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Kılıç, M.; Ensing, B.

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Microscopic Picture of the Solvent Reorganization During Electron Transfer to Flavin in Water

Murat Kılıç^{*,†} and Bernd Ensing[‡]

Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Park 904, 1098 XH, Amsterdam, The Netherlands

Supporting Information

ABSTRACT: The redox potential of molecular species is largely modulated by its molecular environment so that a change of the environment will lead to a different redox potential. However, a detailed molecular picture of reorganization of the environment upon reduction is still unclear. To unravel the details of the solvent reorganization during electron transfer, we have performed density functional theory-based molecular dynamics (DFT-MD) and hybrid quantum mechanics/molecular mechanics (QM/MM) simulations of the reduction of lumiflavin. Previously, we have calculated the reduction free energy curves of the redox half reactions of lumiflavin in water as a function of the instantaneous gap energy (ΔE) (*J. Chem. Theory Comput.* **2013**, 9, 3889–3899). In this work, we focus on finding the changes in the solvent environment that correlate with this ΔE reaction coordinate. Comparing the QM/MM simulations, in which the solvent is modeled with an empirical force field, with the (full) DFT-MD simulations, we find that the response through electronic polarization plays a significant role in the latter case. Also a small charge transfer between flavin and solvent is observed in the full DFT treatment. As a result, we find only in the case of the QM/MM model a strong correlation between ΔE and the (pairwise computed) electrostatic potential (ESP) at the flavin due to the solvent. By analyzing the contribution of the ESP at the flavin per solvent molecule, we cannot only distinguish between the different modes of hydration by solvent molecules that coordinate at the hydrophilic and hydrophobic sides of the flavin molecule but also quantify their contribution to the reorganization free energy by measuring the ESP fluctuations per solvent molecule.

INTRODUCTION

Electron transfer (ET) is a fundamental phenomenon in chemistry, physics, and biology.^{1–5} ET is ubiquitously found in nature, for example, in the photosynthetic reaction centers of plants and bacteria, while in redox chemistry, ET takes place between reacting species, for example, molecular oxygen and a metal surface undergoing corrosion. Also solid state electronics and novel molecular electronics depend on the control of ET in semiconductors and between conductive molecules.

The redox potential is the degree to which a molecular species in the condensed phase can accept or donate electrons. The redox potential is modulated by the molecular environment, which is often a solvent. The environment is polarized by the initial charge distribution of the reacting species and responds to the ET by a reorganization, which in turn affects the ET between the species. The environment reorganization involves both changes in the electronic polarization and changes in the positions and orientations of atoms and molecules. In water solvent, this may lead to changes in the coordination of the electron donor and acceptor species as well as more subtle rearrangements in the hydrogen bond network around the solutes. Understanding how different molecular environments affect the redox potential of a molecule requires a detailed picture of these complex rearrangements that make up the environment response. However, the microscopic

details of the environment reorganization are still poorly understood.

In Marcus theory of electron transfer, the complex role of the environment in the ET process is described by a single well-chosen parameter: the vertical energy gap, ΔE .^{6,7} The vertical energy gap is the energetic cost to transfer the electron at fixed nuclear positions, which largely depends on the environment polarization (the instantaneous geometry of the solutes themselves may also have an influence). By using the energy gap as the reaction coordinate, the free energy profiles of the two reactant and product states can be represented by a pair of crossing parabolas with identical curvature. Marcus theory assumes that the molecules undergoing ET are surrounded by an environment that responds in a linear manner to the amount of charge transferred. Previously, we have computed the free energy profiles as a function of ΔE for the first and second reduction reactions of lumiflavin in water using density functional theory based molecular dynamics (DFT-MD) simulations.⁸ Lumiflavin is one of the smallest members of the class of flavin derivatives that all contain an aromatic heteronuclear three-ring (isoalloxazine) motif, as

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Figure 1. Chemical structure of lumiflavin (LF) and its protonated form (LFH). The M10 methyl group is replaced by longer tail units in riboflavin, flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN). In the reduced states of the flavin, LFH, the N5 atom is readily protonated.

illustrated in Figure 1. Flavins can accept up to two electrons and two protons and play as such an important role in a wide range of biological redox processes. Their redox properties and protonation states have been the topic of several experimental^{9–13} and numerical^{14–19} studies. Our DFT-MD simulations differ from previous calculations in that we sample explicit solvent configurations. This way we discovered that for the second flavin reduction, the solvent responds in a nonlinear fashion through a coupling with the so-called butterfly bending of the flavin molecule.⁸

In the current work, we focus our attention on a detailed analysis of the aqueous solvent response upon flavin reduction. In addition to our previous DFT-MD simulations of lumiflavin in the oxidized, singly reduced (semiquinone), and fully reduced states, we perform simulations at intermediate, fractionally charged states to assess the solvent reorganization in smaller steps. We also perform hybrid QM/MM simulations, in which the solvent is modeled using a (MM) force field of pairwise potentials that couples to the DFT (QM) treated flavin. By comparing the QM/MM results to those from the (full) DFT model, we can assess the electronic solvent response, which is only present in the latter. The hybrid QM/MM approach also allows for larger systems and thus for analysis of the longer-range solvent response. Hereafter, we first briefly describe the background of Marcus' theory of electron transfer that underlies our calculations and the computational details of the QM and QM/MM simulations. The Results section describes first our calculations of lumiflavin in the gas phase, then we characterize the structure of the water solvent around lumiflavin in the different oxidation states with QM/MM and QM, and finally we present the role of electrostatic potential of the first and second reduction processes of lumiflavin in water.

METHODS

Calculation of Redox Potential. Redox potentials are directly proportional to the free energy difference between the oxidized and the reduced state.^{20,21} This free energy difference can be calculated using a free energy perturbation approach that was pioneered by Warshell et al.²² and in recent years further developed in the context of DFT-MD simulation by Sprik and co-workers.^{23–30} Here, the free energy profile is calculated by measuring the vertical energy gap, ΔE , which is the energy needed to add an electron to the system,

$$\Delta E = E_{\text{R}}(\mathbf{r}^{\text{N}}) - E_{\text{O}}(\mathbf{r}^{\text{N}}) \quad (1)$$

along a simulation trajectory in the oxidized or reduced state. The free energy $A_{\eta}(\Delta E)$ is computed from the histogram of the gap energy, $P_{\eta}(\Delta E)$:

$$A_{\eta}(\Delta E) = -k_{\text{B}}T \ln[P_{\eta}(\Delta E)] \quad (2)$$

with η indicating either the oxidized or the reduced state. In the case of linear response of the solvent environment, as assumed in Marcus' theory of electron transfer, the probability functions $P_{\eta}(\Delta E)$ are Gaussian and the free energy curves are parabolic with identical curvature. The reaction free energy, ΔA , and the reorganization free energy, λ , are then given by the following well-known equations,

$$\Delta A = \frac{1}{2}(\langle \Delta E \rangle_{\text{R}} + \langle \Delta E \rangle_{\text{O}}) \quad (3)$$

and

$$\lambda = \frac{1}{2}(\langle \Delta E \rangle_{\text{O}} - \langle \Delta E \rangle_{\text{R}}) \quad (4)$$

respectively, in which the brackets, $\langle \dots \rangle_{\eta}$, denote the average value in oxidation state η . The reorganization free energy can in principle also be obtained from the fluctuations in the vertical energy gap,

$$\lambda_{\eta} = \frac{\sigma_{\eta}^2}{2k_{\text{B}}T} \quad (5)$$

with σ^2 the variation in ΔE , with T the absolute temperature, and with k_{B} the Boltzmann constant. Although we previously established that the amplitude of the fluctuations is systematically underestimated by our relatively short DFT-MD simulations,⁸ we can nevertheless use this quantity to compare QM versus MM solvent as well as to assess the contributions from different solvent molecules.

Adding the solvent reorganization free energy from eq 5 to the average energy gap provides an alternative means to eq 3 to obtain the reaction free energy:

$$\begin{aligned} \Delta A_{\text{O}} &= \langle \Delta E \rangle_{\text{O}} - \lambda_{\text{O}} \quad \text{and} \\ \Delta A_{\text{R}} &= \langle \Delta E \rangle_{\text{R}} + \lambda_{\text{R}} \end{aligned} \quad (6)$$

although the limited statistics of the DFT-MD estimate of λ_{η} from the fluctuations makes these numbers less reliable than when only the first moments of ΔE are used as in eq 3.

Apart from studying lumiflavin in the oxidized and reduced states, we can use computer simulations also to examine the system at (fictitious) fractional electron transfer states. This

Figure 2. Top panels: distributions of the vertical energy gap from DFT-MD simulations of lumiflavin in the vacuum at six values of the coupling parameter, η , at $T = 300$ K (left) and $T = 350$ K (right). Bottom left panel: free energy curves at different temperatures. The inset shows the extrapolation to the “transition state” at $\Delta E = 0$ (see legend). Bottom right panel: free energy curves obtained at three levels of approximation (ΔE_O and ΔE_R , Gaussian fits, and WHAM). See text for further details.

allows us to scrutinize in more detail how the solvent responds to a (partial) change of the solute charge. To model a partially charged system, the simulation is performed using a mixed Hamiltonian that is a linear superposition of the neutral (oxidized) and anionic (reduced) Hamiltonians:

$$H_\eta = \eta H_R + (1 - \eta) H_O \quad (7)$$

With the coupling parameter η equal to zero, the Hamiltonian is that of the lumiflavin in the neutral oxidized state, whereas with $\eta = 1$, the Hamiltonian is that of the anionic reduced state. An intermediate value allows for simulation on a fictitious potential that is a superposition of the two redox states, which corresponds to a state with a fractional electron. Thus, every MD step two DFT calculations are performed for the nuclear configuration in the electronically oxidized and reduced states (note that one state has an unpaired electron, so that one of the two calculations is an unrestricted DFT calculation), after which the system is propagated by mixing the nuclear forces from the two states analogous to eq 7. The ΔE is obtained from the H_R and H_O energies, which are printed to output in addition to the H_η energy. The $P_\eta(\Delta E)$ distribution obtained at a fictitious fractional charged state can be unbiased and used to improve the computed free energy landscape in the otherwise poorly sampled transition state region where the parabolas cross. To unbiased a probability distribution, $P_{\eta_2}(\Delta E)$, measured at a fractional value η_2 , to obtain the probability distribution, $P_{\eta_1}(\Delta E)$, at the fully oxidized or fully reduced state η_1 , requires a reweighting using

$$P(\Delta E)_{\eta_1} = e^{-\beta(\eta_2 - \eta_1)\Delta E} \langle P(\Delta E) \rangle_{\eta_2} \frac{Z_{\eta_2}}{Z_{\eta_1}} \quad (8)$$

in which the ratio of the partition functions, $\frac{Z_{\eta_2}}{Z_{\eta_1}}$, can simply be obtained by normalization of $P_{\eta_1}(\Delta E)$. Similarly, the free energy profile at η_2 is unbiased using

$$A(\Delta E)_{\eta_1} = (\eta_2 - \eta_1)\Delta E - k_B T \ln[\langle P(\Delta E) \rangle_{\eta_2}] + c \quad (9)$$

A derivation of the above two reweighting formulas is given in the [Supporting Information](#). The piecewise free energy profiles obtained at different values of η are combined to the profiles of the oxidized and reduced states using the weighted histogram analysis method (WHAM).³¹

Computational Details. We have performed classical force field (MM) simulations, DFT-based (QM) molecular dynamics simulations, and hybrid QM/MM simulations of lumiflavin in water. The classical MD and QM/MM models contained a single lumiflavin (LF) or protonated lumiflavin (LFH) molecule and about 10 465 solvent water molecules in a cubic, periodically replicated box of length 68.57 Å. The (full) QM representation of these systems contained the flavin with 102 water molecules in a cubic box of length 15.148 Å.

The Amber ff99SB force field³² with the flexible SPC water model was used for the MM representation of the system. The MM system was equilibrated using the Gromacs (4.5)³³ classical MD program. The final configurations were used as input for subsequent QM/MM calculations, in which the flavin was treated at the QM level of theory and the water solvent MM. The QM/MM simulations were performed with the CP2K program (v.2.2).^{34,35} The cubic QM box size was 18.0 Å in the QM/MM representation.

The QM representation employed DFT with the Perdew–Burke–Ernzerhof (PBE)³⁶ exchange–correlation functional and pseudopotentials of the Goedecker–Teter–Hutter (GTH) type, based on the parametrization of Hartwigsen–Goedecker–Hutter.^{37,38} We used a Gaussian basis set designed specifically for these pseudopotentials, of double- ζ quality

Figure 3. Vertical energy gap distributions (top panels) and free energy curves (bottom panels) of the first reduction of lumiflavin in water computed in full QM (left-hand-side) and using the hybrid QM/MM model (right-hand-side). The blue and red free energy curves are obtained from the corresponding fully oxidized ($\eta = 0$) and reduced ($\eta = 1$) Gaussian fits of $P(\Delta E)$ using eq 2. The black dots show the result combining all five distributions using eq 9 and the weighted histogram analysis method (WHAM).

with polarization functions (DZVP³⁹). An auxiliary planewave basis was used to expand the electron density with a cutoff of 280 Ry. Unrestricted DFT was used to describe the electronic doublet state of the singly reduced flavin molecule.

The DFT-MD simulations used the Born–Oppenheimer method with a time step of 0.5 fs in the canonical (NVT) ensemble. The temperature was controlled with a canonical sampling through velocity rescaling (CSVR)⁴⁰ thermostat with a characteristic frequency of 100 cm^{−1}.

RESULTS

Reduction of Lumiflavin in Vacuum. We performed 30 ps long DFT-MD simulations of lumiflavin in vacuum at two different temperatures $T = 300$ and 350 K and six different values of the coupling parameter $\eta = (0, 0.2, 0.4, 0.6, 0.8, 1.0)$, in which $\eta = 0$ represents the neutral oxidized species and $\eta = 1$ the anionic reduced species (see also eq 7). Figure 2 shows in the top panels the probability functions of the vertical energy gap, $P(\Delta E)$, which show the expected Gaussian shape, although at $T = 350$ K some variation in the width of the distributions is seen, mostly likely due to the reduced ergodicity of this low-dimensional system. The solid lines are obtained by fitting the data (shown in circles) with a Gaussian curve. We can compute the free energy curves from the energy gap distributions at three different levels of approximation. In the first manner, we only use the values obtained for the average energy gap for the neutral lumiflavin, $\langle \Delta E \rangle_{\text{O}}$, and the anionic molecule, $\langle \Delta E \rangle_{\text{R}}$. Using Marcus' assumption that the two curves should be parabolic with the same curvature and vertically shifted by ΔE , the curves can be written as

$$\Delta A_{\text{O}} = \frac{(\Delta E - \langle \Delta E \rangle_{\text{R}})^2}{2(\langle \Delta E \rangle_{\text{O}} - \langle \Delta E \rangle_{\text{R}})} - \Delta E \quad (10)$$

and

$$\Delta A_{\text{R}} = \frac{(\Delta E - \langle \Delta E \rangle_{\text{R}})^2}{2(\langle \Delta E \rangle_{\text{O}} - \langle \Delta E \rangle_{\text{R}})} \quad (11)$$

Second, we can obtain the two free energy curves from the Gaussian fits to the distribution functions, $P_0(\Delta E)$ and $P_1(\Delta E)$, using eq 2. In the third manner, we do not assume that the distribution should be Gaussian and thus that the curves should be parabolic. Instead, we reweight the measured histograms of ΔE to correct for the bias induced by the mixed Hamiltonian approach to recover the free energy curves using eq 9 and WHAM³¹ to combine the six curves to either the $\eta = 0$ curve or the $\eta = 1$ curve. The bottom right panel in Figure 2 shows that the three levels of approximation result in very similar free energy curves for the reduction of lumiflavin in the gas phase. The bottom left panel shows that the free energy curves at $T = 300$ and $T = 350$ K do not differ from the zero Kelvin potential energy curves obtained from geometry optimizations. These results will serve as a reference in the discussion of the results for the reduction reactions of lumiflavin in water solvent presented next.

Reduction of Lumiflavin in Water Solvent. Figure 3 shows the results for the (first) reduction reaction of lumiflavin in aqueous solution, with on the left-hand-side the results from the full QM simulations and on the right-hand-side the result from the hybrid QM/MM model. Here, we compare the results from the QM/MM simulations performed at $T = 300$ K with QM simulations done at a somewhat elevated temperature of $T = 350$ K to compensate for the previously observed overstructuring in DFT-PBE water (see also ref 8). The top panels in Figure 3 show the normalized $P(\Delta E)$ distributions that we computed at five values of the coupling parameter $\eta = (0.00, 0.25, 0.50, 0.75, 1.00)$. The histograms (shown by dots) are well fitted by Gaussian functions (solid lines). The fluctuations in ΔE , as represented by the widths of the

Table 1. First Four Rows: First and Second Moment of the Vertical Energy Gap Distributions Obtained from the Gaussian Fit Functions, With in Parentheses an Error Estimate in the Last Two Digits^a

method	QM/MM	LF → LF [−]		LFH → LFH [−]		QM	eq
		solute only	QM	vacuum	QM/MM		
T/K	300	300	350	300	300	350	
⟨ΔE⟩ _O	−1.34 (02)	−1.62 (01)	0.47 (02)	−1.62 (00)	−1.12 (06)	0.42 (05)	
⟨ΔE⟩ _R	−4.28 (02)	−1.97 (00)	−0.87 (03)	−1.95 (00)	−4.06 (06)	−0.89 (05)	
σ _O	0.26 (00)	0.14 (01)	0.19 (00)	0.09 (00)	0.28 (02)	0.17 (01)	
σ _R	0.26 (00)	0.10 (00)	0.18 (01)	0.09 (00)	0.28 (02)	0.24 (01)	
λ _O	1.28	0.38	0.59	0.16	1.55	0.49	5
λ _R	1.27	0.19	0.55	0.16	1.50	0.95	5
λ	1.47	0.18	0.67	0.17	1.47	0.65	4
ΔA _O	−2.62	−1.52	−0.12	−1.78	−2.67	0.07	6
ΔA _R	−3.01	−1.87	−0.31	−1.79	−2.56	0.06	6
ΔA	−2.81	−1.80	−0.20	−1.79	−2.59	−0.24	3
ΔA′	−2.81		−0.20	−1.80	−2.59	−0.24	2

^aLast seven rows: redox properties derived from the ΔE distributions using the equations listed in the last column. See text for further details.

distributions, are seen to be very similar for each of the five (fractional) oxidation states of lumiflavin. However, comparing the QM with the QM/MM results, we note a large shift of the distributions along the x -axis as well as broader $P(\Delta E)$ distributions in the QM/MM case (note the different scales on the x -axis). As a result, also the free energy profiles shown in the bottom panels are significantly different, with a double-well shaped profile for the QM case on the left, while on the right-hand-side the QM/MM result showing the oxidized state curve (blue) always above the reduced state curve (red). The latter shape of the free energy landscape, which we already saw in the gas-phase (see the inset in the bottom-left panel of Figure 2) is known as the “Marcus inverted region”.

Apart from the different representation of the solvent in the QM and QM/MM simulations, an important difference between the two models is the size of the periodic box and the number of solvent water molecules in it. The QM box has an edge of about 15 Å, which is very small so that the electron added to the system feels a strong interaction with its infinite array of periodic images. Also the polarized solvent is more affected by the finite size effects in the QM case. The periodic QM/MM box is much larger, with an edge of almost 70 Å and as a first approximation we could consider to neglect the finite size effects on the QM/MM results. The QM/MM free energy landscape can then be seen as resulting from the gas phase profiles but with a significant lowering of the relative free energy of the aqueous reduced state. That is, in the reduced state, the polarized solvent interacts strongly with the anionic flavin, making removal of the electron energetically costly, and resulting in an average ΔE of −4.28 eV. Indeed, without solvent the average ΔE is −1.95 eV (these numbers are also found in the second row in Table 1). In the neutral oxidized state, the solvent is (on average) not polarized and should therefore ideally not affect the cost to add an electron with respect to the gas phase. In practice, we find that ΔE = −1.34 eV in the QM/MM solvent, somewhat less negative than in the gas phase (−1.62 eV). This means that the solvent structure is still somewhat polarized by the neutral amphiphilic flavin molecule and stabilizing the oxidized state more than the reduced state.

The finite size effects on the solvation free energy, electron transfer free energy, and reorganization free energy have been investigated using continuum theory and simulations with explicit solvent molecules in several studies.^{41–43} The

reorganization free energy was found to effectively scale inversely proportional with the box size, $\lambda \propto L^{-1}$ (although Seidel et al.⁴³ also found a good fit taking $\lambda \propto L^{-1/2}$), while ΔA effectively scales inversely proportional with the box volume, $\lambda \propto L^{-3}$. Alaya and Sprik⁴² use the following dependence of ΔA and λ on the box size based on the continuum Born model:

$$\Delta A(L) = \Delta A_{\infty} - (q_{\text{O}}^2 - q_{\text{R}}^2) \frac{2\pi R^2}{3L^3} = \Delta A_{\infty} - \gamma_3 L^{-3} \quad (12)$$

$$\begin{aligned} \lambda(L) &= \frac{1}{2}(q_{\text{O}} - q_{\text{R}})^2 \left(\frac{1}{\epsilon_{\text{opt}}} - \frac{1}{\epsilon_{\text{stat}}} \right) \left(\frac{1}{R} + \frac{\xi_{\text{EW}}}{L} \right) \\ &= c_1 + \gamma_1 L^{-1} \end{aligned} \quad (13)$$

in which R is the effective spherical radius of the solute, q is its charge, and ϵ_{opt} and ϵ_{stat} are the optical and static dielectric constants, respectively. On the right-hand-side, we have simplified these expressions, using the proportionality constants γ_3 and γ_1 and the constant c_1 . Ayala and Sprik find for γ_3 a value of −439 for a $\text{M}^{1+}/\text{M}^{2+}$ metal ion oxidation and a value of −1080 for a $\text{M}^{2+}/\text{M}^{3+}$ oxidation. For γ_1 , which should not be dependent on the value of the initial charge as seen in eq 13, they find −13.3 and −14.3, respectively.

When we now turn to the QM case with the much smaller box size, we find $\langle \Delta E \rangle_{\text{R}} = -0.87$ and $\langle \Delta E \rangle_{\text{O}} = 0.47$ eV, resulting in a much less negative ΔA = −0.20 eV compared to the QM/MM result of ΔA = −2.81 eV. If we were to assume that this difference were solely due to the difference in box size, this would give us a proportionality constant of $\gamma_3 = -1303$. The QM/MM and QM values for the reorganization free energy of 1.47 and 0.67, respectively, would give $\gamma_1 = -9.9$. Not surprisingly, these numbers are rather different from the numbers found by Ayala and Sprik, because also the difference in the models has a significant effect on ΔA and λ. The main difference is of course the electronic polarization of the solvent that is only taken into account in the QM case. Neglecting the electronic polarization is expected to lead to a too high reorganization free energy, as $\epsilon_{\text{opt}} = 1$ for the static MM charges, rather than $\epsilon_{\text{opt}} \approx 2$. There may also be differences in the first coordination shell structure and hydration between the two models.

A final remark to make about the QM and QM/MM free energy landscapes shown in Figure 3 is that the results from the simulations at intermediate (fractional) oxidation states, η , confirm the linearity of the solvent response during the first reduction reaction. This is shown by the very similar widths of the distributions, $P_\eta(\Delta E)$, and also by the averages, $\langle \Delta E \rangle_\eta$, which we have plotted as a function of η in Figure 4. Indeed

Figure 4. Vertical energy gap, $\langle \Delta E \rangle_\eta$ of the half reaction $\text{LF} + \text{e}^- \rightarrow \text{LF}^-$ in vacuum ($T = 0$ and $T = 300$ K) and in water ($T = 300$ K and $T = 350$ K) as a function of the amount of “lumiflavin reduction”, η . The curves are almost perfectly linear as expected for systems that follow Marcus behavior. The slopes equal twice the reorganization free energy.

the response of the environment, represented by ΔE as the reaction coordinate, is almost perfectly linear with the amount of added charge. But what are the specific changes in the solvent structure that make up the response of the system? In the rest of this paper, we will answer this question by analyzing (1) the inner sphere reorganization of the lumiflavin molecule itself, (2) the reorganization of the first coordination shell structure, (3) the electronic polarization of the solvent, and (4) the changes of the electrostatic potential at the flavin due to the solvent.

Inner Sphere and Solvent Reorganization, Solvation Shell Structure Rearrangement. The response of the lumiflavin molecule (i.e., the inner sphere reorganization) upon adding an electron was already discussed previously for the flavin in vacuum, where we showed that the structural changes largely match the structure of the lowest unoccupied molecular orbital (LUMO) that becomes filled.⁸ This leads to a small reorganization (free) energy of $\lambda = 0.17$ eV. Is the inner sphere response the same in aqueous solution? To answer this question, we examined the structures of the lumiflavin molecule taken from the QM/MM aqueous solution trajectories and recomputed $P_\eta(\Delta E)$ after stripping the flavin from its solvent environment, i.e., in vacuum and without periodic boundaries. The results are compiled in Table 1 in the column labeled “Solute only”. The close match of $\langle \Delta E \rangle_\eta$, ΔA , and λ with those for the lumiflavin in vacuum, indicates that the distribution of lumiflavin geometries is very similar in vacuum and in solution.

The reorganization of the solvation shell structure can be analyzed by comparing the radial distribution functions of water atoms around the lumiflavin in the oxidized and reduced states. This detailed analysis was done for the QM model in ref 8, in which we reported a number of changes that take place in the hydrogen bonded network at the hydrophilic side of the lumiflavin. For the QM/MM model the hydration, and in

particular the changes in hydration upon flavin reduction, of the QM lumiflavin by the MM water depends critically upon the parametrization of the coupling between the two representations. An initial QM/MM simulation of lumiflavin in water, using the default parametrization of the cp2k program did not show the expected hydrogen bonding of the solvent toward the O2, O4 and N5 atoms. Optimization of the radial cutoff parameter for hydrogen to a value of 0.25 Å and for oxygen to a value of 0.78 Å resulted in the expected solvation shell structure, using the QM result as a reference. A comparison of the radial distribution functions is shown in Figure 5. The QM/MM recovers qualitatively the same first solvation shell structure in the neutral and anionic states. There is a somewhat greater structure at the carboxylic oxygens (O2 and O4) in both reduction reactions and the N5 in the LF/LF[−] state. Also plotted are the radial distribution functions obtained from the intermediate η value simulations. It would be interesting to see whether the changes in the first solvation shell also take place in a linear degree, as the linearity of $\Delta E \propto \eta$ suggests. Unfortunately, this first shell reorganization is rather subtle and second our simulations may be too short to distill accurate distributions. However, a significant change takes place at the N5 atom (its coordination number increases from 0.6 to 1.9 during the first reduction reaction⁸), and a gradual increase of the first peak is indeed seen from the intermediate distribution functions.

Solvent Reorganization, Electronic Effects. Part of the solvent response to addition of an electron to the solute is through electronic polarization. To examine the extend of this electronic polarization and how it is distributed around the lumiflavin, we have computed the Mulliken charges of the QM system (lumiflavin and 102 water molecules) for each time frame, before and after adding (or subtracting) the electron. This was done for the simulation in the oxidized (LF) and first reduced (LF[−]) states as well as for the second reduction, LFH and LFH[−], states.

Figure 6 shows snapshots from simulations of the aqueous lumiflavin in the different states, in which the solvent atoms are color coded to represent the time averaged change in the Mulliken charges upon the vertical excitation (i.e., adding or subtracting the electron). Note that this representation is only possible because the molecules do not significantly diffuse during the short time span of 2.5 ps over which the average is taken. The main polarization is seen to take place at the hydrogen bonded solvent atoms, which we therefore show in ball–stick representation. The dark blue to dark red color code represents a change in Mulliken charge ranging from −0.009 to 0.068 electron. In each of the four states, a similar pattern is seen: a strong electronic polarization takes place at almost all first coordination shell water molecules, especially at the H-bonded molecules at the hydrophilic side of the flavin but also at several first shell molecules that flank the ring-system at the side. The charge shift is away from the flavin upon adding an electron, making the H-bonded atom more positive and the other atoms more negative. Note that some of the polarized solvent molecules show significantly more negative charge (blue) than positive charge (red), which indicates an additional change in the net charge of the molecule due to intermolecular charge transfer.

Most likely, this charge transfer takes place between H-bonded solvent molecule and the lumiflavin, which could spill out some electron charge, after adding the electron, to nearby water molecules. Note that the surplus blue coloring could also

Figure 5. Radial distribution of solvent water around selected atoms of lumiflavin at $T = 350$ K for QM(QM) and at $T = 300$ K for QM/MM. Left panels: neutral lumiflavin (black lines) and reduced anionic lumiflavin (magenta). Right panels: neutral semiquinone state, LFH (black) and doubly reduced anionic flavin, LFH^{•−} (magenta).

Figure 6. Average change of the atomic (Mulliken) charges upon adding an electron to the neutral flavin (left panels) and upon removing an electron from the anionic species (right panels), shown using snapshots from the full QM simulations of the first reduction reaction of flavin (top) and the second reduction (bottom) at $T = 350$ K. The color coding ranges from -0.009 (dark blue) to 0.068 electron (dark red). The images are generated with VMD.⁴⁴

be explained by a charge transfer from solvent molecules to lumiflavin *before* adding the electron, as this state is the reference state to compute the difference in the Mulliken charges. Indeed, the latter is the case, as is seen from the total Mulliken charge on the lumiflavin atoms shown in Figure 7. Irrespective of the oxidized ($\eta = 0$) or reduced ($\eta = 1$) state of the system, the total charge on the flavin is fluctuating around -1 when the electron is added but somewhat negative by -0.2 electron for the neutral flavin. Clearly, both the electronic polarization of solvent molecules and the charge transfer between solute and solvent are not taken into account in the QM/MM model, so that a difference in the results for the redox properties should be expected between the QM and QM/MM models.

Solvent Reorganization, Electrostatic Potential, and Fluctuations. The solvent response through a change in the static polarization (here “static” means “not electronic”) is due to a reorientation of the solvent molecules, which carry a nonuniform electronic charge distribution. This in turn changes the electrostatic potential experienced by the solute, which couples directly to ΔE . In the case of the QM/MM model, we can compute the electrostatic potential, V^{elec} , at the lumiflavin due to the solvent using

Figure 7. Charge of lumiflavin computed as the sum of the Mulliken charges of the lumiflavin atoms during 2.5 ps of the QM simulations of LF (black) and LF[−] (red) in water, before and after adding or subtracting the electron. Note the systematic deviation from zero charge at the neutral LF due to a small charge transfer from the solvent.

$$V^{\text{elec}} = \frac{1}{4\pi\epsilon} \sum_i \frac{q_e q_i}{|\mathbf{r}_c - \mathbf{r}_i|} \quad (14)$$

in which the sum runs over all solvent atoms, with q_i the MM charge of the oxygen or hydrogen atom i and $\mathbf{r}_i$ its position. For simplicity, we compute V^{elec} at the center of mass of the lumiflavin, $\mathbf{r}_c$, and since we are interested in the potential felt by the added electron, q_e equals -1 . Long range contributions are included using Ewald summation.

The left panel in Figure 8 shows the electrostatic potential at the flavin center versus ΔE in the QM/MM case. The plots combine the data taken from all five simulations at different fractional oxidation states, for both the first and second reduction reactions. The correlation between V^{elec} and ΔE is very high, with a correlation coefficient of 0.99. Such a strong correlation is even somewhat surprising, because we only consider V^{elec} at the lumiflavin center, whereas we know that the electron added to the flavin is rather delocalized over the three-ring system.⁸

For the QM solvent, it is less trivial how to compute the electrostatic potential at the flavin due to the solvent. We have approximated V^{elec} in this case in two manners, which give qualitatively the same result. We first computed V^{elec} using eq 14 and taking for the q_i the MM charges for the solvent oxygen

and hydrogen atoms. In the second manner, we use instead the Mulliken charges (before adding or removing the electron) for q_i . The latter V^{elec} is shown versus ΔE with black dots in the right-hand-side panel of Figure 8. Clearly, the correlation, indicated with the straight line fitted through the data points, is much weaker compared to that of the QM/MM case. Apparently, the electrostatic potential computed by summing over MM or Mulliken charges does not capture well the static solvent polarization in the QM case.

For the QM/MM case, the electrostatic potential captures the solvent reorganization rather well. Since V^{elec} is computed by simply adding the contributions from each of the solvent molecules, we can examine the contributions from the separate water molecules. Figure 9 shows the time average contribution

Figure 9. Snapshots of aqueous lumiflavin in which each solvent molecule is color coded to illustrate its contribution to the average electrostatic potential at the lumiflavin center. The color range runs from -0.08 eV (dark red) to 0.04 eV (dark blue). The averages are taken over the last 2.5 ps of the QM simulation. The images are generated with VMD.⁴⁴

of each water molecule to the electrostatic potential experienced by the lumiflavin in the different oxidation states. Here the color-coding of bright red to bright blue runs from -0.08 to 0.04 eV. Perhaps, not surprisingly, the water molecules in the first coordination shell display the strongest

Figure 8. Electrostatic potential, V^{elec} , at the center of mass of lumiflavin due to the water solvent versus ΔE in the QM/MM simulations (left panel) and the QM simulations (right panel). V^{elec} was computed with eq 14 and summing over all SPC water model charges in the QM/MM model, whereas in the full QM case the oxygen and hydrogen Mulliken charges were used.

contribution to V^{elec} , with molecules that donate a hydrogen bond giving a positive contribution (blue) and molecules that are more oriented with the oxygen toward the flavin contributing negatively to V^{elec} . Of course, this does not imply that V^{elec} is short-ranged as beyond the first shell the contribution is distributed over larger and larger shells of water molecules. Comparing the oxidized state (left-hand-side) to the reduced state (right-hand-side), we also observe the (average) reorientation of water molecules upon reduction as a color shift from predominantly red to more blue. Note that solvent molecules that contribute strongly to the electrostatic potential at the flavin directly affect ΔE and therefore ΔA and the redox potential. Moreover, solvent molecules that display a change in electrostatic potential upon flavin reduction contribute to the reorganization free energy.

The contribution to the reorganization free energy per solvent molecule can also be visualized by computing the second moment of the V^{elec} per molecule. This is shown in Figure 10, in which the color coding from white to bright blue

Figure 10. Average fluctuations (σ) of the electrostatic potential that each water molecule induces at the lumiflavin center illustrated by coloring the solvent molecules of a representative snapshot. The color range runs from 0 (white) to 0.136 meV (dark blue).

refers to average fluctuations (here taken as the standard deviation, σ) in V^{elec} that run from 0 to 0.136 meV respectively. Again, the major distribution arises from solvent molecules in the first coordination shell. Interestingly, however, the molecules that are H-bonded to the hydrophilic side of the flavin are seen to contribute much less to the solvent reorganization than the molecules flanking the three-ring system from the side. Apparently, the hydrophilic side of the solute, which takes part of the hydrogen bond network of the solvent, is hydrated by solvent molecules that then contribute to the average electrostatic potential but hardly to the reorganization free energy as they cannot reorient. Instead the under-coordinated solvent molecules at the hydrophobic sides of the lumiflavin are much more flexible to reorient their dipole and thus contribute much more to the solvent reorganization.

CONCLUSIONS

We argued at the beginning of this article that a detailed molecular picture of the environment reorganization during a

reduction reactions was still unclear. In order to unravel the environment response to an electron transfer reaction, we have studied the first and second reduction reactions of lumiflavin in gas phase and water solution at $T = 300$ K and $T = 350$ K, using DFT-based molecular dynamics simulations at the DFT-PBE level of theory and hybrid quantum mechanics/molecular mechanics (QM/MM). Comparing these two methods, we have found that the response through electronic polarization plays a significant role in the simulations. Moreover, the QM/MM model allows for larger systems and thus for analysis of the longer-range solvent response. We find that the curvature of the parabolic free energy profiles does not depend on the (fractional) charge of the flavin, which is in perfect agreement with the linear response assumption that underlies Marcus's theory of electron transfer. However, the classical treatment of the solvent in the QM/MM model leads to an almost twice as large reorganization free energy compared to that in the fully QM case. Although this can be largely explained by the difference in system size, we suspect that also the lack of the electronic polarization (which provides an instantaneous relaxation of the electrostatic interaction upon a change in the flavin charge) in the MM solvent can result in an increased solvent reorganization.

Lumiflavin is an amphiphilic molecule with on one side a hydrophobic methyl substituted carbon ring and at the other end a heteroatomic ring with nitrogen atoms and carbonyl groups that form hydrogen bonds with the water solvent. Radial distribution functions of the flavin atoms with respect to the solvent atoms reveal a pronounced solvent structure at the hydrophilic part of the flavin, which shows small differences between the neutral and anionic states of the molecule. In the reduced state, a small increase of the first RDF peaks indicate a strengthening of the hydrogen bonds with respect to that in the oxidized state. At the N5 position in the middle ring, a more significant reorganization takes place: this nitrogen atom is not involved in hydrogen bonding in the oxidized state but can accept up to two hydrogen bonds after reduction. Simulations of partially charged lumiflavin show that this H-bond affinity increases gradually upon charging. The radial distribution functions of the full DFT and the hybrid QM/MM models show a similar picture, although the RDFs of the carbonyl groups is somewhat more structured in the QM/MM model.

Apart from the changes in the solvent coordination and the hydrogen-bonding with the flavin, the solvent reorganization occurs mainly through changes in the electronic and static polarization of the solvent. By computing the average difference in the Mulliken charges of the solvent atoms before and after adding (or subtracting) an electron to (or from) the flavin, we show that the electronic polarization is indeed significant, especially at the first coordination shell solvent molecules that form hydrogen bonds to the flavin, but in a lesser extend also at the water molecules further away. The Mulliken charges also reveal a small but significant charge transfer that takes place from the solvent to the flavin in the oxidized state.

For the QM model, we have computed the electrostatic potential at the lumiflavin due to the solvent atoms using either the Mulliken charges or taking fixed MM charges at the solvent atom positions. In both cases, the electrostatic potential correlates poorly with the vertical gap energy, ΔE . Instead, in the QM/MM case, the correlation between ΔE and the electrostatic potential at the flavin computed by summing over

the solvent MM charges is very strong. This shows that the solvent reorganization is captured very well by regarding only the change in the electrostatic potential in the QM/MM case, whereas in the QM case this relation is less clear, most likely due to additional relaxation due to the electronic polarization and charge transfer.

By further partitioning the average electrostatic potential at lumiflavin in its contributions per solvent molecule, we find that the first coordination shell water molecules that donate a hydrogen bond to the flavin induce a positive potential, whereas the water molecules that accept a H-bond and also the non-hydrogen bonded solvent molecules that flank the aromatic ring system mainly contribute a negative potential. As the average electrostatic potential directly affects the average ΔE , and thus the redox potential, we predict that there is a relation between the number of H-bond accepting or donating substituents of a compound and its redox potential. Finally, by mapping the standard deviation in the electrostatic potential contribution per solvent molecule, we find that the fluctuations are small for the hydrogen bonded water molecules. This confirms that these molecules contribute little to the reorganization free energy, in agreement in the small changes seen in the RDFs. Instead the water molecules that flank the lumiflavin on the side are seen to show the largest contribution to the fluctuations, together with the water molecules that form H-bonds at the N5 upon flavin reduction.

■ ASSOCIATED CONTENT

Supporting Information

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Derivation of unbiasing the fractional electron transfer state simulations (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: murat.kilic@epfl.ch.

ORCID

Murat Kılıç: 0000-0002-1249-0460

Bernd Ensing: 0000-0002-4913-3571

Present Address

[†]M.K.: Laboratory of Computational Chemistry and Biochemistry (LCBC), Institute of Chemical Sciences and Engineering, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

Notes

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