

Faraday Discussions

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Spiers Memorial Lecture

Interplay of theory and computation in chemistry—examples from on-water organic catalysis, enzyme catalysis, and single-molecule fluctuations

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In this lecture, several examples are considered that illustrate the interplay of experiment, theory, and computations. The examples include on-water catalysis of organic reactions, enzymatic catalysis, single molecule fluctuations, and some much earlier work on electron transfer and atom or group transfer reactions. Computations have made a major impact on our understanding and in the comparisons with experiments. There are also major advantages of analytical theories that may capture in a single equation an entire field and relate experiments of one type to those of another. Such a theory has a generic quality. These topics are explored in the present lecture.

1 Introduction

It is a pleasure to participate in this Faraday Discussion. The topics are a blend of physical organic, computational and theoretical chemistry. New avenues of research have opened up, stimulated in part by new techniques. In this lecture I would like to consider several recent initially-puzzling topics related to this broad area. They are (1) the “on-water” catalysis of some organic reactions, for example, a particular cycloaddition reaction that shows a striking acceleration of the reaction rate when the organic reactants are stirred vigorously with excess water, (2) some unusual features of enzyme catalysis, and (3) single molecule fluctuations in enzymes. I conclude with several comments on the interplay of experiments, theoretical equations, and computational chemistry, recalling in the process some earlier history for various transfer reactions.

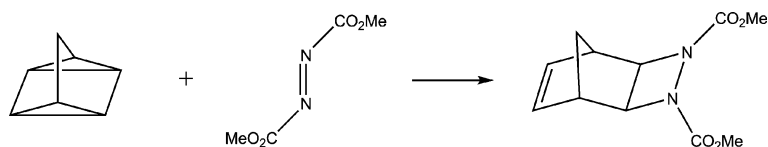
In the Spiers Memorial Lecture itself the various topics described here were extensively illustrated. Many of these illustrations, and more, including some on anomalous mass-independent isotope fractionation and on intermittently fluorescing quantum dots, appeared in an article I wrote in celebration of an ACS Centennial of *The Journal of Physical Chemistry*. This article, which was published earlier this year in *The Journal of Physical Chemistry C*, is cited as ref. 12 in the present article. Rather than duplicate those many illustrations (two examples do appear in a Discussion comment), I would like to refer the reader to ref. 12 for examples.

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2 Results and discussions

On-water catalysis

We consider first a cycloaddition reaction involving approximately a one-to-one mixture of two miscible organic reactants stirred strongly in excess water,¹ forming an emulsion. A particular reaction between quadricyclane and diethylazodicarboxylate that normally requires 48 h for completion in the “neat” reaction took only 10 min in the emulsion:



To interpret this initially puzzling result, Dr Yousung Jung and I recalled² some experiments^{3,4} on sum-frequency generation (SFG) measurements of air/water and oil/water interfaces. In this SFG work, visible and infrared laser beams are mixed and used to study the nature of these surfaces. The merit of the technique is that it is surface-sensitive rather than bulk sensitive.

The SFG results showed that approximately 25% of the OH groups of the water molecules at the surface were “dangling”, that is, they were not hydrogen bonded to other water molecules.³ Their presence could be detected by their infrared absorption at 3700 cm^{-1} , which is at a higher frequency than that for a hydrogen-bonded OH. This result provided a clue to the catalysis: catalysis should occur if these dangling OHs are attracted more to the transition state (TS) of the reaction than to the reactants themselves. Dr Jung treated this phenomenon using density functional theory and found that in the calculations, as in the experiments, catalysis occurs.² The results also provided a comparison with the reaction rate in dilute aqueous solution, a reaction studied much earlier.⁵ For this in-water reaction, if the hydrogen-bonded structure around the small organic reactants in-water were largely intact, then to bond to the TS some H-bonds in the water would need to be broken and so there would be an addition to the activation energy barrier, resulting in a reduced “intrinsic” rate of reaction. (Evidence for an intact hydrogen-bonded structure surrounding a small molecule, CH_4 , was seen in neutron diffraction data.⁶)

To compare the reaction rates for all three different reaction conditions, on-water, in-water, and no-water, it was necessary to reduce the three rates to the same basis. To this end we used an approximate statistical mechanical method to obtain from the rate the probability of reaction of two nearest-neighbor organic reactants (in particular, also at the water/organic surface in the case of the on-water reaction).² That analysis served to reduce all the rate constants to the same units (s^{-1}), yielding an “intrinsic” rate constant for each system. We were able to explain the qualitative trends in the different rates in this way and make some approximate estimates of the rates.² The “intrinsic” rates for the “neat” and the on-water reactions differ much more than the values that are reflected in the 10 min compared to 48 h, because in the emulsion some of the organic reactants are not near the water/organic interface and so should not be counted in estimating the “intrinsic” rate constant for the on-water reaction from the experimental rates.

A missing quantity was some estimate of the typical size of an emulsion particle. In the absence of a direct measurement, a value was assumed, but actual measurements would remove that approximation. The phenomenon and the model are not restricted to water/oil surfaces with dangling OHs, and indeed more recently such an example has been found involving catalysis by OHs on metal oxides.⁷ Catalysis occurred when an oxide also had OHs on its surfaces.

In the present example, the underlying theory is partly analytical (the statistical mechanical analysis that reduced all three rates to the same basis), and in the qualitative physical picture of the catalysis. However, for a calculation of the potential energy surface for the reaction and a theoretical estimate of the rate from it, computations play a vital role.

Enzyme catalysis

For the topic of enzyme catalysis, we consider two unusual experimental results that reveal another side of the interplay of experiment, theory and computations. The first of these experiments involves the temperature-independent kinetic H/D isotope effect, $k_{\text{H}}/k_{\text{D}}$, that is observed when some enzymes operate on their natural substrates under their natural conditions.^{8–10} The second involves the Arrhenius pre-exponential factor A in the rate constant for a particular thermophilic enzyme above and below its “break point” temperature T_{b} .^{9,10} In the latter, a plot of $\log k_{\text{H}}$ versus $1/T$ has a larger slope below T_{b} than above, yielding a larger activation energy E_{a} in $k_{\text{H}} = A \exp(-E_{\text{a}}/kT)$. Correspondingly, there was a change of many orders of magnitude in A at T_{b} .

Temperature-independent $k_{\text{H}}/k_{\text{D}}$. In the first of these effects, typically seen for an enzyme acting on its natural substrate at its natural temperature, a kinetic isotope effect (KIE), the ratio $k_{\text{H}}/k_{\text{D}}$, is independent of temperature.^{8–10} The simplest explanation of this widespread phenomenon is not that it is a coincidence, the balance of two conflicting effects that happen to cancel. Instead, the simplest interpretation, which could be explored by computations with a sufficiently accurate potential energy surface, is that an H transfer, $\text{AH} + \text{B} \rightarrow \text{A} + \text{HB}$, between the substrate and co-factor requires very little stretching of the H bond length in A (*e.g.* in a CH or OH distance) prior to the H transfer. Here, the H may be H^+ , H^- or H, and when it is H it may be a proton-coupled electron transfer, with the electron and the proton coming from different sites.

An AH stretching would lead to a change in the zero-point energy prior to the “H jump”. Such a change would be different for H and D, and so cause an activation energy for the ratio $k_{\text{H}}/k_{\text{D}}$, that is, the difference of activation energies of the H and D transfers. Prior to that transfer there is also a “reorganization” of the various dipoles and charges in the system, until the entire system containing $\text{AH} + \text{B}$ has the same total energy as that for $\text{A} + \text{HB}$, *i.e.* these two systems are in “resonance”. In effect, in this nonadiabatic model for an H transfer (an H jump) the Franck–Condon principle is assumed for the transfer of the relatively light particle H. The reorganization is of the dipoles and charges and is expected to be isotopically insensitive and so not affect the $k_{\text{H}}/k_{\text{D}}$ ratio. The net result is that the KIE, $k_{\text{H}}/k_{\text{D}}$, is temperature-independent under the natural conditions mentioned.

Abnormal A factors. For the thermophilic enzyme, in the present case an *ht* alcohol dehydrogenase,^{9,10} there is a break in the slope of the plot of $\log k$ versus $1/T$, and since k is a continuous function of T , there is marked change in the Arrhenius pre-exponential factor A . Above T_{b} the value of A was “normal”, that is, of the order 10^{12} to 10^{13} s^{-1} . This value is the expected one when no reorientation of the reactants is required to reach the transition state and when there is no large standard entropy of reaction, ΔS^\ddagger , for the transfer step. For H transfer in this particular system, the A below T_{b} is about a factor 10^5 higher than above T_{b} , while for D transfer it is a factor of about 10^{10} higher!

This result for the change of a pre-exponential factor for the H-transfer reminds one of a somewhat similar effect seen in the viscosity η of silica at its glass temperature T_{g} . In a plot of $\log \eta$ versus $1/T$ the pre-exponential factor below T_{g} is a factor 10^5 higher than above T_{g} .¹¹ The observation suggests that below T_{b} the enzyme is much less flexible than above T_{b} . In fact, H/D exchange rates below T_{b} are slower than above, suggesting a decrease in flexibility of the enzyme.¹⁰ In some conventional

phase transitions the transition occurring at T_b is not a first-order transition, since that would entail a discontinuity in k , but rather the equivalent of a second-order transition that involves a change in the derivative (slope) of k .

The interpretation of the various results using molecular dynamics computations (MD) does not appear to be an option at present, since the relevant time scale is of the order milliseconds to seconds, too long compared with the typical pico- to nanoseconds used for MD for large systems. Thus, any computational analysis will need to seek some different route for treating the slow conformational or hydrogen bonding changes. An analytical treatment below T_b in the thermophilic protein might entail some Kramers-like equation for the sluggish system. We have commented on this possibility for the H-transfer elsewhere.¹² However, if the H in AH has to stretch in the now unnatural rather than natural environment below T_b , then a two-coordinate extension of the Kramers equation is needed, the protein coordinate being the slow coordinate. This additional coordinate offers a possibility of explaining the large difference in activation energies of the H and D transfers and hence in A factors when the system is below T_b . We have briefly commented on the possibility of using a two-dimensional Kramers equation elsewhere.¹²

Single molecule fluctuations in enzymes and autocorrelation functions

In single molecule studies of enzymes it has been found that various autocorrelation functions of fluctuations, such as those in enzyme catalysis rates, in fluorescence lifetimes, and spectral diffusion, are not simple exponential decays but rather highly nonexponential decays over periods of milliseconds to seconds.^{13–15} An approximate analytical theory was introduced to interpret this behavior.¹⁶ In the model it was assumed that the fluctuations were due to those of the local electrostatic field at the chromophore or substrate, arising from fluctuations of the various dipoles and charges in the enzyme and its surroundings. A consequence of the model was that all three normalized correlation functions (except the fluorescence one for a system with no quenchers), were approximately equal and equal to an electrostatic energy autocorrelation function. We found that the experimental data for such a comparison was very limited—for only one enzyme was any comparison possible: the cholesterol oxidization of FAD, for which two of the autocorrelation functions had been measured.¹³ The two correlation functions agreed within the error bars. The model can be tested further when more experimental data become available.

We also approximately related this electrostatic autocorrelation function to the dielectric dispersion of the enzyme system¹⁷ using the fluctuation–dissipation theorem. There are data on dielectric dispersion of some enzymes but no *detailed* data on those for which the autocorrelations for the fluctuations have been measured. Use of a standard equation, the Havriliak–Nagami equation,¹⁸ for the dielectric dispersion of complex systems reproduced the autocorrelation functions using typical parameters.¹⁷ However, data fitting with parameters is not an adequate substitute for the actual dielectric dispersion data. Also, any comparison using the dispersion data requires some caution since the enzyme and its immediate environment are not dielectrically homogenous. Nevertheless, the comparison could be instructive. Relating one autocorrelation function to another, even if approximate, demonstrates a complementarity of analytic theory and computations, since MD computations are not presently feasible for this time scale of milliseconds to seconds.

This type of analysis was used by Hsu *et al.*¹⁹ to treat the time-dependent Stokes shift for a coumarin dye in water. The latter was calculated from the dielectric dispersion data for water using the fluctuation dissipation theorem, and compared with the experimental data. This comparison of theory and experiment²⁰ gave good agreement without adjustable parameters.¹⁹ The relaxation of the water solvent covered a much shorter time scale than that in the enzyme studies, since water relaxes in picoseconds rather seconds. Of course, one may not expect such good agreement for a more complex inhomogeneous system, such as enzymes in water.

Comments on the interplay of experiment, theoretical equations and computations

We have considered in the preceding sections several examples of an interplay between experiments, theoretical equations, and computations. A particular example of this interaction that encompasses all three and that we did not consider is the cross-relation that was derived for electron transfer reactions.²¹ It is a generic expression, expressing the rates of electron transfer reactions in terms of those of related self-exchange reactions and the equilibrium constant. Insight into the cross-relation can be based on a two-parabola approximation, one parabola for a plot of the free energy of the reactants and environment along a reaction coordinate, and the other parabola for the free energy of the products and their environment. The origin of the cross-relation was additionally seen by relating the reorganization energy λ for the cross-reaction to those for the two self-exchange reactions involved.²¹ Largely reasonable agreement with experimental data was obtained when the conditions of the reaction satisfied the assumptions. Computations supported the parabolic approximation.²²

However, Sutin²³ applied the cross-relation to a reaction that was not an electron transfer, an atom transfer, and it worked. There was approximate agreement of the theoretical and the measured rate constant. This unexpected result prompted me to develop a different method of deriving that relation. The intersecting parabolas are particularly suitable for a system where the electronic interaction of the reactants is weak, such as a weak-overlap electron transfer, though they have been used as a basis for strongly interacting systems as a start in a perturbation approach, as in the empirical valence bond method. Instead, I used Johnston's "bond energy-bond order" method that assumes a conservation of bond order along the reaction coordinate.²⁴ A cross-relation was obtained, a hyperbolic tangent equation that reduces to the simple quadratic equation similar to that for electron transfer, as long as $\Delta G^\circ/\lambda$ is not large.²⁴ (As expected from the topography of the surface, it did not have the inverted region that plays a key role in the weak-overlap electron transfers.²¹) The experimental data of Lewis and Hu²⁵ for methyl transfers between arenesulfonates showed good agreement between experimental and calculated rate constants. Computational tests of the cross-relation were also made for these strongly interacting systems.²⁶

Unfortunately not every organic reaction is of this simple type. That is, they need not have self-exchange reactions with which to predict the rate of a cross-reaction. In fact, most organic reactions do not. If this particular method is to have some broader applicability it would need to be extended. A step in that direction was taken by Chen and Murdoch.²⁷

An example of generic *versus* the specific, in earlier days and in this symposium, is seen in the Hammett ρ - σ relation. It serves to correlate the rates of many reactions. It will be interesting to see whether computer-based calculations and experiments will stimulate by their results some new theoretically-based expressions that correlate a large body of data. In the meanwhile, as this Faraday Discussion amply demonstrates, the computations are providing detailed comparisons and analyses.

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References

- 1 S. Narayan, J. Muldoon, M. G. Finn, V. V. Fokin, H. C. Kolb and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2005, **44**, 3275.
- 2 Y. S. Jung and R. A. Marcus, *J. Am. Chem. Soc.*, 2007, **129**, 5492.
- 3 Y. R. Shen and V. Ostroverkhov, *Chem. Rev.*, 2006, **106**, 1140.

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- 4 F. G. Moore and G. L. Richmond, *Acc. Chem. Res.*, 2008, **41**, 739.
 - 5 D. C. Rideout and R. Breslow, *J. Am. Chem. Soc.*, 1980, **102**, 7816.
 - 6 P. H. K. de Jong, J. E. Wilson, G. W. Neilson and A. D. Buckingham, *Mol. Phys.*, 1997, **91**, 99.
 - 7 F. Niu, C. Liu, Z. Cui, J. Zhai, L. Jiang and W. Song, *Chem. Commun.*, 2008, 2803.
 - 8 R. J. Harris, R. Meskys, M. J. Sutcliffe and N. S. Scrutton, *Biochemistry*, 2000, **39**, 1189; J. Basran, M. J. Sutcliffe and N. S. Scrutton, *J. Biol. Chem.*, 2001, **276**, 24581; J. L. Abad, F. Camps and G. Fabriàs, *Angew. Chem., Int. Ed.*, 2000, **39**, 3279; N. Agrawal, B. Hong, C. Mihai and A. Kohen, *Biochemistry*, 2004, **43**, 1998; R. S. Sikorski, L. Wang, K. A. Markham, P. T. R. Rajagopalan, S. J. Benkovic and A. Kohen, *J. Am. Chem. Soc.*, 2004, **126**, 4778; B. Hong, F. Maley and A. Kohen, *Biochemistry*, 2007, **46**, 14188; M. J. Knapp, K. Rickert and J. P. Klinman, *J. Am. Chem. Soc.*, 2002, **124**, 3865.
 - 9 A. Kohen, R. Cannio, S. Bartolucci and J. P. Klinman, *Nature*, 1999, **399**, 496; Z.-X. Liang, T. Lee, K. A. Resing, N. G. Ahn and J. P. Klinman, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 9556. A break-point T_b is also seen for the thermophilic hydrofoliate reductase in: G. Maglia and R. K. Allemann, *J. Am. Chem. Soc.*, 2003, **125**, 13372.
 - 10 Z.-X. Liang and J. P. Klinman, *Curr. Opin. Struct. Biol.*, 2004, **14**, 648; Z.-X. Liang, I. Tsigos, V. Bouriotis and J. P. Klinman, *J. Am. Chem. Soc.*, 2004, **126**, 9500.
 - 11 R. H. Doremus, *J. Appl. Phys.*, 2002, **92**, 7619.
 - 12 R. A. Marcus, *J. Phys. Chem. C*, 2009, **113**, 14598.
 - 13 H. P. Lu, L. Xun and X. S. Xie, *Science*, 1998, **282**, 1877.
 - 14 O. Flomenbom, K. Velonia, D. Loos, S. Masuo, M. Cotlet, Y. Engelborghs, J. Hofkens, A. E. Rowan, R. J. M. Nolte, M. Van der Auweraer, F. C. de Schryver and J. Klafter, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 2368.
 - 15 B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. B. Luo, H. Y. Sun, B. J. Cherayil, S. C. Kou and X. S. Xie, *Nat. Chem. Biol.*, 2006, **2**, 87.
 - 16 M. K. Prakash and R. A. Marcus, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 15982.
 - 17 M. K. Prakash and R. A. Marcus, *J. Phys. Chem. B*, 2008, **112**, 399.
 - 18 S. Havriliak and S. Nagami, *Polymer*, 1967, **8**, 161.
 - 19 C.-P. Hsu, X. Y. Song and R. A. Marcus, *J. Phys. Chem. B*, 1997, **101**, 2546.
 - 20 R. Jimenez, G. R. Fleming, P. V. Kumar and M. Maroncelli, *Nature*, 1994, **369**, 471.
 - 21 R. A. Marcus, *Discuss. Faraday Soc.*, 1960, **29**, 21; R. A. Marcus, *J. Phys. Chem.*, 1963, **67**, 853; R. A. Marcus, *J. Chem. Phys.*, 1965, **43**, 679.
 - 22 A. Warshel, *J. Phys. Chem.*, 1982, **86**, 2218; R. A. Kuharski, J. S. Bader, D. Chandler, M. Sprik, M. L. Klein and R. W. Impey, *J. Chem. Phys.*, 1988, **89**, 3248.
 - 23 N. Sutin, *Proc. Symp. Exchange Reactions, Upton, N. Y.*, 1965, p. 7; A. Haim and N. Sutin, *J. Am. Chem. Soc.*, 1966, **88**, 434; N. Sutin, *Annu. Rev. Phys. Chem.*, 1966, **17**, 119.
 - 24 R. A. Marcus, *J. Phys. Chem.*, 1968, **72**, 891. Johnston's method is described in: H. S. Johnston, *Gas Phase Reaction Rate Theory*, Ronald Press Company, New York, 1966.
 - 25 E. S. Lewis and D. D. Hu, *J. Am. Chem. Soc.*, 1984, **106**, 3292.
 - 26 S. Wolfe, D. J. Mitchell and H. B. Schlegel, *J. Am. Chem. Soc.*, 1981, **103**, 7694; J. Donnelly and J. R. Murdoch, *J. Am. Chem. Soc.*, 1984, **106**, 4724.
 - 27 M. Y. Chen and J. R. Murdoch, *J. Am. Chem. Soc.*, 1984, **106**, 4735.