Importance of Many-Body Effects in the Kernel of Hemoglobin for Ligand Binding
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Metalloporphyrin systems, such as heme, play a central role in biochemistry. The ability of such molecules to reversibly bind small ligands is of great interest, particularly in the case of heme which binds diatomic ligands such as oxygen and carbon monoxide. Heme acts as a transport molecule for oxygen in human respiration, while carbon monoxide inhibits this function. Despite intensive studies [1–3], the binding of the iron atom at centre of the heme molecule to O₂ and CO ligands remains poorly understood. In particular, one problem obtained with density functional theory [4] (DFT) approaches to ligand binding of heme is that the difference in the binding energy ($\Delta\Delta E$) of carboxy-heme and oxyheme is very large, and the theory predicts an unrealistic binding affinity to CO, several orders of magnitude larger than to O₂ [5, 6].

Recent progress has been made to cure this problem using DFT+U for the molecular systems [7, 8], with which it was found that the inclusion of many body effects in the calculations reduced the imbalance between O₂ and CO affinities [9]. Inclusion of conformal modifications, such as the Fe-C-O binding angle [10], or the deviation of the Fe atom from the porphyrin plane, were also shown to affect CO and O₂ binding energies.

A general problem encountered by DFT is the strong dependence of the energetics and the spin state on small changes in the geometry. In particular, traditional DFT fails to describe the correct high-spin ground state of heme molecules. DFT+U provides an improved description [7, 11], but is known to overestimate magnetic moments and gives often artificial and non physical spin-symmetry-broken states. Moreover, the rotationally-invariant DFT+U methodology does not capture well the effect of the Hund’s coupling $J$, which is known to be large in iron based systems. It was recently shown that the effect of strong correlations are not always driven by the Coulomb repulsion $U$ alone, but in some cases act in combination with the Hund’s coupling $J$ [12–14]. Understanding the effect of strong correlations in heme, and in particular how the symmetry of the highest occupied molecular orbital (HOMO) is affected by $U$ and $J$, is important in the context of describing the CO binding, which was shown to be strongly dependent on the HOMO symmetry [15].

Recent progress has been made in this direction by dynamical mean field theory [16] (DMFT), combined with DFT (DFT+DMFT) which can refine the description of the charge and spin of correlated ions, and describes in a remarkable way the strong correlations, induced by both $U$ and $J$. Also, DFT can only describe a static magnetic moment associated with a spin symmetry broken state, and requires the inclusion of the spin-orbit interaction to explain a change of spin states [17]. This is not necessary at the DMFT level, which describes both static and fluctuating magnetic moments within the same framework.

In this work, we extend the DFT+U analysis by means of the combination of state-of-the-art linear scaling DFT [18] with DMFT, and apply this methodology to heme. The methodology builds upon our earlier works [19] and is described in detail in the supplementary material.

Although DFT+DMFT has been widely used to study solids, in this study we apply our real-space DFT+DMFT implementation to a moderately large molecule, extending the scope of applicability of DMFT to biology in an unprecedented manner. DMFT allows the quantum and thermal fluctuations, missing in zero-temperature DFT calculations, to be recovered. Moreover, it includes within the calculation both the Coulomb repulsion $U$ and the Hund’s coupling $J$. Which of $U$ or $J$ drives the many body effects in heme [14] remains an open question, paramount to understanding ligand bind-
on we find a very sharp dependence of the electronic density on the Hund’s coupling for iron \(3\). Since the iron occupation is a local observable, and hence strained to integer values in DFT and DFT+DMFT, the occupancy (Fig. 1.a) is shown for the real-space representation of the electronic spectral density of the HOMO of FeP-d for \(J = 0\) eV and \(J = 0.8\) eV. The large central sphere shows the location of the iron atom, and the four blue spheres indicate nitrogen atoms.

In our view, this relates to the Fe-O-O angle obtained in the tetraphenylporphine configuration, a triplet with different orbital symmetry in the octaethylporphine configuration [25], and a quintet in the octamethylnitrobenezporphine configuration [26]. The strong dependence of the spin state with respect to small modifications in the structure is consistent with an entangled spin state.

In our calculations, we find that both oxyheme and carboxyhemoglobin adopt a low spin state for \(J < 0.25eV\) and larger multiplicities in the physical region of \(J \approx 0.8eV\), while in both cases the spin state is very close in character to that of unligated deoxyheme. Significantly, we observe only subtle differences between FeP(O\(_2\)), FeP(O) and FeP-d for \(J = 0.8\) eV, while the DFT and DFT+U treatment yields ground-states for carboxyhemoglobin and oxyheme of pure closed-shell and open-shell singlet configurations, respectively [6, 7, 9].

Moreover, we find that the symmetry of the highest occupied molecular orbital (HOMO) of FeP-d, as estimated from the real-space spectral density of the prominent feature below the Fermi level, is highly dependent on the Hund’s coupling \(J\). In particular, for \(J = 0\) eV, the HOMO is an admixture of orbital characters (see vertical labels in Fig. 1.a). However, the Hund’s coupling drives a rather complex orbital selection, such that for the region of greatest interest, \(J \approx 0.8\) eV, the HOMO predominantly exhibits \(d_{3z^2-r^2}\) symmetry. The orbital selection process also induces a pinning of the Fermi density to the quantum impurity, such that it is delocalized for \(J = 0\) eV (see Fig. 1.c), while for \(J = 0.8\) eV (Fig. 1.d) it is instead localized to the iron 3d sub-shell.

In our view, this relates to the Fe-O-O angle obtained for FeP-d for c) \(J = 0\) eV and d) \(J = 0.8\) eV.

FIG. 1: **Orbital selection scenario:** Dependence of a) the iron 3d subspace occupancy \(n_d\) and b) the effective spin quantum number \(s\) on the Hund’s coupling \(J\), for both unligated and ligated heme models. The physically relevant region \(0.5 eV < J < 1 eV\) is highlighted in yellow. Isosurfaces of the real-space representation of the electronic spectral density of the HOMO of FeP-d for c) \(J = 0\) eV and d) \(J = 0.8\) eV. The large central sphere shows the location of the iron atom, and the four blue spheres indicate nitrogen atoms.

In our view, this relates to the Fe-O-O angle obtained for FeP-d for c) \(J = 0\) eV and d) \(J = 0.8\) eV.
in FeP(O$_2$) [27]. Indeed, the bent geometry of FeP(O$_2$)
can be explained by a favorable interaction between the
p*-orbital of the O$_2$ and the d$_{3z^2−r^2}$ orbital on Fe [27]:
the O$_2$ p*-orbital is closer in energy to d$_{3z^2−r^2}$ compared
to the p*-orbitals in CO, and hence it gains more energy
by bending, which increases the overlap. For FeP(CO)
the situation is opposite, and there is no stabilization
gained by bending [27]. On the contrary, the bending in
FeP(CO) is induced by the strain of the protein and it
reduces the binding energy. Naively, the orbital selec-
tion of the d$_{3z^2−r^2}$ orbital is hence expected to go
in the direction of curing the strong O$_2$ and CO im-
balance. Moreover, the charge localization at the Fermi
level suggests that other artificial binding between the non-
etallic atomic orbitals of heme and strong electroneg-
tive O$_2$ will not be obtained, and hence will protect heme
from undesired charge transfer.

We now discuss the degree of quantum entanglement
exhibited by FeP-d and FeP-p (see Fig. 2.a). We com-
puted the von Neumann entropy Λ = −tr(ρd log(ρd)),
where ρd is the reduced finite-temperature density-
matrix of the iron 3d impurity subspace, traced over the
states of the AIM bath environment. The entropy quanti-
tifies to what extent the wave-function consists of an en-
tangled superposition.

We observe that the entropy rises sharply at J ≈
0.25 eV, corresponding to the transition from the dou-
blet spin state to the triplet/quintet entangled state. As
expected, the entropy is small in the low-spin region
(J < 0.25 eV) and also in the fully-polarized limit. At
J = 0 eV (Fig. 2.b), we find that the dominant con-
figuration consists of the doublets (d$_{3z^2−r^2}$)$^2$(d$_{xy}$)$^2$(d$_{xz}$)$^2$,
with a single electron in the d$_{xy}$−p$^z$ orbital. The lat-
ter hybridizes strongly with the nitrogen 2p orbitals, but
all other orbitals are mostly filled or empty, so this con-
figuration is, essentially, a classical state with a finite
magnetic moment.

At larger J values, however, such as J = 0.8eV
(Fig. 2.c), all orbitals are partially filled, and an increas-
ing number of electronic configurations, with different
valence and spin, contribute to the statistics, and thus
the iron impurity wave-function is fluctuating. Although

![FIG. 2: Valence fluctuations: a) Von Neumann entropy Λ obtained by DFT+DMFT for FeP-p (circles) and FeP-d (squares). Histograms of the dominant electronic configurations for FeP-d for b) J = 0 eV and (c) J = 0.8 eV. The pie wedge labelled other contains configurations with a weight smaller than 3%. The iron 3d spin S$^z$ and iron 3d occupancy n$_d$ of the dominant configurations is indicated.](image)

the valence fluctuation are captured to some extent at
the DFT level (Λ$_{DFT}$ ≈ 0.75), we find that many body
effects contribute significantly to the entropy.

Our results indicate that as FeP-d and FeP-p molecules
approach a regime with large entanglement for J ≈ 0.5,
with a concomitant orbital selection close to the Fermi
level. The orbital selection close to the Fermi level in
turn induces a charge-localization effect. The latter ef-
effect of the Hund’s coupling can be understood with a
simple picture: a large Hund’s coupling partially empties
the d$_{3z^2−r^2}$ orbital and brings the weight of this orbital
closer to the Fermi level, thereby reducing the hybridiza-
tion between the iron 3d states and the nitrogen 2p states
close to the Fermi level. The subtle interplay between
the charge-localization induced by the Hund’s coupling
(orbital selection close to the Fermi energy) and the de-
localization induced by strong correlations (the tendency
for electrons to escape the iron 3d orbitals in order to reduce
the Coulomb energy) is captured by the DFT+DMFT
methodology but is absent in Kohn-Sham DFT. We em-
phasize that these ingredients are paramount to an es-
timation of the charge transfer and binding properties
between the iron atom and the ligand in oxyheme and
carboxyhem.

![FIG. 3: Energetics: a) Difference in CO and O$_2$ binding energies Δ∆E. The binding to CO is always favored, however the imbalance is strongly reduced for J > 0.5eV. b) Total energy of FeP as a function of J. The minimum of the total energy is obtained for J = 0.9eV.](image)
data is obtained for our calculations done at note that experimental spectra for FeP(CO) and FeP(O) principles calculations, in combination with DMFT, on state of heme. spectroscopy may be used as a sensitive probe for the ligation (see sup. material). We propose that time-resolved spec-
of the iron atom after an initial quench in polarization calculations to the time dependence of the magnetization ω doming effect of the iron-intercalated porphyrin model FeP-p. Hence, the symmetry breaking associated present in FeP-d, is absent in the planar theoretical feature (HOMO) below the Fermi level and the LUMO 3 2− $r \approx 0$eV. This scenario sheds some light on the strong CO and O2 binding imbalance problem obtained by extracting the binding energies in simpler zero temperature and J = 0eV DFT calculations. The difference in binding energies is dramatically reduced for physical value of J ≈ 0.8. The smaller remaining imbalance might be further explained by the strain energy contained in the protein structure [9] or by the contribution from the entropic term. Finally, the relevance of a finite Hund’s coupling in heme is confirmed by the total energy extracted from the DFT+DMFT of unligated heme, which shows a minima for $J = 0.9eV$.

We have proposed a new mechanism for ligand binding to heme based on an orbital selective process, on this basis, a scenario which we term bonding determined by local valence fluctuations. Finally, we have obtained a reasonable agreement between experimental and our theoretical optical absorption spectra, our description accounting for the observation of optical transitions in the infrared regime and the double peaked structure of the optical response at ω ≈ 2eV.

At the time of writing, we became aware of related application of DMFT to an organometallic crystal [31]. We are grateful to R.H. McKenzie for comments and bringing Ref. [23] to our attention, and to D. Cole for many insightful discussions. C.W. was supported by the Swiss National Foundation for Science (SNFS). D.D.O’R. was supported by EPSRC. N.D.M.H was supported by EPSRC grant number EP/G055882/1. P.B.L is supported by the US Department of Energy under FWP 70069. Calculations were performed on the Cambridge High Performance Computing Service under EPSRC grant EP/F032773/1. Correspondence and requests for materials should be addressed to C.W.

FIG. 4: Optical measurements: Optical conductivity of FeP-d (bold line) and FeP(CO) (solid) and FeP(O2) (dashed line). The vertical arrow indicates the energy of the experimental peak associated to the Fe-N charge transfer. Inset: experimental measurements [28] for unligated heme (bold line), oxy- (dashed line) and carboxy- (solid) heme are shown for comparison. J = 0.8eV was used for all calculations above.

(3.3b), suggesting further that the heme molecule is particularly well suited to host metallic d atoms, which tend to have a large screened J interaction when hybridising to light elements such as nitrogen or oxygen.

We now move to our calculations of the optical absorption spectra of heme (Fig. 4). Our theoretical absorption spectra, shown in Fig. 4, are in reasonable agreement with experimental data [28], in particular for the optical transitions at ω ≈ 2 eV. We attribute this spectral feature to charge-transfer excitations from iron to nitrogen-centered orbitals. The spectrum is dominated by the characteristic porphyrin Q-bands (those at ≈ 2eV), and Soret bands [29] (at ≈ 4eV). Our results offer insight into the infrared absorption band present at ≈ 1eV, in our calculation, and observed in experiments at 0.6 eV [30]. This infrared peak is described, in our calculations, as arising from transitions between the d_{xz}\text{-}d_{yz} spectral feature (HOMO) below the Fermi level and the LUMO (quasi-degenerate d_{xz} and d_{yz}) above the Fermi level.

Interestingly, we find that the infrared optical weight in unligated heme, associated with d-d transitions and present in FeP-d, is absent in the planar theoretical model FeP-p. Hence, the symmetry breaking associated with the doming effect of the iron-intercalated porphyrin macrocycle permits d-d optical transitions, and is responsible for the spectral weight in the infrared regime. We note that experimental spectra for FeP(CO) and FeP(O2) exhibit a double peak structure at ω ≈ 2eV, absent from our calculations done at J = 0eV, but recovered for J > 0.8eV. The best agreement with the experimental data is obtained for J = 0.9eV. Finally, we extended our calculations to the time dependence of the magnetization of the iron atom after an initial quench in polarization (see sup. material). We propose that time-resolved spectroscopy may be used as a sensitive probe for the ligation state of heme.

In conclusion, we have carried out linear-scaling first-principles calculations, in combination with DMFT, on both unligated and ligated heme. We have presented a newly-developed methodology applied to a molecule of important biological function, exemplifying how subtle quantum effects can be captured by our methodology. In particular, we have found that the Hund’s coupling J drives an orbital selection process in unligated heme, which enhances the bond in the out-of-plane direction. The von Neumann entropy quantifying valence fluctuations in the iron 3d subspace is large for the physical values of J ≈ 0.8eV. This scenario sheds some light on the strong CO and O2 binding imbalance problem obtained by extracting the binding energies in simpler zero temperature and J = 0eV DFT calculations. The difference in binding energies is dramatically reduced for physical value of J ≈ 0.8. The smaller remaining imbalance might be further explained by the strain energy contained in the protein structure [9] or by the contribution from the entropic term. Finally, the relevance of a finite Hund’s coupling in heme is confirmed by the total energy extracted from the DFT+DMFT of unligated heme, which shows a minima for $J = 0.9eV$.

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