Unraveling Low-Barrier Hydrogen Bonds in Complex Systems with a Simple Quantum Topological Criterion

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The importance of low-barrier hydrogen bonds (LBHBs) in enzyme catalysis has remained a controversial topic. LBHBs are defined as protons that are delocalized between two heavy atoms. Since the 1990s LBHBs have been proposed to play a crucial role in several enzyme-catalytic pathways by stabilizing transition states or reaction intermediates.[1–3] However, more recently, with the development of improved theoretical tools for enzymatic studies, such as the hybrid quantum-mechanic/molecular-mechanic (QM/MM) method,[4] several theoretical studies have investigated such systems from a quantum-chemical point of view. It appears that the existence of the LBHB is not that clear and several enzymes, such as ketosteroid isomerase,[5] triosephosphate isomerase,[6,7] and citrate synthase[8–10] have been proven to use short and strong hydrogen bonds (SSHBs), but not LBHBs. This controversy arises from the lack of tools to clearly identify a LBHB. Indeed, the existence of a LBHB is usually invoked when a SSHB is localized between two residues with matching pK_a values; this differs from the initial LBHB definition. QM calculations may help to provide the answer. However, those calculations are very expensive in enzymes due to the large size of the systems. Herein, we aim to present a simple procedure to visually assess the presence of such a peculiar type of hydrogen bond (HB) by using the electron localization function (ELF). First we will define and compare the usual LBHB characteristics to other strong HBs, namely, the single-well HBs (SWHBs) and SSHBs. We will then describe the methodology used on a small homonuclear hydrogen-bond system, the $[H_5O_2]^+$-protonated water dimer, which enables us to discuss the different types of hydrogen bonding. The last part is devoted to larger systems, namely, the heteronuclear trichloroacetate (Tca)–4-methyl-1H-imidazole (HMim) system,[11] the carboxylic acid/carboxylate anionic system,[12] the $[N_2H_7]^+$ cation,[13] the proton sponge of intra-bridgeheads,[12,13] the phenol/phenolate anionic system,[12] the hydrogen maleate[12,14–16] and the hydrogen difluoride anions,[17] which are treated purely by QM methods, and the complete phosphorescent yellow protein (PYP)[18] analyzed by ab initio QM/MM methods.

The proton-transfer reaction between two electronegative atoms (X and Y) can be described by Marcus' theory. Let us consider a proton-transfer reaction between two electronegative atoms (X and Y). The distance between H and (for example) X may be used as a reaction coordinate (RC) for this system. The H−X or H−Y bonds can be modeled by considering the proton as being harmonically bonded to X or Y. The usual representation of a HB is shown in Figure 1a. It is a double-well profile in which the barrier is high
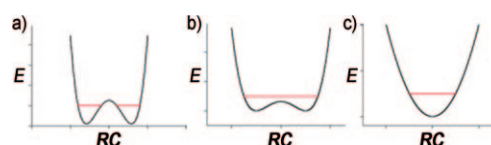

Figure 1. Profiles of the different types of HBs studied herein: a) normal HB, b) LBHB, and c) SWHB.

enough to block the proton in one of the specific wells. If the two heavy atoms get closer, the barrier decreases and the zero-point energy (ZPE) of the proton is raised above the transfer barrier. That is, even if a barrier exists, the proton can be delocalized between the two heavy atoms. This is the signature for a LBHB (Figure 1b). Finally, when the two heavy atoms are even closer, the barrier disappears and the double-well profile turns into a single-well profile, which is characteristic of a specific LBHB, namely, SWHB (Figure 1c).

The ELF function[19–21] is associated with a measure of the Pauli repulsion in the atomic or molecular space and ena-

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bles access to the probability of finding two electrons with the same spin. ELF values are restricted between 0 and 1 and can be interpreted as a signature of the electronic pair distribution, but, in contrast to pair functions, it can easily be calculated and interpreted. The ELF function can be partitioned into an intuitive chemical scheme once it has been computed on a 3D grid from a given ab initio wave function. Indeed, core regions, denoted C(X), can be determined for any atom. This is also the case for valence regions associated to lone pairs, denoted V(X), and for chemical bonds V-(X,Y). These ELF regions, the so-called basins, closely match the domains of Gillespie's valence-shell electron-pair repulsion model.[22] Usually, hydrogen-atom basins are not explicitly visualized as isolated domains, V(H), but are included within protonated basins, V(X,H), in the usual case of X−H bonds (X = C, N, O, S etc).

A previous study describing the ELF topology of HBs mentioned the appearance of an isolated domain for a hydrogen basin V(H) for strong HBs.[23] The emergence of that V(H) basin was also reported when studying the proton transfer within the $[H_5O_2]^+$ system.[24] The aim of the present study is to clearly identify such a proton basin, V(H), as a topological signature of a LBHB. We will first look at the already studied model system $[H_5O_2]^+$.

Figure 2 (Figure S1 in the Supporting Information) present the 3D scan of the energy of the complex depending on the distance between the two oxygen atoms (d(O–O)) and between the hydrogen and oxygen atoms (d(O–H), the remaining coordinates are kept frozen) of the B3LYP functional and the 6-31++G** basis set (the values are given in Table S2 in the Supporting Information). A CCSD-T/aug-cc-pVTZ profile was also performed and is provided in Figure 2 c,d. Both surfaces are relatively similar except for the equilibrium distance between oxygen and hydrogen atoms
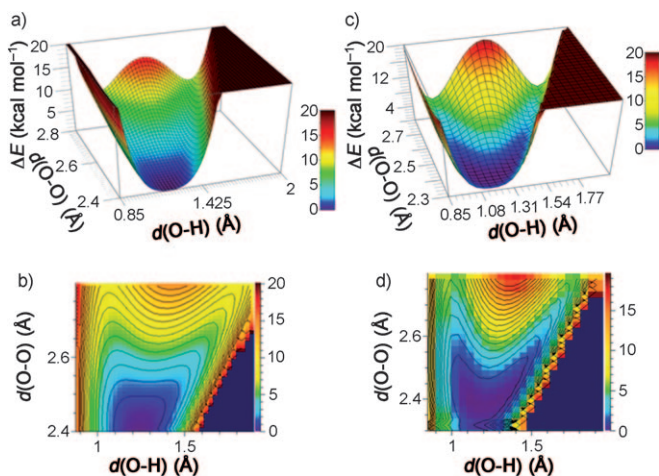
that appears slightly closer to the oxygen atom in the higher level of theory. A previous study calculated the potential energy surface (PES) with more degrees of freedom,[25] which are necessary to describe complex motions and vibrations.[26] The goal here is not to obtain the best possible surface but to be able to compare the appearance of the proton basin in relation to the energy barrier. The relative energy, ΔE, plotted in Figure 2 is the difference between the energy of the complex at a given geometry and the energy of the minimum: $\Delta E = E - E_{min}$ (the energy values are given in Table S2 in the Supporting Information). For a given d(O–O), we have at a minimum, a system made of an oxonium ion stabilized by a water molecule through a HB. ELF calculations were performed along this 3D profile and several representative structures are presented in Figure 3 (and Figure S3 in the Supporting Information). Tests performed with the correlated ELF formulation[27] exhibit no topological differences between DFT and post Hartree–Fock levels of computation (see Figure S4 in the Supporting Information) and choice of the basis set (this latter point being well-documented as ELF populations are less sensitive to basis sets than usual Mulliken analyses).[21,28]
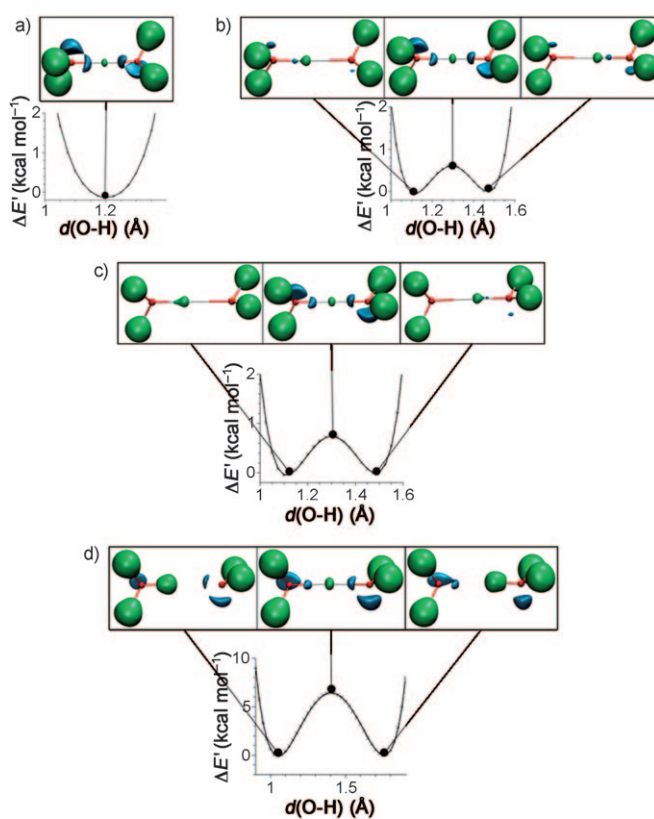

Figure 2. 3D graph and surface maps of the relative energy, (ΔE, kcal mol^{-1}) with respect to the distance (in Å) between the two oxygen atoms (d(O–O)) and between hydrogen and oxygen atoms (d(O–H)) at the B3LYP/6-31++G** (a and b) and CCSD-T/aug-cc-pVtz (c and d) levels of theory. ΔE is an arbitrary energy difference taken as $\Delta E = E - E_{min}$, where E_{min} is the lowest point on the PES. The contour lines in (b) represent isoenergetic lines of steps of 1 kcal mol^{-1}.

Figure 3. Variation of $\Delta E'$ with the distance between hydrogen and oxygen atoms for a) d(O–O) = 2.4, b) 2.58, c) 2.59, and d) 2.8 Å at the B3LYP/6-31++G** level of theory. $\Delta E' = E - E'_{min}$ where E'_{min} is the minimum for a given oxygen–oxygen distance. On the top of each profile, a representation of the ELF basins for key structures (the maximum and the minima) is provided. Lone pairs appear in blue and proton basins in green. Larger pictures are provided in Figure S3 in the Supporting Information.

Up to $d(\text{O–O}) = 2.49$ Å the proton-transfer profile is a single-well profile and so the HB can be described as a SWHB. As shown in Figure 3a, for such profiles, at the equilibrium distance, the proton is delocalized between the two oxygen atoms and has its own ELF basin.

For $2.50 \leq d(\text{O–O}) \leq 2.58$ Å, the barrier is still very low $\Delta E^{\neq} < 0.65$ kcal mol^{-1} even if the proton transfer shows a double-well profile. The ELF representation of the minima of the system shows a basin for the proton distinct from the oxygen-atom lone pair (Figure 3b). For these profiles, the HBs can be considered as LBHB.

For $d(\text{O–O}) = 2.59$ Å, we reach the limitations of the method, because at one minimum the proton has its own basin and at the other one it does not (Figure 3c). This means, that at this point on the PES, a very small change of energy transforms the HB from a LBHB into a certainly short and strong, but normal HB.

For $d(\text{O–O}) > 2.80$ Å, the proton transfer exhibits a double-well profile with a barrier high enough to localize the proton in one of the two wells. The HB is no longer a LBHB. The proton is purely localized on an oxygen atom and does not have its own ELF basin (Figure 3d). Two animated gif movies illustrate this. The first movie (see Supporting Information 2) focuses on the proton transfer and illustrates the change of color code between a protonated basin and a V(H) basin. The second movie follows the increase of the isosurface values for a fixed distance of the dimer (2.4 Å), showing that the topology does not depend on a specific isosurface choice. The results clearly show that the ELF topological signature of the LBHB can be associated with the V(H) basin. Such a transition between a V(OH) to a well-defined V(H) basin appears to provide more insights to uncover LBHBs than usual quantum theory of atoms in molecules (QTAIM)[29] studies in which the H basin always exists due to the atomic nature of such a partition. Indeed, existing studies tend to show that even if the Laplacian of density is a useful quantity to study such systems, it mainly leads to a qualitative correlation with the experiment,[30] whereas the ELF criterion could be a more predictive tool of the nature of distinct HBs with similar densities. To extend our study, we will now investigate if such a topological signature can be observed in more realistic systems.

The second example of this study is an example of two heteronuclear HBs linked through the π system of an imidazole ring (see Figure 4). The increasing complexity of such a system arises from the fact that the equilibrium distances for one HB depend on the equilibrium distances of the other and that the HBs are now heteronuclear. In a previous work by Lankau and Yu,[11] only the HB parameterized with the distances d_1 and d_2 (defined in Figure 4a) appeared as a LBHB. The other one was a purely localized HB. We performed a 3D PES for the same system by scanning d_1 from 0.8 to 1.6 Å by steps of 0.05 Å and d_2 from 2.48 to 2.60 Å by steps of 0.01 Å (see Figure 4b and Figure S5B in the Supporting Information) and a larger one scanning d_1 from 0.8 to 2.0 Å and d_2 from 2.45 to 2.80 Å (see Figure S5B). As
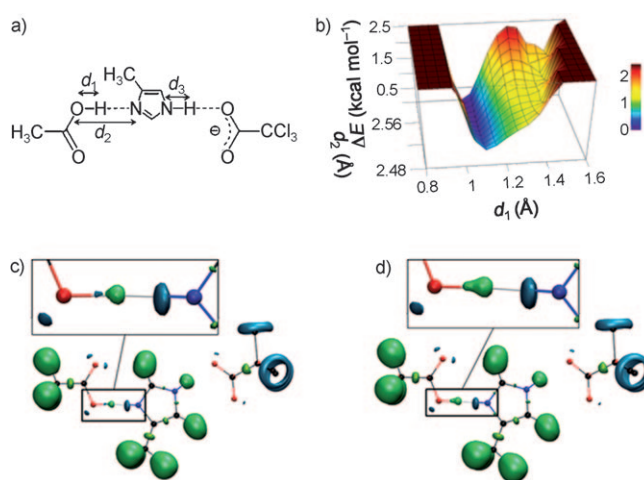

Figure 4. a) Representation of the Tca–HMim complex and of different important distances involved in the two HBs. b) The 3D-relaxed PES for proton transfer in Tca–HMim for increasing distances between the oxygen and nitrogen atoms. The d_1 and d_2 distances are defined in (a). c,d) ELF representations of the Tca–HMim equilibrium geometry for $d_2 = 2.55$ (c) and 2.60 Å (d). A close-up of the important HB is also provided to distinguish the LBHB from the normal HB. Larger pictures are provided in Figure S5 in the Supporting Information.

shown in the Supporting Information (Figure S5A), the energy of the LBHB depends largely on the second HB, and modifying d_3 would change the PES. This is why a relaxed scan of the PES was performed (see the Supporting Information for details). The level of theory remained B3LYP with the 6-31++G** basis set. The profile, here, is not perfectly symmetric and the O–H form is usually preferred to the H–N form. It is then important to look at the presence of a proton basin for the deepest well to ascertain the LBHB.

As with the $[\text{H}_5\text{O}_2]^+$ complex, at short d_2 distances ($d_2 \leq 2.53$ Å), the HB is a SWHB and the proton has its own basin. When d_2 is increased, the HB is modified into a LBHB (at $2.54 \leq d_2 \leq 2.57$ Å and $\Delta E^{\neq} = 1.37$ kcal mol^{-1}) with a basin for the proton. Finally, for $d_2 \geq 2.58$ Å, it turns into a normal HB. These results are slightly different from those of Lankau and Yu,[11] because the SWHB vanishes (and then a maximum appears) at $d_2 = 2.49$ Å, which is slightly shorter than the results herein. This might be due to the methodology and basis sets used in the different studies.

Other well-referenced small systems were also tested.[12,14,17,31,32] The hydrogen maleate anion (Figure 5a)[12,14–16] and the hydrogen difluoride anion (Figure 5b)[17] are explained in the main text and four other examples, including the carboxylic acid/carboxylate anionic system, the $[\text{N}_2\text{H}_7]^+$ cation, the proton sponge of intrabridgeheads, and the phenol/phenolate anionic system can be found in the Supporting Information (Figure S7). This series is not exhaustive, but proposes representative examples of known LBHBs on very different types of systems (cations or anions; alcohol, amine, or carboxylic acid groups etc) to validate our theory. In all the proposed systems, the V(H) basin does appear for the optimized structures.
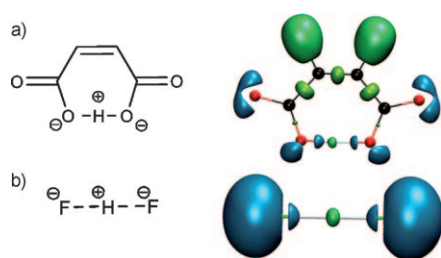
Figure 5. Scheme and ELF representation of a) hydrogen maleate and b) hydrogen difluoride anions.

The final example aims to test our methodology on a large system—the phosphorescent yellow protein (PYP) enzyme. In a recent publication, Yamaguchi et al.[18] used a neutron-diffraction structure of the PYP to show the existence of a LBHB between the chromophore and Glu46. They also found that the HB between the chromophore and Tyr42 was not a LBHB, but a short ionic HB (SIHB). Herein, we defined the QM system as the chromophore, Tyr42, Glu46, and Cys69 residues.

We performed a QM/MM[33] single-point calculation of the system with the neutron-diffraction geometry given in ref. [18]. The boundary limits were treated by using the pseudobond approach.[34–36] As shown in Figure 6 (Figure S8 in the Supporting Information provides a larger representation of the same system), the hydrogen basin appears for the chromophore–Glu46 HB, but not for the chromophore–Tyr42 HB. This suggests that the former is a LBHB, whereas
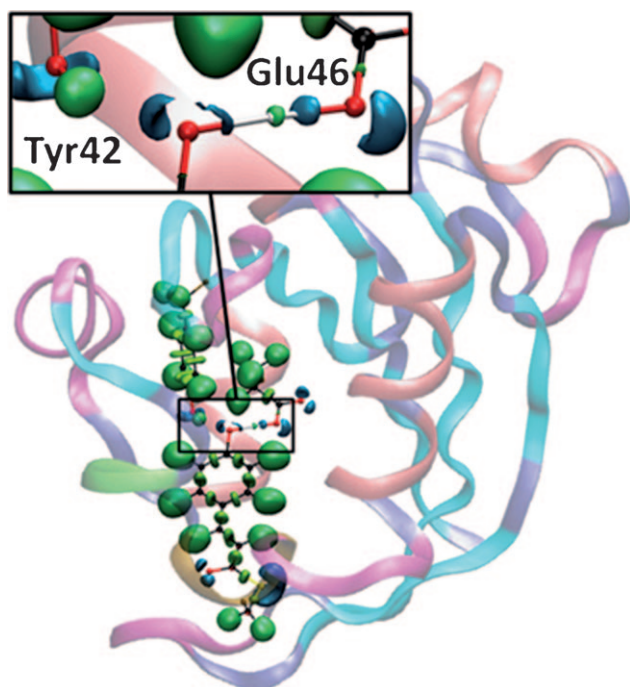

Figure 6. QM/MM representation of the protein chromophore ELF and a close-up of the HB between Glu46 or Tyr42 and the chromophore, calculated with the parm99 force field from AMBER for the MM subsystem and B3LYP/6-31++G** for the QM subsystem.

the latter is not. Our results are in agreement with the experimental results for the assignment of the LBHB compared to the normal HB.

To conclude, during the last decades many experimental and theoretical studies have investigated the possible existence of LBHBs. The usual theoretical procedures build a profile of the proton transfer and decide whether the barrier is too high or not for a LBHB. Such an approach is time consuming and the results are quite difficult to obtain, especially for the free energy profiles. Herein, we propose a simple theoretical alternative based on the ELF topological analysis allowing for discrimination of a LBHB from a normal HB. We show that LBHBs are associated with a simple ELF quantum topological criterion: the presence of a V(H) localization domain. Indeed, for such specific HBs, the proton is delocalized between the two heavy atoms resulting in the emergence of the very unusual V(H) basin. The possibility of a clear topological signature of a LBHB was first shown on the small $[H_5O_2]^+$ cluster that presents a homonuclear LBHB. We then tested our methodology on a more complex system, the Tca–HMim anion in which two heteronuclear HBs are linked through a π system. We found that a basin appears at short d_2 distances for LBHBs. Finally, after testing our hypothesis on four other well-defined systems we applied our topological criteria to the HB patterns of the PYP. By using a high-level QM/MM calculation, we were able to discriminate between a LBHB and normal HB in agreement with state-of-the-art experimental results. As such a topological criterion has been verified to be valid by methods ranging from DFT to coupled-cluster. This type of study opens the possibility to easily unravel the presence of LBHB in complex systems, because this method appears as a new tool able to discriminate an LBHB from a normal HB in various chemical and biochemical systems.

Computational Details

Several density functionals were tested. Results from the B3LYP[37,38] functional are presented herein. Following the discussion on the $[H_5O_2]^+$-protonated water scans at the CCSD(T) and B3LYP levels, additional results comparing B3LYP to MO6-2X[39] and B97-D[40] are presented in the Supporting Information and exhibit a conservation of the main B3LYP topological features. The latter two functionals were shown to be accurate compared to high-level experiments concerning structure and spectroscopy.[41] In other words, if the choice of the quantum method provides a reasonable energy surface, the ELF quantum topological criterion for the LBHBs identification stands.

The B3LYP/6-31++G**[42] scan was performed with Gaussian 09,[43] freezing all internal coordinates of the complex except d(O–H) and d(O–O). d(O–H) was scanned from 0.85 to 2.00 Å by steps of 0.025 Å, whereas d(O–O) was scanned from 2.4 to 2.8 Å by steps of 0.01 Å. Frozen internal coordinates were taken from the optimized structure. For the CCSD-T/aug-cc-pVTZ[44] scan, d(O–H) was increased from 0.85 to 2.00 Å by steps of 0.05 Å and d(O–O) from 2.2 to 2.8 Å by steps of 0.02 Å.

All QM/MM calculations were performed with a modified version of Gaussian 09 interfaced to a modified version of TINKER.[45] The AMBER95[46] all-atom force field parameter set and the TIP3P[47] model for water were used. In both cases, we used the pseudobond[34–36] ap-

proach to model the QM/MM boundary. Pseudobonds are essential to obtain a good representation of density/ELF at the boundary between QM and MM systems. All ELF computations and topological analyses were performed with a modified TopMod package,[28,48] which includes the capability of using correlated wave functions.[27] By using TopMod, the ELF topological analysis allows an automatic partition of space within domains related to specific points called attractors (the ELF equivalent of the centroids of the localized orbitals), which can be localized (and associated to *xyz* coordinates). The interested reader could find more detail in a review dedicated to the use of ELF in biological systems.[21]

Acknowledgements

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