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Review

Quantum Tunneling in Biological Reactions: the Interplay between Theory and Experiments

José N. Onuchic,*[,a] Chigusa Kobayashi[b] and Kim K. Baldridge[a,c]

[a]*Center for Theoretical Biological Physics, University of California at San Diego, La Jolla, CA 92093, USA*

[b]*Department of Theoretical and Computational Molecular Science, Institute for Molecular Science, Myodaiji, Okazaki 444-8585, Japan*

[c]*University of Zürich, Organic Chemistry Institute, Winterthurestrasse 190, 8050 Zürich, Switzerland*

Ricardo Ferreira foi o primeiro cientista brasileiro a compreender a necessidade de aproximações teóricas sólidas para explicar os mecanismos que governam as ciências da vida. Nesta edição em sua homenagem, descrevemos como modelos teóricos têm sido aplicados para guiar o entendimento do fenômeno de tunelamento de elétrons em biologia. Durante quase vinte anos, o nosso modelo de "Pathways" tem se mostrado o mais bem sucedido modelo teórico para descrever o mecanismo de tunelamento no processo de transferência de elétrons em sistemas biológicos, particularmente em proteínas. Recentemente, o modelo de Pathways foi generalizado para incluir o efeito da dinâmica de proteínas na modulação do Fator Franck-Condon e dos elementos de tunelamento da matriz. A interferência entre diferentes trajetórias modula as interações de tunelamento de elétrons em proteínas (particularmente interferências destrutivas), e efeitos resultantes da dinâmica de proteínas são de importância crítica. O tunelamento de elétrons pode ser controlado por conformações de equilíbrio da proteína, as quais parecem ser necessárias para minimizar os efeitos de interferência destrutiva. Em contraste, quando configurações de equilíbrio apresentam pouca interferência destrutiva, o tunelamento de elétrons é promovido por uma (ou algumas) etapa(s) construtivamente interferente e os efeitos dinâmicos são modestos. Este novo mecanismo resultou na previsão de várias constantes de velocidades para reações de transferência de elétrons que foram confirmadas experimentalmente.

Ricardo Ferreira was the first Brazilian scientist to understand the need of solid theoretical approaches to obtain quantitative understanding mechanisms governing the life sciences. Therefore, in this issue in his honor, we decided to describe how theory has been able to guide the understanding of electron tunneling in biology. During almost twenth years, our *Pathway* model has been the most powerful model in terms of predicting the tunneling mechanism for electron transfer in biological systems, particularly proteins. Recently, we have generalized the conventional *Pathway* models to understand how protein dynamics modulate not only the Franck-Condon Factor but also the tunneling matrix element. The interference among pathways modulates the electron tunneling interactions in proteins (particularly destructive interference), and dynamical effects are of critical importance. Tunneling can be controlled by protein conformations from equilibrium, which may be needed to minimize the effect of destructive interference during tunneling. In contrast, when equilibrium configurations have small destructive interference, electron tunneling is mediated by one (or a few) constructively interfering pathway tubes and dynamical effects are modest. This new mechanism has predicted several experimental rates that were later confirmed by experiments.

Keywords: electron transfer, protein dynamics, quantum interference

1. Introduction

Understanding the mechanisms governing biomolecular machines requires more than simple hypothesis. There is a need for solid theoretical approaches and model development. Ricardo Ferreira realized this need early in his career. He has made seminal contributions to many problems, including enzymatic catalysis, biogenesis, and chirality in proteins. Therefore, to his honor, in this paper we decided to describe how thermal motions are important for control in biomolecular machines. Thermal motions may be global, affecting phenomena such as allostery, and

*e-mail: jonuchic@ucsd.edu

folding, or local, such as in electron transfer (ET). In ET, structural fluctuations on the picosecond time scale and sub-angstrom structural fluctuations may be sufficient for substantial changes in quantum interference.

ET reactions occur in many important mechanisms in living systems. ET is involved in the early steps of bioenergetic processes, such as photosynthesis and oxidative phosphorylation, enzymatic catalysis, DNA damage and drug metabolism. Typically, the protein environment mediates biological ET and tunneling takes place over large separations (5 to 20 Å), leading to a weak tunneling coupling between the donor (D) and the acceptor (A) sites. This coupling is termed the electronic matrix element (T_{DA}). In this weak coupling regime, the ET rates are in the nonadiabatic regime and may be written in a perturbation theory expression as the product of the square of T_{DA} and the probability of the donor and acceptor forming a resonant activated complex.[1,2] This latter term is the well-known Marcus theory for electron transfer.[3] In the early 1990's, Beratan and Onuchic developed the *Pathways* method to estimate values of T_{DA}, and to explain the physics behind the mechanism of tunneling through proteins.[4] *Pathways* demonstrates that tunneling occurs via a dominant pathway tube (a family of similar pathways), and that the decay through this tube can be quantified as a product of contributions from covalent bonds, hydrogen bonds, and through-space jumps.

This original *Pathways* description, although very successful in explaining many experimental results and enabling design of novel ET proteins, is only applicable when a single tube dominates the reaction. In 2000, theoretical work by Balabin and Onuchic demonstrated that, when ET reactions are dominated by a single pathway tube (or a few tubes that interfere constructively), the protein can be treated "statically" and effects due to protein dynamics are minor and therefore can be ignored.[5] The situation is exactly the opposite when ET involves multiple tubes with destructive interference. T_{DA}'s become extremely sensitive to structural details and therefore protein dynamics cannot be ignored. If only the crystallographic conformation is included, the incorrect tunneling matrix element will be computed. In this case, tunneling is dominated by far-from-equilibrium conformations that minimize destructive interference; in this case again one or few tubes dominate ET. Marcus theory explains how protein dynamics is the key in modulating the energy gap between donor and acceptor sites in protein electron transfer reactions. Complementary to this view, we have now shown how thermal control goes beyond Marcus theory and can actually be needed when determining the tunneling matrix element. To exemplify this result, below we show calculations of tunneling matrix elements in the protein Azurin.

2. Electron Tunneling Mediated by Azurin Protein

In the early work by Balabin and Onuchic,[5] we have shown a real biological situation where fluctuations far-from-equilibrium are important in explaining experimental results. How have these calculations been successful using very simplified quantum chemistry approaches? The goal here is to answer this question. We wish to show that the dominating tunneling elements occur when destructive interference is dominant, and in this situation a single pathway tube (or a few tubes that interfere constructively) control ET. In this regime, even simplified quantum chemistry methods are sufficient to compute the tunneling matrix element. The simple Azurin is a great system to demonstrate this result. Since it is simple enough, it permits calculations at different levels of complexity. This conclusion not only explains the success of the Pathways method but give us hope that tunneling calculations in more complex protein systems are now possible.

The answer to this question came in 2003 in paper by Kobayashi and collaborators.[6] Combining molecular dynamics with semi-empirical and *ab initio* (Hartree-Fock) quantum chemistry calculations, we have tested quantitatively how differences in protein dynamics and the quality of the electronic Hamiltonian impact the value of the tunneling matrix element. As described above, Azurin has been chosen because it is a system for which the Pathways results are well understood and is small enough for sophisticated quantum chemistry calculations. We have chosen tunneling through Azurin that is dominated by either one, or at most, a few constructively interfering pathway tubes.

Before presenting the results, we summarize the formalism that is used to quantify these results.[6] In the electron transfer (ET) process, the medium between donor and acceptor sites is referred as the bridge. The Green's function matrix of this isolated bridge system describes the "virtual" propagation of the tunneling electron between the donor and acceptor sites of the protein. To explore how the protein bridge affects the tunneling matrix element, we calculate the Green's function matrix as a function of the bridge nuclear coordinates and electronic states. The tunneling matrix (T_{DA}) is calculated using the standard Lowdin partition[7] as proposed earlier by Beratan and collaborators,[8]

$$\mathbf{T}_{DA}(E_{tun}) = \sum_{d,a}^{bridge} \beta_{Dd} \left(E_{tun}\mathbf{S} - \mathbf{H}_{bridge} \right)^{-1} \beta_{Aa}$$

where β_{Dd} and β_{Aa} are the electron interactions between the D(A) orbitals and the bridge orbitals. And H_{bridge} is the Hamiltonian of isolated bridge system. The Green's

function of the isolated system is defined as,

$$\widetilde{\mathbf{G}} = \left(E_{tun}\mathbf{S} - \mathbf{H}_{bridge}\right)^{-1}$$
$$\mathbf{G} = \mathbf{S}\widetilde{\mathbf{G}}\mathbf{S}$$

In effectively chainlike bridges, ET coupling can be characterized by observing the decay per bond, which is represented by,[9,10]

$$\varepsilon_j = \frac{\mathbf{G}_{i,j+1}}{\mathbf{G}i,j} \quad (j > i)$$
$$\approx \frac{\widetilde{\mathbf{G}}_{i,j+1}}{\widetilde{\mathbf{G}}i,j}$$

As such, we can use the decay of the Green's function to estimate how propagation of the tunneling electron actually occurs though the bridge system.

As discussed above, many interesting protein systems are too large to perform full *ab initio* calculations. Therefore, how can we be sure that more simplified methods are sufficient? With this goal in mind, we have work towards developing a computational protocol that, while computationally affordable, is numerically reliable. Azurin has proven to be the perfect test system for this work. Our results have shown that reliable calculations can be performed with a carefully chosen semi-empirical methodology that is coupled together with Molecular Dynamics. We concluded that a modified-AM1 semi-empirical methodology method is sufficient. In a standard AM1 method, a neglect of diatomic differential overlap is observed, often implying the use of a unit overlap matrix, which can be a rather severe approximation, particularly for applications of the type considered here. To perform these calculations we have reformulated the AM1 implementation in the GAMESS,[12] program to include differential overlap, in order to carry out more accurate Green's function calculations. This implementation was successfully tested and validated with the Azurin calculations.[6]

These calculations showed that tunneling through these beta strands in Azurin is controlled by a single pathway tube or a few tubes constructively interfering. Figures 1 and 2 show that, although the tunneling matrix elements vary wildly, the conformations with larger coupling dominate the rate. Recall that the rate varies quadratically with the coupling. For these dominant configurations, the value for the electronic coupling is robust and calculations performed with

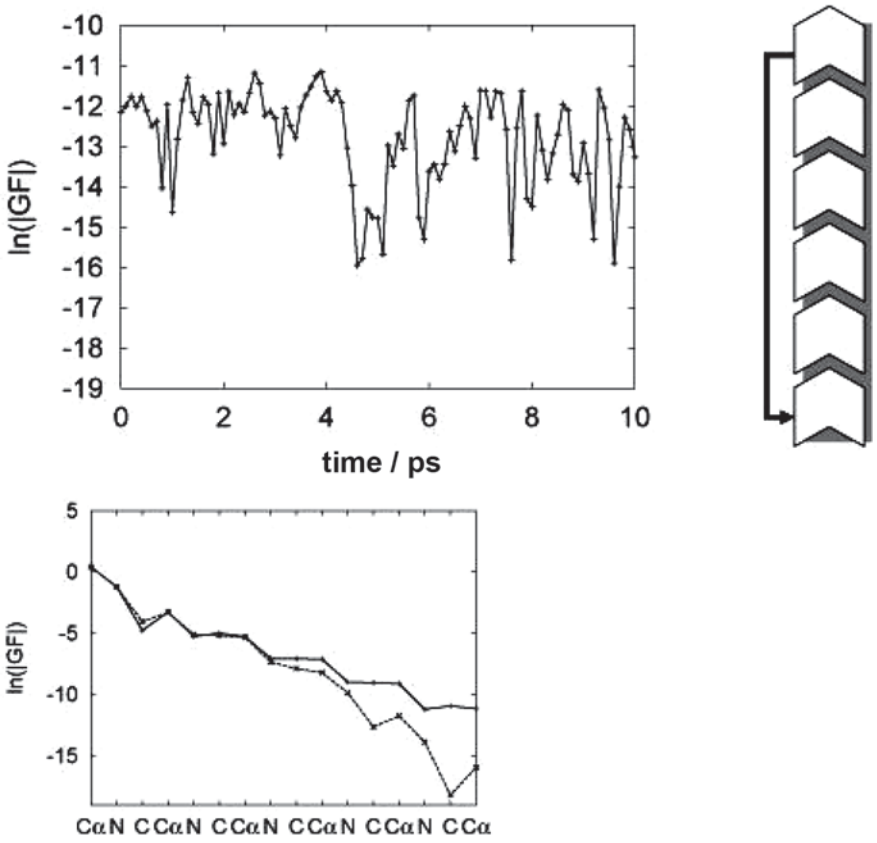

Figure 1. Green's function matrix elements for the bridge system through a single beta strand (mostly mediated by covalent bonds): The top figure shows the Green's function matrix elements that have been calculated for several different conformations obtained from a molecular dynamics trajectory. The bottom graphics shows the matrix elements decays for the strongest and the weakest element conformations.

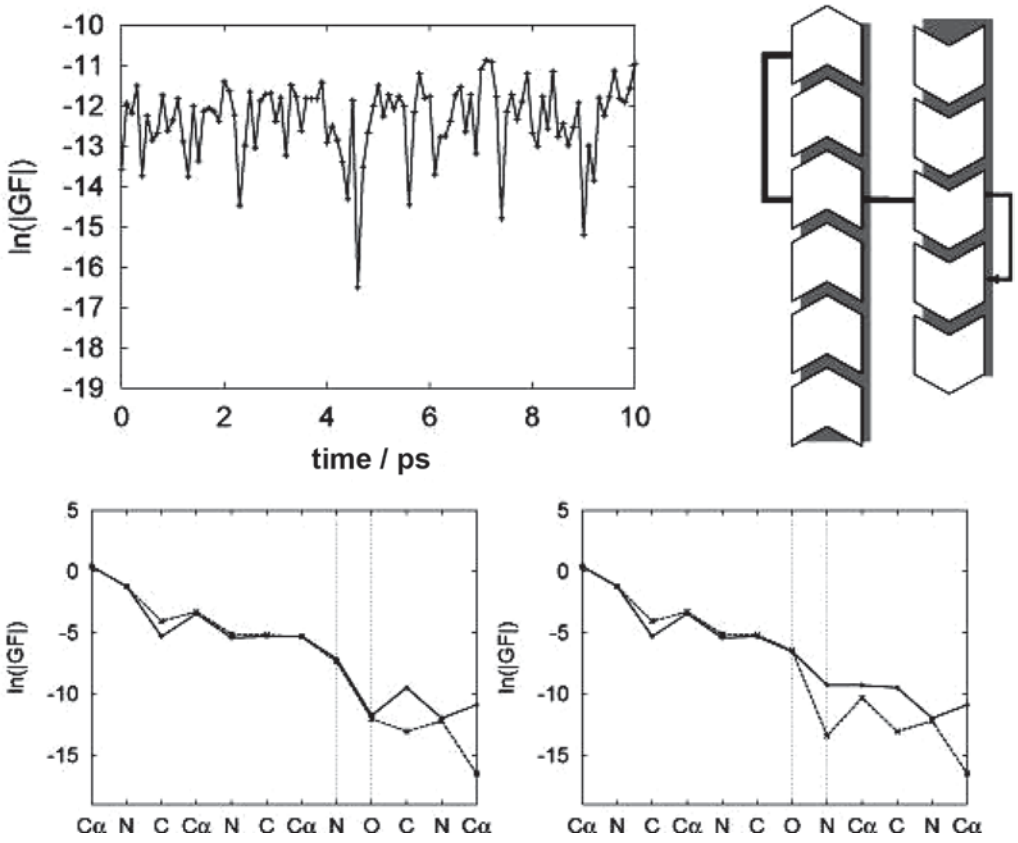

Figure 2. Green's function matrix elements for the Azurin bridge system composed of two neighboring beta strands. As shown in the figure, the dominant pathway has to include a hydrogen bond in addition to the covalent bonds. The top figure shows the Green's function matrix elements that have been calculated for several different conformations obtained from a molecular dynamics trajectory. The two bottom figures show the matrix elements decays for the strongest and the weakest element conformations for the pathway through two different hydrogen bonds, respectively.

simple Hamiltonians quantitatively agree with sophisticated calculations utilizing a full *ab initio* approach;[6] calculations performed with full Hartree-Fock are in good agreement with semi-empirical results using AM1 non-ZDO.

Another important result from Figures 1 and 2 is the dependence of the electronic coupling on the protein dynamical fluctuations. The Green's function elements were calculated for the two pathways. In Figure 1, the pathway is through a single beta strand and is basically dominated by covalent bonds. In Figure 2, that pathway needs to jump between two neighboring strands and therefore needs to include hydrogen bonds across the strands to facilitate this motion. The Green's function elements fluctuate wildly for different protein conformations; the coupling varies by about 2-3 orders of magnitude. In this particular test system, the dominant coupling conformations are equilibrium ones, but in more interesting biological systems, where most of the equilibrium conformations interfere destructively, tunneling will mostly occurs in less-likely structures that offset this effect. These far-from-equilibrium configurations dominate the rate.

The calculations for Azurin enable us to develop a new protocol that combines AM1 non-ZDO semi-empirical calculations with molecular dynamics to quantitatively calculate electron tunneling matrix elements in more interesting proteins and protein complexes where relevant biological electron transfers take place. These calculations would be impractical if a full *ab initio* approach was needed, but our results show that for the dominant pathways a simpler method is sufficient.

3. Conclusion

This manuscript in honor of Ricardo Ferreira shows an example of how theory can be used to understand biological processes that require the level of quantum mechanics for sufficient detail in molecular processes, as well as inclusion of protein motion *via* molecular dynamics. Ricardo has pointed out the importance of theory to help understand biology many years ago. Today, this is accepted by most of the scientific community, but Ricardo was the visionary that predicted this need many years ago and has performed some of the seminal contributions of theoretical applications in biology.

Our ET example demonstrates how biology in some cases may utilize thermal fluctuations to maximize the tunneling rate. This need was shown for some ET in photosynthesis by Balabin and Onuchic.[5] In this manuscript we show that, since for dominant ET conformations the rate is controlled by a single pathway tube or a few tubes constructively interfering, the procedure developed above is sufficient for theoretically studying the biologically relevant processes, thus providing a reasonable theoretical strategy for studying large interesting protein systems that are typically beyond full out ab initio methods.

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José Nelson Onuchic *is a Professor of Physics at the University of California, San Diego and is the co-Director of the NSF-sponsored Center for Theoretical Biological Physics. He did his undergraduate work at the University of São Paulo, Brazil, and received his PhD from Caltech at 1987 working with John J. Hopfield. His thesis focuses the theory of electron transfer reactions in biology. He then returned to the University of São Paulo as an Assistant Professor for two and half years. He came to UCSD in 1990. In 1989 he was awarded the International Centre for Theoretical Physics Prize in honor of Werner Heisenberg in Trieste, Italy, in 1992 he received the Beckman Young Investigator Award, and he is a fellow of the American Physical Society. In 2006 he was elected to the National Academy of Sciences, USA. His research interests also include protein folding, function and dynamics and more recently stochastic effects in genetic networks.*

Chigusa Kobayashi *got her B. Sci. from Nagoya University in 1996 and a master in science from the same university in 1998. She obtained her Doctor in Science from the Nagoya University in 2000. After starting her postdoctoral studies also in Nagoya, she moved to the University of California at San Diego in 2001 and to Kobe University in 2003. She is currently at postdoctoral fellow at the Institute for Molecular Science, Okazaki, Japan.*

Kim K Baldridge *is a theoretical chemist with expertise in development, implementation and application of QM-based methodology, with applications in (bio)chemical reaction processes in environment. Efforts include development of associated grid and middleware technologies. Baldridge has over 125 publications, is PI in several (inter)national interdisciplinary projects and personal research grants. Baldridge holds adjunct professorships at UCSD & SDSU, California. She is originator of 2 annual Symposia (MGM, 12 yrs) & DCH (3 yrs), holds the 2000 Agnes Fay Morgan Award, is Fellow of AAAS & APS. After 16 yrs at SDSC, California, Baldridge holds a chaired position at U. Zurich, Switzerland, as professor of theoretical chemistry and co-director of the organic chemistry institute.*

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