DETERMINATION OF PROTON TRANSFER RATE CONSTANTS USING AB INITIO, MOLECULAR DYNAMICS AND DENSITY MATRIX EVOLUTION CALCULATIONS.

DAVID VAN DER SPOEL and HERMAN J.C. BERENDSEN
BIOSON Research Institute, Department of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

In this work we give an overview of the methodologies required to compute the rate of proton transfer in hydrogen bonded systems in solution. Using ab initio or density functional methods we determine proton potentials of a truncated system as a function of proton-donor proton-acceptor distance as well as nonbonding parameters. By classical molecular dynamics we evaluate a swarm of proton potentials with the proton fixed in the reactant well. The rate of proton transfer is calculated perturbatively using the Density Matrix Evolution (DME) method, going beyond the Born-Oppenheimer approximation. The method is illustrated by two examples: hydrogen malonate and the active center of HIV-1 protease.

1 Introduction.

Hydrogen bonding is of prime importance in chemistry and biochemistry. Intramolecular hydrogen bonds stabilize the folded form of proteins as well as small organic molecules. Intermolecular hydrogen bonding is often important for specificity of binding between molecules in a complex [1] and it determines the structure and properties of water and many other polar molecules such as alcohols. For molecular dynamics (MD) and Monte Carlo simulations of liquid water, where we are interested in thermodynamics and structure, hydrogen atoms can be described classically and statically [2–4]. When proton dynamics is of interest, eg. when proton transfer from donor to acceptor is non-negligable, a quantum mechanical description of the proton is required [5]. It was recently proposed for instance, that hydrogen bonds in a β-sheet may be of N^- ... HOC^+ character rather than NH ... OC [6]. Hydrogen bonds in small organic molecules, such as hydrogen malonate, have been studied extensively by spectroscopic techniques [7] as well as theoretical methods [8], and see below.

Proton transfer is often important in chemical reactions, especially in biological systems such as proteins where hydrogen bonds are numerous. An interesting example of the latter are the aspartic proteases, proteins which have a strong hydrogen bond between a charged and uncharged aspartate residue in the active site. It is thought that proteolysis is initiated by a proton transfer reaction in this hydrogen bond [9]. Experimental determination of proton transfer rates is difficult, although it has been done for some cases, eg. the association of H_3O^+ and OH^- in water[10], benzoic acid dimers in a crystal[11] or in deuteron glasses[12]. In this work we will show how the rate of proton tranfer can be determined, even in complex systems such as an enzyme, by a combination of classical MD and quantum mechanical methods, taking solvation into account.

It is in principle possible to couple classical mechanics (in this case, molecular dynamics) and quantum dynamics directly[13–17] and compute the rate of proton transfer by counting the number of proton transfers during a long simulation or using the reactive flux correlation mechanism[18]. This is however not practical for many highly polar systems like proton transfer in aqueous solution and enzymatic environments, because very long simulations, $O(\mu s)$, would be necessary to get a reliable estimate of the rate constant. We have therefore used a perturbational approach, in which an entirely classical simulation is performed where the proton is fixed in the reactant well. The simulation then delivers an ensemble of proton potentials that we use as starting point for the quantum-dynamical computation of the rate constant by the Density Matrix Evolution (DME) method[19].

The hydrogen bonds in systems such as hydrogen malonate and the active site of HIV-1 protease are essentially symmetric. The proton potential in such a system may be single well or double well depending on the distance between donor and acceptor atoms. However, when a proton is bound to one of the oxygen atoms that can function as proton donor, the symmetry will be broken due to polarization of the environment. There are two contributions to this phenomenon, first solvent molecules will stabilize the hydrogen bond by orientational as well as electronic polarization, second the C-O bond length of the proton acceptor will be elongated because of the hydrogen bond. For the proton this means that the reactant well is stabilized with respect to the product well. This environment-induced asymmetry is not constant however. Thermal fluctuations in the solvent around the hydrogen bond and in O-O distance may lead to a (temporarily) decreased stabilization of the reactant well. Since a proton is essentially a quantum particle, it may tunnel through a barrier and the product well will be populated significantly.

In this paper we will give a schematic overview of the computational procedure without going into details which have been published elsewhere[19–22]. The procedure will be illustrated with two examples that we have studied recently, hydrogen malonate[22] and HIV-1 protease[23].

2 Methodology.

Five basic computational tools are essential for our work:

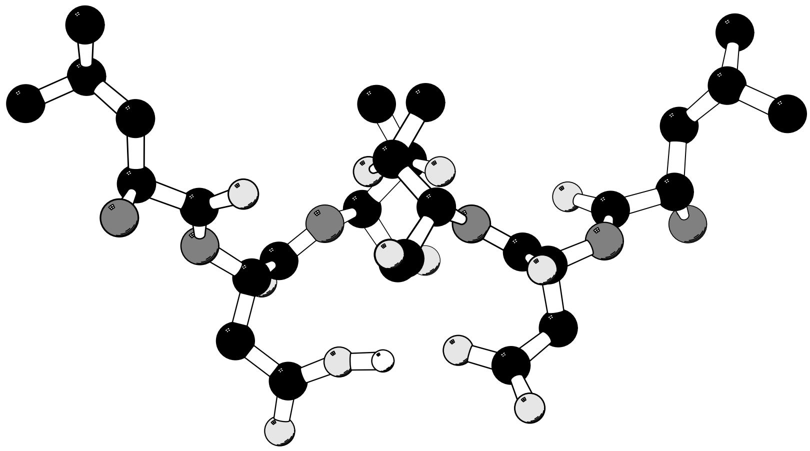

Figure 1: Structure of the active site of HIV-1 protease, of both monomers residues Leu24-Asp25-Thr26 are plotted. Carbon atoms are black, nitrogen atoms are dark grey, oxygen atoms are light grey, the proton is white. The plot was made using MOLSCRIPT [27]

1. Ab Initio calculations which we performed using the Gaussian-92 suite of programs [24].
2. Molecular dynamics simulations using the **GROMACS** software [25,26].
3. Computation of the classical contribution to the proton potential.
4. Computation of the total proton potential.
5. Computation of density matrix evolution [19].

Each of these has its own specific problems such as the selection of level of theory for the ab-initio computations, the force field for the MD simulations and the basis functions for the DME calculation. Although these questions are important, we will not discuss them here; it is clear that methodological improvements or improved parametrization of force fields and basis sets will enhance the accuracy of the result.

Instead, we will focus on a “recipe” for computing the rate of proton transfer in hydrogen bonded systems. One of our example systems, (HIV-1 protease), is depicted in Figure 1. It can be seen here that the system is very symmetric. A flow chart that gives a quick overview of the procedure is given in Figure 2. The computational units in the flowchart can almost be used like a black box. If one is aware of the limitations and possibilities of each of them, they can be used without deep insight in algorithms etc. We will describe these

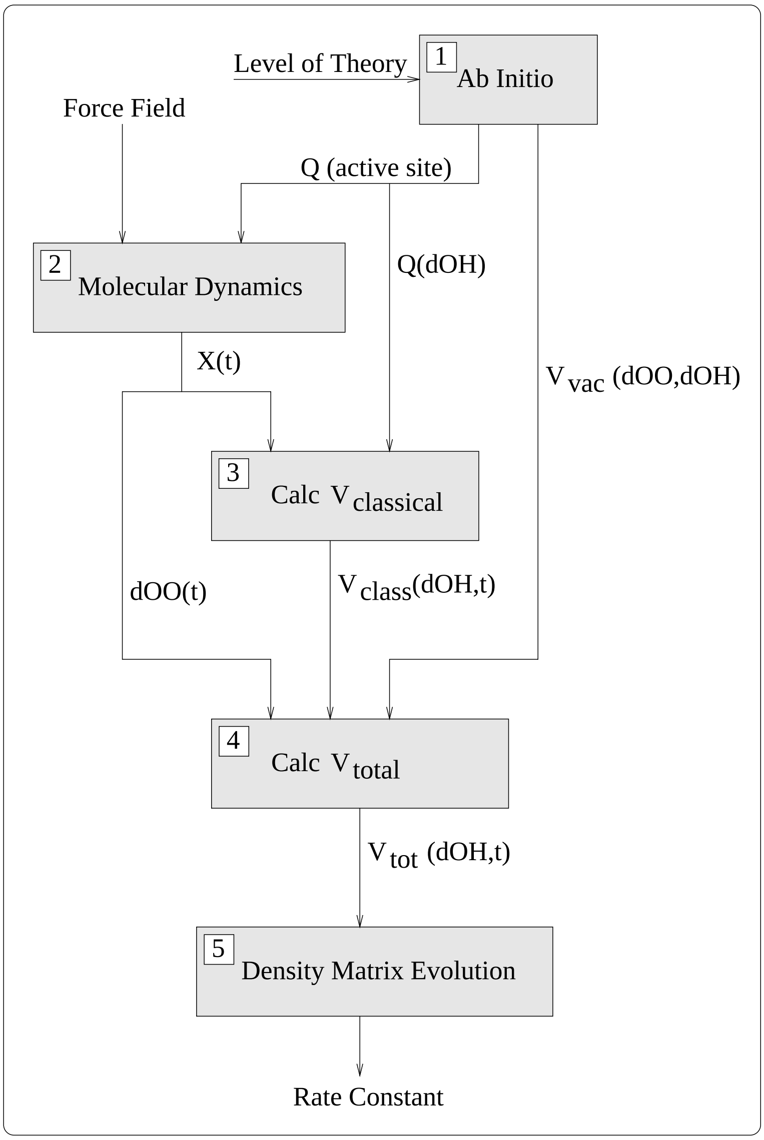

Figure 2: Flowchart for computation of the rate constant of proton transfer in hydrogen bonded systems. dOO is the distance between proton donor and acceptor, dOH is the distance between proton and proton donor. The five boxes denote the basic computations in our scheme.

5 items below.

Step 1. Ab Initio

Ab Initio calculations were performed on the complete hydrogen malonate ion, for the HIV-1 protease it was necessary to truncate the system to hydrogen diformate, which is similar to hydrogen malonate. It is well known that the calculated proton potentials are very much dependent on the applied level of theory. Flexible basis sets are required and inclusion of electron correlation is especially important [28]. Density functional theory calculations are very attractive since they yield results close to the MP2 level of theory at only a fraction of the computational cost. In hydrogen malonate ab initio calculations were not conclusive about the height of the barrier for the proton potential, for hydrogen diformate we used B3LYP/6-311+G(2d,2p) [29,30] as implemented in the Gaussian-92 suite of programs [24]. Proton potentials $V_{vac}(dOO, dOH)$, i.e. the proton energy as a function of oxygen-oxygen distance (dOO) and proton-oxygen donor distance (dOH) were computed for 3 different dOO and 7-9 different dOH distances. At each point the angle $\angle$DHA was optimized while the other degrees of freedom were frozen because the proton is much faster than the other nuclei. The resulting *in vacuo* proton potentials are plotted in Figure 3 for the HIV-1 protease truncated system.

There are several methods to compute point charges from ab initio data, the most well known being the Mulliken population analysis. For the charges on hydrogen malonate we used the method that conserves the dipole moment [31]. In the truncated system of HIV-1 protease we used the Merz-Kollman procedure, that fits the charges to the electrostatic potential [32] using a solvent reaction field. We think the latter method is the method of choice at this point in time. For the purpose of the computing the classical part of the proton potential the charges were computed for 3 different dOH at an average dOO distance, since the charges do not depend on dOO very much, but they strongly depend on dOH.

Step 2. Molecular Dynamics

MD calculations of the hydrogen bonded system in explicit solvent (SPC water [2]) were performed, using periodic boundary conditions. We used the GROMOS forcefield [33] with modifications as suggested in [34, 35] and explicit hydrogen atoms on aromatic residues in the HIV-1 simulation [36]. The complete hydrogen malonate system contained 255 water molecules in a truncted octahedron box, while the HIV-1 protease dimer was dissolved in a rectangular box with 5795 water molecules giving rise to 19290 atoms. Full details of the MD simulations are given in refs. [20] and [23]. For the computation of the proton potentials it is necessary to have a large number of time origins, therefore the coordinates of all atoms including solvent were stored every 10 fs during 70 ps MD (HIV-1) resp. every 2 fs during 100 ps MD (hydrogen malonate). The highest frequencies that can be extracted from the molecular dynamics trajectory of a protein simulation are typically not higher than 3000 cm^{-1} which corresponds to about 10 fs. Therefore it is not useful to store coordinates more often. The MD simulations of the solvated HIV-1 protease dimer took 30h/10 ps on a SGI Indigo^2 computer.

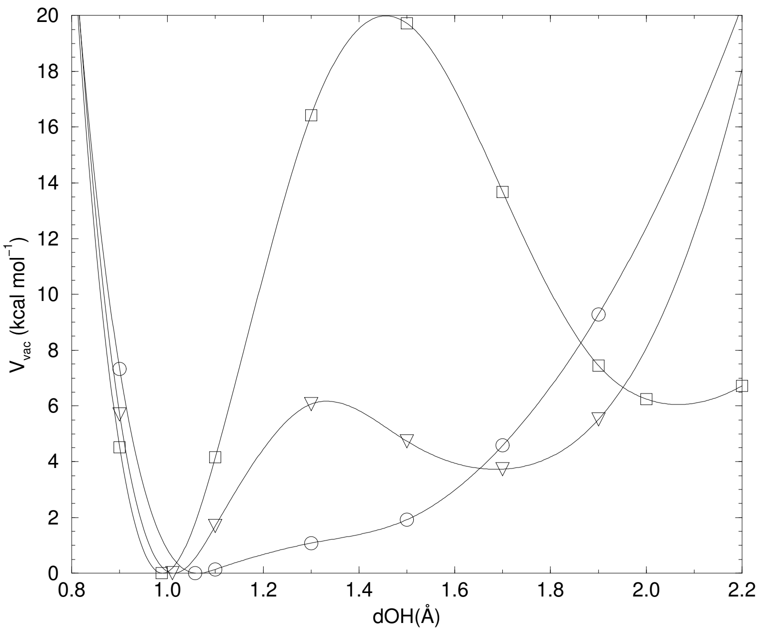

Figure 3: Proton potentials for the hydrogen diformate for various OO distances calculated on the B3LYP/6-31+G(2d,2p) level. Orientation of the carboxylic groups was corresponding to the x-ray structure. Note the asymmetry because of the separation of time scales, because the proton is much faster than the other particles. The calculations were performed at OO distances of 2.350Å (○), 2.537 Å (▽) and 2.815Å (□).

Step 3. $V_{classical}$

Using the coordinate frames from MD and the charges from ab-initio computations, the classical contribution V_{class} to the proton potential was determined as follows:
At each coordinate frame the coulomb interaction of the truncated HIV-1 system resp. hydrogen malonate were computed with the surrounding solvent, while the donor-proton distance (dOH) was varied. The charges from ab initio were interpolated using a spline procedure since more points (different dOH) on the proton potential were computed than charges. Note that in the HIV-1 case solvent includes the remainder of the protein and water molecules.
This leads to a table of time-dependent classical proton potentials V_{class}(t,dOH). When the proton hops from donor to acceptor, electronic polarization will be immediate, i.e. the proton is much slower than electrons. The solvent on the other hand is much slower than the proton, so that we can safely assume that solvent is frozen during proton transfer. In our classical calculation the electronic polarization is not taken into account, although its contribution is non-negligable. Inclusion of electronic polarization would require polarizable force fields for proteins which are currently not available, although a promising method based on polarizable shell model is under development in our laboratory [37]. Therefore we derived an analytical correction to V_{class} based on the Onsager reaction field model (see ref. [23] for details) which implies a simple scaling of V_{class} by a factor 0.583.

Step 4. V_{total}

The time-dependent *in vacuo* proton potential is computed by filling in dOO(t) from the MD simulation in $V_{vac}(dOO, dOH)$:

$$V_{vac}(t, dOH) \; = \; V_{vac}(dOO(t), dOH) \tag{1}$$

Then this term is summed to V_{class}(t,dOH) to yield the total time dependent proton potential V_{tot}(t,dOH):

$$V_{tot}(t, dOH) \; = \; V_{class}(t, dOH) + V_{vac}(t, dOH) \tag{2}$$

Since we computed $V_{vac}(t, dOH)$ at fewer different dOH than V_{class} we apply spline interpolation to get the proton potential at intermediate dOH. This procedure delivers a table $V_{tot}(t, dOH)$ which can be used in the subsequent quantum dynamical calculation.

Step 5. Density Matrix Evolution

Recently the Liouville-Von Neuman equation

$$\frac{\partial \boldsymbol{\rho}}{\partial t} = -\frac{i}{\hbar}[\boldsymbol{\rho}, \mathbf{H}] \tag{3}$$

was coupled with classical equations of motion in a consistent way to allow for simultaneous integration of classical- and quantum- degrees of freedom and the method was called Density Matrix Evolution [15, 19–23, 38–40]. Here we have used the DME method in a perturbational way: the classical system was considered frozen and the time evolution of the quantum subsystem (i.e. the proton) was computed using many different time origins. Thus, we solve the time-dependent Schrödinger equation by the DME method for all the proton potentials obtained before. Starting from each time origin, the proton is put in the reactant well and the Schrödinger equation is integrated for 1-2 ps with a time step of 1 fs. Since the time-dependent proton potentials do not have such high time-resolution we have used a spline interpolation to obtain the potential at intermediate points. In the hydrogen malonate case two basis functions for the proton were used in the first attempt to compute the rate constant [20]. Later, the calculations were redone with five basis functions, yielding a thirty-fold increase of the rate constant [22]. It was concluded that five flexible basis functions suffice to describe the quantum dynamics of the proton; therefore we have also used five basis functions for the proton in the active site of HIV-1 protease.

The initial increase of the product population Θ can be computed as the expectation value of the heavyside operator concerning the population of the product well:

$$\Theta \;=\; \int_R^\infty \Psi \;\Psi^* dr \tag{4}$$

where R is the $dOO/2$. Θ averaged over many thousand time origins is shown in Figure 4 for the case of HIV-1 protease.

From the slope the rate constant for the proton transfer can be estimated. This part of the calculations was described in great detail in ref. [22]. It is worth to stress that when the swarm of proton potentials is determined, the quantum dynamical calculations could be performed by the DME method, but also by other quantum dynamical methods like wave packet dynamics [13, 41], real time path integration [14, 42] or surface hopping [16, 43].

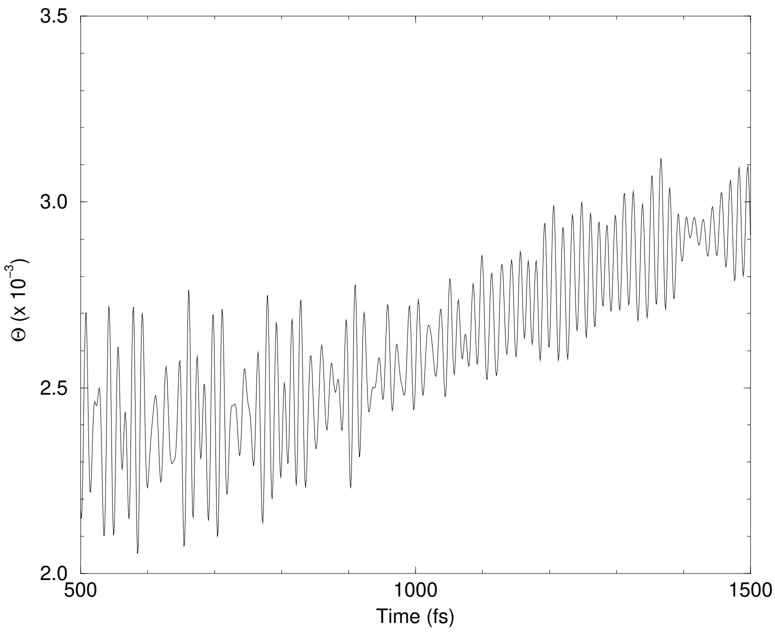

Figure 4: Time course of population of product state Θ in the active site of HIV-1 protease, calculated by the Density Matrix Evolution method obtained by averaging solutions over 6000 time origins.

3 Results & Discussion

With our methodology we are able to calculate the rate of proton transfer in hydrogen bonded systems. For hydrogen malonate [22] we found the rate constant to be $(4.0 \pm 0.9) \times 10^8 \mathrm{s}^{-1}$, for the active site of HIV-1 protease [23] (see Figure 1) we found $(6.7 \pm 0.9) \times 10^8 \mathrm{s}^{-1}$. Both rate constants imply that proton transfer happens on the nanosecond time scale. It should be noted that in the computation of the rate constant in the case of hydrogen malonate the polarization correction to V_{class} (see Step 3 above) was not taken into account; since electronic polarization stabilizes population of the product well, proton transfer will be enhanced and the rate constant will be higher. Although the rate constants of proton transfer depend on many factors, the fact that we find about the same rate constant for the proton transfer between the carboxylates in aqueous solution and in the protein environment indicates that the HIV-1 active center is comparable to water as concerns polarity.

Several other methods are available for the calculation of proton transfer rate in complex systems such as path integral [14, 42], wave packet dynamics [13, 41] and surface hopping [16, 43]. Like path integral and wave packet dynamics, DME using n basis functions giving rise to n levels, represents an approach which takes into account ground state and excited state proton transfer simultaneously. It would be interesting to see whether any of these methods applied to our test systems would yield similar rate constants. If so, this would seriously strengthen the confidence in combined classical-quantum mechanical calculations.

Outlook

A challenging application of our method is in the design of inhibitors (or catalysts) for systems where proton transfer is crucial. From the calculated rate constant for proton transfer between Asp-25 and Asp-125 in the active center of HIV-1 protease, it is evident that the proton transfer takes place on the nanosecond timescale. The hydrogen bond is therefore highly polarizable and the equilibrium can be shifted by binding of an inhibitor. The high polarizability of the hydrogen bond should be taken into account by future design of HIV-1 protease inhibitors using molecular dynamics simulations and thermodynamic integration methods. A number of such studies on the molecular mechanics level have already been done [9, 44–46]. Another application of the combination of the methods above, currently being done in our laboratory, is the computation of the rate of proton transfer from H_3O^+ to OH^- when in contact in aqueous solution [47].

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