

Acidity Constants of Lumiflavin from First Principles Molecular Dynamics Simulations

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Abstract

We have computed the free energy profiles of the deprotonation reactions of lumiflavin in the semiquinone and fully reduced oxidation states using constrained DFT-based molecular dynamics simulations. In the semiquinone state, the N5 nitrogen atom and the N1 nitrogen atom can become protonated. We find, in agreement with experiment, that the N5 site is the predominant proton acceptor, although the computed pK_a value is somewhat smaller than the experimental number. The computed pK_a for the N1 protonation in the fully reduced state is in good agreement with the experimental number. We employ two different, commonly used, reaction coordinates based on the distances between the proton and the donor and acceptor atoms. Further improvement of the accuracy of this type of pK_a calculations may require development of more advanced reaction coordinates that go beyond the description of only the first proton transfer step from a donor atom to a first solvation shell water molecule.

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1 Supplementary Information

1.1 Radial distribution functions

To quantify the solvent structure around the proton donating nitrogen atom of lumiflavin and the accepting first solvation shell water oxygen, we have computed the radial distribution functions (RDF) of the hydrogens around these nitrogen atoms (labeled N1 and N5) and oxygen atom (O_w), see also figure 4 in the paper. The graphs are shown in figure 1 for the deprotonation of the N5 site in the semiquinone state (labeled PT1A, top panels), the N1 deprotonation in the semiquinone state (PT1B, middle panels), and the N1 deprotonation in the fully reduced state (PT2, bottom panels). The different colours show the RDFs at different stages of the proton transfer reactions, with in black the initial flavin protonated state, in red an intermediate state in which the proton is (almost) equidistant from the donor and the acceptor atoms, and in green the final stage at which the second proton transfer, from first to second coordination shell, takes place.

The first steep peaks in the left-hand-side panels are due to the position of the transferring proton, which moves to the right (*i.e.* larger N-H distance) as the reaction proceeds. The smaller second peak is mainly due to the two hydrogens of the accepting water molecule, but overlaps with contributions due to other nearby hydrogens, in particular from a hydrogen that is donated to the accepting O_w by another first shell water molecule (see also figure 4 in the paper) and from one or two water molecules that flank the flavin near the donating N atom. As a result from this overlap, the integrals of the RDFs shown by dashed lines do not show a clear step for the number of hydrogens contributing to the second peak. For all three proton transfer reactions, the peak moves to the left and becomes somewhat more pronounced as the transfer proceeds, which is due to the shortening of the N- O_w distance.

At the accepting water oxygen (right panels), the first peaks are due to the two water hydrogens and the proton. The peak due to the latter moves to the left (*i.e.* shorter O_w -H distance) as the proton transfer occurs. The integral indicates a fourth hydrogen (note the right-hand-axis), which is due to a second shell water molecule that donates an H-bond to the O_w , except in the later stages of the PT2 reaction (bottom

right panel). Comparing the solvent structure of the accepting water molecule between the three proton transfer reactions, we find that in the PT2 case the water molecule is always somewhat under-coordinated as also illustrated in figure 4 in the paper. Why this under-coordination only occurs in the fully reduced state is not clear.

1.2 Free energy profiles using the addition H_3O^+ restraint

As described in the paper, we equilibrated the systems also in the product state, in which the flavin has donated its proton to the nearest water molecule, by using, in addition to the Δd constraint, a restraint that avoids that the two hydrogens, other than the transferred proton, leave the H_3O^+ ion. For this restraint, we used a coordination number variable of the number of hydrogens (other than the proton) within a cutoff distance to the oxygen, subject to a steep wall potential that would become effective if the coordination number would drop below 1.6 when one of the hydrogens would attempt to leave.

We have considered whether it would be a good idea to also perform the production runs with this restraint applied, in an attempt to overcome the problem that the free energy profiles can only be computed up to the point that the proton jumps away into the bulk water. Unfortunately, this approach does not work. The main reason is that the transport of the proton, after it reached the first solvent water and formed a hydronium ion, does not proceed as an intact H_3O^+ ion, but rather hops from the first water to next via an intermediate H_5O_2^+ complex (aka the Grotthus mechanisms), so that the first water maintains its H-bond with the flavin donor atom. As the restraint enforces the hydronium ion to remain as an intact H_3O^+ ion, the H-bond with the flavin is stretched when the Δd reaction coordinate is further increased. This results in a negative force (see the middle right panel in Figure 2, and therefore a too high free energy profile (see the top panel).

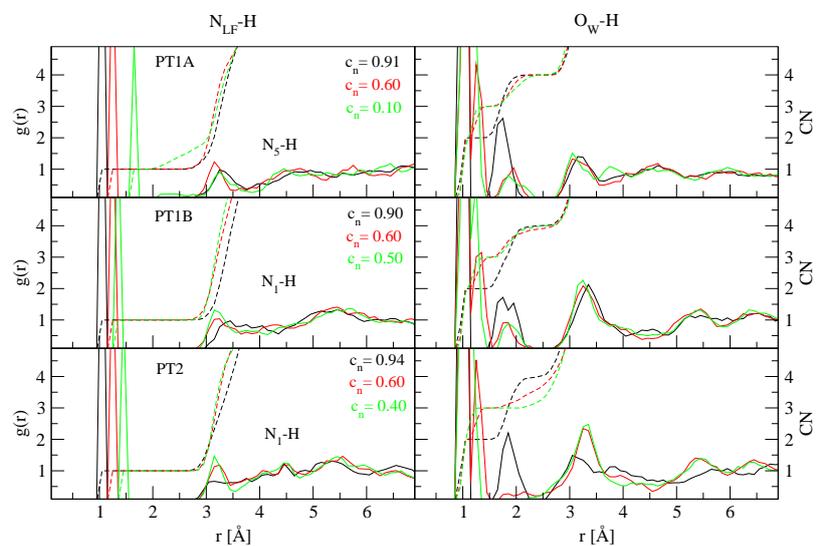


Figure 1: Radial distribution functions of the number of water solvent hydrogen atoms around the proton donating flavin nitrogen atoms (left panels) and around the proton accepting water oxygen atom (right panels), at different stages of the (de-) protonation reactions. Top-to-bottom: N5 deprotonation in the flavin semiquinone state (PT1A), N1 deprotonation in the semiquinone state (PT1B), and N1 deprotonation in the fully reduced state. The dashed lines show the integration of the RDFs from which the coordination numbers can be read, using the right-hand-side axis.

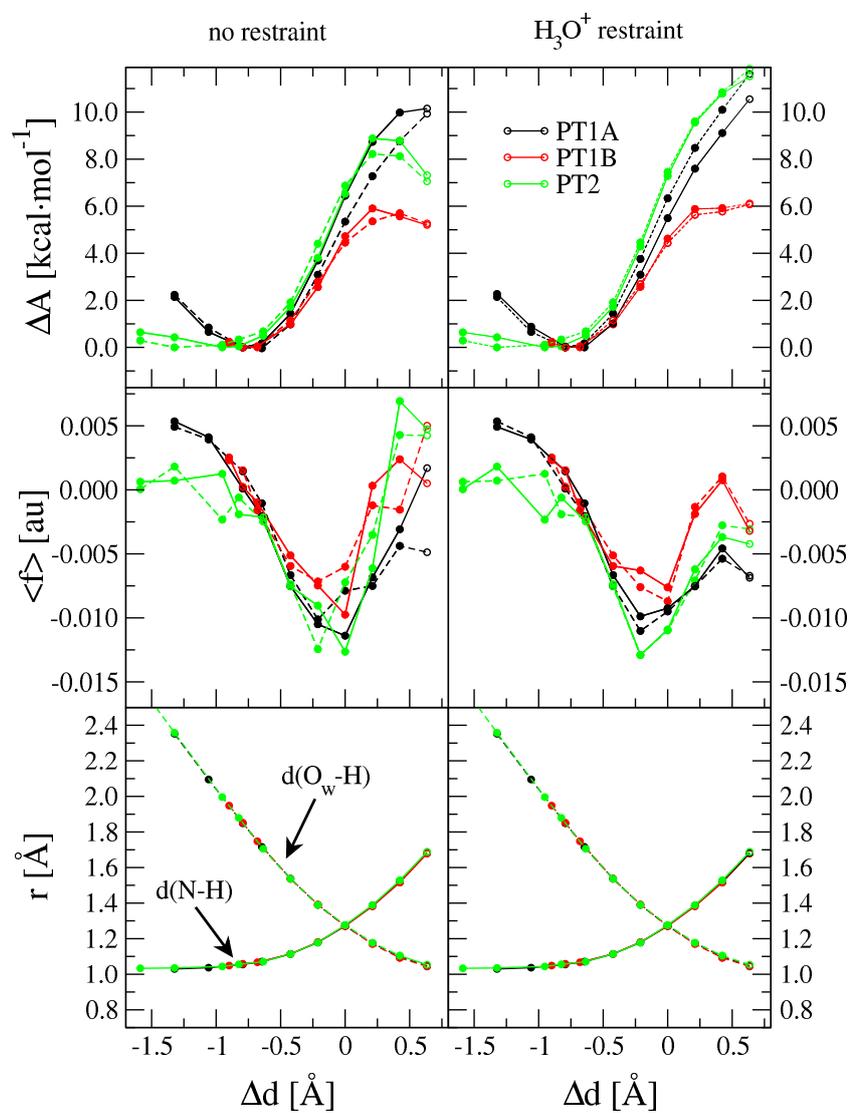


Figure 2: Top panels: free energy profiles for the three protonation reactions computed using the Δd coordinate and an additional H_3O^+ restraint (right panels) and without this restraint (left panels). Note that the latter is thus the same as shown in figure 4 in the paper. Middle panels: average force of constraint. Bottom panels: distance between the proton and the lumiflavin N1 or N5 nitrogen atom and distance between the proton and the accepting water molecule oxygen atom.