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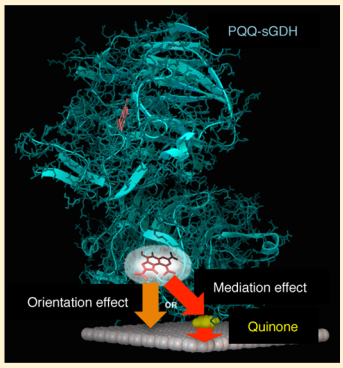
Role of Quinones in Electron Transfer of PQQ–Glucose Dehydrogenase Anodes—Mediation or Orientation Effect

Sofia Babanova,[†,§] Ivana Matanovic,[†,‡,§] Madelaine Seow Chavez,[†] and Plamen Atanassov*[,†]

[†]Chemical and Biological Engineering Department, Center for Micro-engineering Materials, University of New Mexico, Albuquerque, New Mexico 87131, United States

[‡]Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, United States

ABSTRACT: In this study, the influence of two quinones (1,2- and 1,4-benzoquinone) on the operation and mechanism of electron transfer in PQQ-dependent glucose dehydrogenase (PQQ–sGDH) anodes has been determined. Benzoquinones were experimentally explored as mediators present in the electrolyte. The electrochemical performance of the PQQ–sGDH anodes with and without the mediators was examined and for the first time molecular docking simulations were used to gain a fundamental understanding to explain the role of the mediator molecules in the design and operation of the enzymatic electrodes. It was proposed that the higher performance of the PQQ–sGDH anodes in the presence of 1,2- and 1,4-benzoquinones introduced in the solution is due to the shorter distance between these molecules and PQQ in the enzymatic molecule. It was also hypothesized that when 1,4-benzoquinone is adsorbed on a carbon support, it would play the dual role of a mediator and an orienting agent. At the same time, when 1,2-benzoquinone and ubiquinone are adsorbed on the electrode surface, the enzyme would transfer the electrons directly to the support, and these molecules would primarily play the role of an orienting agent.



INTRODUCTION

The utilization of PQQ-dependent enzymes gained significant attention in the last 10 years when researchers started exploring the advantages of enzymes capable of direct electron transfer (DET).[1−5] Among those enzymes is soluble PQQ-dependent glucose dehydrogenase (PQQ–sGDH) from *Acinetobacter calcoaceticus*, which has been previously implemented in the design of enzymatic anodes.[1,6,7] As such, various techniques for PQQ–sGDH immobilization and/or enhanced electron transfer have been developed.[1,8−10] An interesting observation was made by Koto et al., who developed a PQQ–sGDH dependent anode with improved enzyme orientation.[11] They showed that PQQ–sGDH is efficiently oriented when the glucose-binding pocket is facing away from the electrode surface. The PQQ–sGDH they used was isolated from thermophilic *Pyrobaculum aerophilum*. This enzyme has an amino acid sequence with a relatively low identity (26%) with PQQ–sGDH from *A. calcoaceticus*.[12] The native state of the enzyme is a monomer, whereas that of *A. calcoaceticus* is a dimer. In addition, a very important difference between the two PQQ–sGDHs is the position of the enzymes' coenzymes, which are located in the center of *P. aerophilum* PQQ–sGDH and close to the enzyme surface in the case of *A. calcoaceticus* PQQ–sGDH. Thus, the conclusions regarding the proper enzyme orientation of *P. aerophilum* PQQ–sGDH are not entirely applicable for PQQ–sGDH from *A. calcoaceticus*. In both cases, a hypothesis has been stated that if the substrate binding pocket is placed facing the electrode surface, the access of the glucose to the substrate-binding pocket will be blocked, and the oxidation of glucose and subsequent current production will be hindered (Figure 1a).[1,11] However, the close positioning of the substrate binding pocket of *A. calcoaceticus* PQQ–sGDH will bring the enzyme's cofactor closer to the electrode surface and increase the possibility of direct electron transfer. However, when the enzyme molecule is placed on the surface in a way that will fully expose the glucose-binding pocket, a DET from *A. calcoaceticus* PQQ–sGDH toward the electrode surface will be very unlikely due to the long distance electrons should travel (Figure 1b).

In natural conditions, PQQ–sGDH is dissolved into the cytoplasm of the cell without being immobilized.[13] This suggests that the electron donor and the electron acceptor can easily diffuse into the enzymatic molecule and position themselves close to the coenzyme, which are buried inside the PQQ–sGDH.[14] The latter emphasizes that in the design of bioelectrodes based on glucose oxidation by PQQ–sGDH, it is relevant to have an electrochemically active molecule that can be reduced by the enzyme and be explored as a mediator. In other words, as it has been demonstrated many times before in the case of PQQ–sGDH (Figure 2, mediated electron transfer (MET) is the preferable approach for improving the interactions at the bionano interface, although it has been shown that this enzyme is capable of DET.[8,9,15−17] However, this is true only when mediators are dissolved in the electrolyte. Once the mediators are immobilized on the electrode's surface, the question of the enzyme proper orientation arises again.

The factors determining the performance of a system will depend on system's components and their cross-correlations. Let

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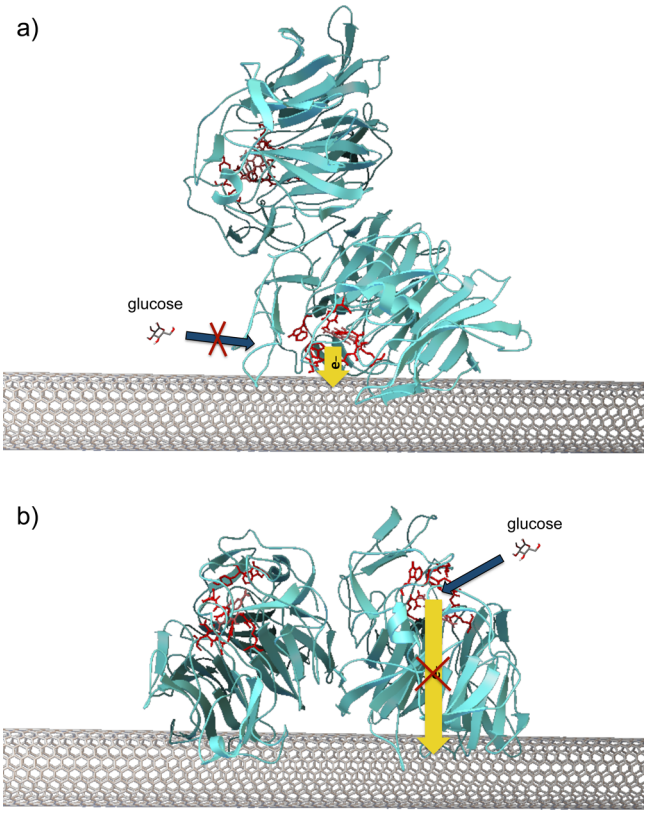

Figure 1. Schematic representation of *A. calcoaceticus* PQQ–sGDH immobilization on carbon nanotubes. The amino acids from glucose-binding pocket are highlighted in red.

us consider the following situations for the enzymatic anode: (i) a MET with freely diffusing mediators, (ii) a MET, where the mediator is attached to the electrode surface, and (iii) DET. During MET, four processes have to take place in order to have an operational anode: (1) Diffusion of the substrate toward the enzyme, (2) oxidation of the substrate by the enzyme coupled with a reduction of the enzyme's coenzyme, (3) oxidation of the coenzyme by the mediator, and (4) oxidation of the reduced form of the mediator at the electrode surface. When the mediator is in a free form (i.e., dissolved in the electrolyte), two additional steps have to occur: diffusion of the oxidized form of the mediator toward the enzyme and diffusion of the reduced form of the mediator from the enzyme toward the electrode surface. Each of the described steps can be a rate-limiting step. Some authors reported enzyme-mediator interactions[18] as the most important factor, while others argue that the oxidation of the mediator is the slowest process among them all.[19] When DET is the mechanism of electron transfer, only two steps take place: (1) Diffusion of the substrate toward the enzymatic molecule and (2) oxidation of the substrate with a concomitant reduction of the enzyme at the electrode surface. Although there are fewer steps during DET, the necessity of appropriate enzyme orientation is significantly decreasing the efficiency of the overall process. The same problem can be observed in the case of MET with immobilized mediator. The importance of the protein immobilization technique, surface properties, and protein orientation on the charge transfer rates and overall electrochemical behavior has been initially studied for cytochrome *c* as a model charge-transfer protein in the 90s,[20,21] yet for the decades to follow, it has been seldom transferred to electrocatalytically active enzymes.

An essential part of the current study is computational docking, which is commonly used in drug design and allows study of the interactions between macromolecules such as enzymes and their substrates or other ligands.[22−24] In this study, we applied a computational approach available through AutoDock Vina Software, which treats molecular docking as a stochastic global optimization of the scoring function on a precalculated grid maps.[20] A scoring function approximates the standard chemical potential of the system, which in turn determines the bound conformation preference and the free energy of binding. The particular implementation of the Autodock Vina scoring function and the evaluation of Autodock Vina's speed and accuracy can be found in ref 22. However, one of the biggest advantages of this computational docking approach is the ability to predict the bound conformations without any knowledge of the ligand's binding site or its location on the macromolecule.

Molecular docking simulations were used here to gain insight into interactions between quinones and PQQ–sGDH in two cases, when mediators are dissolved in solution or immobilized on a carbon support material. The role of two types of quinones, 1,2- and 1,4-benzoquinone, in the operation and mechanism of the electron transfer in PQQ–sGDH anodes has been addressed. Namely, the anode performance was compared in terms of the anode current output and the mediator's effect when 1,2- and 1,4-benzoquinones were used as mediators dissolved in the electrolyte or immobilized on the electrode surface.

MATERIALS AND METHODS

Materials. PQQ-dependent glucose dehydrogenase (PQQ–GDH), E.C. 1.1.5.2 (GLD-321) was purchased from Toyobo Co., Ltd., Osaka, Japan, and used as supplied. All chemicals were obtained from Sigma–Aldrich, St. Louis, MO, unless otherwise stated: β-D-(+)-glucose (99.5% GC), 3-(*N*-morpholino)propanesulfonic acid (MOPS), ≥99.5% (titration), calcium chloride ($CaCl_2$), potassium chloride (KCl), 1,2-benzoquinone and 1,4-benzoquinone, and 1-pyrenebutanoic acid succinimidyl ester (PBSE).

Anode Preparation. Circular piece (d = 0.3 mm) of singe-walled buckeye paper (SWBP) was cut, immersed in a solution of 10 mM PBSE, dissolved in DMSO, and left for 1 h for adsorption of the tether. The paper disc was then washed with DI water and transferred in a solution of 2 mg/mL of PQQ-dependent soluble GDH in 20 mM MOPS (pH 6) with 6 mM $CaCl_2$ and 10 mM KCl, where it was kept at 4 °C for 18 h.

Electrochemical Methods. After the enzyme immobilization, the electrodes were washed again with 20 mM MOPS buffer to take out the unattached enzyme and placed on glassy carbon support held by a plastic cap with an opening of 0.15 mm in diameter. The anodes were tested in a three-electrode electrochemical cell with saturated Ag/AgCl reference and Pt-wire counter electrodes. Twenty mM MOPS (pH 6) with 6 mM $CaCl_2$, 10 mM KCl was used as electrolyte with 10 mM glucose as an enzyme substrate and 2 mM 1,2- or 1,4-benzoquinone as a mediator. Potentiostatic polarization curves of the anodes were carried out applying constant potential for 300 s at each step starting from open circuit potential to 0.30 V, using Ag/AgCl as the reference electrode, at increments of 0.05 V. The generated current was normalized to the area of the electrode opening. After the polarization curves, cyclic voltammetry (CV) at 10 mV/s was carried out to determine the formal redox potentials ($E^{0\prime}$) of the tested benzoquinones in the presence of the enzyme. For that purpose, the potential of the working electrode was swept from −0.6 to 0.6 V vs Ag/AgCl.

Computational Methods. Docking of different quinones was performed using AutoDock Vina software.[22,23] For the docking simulations, the enzyme crystallographic structure was used (1C9U from protein data bank[14]). Autodock Vina provides nine models for each docking simulation. The first model is considered as the best fit

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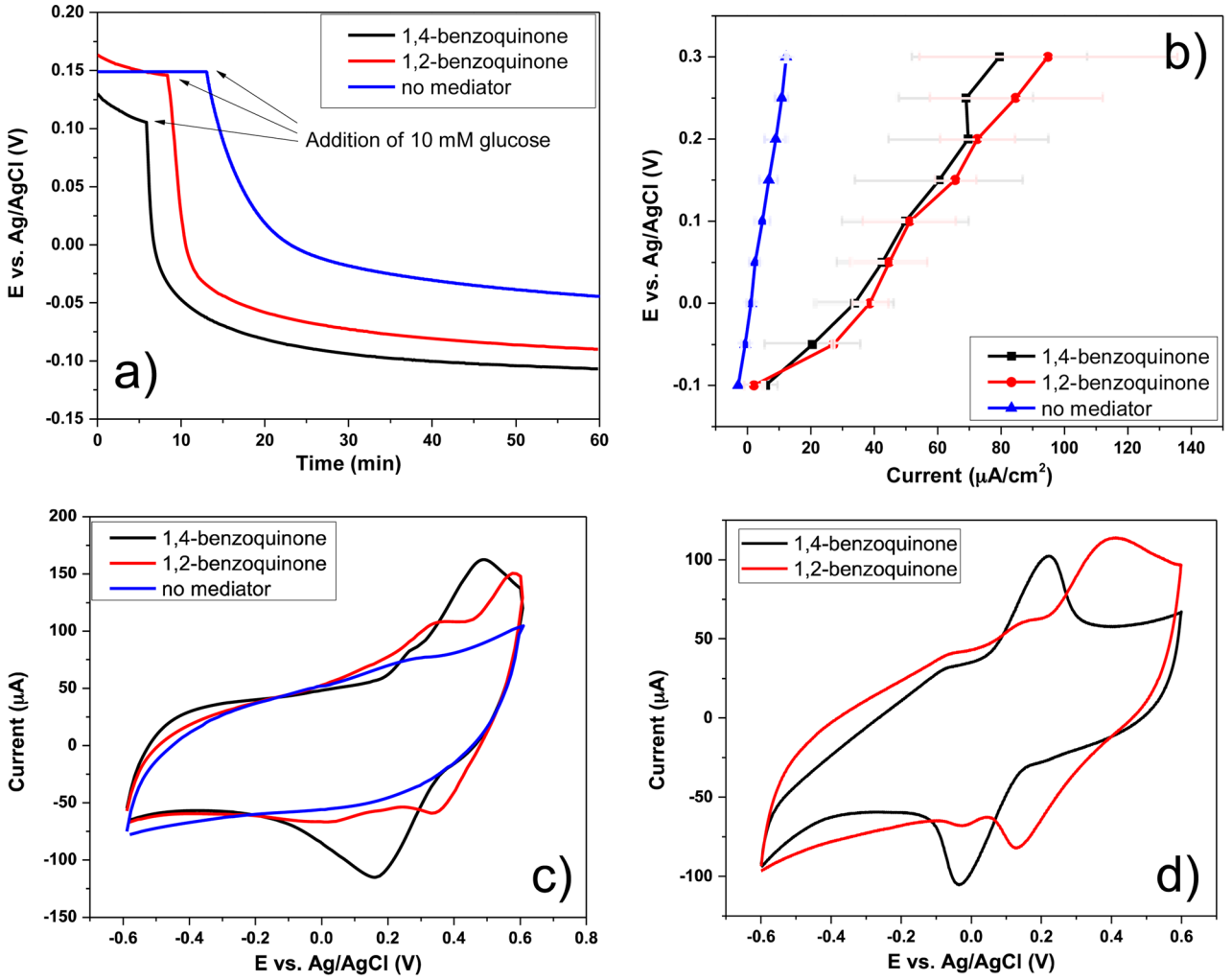

Figure 2. (a) OCP of PQQ–sGDH electrode in the presence of 2 mM 1,2- and 1,4-benzoquinones; (b) Average potentiostatic polarization curves of PQQ–sGDH electrodes without and with 2 mM solutions of 1,2- and 1,4-benzoquinones (n = 3). The error bars show the standard deviation of the current at each potential applied. (c) CV of PQQ–sGDH anodes in the presence of 2 mM solutions of 1,2- and 1,4-benzoquinones. (d) CV of 2 mM solutions of 1,2- and 1,4-benzoquinones in buffer on SWBP, 10 mV/s.

model with the highest affinity. To increase the reliability of the docking, in each case the docking simulations were performed three times. In addition, the convergence was tested by varying various parameters, such as the size of the search space or the exhaustiveness of the search, confirming that model 1, being the best fit with the highest affinity and the most frequently appearing model. Therefore, this model has been shown and used in this study. The structures of quinones in water were optimized using B3LYP/6-31G level of theory and the polarizable continuum model as implemented in the Gaussian 09 quantum chemical package,[25] while the structures of quinones on the graphene support were optimized using Density Functional Theory in the plane wave formalism with the vdW-DF functional proposed by Dion et al.[26,27] as implemented in VASP 5.2.[28−30] During the docking of 1,2- and 1,4-benzoquinones into PQQ–sGDH, both the amino acids from the glucose-binding pocket (GLN 76, ASP 143, HIS 144, LEU 169, GLN 168, ARG 228, TRP 346, and TYR 343[14]) and the benzoquinone molecules were treated as flexible and were allowed to change their conformation (Figures 3 and 4). When modeling the interactions between quinones (1,2- and 1,4-benzquinones and ubiquinone) adsorbed on a graphene sheet and PQQ–sGDH, the whole enzyme molecule was considered as rigid (Figures 5–7).

The crystallographic structure of the enzyme–glucose complex[14] was used when modeling the docking of the two benzoquinones in the presence of glucose. The amino acids from the binding-pocket were treated as flexible during the docking process (Figure 4b). It has to be mentioned that no significant differences in the benzoquinones docking in the presence of glucose were observed when the amino acid residues in the glucose-binding pocket were allowed to be flexible or were set as rigid (data not shown).

RESULTS AND DISCUSSION

In our previous work with soluble PQQ-dependent glucose dehydrogenase, it was established that organic compounds with quinone structure can be successfully explored as mediators.[19] The compounds used were ubiquinone, 1,2- and 1,4-benzoquinones, physically adsorbed on the surface of two types of nanotube paper–single and multiwalled buckeye paper. It was observed that when the mediators are immobilized on an electrode surface, regardless of the type of the nanotube paper used, the electron transfer rate from those compounds to the electrode surface and vice versa is the rate-limiting step in the operation of PQQ–sGDH anodes. The main focus of the study was on the mediator–support interactions assuming that the mediator-enzyme affinity is not the main parameter determining the electrodes' behavior. In this study, the mediator-enzyme interactions were further studied by using both the electrochemical methods and the computational approach for modeling the docking of the mediators into the enzyme molecule.

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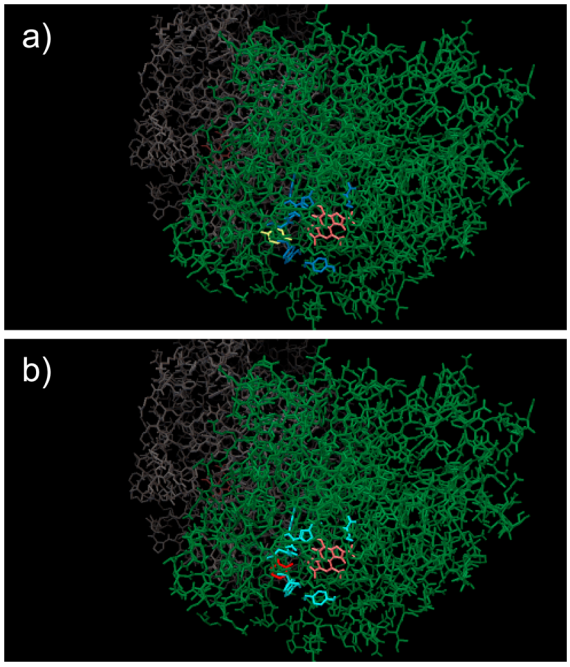

Figure 3. Docking of (a) 1,4-benzoquinone (yellow) and (b) 1,2-benzoquinone (red) in PQQ–sGDH modeled with AutoDock Vina. PQQ (pink) and the amino acids form the substrate-binding pocket (blue and cyan) are highlighted.

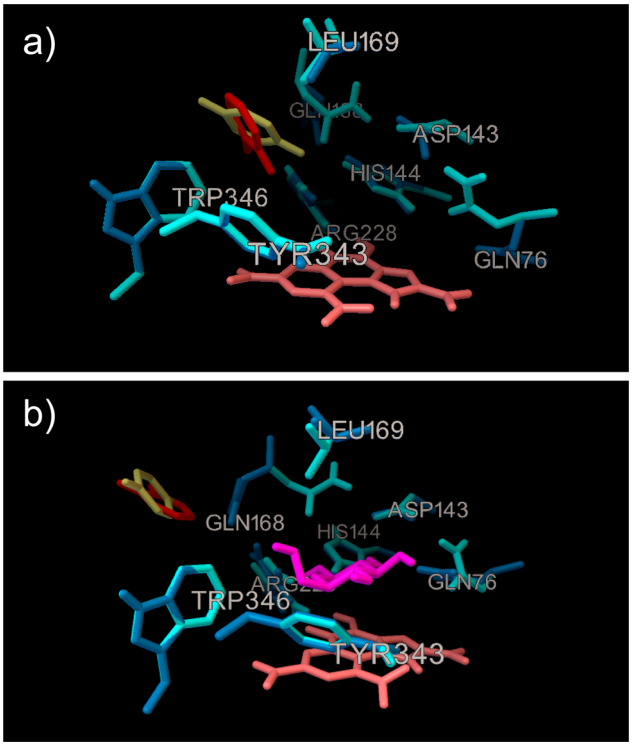

Figure 4. Docking of 1,4-benzoquinone (red) and 1,2-benzoquinone (yellow) in PQQ–sGDH in (a) absence of glucose and (b) in the presence of glucose (purple), modeled with AutoDock Vina. Only the substrate-binding pocket with PQQ (pink) is represented.

For the electrochemical experiments, 1,2- and 1,4-benzoquinones were used as mediators in solution (i.e., the compounds were dissolved in the electrolyte). Only single-walled buckeye paper (SWBP) was used as electrode material since it was

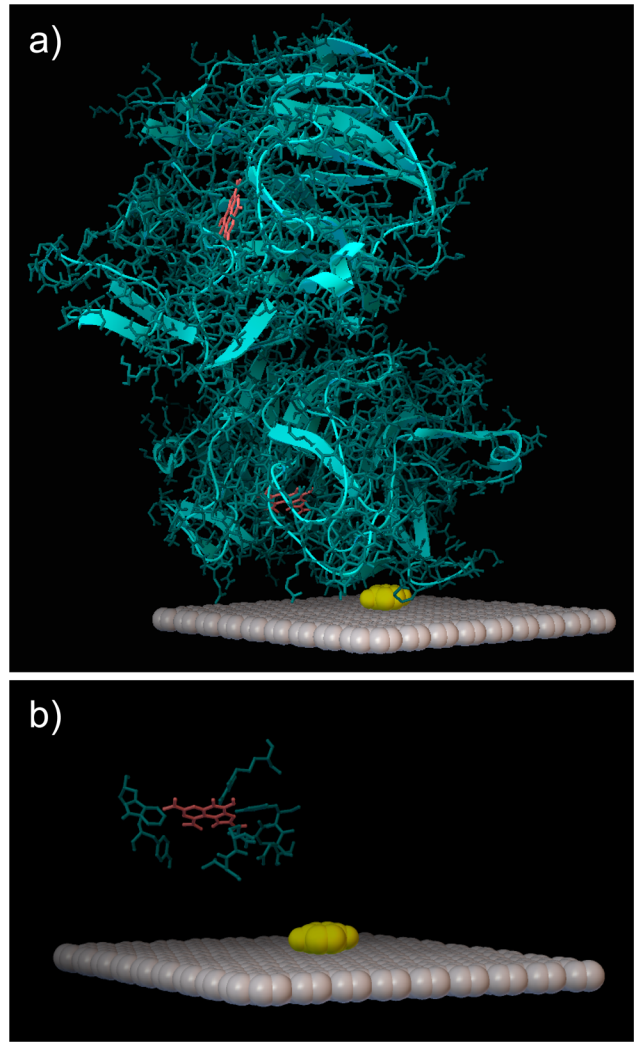

Figure 5. (a) Docking of 1,4-benzoquinone (yellow), adsorbed on a graphene sheet with PQQ–GDH modeled with AutoDock Vina. PQQ is represented as pink. (b) Only the enzyme substrate-binding pocket is shown.

established that PQQ–sGDH interacts better with single-walled than multiwalled nanotubes.[7,19] PQQ–sGDH was tethered to SWBP through the use of 1-pyrenebutanoic acid succinimidyl ester (PBSE), a very well-known enzyme tethering agent.[31−34] The paper with the immobilized enzyme was assembled onto cap glassy carbon electrode and tested in 20 mM MOPS with 6 mM $CaCl_2$, 10 mM KCl, 10 mM D-glucose and 2 mM 1,2- or 1,4-benzoquinone. The glucose was introduced in the electrolyte during the open circuit potential (OCP) measurement (Figure 2a). Obviously the presence of glucose led to a very fast drop in OCP to −0.09 V and −0.110 V vs Ag/AgCl in the presence of 1,2- and 1,4-benzoquinone, respectively. The decrease of OCP indicates preserved enzyme activity and successful glucose oxidation by PQQ–sGDH. The measured OCP of the PQQ–sGDH electrodes was significantly lower than the formal redox potentials of benzoquinones in the same electrolyte (0.260 and 0.090 V vs Ag/AgCl for 1,2- and 1,4-benzoquinone, respectively, Figure 2d) and more similar to the redox potential of PQQ in the enzymatic molecule.[7] Therefore, the observed OCP can be attributed to the direct interaction of the enzyme's cofactor and the electrode surface under open circuit conditions although the OCP in the presence of mediators is lower than just a PQQ–

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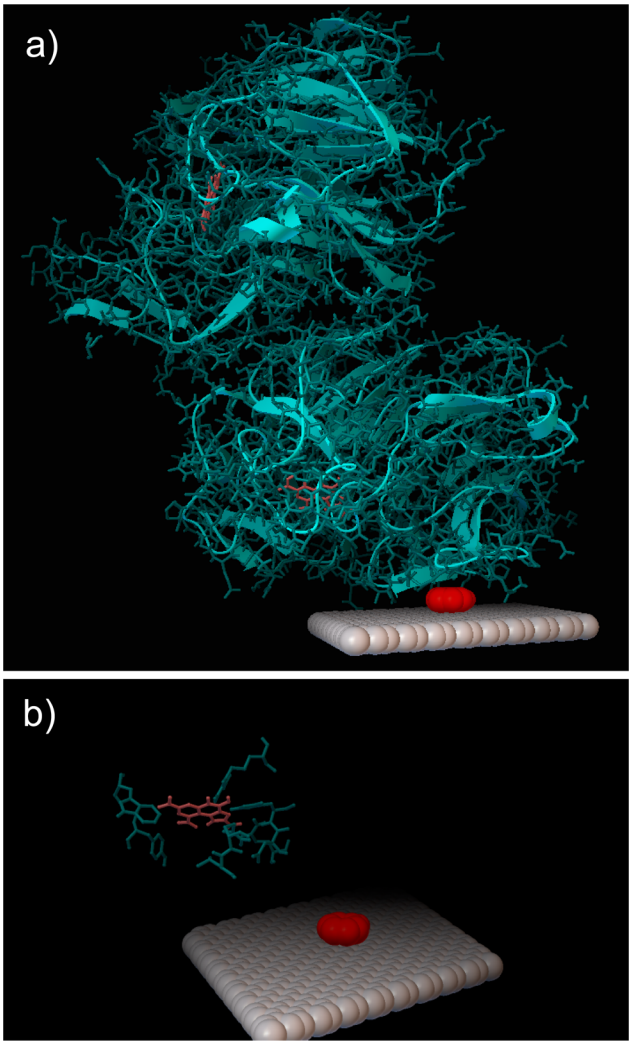

Figure 6. (a) Docking of 1,2-benzoquinone (red), adsorbed on graphene sheet with PQQ–GDH modeled with AutoDock Vina. PQQ is represented in the figure as pink. (b) Only the enzyme substrate-binding pocket is shown.

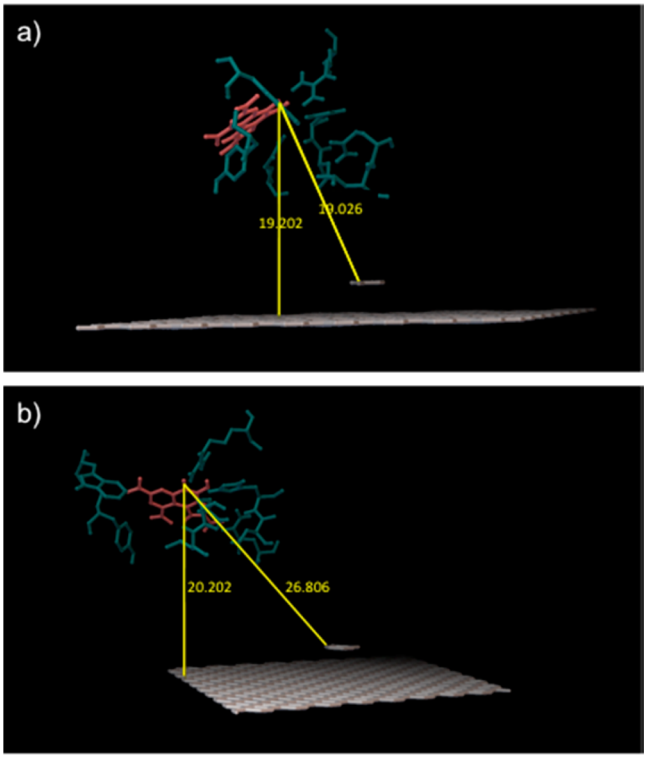

Figure 7. Docking of (a) 1,4-benzoquinone and (b) 1,2-benzoquinone, adsorbed on a graphene sheet with PQQ–sGDH modeled using AutoDock Vina. Only the enzyme substrate-binding pocket is shown. PQQ is colored pink and the amino acids from the pocket are cyan. The distances between the support and PQQ are also given.

sGDH anode. The latter indicates an interaction between PQQ and the mediators in the solution.

Polarization curves were carried out to electrochemically monitor the enzyme–mediator–support interactions under poised potentials (Figure 2b). Surprisingly, the current generated by PQQ–sGDH electrodes in the presence of the two mediators was identical. There was no statistical difference between the polarization curves recorded in the presence of two benzoquinones. In both cases though, the recorded current densities were higher than the control electrode with DET.

The performed cyclic voltammetry of the PQQ–sGDH anode in the presence of the mediators (Figure 2c) showed a pair of peaks for 1,4-benziquinone with a formal redox potential ($E^{0\prime}$) of 0.324 V vs Ag/AgCl. This is significantly higher than the $E^{0\prime}$ observed for 1,4-benzoquinone adsorbed on the same electrode material (0.150 V vs Ag/AgCl[19]) and 1,4-benzoquinone in solution (Figure 2d), both determined in absence of the enzyme.

For 1,2-benzoquinone, two pairs of redox peaks can be observed with $E_1^{0\prime}$= 0.161 V and $E_2^{0\prime}$= 0.458 V vs Ag/AgCl. These peaks can be assigned to the two steps of 1,2-benzoquinone redox reaction, each involving transfer of one electron.[35] The two separate steps in the presence (Figure 2c) and the absence (Figure 2d) of the enzyme can be attributed to a slow second redox step, which is in agreement with our previous observation showing lower electron transfer rate for 1,2-benzoquinone than for 1,4-benzoquinone on the same electrode material.[19] One oxidation peak at 0.240 V vs Ag/AgCl was detected on the CV of the PQQ–sGDH anode without a mediator, which is most likely due to the oxidation of glucose by the enzyme.

In contrast to the results reported herein (mediators in solution), when the mediators were adsorbed on the electrode material, the 1,4-benzoquinone PQQ–sGDH electrode outperformed the anode with 1,2-benzoquinone.[19] To understand the difference in behavior of the system when the mediators are in solution and adsorbed on the electrode surface, computational modeling was used to study: (1) The docking of the tested mediator molecules to PQQ–sGDH (Figure 3) and (2) the interactions of the enzyme with a support material, which has mediators adsorbed on its surface (Figure 4). For the docking calculations, optimized structures of the mediators in the solution or on the support were used.

On the basis of the results of benzoquinones' docking to the PQQ–sGDH molecule, it was established that both benzoquinones bind to the same portion of the enzyme molecule, into the glucose binding pocket and close to PQQ (Figure 3). A closer look reveals that the two benzoquinones position themselves at approximately the same distance from PQQ. The distances of 6.3 and 6.5 Å were calculated for 1,4- and 1,2-bezoqunone, respectively (Figure 4, Table 1). The latter can explain the identical electrochemical performance of the PQQ–sGDH anodes in the presence of the mediators. The small distance between benzoquinones and PQQ (<10 Å) allows an electron

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Table 1. Parameters Associated with the Tested Systems

	affinity kcal/mol	distance Å[a]	$E^{0\prime}$ vs Ag/AgCl, V[19]	diffusion coefficient, cm^2/s	current density[b], $\mu A/cm^2$	K_{ET}, s^{-1}[19]
1,4-benzoquinone	−4.8	6.3	0.324	2.70×10^{-6}[36]	69.6	0.190
1,2-benzoquinone	−4.8	6.5	0.458	4.20×10^{-6}[36]	72.5	0.154
1,4-benzoquinone ads.	−18.2	19.0	0.150		41[19]	0.190
1,2-benzoquinone ads.	−16.9	26.8	0.389		24[19]	0.154
glucose	−5.7	3.9		6.70×10^{-6}	9[a]	

[a]The distance between the docked molecules and the PQQ cofactor. [b]Current densities at 0.2 V vs Ag/AgCl; b Current densities for PQQ−sGDH anode without a mediator.

transfer to occur from the PQQ·H_2 to the mediator, leading to an efficient mediator reduction.

The docking simulations in the presence of glucose (Figure 3b) reveal that the mediators dock into the glucose binding pocket next to the glucose and, thus, both the substrate and the electron acceptor can interact with the enzymatic molecule at the same time leading to fast glucose oxidation and acceptor reduction. The higher diffusion coefficient of glucose as compared to benzoquinones and the higher affinity of the enzyme toward glucose (Table 1) could suggest that PQQ−sGDH first interacts with the glucose molecule and then with benzoquinones.

The calculated affinities of PQQ−sGDH for the two benzoquinones in solution are the same (Table 1). The PQQ−benzoquinone distances upon binding, benzoquinone diffusion coefficients, and the electrochemical performance of PQQ−sGDH anodes in the presence of the two mediators are also similar suggesting that either the mediator diffusion, enzyme affinity, or the distance of the mediator from the enzyme's coenzyme is the rate limiting factor when the mediators are freely diffusing in the electrolyte.

As reported in previous work, this is not the case when the same mediators are immobilized on the electrode surface. When benzoquinones are adsorbed on the support, a different electrochemical output of the enzymatic anode was observed.[19] Therefore, the next step in this study involved modeling of the interactions between the enzyme and the mediators adsorbed on a carbonaceous support (Figure 4).

In contrast to the enzyme-free mediator interactions where both benzoquinones interact with the enzyme in a similar fashion, when the mediators are adsorbed on a support, PQQ−sGDH positions itself relative to the modified support slightly different (Figures 5 and 6). The distance between PQQ and the mediator molecule is greater when 1,2-benzoquinone is used in comparison to 1,4-benzoquinone (Table 1). Namely, the distance between the mediator adsorbed on the graphene sheet and PQQ was determined to be 26.8 and 19.0 Å in the case of 1,2-benzoquinone and 1,4-benzoquinone, respectively. Alternatively, based on the calculated affinities (Table 1), the interaction between the enzyme and the surface modified with 1,4-benzoquinone is higher than the interaction between the enzyme and the surface modified with 1,2-benzoquinone. In general, due to the additional interactions of the enzyme with the support material (one can assume hydrophobic interactions) the binding affinity of PQQ−sGDH for the adsorbed benzoquinones seems higher than the binding affinity for the same molecules in the solution. However, the two are not directly comparable. We can only compare the affinity of the enzyme for the benzoquinones in the solution on the one hand, and the affinity toward the modified surfaces, on another.

Table 1 also compares the affinities of the enzyme toward benzoquinones and the distance of the mediators from PQQ for both mediators in solution and mediators adsorbed on the carbon support. Obviously, the affinity of the enzyme toward the modified carbon material is much higher in comparison to the mediators in solution, which can be attributed to the larger surface of the support material and the large number of amino acid residues able to interact with the support. The distance between PQQ and the mediators is significantly higher when the latter are immobilized on a support material than when they are freely diffusing in the solution. The higher tunneling distance implies lower electron transfer efficiency, which can also explain why 1,2-benzoquinone, adsorbed on the electrode surface, increased the generated current by 2.5 times. The difference in the electron transfer rate also has to be taken into account indicating 1,4-benzoquinone as the preferable mediator when this step is the rate limiting step.

On the basis of the position of PQQ relative to the support material and the mediator (Figure 7), it can be inferred that when 1,4-benzoquinone is adsorbed, the distance between PQQ and the mediator (19.0 Å) is comparable to the distance between PQQ and the support material (19.2 Å). In addition 1,4-benzoquinone has significantly higher electron affinity (the charge transfer was calculated as $-0.58e$ for the first and $-0.94e$ for the second electron being introduced)[19] than the carbon support, which will most likely lead to the facilitated 1,4-benzoquinone reduction and its participation in the electron transfer. Thus, one can conclude that the 1,4-benzoquinone plays a mediation role. At the same time, when 1,2-benzoquinone is adsorbed on the surface, due to the shorter distance between PQQ and the electrode surface (20.2 Å) in comparison to the PQQ/1,2-benzoquinone distance (26.8 Å), the enzyme will most likely transfer the electrons directly to the support surface. Consequently, the modifier will only play the role of an orienting agent by positioning PQQ closer to the electrode surface.

A similar conclusion can be made for the PQQ−sGDH anode modified with physically adsorbed ubiquinone, which in a previous study demonstrated 1.5 times increase in the current performance in comparison to unmodified PQQ−sGDH anode.[19] Figures 8 and 9 show the ubiquinone docking when ubiquinone is adsorbed on a graphene sheet. As it was discussed, two possible adsorption configurations of ubiquinone were considered, configuration 1 (Figure 8), in which the isoprenoid side chain of the ubiquinone is adsorbed on the support, while the quinone moiety is ∼10 Å away from the surface, and configuration 2 (Figure 9), in which both the quinone moiety and the isoprenoid side chain are adsorbed on the graphene surface.[19] As suggested by the modeling results, in both cases, the distance between PQQ of the enzymatic molecule and the support material is shorter than the distance between PQQ and the quinone moiety of ubiquinone. For configuration 1, the distance between PQQ and the graphene sheet was found to be 20 Å versus 23 Å, which was calculated as the distance between PQQ and the quinone moiety of ubiquinone. The latter distance

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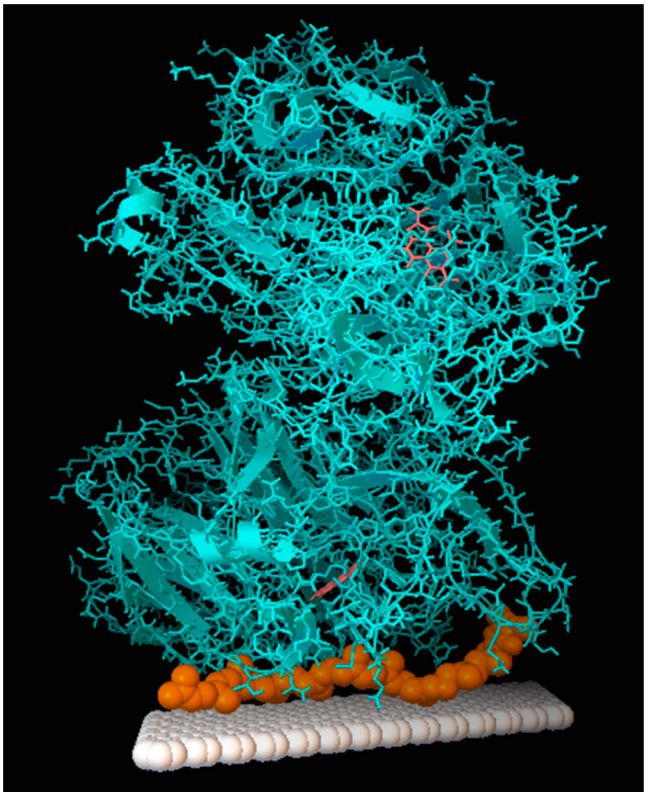

Figure 8. Docking of ubiquinone (configuration 1) adsorbed on a graphene sheet with PQQ–sGDH modeled using AutoDock Vina. PQQ is represented as pink.

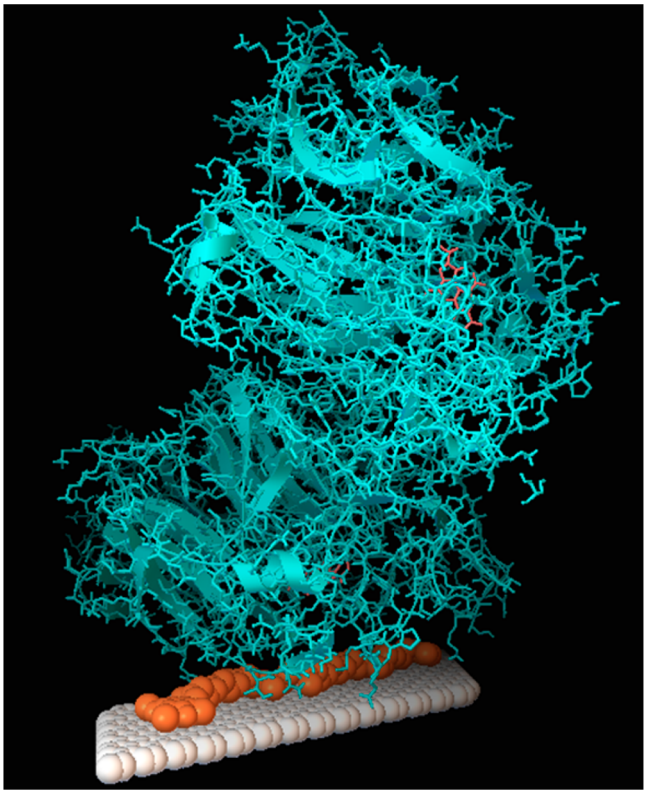

Figure 9. Docking of ubiquinone (configuration 2) adsorbed on a graphene sheet with PQQ–sGDH modeled using AutoDock Vina. PQQ is represented as pink.

is even larger in the case of configuration 2 (34.4 Å), while the PQQ–support surface distance was determined to be 19.7 Å. In a previous study, it was assumed that low electron affinity (charge being transferred to ubiquinone was calculated as $-0.12e$ for the first and $-0.28e$ for the second electron being introduced), accounts for the observance that ubiquinone most likely will not participate in the electron transfer but will provide enzyme orientation, which will position the enzyme's coenzyme closer to the electrode surface.[19] The low electron affinity of ubiquinone can be used as an indirect indicator for the low reduction ability of this compound. The modeling calculations performed in this study further confirm that conclusion and proposes a similar role for ubiquinone as for the adsorbed 1,2-benzoquinone. Unfortunately, due to the hydrophobic character of the isoprenoid side chain, ubiquinone is not soluble in aqueous solutions and cannot be experimentally tested as a mediator in solution. In addition, AutoDock simulations of the docking of free ubiquinone showed that this molecule interacts with a portion of the enzyme molecule positioned far away from PQQ (Figure 10a). This indicates that ubiquinone will not be a good

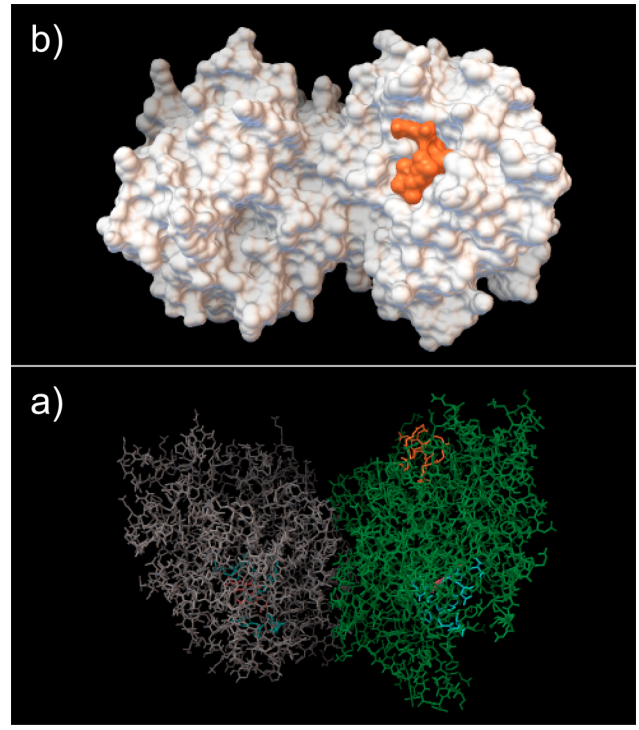

Figure 10. (a) Docking of ubiquinone (orange) in PQQ–sGDH modeled with AutoDock Vina. PQQ (pink) and the amino acids form the substrate-binding pocket (cyan) are highlighted. (b) The van der Waals surfaces of the enzyme and ubiquinone are plotted.

mediator in solution since the electrons have to travel longer distance through the enzymatic molecule in order to reach the mediator. However, a stereospecific pocket that fits the ubiquinone molecule can be seen in Figure 10b, which indicates that internal electron transfer could still be possible as PQQ–sGDH can interact with ubiquinone and use it as an electron acceptor in the natural environment.

When the characteristic parameters of PQQ–sGDH anodes, exploring 1,2- and 1,4-benzoquinones, were processed through Principal Component Analysis (Figure 11), it was established that principal component 1 (PC1) separates the samples with mediators in solution from the samples with mediators adsorbed

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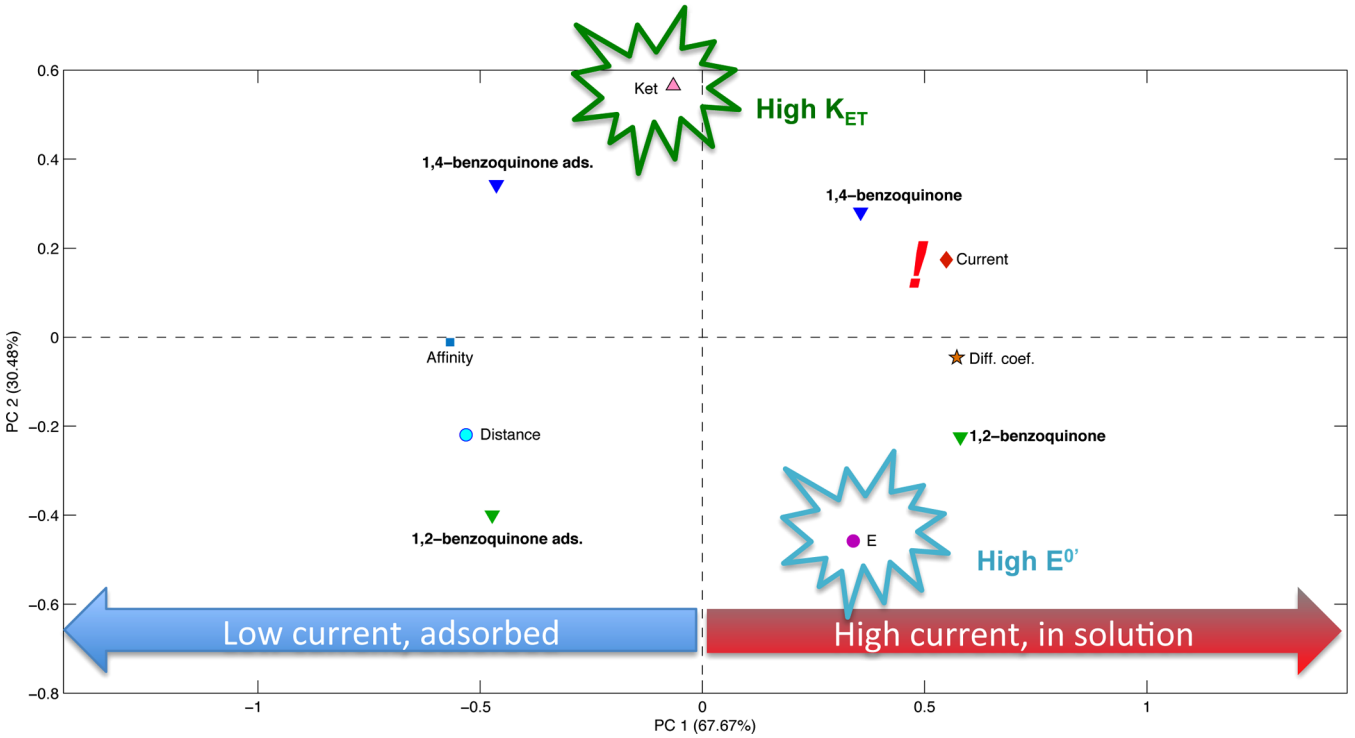

Figure 11. PCA biplot of scores (samples) and loadings (the variables) for PQQ-dependent GDH anodes with different mediators.

on the electrode surface. This separation, based on the mediator position in the system, fits well with the electrode's electrochemical performance. The higher currents observed with the free mediators are most likely due to the smaller distance between PQQ and the mediators when they interact with the enzyme. It also has to be taken into account that in all three cases involving immobilized mediators, the enzyme positions itself with the substrate-binding pocket facing the electrode surface and thus hinders the access of glucose, which can further explain the lower performance of the electrode.

Principal component 2 (PC2) divides the anodes based on the mediator used: 1,2- vs 1,4-benzoquinone. 1,4-Benzoquinone is characterized with higher K_{ET}, which provides faster electron transfer, and 1,2-benzoquinone is characterized with higher $E^{0'}$, determining the higher driving force for the electron transfer. Both parameters have proven to be important when benzoquinones are adsorbed on the electrode surface.

CONCLUSIONS

Molecular docking calculations were used for the first time in combination with experimental electrochemical methods to study the interactions between 1,2- and 1,4-benzoquinones in solution and PQQ-dependent soluble glucose dehydrogenase. The study was further expanded by analyzing the binding energies and interactions of PQQ–sGDH with benzoquinones in solution and with benzoquinones and ubiquinone adsorbed on the graphene sheet. It was established that the higher electrochemical performance of PQQ–sGDH anodes in the presence of free 1,2- and 1,4-benzoquinones is most likely due to the smaller distance between these molecules and PQQ in the enzymatic molecule. The simulations also show that in addition to the larger PQQ–mediators distance, in all three cases involving immobilized mediators, the enzyme positions itself with the substrate-binding pocket facing the electrode surface. This hinders the access of glucose, which further decreases the electrodes performance.

Furthermore, it was proposed that when 1,4-benzoquinone is adsorbed, it plays the role of a mediator. At the same time, when 1,2-benzoquinone is adsorbed on the electrode surface, the enzyme will transfer the electrons directly to the support, and the modifier will mostly play the role of an orienting agent, providing enzyme positioning with the PQQ closer to the electrode surface. A similar conclusion can be made for the PQQ–sGDH anode modified with physically adsorbed ubiquinone, for which a purely orientational role was proposed.

AUTHOR INFORMATION

Corresponding Author

*plamen@unm.edu

Author Contributions

§S.B. and I.M. contributed equally.

Notes

The authors declare no competing financial interest.

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