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## REVISED

# A theoretical study of the energetics of the reactions of triplet dioxygen with hydroquinone, semiquinone, and their protonated forms; relation to the mechanism of superoxide generation in the respiratory chain

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## Abstract

One-electron reduction of the dioxygen molecule by the reduced form of mitochondrial ubiquinones (Q) of the NADH dehydrogenase (complex I) and mitochondrial cytochrome  $bc_1$  (complex III) is believed to be the main source of superoxide anion radical  $O_2^{\bullet-}$  and hydroperoxide radical  $OOH^{\bullet}$ . In this work we modeled the energetics of four possible reactions of the triplet ( $^3\Sigma_g$ ) dioxygen-molecule reduction by fully reduced and protonated ubiquinone ( $QH_2$ ; reaction **1**), its deprotonated form ( $QH^-$ ; reaction **2**), the semiquinone radical ( $QH^{\bullet}$ ; reaction **3**), and the semiquinone anion radical ( $Q^{\bullet-}$ ; reaction **4**), by means of *ab initio* calculations with the 6-31G(d) and 6-31+G(d) basis set in the Restricted Open-Shell Hartree Fock (ROHF), Unrestricted Hartree Fock (UHF), and Complete Active Space Self Consistent Field (CASSCF) with dynamic correlation [at the second-order Møller-Plesset (MP2) or Multiple Reference Møller-Plesset (MRMP), respectively] schemes and the Basis Set Superposition Error (BSSE) correction included, as well as semiempirical AM1 and PM3 calculations in the UHF and ROHF schemes. 2-butene-1,4-dione and p-benzoquinone were selected as model compounds. For the reduced forms of both compounds, reaction **1** turned out to be energetically unfavorable at all levels of theory, this agreeing with the experimentally observed diminished reductive properties of hydroquinone derivatives at low pH. For 2-butene-1,4-dione treated at the most advanced MRMP/CASSCF/6-31+G(d) level, the energies of reactions **1** – **4** are 4.7, -34.3, -15.0, and -4.1 kcal/mol, respectively. This finding suggests that reactions **2** and **3** are the most likely mechanisms of electron transfer to molecular oxygen in aprotic environments and that proton transfer is involved in this process. Nearly the same energies of reactions **2** and **3** were calculated at the MRMP/CASSCF/6-31+G(d) level for reduced forms of p-benzoquinone. Inclusion of diffuse functions in the basis set and dynamic correlation at the CASSCF level appears essential. Because deprotonated ubiquinol is unlikely to exist in physiological environment, reaction **3** appears to be the most likely mechanism of one-electron reduction of oxygen; however, if oxygen can penetrate cytochrome  $bc_1$  as far as the  $Q_o$  center where ubiquinol can be deprotonated, reaction **2** can also come into play. The energies of reactions **2** and **3** calculated at the MRMP/CASSCF/6-31+G(d) level are most closely reproduced in the *ab initio* and semiempir-

ical UHF PM3 calculations. Additional semiempirical calculations on more realistic models of ubiquinone: 2,3-dimethoxy-6-methyl-p-benzoquinone and 2,3-dimethoxy-5-isoprenyl-6-methyl-p-benzoquinone gave qualitatively the same relations between the energies of reactions **2** and **3** as those carried out for p-benzoquinone species, thereby suggesting that this method could be used in studying electron-transfer reactions from reduced quinone derivatives to molecular oxygen in more complex systems, such as a model of the Q<sub>o</sub> site of cytochrome bc<sub>1</sub>, where applying *ab initio* methods is infeasible.

**Key words:** superoxide, ubiquinone, electron transfer, molecular quantum mechanics.

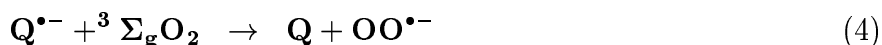
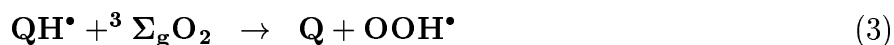
# 1 Introduction

The superoxide anion radical  $O_2^{\bullet-}$  and the hydroperoxide radical  $OOH^{\bullet}$  are harmful to living cells, because they initiate chains of free radical reactions leading to the destruction of cell components either by producing the hydroxyl radicals or by direct interactions with biomolecules.<sup>1</sup> These two reactive oxygen species (ROS) most probably emerge as unwanted by-products during mitochondrial respiration.<sup>2,3</sup> It has recently been suggested<sup>3,4</sup> that the bulk of superoxide production by mitochondria observed in experiments might be an artifact of running most experiments *in vitro* on isolated mitochondria, thus changing their physical state and, moreover, the indicators used to detect the ROS are electron carrier themselves and can, therefore, give false positive or negative results.<sup>5,6</sup> Nevertheless, evidence also exists for the production of superoxide by mitochondria in the *in vivo* conditions.<sup>3</sup> The leakage of ROS was not observed in bioenergetically unaffected mitochondria,<sup>6,7</sup> but is significant on addition of foreign compounds, which may interact with electron carriers of the respiratory chain<sup>8</sup> such as, e.g., antimycin A which inhibits the mitochondrial Q-cycle between high-potential cytochrome b and ubiquinone-binding center N<sup>9</sup> as well as in the conditions of reductive stress<sup>10</sup> or upon addition of cadmium cations.<sup>11</sup> One-electron reduction of dioxygen is probably the main cause of the peroxidating properties and, consequently, the cardiotoxicity of the anticancer drugs of the ametantrone and anthracycline family, which can replace native ubiquinones in the quinone-reducing centers of the redox enzymes, presumably the complex I;<sup>12-14</sup> similar mechanisms might account for general toxicity of quinone-based drugs.<sup>15</sup>

Most experiments suggest that one-electron reduction of the dioxygen molecule is mediated by ubiquinones (Figure 1) from the mitochondrial NADH dehydrogenase (complex I)<sup>12-14,16-20</sup> and the mitochondrial cytochrome  $bc_1$  (complex III).<sup>9,18,21-28</sup> Particularly mutations of cytochrome  $bc_1$  can lead to increased free-radical production resulting in mitochondrial myopathies.<sup>28</sup> It was reported, however, that succinate reductase (complex II)<sup>18</sup> rather than complex I and III might be the major site of one-electron reduction of dioxygen; another report suggests that the flavin mononucleotide (FMN) group rather than the ubiquinone of complex I is mainly involved.<sup>29</sup>

Fig. 1

The possible mechanisms of the ubiquinone-mediated one-electron reduction of dioxygen probably involve dioxygen interaction with reduced form of mitochondrial ubiquinone, namely ubiquinol or ubisemiquinone or their conjugated bases, the possible reaction schemes being shown in Eqs. (1) – (4), **Q** denoting the oxidized ubiquinone.<sup>12–14, 23, 24, 30–32</sup>



Reactions **3** and **4** involving protonated semiquinone and its anion, respectively, are considered in the literature to be the most likely mechanism of electron transfer to dioxygen.<sup>3, 23, 26, 27, 30, 31, 33–39</sup> Such a mechanism appears reasonable, because the electron flow during the Q-cycle in cytochrome bc<sub>1</sub> involves electron bifurcation at the Q<sub>o</sub> center; one electron is transferred to the iron-sulfur cluster (ISP), this resulting in the formation of ubisemiquinone, and the other one to the Q<sub>i</sub> center.<sup>28, 40, 41</sup> The semiquinone species can be accumulated in the Q<sub>o</sub> center of the enzyme, if further electron transfer to the Q<sub>i</sub> center is blocked by replacing the ubiquinone of that center with a foreign molecule (e.g., antimycin A)<sup>27, 28, 39</sup> or by changing the conformation or mobility of the enzyme which hinders the conformational transition necessary for normal electron transfer to proceed.<sup>3, 28</sup> Evidence has also been gathered that the toxicity of cadmium is caused by binding Cd<sup>2+</sup> between the ubisemiquinone of cytochrome b<sub>566</sub> of the the Q<sub>o</sub> site and cytochrome b of complex III, thereby hindering the electron transfer between subunits and resulting in direct electron transfer from ubisemiquinone of the Q<sub>o</sub> site to molecular oxygen.<sup>11</sup> Most likely, diffusion of ubisemiquinone to both the inner and outer side of the mitochondrial membrane subsequently occurs where it can react (in its anionic or protonated form) with molecular oxygen.<sup>27, 39</sup>

Kozlov et al.<sup>36</sup> showed that reaction **4** involving deprotonated semiquinone occurs predominantly in ethanol-water mixture.<sup>36</sup> They ruled out the involvement of both protonated (reaction **1**) and deprotonated (reaction **2**) ubiquinol, as well as that of protonated semiquinone (reaction



3). On the other hand, we demonstrated by electrochemical<sup>31,42</sup> and UV-spectroscopic<sup>31</sup> experiments carried out in aprotic solvents as well as by theoretical calculations<sup>30</sup> that semiquinone anion radicals obtained by one-electron reduction of anthraquinone derivatives bearing hydroxy groups can transfer an electron and a proton to the ground-state triplet dioxygen molecule, which results in the formation of the peroxy radical and the base conjugated to the hydroxyanthraquinone molecule. The reaction was found to be strongly exoenergetic and its occurrence was manifested in shifting the anthraquinone reduction potential peak towards less negative values, lowering the peak corresponding to the back-oxidation of the semiquinone radical, as well as the appearance of the deprotonated form in the reaction mixture.<sup>31</sup> Subsequently,<sup>43,44</sup> we observed the same influence of molecular oxygen on the reduction of lapachol and isolapachol (the hydroxy derivatives of naphthoquinone). The non-hydroxy derivatives of anthraquinone were inert in aprotic solvents,<sup>31</sup> which suggests the involvement of protons in the reaction. This finding does not contradict that of Kozlov et al.,<sup>36</sup> because their experiments were performed in a protic environment.

Wang and Eriksson<sup>32</sup> carried out a Density Functional Theory (DFT) study of the energetics of electron transfer between semiquinone and oxygen and concluded that these reactions are unlikely to occur *in vacuo* or in non-polar environment if triplet oxygen is involved but are exoenergetic for the singlet  $^1\Delta_g$  oxygen.<sup>32</sup> Therefore their results cast doubts on the involvement of semiquinones in superoxide production, unless the oxygen molecule is excited to the  $^1\Delta_g$  state, and also seem to contradict our above-mentioned results obtained with *ab initio* Restricted Open Shell Hartree Fock (ROHF) and Complete Active Space Self Consistent Field (CASSCF).<sup>30</sup> It should be noted, however, that the compounds we studied were derivatives of anthraquinone and not ubiquinone; therefore our results are not necessarily relevant to oxygen reduction by ubiquinones. This prompted us to examine the energetics of reactions (1) – (4) for models of ubiquinone by *ab initio methods* at the ROHF, the Unrestricted Hartree Fock (UHF), and the CASSCF level. The third of them enables us to treat open-shell systems in the most straightforward and accurate manner.



## 2 Methods

### 2.1 Model compounds

In most of the calculations, ubiquinone was modeled by 2-butene-1,4-dione (Figure 2a) or p-benzoquinone (Figure 2b) depending on computational cost. The simpler model compound served to study the energetics of reactions **1** – **4** at high theory level. Once this was accomplished, calculations were carried out on selected reactions for species derived from p-benzoquinone. Additional semiempirical PM3 calculations were carried out on more realistic ubiquinone models shown in Figure 2c and 2d, respectively.

### 2.2 Computational procedures

Calculations were carried out at the *ab initio* and semiempirical levels of theory. The purpose of doing semiempirical calculations was to establish whether semiempirical methods which, in contrast to *ab initio* methods can be used in studying more realistic models of the active centers of the redox enzymes, can reproduce the energetics of the reactions studied at a sufficient level of accuracy. Full geometry optimization with no symmetry constraints was performed. In the *ab initio* calculations the 6-31G(d) as well as 6-31+G(d) basis sets were used. In *ab initio* calculations the geometries were optimized at the CASSCF, UHF, and ROHF levels, respectively. Proper spin multiplicity was rigorously maintained for all reactions (triplet for reactions **1** and **3** and doublet for reactions **2** and **4**) in all but UHF calculations in which multiplicity is defined only approximately. For optimized geometries the energy correction due to dynamic correlation was estimated by carrying out single-point Multireference Møller-Plesset Theory (MRMP)<sup>45</sup> calculations at the CASSCF level and second-order Møller-Plesset perturbation theory (MP2) calculations<sup>46,47</sup> at the ROHF and UHF levels. The active space in the CASSCF calculations consisted of 8 orbitals with 10 (reaction **1** and **2**) or 11 (reaction **3** and **4**) electrons, respectively. These active spaces will be referred to as the (10,8) and the (11,8) active space, respectively. For isolated substrates the active-space orbitals correspond to the HOMO-LUMO pair of organic molecule as well as the  $\pi_{\pm 1} - \pi_{\pm 1}^*$  and the  $\sigma_{O-O} - \sigma_{O-O}^*$  pairs of the oxygen. The AM1 and PM3 methods<sup>48</sup> were used in semiempirical calculations.



The basis set superposition error (BSSE) was estimated by the method of Chałasiński et al.,<sup>49</sup> as in our previous work.<sup>30,50-52</sup> The BSSE corrections were calculated for optimized structures. The method used is the generalized counter-poise method of Boys and Bernardi<sup>53</sup> to treat the cases in which the internal geometry of the components of the interacting systems changes on interaction. In this method, the energy of interaction of species A with species B in system A–B is first decomposed as follows:

$$\Delta E_{AB} = E_{AB} - (E_A + E_B) = [E_{AB} - (\tilde{E}_A + \tilde{E}_B)] + (\tilde{E}_A - E_A) + (\tilde{E}_B - E_B) \quad (5)$$

where  $E_A$ ,  $E_B$ , and  $E_{AB}$  denote the energies of isolated A, isolated B, and the system A–B, respectively,  $\tilde{E}_A$  denotes the energy of A at the geometry that it assumes in A–B, and  $\tilde{E}_B$  denotes the energy of B at the geometry it has in A–B.

The terms  $\tilde{E}_A - E_A$  and  $\tilde{E}_B - E_B$  in Eq. (5) can be regarded as the distortion energies of A and B on the formation of A–B. They are not influenced by the BSSE, because their components are calculated in the basis set of the same size. BSSE affects only the term  $E_{AB} - (\tilde{E}_A + \tilde{E}_B)$ . Because the distorted components have the same geometry as in A–B, the counter-poise procedure of Boys and Bernardi<sup>53</sup> can now be applied. The BSSE correction,  $\delta E_{A-B}$ , to the interaction energy is therefore defined as follows:<sup>49</sup>

$$\delta E_{A-B} = [\tilde{E}_A(\chi_A \cup \chi_B) - \tilde{E}_A(\chi_A)] + [\tilde{E}_B(\chi_A \cup \chi_B) - \tilde{E}_B(\chi_B)] \quad (6)$$

where  $\chi_A$  and  $\chi_B$  denote the basis sets of A and B and  $\chi_A \cup \chi_B$  denotes the basis set of A–B. Thus, in order to estimate the BSSE four additional energy calculations are required: on subsystem A in the geometry it has in A–B with the basis set of A and with the basis set of A–B, respectively, and on subsystem B in the geometry it has in A–B with the basis set of B and with the basis set of A–B, respectively.

Generally, the BSSE correction is considered to be overestimated in cases where the electron correlation is taken into account.<sup>54</sup> Based on the comparison of the calculated well-depths of the interaction potentials of the He<sub>2</sub>, Ne<sub>2</sub>, and He–Ne systems with the experimental data, Bachrach et al.<sup>55</sup> proposed to scale the correction of the interaction energy by the factor of 0.7.



In this work, however, we added the *unscaled* BSSE-correction values, in order to indicate the lower (without BSSE correction) and upper (with BSSE correction) boundaries of the energies.

To estimate the changes of the enthalpy ( $\Delta H$ ) and Gibbs free energy ( $\Delta G$ ; this quantity is directly related to the equilibrium constant of a reaction) of reactions **1** – **4** in *ab initio* calculations we computed the rotational and vibrational contributions to the partition functions of the substrates and the products (the harmonic-approximation approach). The translational contributions are the same for substrates and products when the system under study is treated as a whole. These computations required the calculation of the Hessian matrix for each of the systems studied. The rotational and vibrational partition functions and the respective contributions to  $\Delta H$  and  $\Delta G$  were computed using formulas readily available in any textbook of statistical mechanics (see, e.g., ref. 56).

All calculations were carried out with the use of the GAMESS<sup>57</sup> and the MOPAC 2000<sup>58</sup> packages. Molecular structures were drawn with MOLMOL.<sup>59</sup>

## 3 Results and discussion

### 3.1 *cis*-1,3-butane-1,4-dione

Gradient-optimized structures (at the CASSCF/6-31+G(d) level) of the systems studied (before and after an electron or electron and proton transfer), together with selected geometric parameters and Mulliken charges are presented in Figures 3a-d for reactions **1** – **4**, respectively.

Fig. 3 An analysis of the configuration state functions in the basis of the natural orbitals revealed that electron transfer occurs from one initially doubly-occupied (reaction **1** and **3**) or one initially singly-occupied (reactions **2** and **4**) frontier orbital of the organic donor to one of the  $\pi_{2p}^*$  orbitals of the dioxygen molecule for all reactions. This means that only the occupation number of the orbital of the organic donor changed from nearly 2.0 (reactions **1** and **3** or nearly 1.0 (reaction **2** and **4**) to nearly 1.0 or nearly 0.0, respectively and that of the  $\pi_{2p}^*$  orbital of dioxygen changed from nearly 1.0 to nearly 2.0. The occupation numbers of the remaining orbitals of the active space did not vary remarkably. The frontier orbitals of the organic donors involved in electron transfer are shown in Figure 4.



The energies of the reactions are summarized in Table 1a, while in Table 1b we summarize the sums of rotational and vibrational (calculated within harmonic approximation) contributions to enthalpy ( $\Delta H$ ) and Gibbs free energy ( $\Delta G$ ) as well as the changes of these quantities upon the reactions studied calculated using the energies obtained at the MRMP level with BSSE correction. At all levels of theory the electron transfer from QH<sub>2</sub> (reaction **1**) is energetically unfavorable, this corresponding to the experimental finding of Kozlov et al.<sup>36</sup> Reactions **2** and **3** are definitely more favored than reaction **4**, which suggest essential role of the coupling between electron and proton transfer in oxygen reduction by semiquinones and quinols. However, it should be noted, that both the water molecules and the components of the mitochondrial complex III are sources of protons and, consequently, reaction **4** cannot be dismissed.

The results obtained for reactions **2** and **3** at the most advanced MRMP/CASSCF/6-31+G(d) level suggest that reaction **2** (the transfer of one electron from a model of ubiquinol monoanion) to oxygen is more exoenergetic than the transfer of one electron from a model of neutral ubisemiquinone. It should be noted that this result is obtained only after dynamic correlation is included and both reactions appear almost equally exoenergetic at the CASSCF level. The energy relations between reactions **2** and **3** at the CASSCF/6-31+G(d) and MRMP/CASSCF/6-31+G(d) level are qualitatively reproduced by those between energies calculated at the UHF/6-31+G(d) level without including dynamic correlation. On the other hand, the difference between the energies of reaction **2** and **3** calculated at the UHF/6-31+G(d) level is three times greater than that computed at the most advanced MRMP/CASSCF/6-31+G(d) level. This is not surprising because UHF tends to overestimate the energies of biradical states with separated electron spins, as occurs in reaction **2**. Including dynamic correlation does not bring the energy relation closer to those predicted at the most advanced level; conversely, the energy of reaction **3** becomes almost the same as that of reaction **2** at the UMP2/6-31+G(d) level and reaction **2** becomes endoenergetic at the RMP2/6-31+G(d). Therefore, when a system is too complex to carry out advanced calculations, the most reasonable prediction of the energetics of the reactions of electron transfer from ubiquinol/ubisemiquinone models to dioxygen seems to be provided by UHF calculations without including dynamic correlation.

Table 1



It can also be noted that inclusion of the BSSE does not influence much the relations between the energies of reactions **1** – **4**, regardless of theory level. Similarly, the translational and rotational contributions to  $\Delta H$  and  $\Delta G$  are not significant compared to the differences between the energies of the reactions and, consequently, the qualitative relations between the  $\Delta H$  and  $\Delta G$  values of reactions **1** – **4** are the same as those between the  $\Delta E$  values of these reactions.

### 3.2 Hydroquinone and ubisemiquinone and their derivatives

Gradient optimized geometries of the systems studied (before and after an electron and proton transfer) together with selected geometric parameters (optimized at the CASSCF/6-31+G(d) level) and Mulliken population charges are presented in Figure 5 for reactions **2** and **3** respectively, while the energies of reactions **1** – **3**, partition-function contributions to  $\Delta H$  and  $\Delta G$ , and the values of  $\Delta H$  and  $\Delta G$  of reaction **2** and **3** calculated using the MRMP energies are summarized in Table 2. Reaction **4** was not considered because reaction **3** in which both proton and electron transfer are involved turned out to be more favorable energetically for the simpler model compound. Likewise, reaction **1** was studied only at simpler levels of theory, because it turned out to be endoenergetic for the simpler model compound at all theory levels. Calculations were carried out only with the 6-31+G(d) basis set, because of the necessity of including diffuse functions demonstrated in section 3.1

Fig. 5  
Table 2

As for *cis*-1,3-buta-1,4-dione, electron transfer occurs from an initially doubly-occupied (reactions **1** and **3**) or an initially singly-occupied (reaction **2**) frontier orbital of the organic donor to one of the  $\pi_{2p}^*$  orbitals of dioxygen acceptor. Again reaction **1** is definitely unfavorable. At the most advanced MRMP/CASSCF/6-31+G(d) theory level the energies of reaction **2** and **3** (Table 2) are very similar to those calculated for the simpler model compound (Table 1). Both ROHF and UHF energies are greater for reaction **2** and, as in the case of *cis*-1,3-buta-1,4-dione, the UHF scheme overestimates the difference between the energy of these two reactions predicting that reaction **3** is strongly endoenergetic. Including dynamic correlation in the ROHF or UHF calculations makes the energies diverge more from those calculated at the MRMP/CASSCF/6-31+G(d) level (Table 2), as for the simpler model compound (Table 1).

For realistic calculations on electron-transfer reactions involving reduced ubiquinone species, especially if at least immediate surrounding is to be included, use of the *ab initio* approach is too expensive. We, therefore, have also carried out semiempirical AM1 and PM3 calculations on reactions **1** – **4** involving reduced p-benzoquinone species as well as of more realistic models of mitochondrial ubiquinone depicted in Figure 2c and d. The results are summarized in Table 3. As shown, reaction **1** is predicted endoenergetic by both AM1 and PM3 semiempirical calculations, in agreement with the results of *ab initio* calculations both with involvement of hydroquinone (Table 2) and the simpler model compound (Table 1). Reaction **4** also is predicted to be endoenergetic, though to a lesser extent, which is in agreement with the results of the *ab initio* calculations (Table 1 and 2). AM1 predicts reactions **2** and **3** to be endoenergetic, while PM3 predicts reaction **2** to be exoenergetic at both the ROHF and UHF level. It can be noted (Table 3) that reaction **3** is definitely endoenergetic and reaction **2** is definitely exoenergetic, while the difference in the enthalpy of these two reaction is strongly reduced at the ROHF level. It can be noted that the difference between the enthalpies of reactions **2** and **3** at the UHF/PM3 level is very similar to the energy difference computed at the MRMP/CASSCF/6-31+G(d) level and both energies are shifted up by about 22 kcal/mol. Consequently, use of the PM3 method at the UHF level could be considered when estimating the energetics of electron transfer from reduced forms of ubiquinone to molecular oxygen in more complex systems such as, e.g., models of the whole Q<sub>o</sub> center of cytochrome bc<sub>1</sub> where multiconfigurational *ab initio* calculations are infeasible to carry out. The better performance of the PM3 compared to the AM1 method is in agreement with the results of our earlier study of the reduction of anthracenedione derivatives<sup>42</sup> in which we demonstrated that the PM3-calculated enthalpies of reduction of these compounds correlate very well with their reduction potentials in dimethylsulfoxide determined by cyclic voltammetry.

The results of AM1 and PM3 calculations for reactions **2** and **3** involving reduced forms of 2,3-dimethoxy-6-methyl-p-benzoquinone (Figure 2c) and 2,3-dimethoxy-5-isoprenyl-6-methyl-p-benzoquinone (Figure 2d) are summarized in Table 4. The PM3-calculated enthalpies of these reactions are similar to those involving reduced p-benzoquinone species for

Table 3

Table 4



2,3-dimethoxy-5-isoprenyl-6-methyl-p-benzoquinone and slightly higher for 2,3-dimethoxy-6-methyl-p-benzoquinone. The relations between the enthalpies of reactions **2** and **3** are similar to those in Table 3. Therefore, the p-benzoquinone moiety appears to be the major determinant of the energetics of electron-transfer reactions, while the substituents play a secondary role.

## 4 Conclusions

The results of this work suggest that ubiquinone-mediated one-electron reduction of the triplet-oxygen molecule in non-polar environment is most likely to proceed via electron transfer from the ubiquinol monoanion (reaction **2**) or from neutral ubisemiquinone (reaction **3**), with preference to the first process. Concerted electron and proton transfer appears to be essential for the reaction to occur. Electron transfer from the ubisemiquinone anion appears to be less likely, although it cannot be ruled out especially if proton donors are accessible in neighborhood, as occurs in the redox enzymes. These results agree with those of our earlier theoretical<sup>30</sup> and experimental<sup>31</sup> findings that proton-donor groups are required for anthraquinone-mediated oxygen reduction. Electron transfer from fully protonated ubiquinol appears strongly endoenergetic.

Our results disagree with those obtained by Wang and Eriksson<sup>32</sup> using the DFT methodology who found all four reactions of single-electron transfer to be exoenergetic. As we have shown despite the seemingly trivial calculations on the systems under considerations the calculated energies depend very strongly on the level of theoretical description. The CASSCF level used in this study is the most advanced affordable because of the complexity of the system and unlike DFT does not suffer from the problem of spin contamination. The results obtained in this study should therefore be considered more reliable.

Given the fact that fully reduced quinols are weak acids and deprotonated ubiquinol is, therefore, unlikely to exist at physiological conditions, the most likely mechanism of electron transfer to oxygen is the one that involves ubisemiquinone (reaction **3**) or ubisemiquinone anion if proton donors are accessible. This conclusion conforms with the mechanism postulated by other researchers.<sup>3, 23, 26, 27, 31, 33-39</sup> Nevertheless, if normal electron flow in mitochondrial complex III is impeded, a possibility cannot be excluded that oxygen molecules could reach



the  $Q_o$  center, where ubiquinol molecules are hydrogen-bonded to the histidine and ionized aspartic-acid side chains, thereby creating a partially anionic state. It has been demonstrated earlier<sup>60</sup> by means of molecular dynamics simulations that oxygen diffusion to the interior of redox enzymes is feasible.

## 5 Acknowledgments

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Table 1: a) Energies (kcal/mol) of reactions **1** – **4** involving *cis*-1,3-but-1,4-dione species calculated at various levels of theory.<sup>a</sup>

No <sup>b</sup>	ROHF		RMP2 <sup>c</sup>		UHF		UMP2 <sup>c</sup>		CASSCF		MRMP <sup>c</sup>	
6-31G(d)												
1.	4.8	( 9.5)	25.9	( 42.4)	-0.0	( 2.0)	25.1	( 30.2)	31.1	( 32.6)	2.2	( 5.9)
2.	-33.3	(-32.1)	-18.3	(-14.9)	-17.8	(-16.3)	-4.1	( -1.5)	-12.8	( -9.8)	-39.5	(-34.0)
3.	-20.4	(-19.0)	-6.5	( -3.1)	4.1	( 7.6)	-24.1	(-36.8)	-16.7	(-15.4)	-20.6	(-17.2)
4.	-1.0	( 0.4)	4.9	( 8.3)	11.7	( 13.6)	-3.5	( -1.6)	-1.4	( -0.1)	-8.5	( -5.0)
6-31+G(d)												
1.	4.9	( 6.1)	26.2	(29.2)	0.8	( 8.3)	0.0	( 22.6)	28.8	( 30.1)	1.5	( 4.7)
2.	-16.4	(-15.3)	5.7	( 9.1)	-28.9	(-20.5)	-21.6	(-19.5)	-12.0	( -9.3)	-41.4	(-34.4)
3.	-20.0	(-18.8)	-5.7	(-3.0)	4.4	( 5.5)	-23.4	(-20.4)	-13.6	(-12.5)	-17.9	(-15.0)
4.	-0.2	( 0.6)	3.8	( 6.2)	11.4	( 12.2)	-4.7	( -2.6)	-3.2	( -2.4)	-6.8	( -4.1)

b) Partition-function contributions to  $\Delta H$  and  $\Delta G$  of reactions **1**. – **4**. calculated in the harmonic approximation and the values of  $\Delta H$  and  $\Delta G$  of these reactions calculated using the MRMP energies from Table 1a.

No	$\Delta H_{rot,osc}$	$\Delta G_{rot,osc}$	$\Delta H^a$		$\Delta G^a$	
6-31G(d)						
1.	-0.4	1.0	1.8	( 5.1)	3.2	( 6.9)
2.	2.2	1.5	-37.3	(-31.8)	-38.0	(-32.5)
3.	2.1	0.7	-18.5	(-15.1)	-18.9	(-16.5)
4.	1.6	2.7	-6.9	( -3.4)	-5.8	( -2.3)
6-31+G(d)						
.	2.0	0.5	3.5	( 6.7)	2.0	( 5.2)
.	1.6	0.8	-39.8	(-32.8)	-40.6	(-33.6)
.	2.1	0.6	-15.8	(-12.9)	-17.3	(-14.4)
.	1.8	1.6	-5.0	( -2.3)	-5.2	( -2.5)

Values in parentheses are BSSE-corrected energies.

No. is the number of the reaction (eqs 1 – 4).

MP2: ROHF with dynamic correlation at the MP2 level; UMP2: UHF with dynamic correlation at the MP2 level, MRMP: CASSCF with dynamic correlation at the MRMP level.

Table 2: a) Energies (kcal/mol) of reactions **1** – **3** involving p-benzoquinone species calculated at various levels of theory with the 6-31+G(d) basis set.<sup>a</sup>

No <sup>b</sup>	ROHF		RMP2 <sup>c</sup>		UHF		UMP2 <sup>c</sup>		CASSCF		MRMP <sup>c</sup>	
1.	18.8	( 20.0)	35.5	( 38.6)	10.0	( 11.1)	49.9	( 53.1)	<i>-d</i>	<i>-d</i>	<i>-d</i>	<i>-d</i>
2.	-20.0	(-18.8)	-3.5	( -0.0)	-19.0	(-17.9)	-2.2	( 1.3)	-2.5	( 0.2)	-36.7	(-31.5)
3.	-11.0	( -9.8)	-3.4	(-0.4)	17.7	( 18.8)	-19.0	(-15.8)	-8.8	( -7.5)	-17.1	(-13.9)

b) Partition-function contributions to  $\Delta H$  and  $\Delta G$  of reactions **1**. – **4**. calculated in the harmonic approximation and the values of  $\Delta H$  and  $\Delta G$  of these reactions calculated using the MRMP energies from Table 2a.

No	$\Delta H_{rot,osc}$	$\Delta G_{rot,osc}$	$\Delta H^a$		$\Delta G^a$	
6-31G(d)						
2.	1.4	2.7	-35.3	(-30.1)	-34.0	(-28.8)
3.	2.0	-0.3	-15.1	(-11.9)	-17.4	(-14.2)

<sup>a</sup>Values in parentheses are BSSE-corrected energies.

<sup>b</sup>The number of the reaction (eqs 1 – 4).

<sup>c</sup>MP2: ROHF with dynamic correlation at the MP2 level; UMP2: UHF with dynamic correlation at the MP2 level, MRMP: CASSCF

<sup>d</sup>h dynamic correlation at the MRMP level.

<sup>e</sup>reaction **1** was not studied at the CASSCF/MRMP level.

Table 3: Enthalpies (kcal/mol) of reactions **1** – **4** involving p-benzoquinone calculated at the ROHF and UHF levels of theory with the use of the semiempirical AM1 and PM3 methods.

No <sup>a</sup>	AM1		PM3	
	ROHF	UHF	ROHF	UHF
1.	28.8	22.4	27.2	18.5
2.	4.0	1.9	-4.3	-7.5
3.	10.0	18.5	0.8	9.3
4.	23.7	30.7	23.0	7.4

<sup>a</sup>The number of the reaction (eqs 1 – 4).



Table 4: Enthalpies (kcal/mol) of reactions **2** and **3** involving 2,3-dimethoxy-6-methyl-p-benzoquinone (Figure 2c) and 2,3-dimethoxy-5-isoprenyl-6-methyl-p-benzoquinone (Figure 2d) calculated at the ROHF and UHF levels of theory with the use of the semiempirical AM1 and PM3 methods.

No <sup>a</sup>	AM1		PM3	
	ROHF	UHF	ROHF	UHF
2,3-dimethoxy-6-methyl- p-benzoquinone				
2.	17.7	14.3	-1.4	-4.9
3.	17.8	28.3	2.1	11.5
2,3-dimethoxy-5-isoprenyl-6-methyl- p-benzoquinone				
2.	20.6	17.5	-0.1	-8.9
3.	18.5	61.8	1.3	8.3

<sup>a</sup>The number of the reaction (eqs 2 and 3).

## Figure legends

**Fig. 1.** The structure of mitochondrial ubiquinones optimized with the PM3 semiempirical method. The structure has been drawn with MOLMOL.<sup>59</sup>

**Fig. 2.** The structures of the compounds selected to model ubiquinone: (a) *cis*-1,3-buta-1,4-dione, (b) and p-benzoquinone, (c) 2,3-dimethoxy-6-methyl-p-benzoquinone, and (d) 2,3-dimethoxy-5-isoprenyl-6-methyl-p-benzoquinone.

**Fig. 3.** MRMP/CASSCF/6-31+G(d)-optimized geometries and partial atomic charges of the substrates and the products of reaction (a) **1**, (b) **2**, (c) **3**, and (d) **4** for *cis*-1,3-buta-1,4-dione. The pictures have been drawn with MOLMOL.<sup>59</sup>

**Fig. 4.** The frontier orbitals of the organic-donor molecules involved in electron transfer: (a) reaction **1**, (b) reaction **2** (c) reaction **3** and (d) reaction **4** involving the species derived from *cis*-1,3-buta-1,4-dione.

**Fig. 5.** MRMP/CASSCF/6-31+G(d)-optimized geometries and partial atomic charges of the substrates and the products of reaction (a) **2** (b) and **3** for p-benzoquinone. The pictures have been drawn with MOLMOL.<sup>59</sup>



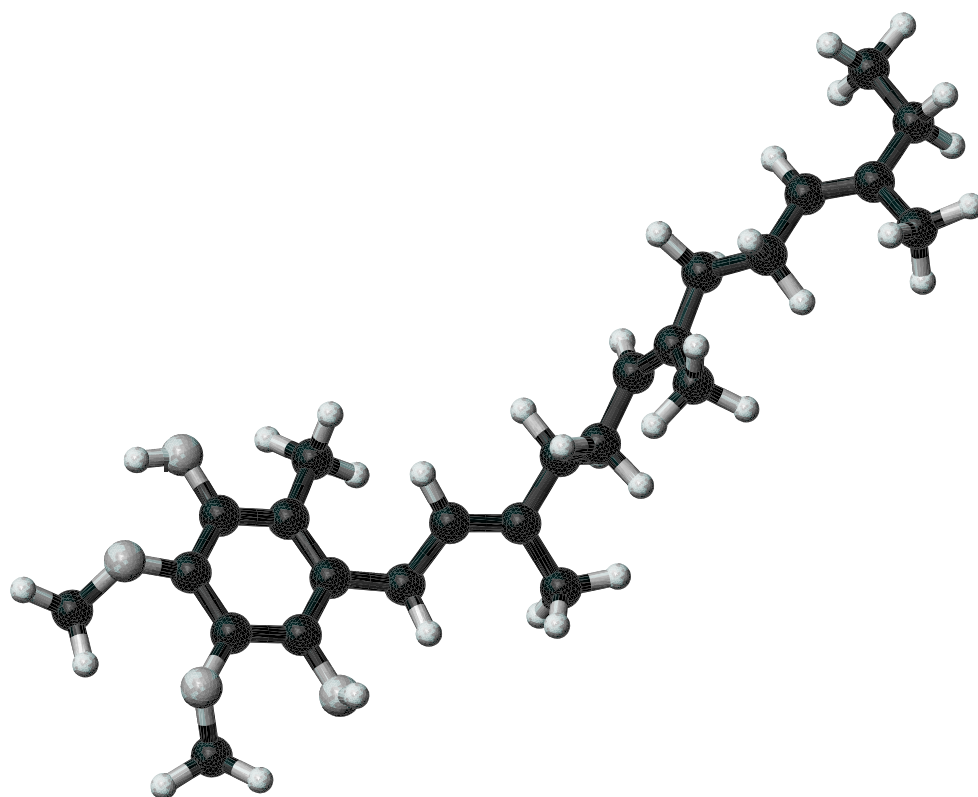
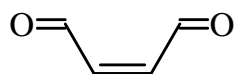
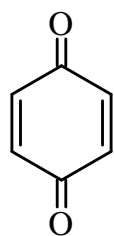


Figure 1

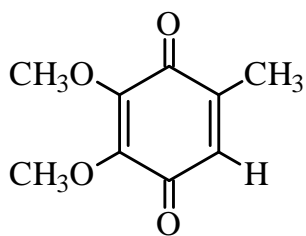




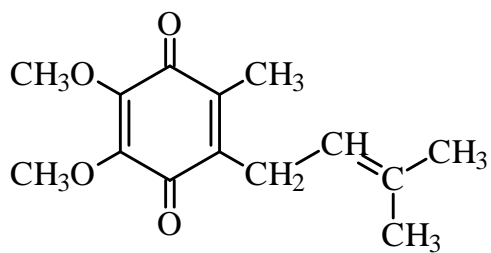
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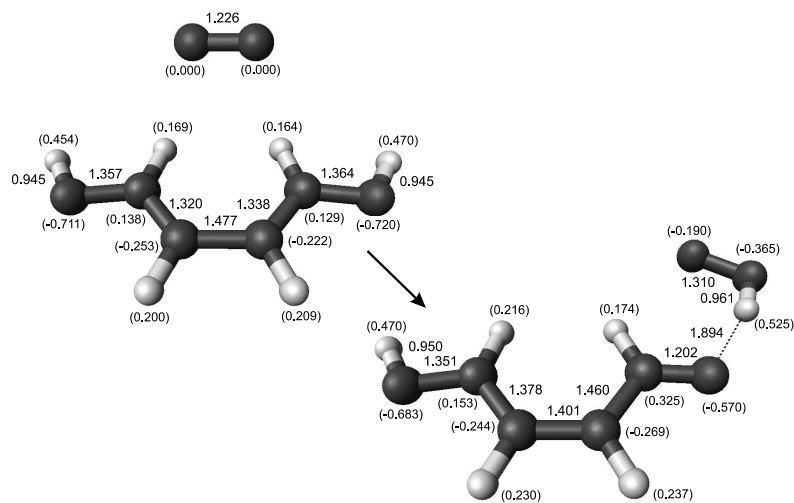
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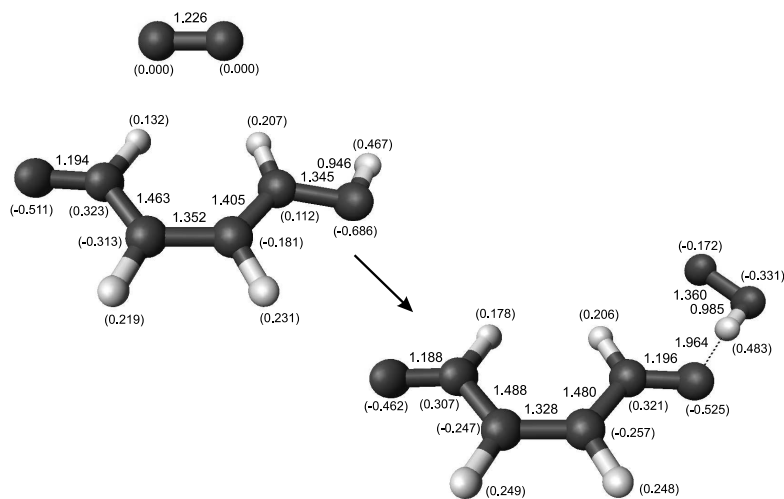
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d



a



b

Figure 3

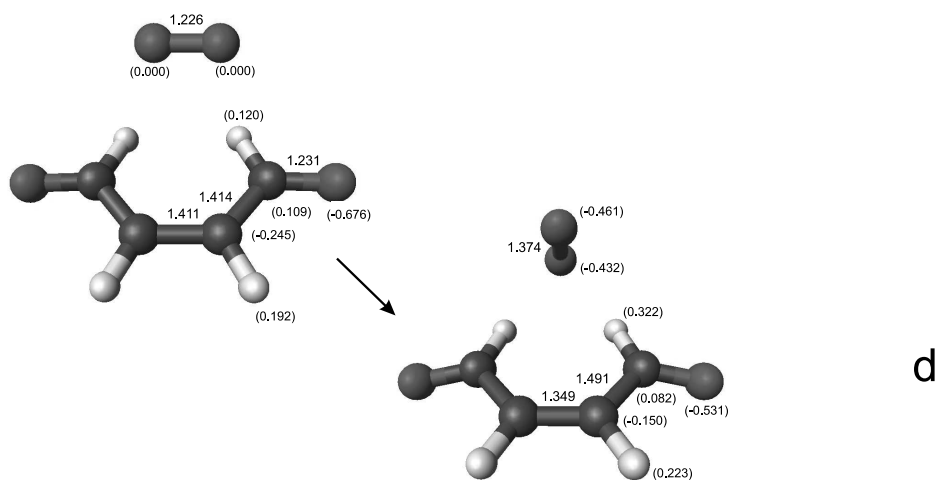
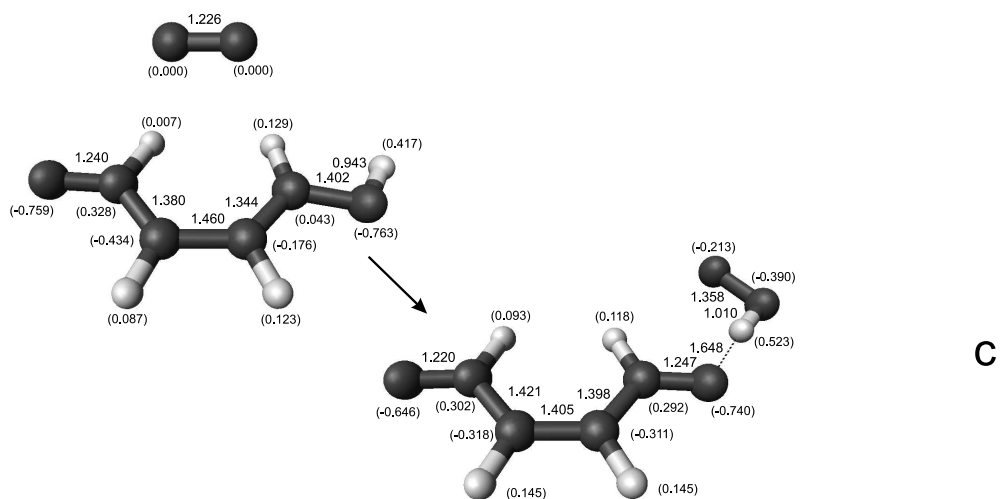


Figure 3 (contd)



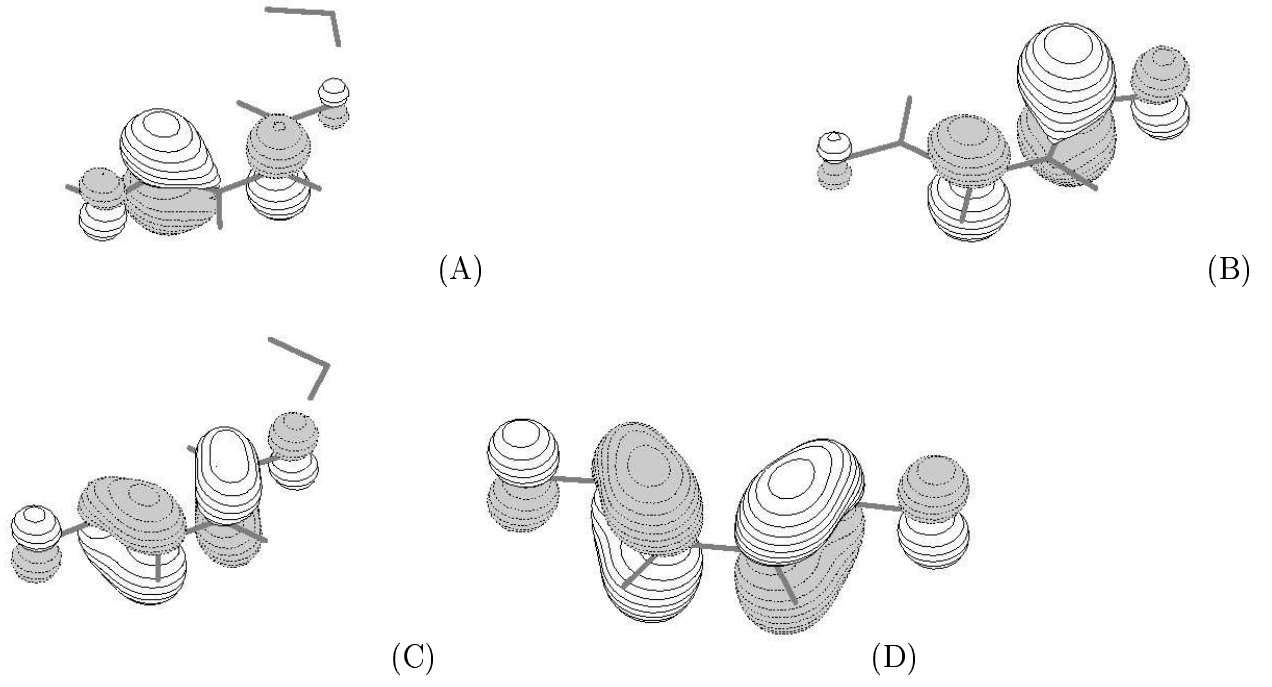
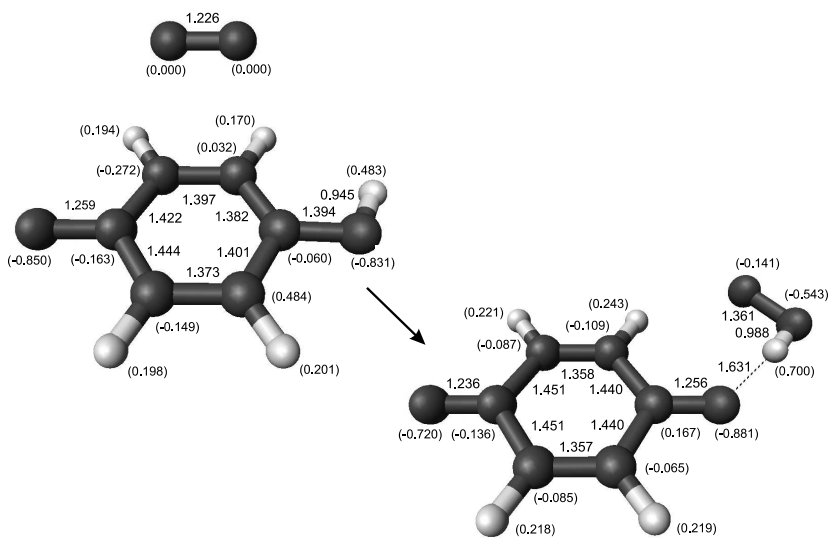
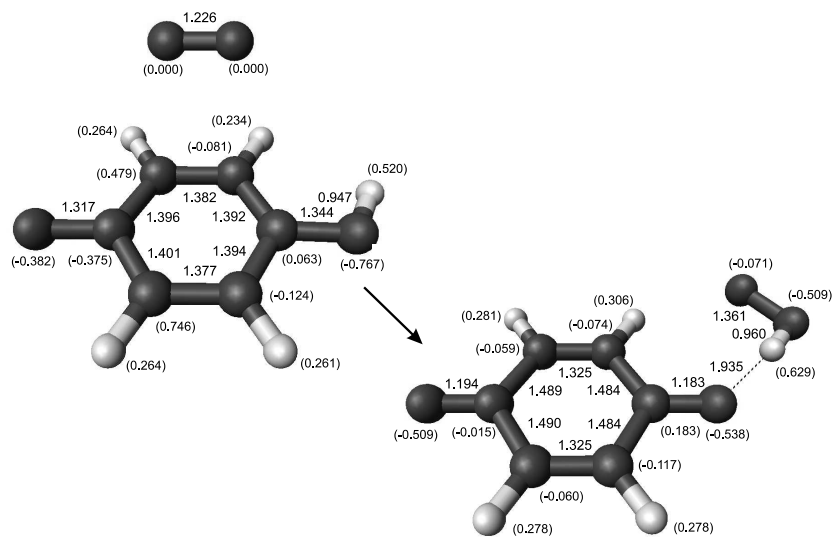


Figure 4



a



b

Figure 5

