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# Simulating biological charge transfer: Continuum dielectric theory or molecular dynamics?

David Gnandt, Sehee Na, Thorsten Koslowski*

*Institut für Physikalische Chemie, Universität Freiburg, Albertstraße 23a, 79104 Freiburg im Breisgau, Germany*

A B S T R A C T

We study the thermodynamic parameters of Marcus's theory of charge transfer, the driving forces and the reorganization energies, using two widely applied approaches to bioenergetic problems that seem to be radically different: continuum dielectric theory via a numerical solution of Poisson's equation, and the thermodynamic integration approach based upon classical Newtonian molecular dynamics, as perfomed by Na et al., PCCP 19, 18,938 (2017). With application to a nitrite reductase NrfHA protein heterodimer, we obtain an excellent agreement between the respective driving forces with an r.m.s. deviation of 1.7 kcal/mol, and a lower limit to the reorganization energies. The computational methods turn out to be mutually supportive: molecular dynamics can be used to determine the parameters of a dielectric theory computation, which on the other hand can be used to properly rescale the reorganization energies and partition them into aqueous and protein contributions. In addition, we use the electrostatic approach to study the influence of $Ca^{2+}$ ions on the free energy landscape of charge transfer.

## 1. Introduction

Electron transfer is a paradigmatic, apparently simple reaction that has fascinated experimentalists and theorists alike. The synthesis of the Creutz-Taube mixed valence ruthenium complex [1, 2], Marcus' theory of charge transfer [3, 4], the first structural characterization of a membrane protein performing photosynthesis [5, 6], or the understanding of charge transport in doped polymers [7, 8] are landmarks of chemistry. Electron transfer plays an essential role for all living organisms. Photosynthesis, respiration, sensing, signalling, or DNA damage and repair are examples of charge transfer processes inherent to many – and sometimes all – forms of life on our planet. Biopolymers undergoing charge transfer provide important benchmarks for theoretical approaches, such as free energy methods, that find ubiquitous application in the de novo design of drugs [9, 10, 11].

From a theoretical perspective, charge transfer reactions are described by Marcus' seminal theory and its extensions [3, 4, 12, 13]. Marcus' perspective is a combined thermodynamic and quantum mechanical one, expanding the free energy of the reaction, $\Delta G$, parabolically around the electron donor and the acceptor states, as depicted in Fig. 1.

The curvature of the parabola is proportional to the reorganization energy $\lambda$, the minima show an energy difference given by the thermodynamic driving force, $\Delta G$, and the two centers of electron localization are coupled by the electronic tunnel splitting, $t$. In the nonadiabatic or small-coupling regime, the electron transfer rate can be computed by

$$k_{CT} = \frac{t^2}{\hbar}\sqrt{\frac{\pi}{\lambda k_B T}}\exp\left\{-\frac{(\Delta G+\lambda)^2}{4\lambda k_B T}\right\} \quad (1)$$

Knowledge of the donor-acceptor tunnel splitting $t$ requires a quantum chemical calculation [14, 15, 16, 17, 18] or the application of phenomenological schemes such as the Dutton-Moser rule [19, 20, 21] or the pathways concept of Beratan, Onuchic and Gray [22, 23, 24].

On the other hand, $\Delta G$ and the outer sphere reorganization energy can be computed within a classical framework. Here, concepts from two realms of classical physics reign. The first makes use of dielectric continuum theory, as expressed by the Poisson equation, which is equivalent to Gauss' law or one of Maxwell's equation of classical electrodynamics [25]. In Marcus' original approach [4], $\lambda$ and $\Delta G$ have been estimated analytically for a simple model of two spheres immersed in a polarizable dielectric environment. For this arrangement, analytical results have also been obtained for the Poisson-Boltzmann equation, which extends the Poisson equation by an additional source term, stemming from an ionic cloud. Whereas it is painstakingly difficult to handle more complex environments in this manner [26], a numerical treatment of the Poisson-Boltzmann equation has opened the way towards the computation of thermodynamic properties for models of biopolymers [27, 28, 29]. Here, protein models and their environments are subdivided into regions carrying charges and those characterized by a non-uniform dielectric medium. Discretized on a lattice, the Poisson equation can be solved very efficiently by numerical methods appropriate to large sparse systems of equations. This concept has been implemented into widely used program packages such as DelPhi [30],

* Corresponding author.
*E-mail address:* thorsten.koslowski@physchem.uni-freiburg.de (T. Koslowski).

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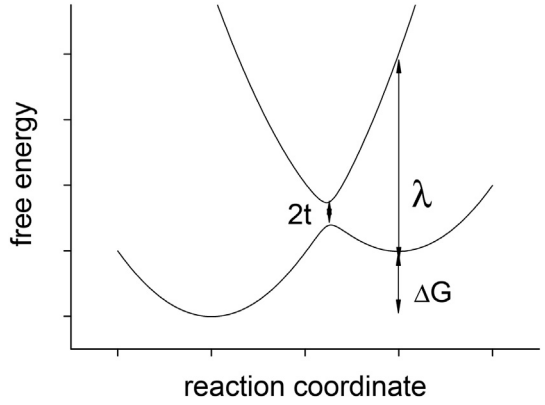


**Fig. 1.** Free energy of a charge transfer reaction according to Marcus' theory. Minima of the free energy curve are expanded parabolically, and are coupled by a tunnel matrix element *t*. Further labels refer to the reorganization energy, λ, and the thermodynamic driving force, Δ*G*.

APBS [31, 32], GMCT [33], a list by no means exhaustive [28, 29]. The continuum approach does, however, lack at least some of the local response, dynamics and flexibility of an atomistic view. Usually, protein dielectric constants are used as uniform parameters, they range from 1 to 2 [34, 35] over 8–10 [36, 37], 11 [38] to 20 [39], to give some examples. Dry protein powder exhibits an experimental dielectric constant of $\epsilon = 4$ [40]. Often, proteins are partitioned into regions characterized by different dielectric constants, see e.g. [41, 42]. A Gaussian smoothing approach to protein dielectrics has been suggested by Li et al. [43].

The second approach to the computation of the thermodynamic properties relevant to biological charge transfer is based upon classical Newtonian mechanics. Biopolymers and their environment are described by atomistic, classical force fields, and the atomic coordinates are propagated in time. Here, the thermodynamic integration approach has turned out to be both effective and statistically accurate [44], particularly if supplemented by a least-squares thermodynamic network analysis [45, 46]. As a drawback, molecular dynamics usually requires the full atomistic resolution of an x-ray or NMR structure or of a corresponding homology model as an initial structure. Computed Δ*G* and λ values can be benchmarked against experimental information: the driving forces correspond to differences in midpoint potentials (at least for weakly interacting electrons), and λ values are available indirectly from a variety of experiments, such as the measurements of charge transfer rates against driving forces [47]. The reorganization energy is ususally split into an inner sphere molecular and an outer sphere polarization part [48]. For the system studied here, the latter contribution will turn out to be dominant, and we will focus on this quantity.

As an example, we study the nitrite reductase membrane protein complex of a sulphate-reducing bacterium, *Desulfovibrio vulgaris Hildenborough* [49, 50]. Bacteria of this type play an important role in a global biogeochemical process, the nitrogen cycle [51, 52]. It involves the conversion of nitrogen compounds covering a variety of oxidation states – e.g. ammonia, molecular nitrogen, nitrite and nitrate – that are interconnected by reactions that transfer electrons and protons [53, 54]. The growth of organisms is often restricted by to the lack of nitrogen in an accessible form [53], although the element is the most abundandant in the atmosphere of the earth. Many bacteria catalyze the reaction of nitrogen compounds to ammonia,

$$NO_2^- + 6\,e^- + 8\,H^+ \rightleftharpoons NH_4^+ + 2\,H_2O \qquad (2)$$

using nitrite reductase enzymes and are thus able to solely rely on this substrate [55, 49]. In these enzymes, electrons and protons have to be transferred sequentially to the reactive site. For certain classes of protobacteria, NrfA builds a complex with NrfH, a cytochrome *c* containing four heme cofactors [56, 50]. The resulting complex can be found on

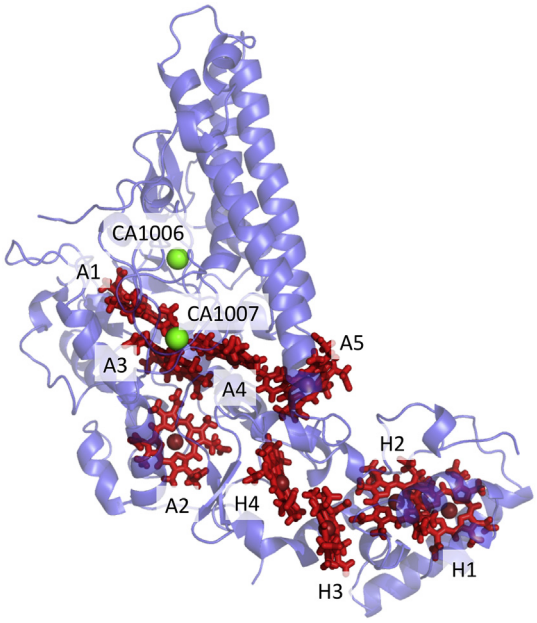


**Fig. 2.** Cartoon model of the $NrfH_2A_4$ aggregate following the x-ray structure of Rodrigues et al. [50]. The NrfA and NrfH protein chains are colored in purple, the heme cofactors in red, and the $Ca^{2+}$ ions in green.

the periplasmic side of the the membrane [56, 50, 51, 57]. Here, we study $NrfH_2A_4$ from *D. vulgaris*, which consists of six protein chains that host a total of 28 heme molecules [50]. We study one of the NrfH chains that contains four heme cofactors, and a NrfA subunit that hosts five hemes, including the active center, A1. The simulated system is shown in Fig. 2. The hemes are in a low spin state, with the exception of the unusually coordinated high spin cofactors A1 and H1. To our knowledge, this structure is at present the only one that has been solved by x-ray diffraction for a protein complex that contains both NrfA and NrfH chains, it is available in the absence [50] and in the presence [58] of an inhibitor. Isolated NrfA chains, on the other hand, have been characterized structurally for a number of organisms: *Sulfurospirillum deleyianum* [59], *Thioalkalivibrio paradoxus* [60], *Desulfovibrio desulfuricans* [61], *Thioalkalivibrio nitratireducens* [62, 63], *E. coli* [64, 65, 66], *Shewanella oneidensis* [67, 68], *Wolinella succinogenes* [69, 70, 71] and *Desulfovibrio vulgaris* [50, 58]. The four heme molecules of the NrfH protein chain are believed to gather and conduct electrons, which enter the system from the quinone pool [56, 58]. The NrfA hemes are supposed to represent the active site, or participate in electron storage and transfer [56]. Molecular dynamics simulations of the thermodynamic integration type indicate that the free energy surface within the NrfA unit changes from organism to organism, despite a considerable structural similarity between the proteins [72, 46].

The remaining part of this work is organized as follows. The methods are described in the next section, including a brief recall of the molecular dynamics simulations and a more detailed description of the dielectric continuum theory computations. In the third section, we compare thermodynamic driving forces and reorganization energies, and we apply the computational scheme to investigate the influence of $Ca^{2+}$ ions on the free energy surface. Conclusions will be derived in the final section.

## 2. Methods

### 2.1. Dielectric theory

Both the molecular dynamics simulation and the dielectric theory computations are based on the x-ray crystal structure of the $NrfH_2A_4$ aggregate [50], with the protein database identification 2J7A.