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On the Overwhelming Complexity of Mechanochemical Disulphide Bond Reduction in Alkaline Solution

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The coupling between mechanical stress and the reactivity of disulphide bridges has recently received a great deal of attention due to its broad relevance in biochemistry and materials science. Here, we will highlight the main findings of our computational studies on mechanochemistry of disulphide bridges, which have been carried out in the past few years in the framework of GCS Large Scale Projects. Our investigations have disclosed a very complex mechanistic scenario for the mechanochemistry of disulphides in aqueous alkaline solution. In the low-force regime, external forces play a dual role in the reduction of disulphide bridges via a bimolecular S_N2 attack of a hydroxide ion at a sulphur atom. On the one hand, the external tensile force accelerates the reaction by virtue of the mechanical work performed on the system as the reaction proceeds. On the other hand, tensile forces can induce a conformational distortion of the disulphide moiety that drives the system into a spatial arrangement that is less prone to a nucleophilic attack due to steric hindrance. In the high-force regime, in turn, the tensile force gives rise to a competition between bimolecular S_N2 and unimolecular C–S bond breaking mechanisms as well as to drastic changes in the free energy landscape of the system as a result of which bimolecular reaction pathways transform into pure bond-breaking processes. Our results not only provide a rationale for the enigmatic outcome of certain single-molecule force spectroscopy experiments but also suggest new experiments to continue unravelling the intricacies of the mechanochemistry of disulphide bridges.

1 Introduction

Chemical reactions are commonly initiated by means of heat, light or electricity. However, thanks to the development of new experimental techniques in the past two decades, such as mainly sonochemical and atomic force microscopy techniques, the use of tensile mechanical forces has recently emerged as a new way of triggering reactions. This has given rise to a new research field that is known as ***covalent mechanochemistry***[1–6], which focuses on the controlled investigation and specific manipulation of chemical bonds by mechanical means. This research field holds great promise because it could provide new ways of synthesising molecules and it could lead to new polymers with advanced functionalities, such a polymers that strengthen under stress or polymers that signal when they are close to mechanical failing through a colour change.

Disulphide bond reduction in particular has recently received a great deal of attention in the context of covalent mechanochemistry[7–12]. This is because this class of reactions constitutes an excellent system for exploring the fundamental aspects of the coupling between mechanical stress and reactivity and, in addition, because the mechanochemistry

of disulphides is highly relevant in biochemistry as well as in polymer-based materials science and technology. In biochemistry, the reduction of disulphide bridges located in regions of mechanical strain of proteins is increasingly recognised as a key element in the regulation of the activity of proteins[13,14]. In polymer science and technology, in turn, the mechanochemistry of disulphides can find key applications in the recycling of rubber waste, which is an environmental challenge faced by industry worldwide. Indeed, it has already been demonstrated that devulcanisation of rubber waste can be sustainably achieved by means of mechanochemical procedures in which mechanical stress induces the cleavage of di- or polysulphide links[15]. In addition to this, recent work strongly suggests that disulphide bridges might well be employed as dynamic linkages in the development of self-healable polymers[16].

Force-clamp atomic force microscopy[17] (AFM) is one of the most relevant type of experimental techniques that have been employed so far to investigate the impact of tensile forces on the rate of disulphide bond reduction. This technique allows one to apply a constant stretching force to a reactive molecular system as the reactants transform into products. In a landmark set of force-clamp AFM experiments[9], the cleavage of a protein disulphide bond was probed in aqueous alkaline solution as a function of the applied tensile force up to forces of 2 nN. These experiments revealed an abrupt change in the reactivity of this disulphide bond with hydroxide anions at a force of around 0.5 nN, above which the accelerating effect of tensile force on the reaction rate was found to be considerably diminished[9]. The discovery of this "*mechanochemical switch*" is a very relevant finding because it shows that reactivity and mechanical stress can couple in complex ways. Yet, the origin of this enigmatic switch could not be established on the basis of the experimental data alone.

Herein, we present the results of our computational investigations that were carried out on the JUQUEEN supercomputer at Forschungszentrum Jülich with the goal of deciphering the origin of the mechanical switch and achieving a better molecular understanding of the mechanochemistry of disulphide bridges. The rest of this feature article is organised as follows. We shall first introduce in a nutshell the methodology employed in our simulations before disclosing the origin of the mechanochemical switch. Subsequently, we shall reveal that disulphide bond cleavage occurs via two competing mechanisms for forces around 2 nN. Finally, we shall show that the drastic changes brought about by external forces in the free energy landscapes of disulphide bond reductions lead to profound changes in the reaction mechanisms. Last but not least, we also provide some outlook on future computational work.

2 Methodology

The model system employed to carry out the computational study of the mechanism of disulphide bond cleavage by hydroxide ions in aqueous solution consisted of one diethyl disulphide molecule (see Fig. 1a) solvated by 70 explicit water molecules in a periodic simulation box that contained one additional hydroxide anion. This model system was particularly suited to properly model the behaviour of the system in bulk water and the critical de- and re-solvation effects around the disulphide bridge as its cleavage takes place. The explicit consideration of the protein environment in the simulations was not necessary because in the force-clamp AFM experiments the disulphide bridge was totally exposed to the solvent molecules.

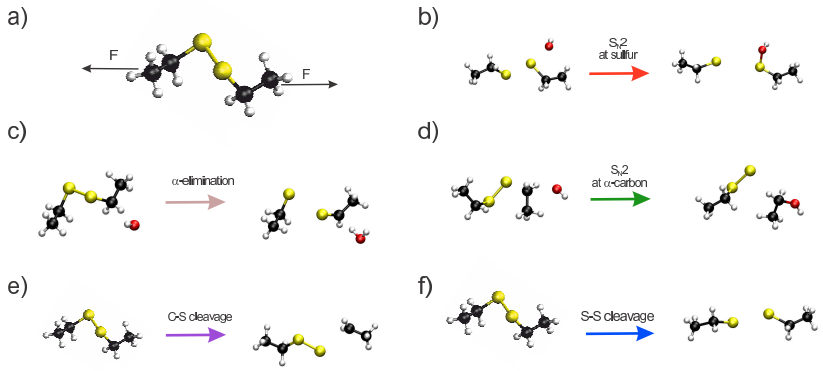

Figure 1. *a)*: The diethyl disulphide molecule where the external forces that were applied to its terminal carbon atoms in a fixed direction in space are visualised. *b-f*: Scheme of the five different reaction pathways considered in this study (see text for a detailed explanation).

Several distinct reaction mechanisms were investigated for the disulphide bond cleavage in alkaline solution. We first considered the "S_N2 at S" mechanism (see Fig. 1b), which is characterised by a direct attack of the solvated hydroxide ion, OH^-(aq), to one of the sulphur atoms of the disulphide bridge, because decomposition of cystine and other aliphatic disulphides is known to proceed via this mechanism upon thermal activation in alkaline solution, i.e. in the absence of any external forces.

We also considered two alternative mechanisms that have been proposed to be operative in the thermal cleavage of some disulphides in basic media, namely the α-elimination mechanism and a "S_N2 at C" mechanism. The α-elimination mechanism is initiated by the abstraction of a proton from the disulphide (Fig. 1c), while the "S_N2 at C" channel is a nucleophilic attack of the OH^- ion to one of the β-carbon atoms that neighbours the disulphide bridge (Fig. 1d). In addition to these reaction pathways, we also investigated two other mechanisms that might well come into play when the reaction is promoted by mechanical means, namely the C–S (Fig. 1e) and S–S bond rupture (Fig. 1f) pathways without the direct intervention of OH^-.

The mechanochemistry of the disulphide bond cleavage was explored through the evaluation of the activation free energies of all different mechanisms displayed in Fig. 1 as a function of the value of the applied external tensile force. The activation free energies were evaluated by means of a set of so-called isotensional[19] *ab initio* molecular dynamics (AIMD)[18] simulations in the presence of a constant external tensile force acting on the two β-carbon atoms of the disulphide and applied along a fixed direction in space to mimic force-clamp conditions (Fig. 1a). The setup with external forces is based on the computational framework that we had previously devised to study mechanochemistry using force-transformed potential energy surfaces[19] and force-transformed free energy surfaces[20,21]. The electronic structure of the entire aqueous system, i.e. of all solute and solvent molecules included in the simulation box, was taken into account efficiently using density functional theory. The AIMD simulations were performed using the Car-Parrinello propagation scheme[22] as implemented in the CPMD package[23], which scales very effi-

ciently in supercomputers that allow for massive parallelisation such as JUQUEEN. Even if the disulphide bond cleavage is promoted by mechanical means, the timescale of this process is typically orders in magnitude larger than the typical timescale of an AIMD simulation, which usually spans timescales on the order of 100 ps. For this reason, the activation free energies for all the reaction channels of Fig. 1 were evaluated using enhanced sampling techniques. Specifically, we used metadynamics[24] and the thermodynamic integration-based "blue moon" technique[25]. Overall, the *ab initio* trajectories that were generated for all the different reaction pathways and for different forces spanned the multi-nanosecond timescale. It is thus clear that the *in silico* investigation of the mechanochemistry of disulphide bridges would not have been possible without the sustained allocation of supercomputing time for massively parallel jobs on JUQUEEN at the Jülich Supercomputing Centre in the framework of GCS Large Scale Projects.

3 Origin of the Mechanochemical Switch

The first reaction mechanism considered in our study was the "S_N2 at S" mechanism because this was expected to be the lowest energy pathway at zero force (i.e. in purely thermal conditions). The force-dependence of the reaction rates obtained in our simulations (top panel of Fig. 2a) shows that the tensile force is able to accelerate the "S_N2 at S" reaction pathway. Most remarkably, however, these results reveal that there is a critical force around 0.3 nN above which the susceptibility of the reaction rate to the applied force

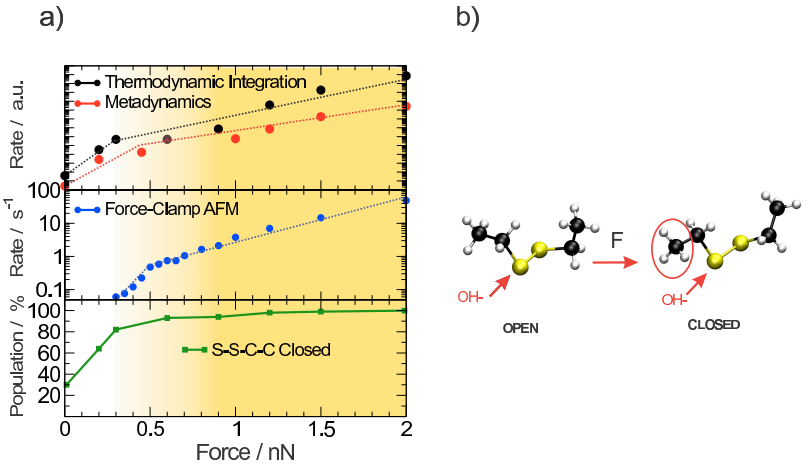

Figure 2. *a)*: Top panel: Force-dependence of the reaction rates obtained from the computed activation free energies ($\Delta A^{\ddagger}(F)$) via $k(F) \sim \exp[-\Delta A^{\ddagger}(F)/k_{\mathrm{B}}T]$, plotted on a logarithmic scale using arbitrary units; Middle panel: reaction rates from force-clamp AFM experiments on a logarithmic scale (data extracted from Figure 2**c** of Ref. 9; Bottom panel: Relative populations of the *closed* conformer of the disulphide molecule in the reactant state as a function of force (determined by computing the normalised number of configurations where the S-S-C-C dihedral angles are in the range $(180 \pm 50)^{\circ}$). *b)* Force-induced transition from the *open* (left) to the *closed* (right) diethyl disulphide conformer and the resulting shielding effect with respect to a collinear approach of the nucleophile, OH^{-}, relative to the S–S bond; note that all surrounding water molecules have been omitted for clarity.

greatly diminishes. Therefore, the mechanochemical switch observed in experiments (see middle panel of Fig. 2a) was indeed captured in our simulations[26].

A detailed analysis of the molecular dynamics trajectories revealed that the mechanochemical switch originates in a force-induced conformational change of the disulphide moiety involving its S–S–C–C dihedral angle. The conformational profile along this dihedral angle exhibits three different minimum-energy configurations at the following values of the dihedral: 60°, 180° and 300°. The conformations with a dihedral angle of 60° or 300° can be called *open* conformations, while those with a dihedral angle of 180° can be called *closed* conformations These names reflect the fact that in the *closed* conformer the β carbon atom is reoriented by rotation such that it hinders the nucleophilic attack of the OH^- ion (see Fig. 2b). Note that a S_N2 mechanism is highly directional and that in this particular case it requires the two sulphurs and the approaching oxygen to be collinear, thus forming an ideal attack angle close to 180°. At zero force, the most stable conformer is the *open* conformer with a dihedral angle of 60°. However, our simulations revealed that forces as small as 0.2 nN are able to revert the energetic ordering of the conformers such that the *closed* conformer becomes the more stable one. Accordingly, the population of the *closed* conformer steeply increases with the force (see bottom panel of Fig. 2a). In fact, at a force of 0.3 nN the population of the *closed* conformer is higher than 80% (see bottom panel of Fig. 2a). For forces larger than 0.3 nN, the population of the closed conformer still increases but at a much slower pace. The force at which this abrupt change of pace occurs coincides with the force at which the mechanochemical switch observed in simulations occurs at the level of the force-dependence of the reaction rate. This provides solid evidence that this mechanochemical switch arises from a force-induced conformational rearrangement of the disulphide moiety involving its S–S–C–C dihedral angle that drives the disulphide into a conformation that is shielded against nucleophilic attack due to steric hindrance[26]. It is worth mentioning that the same mechanically-assisted conformational change observed for the diethyl-disulphide molecule was also observed in larger model systems such as cystine and a polypeptide molecule[27].

Overall, the large-scale isotensional metadynamics AIMD simulations allowed us to unravel the enigmatic origin of the mechanochemical switch observed in the mechanically-assisted disulphide bond reduction in alkaline media. This switch stems from a Janus-like role played by the mechanical force, which leads to antagonistic effects. On the one hand, the force performs work on the system and thereby accelerates the reaction. On the other hand, the force also induces a significant structural distortion that drives the disulphide into a conformation that is less susceptible to be attacked by a nucleophile due to steric hindrance effects.

4 Competition of Mechanisms in the High-Force Regime

In the previous section we have demonstrated that the mechanochemical switch of the disulphide bond cleavage in alkaline solution can be rationalised on the basis of a "S_N2 at S" mechanism. Yet, there is still a crucial question open, namely does the same reaction mechanism still operate in the high-force regime, or do forces exceeding about one nN lead to different scenarios? In order to provide an answer to this question, we computed the force-dependence of the activation free energies of all reaction channels included in Fig. 1. The results of these simulations are summarised in Fig. 3. The "S_N2 at S" pathway

turns out to indeed remains the lowest one in free energy for most of the range of forces. However, even if the activation free energies of the pure S–S and C–S bond rupture channels lie significantly higher in energy than that of the "S_N2 at S" pathway at zero force, the curves of Fig. 3a show that the effect of tensile force on S–S and C–S bond rupture is much more pronounced than on nucleophilic attack at sulphur. As a result of this, at a force of about 2 nN, the activation free energy of the C–S bond-scission process is slightly lower than that of the "S_N2 at S" process. It thus follows that the unimolecular C–S bond rupture channel competes with the mechanism based on a bimolecular S_N2 attack of OH^- at sulphur in a range of high forces that is relevant to experiments[28].

5 Confluence of Reaction Mechanisms for Disulphide Bond Cleavage

Besides the competition between the C–S bond rupture and the "S_N2 at S" pathways, our simulations also revealed that the external force is able to transform concerted pathways into two-step mechanisms. As shown in Fig. 3b, at forces around 2.25 nN, the "S_N2 at S" pathway, which is based on a nucleophilic attack of the OH^- ion at a sulphur atom in concert with S–S bond cleavage, transforms into a direct S–S rupture process, followed by addition of the OH^- to the generated cation. In this two-step mechanism, the S–S bond rupture is the rate determining step, while the second step is virtually barrierless, and thus, it does not play any role in the kinetics of the process. In the simulations carried out at and above the critical force of 2.25 nN, the reaction pathway associated with the bimolecular

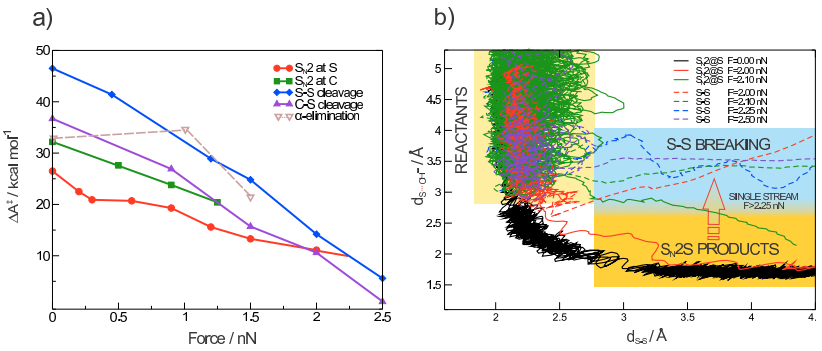

Figure 3. *a)*: Force dependence of the activation free energies at 300 K for all reaction channels shown in Fig. 1. The colour code used to label these curves corresponds to that introduced in Fig. 1. Note that the computed data (displayed using different symbols) are connected with straight lines to guide the eye. *b)*: Reactive pathways obtained in the isotensional *ab initio* metadynamics simulations at different values of constant tensile forces at indicated. These pathways are displayed in terms of trajectories in the reactive subspace that is spanned by those two generalised coordinates d_{S-S} and $d_{S\ldots OH}$ which are required to describe both the nucleophilic attack at S by OH^- as well as S–S cleavage. The trajectories depicted with solid and broken lines correspond to pathways following the "S_N2 at S" and S–S cleavage mechanisms, respectively, from reactants to products. The reactive pathways at 0 and 2 nN were obtained by means of multiple-walker metadynamics simulations using the two following collective variables (CVs): $d_{S-S} - d_{S\ldots OH}$ and the S–S–C–C torsion angle. The reactive pathways at 2.10, 2.25 and 2.50 nN were also obtained by means of multiple-walker metadynamics simulations using these two different CVs: d_{S-S} and $d_{S\ldots OH}$. Therefore, in the metadynamics simulations performed at larger forces, the crucial d_{S-S} and $d_{S\ldots OH}$ generalised coordinates are treated as independent CVs in order to endow the system with even more flexibility in the regime close to the critical force of confluence.

concerted S_N2 mechanism was never observed, although our set-up was flexible enough to reveal this very pathway at only somewhat lower forces (see Fig. 3b). This strongly suggests that the transition state of the "S_N2 at S" pathway no longer exists above the critical force. In other words: this very channel ceases to exist above this force[28]. Therefore, our simulations revealed a very intriguing topological scenario. Below a force of 2.25 nN, both "S_N2 at S" and S–S bond rupture pathways exist as independent reactions channels (see Fig. 1). At and above this critical force, the "S_N2 at S" pathway collapses into a pure S–S bond breaking process. Note that the lengthening of the S–S bond associated with the S–S bond breaking pathway is, in fact, one component of the reaction coordinate associated with the "S_N2 at S" pathway. It can thus be stated that only one of the components of the reaction coordinate of the bimolecular S_N2 mechanism survives beyond 2.25 nN. For these reasons, it is concluded that the external force brings about a confluence of the "S_N2 at S" and S–S bond rupture mechanisms[28]. It should be mentioned that our simulations also revealed that there is an additional confluence between the "S_N2 at C" and C–S bond rupture mechanisms[28].

6 Force-Induced Reversal of β-Eliminations of Disulphides in Alkaline Solution

As shown in Fig. 1, disulphide bond cleavage can occur via elimination mechanisms which involve the abstraction of a proton of the disulphide moiety. Besides the α-elimination pathway considered in Fig. 1, there is yet another type of elimination mechanism that is operative for some particular disulphides in alkaline solution. It is the so-called β-elimination mechanism, which occurs via the abstraction of a β-proton of the disulphide moiety and a C–S bond breaking. The model system employed to investigate the mechanical response of this mechanism (Fig. 4a) is necessarily larger than the diethyl disulphide species used so far for all other reaction channels in order to ensure that the acidity of the β-proton is properly represented.

The simulations of the β-elimination channel at zero force demonstrate that this is a two-step mechanism in which the abstraction of a β-proton is followed by a C–S bond breaking of the carbanion generated in the first step. The deprotonation step is the rate-determining step whereas subsequent C–S bond rupture is virtually a barrier process[29]. The activation free energy of the deprotonation step at zero force is higher than that of the "S_N2 at S" pathway (Fig. 3a). The force-dependence of the activation energy of this reaction channel (being the so-called carbanion pathway) demonstrates that the external force does not result in any acceleration of the reaction (see Fig. 4b), which means that the β-deprotonation step is decoupled from the mechanical force[26]. When reaching a tensile force of 1 nN, the reactivity scenario found at zero and small forces drastically changes because the external force is able to create a second reaction channel (the so-called carbocation pathway), where now the β-deprotonation step becomes a barrierless process that follows the C–S bond cleavage (see Fig. 4c and Fig. 4d). The external force is thus able to revert the order in which the two steps of the β-elimination mechanism take place, thereby transforming a slow and force-independent process with second-order kinetics into a unimolecular reaction that is greatly accelerated by mechanical forces[29].

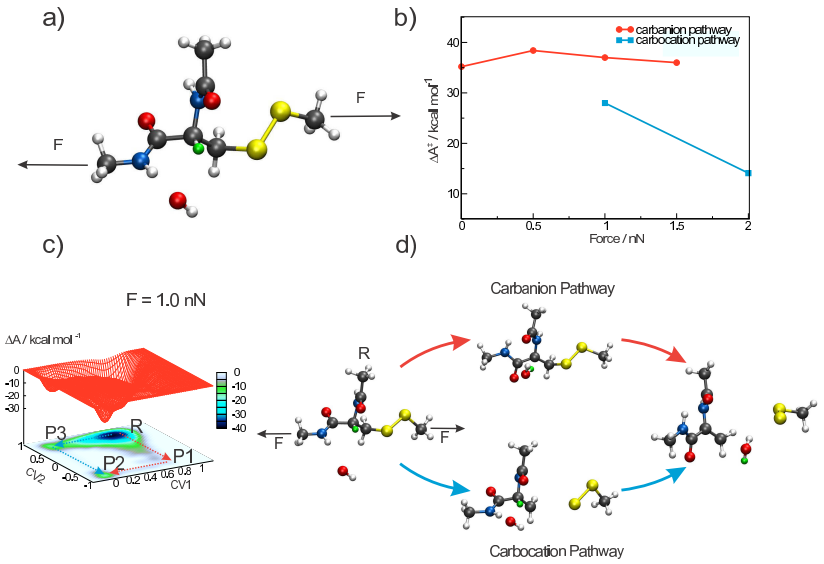

Figure 4. *a*: Model system to study the β-elimination pathway. *b*: Force-dependence of the activation free energy for β-elimination of the disulphide bond corresponding to the rate-determining bimolecular and unimolecular first step of the reaction according to the carbanion (red circles) and carbocation (blue squares) pathways as preferred at low and higher forces, respectively. *c*: Force-transformed free energy landscape for β-elimination at a constant force of $F = 1$ nN where the carbocation channel is energetically preferred over the still existing carbanion pathway. Red and blue arrows indicate the carbanion and carbocation pathways, respectively, whereas R and Pn, denote reactant and (intermediate) product. *d*: Representative snapshots sampled from isotensional *ab initio* metadynamics simulations of the carbocation and carbanion channels at $F = 1$ nN.

7 Conclusions and Outlook

Our *in silico* investigations carried out over the course of the past few years on the mechanochemistry of disulphide bridges have led to the discovery of an astonishingly complex mechanistic scenario thanks to the long-time support received in the framework of GCS Large Scale Projects. The finding that external mechanical forces are able to stabilise a conformer that is less prone to be attacked by nucleophiles not only explains the observations of single-molecule force spectroscopy experiments, but also announces the possibility of designing disulphide derivatives whose stability increases under mechanical strain. Furthermore, the competition between mechanisms and the force-induced transformations of bimolecular mechanisms into unimolecular bond-breaking processes unveiled in the moderate and high-force regimes strongly suggests that more "*mechanochemical switches*" might be detected in upcoming single-molecule force spectroscopy experiments using properly designed disulphides in alkaline solution.

In future computational work, it would be highly interesting to explore whether the mechanochemical switch observed when using OH^- as a nucleophile is also observed when using more complex nucleophiles, such as the 1,4-DL-dithiothreitol molecule. This, in turn, also necessitates to construct yet larger disulphide models to keep the steric balance between the reactant and the attacking reagent. Larger scale simulations in which the exter-

nal forces will be applied in the frame of a QM/MM setup to afford a realistic description of systems of ever increasing complexity are an interesting option to follow.

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