Large and Fast Relaxations Inside a Protein: Calculation and Measurement of Reorganisation Energies in Alcohol Dehydrogenase[1]

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Abstract

CIS electronic structure calculations have been employed to derive the fluctuation in the ground to excited state energy gap of dihydro-Nicotinamide Adenine Dinucleotide (NADH) embedded in the Horse Liver Alcohol Dehydrogenase (LADH *figure 1.*) protein matrix as a function of time.

Classical molecular dynamics trajectories of 130 ps in length have been generated for an LADH+NADH dimer in water to elucidate the dynamics of equilibrated LADH over a 5ps timescale. Steady-state absorption and emission spectra have then been calculated to an accuracy within 10 % of experiment *(figure 2)* by utilising *ca.* 10,000 CIS QM/MM calculations per simulation coupled to our framework for predicting optical spectra based on linear response theory.[1,2,3] It has been found that the accuracy of the results obtained are very dependent on the quality of the molecular dynamics trajectory, especially in the parameterisation of the nicotinamide moiety. This can be explained by considering the orbitals involved in the excitation leading to the observed emission. *Figure 3* shows a snapshot from one of the CIS calculations. The orbitals are localised on the nicotinamide moiety and so it is fluctuations in this section of the NADH that are responsible for the emission spectra of LADH.

The results have shown that applications of linear response theory can work for systems, such as NADH, which have very large reorganisation energies (Stokes Shift ≈ 1 eV) yielding predicted spectra that are within 10 % of experiment. Previous work with different NADH parameters yielded poor results implying that the method employed in this work provides an independent method for validating certain force field parameters.

Figure 1. Cartoon representation of the crystal structure[4] of Horse Liver LADH (1LDY[4]) at 2.5 Å.
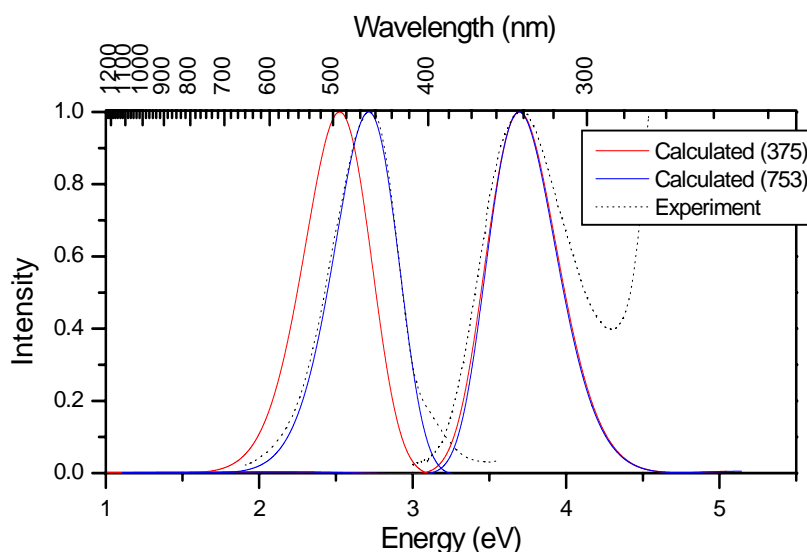

Figure 2. Comparison of average calculated (solid) and experimental (dotted) absorption and emission spectra for LADH.

Figure 3. Snapshot from one of the CIS/3-21G* calculations showing the ground state orbitals that are excited from. This clearly shows that the excitation is centred on the nicotinamide end of the NADH.
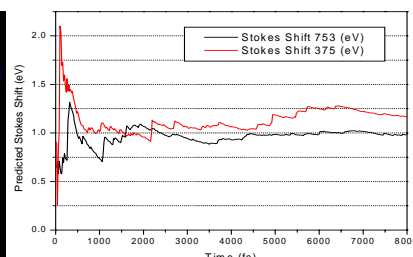

Figure 4. Plot showing predicted Stokes shift as a function of run length for each NADH residue within the LADH dimer.

Methodology

The methodology used is discussed in reference 2 but can be summarised as follows:

1) A equilibrated classical molecular dynamics trajectory at 300K is generated using the Amber force field[5] and AMBER suite[6] of molecular dynamics programs. Snapshots of the structure are taken at 2fs intervals over a production run length of 10ps.

2) *Ca.* 10,000 time ordered QM/MM input structures for the *ab initio* package Gaussian 98A7[7] are then produced from the MD trajectory. A single point CIS/3-21G* calculation is then performed on each structure with the NADH coenzyme forming the QM section with the point charges of the surrounding protein and solvent included in the one electron Hamiltonian. This yields a fluctuating ground to excited state energy gap.

3) The autocorrelation of this fluctuating energy gap $M(t)$ is then found using equation 1.

$$M(t) = \frac{1}{\Delta^2} \int_{-\infty}^{\infty} \delta\omega_{ab}(t+\tau)\delta\omega_{ab}(\tau)d\tau \qquad (1)$$

The Fourier transform of this then yields the spectrum of oscillators.

4) From the spectrum of oscillators we can, assuming linear response, construct the optical absorption spectra. The emission spectrum is found by assuming that the excited state potential energy surface has the same profile as that of the ground state, but with the minimum shifted along the relaxation coordinate. In order to introduce a Stokes shift, $M(t)$, is modified to satisfy detailed balance, imparting a complex component. This makes the response of the system conform to the fluctuation-dissipation theorem[8]. Detailed balance is included by operating on the spectrum of oscillators $J(\omega)$, given by the Fourier transform of $M(t)$, so that positive frequencies are related to their negative counterparts by a Boltzman coefficient[9] such that

$$J(-\omega) = \exp(-\hbar\omega/kT)J(\omega) \qquad (2)$$

A modified semi-classical form of the spectral density is formed to satisfy this relationship which can then be back Fourier transformed to yield a modified form of $M(t)$ which imparts a complex component for the linear response function resulting in a Stokes shift between the calculated absorption and emission spectra.

Stokes Shift Convergence

Since the predicted Stokes shift is based on an average, as the number of points used to calculate it increases so the predicted Stokes shift will converge. Thus it is necessary to include enough points such that the predicted Stokes shift can be said to be converged. Since LADH is a dimer containing two NADH residues it was necessary to calculate the Stokes shift for both sites as a function of run length. *Figure 4* shows the predicted Stokes shift vs run length for each NADH. If both NADH sites occupy identical environments then given a long enough time sufficient phase space should be sampled such that the two sites exhibit identical Stokes shifts. In this work it can be seen that the two sites differ by approximately 0.18 ev, hence the two different emission spectra observed. This could be due to a number of reasons.

- Insufficient sampling, a longer run length may be required to sample sufficient phase space.

- MD trajectory not sufficiently equilibrated. More equilibration time may be required before production sampling.

- True in-equivalency between the sites. The two sites may actually be in-equivalent over the period of the molecular dynamics simulation. For example it has been speculated that LADH may exhibit "Half-the-Site activity."

Conclusions

We have:

· Shown that an *ab initio* QM/MM method is capable of reproducing the experimental optical absorption and emission spectra for NADH complexed with LADH in water.

· Found that through vibrations alone and employing just ground state equilibrium dynamics it is possible to accurately predict the existence of a Stokes shift between the absorption and emission spectrum as large as 1 eV.

· Shown that the theory of linear response and the associated approximations made still holds for systems where the reorganisation energy is very large.

· Found that good parameterisation of the NADH moiety is essential to obtaining accurate results and through this have found that the reorganisation upon optical excitation is largely independent of solute-solvent interactions and instead is due to internal vibrations and rotations within the nicotinamide moiety.

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