How the disulfide conformation determines the disulfide/thiol redox potential

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Protein disulfides can adopt a wide variety of conformations, each having different energies. Limited experimental data suggest that disulfides adopting a high energy have an enhanced likelihood for reduction, but the exact nature of this relation is not clear. Using a computational approach, we give insight on the conformational dependence of the redox behavior of the disulfide bond, which relates structure to reactivity. The relative energy of different conformations of the diethyl disulfide model system correlates with the disulfide/thiol redox potential $E^\circ$. Insight in the calculated redox potentials is obtained via quantitative molecular orbital theory, and via the decomposition of $E^\circ$ into a vertical electron affinity and a subsequent reorganization term. We have identified the determinants of the disulfide conformational energies and characterized the barrier to rotation around the disulfide bond. Our findings on the diethyl disulfide model system can be transferred to examples from the Protein Data Base. In conclusion, strained disulfide conformations with a high conformational energy have a large tendency to be reduced. Upon reduction, unfavorable interactions are released. This explains why reorganization effects and not a higher tendency to accept electrons account for the high reduction potential of high-energy disulfides.

Keywords: disulfide; conformation; quantitative MO theory; disulfide/thiol redox potential; structure–reactivity relations

Introduction

Disulfides are among the most important bonds in proteins (Antonello, Benassi, Gavioli, Taddei, & Maran, 2002; Benassi & Taddei, 1998; Depuydt, Messens, & Collet, 2011; Gamez, Serrano-Andres, & Yanez, 2010a; Gamez, Serrano-Andres, & Yanez, 2010b; Rickard, Berges, Houee-Levin, & Rauk, 2008). They not only stabilize protein structures, but they are also central players in essential thiol-disulfide pathways (Depuydt et al., 2011; Marino & Gladyshev, 2012). Insight in disulfide/thiol redox potentials is of pivotal importance, for example to understand the specificity and mechanism of thiol-disulfide switches in cellular redox pathways. In the thioredoxin (Trx) superfamily, it is well documented that the disulfide reduction potential is related to the $pK_a$ of the nucleophilic cysteine (Roos, Foppe, & Messens, 2013). This was shown for Trx active site mutants and disulfide binding protein A (DsbA) for which a linear correlation between $pK_a$ and reduction potential was found (Huber-Wunderlich & Glockshuber, 1998; Roos et al., 2007). Empirical relations between the cysteine $pK_a$ and the disulfide/thiol reduction potential have been proposed for Trx-type oxidoreductases (Mossner, Iwai, & Glockshuber, 2000).

However, also non-pH dependent factors as intramolecular strain and conformational space due to the protein chain are suggested to influence the disulfide/thiol redox potential (Wouters, Fan, & Haworth, 2010). Protein disulfides can adopt a wide variety of conformations, each having different energies. Limited experimental data suggest that disulfides adopting strained conformations have an enhanced likelihood for reduction to dithiol (Wouters, George, & Haworth, 2007), but the exact nature of this relation is not clear. For example, disulfides bridging $\beta$-sheets (cross strand disulfides) have recently been categorized as ‘forbidden disulfides’. This concept was introduced to identify disulfides that disobey the ‘rules’ defining the constraints imposed by protein structure on disulfide formation (Wouters et al., 2010). These forbidden disulfides are suggested to adopt strained conformations necessary for their function as redox switches (Haworth, Feng, & Wouters, 2006; Wouters et al., 2010).
We will investigate if the disulfide/thiol redox potential ($E^\circ$) can be related to the disulfide conformation and how the redox potential exactly depends on disulfide conformation. To this end, the conformational dependence of calculated $E^\circ$ values will be scrutinized via quantitative molecular orbital (MO) theory and via the decomposition of $E^\circ$ into a vertical electron affinity and a subsequent geometry reorganization term, in analogy with earlier work (Roos, De Proft, & Geerlings, 2013). We will identify the structural determinants of the disulfide energies and characterize the barrier to rotation around the S–S bond. As such, insight in non-pH dependent factors determining the disulfide redox potential will be given.

**Molecular models and methods**

Diethyl disulfide with different conformations (Supplementary Table S1) are fully optimized (R) or fully optimized with fixed dihedral angles (F) at the MP2/6-31+G(d,p) level Gaussian09 (Frisch et al., 2009). All subsequent energy calculations are performed at the MP2/6–311++G(d,p) level. The MP2 level of theory has been shown to perform well for describing electron capture by disulfides in among other studies (Gamez et al., 2010a; Gamez et al., 2010b; Roos, De Proft et al., 2013). If indicated, the IEF-PCM model with UFF radii and the default cavity in Gaussian03 at the hf/6-31+G(d) level (Frisch et al., 2009) was used for simulating aqueous solution. Redox potentials are calculated at the optimum geometry at the MP2/6-31++G(d,p) level Gaussian09 (Frisch et al., 2009). All subsequent energy calculations are performed at the MP2/6–311++G(d,p) level. The MP2 level of theory has been shown to perform well for describing electron capture by disulfides in among other studies (Gamez et al., 2010a; Gamez et al., 2010b; Roos, De Proft et al., 2013). If indicated, the IEF-PCM model with UFF radii and the default cavity in Gaussian09 (Frisch et al., 2009) was used for simulating aqueous solution. Redox potentials are calculated as described in (Billiet, Geerlings, Messens, & Roos, 2012; Ho, Klamt, & Coote, 2010; Roos, De Proft et al., 2013). Gas phase free energies $\Delta G_{\text{gas}}$ are calculated at the optimum geometry at the MP2/6–31+G(d,p) level from the vibrational frequencies, assuming that reaction species behave as an ideal gas within the rigid rotator harmonic oscillator approximation. Free energies of solvation $\Delta G_{\text{solv}}$ are calculated using the IEF-PCM solvent model with UAHF radii and the default cavity in Gaussian03 at the hf/6-31+G(d) level (Frisch et al., 2003).

The energy decomposition analysis (Bickelhaupt & Baerends, 2000; Bickelhaupt, Diefenbach, de Visser, de Koning, & Nibbering, 1998; Bickelhaupt, Nibbering, Vanwezenbeek, & Baerends, 1992) (see also Results section) of the barrier to rotation around the S–S bond in CH$_3$SSCH$_3$ was performed at the BLYP-D3(BJ)/TZ2P level of dispersion corrected density functional theory using the Amsterdam density functional (ADF) package (Baerends et al., 2012; te Velde et al., 2001). The geometry of CH$_3$SSCH$_3$ was optimized without symmetry at the MP2/6-31+G(d,p) level using Gaussian09 (Frisch et al., 2009). Cs symmetry of the CH$_3$S$^-$ fragments was enforced during geometry optimization by fixing appropriate bond lengths and angles.

**Results and discussion**

The conformation of diethyl disulfide CH$_3$CH$_2$S–SCH$_2$CH$_3$ can be characterized by the dihedral angels: $\chi_2$, $\chi_3$, and $\chi_2'$ (see Scheme 1). Based on the scatter plot of experimental $\chi_3$, $\chi_2$, and $\chi_2'$ angles (Haworth, Gready, George, & Wouters, 2007), we select a $\chi_3$ angle of 90° and generate three series of structures by internal rotation around the S$_2$–C$_6$ bond. The dihedral angels $\chi_2$, $\chi_3$ are kept frozen, while $\chi_2'$ is varied in steps (see (a) – (c)):

(a) (60, 90, 60) → (60, 90, −60) → (60, 90, −120) → (60, 90, 180)
(b) (−60, 90, 60) → (−60, 90, −25) → (−60, 90, −60)
(c) (180, 90, 180) → (180, 90, 120) → (180, 90, 75)
→ (180, 90, 0) → (180, 90, −65) → (180, 90, −120)

Note that the structure of CH$_3$CH$_2$S–SCH$_2$CH$_3$ is symmetrical with equivalent $\chi_2$ and $\chi_2'$. Therefore, the conformations ($\chi_2$, $\chi_3$, $\chi_2'$) and ($\chi_2'$, $\chi_3$, $\chi_2$) have the same energy. Apart from these conformations, some extra structures are considered in order to represent most of the experimentally found conformations (Wouters et al., 2007). The structures are fully optimized (R) or fully optimized with fixed dihedral angles (F) as neutral disulfides and as disulfide anions (See Material and Method section and Table S1). The (68,87,68)(R) conformation has the lowest energy and is denoted as the disulfide with the lowest energy conformation. Redox potentials $E^\circ$ (vs. the standard hydrogen electrode) for the disulfide/thiol reduction (reaction (1)) are calculated as described in (Ho et al., 2010; Billiet et al., 2012; Roos, De Proft et al., 2013) (see Table S2).

$$\text{CH}_3\text{CH}_2\text{S} + \text{SCH}_2\text{CH}_3 + 2e^- + 2\text{H}^+ \rightarrow 2\text{CH}_3\text{CH}_2\text{SH} \quad (1)$$

The standard reduction potentials ($E^\circ$) can be obtained via the Nernst equation:

$$E^\circ = -\frac{\Delta G^\circ}{nF} - E^\circ_{\text{SHE}} \quad (2)$$
with \( \Delta G^\circ \) the Gibbs free energy for reaction (1) in aqueous solution, \( E^\text{SHE} \) the standard hydrogen electrode potential, \( n \) the number of transferred electrons (here 2), and \( F \) the Faraday constant. For \( E^\text{SHE} \), a value of 4.47 V is used, compatible with the IEF-PCM solvent model (Ho, Coote, Cramer, & Truhlar, in press).

\[ \Delta G^\circ = \Delta G^\circ_{\text{gas}} + 2\Delta G^\circ_{\text{solv}, \text{CH}_2\text{SH}} - \Delta G^\circ_{\text{solv}, \text{CH}_2\text{SSCH}_2\text{CH}_3} - 2G^\circ (H^+, \text{aq}) + RT \ln \left( \frac{RT}{P} \right) \]

with \( G^\circ (H^+, \text{aq}) = 272.2 \text{ kcal/mol} \), obtained from (Tissandier et al., 1998). The \( RT \ln (RT/P) \) term makes the conversion between the gas phase standard state of 1 atm and the solution phase standard state of 1 mol/l.

**Disulfide/thiol reduction potential correlates with the disulfide conformational energy**

The decomposition of the global reduction process in reaction (1) into: (i) the reorganization of diethyl disulfide from the lowest energy conformation \((68,87,68)\) (R) with \( \Delta E_{\text{rel}} = 0 \) to the desired conformation \( \Delta E_{\text{rel}} \); (ii) the electron uptake \( \Delta E_{\text{EA}} \) and the reorganization to the anion geometry \( \Delta E_{\text{reorg}} \); and (iii) the dissociation of the disulfide anion by which two thiol molecules are formed (Antonello et al., 2002) gives insight (Roos, De Proft et al., 2013) into the relationship between the diethyl disulfide conformation and its ability to be reduced (see Figure 1 and Scheme S1).

\[ \Delta E_{\text{rel}} \] and the adiabatic electron capture \( \Delta E_{\text{adiab}} \) (which is the sum of \( \Delta E_{\text{EA}} \) and \( \Delta E_{\text{reorg}} \)) are different for each disulfide conformation. The disulfide anion dissociation via a three-step process (see reactions (6), (7), and (8) in Scheme S1) is the common part of the stepwise two-electron reduction process (Antonello et al., 2002). \( \Delta E_{\text{rel}} \) and \( \Delta E_{\text{adiab}} \) correlate with \( E^\circ \) with a positive and negative slope, respectively (Figure 2). This means that disulfide structures with a high conformational energy \( \Delta E_{\text{rel}} \) have a high \( E^\circ \) and thus a large tendency to be reduced. This is coupled to a high adiabatic electron affinity \( \Delta E_{\text{adiab}} \).

\[ \Delta E_{\text{EA}} \] nor \( \Delta E_{\text{reorg}} \) correlate with \( E^\circ \) (Figure 2). A low correlation is found between the relative anion energy \( \Delta E_{\text{reorg}}2 \) and \( E^\circ \) (see Figure S1).

![Figure 1. Disulfide/thiol reduction via different steps, according to Supplementary scheme S1. Reorganization of diethyl disulfide (RSSR) from the lowest energy conformation RSSR_{(ox,ox)\text{L}} to the considered conformation RSSR_{(ox,ox)\text{L}}, leading to \( \Delta E_{\text{rel}} \) in path A. In path B, no such reorganization takes place and thus the path of the reference disulfide having the lowest energy conformation is followed. 2 Electron uptake (RSSR_{(red,ox)}) and reorganization step to the anion geometry RSSR_{(red,red)\text{L}}, giving \( \Delta E_{\text{adiab}} \). \( \Delta E_{\text{EA}} \) is the vertical electron affinity: In path A: \( \Delta E_{\text{EA}} = E_{\text{opt}} \) (neutral) - \( E_{\text{opt}} \) (anion with same conformation as neutral), calculated for disulfides with a conformation different from the lowest energy conformation (68,87,68) (R). In path B: \( \Delta E_{\text{EA}} = E_{\text{opt}} \) (neutral) - \( E_{\text{opt}} \) (anion with same conformation as neutral), calculated for the disulfide adopting the lowest energy conformation (68,87,68)(R). Dissociation of the disulfide anion to dithiol 2RSH. Step 3 is the common part of the reduction process for each disulfide conformation and is not considered for analysis since this step does not explain trends in \( E^\circ \). \( \Delta E_{\text{rel}} \) and \( \Delta E_{\text{adiab}} \) correlate with \( E^\circ \). Subscript (left/right) refers to oxidation state/geometry; for example, red/ox = reduced form of the species having the geometry of the oxidized form. L: lowest energy conformation.](image-url)
Reorganization effects determine the disulfide/thiol reduction potential

Further insight in $E^\circ$ can be obtained from the analysis of $\Delta E_{\text{adiab}}$, which is the sum of $\Delta E_{\text{reorg}}$ and $\Delta E_{\text{EA}}$. $\Delta E_{\text{reorg}}$ ranges from $-34$ to $-44$ kcal/mol and contributes most to $\Delta E_{\text{adiab}}$. $\Delta E_{\text{EA}}$ accounts only for $-12$ to $-18$ kcal/mol (see Table S3). Compared to the reference structure with the lowest energy conformation, $\Delta E_{\text{reorg}}$ is up to $10$ kcal/mol more negative (purple bar towards negative side in Figure 3), while $\Delta E_{\text{EA}}$ is up to $6$ kcal/mol less negative (green bar towards positive side in Figure 3), for disulfides with high-energy conformations. This means that the large tendency of high-energy disulfides to be reduced originates from favorable reorganization effects upon reduction and not from an increased ability to take up an electron.

The disulfide conformations with $\chi^2$ (or $\chi^2'$) $\sim 120^\circ$ or $-120^\circ$ ((67,86,125) (R) and (−67,−86,125) (R)) are transition states for rotation around the disulfide bond, explaining their high conformational energy and high $E^\circ$.

In these structures, similar intramolecular interaction distances as in the reference structure with the lowest energy conformation are found (see Table S4). Accordingly, upon reduction, no unfavorable interactions need to be broken and $\Delta E_{\text{reorg}}$ is comparable to this of the reference structure (green circle in Figure 3).

Non-covalent interactions determine $\Delta E_{\text{rel}}$

Thus, we find that reorganization effects determine $E^\circ$. Insight in why reorganization effects determine $E^\circ$ and therefore in $\Delta E_{\text{rel}}$ can be obtained from the analysis of the disulfide bond formation from two CH$_3$CH$_2$S$^-$ fragments (see Eqs. (4)–(6)):

$$2\text{CH}_3\text{CH}_2\text{S}^- \rightarrow \text{CH}_3\text{CH}_2\text{S}^- \text{SCH}_2\text{CH}_3 \quad \Delta E_{\text{bind}} = \Delta E_{\text{rel}}$$

(4)

$$2\text{CH}_3\text{CH}_2\text{S}^- \rightarrow \text{CH}_3\text{CH}_2\text{S}^-_{\text{frag1}} + \text{CH}_3\text{CH}_2\text{S}^-_{\text{frag2}} \quad \Delta E_{\text{prep}}$$

(5)

Figure 2. $\Delta E_{\text{rel}}$ and $\Delta E_{\text{adiab}}$ correlate with $E^\circ$, but $\Delta E_{\text{reorg}}$ and $\Delta E_{\text{EA}}$ do not.

Figure 3. (a-b) Relative vertical electron affinity $\Delta E_{\text{EA}}$ (green) and relative reorganization energy $\Delta E_{\text{reorg}}$ (purple) in function of the relative energy $\Delta E_{\text{rel}}$ of the structures. The relative energies are calculated with the lowest energy conformation as reference ($\Delta E_{\text{relative}} = E - E_{\text{reference}}$) at the MP2/6–311++G(d,p) level in aqueous solution. b) Identification of different groups (See Table S4) – correspondence with Figure 4.
CH$_3$CH$_2$S$_{\text{frag1}}$ + CH$_3$CH$_2$S$_{\text{frag2}}$

\[ \rightarrow \text{CH}_3\text{CH}_2\text{S} - \text{SCH}_2\text{CH}_3(\text{ox/ox}) \Delta E_{\text{int}} \quad (6) \]

The interaction energy $\Delta E_{\text{int}}$ between two CH$_3$CH$_2$S fragments contributes with $\sim$60 kcal/mol to the total disulfide bond formation energy $\Delta E_{\text{bind}}$. The reorganization of the fragments from the equilibrium geometry of CH$_3$CH$_2$S$^\cdot$ to the geometry they adopt in the disulfide $\Delta E_{\text{prep}}$ contributes only with $\sim$1 kcal/mol to $\Delta E_{\text{bind}}$ (see Table S5). Compared to the reference structure with the lowest energy conformation, $\Delta E_{\text{prep}}$ differs less than 1 kcal/mol (Figure 4, brown bars), while $\Delta E_{\text{int}}$ is up to 5 kcal/mol less negative (blue bar towards positive side in Figure 4), for disulfides with high-energy conformations.

Therefore, $\Delta E_{\text{bind}}$ is determined by the disulfide bond formation energy $\Delta E_{\text{int}}$ associated to reaction (6).

The disulfide conformations with $\chi$2 (or $\chi$2$^\prime$) $\sim$ 180° (orange circles, Figure 3b and 4) have a relatively high $\Delta E_{\text{prep}}$ term. In the CH$_3$CH$_2$S$^\cdot$ fragments of these structures, a CCS angle of 109° is found, while in the equilibrium structure of CH$_3$CH$_2$S this angle is 115° (see Table S6). Accordingly, the large negative $\Delta E_{\text{reorg}}$ term found upon reduction, even for low-energy structures having $\chi$2 (or $\chi$2$^\prime$) $\sim$ 180° (see Figure 3), can thus be assigned to the high $\Delta E_{\text{prep}}$ term originating from the unfavorable CCS angle of 109° in the CH$_3$CH$_2$S$^\cdot$ fragments. In the further analysis, the disulfides with $\chi$2 (or $\chi$2$^\prime$) $\sim$ 180° will be considered as a separate group in which the same effects apply as will be discussed for disulfides with $\chi$2 and $\chi$2$^\prime$ $\neq$ 180°.

$\Delta E_{\text{bind}}$ is determined by $\Delta E_{\text{int}}$ and thus, since $\Delta E_{\text{bind}}$ equals $\Delta E_{\text{rel}}$, also $\Delta E_{\text{rel}}$ is determined by $\Delta E_{\text{int}}$. $\Delta E_{\text{int}}$ is the interaction energy between two CH$_3$CH$_2$S$^\cdot$ fragments and can be estimated as the sum of the disulfide binding energy $\Delta E_{\text{SS,bind}}$ (Eq. (8)) and the non-covalent intramolecular interaction energy $\Delta E_{\text{non-cov}}$ (Eq.(7)), e.g. between the CH$_3$ groups in each of the two CH$_3$CH$_2$S$^\cdot$ fragments, calculated as follows (see Table S7, Figure 4b):

$$\Delta E_{\text{non-cov}} = \Delta E_{\text{int}} - \Delta E_{\text{SS,bind}}$$

$$\Delta E_{\text{SS,bind}} = E_{\text{SS}} - E_{\text{S,trip}} - E_{\text{S,sinn}}$$

with $E_{\text{S,trip}}$ and $E_{\text{S,sinn}}$ the energy of the biradical sulfur atom S in, respectively, triplet and singlet state and $E_{\text{SS}}$ the energy of S$_2$ in triplet state, adopting the same S–S length as in diethyl disulfide.

Figure 4b shows relative $\Delta E_{\text{non-cov}}$ values as high as $\sim$4 kcal/mol (green bars), while the relative $\Delta E_{\text{SS,bind}}$ values are lower than 1 kcal/mol (red bars). As such, the trend in $\Delta E_{\text{int}}$ (Figure 4a) originates from differences in non-covalent intramolecular interactions among different disulfide conformations and not from differences in disulfide binding energy. This corresponds with the similar S–S bond length of 2.06–2.07 Å found in all structures (with the (60,–140,60) (F) structure being an exception, see further, purple circle in Figure 3, 4). All d (S$_1$–S$_2$), d(Ca$_1$–C$_2$), d(Cb$_1$–C$_2$), d(Ca$_1$–C$_2$), d(S$_1$–C$_2$) and d(S$_2$–C$_1$) distances can be found in Table S4 (see Scheme 1 for the numbering of the C and S atoms).

Pauli repulsion, electrostatic, and orbital interactions are at the basis of $\Delta E_{\text{rel}}$.

To further understand $\Delta E_{\text{rel}}$, an energy decomposition analysis (EDA) for the rotation around the S–S bond in CH$_3$S–SCH$_3$ is performed, in analogy with Ref. (Bickelhaupt & Baerends, 2003; discussion Weinhold, F. Angew. Chem. Int. Ed. 42, 4188–4194, 2003; Poater, Sola, & Bickelhaupt, 2006). EDA is a widely used and straightforward electronic-structure analysis method, using well-defined, physically relevant terms (Bickelhaupt & Baerends, 2000; Krapp, Bickelhaupt, &
Frenking, 2006). The total bond energy between two CH₃S fragments A and B, ΔE_int is given by:

\[ ΔE_{\text{int}} = ΔE_{\text{Pauli}} + ΔV_{\text{elstat}} + ΔE_{\text{oi}} \]  

with ΔE_{Pauli} the Pauli repulsive orbital interaction arising from anti-symmetrisation and renormalization of the product function \( \Psi^A \Psi^B \), giving \( \Psi^0 = \sqrt{A}[\Psi^A \Psi^B] \). ΔE_{Pauli} comprises the destabilizing interactions between occupied orbitals. ΔV_{elstat} is the classical electrostatic interaction and ΔE_{oi} is the attractive orbital interaction arising from the energy relaxation from \( E[\Psi^A \Psi^B] \) to \( E[\Psi^{AB}] \) of the total wave function \( \Psi^{AB} \). ΔE_{oi} accounts for charge transfer and polarization.

The plot of ΔE_{Pauli}, ΔV_{elstat} and ΔE_{oi} in function of the dihedral CSSC angle \( \chi^3 \) of CH₃S–SCH₃ (this corresponds to \( \chi^3 \) in CH₃CH₂S–SCH₃(C₂H₅), calculated at the geometry of the lowest energy conformation of CH₃S–SCH₃ (\( \chi^3 = 83° \)), shows a region of favorable \( \chi^3 \) angles between 70° and 120° (Figure 5). Pauli repulsion dominates when \( \chi^3 < 70° \), while, compared to the reference disulfide with the lowest energy conformation, less favorable electrostatic and orbital interactions are present when \( \chi^3 > 120° \).

The high ΔE_{rel} of 5.40 kcal/mol of the (60,–140,60) (F) structure (purple circle in Figures 3b and 4) can now be assigned to less favorable electrostatic and orbital interactions found in the (60,–140,60) (F) structure compared to the lowest energy structure, with the effect of the orbital interaction being the largest (Figure 5). This corresponds to a less negative ΔE_{SSbind} (Figure 4b) and shows up in the large S–S bond length of 2.09 Å. The (60,–140,60) (F) structure relaxes to the (71,–111,71) (R) structure during full geometry optimization, having a lower ΔE_{rel} of 2.05 kcal/mol, in accordance with a \( \chi^3 \) angle in the favorable \(-70° < \chi^3 < -120°\) region (for symmetry reasons the \(-120° < \chi^3 < -70°\) and \(70° < \chi^3 < 120°\) of CH₃S–SCH₃ are identical).

The high relative energy (–6 kcal/mol) of the so-called staple structures (–60,90,–60) (F) and (–60,90,–25) (F), in which a short d(Ca₁–Ca₂) of ~3.3 Å is found (Figure 6a), can be assigned to Pauli repulsion causing steric hindrance between the terminal –CH₃ groups. The (–60,90,–60) (F) and (–60,90,–25) (F) structures relax to (–70,111,–70) (R) during full geometry optimization by which the d(Ca₁–Ca₂) increases to ~3.6 Å. The decom- position of the interaction energy between two CH₄ molecules as a function of the C–C distance shows that the Pauli repulsion decreases substantially when the C–C distance increases from 3.3 Å to 3.6 Å (see Figure S2). Accordingly, ΔE_{rel} decreases to 2.05 kcal/mol in the relaxed structures.

The terminal –CH₃ group and the sulfur atom in the structure (60,90,–5) (F) and (66,91,–4) (R) are in an eclipsed conformation (Figure 6b) which causes the S₁–Ca₂ distance to become as short as ~3.2 Å. In analogy with ethane (Bickelhaupt & Baerends, 2003; discussion Weinhold, F. Angew. Chem. Int. Ed. 42, 4188–4194, 2003), Pauli repulsion causes the high energy of these conformations. Upon reduction, the Pauli repulsion will be released, explaining the favorable ΔE_{reorg} term causing the high \( E^o \) of staple and eclipsed conformations (red and blue circles, respectively, in Figure 3).

**How the S–S bond length determines ΔE_{rel}**

In disulfide proteins, S–S bond lengths can be substantially different from the equilibrium S–S bond distance (~2.05 Å), see for example Cys426–Cys473 (1crw) and Cys3–Cys31 (1dfn) having a S–S bond length of ~1.96 Å (Table 1).

The plot of ΔE_{Pauli}, ΔV_{elstat} and ΔE_{oi} in function of the S–S bond length (Figure 7), calculated at the geometry of the lowest energy conformation of CH₃SSCH₃, shows that the decrease in S–S bond length from 2.05 Å to 1.9 Å causes an increase in Pauli repulsion of ~65 kcal/mol, while the stabilization due to electrostatic attraction and orbital interaction increases only by ~31 kcal/mol each. This results in a weakening of the interaction energy of ~3 kcal/mol (Figure 7). Therefore, the high relative energy of disulfides having a short S–S bond length originates from the high Pauli repulsion between the binding sulfur atoms.

**Examples from the PDB**

Our findings nicely agree with and explain a vast body of experimental evidence provided by the Protein Data Bank.
Base (PDB). The latter contains various examples that point to a link between disulfide conformation and redox potential. Thioredoxin (Trx) is a reductase with a low disulfide redox potential (e.g., –270 mV for Escherichia coli Trx (Aslund, Berndt, & Holmgren, 1997)). Trx crystallizes preferentially in its oxidized form, in which the disulfide adopts a low-energy conformation (Table 1). Disulfide binding protein A (DsbA) is a strong oxidant having a high redox potential (e.g., –80 mV for DsbA1 of Neisseria meningitides (Lafaye et al., 2009)). DsbA crystallizes preferentially in its reduced form. The disulfide conformation in the oxidized structure of the Thr176Val mutant of DsbA1 of Neisseria meningitides lies ~2 kcal/mol higher in energy than the Trx disulfide (Table 1), consistent with its higher redox potential of –115 mV (Lafaye et al., 2009). For Trx, DsbA, DsbD, and glutaredoxin 1 (Grx1), a linear correlation is found between the disulfide conformational energy and the redox potential (Figure 8). This shows the predictive potential of the relation between conformation and redox potential. Other examples than the ones reported here are difficult to find, as either no redox potentials are measured or no structural information is available.

By performing our study in the assumption that all disulfides reduce to the same reaction product, namely, dithiol (see reaction 1), only the conformational dependence of the redox potential was considered. However, the NMR structure 2lqq of Mycobacterium smegmatis mycoredoxin 1 (Mrx1) (Van Laer et al., 2012) illustrates that disulfide conformation is not the only factor determining the disulfide redox potential. The M. smegmatis Mrx1 Cys14–Cys17 disulfide is present in nine different conformations. Some of them are clearly much higher in energy than expected from the redox potential of –218 mV (Van Laer et al., 2012) (Table 1). Therefore, also other factors, as for example, the well-documented pKₐ dependence (Huber-Wunderlich & Glockshuber, 1998; Mossner et al., 2000; Roos et al., 2007; Roos, Foloppe et al. 2013) of disulfide redox potentials, might play a role. In the scope of this manuscript and thus to demonstrate and to give insight in the conformational dependence of the disulfide/thiol redox potential, the simplified molecular model is very insightful, as all intramolecular interactions (i.e. the interactions on which the relative disulfide energy depends) are present. How the interplay between conformation and other factors from the protein environment determines the protein disulfide E° will be the subject for further research.

The link between the disulfide/thiol conformation and its reduction potential constitutes a structure–reactivity relationship. This combined with the well-known pKₐ dependence of the disulfide redox potential might result in quantitative structure activity relationships (QSAR) by which oxidation potentials can be predicted from structural data and vice versa the disulfide conformations found in proteins might be explained. This can be important, for example, in the case of DsbA. The latter preferentially crystallizes in its reduced form and thus structures of its oxidized form are not well represented in the PDB. Therefore, our study significantly contributes to a deeper understanding of the disulfide redox biochemistry.

In proteins, the S–S bond length is significantly affected by the steric constraints associated with the 3D structure of the polypeptide backbone. In these systems, the S–S bond length is the primary determinant of the disulfide conformational energy ΔE_rel. This is illustrated in the PDB by the Cys426–Cys473 (1crw) disulfide of human Cdc25B and the Cys3–Cys31 (1dfn) disulfide of human defensin HNP-3 having a high conformational energy in accordance with their low S–S bond length of

Figure 6. (a) Staple and (b) eclipsed conformation of the (–60,90,–60) (F) and (60,90,–5) (F) of diethyl disulfide conformation, respectively.
Table 1. Examples from the Protein Data Base.

| Protein | PDB | $E_{ref}$ (kcal/mol) | $E^\circ$ (mV) | Dihedral angles | d (S1–S2) | d (Ca1–Ca2) | d (S1–Ca2) |
|---------|-----|----------------------|----------------|----------------|-----------|-------------|------------|
| Reference diethyl disulfide | (60,90,60) (F) | 0.0 | $-575$ | 60,90,60 | 2.058 | 5.59 | 3.41 |
| E. coli Trx | 1xoa | 2.6 | $-270^a$ (Aslund et al., 1997) | 74,76,–146 | 2.070 | 5.24 | 3.72 |
| S. aureus Trx (WT) | 2o7k | 3.1 | $-268^a$ (Roos et al., 2007) | 81,74,–146 | 2.036 | 5.32 | 3.70 |
| S. aureus Trx (P31T) | 2o85 | 3.2 | $-236^a$ (Roos et al., 2007) | 77,75,–146 | 2.034 | 5.25 | 3.68 |
| S. aureus Trx (P31S) | 2o87 | 3.2 | $-244^a$ (Roos et al., 2007) | 80,74,–146 | 2.034 | 5.31 | 3.71 |
| E. coli DsbD-alpha | 1jpe | 3.0 | $-232^b$ (Rozhkova et al., 2004) | $-77,93,–112$ | 2.038 | 3.88 | 3.68 |
| Human Hemochromatosis protein | 1oe4 (C) | 3.3 | n. a. | 70,92,–127 | 2.049 | 5.42 | 3.77 |
| C. botulinum neurotoxin type B | 1epw | 3.6 | n. a. | $-106,98,–81$ | 2.031 | 3.98 | 3.77 |
| E. coli Grx1 | 2c1r | 3.6 | $-233^a$ (Aslund et al., 1997) | $-142,80,71$ | 2.064 | 5.21 | 3.69 |
| N. meningitides DsbA1 | 3h28 | 4.3 | $-115^a$ (Lafaye et al., 2009) | 71,75,–130 | 2.091 | 5.16 | 3.67 |
| Anguilla Anguilla agglutinin | 1k12 | 5.5 | n. a. | 91,–88,83 | 2.053 | 3.74 | 3.97 |
| Human Cdc25B | 1ewr | 19.6 | n. a. | 43,–142,78 | 1.957 | 5.09 | 3.57 |
| Humandefensin HNP-3 | 1dfm | 7.9 | n. a. | $-97,104,–90$ | 1.966 | 4.29 | 3.83 |
| M. smegmatis Mrx1(a)c | 2lqq | 10.1 | $-218^a$ (Van Laer et al., 2012) | 86,153,46 | 2.014 | 5.82 | 3.26 |
| M. smegmatis Mrx1(b) | 2lqq | 2.9 | $-218^a$ (Van Laer et al., 2012) | $-149,75,79$ | 2.023 | 5.41 | 3.66 |
| M. smegmatis Mrx1(c) | 2lqq | 5.3 | $-218^a$ (Van Laer et al., 2012) | $-134,116,75$ | 2.026 | 5.91 | 3.64 |

*Coordinates of diethyl disulfide taken from the PDB. Hydrogen atoms were placed and optimized at B3LYP/6-311++G(d,p) level. Energies were calculated at the MP2/6-311++G(d,p) level relative to the lowest energy conformation of diethyl disulfide optimized with fixed dihedral angles (60,90,60) (F).

n. a.: not available.

$^a$Equilibrated against GSSG/GSH.

$^b$Equilibrated against DTT.

$^c$The analyzed disulfide conformations are more than 2 times present in the ensemble of 20 NMR conformers. Three conformations, each present 1 time, and three conformations, each present 2 times, are not analyzed here.
\( \sim 1.9 \, \text{Å} \) (Table 1). The \( \chi^3 \), \( \chi^2 \), and \( \chi^2' \) angles, which determine the intramolecular distances are the secondary factors determining \( \Delta E_{\text{rel}} \). Together with the S–S bond length, they determine the Pauli repulsion and electrostatic and orbital interactions between two \( \text{CH}_3\text{CH}_2\text{S} \) fragments and as such \( \Delta E_{\text{rel}} \) of the different disulfide conformations. Mainly Pauli repulsion is at the basis of the unfavorable interactions in strained conformations having, e.g. a short S–S bond length or short \( d(\text{Ca}_1–\text{Ca}_2) \) and \( d(S_1–\text{Ca}_2) \) distances (Figure 7, Supplementary Figure S2). Upon reduction, the S–S bond is broken and the structure can relax such that the destabilizing Pauli repulsion can be relieved. This is reflected by the favorable \( \Delta E_{\text{reorg}} \) term causing the high \( E^0 \) of high-energy disulfides (red, blue, and purple circles in Figure 3).

The disulfide conformations in which \( \chi^2 \) (or \( \chi^2' \)) adopts a value of \( \sim 120° \) or \( -120° \) constitute an exception. These conformations are transition states for rotation around the S–S bond (green circles in Figure 3). Their high \( E^0 \) does not originate from reorganization effects because similar intramolecular interaction distances are present as in the reference structure with the lowest energy conformation. In proteins, these conformations are stabilized by the protein scaffold, for example in the PDB structures 1de4, 3hz8 and in some conformers of 2lqq (Table 1).

Based on the energy decomposition analysis of the barrier to rotation around the S–S bond in \( \text{CH}_3\text{SSCH}_3 \), a region of favorable \( \chi^3 \) angles between 70° and 120° could be identified (Figure 5). Previously, it has been shown that electronic factors favor a dihedral angle \( \chi^3 \) of 90° and that steric repulsion is in general responsible for larger angles \( \chi^3 \) (Bickelhaupt, Sola, & Schleyer, 1995; El-Hamdi, Poater, Bickelhaupt, & Sola, 2013). The existence of such a low-energy region makes it possible that, in practice, \( \chi^3 \) can adopt values between \( \sim 87° \) (in the lowest energy conformation) and \( \sim 100° \) in which case the \( d(\text{Ca}_1–\text{Ca}_2) \) distance increases to more than 3.6 Å which relieves Pauli repulsion between the terminal \( -\text{CH}_3 \) groups. This is observed in the diethyl disulfide model system and in the disulfides of the 1k12, 1lpe and 1epw protein structures (Table 1).

Further, the favorable 70° < \( \chi^3 < 120° \) region (see Figure 5) rationalizes the high energy of the Cys426–Cys473 (1cwr) disulfide of human Cdc25B and of some of the conformers of the Cys14–Cys17 (2lqq) disulfide of \( M. \text{smegmatis} \) Mrx1 (Table 1). These disulfides have \( \chi^3 \) angles > 120° (or < -120°) resulting in less favorable electrostatic and orbital interactions compared to structures having \( \chi^3 \) angles within the 70° < \( \chi^3 < 120° \) region.

It might also explain the very low number of experimental protein disulfides with \( \chi^3 \) angles below 70° and above 120° (Haworth et al., 2007).
Conclusion
The pKa dependence of the disulfide/thiol redox potential is well documented but, hitherto, its conformational dependence is not. Our computational studies clearly show that the disulfide redox potential is linked to the disulfide conformation, both in model systems and in examples from the PDB. Strained disulfide conformations with a high conformational energy $\Delta E_{\text{rel}}$ have a strong tendency to be reduced. We show that this is not caused by a higher electron affinity of the strained disulfide conformation. Instead, the motor behind its facile reduction is the release of intramolecular strain as the thiol is formed.

Supplementary material
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2013.851034.

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List of abbreviations
- Dsb: Disulfide binding protein
- DTT: Dithiothreitol
- EDA: Energy decomposition analysis
- Grx: Glutaredoxin
- GSH: Glutathione
- GSSG: Glutathione disulfide
- F: Disulfide optimized with fixed dihedral angles
- MO: Molecular orbital
- Mrx: Mycoredoxin
- PDB: Protein data base
- R: Disulfide optimized with relaxed dihedral angles
- Trx: Thioredoxin
- WT: Wild type
- C. botulinum: Clostridium botulinum
- E. coli: Escherichia coli
- M. smegmatis: Mycobacterium smegmatis
- N. meningitides: Neisseria meningitides
- S. aureus: Staphylococcus aureus
- Trx: Thioredoxin
- F: Disulfide optimized with relaxed dihedral angles
- R: Disulfide optimized with fixed dihedral angles
- MO: Molecular orbital
- Mrx: Mycoredoxin
- PDB: Protein data base
- R: Disulfide optimized with relaxed dihedral angles
- Trx: Thioredoxin
- WT: Wild type
- C. botulinum: Clostridium botulinum
- E. coli: Escherichia coli
- M. smegmatis: Mycobacterium smegmatis
- N. meningitides: Neisseria meningitides
- S. aureus: Staphylococcus aureus

References
Antonello, S., Benassi, R., Gavioli, G., Taddei, F., & Maran, F. (2002). Theoretical and electrochemical analysis of dissociative electron transfers proceeding through formation of loose radical anion species: Reduction of symmetrical and unsymmetrical disulfides. Journal of the American Chemical Society, 124, 7529–7538.

Bickelhaupt, F. M., & Baerends, E. J. (2000). Kohn-Sham density functional theory: Predicting and understanding chemistry. In K. B. Lipkowitz & D. B. Boyd (Eds.), Reviews in computational chemistry Vol. 15 (pp. 1–86). New York, NY: Wiley-VCH.

Bickelhaupt, F. M., & Baerends, E. J. (2003; discussion Weinhold, F. Angew. Chem. Int. Ed. 42, 4188–4194, 2003). The case for steric repulsion causing the staggered conformation of ethane. Angewandte Chemie International Edition, 42, 4183–4188.

Bickelhaupt, F. M., Diefenbach, A., de Visser, S. P., de Koning, L. J., & Nibbering, N. M. M. (1998). Nature of the three-electron bond in $\text{H}_2\text{S}_2\text{H}_2$. Journal of Physical Chemistry A, 102, 9549–9553.

Bickelhaupt, F. M., Nibbering, N. M. M., Vanwezenbeek, E. M., & Baerends, E. J. (1992). Central bond in the 3 CN dimers NC-CN, CN-CN, and CN–NC - electron pair bonding and pauli repulsion effects. Journal of Physical Chemistry, 96, 4864–4873.

Bickelhaupt, F. M., Sola, M., & Schleyer, P. V. (1995). Theoretical investigation of the relative stabilities of XSSX and X2SS isomers (X=F, Cl, H, and CH3). Journal of Computational Chemistry, 16, 465–477.

Billiet, L., Geerlings, P., Messens, J., & Roos, G. (2012). The thermodynamics of thiol sulfenylation. Free Radical Biology and Medicine, 52, 1473–1485.

Depuydt, M., Messens, J., & Collet, J. F. (2011). How proteins form disulfide bonds. Antioxid. Redox Signaling, 15, 49–66.

El-Hamdi, M., Poater, J., Bickelhaupt, F. M., & Sola, M. (2013). $X_2Y_2$ isomers: Tuning structure and relative stability through electronegativity differences (X = H, Li, Na, F, Cl, Br, I; Y = O, S, Se, Te). Inorganic Chemistry, 52, 2458–2465.

Frisch M. J., Trucks G. W., Schlegel H. B., Scuseria G. E., Robb M. A., Cheeseman J. R., … Pople J. A. (2003). Gaussian 03, Revision A.1. Wallingford, CT: Gaussian Inc.

Gaussian 09, Revision A.1. Wallingford, CT: Gaussian Inc.

Gaussian 09, Revision A.1. Wallingford, CT: Gaussian Inc.

Gomez, J. A., Serrano-Andres, L., & Yanez, M. (2010a). Asymmetry and non-adiabaticity in fragmentation of disulfide bonds upon electron capture. Chemical Physics and Physical Chemistry, 11, 2530–2538.

Gomez, J. A., Serrano-Andres, L., & Yanez, M. (2010b). Electron capture activation of the disulfide bond. The role of the asymmetry and electronegativity. Physical Chemistry and Chemical Physics, 12, 1042–1050.
Haworth, N. L., Feng, L. L., & Wouters, M. A. (2006). High torsional energy disulfides: relationship between cross-strand disulfides and right-handed staples. Journal of bioinformatics and computational biology, 4, 155–168.

Haworth, N. L., Gready, J. E., George, R. A., & Wouters, M. A. (2007). Evaluating the stability of disulphide bridges in proteins: a torsional potential energy surface for diethyl disulphide. Molecular Simulations, 33, 475–485.

Ho, J., Coote, M. L., Cramer, C. J., & Truhlar, D. G. (2012). Theoretical Calculation of Reduction Potentials. In B. Speiser & O. Hammerich (Eds.), Organic Electrochemistry (5th ed.). Boca Raton, FL: CRC Press.

Huber-Wunderlich, M., & Glockshuber, R. (1998). A single dipeptide sequence modulates the redox properties of a whole enzyme family. Folding and Design, 3, 161–171.

Krapp, A., Bickelhaupt, F. M., & Frenking, G. (2006). Orbital overlap and chemical bonding. Chemistry A European Journal, 12, 9196–9216.

Van Laer, K., Buts, L., Foloppe, N., Vertommen, D., Van Belle, K., Wahi, K., … Messens, J. (2012). Mycoredoxin-1 is one of the missing links in the oxidative stress defense mechanism of Mycobacteria. Molecular Microbiology, 86, 787–804.

Lafaye, C., Iwema, T., Carpentier, P., Jullian-Binard, C., Kroll, J. S., Collet, J. F., & Serre, L. (2009). Biochemical and structural study of the homologues of the thiol-disulphide oxidoreductase DsbA in Neisseria meningitidis. Journal of Molecular Biology, 392, 952–966.

Marino, S. M., & Gladyshev, V. N. (2012). Analysis and functional prediction of reactive cysteine residues. Journal of Biological Chemistry, 287, 4419–4425.

Mossner, E., Iwai, H., & Glockshuber, R. (2000). Influence of the \( pK_a \) value of the buried, active-site cysteine on the redox properties of thioredoxin-like oxidoreductases. FEBS letters, 477, 21–26.

Poater, J., Sola, M., & Bickelhaupt, F. M. (2006). Hydrogen-hydrogen bonding in planar biphenyl, predicted by atoms-in-molecules theory, does not exist. Chemistry: A European Journal, 12, 2889–2895.

Rickard, G. A., Berges, J., Houee-Levin, C., & Rauk, A. (2008). Ab initio and QM/MM study of electron addition on the disulphide bond in thioredoxin. Journal of Physical Chemistry B, 112, 5774–5787.

Roos, G., Foloppe, N., & Messens, J. (2013). Understanding the \( pK_a \) of redox cysteines: the key role of hydrogen bonding. Antioxid Redox Signal, 18, 94–127.

Roos, G., Garcia-Pino, A., Van Belle, K., Brosens, E., Wahi, K., Vandenbussche, G., Wynn, L., Loris, R., & Messens, J. (2007). The conserved active site proline determines the reducing power of Staphylococcus aureus thioredoxin. Journal of Molecular Biology, 368, 800–811.

Roos, G., De Proft, F., & Geerlings, P. (2013). Electron capture by the thiol radical and disulphide bond: Ligand effects on the reduction potential. Chemistry: A European Journal, 19, 5050–5060.

Rozhkova, A., Stimimann, C. U., Frei, P., Grauschopf, U., Brunisholz, R., Grutter, M. G., … Glockshuber, R. (2004). Structural basis and kinetics of inter- and intramolecular disulphide exchange in the redox catalyst DsbD. EMBO Journal, 23, 1709–1719.

Tissandier, M. D., Cowen, K. A., Feng, W. Y., Gundlach, E., Cohen, M. H., Earhart, A. D., … Tuttle, T. R. (1998). The proton’s absolute aqueous enthalpy and Gibbs free energy of solvation from cluster-ion solvation data. Journal of Physical Chemistry A, 102, 931–937.

e Velde, G., Bickelhaupt, F. M., Baerends, E. J., Fonseca Guerra, C., Van Gisbergen, S. J. A., Snijders, J. G., & Ziegler, T. (2001). Chemistry with ADF. Journal of Computational Chemistry, 22, 931–967.

Wouters, M. A., Fan, S. W., & Haworth, N. L. (2010). Disulphides as redox switches: From molecular mechanisms to functional significance. Antioxid Redox Signal, 12, 53–91.

Wouters, M. A., George, R. A., & Haworth, N. L. (2007). “Forbidden” disulphides: Their role as redox switches. Current protein & peptide science, 8, 484–495.