Theoretical and Computational Models for Organic Chemistry

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Kluwer Academic Publishers
Dordrecht / Boston / London

Published in cooperation with NATO Scientific Affairs Division
EXCITED STATE PROTON TRANSFER REACTIONS

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"Let us descend to the garden
Things seen from there one does not see from here"
An Israeli folk song.

ABSTRACT. Proton dissociation in solution is traditionally described via a two step kinetic mechanism of complex formation and proton escape. This mechanism leads to an ultimate exponential decay of the acid concentration. In contrast, we find that the fluorescence signal from an excited, negatively charged hydroxypyrene derivative in solution has a non-exponential tail, attributed to reversible geminate recombination in the excited state. The theory of reversible diffusion influenced reactions predicts a $t^{-3/2}$ asymptotic decay, in quantitative agreement with experiment.

The addition of inert salts screens the Coulombic attraction thus diminishing geminate recombination. As a result, the non-exponential tail and the steady-state quantum yield decrease with increasing ionic strength. This contrasts with the classical theories of Brönsted and Bjerrum which predict zero salt effect on the dissociation direction. We model the screening effect by a "Naive Approximation", assuming zero screening beyond the separation of the nearest, oppositely charged ion. For interpreting pH effect on the proton transfer reaction, we approximate the many-body problem by introducing a bimolecular boundary condition in the diffusion equation. This is compared with one dimensional stochastic simulations.

315
Proton transfer is a fundamental process in both chemistry [1-3] and biology [4]. In particular, proton dissociation namely, proton transfer to solvent, from aromatic dye molecules in their excited electronic state [5] can be easily studied by virtue of their strong fluorescence signal [6]. The older fluorescence measurements did not possess time resolution: It was only possible to obtain steady-state quantum yields under conditions of constant illumination [6]. The conventional interpretation of the experimental data assumed a chemical kinetic scheme, such as [3]

$$R^*OH = R^*O^- \cdot H^+ \rightarrow R^*O^- + H^+ \quad (1)$$

In this scheme the proton first dissociates to form a geminate ionic complex and then separates from the parent anion. In the time domain, the rate-equations for this two-step mechanism leads to bi-exponential decay of the excited acid (R*OH) population.

At low pH values, when additional protons are present, the separation step becomes reversible and one observes homogeneous proton recombination. The reaction under these conditions undergoes a transition from unimolecular (correlated pairs) to a bimolecular (or pseudo-unimolecular) reaction. The rate of this recombination reaction is expected to diminish with increasing concentration of inert salt, which screens the Coulombic attraction between the proton and the anion. In fact, the classical Brönsted-Bjerrum theory of salt effects puts all of the effect in the recombination reaction while predicting zero salt effect on the dissociation direction [7].

Time resolved measurements of ultrafast proton dissociation in the excited state are challenging these classical views [8-13]. Our picosecond fluorescence experiments have shown that the kinetic scheme, eq 1, does not accurately describe the kinetic data. Firstly, the R*OH fluorescence decays asymptotically as a power law, rather than exponentially [10]. The area under the non-exponential tail contributes to the observed quantum yield. In addition, there is a marked salt effect on the elementary dissociation process:


In order to a conventional rate equation the potential of mutual R*O-/-H+ distance dist differential equation fo the observed reaction development of the the (almost obvious) extrered reactions [15, 16].

The aim of thin reversible proton tran theory of diffusion inf industrial procedures may be fou asymptotic power-law o salt effect [11] and the [11] and (iii) an exten competing geminate at pH values.

2. The Experimental S

Our work [9-13] has ce in Figure 1. This hydroxypyrrole 1,3,6-tr as pyranine or HPTS Dissolved as its trisodium in its ground state, who is around 8. In the fis group becomes highly a
The relative $R^+\text{OH}/R^+\text{O}^-$ quantum yield decreases with increasing ionic strength [11].

In order to account for the above behavior we have replaced the conventional rate equations by a Smoluchowski equation, describing diffusion in the potential of mutual interaction. In this picture one introduces explicitly the $R^+\text{O}^-/H^+$ distance distribution, thus obtaining a partial (instead of an ordinary) differential equation for the time evolution of proton dissociation. The fact that the observed reaction is reversible in the excited-state, has promoted the development of the theory of reversible diffusion influenced reactions [14], as an (almost obvious) extension of the theory of (irreversible) diffusion influenced reactions [15, 16].

The aim of this lecture is to provide a qualitative description of reversible proton transfer reactions in the excited-state, using the extended theory of diffusion influenced reactions. The complete equations and numerical procedures may be found in the literature [10-14]. Major results include (i) the asymptotic power-law decay and the evidence for diffusive kinetics [10]; (ii) The salt effect [11] and the "Naive Approximation" for the screening function [17, 11] and (iii) an extension [18] of the theory for approximating the effect of competing geminate and homogeneous proton recombination expected at low pH values.

2. The Experimental System

Our work [9-13] has centered on the dye molecule shown in Figure 1. This molecule is officially called 8-hydroxyppyrene 1,3,6-trisulfonate and colloquially known as pyranine or HPTS. We also denote it by ROH. Dissolved as its trisodium salt, it is already triply charged in its ground state, where the $pK^+$ value of the OH group is around 8. In the first excited singlet state the hydroxy group becomes highly acidic [5], with a $pK^+$ value of 1.4.

Figure 1. Pyranine.
HPTS shows a strong fluorescence signal, with an absolute quantum yield close to unity (hence it is a good laser dye). In water at pH=6, R^*OH fluorescence peaks around 445 nm and is about 1/20 of the R^*O^- fluorescence signal, which peaks at 510 nm. A steady-state (constant illumination) fluorescence spectrum [13] is shown in Figure 2. The small R^*OH/R^*O^- quantum yield, 1/23, is due to the fast proton dissociation. As demonstrated by Weller [5], the fluorescence spectrum can be titrated by the addition of strong acids. This effect, shown in Figure 2, indicates that the reverse reaction of proton recombination in the excited state cannot be negligible [5].

![Fluorescence spectra of HPTS in water at room temperature](image)

Figure 2. Steady-state fluorescence spectra of HPTS in water at room temperature [13]. Curve labelled 0 is at neutral pH. Other spectra are at low pH and are labelled by the volume fraction of 70% perchloric acid added. Note the isoemissive point at 488 nm.

Early time resolved kinetics within the short lifetime of about 20 ns have been probed (HPTS absorption peaks at 400 nm). Ground state recombinant quenching on the ground state diffusion-controlled rate constant for recombination of the proton-anion complex is quenched by the addition of perchloric acid. The effect is milder than would be expected. We found a correlation between the increase in fluorescence intensity and the Naive Aj

![Graph of log(K_on)](image)

Figure 3. Steady-state proton on function of HClO_4 (+) as an acceptor containing one salt of perchloric acid.
Early time resolved measurements could not follow the excited-state kinetics within the short HPTS fluorescence lifetime \(\tau_f = 5-6\,\text{ns}\). Laser pulses of about 20 ns have been used in conjunction with absorption spectroscopy (HPTS absorption peaks at 403 nm and its anion at 454 nm) to determine the ground state recombination rate coefficient [19] and the effect of fluorescence quenching on the ground-state recombination yields [20]. As expected, the exothermic ground-state recombination reaction \(\text{pK}^* \approx 8\) occurs at a nearly diffusion-controlled rate, \(1.9 \times 10^{12}\,\text{M}^{-1}\,\text{s}^{-1}\) [19]. The dependence of this recombination rate on ionic strength is shown in Figure 3: Increased screening of the proton-anion Coulombic attraction decreases the recombination rate. The effect is milder than predicted by the Debye-Hückel theory [7, 21], which could fit experiment only by invoking the empirical Davies correction [19].

![Graph showing log10 K_on vs. 100/R_0v](image.png)

**Figure 3.** Steady-state protonation rate coefficients for ground-state HPTS [19], as a function of HClO₄ (+') and KClO₄ (X's) concentration, \(c\) (or the average radius of a sphere containing one salt ion, \(R_{av}\)), compared with the asymptotic Debye-Hückel expression and the Naive Approximation [11c], see below.
It was not until the development of picosecond fluorescence spectroscopy that the fate of the excited acid could be followed in the time domain. From the nearly exponential decay profile it was verified [9] that in the excited-state the hydroxyl proton is ejected at an ultrafast rate (100ps). It has occurred to the researchers [9] that, since the fluorescence spectrum can be titrated (Figure 2), there should be an observable recombination process also for the geminate, correlated pair. Indeed, when they looked closely at the decay of the R*OH fluorescence, shown in Figure 4, they discovered a long time, non-exponential, tail which they have attributed to reversible rebinding of the geminate proton, occurring (almost) without quenching of the excited-state [9].

![Non-Exponential HPTS Decay](image)

Figure 4. One of the first non-exponential decays of (acidic) HPTS fluorescence was observed using a streak-camera [10a, 13].

The pH effect on the steady-state fluorescence spectrum, Figure 2, can also be seen in the time domain: Between pH=6 and 3 there is no change in the time resolved data, but at lower pH values the long time tail increases (Figure 5). This corresponds to the pH range for which the R*OH peak in Figure 2 increases in amplitude. The increase in the tail in strongly acidic solvents shows that it independence at neutral not due to homogeneous interpretation of the gem

![pH dependence at 430nm](image)

Figure 5. pH dependence at 430nm [10a]. pH values

3. The Long Time Tail a

The traditional analysis scheme e.g., eq 1, and the dependence of bulk cone time tail. The minimal l of the proton in the ele dependent Smoluchowski "back-reaction" boundary
fluorescence spectroscopy in the time domain. From that in the excited-state ps). It has occurred to can be titrated (Figure also for the geminate, the decay of the R^*OH time, non-exponential, of the geminate proton, [9].

\[ \frac{[\text{acid}]}{[\text{neutral}]} \]

Figure 5. pH dependence of HPTS (acidic form) transient fluorescence signal at 430nm [10a]. pH values (top to bottom) are: 1.3, 1.7, 2.0, 3.6 and 6.0.

3. The Long Time Tail and Proton Diffusion

The traditional analysis of proton transfer reactions [3, 5-7] is by a kinetic scheme e.g., eq 1, and the ensuing chemical rate equations describing the time dependence of bulk concentrations. This is insufficient for explaining the long time tail. The minimal level of complexity has to involve the spatial diffusion of the proton in the electrostatic field of the anion, as depicted by the time-dependent Smoluchowski equation [15], but with a boundary condition (the "back-reaction" boundary condition) which describes reversible reactions [10].
Additional complications to the kinetics besides proton diffusion are possible. These could include vibrational relaxation of the excited pyrene derivative, dielectric relaxation of the solvent (water) and heat transfer. These processes in water occur on timescales which are typically shorter than 10 ps, hence they are over by the time the proton is ejected (100 ps). Rotational diffusion of the HPTS anion (hence of the rebinding site) could also play a role. In water at room temperature it takes some 150 ps. Hence before the proton has had a chance to recombine, the rebinding site is uniformly smeared around a sphere. One is left with the relative proton-anion translational diffusion to consider.

How does a diffusional mechanism differ from the kinetic scheme in eq 1? Both are two-step mechanisms with an identical first step: The reversible dissociation to form a "contact pair". In the diffusion equation it appears as a boundary condition (the back-reaction boundary condition). The second step, separation of the complex, is assumed in ordinary kinetics to be a single, elementary step. In the diffusional mechanism it involves the random motion of the proton as it separates to ever increasing distances. This motion is analogous to the spreading of an ink droplet: As time goes by, its bell shaped density profile decreases in amplitude and increases in width. In the case of the proton, this random motion occurs in a potential field which attracts it to the quadruply charged HPTS anion, resulting in enhanced geminate recombination, detectable through the non-exponential tail in the fluorescence decay. The diffusion equation is therefore generalized to a spherically symmetric Smoluchowski equation. Its numerical solution, convoluted with an instrument response function of about 100 ps, is compared in Figure 6 [10c] with single photon counting data points (multiplied by \( \exp(t/\tau_1) \)).

![Figure 6. HPTS fluorescence decay.](image)

The solution of \( -R_D \tau_a \), depends on the temperature, \( T \), and the diffusion coefficient, \( D_\eta \), which is nearly an order of magnitude higher. The Grotthuss mechanism between neighboring water molecules was shown experimentally to determine the recombination rate parameter in the probability for obs...
The solution of the Smoluchowski equation for a Coulomb potential, $-R_D/r$, depends on the "Debye radius", $R_D = \sqrt{|z_1 z_2|/(\epsilon k_B T)}$, whose magnitude (nearly 30 Å for HPTS in water) is determined by the charges, $z_1$ and $z_2$, the temperature, $T$, and the dielectric constant $\epsilon$ of the solvent. The relative diffusion coefficient, $D$, approximately equals the proton diffusion coefficient, which is nearly an order of magnitude faster than any other ion. This is due to the Grotthuss mechanism of proton diffusion by a chain of proton transfers between neighboring water molecules. All the above quantities are experimentally determinable. The only adjustable parameters are the two rate constants for the first kinetic step: The dissociation rate parameter, $\kappa_d$, and the recombination rate parameter, $\kappa_r$. The effect of some of the above parameters on the probability for observing a bound pair is shown in Figure 7.

Figure 7. Effect of various parameters on the solution of the spherically symmetric (3d), time-dependent Smoluchowski equation with back reaction [10a]. In each panel one parameter is varied while the others are held constant. In all panels, the third curve from the top is identical and corresponds to the observed HPTS signal, multiplied by $\exp(t/\tau_f)$. 

proton diffusion are the excited pyrene heat transfer. These y shorter than 10 ps, (100 ps). Rotational could also play a role. ice before the proton rmly smeared around ional diffusion to

kinetic scheme in eq step: The reversible uation it appears as a n). The second step, etics to be a single, is the random motion ces. This motion is es by, its bell shaped th. In the case of the high attracts it to the

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HPTS fluorescence decay.
The kinetic scheme, eq 1, can be solved analytically. This solution involves one additional rate parameter for the separation of the ion pair. Using the steady-state solution of the Smoluchowski equation, one may express this rate coefficient in terms of $R_D$ and $D$. As seen in Figure 8a, the resulting solution (dashed curve) does not fit the transient data (full curve). These two curves possess however the same area, thus predicting the same quantum yield [10b]. If the third rate coefficient is treated as an adjustable parameter we obtain the bi-exponential fit shown in Figure 8b [10b]. It agrees with the transient data up to about 1ns but, because it decays exponentially rather than as a power law (see below), it underestimates the area under the curve by about 30%. The relative $R^*OH/R^*O^-$ quantum yield that we obtain from the ratio of the two peaks in the steady state fluorescence spectrum (Figure 2) is 0.043. From the area under the transient decay (divided by the fluorescence lifetime, $\tau_f=5.2$ns) we find 0.037. This small discrepancy may be due to some degree of unaccountable quenching, but is still within the experimental error bars. The relative quantum yield obtained from the biexponential fit shown in Figure 8b drops to about 0.03, the discrepancy now being larger than the estimated error.

Figure 8. Two solutions for the ordinary kinetic equations of the Eigen mechanism [3], eq 1 (dashed curves), as compared with the exact numerical solution for the Smoluchowski equation (full curves). Both models have the same $\kappa_s$ and $\kappa_r$, but different values for the complex separation rate constant in the kinetic scheme were employed: (a) giving the same area or (b) the same initial transient behavior as compared with the exact solution for the $R^*OH$ decay [10b].

The smallness decay is one indicator of the diffusional scheme on state should be a power law as $t^{-3/2}$, with Figure 7 demonstrates diminishes with increasing fluorescence signal due to single-step by $\exp(t/\tau_f)$, to correct points, shown on a magnitude in intensit Smoluchowski equation (a power law becomes

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Figure 9. The (for an infinite 1 only on a log-log scale).

Figure 9 is a demonstration of the situation when it involves one a
The smallness of the quantum yield predicted from the bi-exponential decay is one indication that the long-time behavior is not exponential. In the diffusional scheme one can show [14] that the asymptotic decay of the bound state should be a power law. In a three dimensional space it is expected to behave as $t^{-3/2}$, with a prefactor which depends on $R_D$, $D$, $\kappa_d$ and $\kappa_r$. As Figure 7 demonstrates, this factor is enhanced by increasing $R_D$ and $\kappa_r$ and diminishes with increasing $D$ and $\kappa_d$. To test the asymptotic behavior, the fluorescence signal has been followed [10c] over a large time range using the time correlated single photon counting technique. The transient decay is multiplied by $\exp(t/\tau_f)$, to correct for the finite fluorescence lifetime, $\tau_f$. The data points, shown on a log-log scale in Figure 9, covers nearly three orders of magnitude in intensity. The full curve is the exact numerical solution to the Smoluchowski equation, while the dashed line is the asymptotic $t^{-3/2}$ behavior (a power law becomes a straight line in a log-log scale).

![Graph](image)

Figure 9. The asymptotic behavior of HPTS fluorescence decay (for an infinite fluorescence lifetime): Same data as in Figure 6, only on a log-log (base 10) plot [10c].

Figure 9 is a direct experimental proof for the inadequacy of the rate equation, as opposed to the diffusional approach. A bi-exponential decay, even when it involves one additional adjustable parameter, fits only up to about 1ns.
(half of the time scale shown). Approximately at this time the transient data approaches the asymptotic behavior which it subsequently follows for more than an order of magnitude in intensity. We expect the range of this power-law behavior to be limited only by homogeneous recombination with the very small concentration of protons available at the neutral pH values of the experiment.

4. Salt Effects on Steady State and Transient Kinetics

For dissociation reactions in which the reverse, recombination step is slow, dissociation is virtually complete once the pair has separated to the contact distance. Under these conditions the magnitude of the interaction in the dissociated pair, in particular the screening of this interaction by ions, would not affect the dissociation rate. In terms of the Hammond postulate \[22\] and its extensions \[23\] an exothermic dissociation process would have its transition state close to the bound state, so that the equilibrium and recombination rate coefficients would change in parallel. In such a case one expects no salt effect on the dissociation reaction, in agreement with the classical picture of Brønsted and Bjerrum for kinetic salt effects \[7\].

The situation is completely different for fast, diffusion influenced, reversible dissociation processes such as proton dissociation from excited HPTS \[11\]. Due to the fast recombination rate and the strong Coulomb attraction (HPTS anion is quadruply charged) dissociation is not complete once the OH bond is cleaved: The dissociated proton may return to the origin of its random walk and rebind. In water, we find that each dissociated proton rebinds at least once \[10a\]. The number of rebindings, defined as the area under the non-exponential tail divided by the area under the initial exponential decay, is determined by the magnitude of the proton/anion attraction. Screening of this interaction by inert salts is expected to decrease geminate recombination and tilt the balance towards the dissociated state. A particularly large effect is expected in low dielectric solvents, where the Coulomb attraction is especially large. As Figure 10 shows, there is a dramatic effect of added salt (NaNO₃) on the steady state fluorescence spectrum of HPTS in a 50/50%(v) mixture of methanol/water: With it in this solvent) decreases Note also the nice isosom states (acidic and basic quenching effect on the e

Figure 10. Steady state methanol/water as a func a-h correspond to 0, 5, 14,
methanol/water: With increasing salt concentration the acidic peak (at 432nm in this solvent) decreases while the basic peak (508nm) increases in amplitude. Note also the nice isommisive point obtained (482nm), implying that only two states (acidic and basic) are involved and that the salt has practically no quenching effect on the excited HPTS.

![Figure 10. Steady state fluorescence spectra of HPTS in 50/50% (by volume) methanol/water as a function of inert salt (NaNO₃) concentration [11c]. Curves a-h correspond to 0, 5, 14, 27, 50, 83, 130 and 222 mM NaNO₃, respectively.](image-url)
The decrease in the $R^{\ast}OH$ quantum yield at this salt concentration range is entirely due to the suppression of the non-exponential tail by the screening effect which diminishes the geminate recombination process. This is confirmed by direct time resolved measurements [11b] shown in Figure 11.

![Figure 11. Suppression of the long-time tail, by the addition to water of an inert salt (NaNO₃). Streak-camera data [11b].](image)

The simplest way to treat the salt effect is to multiply the bare Coulomb attraction, $-R_D/r$, by a "screening function". Within the framework of our diffusional approach, a screened potential is used in the transient Smoluchowski equation to obtain the transient decay and its time integral namely, the quantum yield. The values for the rate parameters in the back-reaction boundary condition are the same as at zero salt (this assumption will break down at very large salt concentrations, not applied here). We have tested [11c] two screening functions against steady state quantum yields obtained from fluorescence spectra such as those in Figure 10. The first is the well known exponential screening function of Debye and Hückel [21], with the finite ion size correction. It is shown as the dashed curve in Figure 12. As concluded earlier from the fit to the ground-state recombination rate coefficient (Figure 3), the Debye-Hückel potential overestimates the screening effect.

The full curves in [17], which assumes no dissociation, $R_{eq}$, and full screening, $V_{NA}(r) = -(1/3)\epsilon/\epsilon_0$ and zero otherwise. To correct for concentration, we conclude $c^{-1/3}$, rather than exhibit Hückel theory [7, 21]. Th

![Figure 12. Salt effect on quantum yield in different solvents: Water (OH), methanol/water [11c], relative height ratio of the e.g., Figure 10. Dash expression (with finite ion size correction) [17, 11c], respectively. B](image)

The difference be

The main difference between the two functions is the depth. The narrower NaI is closer to the parent anion and has a larger probability for the
this salt concentration exponential tail by the nation process. This is own in Figure 11.

\[ V_{NA}(r) = -\left( \frac{R_D}{r} - \frac{R_D}{R_{av}} \right), \quad r \leq R_{av} \] (2)

and zero otherwise. Taking \( 4\pi c R_{av}^2 / 3 = 1 \), where \( c \) is a uniform salt concentration, we conclude that the quantum yield in the NA is proportional to \( c^{-1/2} \), rather than exhibiting the \( c^{-1/2} \) dependence predicted by the Debye-Hückel theory [7, 21]. This agrees with experiment above about 20 mM salt.

![Graph](image)

Figure 12. Salt effect on the relative \( R^+OH/R^+O^- \) quantum yield in two different solvents: Water (left panel) and a 50/50% (by volume) mixture of methanol/water [11c]. Circles are experimental data obtained from the relative height ratio of the two peaks in the steady-state fluorescence spectrum e.g., Figure 10. Dashed and full curves correspond to the Debye-Hückel expression (with finite ion-size correction) [21] and the Naive Approximation [17, 11c], respectively. Both models employ the zero-salt kinetic parameters.

The difference between the two screened potentials is illustrated by Figure 13. Both predict a remarkably similar value at the contact distance, \( a \). The main difference between the potentials is in their width rather than their depth. The narrower Naive Approximation potential restricts the proton to be closer to the parent anion thus leading to more geminate recombination and larger probabilities for the bound (acidic) state.
Figure 13. The screened Coulomb potentials [11c]: The Naive Approximation (full curve) is compared with the Debye-Hückel potential (dashed curve), both at c=50 mM. The unscreened potential (wide full curve) is also shown for comparison.

5. The pH Effect as a Many-Body Problem

As seen in Figure 5 above, the addition of homogeneous protons results in enhanced proton/anion recombination and therefore an enhanced long time tail. Once the geminate proton dissociates, it competes with the homogeneous protons over the binding site. This many body effect may be treated within the framework of reversible diffusion influenced reactions.

The complete analysis involves the solution of a multidimensional diffusion (Smoluchowski) equation in the coordinates of all of the available protons. At high proton searches for simple but convolution relations do a simpler idea is to extend a "bimolecular bounda rebinding, $r$, by the pr of the other protons [18 dimensional stochastic unbound site is shown excellent when dissoci recombination.

Figure 14. The equation (dashed curves) [18a], unbiased (zero 1 walker on 100 randomly over walker (at most equal to the ra)
protons. At high proton concentrations this becomes impractical and one searches for simple but useful approximations. One approximation, using convolution relations derived for isolated pairs, is given in [14]. An even simpler idea is to extend the back-reaction (reversible) boundary condition into a "imolecular boundary condition", by multiplying the rate coefficient for rebinding, \( \kappa_r \), by the probability that the binding site is unoccupied by any of the other protons [18]. A comparison of this approximation with one dimensional stochastic simulations [18a] for free diffusion and an initially unbound site is shown in Figure 14. It is seen that the approximation is excellent when dissociation is fast, but deteriorates in the limit of irreversible recombination.

![Diagram](image)

Figure 14. The bimolecular boundary condition for the Smoluchowski equation (dashed curves) compared with an exact simulation (full curves) [18a]. The simulation involves 100,000 realizations of unbiased (zero potential), one dimensional random walks involving 20 walkers on 100 lattice sites. The walkers are initially distributed randomly over all sites except the binding site. At later times one walker (at most) can occupy the binding site. The diffusion rate is equal to the rate of entering the site (\( \kappa_r \)), and both are set to unity. The rate for leaving the site (\( \kappa_d \)) varies.
6. Diffusion in Restricted Geometries

The discussion so far centered on proton diffusion in an infinite space. Hence, a spherically symmetric diffusion (Smoluchowski) equation in three dimensional space has been employed in the data analysis. An inner boundary condition (at the contact distance) has been imposed to describe reaction, but no outer boundary condition. Almost all of the interesting biological applications [4] involve proton diffusion in cavities and restricted geometries. These may include the inner volume of an organelle, the water layers between membranes or pores within a membrane.

HPTS fluorescence has been employed in the study of proton diffusion within inverted micelles [24], in liposomes [4a], in apomyoglobin and the inter-membranal hydration layers of multi-lamellar vesicles [4b]. The simplest case for analysis involves the HPTS molecule in the center of a sphere (inverted micelle, liposome) whose walls are impermeable to protons on the timescale of the experiment. This outer wall is therefore described by an additional reflective boundary condition. Inside such a sphere, even a single proton/anion pair ultimately reaches an equilibrium situation: The long-time tail approaches a plateau, rather than decaying to zero. The smaller the radius of the sphere, the higher the expected asymptotic plateau.

A representative calculation [12a] for the probability of observing a bound \( R^+ OH \) molecule inside such a sphere is shown in the left panel of Figure 15. The calculation uses the kinetic parameters from water and assumes a sphere radius of 250\( \AA \). The right panel shows a preliminary experiment [4a] monitoring HPTS inside liposomes (curve labelled B) as compared with its fluorescence trace in pure water (curve A). It is seen that the long-time tail is indeed enhanced when the fluorophor is located inside a liposome. A more quantitative determination of liposome size distribution will allow a quantitative comparison between experiment and theory.

It is important to find good model systems to test the applicability of the theory in restricted geometries. Once the method is calibrated against the radius (or volume) of the cavity, the HPTS fluorescence decay trace could be used as a "microscopic ruler" to determine cavity size [12a].

7. Acknowledgements

I am indebted to my col Pines and A. Szabo, who supported in part by gr Science Foundation (BSF) is supported by the Min.

8. References

[3] Eigen, M. *Angew. C.*
[4] (a) Gutman, M. *Melt*
infinite space. Hence, a boundary condition (at the reaction, but no outer geometries. These may

Figure 15. Reversible proton dissociation inside a finite cavity. Left panel [12a] is a representative calculation for the probability of observing a bound pair (i.e., assuming an infinite radiative lifetime, \( \tau_f \)). Right panel [4a] compares transient HPTS fluorescence in water (trace A) with its signal when located inside a liposome (trace B). The insert shows the same data on a semi-logarithmic scale.

7. Acknowledgements

I am indebted to my colleagues and collaborators: A. Blumen, D. Huppert, E. Pines and A. Szabo, whose talents made this exposition possible. This work is supported in part by grant number 86-00197 from the US-Israel Binational Science Foundation (BSF), Jerusalem, Israel. The Fritz Haber Research Center is supported by the Minerva Gesellschaft für die Forschung, München, BRD.

8. References

AN EXPLORATORY AND THEORETICAL APPROACH TO THE PROBLEM OF \textit{Ab initio} \textit{Ab initio} ELECTRONIC STRUCTURE CALCULATIONS ON BIOMOLECULES

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ABSTRACT. After a brief review of theoretical approaches to the prediction of the electronic structure of biomolecules, the results of \textit{ab initio} calculations on several selected systems are presented. The methods employed include density functional theory (DFT) and \textit{ab initio} molecular orbital (MO) methods. The results are compared with experimental data and other theoretical calculations, and the limitations and potential of these methods are discussed.

1. Introduction

The ionization constants of biomolecules are crucial for understanding their behavior in biological systems. The ionization of water-soluble biomolecules can be described by the following equilibrium:

\[ \text{H}_2\text{O}^+ + \text{R}^\text{-} \rightleftharpoons \text{H}_3\text{O}^+ + \text{R}^- \]

where \( \text{R}^- \) represents the negatively charged group of the biomolecule. The ionization constant \( K_{\text{a}} \) of water is given by:

\[ K_{\text{a}} = \frac{[\text{H}_3\text{O}^+][\text{R}^-]}{[\text{H}_2\text{O}^+][\text{R}^-]} \]

In practice, one tends to use the \( K_{\text{b}} \) constant instead, which is defined as:

\[ K_{\text{b}} = \frac{[\text{H}_2\text{O}^+][\text{R}^-]}{[\text{H}_3\text{O}^+]} \]

was 1923 Brønsted extended the concept of ionization constants to the \( pK_{\text{a}} \) and \( pK_{\text{b}} \) values. These constants are defined as:

\[ pK_{\text{a}} = -\log K_{\text{a}} \] and \[ pK_{\text{b}} = -\log K_{\text{b}} \]

In practice, one tends to use the \( pK_{\text{a}} \) and \( pK_{\text{b}} \) values for their convenience. The \( pK_{\text{a}} \) value of a biomolecule is given by:

\[ pK_{\text{a}} = -\log K_{\text{a}} = \log \frac{[\text{H}_2\text{O}^+][\text{R}^-]}{[\text{H}_3\text{O}^+]} \]

In heterocyclic chemistry, the \( pK_{\text{a}} \) values may outline some examples of weak acids and bases. In order to ensure the desired concentration range of the compound, For example,

\[ S. J. Formosinho et al. (eds.), Theore... 1991 Kluwer Academic Publishers. \]