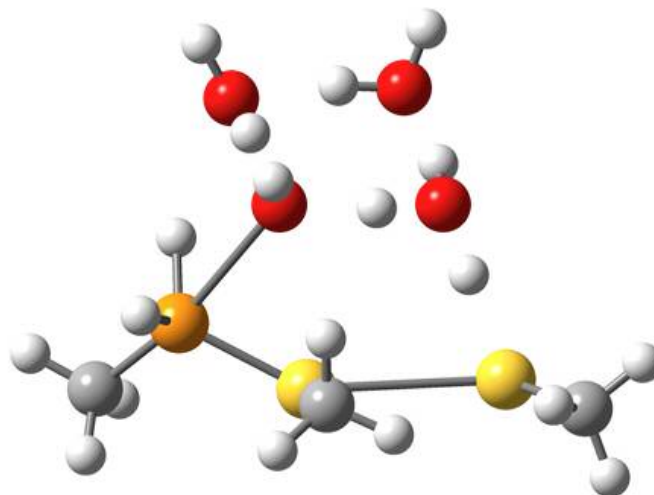


Figure S4. TS for reduction by phosphine

Disulfide bond reduction in glycerol/water mixture

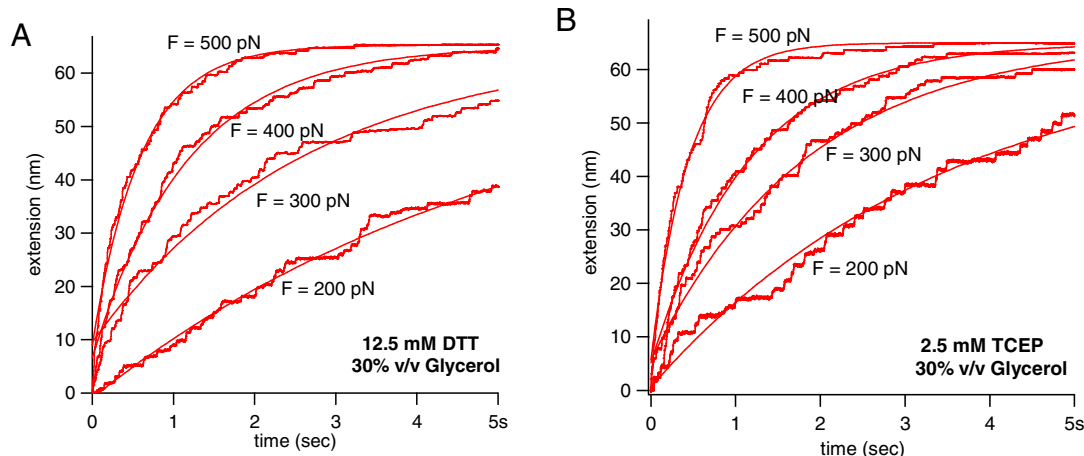


Figure S5. Force-dependent disulfide reduction kinetics in 30% v/v glycerol containing PBS obtained in (A) 12.5 mM DTT and (B) 2.5 mM TCEP. Each extension versus time curve is an average of stage 2 (see Figure 1) from many single-molecule recordings (10-50) containing disulfide reduction events.

Reference 24 in the main text: Frisch, M. J., G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. Montgomery, J. A., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Gaussian 03, Revision C.02*. Gaussian, Inc.: Wallingford CT, 2004.

References

1. Pappas, J. A., *J. Am. Chem. Soc.* **1977**, *99*, 2926-2930.
2. Fernandes, P. A.; Ramos, M. J., *Chemistry* **2004**, *10*, 257-66.