

# Density Functional Theory Investigation of the Active Site of Fe-Hydrogenases. Systematic Study of the Effects of Redox State and Ligands Hardness on Structural and Electronic Properties of Complexes Related to the $[2\text{Fe}]_{\text{H}}$ Subcluster

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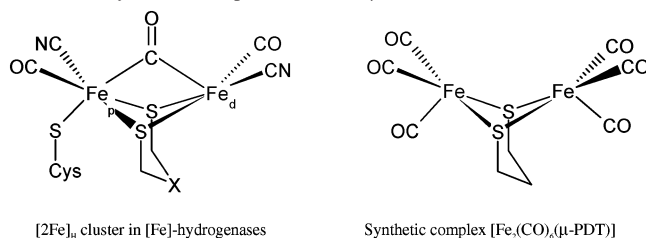
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Density functional theory has been used to investigate complexes related to the  $[2\text{Fe}]_{\text{H}}$  subcluster of  $[\text{Fe}]$ -hydrogenases. In particular, the effects on structural and electronic properties of redox state and ligands with different  $\sigma$ -donor  $\pi$ -acceptor character, which replace the cysteine residue coordinated to the  $[2\text{Fe}]_{\text{H}}$  subcluster in the enzyme, have been investigated. Results show that the structural and electronic properties of fully reduced  $\text{Fe}^{\text{I}}\text{Fe}^{\text{I}}$  complexes are strongly affected by the nature of the ligand L, and in particular, a progressive rotation of the  $\text{Fe}_d(\text{CO})_2(\text{CN})$  group, with a CO ligand moving from a terminal to a semibridged position, is observed going from the softest to the hardest ligand. For the partially oxidized  $\text{Fe}^{\text{I}}\text{Fe}^{\text{II}}$  complexes, two isomers of similar stability, characterized either by a CO ligand in a terminal or bridged position, have been observed. The switching between the two forms is associated with a spin and charge transfer between the two iron atoms, a feature that could be relevant in the catalytic mechanism of dihydrogen activation. The structure of the fully oxidized  $\text{Fe}^{\text{II}}\text{Fe}^{\text{II}}$  models is extremely dependent on the nature of the L ligand; one CO group coordinated to  $\text{Fe}_d$  switches from terminal to bridging position going from complexes characterized by neutral to anionic L ligands.

## Introduction

The experimental and theoretical investigation of coordination compounds related to the active site of Fe-hydrogenases has been recently invigorated by the disclosure of the structural features of the cofactor involved in  $\text{H}_2$  activation (the so-called H cluster),<sup>1</sup> which is formed by a regular  $[\text{Fe}_4\text{S}_4]$  cluster bridged by a cysteine residue to a binuclear subcluster ( $[2\text{Fe}]_{\text{H}}$ ). The two iron atoms of  $[2\text{Fe}]_{\text{H}}$ , referred as proximal ( $\text{Fe}_p$ ) and distal ( $\text{Fe}_d$ ) with respect to the cysteine residue, are coordinated by CO and  $\text{CN}^-$  ligands and linked by a chelating  $\text{S}-\text{X}_3-\text{S}$  group, where  $\text{X}_3$  is composed of covalently bound light atoms (Chart 1).<sup>1,2</sup>

**Chart 1.** Schematic Structures of the  $[2\text{Fe}]_{\text{H}}$  Cluster in Fe-Hydrogenases, As Observed in the Oxidized Form of the Enzyme,<sup>1</sup> and of the Synthetic Complex  $[\text{Fe}_2(\text{CO})_6(\mu\text{-PDT})]^\ddagger$



Different redox states of the  $[2\text{Fe}]_{\text{H}}$  cluster have been characterized spectroscopically.<sup>3</sup> The fully oxidized and reduced forms of the enzyme are EPR silent and have been proposed to correspond to  $\text{Fe}(\text{II})\text{Fe}(\text{II})$  and  $\text{Fe}(\text{I})\text{Fe}(\text{I})$  species, respectively, while the partially oxidized form is paramagnetic.

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The binuclear cluster has also been the subject of intense theoretical research.<sup>4</sup> In particular, we have recently shown that H<sub>2</sub> can be cleaved on Fe(II)Fe(II) species,<sup>5</sup> in agreement with previous proposals.<sup>6</sup> Moreover, the structural and electronic features of  $\mu$ -H Fe(II)Fe(I) and  $\mu$ -H Fe(II)Fe(II) species suggest that H<sub>2</sub> activation paths involving both metal ions are possible.<sup>7</sup> However, despite experimental and theoretical efforts, some aspects related to the structural and catalytic features of the enzyme remain unclear. One of the most puzzling aspects related to the structure of the dinuclear cluster is the observation that even though the [2Fe]<sub>H</sub> subunit of Fe-hydrogenases is similar to dinuclear organometallic complexes which have been recently synthesized and characterized experimentally,<sup>8</sup> the two-edge-bridged square pyramids formed by the iron ligands are oriented differently (Chart 1). The synthetic model complexes are characterized by an Fe–Fe bond and symmetrical edge-bridged square pyramids, whereas in the [2Fe]<sub>H</sub> cluster one square pyramid is inverted with respect to the other, resulting in a vacant coordination site on Fe<sub>d</sub>. This site is most probably occupied by a water molecule in the oxidized form of the enzyme<sup>1b</sup> and by a CO group in the CO-inhibited, oxidized form.<sup>9</sup> Remarkably, X-ray and IR studies indicate that the CO group that bridges the two metal centers in the enzyme switches to a semibridging or terminal position going from the oxidized to the reduced form of the enzyme.<sup>2</sup> The cause of the structural difference between synthetic models and the enzyme active site is still not well understood, and it can depend on several factors such as the electronic properties of the iron ligands, as well as on steric and electronic constraints imposed by the protein environment in the enzyme active site.<sup>10</sup> With the aim of shedding some light on these crucial aspects, we have carried out a DFT investigation of models of the [2Fe]<sub>H</sub> subcluster where the cysteine residue coordi-

nated to Fe<sub>p</sub> in the enzyme has been systematically replaced by groups characterized by different  $\sigma$ -donor and  $\pi$ -acceptor character (CO, P(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>SH, CN<sup>-</sup>, CH<sub>3</sub>S<sup>-</sup>, and CH<sub>3</sub>O<sup>-</sup>). We have also studied the effect of the redox state of the binuclear cluster on its structural and electronic properties. Results allow a better understanding of the molecular properties of complexes related to the [2Fe]<sub>H</sub> subcluster and may help in driving the design of new catalysts.

## Computational Methods

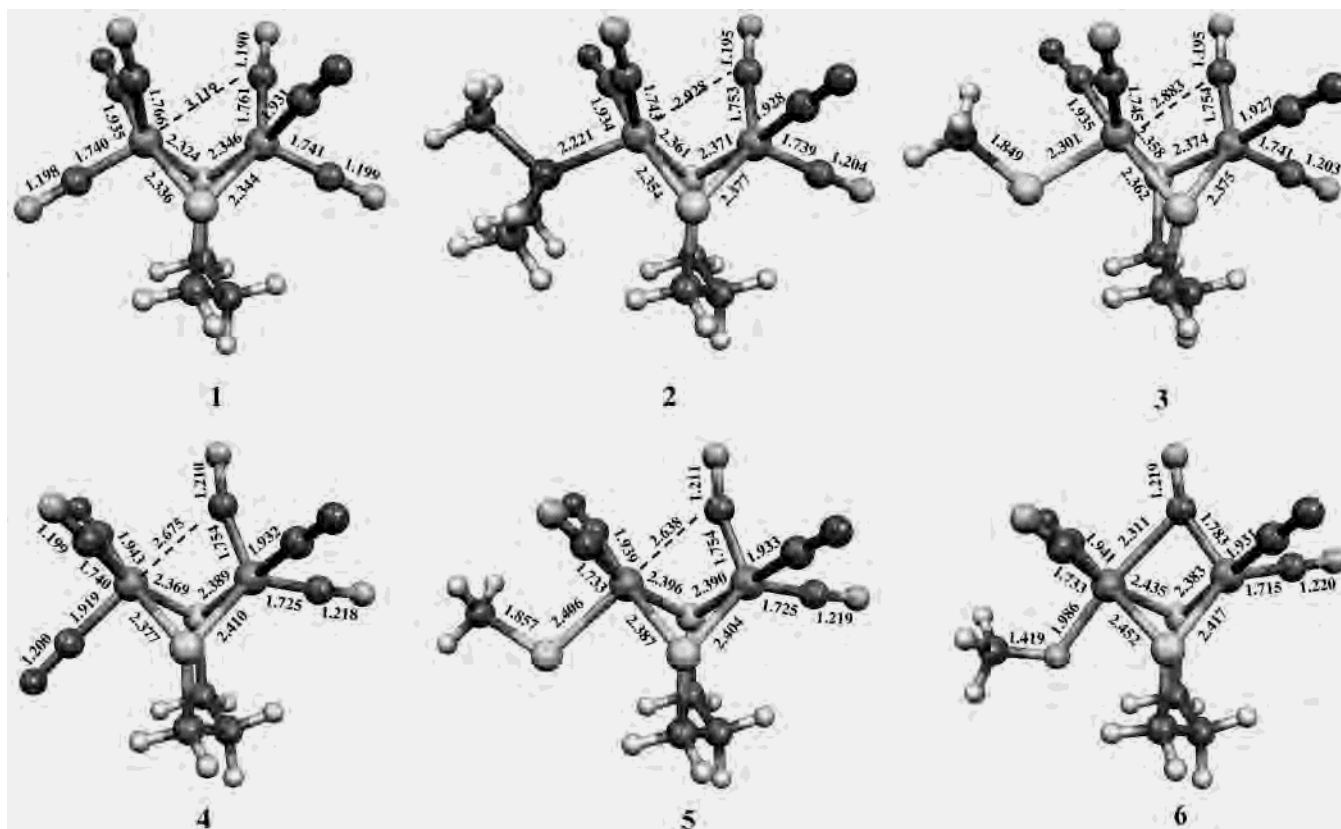
The computational models of the [2Fe]<sub>H</sub> cluster are characterized by the general structure [( $\mu$ -PDT)Fe<sub>2</sub>(CO)<sub>3</sub>(CN)<sub>2</sub>(L)], where L = CO, P(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>SH, CN<sup>-</sup>, CH<sub>3</sub>S<sup>-</sup>, and CH<sub>3</sub>O<sup>-</sup>.

DFT calculations were carried out using the hybrid B3LYP exchange-correlation functional<sup>11</sup> and the effective atomic core potential derived by Hay and Wadt<sup>12</sup> for iron, phosphorus, and sulfur atoms. The adopted basis set is of augmented double- $\zeta$  type: one set of f polarization functions is added to Fe atoms, and one set of d functions is added to S and P atoms.<sup>13</sup> All other atoms are described by a standard double- $\zeta$  basis set.<sup>14</sup> This relatively small basis set was already shown to give molecular geometries in satisfactory agreement with experimental data.<sup>5,7</sup> Spin unrestricted DFT calculations have been carried out for Fe(I)Fe(II) species.

Stationary points on the energy hypersurface have been located by means of energy gradient techniques as implemented in the Gaussian 98 set of programs.<sup>15</sup> Full vibrational analysis has been carried out to further characterize each stationary point. Computed vibrational frequencies for CO and CN<sup>-</sup> groups are available as Supporting Information. Partial atomic charges have been computed according to the natural atomic orbital (NAO) scheme.<sup>16</sup>

In the framework of natural bond orbital (NBO) theory, a Lewis representation of the compounds under investigation has been defined assuming that the electrons of the ligand–metal bonds belong to the ligand. In other words, the bonds between ligands and metal centers are modeled as donor–acceptor interactions in which charge flows from donor orbitals (e.g., the lone pairs of the ligands) to acceptor orbitals (e.g., the empty d orbitals of metal

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**Figure 1.** DFT optimized geometries and bond distances (Å) of fully reduced model complexes  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{2-/3-}$  where  $\text{L} = \text{CO}$ ,  $\text{P}(\text{CH}_3)_3$ ,  $\text{CH}_3\text{SH}$ ,  $\text{CN}^-$ ,  $\text{CH}_3\text{S}^-$ , and  $\text{CH}_3\text{O}^-$ . Fe–Fe distances (Å) are 2.555, 2.543, 2.528, 2.653, 2.655, and 2.634 in **1**, **2**, **3**, **4**, **5**, and **6**, respectively.

atoms). The analysis of the electron occupancies of these localized bonding and antibonding orbitals, as well as lone pairs, has been shown to give a clearer picture of the electronic structure of coordination compounds.<sup>17</sup>

All the complexes investigated in the present study have been assumed to be in low spin state, due to the characteristics of the ligands and in agreement with available experimental evidence.

Only isomers that have a molecular structure related to the  $[\text{2Fe}]_{\text{H}}$  subcluster have been explicitly reported and discussed in the present contribution. We have identified also a few isomers differing from those reported in this paper for the disposition of the L ligand. The discussion of these species will be the subject of a forthcoming report. It should also be noted that the structural parameters of some models (**1**, **3**, **5**, **9**, **11**, **17**) were already published in previous contributions that focused on other aspects of the binuclear cluster chemistry.<sup>5,7</sup> Nevertheless, for the sake of clarity of the discussion, these data have been explicitly reported in the text.

## Results

Results are presented starting with the fully reduced models, which have been often classified as Fe(I)Fe(I) species on the basis of simple electron counting rules. However, as it will be discussed in the following, such an assignment of oxidation numbers is far from the computed net charges on the metal centers, and analogous considerations hold true for the partially oxidized Fe(I)Fe(II) and the fully oxidized Fe(II)Fe(II) forms. For this reason we will often refer simply to fully reduced and partially and fully oxidized model complexes, respectively.

In the following, the general structure of the models is indicated as  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{q-}$  to

highlight ligands coordinated to the proximal ( $\text{Fe}_p$ ) and distal ( $\text{Fe}_d$ ) iron atoms, respectively.

**Fully Reduced  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{q-}$  ( $q = 2, 3$ ) Models.** The DFT structures of the  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{q-}$  ( $q = 2, 3$ ;  $\text{L} = \text{CO}$ ,  $\text{P}(\text{CH}_3)_3$ ,  $\text{CH}_3\text{SH}$ ,  $\text{CN}^-$ ,  $\text{CH}_3\text{S}^-$ , and  $\text{CH}_3\text{O}^-$ ) complexes (**1–6**), as well as selected geometrical parameters, are shown in Figure 1.

In complex **1**, in which the ligand L is the  $\pi$ -acceptor CO group, the two Fe atoms have a distorted square pyramidal arrangement. The  $\text{Fe}_p(\text{CO})_2(\text{CN})$  and  $\text{Fe}_d(\text{CO})_2(\text{CN})$  groups are slightly rotated with respect to the  $\text{FeL}_3$  groups in  $[\text{Fe}_2(\mu\text{-PDT})(\text{CO})_6]$ .<sup>8</sup> In particular, the CO ligands coordinated axially to  $\text{Fe}_p$  and  $\text{Fe}_d$  are rotated with respect to the plane defined by the two metal centers and the midpoint between the two S atoms by about  $-14^\circ$  and  $23^\circ$ , respectively. The different extent of rotation of the two  $\text{Fe}(\text{CO})_2(\text{CN})$  groups is due to the conformation of the propyl chain of PDT, which sterically affects only one  $\text{FeL}_3$  group. In complex **2** ( $\text{L} = \text{P}(\text{CH}_3)_3$ ), the  $\text{Fe}_d(\text{CO})_2(\text{CN})$  group is rotated by about  $35^\circ$ . One CO ligand coordinated to the  $\text{Fe}_d$  atom is slightly bent ( $\angle\text{Fe}_d\text{CO} = 173^\circ$ ) and approaches the  $\text{Fe}_p$  center. In fact, the  $\text{Fe}_p\text{--CO}$  distance (2.928 Å) is shorter than in **1** (3.119 Å), suggesting weak interaction between  $\text{Fe}_p$  and the carbonyl ligand. Complex **3**, in which L is  $\text{CH}_3\text{SH}$ , is structurally similar to **2**; the  $\text{Fe}_d(\text{CO})_2(\text{CN})$  group is rotated by  $36^\circ$ , and the  $\text{Fe}_p\text{--CO}$  distance is 2.883 Å.

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**Table 1.** Charges of Selected Fragments of the Fully Reduced  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{2-\beta-}$  Complexes<sup>a</sup>

	1	2	3	4	5	6
Fe <sub>p</sub>	-0.280	-0.249	-0.151	-0.113	-0.022	0.010
Fe <sub>d</sub>	-0.263	-0.234	-0.240	-0.240	-0.249	-0.224
L	-0.035	0.283	0.213	-0.572	-0.530	-0.641
S <sub>2</sub> C <sub>3</sub> H <sub>6</sub>	-0.314	-0.512	-0.527	-0.549	-0.612	-0.651
CO <sub>d</sub> <sup>br</sup>	-0.014	-0.061	-0.063	-0.209	-0.208	-0.246
CO <sub>d</sub> <sup>ax</sup>	-0.058	-0.096	-0.088	-0.204	-0.204	-0.174
CN <sub>d</sub>	-0.517	-0.523	-0.518	-0.566	-0.565	-0.574
CO <sub>p</sub>	0.003	-0.030	-0.034	0.008	-0.019	-0.014
CN <sub>p</sub>	-0.521	-0.578	-0.593	-0.555	-0.591	-0.575

<sup>a</sup> The subscripts p and d stand for proximal and distal positions with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts br and ax are used as labels of CO groups than have bridged/semibridged and axial positions, respectively.

The model **4** includes the anionic CN<sup>-</sup> ligand, and consequently, the negative charge of the complex is changed to -3. The Fe<sub>d</sub>(CO)<sub>2</sub>(CN) group is rotated by more than 58°; one CO ligand coordinated to Fe<sub>d</sub> is significantly bent ( $\angle\text{Fe}_d\text{CO} = 164.2^\circ$ ) and approaches the Fe<sub>p</sub> atom up to 2.675 Å. Notably, the arrangement of ligands around Fe<sub>d</sub> is more similar to a pseudo-trigonal-bipyramid, with the CN<sup>-</sup> group and one S atom of PDT occupying the axial positions. Complex **5**, in which L is the anionic CH<sub>3</sub>S<sup>-</sup> ligand, is structurally similar to **4**; the Fe<sub>d</sub>(CO)<sub>2</sub>(CN) group is rotated by about 60°, and the distance between the semibridging CO and Fe<sub>p</sub> atom is equal to 2.638 Å. Complex **6** contains the hardest anionic ligand (CH<sub>3</sub>O<sup>-</sup>), and the effects on the Fe<sub>d</sub> coordination geometry observed in **4** and **5** are even more pronounced. The Fe<sub>d</sub>(CO)<sub>2</sub>(CN) unit is strongly rotated (by about 87°); one CO ligand is bent ( $\angle\text{Fe}_d\text{CO} = 152.2^\circ$ ) and asymmetrically bridges the two metal centers (Fe<sub>d</sub>-CO = 1.783 Å and Fe<sub>p</sub>-CO = 2.311 Å). The Fe-Fe distance is not significantly affected by L and differs by less than 0.1 Å going from **1** to **6**. On the other hand, other geometrical parameters change systematically in the series **1-6**. In particular, the average Fe-S bond length increases significantly (2.338, 2.366, 2.367, 2.386, 2.394, and 2.422 Å in **1, 2, 3, 4, 5,** and **6**, respectively). Also, the C-O bond lengths increase in the series **1-6**; the semibridging CO bond length varies from 1.190 Å in **1** to 1.219 Å in **6**, and the CO bond length of the carbonyl group terminally coordinated to Fe<sub>d</sub> increases progressively, going from 1.199 Å in **1** to 1.220 Å in **6**.

Differences in the electronic structure of the complexes **1-6** can be highlighted by the values of net atomic charges (Table 1). In this context, it is worth noting that the charge difference between Fe<sub>p</sub> and Fe<sub>d</sub> increases with the hardness of L; the Fe<sub>d</sub> charge is roughly constant in the series **1-6** (about -0.24), whereas the Fe<sub>p</sub> partial charge changes systematically (from -0.280 to 0.010). Remarkably, the electrophilicity of Fe<sub>p</sub> increases even going from neutral to anionic L ligands.

If the net charge transferred from the ligand to the complex is defined as the difference between the charge of the free ligand and its charge when coordinated to Fe<sub>p</sub>, it turns out that in **1** 0.035 electrons are donated from the complex to L = CO, whereas 0.283 and 0.213 electrons are donated to the complex in **2** and **3**, respectively. As expected, ligand to

complex donation increases when L is anionic and is equal to 0.428, 0.470, and 0.359 electrons for **4, 5,** and **6**, respectively. To have a more detailed picture of the electronic structure of complexes **1-6**, NBO orbital occupancies have been analyzed according to the procedure described in the Computational Methods section. This approach allows one to study single orbital contributions to intramolecular charge transfer. In particular, for the bonding interaction between L and Fe<sub>p</sub>, it allows one to split the net charge transferred from L to the metal center in donation and back-donation contributions. Interestingly, for complexes with neutral L, the donation from L to Fe<sub>p</sub> decreases moving from **1** to **3** (Table 2). However, the degree of back-donation from Fe<sub>p</sub> to L decreases in a more pronounced way. Donation from L to Fe<sub>p</sub> decreases also moving from **4** to **6**. The population of the π\* orbitals of the semibridging CO increases along the series **1-6**, with the largest variation observed switching from neutral to anionic L ligands, as observed also for the other CO group coordinated to Fe<sub>d</sub>.

**Partially Oxidized [L(CO)(CN)Fe<sub>p</sub>(μ-PDT)Fe<sub>d</sub>(CO)<sub>2</sub>(CN)]<sup>q-</sup> (q = 1, 2) Models.** One-electron oxidation of complexes **1-6** leads to paramagnetic species (**7-12**, Figure 2) that correspond to the partially oxidized and EPR active form of the [2Fe]<sub>H</sub> subcluster.

For this class of compounds, two different isomers were obtained sampling the potential energy hypersurface. The first series of isomers can be viewed as a pseudosymmetrical edge-bridged square pyramid, whereas in the second series one square pyramid is inverted relative to the other and one CO group coordinated to Fe<sub>d</sub> is bridged or semibridged between the two Fe atoms. In the following, the former and latter isomers will be referred as the open (**a**) and bridged (**b**) forms, respectively.

As can be observed in Figure 2, the nature of the ligand L affects significantly only the structure of the bridged isomers. In **7b** (L = CO) the Fe<sub>d</sub> atom has a distorted trigonal bipyramidal arrangement with one CO, one CN<sup>-</sup>, and one S atom that occupy the equatorial positions. The arrangement of ligands around Fe<sub>d</sub> progressively approaches a square pyramidal configuration in **8b** and **9b**, with a corresponding movement of one CO group toward Fe<sub>p</sub>. In the complexes with anionic L (**10b, 11b,** and **12b**), Fe<sub>d</sub> has a distorted square pyramidal arrangement, the distance between Fe<sub>p</sub> and the bridging CO shortens as the hardness of L increases, and a corresponding lengthening of the Fe<sub>d</sub>-CO bond is observed.

NAO charges for the open and bridged isomers **7-12** are collected in Tables 3 and 4, respectively. In the open isomers, Fe<sub>p</sub> is always more electrophilic than Fe<sub>d</sub>, whereas the opposite holds true in the bridged forms.

The analysis of orbital populations shows that, in the open isomers, the occupancy of the π\* orbitals of the CO groups coordinated to Fe<sub>d</sub> is only slightly affected by L, the largest difference being observed switching from neutral to anionic L ligands (Table 5). The same analysis carried out on bridged isomers (Table 6) reveals that the occupancy of the π\* orbital of the CO group coordinated to Fe<sub>d</sub> and characterized by a suitable orientation for efficient back-donation from Fe<sub>p</sub> is

**Table 2.** Selected Orbital Populations of the Fully Reduced  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{2-3-}$  Complexes<sup>a</sup>

	1	2	3	4	5	6
$d_{z^2}$ Fe <sub>p</sub>	1.289	1.195	1.095	1.047	0.947	0.901
$d_{x^2-y^2}$ Fe <sub>p</sub>	1.091	1.028	0.997	1.017	1.012	0.999
Lp C(O) <sub>d</sub> <sup>br</sup>	1.495	1.499	1.497	1.530	1.524	1.502
$\pi^*$ C(O) <sub>d</sub> <sup>br</sup>	0.195, 0.259	0.224, 0.250	0.253, 0.243	0.359, 0.228	0.355, 0.244	0.393, 0.294
Lp C(O) <sub>d</sub> <sup>ax</sup>	1.520	1.528	1.525	1.516	1.510	1.472
$\pi^*$ C(O) <sub>d</sub> <sup>ax</sup>	0.264, 0.241	0.262, 0.271	0.269, 0.260	0.261, 0.389	0.258, 0.298	0.226, 0.438
$L_{\text{donation}}$	0.483	0.464	0.259	0.528	0.470	0.292
$L_{\text{back-donation}}$	0.518	0.185	0.063	0.100	-0.002	-0.060

<sup>a</sup>  $L_{\text{donation}}$  is defined as  $L_{\text{donation}} = \sum_i(p_i^{\text{L,free}} - p_i^{\text{L,complex}})$  where  $p_i$  values are populations of occupied orbitals of ligand L in free and in complexed form.  $L_{\text{back-donation}}$  is defined as  $L_{\text{back-donation}} = \sum_i(p_i^{\text{L,complex}} - p_i^{\text{L,free}})$  when  $i$  runs over antibonding orbitals of L. Lp stands for lone pair. The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts br and ax are used as labels to distinguish between the two CO groups coordinated to Fe<sub>d</sub>, which in some complexes have bridged/semibridged and axial positions, respectively.

affected by L and increases significantly from **7b** to **9b**. This effect is much less evident when L is anionic (**10b–12b**).

Another difference between the electronic properties of open and bridged isomers is related to the localization of the electron spin, which is mainly localized on Fe<sub>d</sub> in bridged isomers and is either evenly distributed on the Fe centers (as in the pseudosymmetric complex **7a**) or mainly localized on Fe<sub>p</sub> in open forms (data not shown).

Considering relative stabilities, it turns out that the open and bridged isomers are almost isoenergetic. The largest energy difference is observed for the complexes **7a** and **7b** (L = CO), with the bridged isomer more stable than the open one by about 5.5 kcal mol<sup>-1</sup>. The energy difference decreases considering **8** (L = P(CH<sub>3</sub>)<sub>3</sub>;  $\Delta E = 4.93$  kcal mol<sup>-1</sup>) and **9** (L = CH<sub>3</sub>SH,  $\Delta E = 1.54$  kcal mol<sup>-1</sup>). A similar trend is observed when L is anionic, with  $\Delta E = 5.48$ , 3.62, and 1.92 kcal mol<sup>-1</sup> when L is CN<sup>-</sup>, CH<sub>3</sub>S<sup>-</sup>, and CH<sub>3</sub>O<sup>-</sup>, respectively.

**The Fully Oxidized [L(CO)(CN)Fe<sub>p</sub>( $\mu$ -PDT)Fe<sub>d</sub>(CO)<sub>2</sub>(CN)]<sup>q-</sup> ( $q = 0, 1$ ) Models.** The further one-electron oxidation of the binuclear cluster leads to the fully oxidized species **13–18**, whose molecular geometries and selected geometrical parameters are shown in Figure 3.

In complexes **13**, **14**, and **15**, both Fe atoms are in a distorted square pyramidal arrangement (Figure 3). The CO group coordinated axially to Fe<sub>d</sub> is almost linear (Fe–C–O angle larger than 174°), and the corresponding CO–Fe<sub>p</sub> distance is longer than 2.9 Å, suggesting weak interaction. Remarkably, the geometry of PDT is strongly distorted, and one  $\gamma$ -hydrogen atom of PDT approaches the vacant site of Fe<sub>d</sub>. In fact, the H–Fe<sub>d</sub> distance is equal to 2.177, 2.143, and 2.180 Å in **13**, **14**, and **15**, respectively, suggesting that the C–H bond, which is elongated by about 0.05 Å, is involved in a weak agostic interaction. The Fe–Fe distance is very similar in the three complexes (about 2.84 Å) and significantly longer than the corresponding value computed for more reduced species.

Despite the fully oxidized redox state of the complexes, the net charge of Fe<sub>p</sub> is negative in **13** and **14** (−0.131 and −0.146, respectively; see also Table 7), and only slightly positive in **15** (0.027). It is also worth noting that the occupancy of the  $\pi^*$  orbitals of CO ligands is significantly smaller than the corresponding values computed for more reduced species (Table 8).

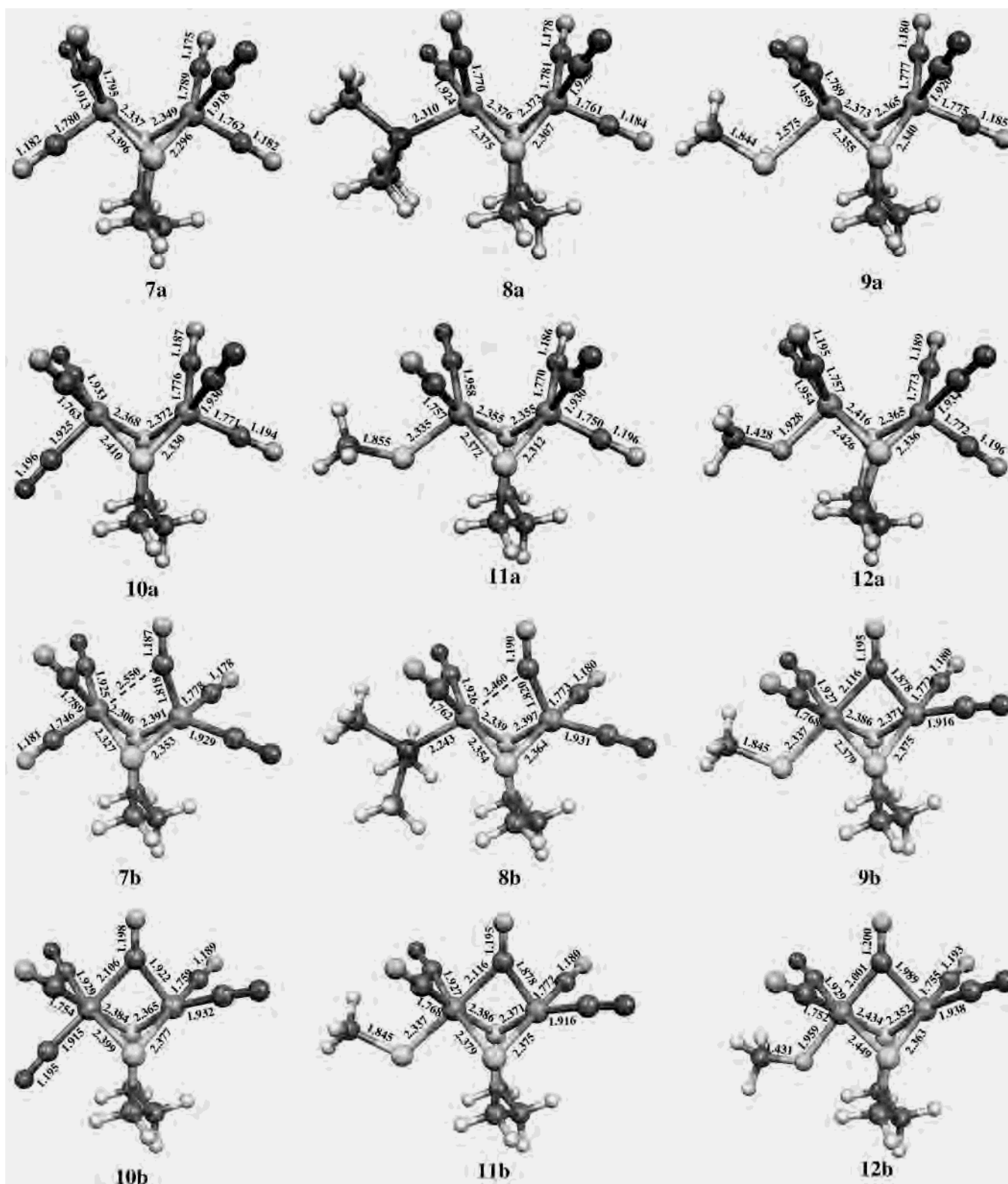
The structures of complexes **16**, **17**, and **18**, where L is anionic, are remarkably different from those computed for species with neutral L. In particular, one CO ligand is asymmetrically bridged between the two Fe atoms, and Fe<sub>p</sub> assumes a pseudo-octahedral arrangement. Moreover, the agostic interaction that was observed in complexes with neutral L is not present in **16**, **17**, and **18**.

The analysis of net atomic charges (Table 7) reveals that the polarization along the Fe–Fe axis decreases moving from **16** to **18**, with Fe<sub>d</sub> remaining always more electrophilic than Fe<sub>p</sub>.

## Discussion

In the first part of this work, we have investigated structural and electronic properties of complexes characterized by the same redox state of the fully reduced form of the enzyme and by the general structure  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{2-3-}$ , where L = CO, P(CH<sub>3</sub>)<sub>3</sub>, CH<sub>3</sub>SH, CN<sup>-</sup>, CH<sub>3</sub>S<sup>-</sup>, and CH<sub>3</sub>O<sup>-</sup>, respectively (**1–6**; Figure 1). In this series of complexes, a progressive rotation of the Fe<sub>d</sub>(CO)<sub>2</sub>(CN) group, whose geometry changes from pseudo-square-pyramidal to trigonal bipyramidal, is observed going from the softest (CO) to the hardest (CH<sub>3</sub>O<sup>-</sup>) L ligand. Concomitantly, a CO group coordinated to Fe<sub>d</sub> approaches Fe<sub>p</sub>, leading to semibridged structures that, especially in the case of L = CH<sub>3</sub>S<sup>-</sup>, closely resemble the structure of the [2Fe]<sub>H</sub> cluster in the reduced form of the enzyme.<sup>2</sup> Interestingly, Darensbourg and co-workers, in light of the key observation that the structure of the [2Fe]<sub>H</sub> cluster in the reduced enzyme is similar to the transition state structure associated to the rotation of one FeL<sub>3</sub> group in [Fe<sub>2</sub>( $\mu$ -PDT)(CO)<sub>6</sub>] and [Fe<sub>2</sub>( $\mu$ -PDT)(CO)<sub>5</sub>(CN)]<sup>1-</sup> complexes, suggested the possibility that the protein could “fix” the cofactor in an entatic state.<sup>4d,10</sup> Indeed, they suggested also that a good electron-donating ligand in trans position to the incipient bridging CO group could better stabilize the semibridged structure.<sup>10</sup> In fact, Pickett et al. have reported spectroscopic data on cleverly designed [Fe<sub>2</sub>S<sub>3</sub>] complexes indicating that  $\mu$ -CO groups are stabilized when two CN<sup>-</sup> and a thioether ligand are coordinated to the metal centers.<sup>18</sup> Our results confirm and extend

(18) George, S. J.; Cui, Z.; Razavet, M.; Pickett, C. J. *Chem. Eur. J.* **2002**, *8*, 4037–4046. Razavet, M.; Davies, S. C.; Hughes, D. L.; Barclay, J. E.; Evans, D. J.; Fairhurst, S. A.; Liu, X.; Pickett, C. J. *J. Chem. Soc., Dalton Trans.* **2003**, 586–595.



**Figure 2.** DFT optimized geometries and bond distances (Å) of partially oxidized model complexes  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_q(\text{CO})_2(\text{CN})]^{1-2-}$  where  $\text{L} = \text{CO}, \text{P}(\text{CH}_3)_3, \text{CH}_3\text{SH}, \text{CN}^-, \text{CH}_3\text{S}^-,$  and  $\text{CH}_3\text{O}^-$ . Fe–Fe distances (Å) are 2.589, 2.513, 2.480, 2.681, 2.587, 2.748, 2.612, 2.607, 2.581, 2.596, 2.597, and 2.607 in **7a**, **8a**, **9a**, **10a**, **11a**, **12a**, **7b**, **8b**, **9b**, **10b**, **11b**, and **12b**, respectively.

this latter observation, showing that the substitution of three CO groups in the parent complex  $[\text{Fe}_2(\mu\text{-PDT})(\text{CO})_6]$  with two  $\text{CN}^-$  and a relatively hard L ligand such as  $\text{CH}_3\text{S}^-$  is sufficient to modify the structural features of the bimetallic

cluster from a pseudosymmetrical edge-bridged square pyramid to a  $\mu\text{-CO}$  species which is extremely similar to the structure of the cluster in the active site of the enzyme and is characterized by a vacant coordination site on  $\text{Fe}_d$ . This

**Table 3.** Charges of Selected Fragments of the Open Form of the Partially Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{1-2-}$  Complexes<sup>a</sup>

	7a	8a	9a	10a	11a	12a
Fe <sub>p</sub>	-0.098	0.000	0.118	0.078	0.155	0.387
Fe <sub>d</sub>	-0.160	-0.171	-0.212	-0.142	-0.236	-0.146
L	0.045	0.326	0.182	-0.533	-0.483	-0.586
S <sub>2</sub> C <sub>3</sub> H <sub>6</sub>	-0.156	-0.341	-0.303	-0.351	-0.512	-0.450
CO <sub>d</sub> <sup>eq</sup>	0.121	0.107	0.124	0.001	0.038	-0.019
CO <sub>d</sub> <sup>ax</sup>	0.078	0.066	0.086	-0.057	-0.040	-0.079
CN <sub>d</sub>	-0.472	-0.476	-0.470	-0.524	-0.518	-0.535
CO <sub>p</sub>	0.115	0.018	0.032	0.076	0.003	0.004
CN <sub>p</sub>	-0.474	-0.531	-0.557	-0.527	-0.568	-0.576
$\langle S^2 \rangle^b$	0.844	0.866	0.789	0.985	0.908	1.251

<sup>a</sup> The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts eq and ax are used as labels to distinguish between CO groups that have equatorial and axial positions, respectively. <sup>b</sup> Expectation value of the squared spin operator before annihilation. The spin projection technique applied to spin unrestricted wave functions led to a mainly pure doublet state (0.75), with the exception of that for **12a**, which is contaminated by high-spin contributions.

**Table 4.** Charges of Selected Fragments of the Bridged Form of the Partially Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{1-2-}$  Complexes<sup>a</sup>

	7b	8b	9b	10b	11b	12b
Fe <sub>p</sub>	-0.241	-0.211	-0.164	-0.217	-0.154	0.030
Fe <sub>d</sub>	0.022	0.030	0.032	0.035	-0.005	0.042
L	0.140	0.468	0.327	-0.488	-0.383	-0.565
S <sub>2</sub> C <sub>3</sub> H <sub>6</sub>	-0.139	-0.343	-0.359	-0.360	-0.408	-0.457
CO <sub>d</sub> <sup>br</sup>	-0.073	-0.080	-0.055	-0.105	-0.111	-0.100
CO <sub>d</sub> <sup>ax</sup>	0.119	0.103	0.108	0.029	0.038	0.004
CN <sub>d</sub>	-0.519	-0.530	-0.489	-0.546	-0.542	-0.558
CO <sub>p</sub>	0.153	0.080	0.114	0.133	0.084	0.116
CN <sub>p</sub>	-0.462	-0.518	-0.515	-0.480	-0.510	-0.513
$\langle S^2 \rangle^b$	0.792	0.789	0.796	0.792	0.794	0.803

<sup>a</sup> The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts br and ax are used as labels to distinguish between CO groups that have bridged/semibridged and axial positions, respectively. <sup>b</sup> Expectation value of the squared spin operator before annihilation. The spin projection technique applied to spin unrestricted wave functions led to mainly pure doublet state (0.75).

**Table 5.** Selected Orbital Populations of the Open Form of the Partially Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{1-2-}$  Complexes<sup>a</sup>

	7a	8a	9a	10a	11a	12a
d <sub>z<sup>2</sup></sub> Fe <sub>p</sub>	1.237	1.178	1.231	0.803	0.765	0.626
d <sub>x<sup>2</sup>-y<sup>2</sup></sub> Fe <sub>p</sub>	0.890	0.784	0.775	1.036	1.114	1.005
Lp C(O) <sub>d</sub> <sup>eq</sup>	1.503	1.499	1.488	1.525	1.493	1.528
π* C(O) <sub>d</sub> <sup>eq</sup>	0.181	0.197	0.193	0.255	0.271	0.269
	0.150	0.155	0.158	0.166	0.206	0.173
Lp C(O) <sub>d</sub> <sup>ax</sup>	1.501	1.503	1.485	1.567	1.530	1.579
π* C(O) <sub>d</sub> <sup>ax</sup>	0.195	0.205	0.206	0.231	0.243	0.235
	0.195	0.196	0.196	0.219	0.232	0.223
L <sub>donation</sub>	0.481	0.483	0.219	0.576	0.511	0.334
L <sub>back-donation</sub>	0.407	0.137	0.025	0.109	0.007	-0.070

<sup>a</sup> L<sub>donation</sub> is defined as  $L_{\text{donation}} = \sum_i (p_i^{\text{L,free}} - p_i^{\text{L,complex}})$  where  $p_i$  values are populations of occupied orbitals of ligand L in free and in complexed form. L<sub>back-donation</sub> is defined as  $L_{\text{back-donation}} = \sum_i (p_i^{\text{L,complex}} - p_i^{\text{L,free}})$  when  $i$  runs over antibonding orbitals of L. Lp stands for lone pair. The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts eq and ax are used as labels to distinguish between CO groups that have equatorial and axial positions, respectively.

strongly suggests that the protein environment does not affect significantly the structural properties of the reduced bimetallic cofactor.

**Table 6.** Selected Orbital Populations of the Bridged Form of the Partially Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{1-2-}$  Complexes<sup>a</sup>

	7b	8b	9b	10b	11b	12b
d <sub>z<sup>2</sup></sub> Fe <sub>p</sub>	1.097	1.045	1.075	1.102	1.058	0.950
d <sub>x<sup>2</sup>-y<sup>2</sup></sub> Fe <sub>p</sub>	1.153	1.082	0.989	1.060	1.028	1.044
Lp C(O) <sub>d</sub> <sup>br</sup>	1.559	1.551	1.477	1.537	1.517	1.513
π* C(O) <sub>d</sub> <sup>br</sup>	0.235	0.258	0.375	0.375	0.380	0.396
	0.230	0.226	0.177	0.166	0.187	0.165
Lp C(O) <sub>d</sub> <sup>ax</sup>	1.485	1.484	1.494	1.502	1.502	1.508
π* C(O) <sub>d</sub> <sup>ax</sup>	0.184	0.192	0.177	0.233	0.229	0.247
	0.187	0.195	0.180	0.208	0.204	0.213
L <sub>donation</sub>	0.582	0.625	0.373	0.641	0.613	0.355
L <sub>back-donation</sub>	0.414	0.134	0.034	0.109	-0.006	-0.069

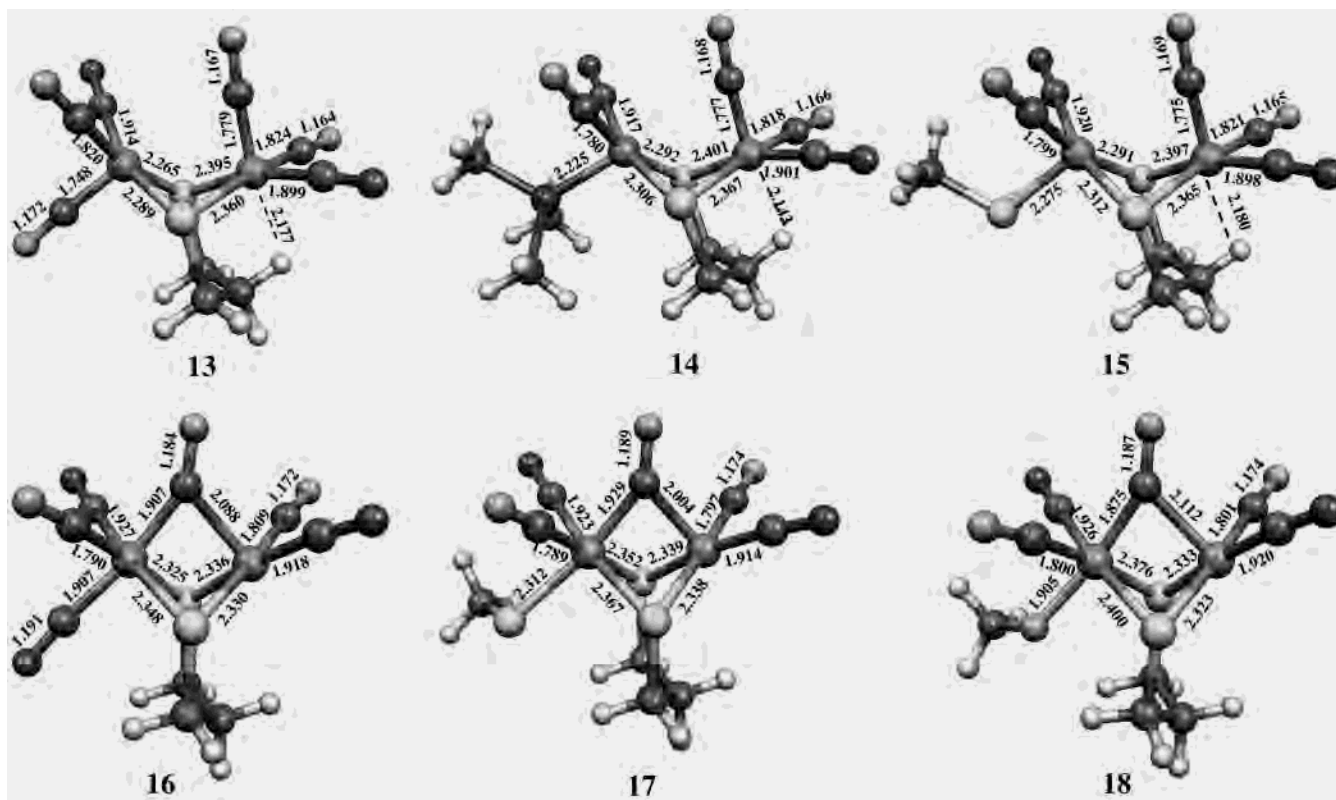
<sup>a</sup> L<sub>donation</sub> is defined as  $L_{\text{donation}} = \sum_i (p_i^{\text{L,free}} - p_i^{\text{L,complex}})$  where  $p_i$  values are populations of occupied orbitals of ligand L in free and in complexed form. L<sub>back-donation</sub> is defined as  $L_{\text{back-donation}} = \sum_i (p_i^{\text{L,complex}} - p_i^{\text{L,free}})$  when  $i$  runs over antibonding orbitals of L. Lp stands for lone pair. The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the [Fe<sub>4</sub>S<sub>4</sub>] and the [2Fe]<sub>H</sub> clusters, respectively. The superscripts br and ax are used as labels of CO groups that have bridged/semibridged and axial positions, respectively.

The analysis of the electronic properties of the model complexes related to the fully reduced form of the enzyme indicates that a significant charge dipole develops along the Fe<sub>p</sub>–Fe<sub>d</sub> axis moving from **1** to the semibridged model **6**. In particular, the nucleophilicity of Fe<sub>p</sub> decreases going from soft to hard L ligands, whereas the partial charge value on Fe<sub>d</sub> is negative and remains almost constant along the series, being compatible with binding of electrophilic species. This observation is relevant because Fe<sub>d</sub>, which in the reduced form of the enzyme has a vacant coordination position in trans position to the semibridging CO group, has been proposed to be the binding site of H<sup>+</sup>.<sup>2</sup> However, it should be noted that oxidative addition of H<sup>+</sup> to Fe(I)Fe(I) complexes leads to species in which the hydride assumes a bridging position between the two iron centers.<sup>19</sup> Our results indicate that when soft ligands are coordinated to the Fe atoms both metal ions have a pronounced nucleophilic character, a characteristic that coupled with the “open” structure of the complexes should favor μ-H coordination. On the other hand, hard L ligands favor complexes where a CO group bridges the two iron atoms, with a concomitant strong reduction of Fe<sub>p</sub> nucleophilicity, favoring protonation of the vacant coordination site of Fe<sub>d</sub>. In this respect, the synthesis of complexes characterized by strategically placed donor ligands is suggested to be crucial to modulate the reactivity of these species and better reproduce the structural features observed in the enzyme.

Models obtained by one-electron oxidation of the fully reduced complexes (**7–12**; Figure 2) should correspond to the paramagnetic form of the enzyme and can exist in two different stable forms, characterized either by the presence (bridged isomers) or absence (open isomers) of a CO group bridging the two metal centers. Similarly to fully reduced models, in the bridged isomers the rotation extent of the Fe<sub>d</sub>(CO)<sub>2</sub>(CN) group is parallel to the hardness of L.

The electronic structure of the partially oxidized models reveals that in the open isomers the Fe<sub>p</sub> atom is more

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**Figure 3.** DFT optimized geometries and bond distances (Å) of fully oxidized model complexes  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{0/1-}$  where L = CO,  $\text{P}(\text{CH}_3)_3$ ,  $\text{CH}_3\text{SH}$ ,  $\text{CN}^-$ ,  $\text{CH}_3\text{S}^-$ , and  $\text{CH}_3\text{O}^-$ . Fe–Fe distances (Å) are 2.843, 2.846, 2.841, 2.513, 2.516, and 2.516 in **13**, **14**, **15**, **16**, **17**, and **18**, respectively.

**Table 7.** Charges of Selected Fragments of the Fully Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{0/1-}$  Complexes<sup>a</sup>

	13	14	15	16	17	18
$\text{Fe}_p$	-0.131	-0.146	0.027	-0.304	-0.218	-0.013
$\text{Fe}_d$	-0.058	-0.058	-0.065	0.205	0.112	0.200
L	0.226	0.604	0.427	-0.394	-0.157	-0.410
$\text{S}_2\text{C}_3\text{H}_6$	0.100	-0.089	-0.078	-0.023	-0.151	-0.187
$\text{CO}_d^{\text{br}}$	0.249	0.244	0.241	0.053	0.037	0.040
$\text{CO}_d^{\text{ax}}$	0.231	0.221	0.222	0.137	0.144	0.122
$\text{CN}_d$	-0.401	-0.415	-0.405	-0.501	-0.489	-0.510
$\text{CO}_p$	0.212	0.131	0.136	0.230	0.175	0.226
$\text{CN}_p$	-0.427	-0.489	-0.504	-0.404	-0.462	-0.449

<sup>a</sup> The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the  $[\text{Fe}_4\text{S}_4]$  and the  $[\text{2Fe}]_{\text{H}}$  clusters, respectively. The superscripts br and ax are used as labels of CO groups that have bridged/semibridged and axial positions, respectively.

electrophilic than  $\text{Fe}_d$  and therefore, at least in principle, more suited to bind  $\sigma$ -donor species such as  $\text{H}_2$  and  $\text{H}^-$ . Moreover, the electrophilicity of  $\text{Fe}_p$  is increased moving from soft to hard L ligands. The same trend is observed in bridged isomers. However, for this class of models,  $\text{Fe}_d$  remains always more electrophilic than  $\text{Fe}_p$ , suggesting that the former is more suited to bind nucleophilic species. The comparison of open and bridged isomers reveals also that when considering complexes with relatively hard L ligands, the partial charge of  $\text{Fe}_p$  in open forms is always significantly more positive than the corresponding value computed for  $\text{Fe}_d$  in bridging isomers, suggesting stronger binding of nucleophilic species to open forms. Of course, this can be either functionally advantageous, facilitating the binding of  $\sigma$ -donor

**Table 8.** Selected Orbital Populations of the Fully Oxidized  $[\text{L}(\text{CO})(\text{CN})\text{Fe}_p(\mu\text{-PDT})\text{Fe}_d(\text{CO})_2(\text{CN})]^{0/1-}$  Complexes<sup>a</sup>

	13	14	15	16	17	18
$d_{z^2} \text{Fe}_p$	0.936	0.947	0.758	1.190	1.154	1.011
$d_{x^2-y^2} \text{Fe}_p$	1.151	1.076	1.075	1.154	1.119	1.120
$\text{Lp C}(\text{O})_d^{\text{br}}$	1.409	1.404	1.405	1.443	1.412	1.422
$\pi^* \text{C}(\text{O})_d^{\text{br}}$	0.140	0.128	0.143	0.242	0.263	0.254
$\text{Lp C}(\text{O})_d^{\text{ax}}$	1.510	1.510	1.509	1.547	1.526	1.539
$\pi^* \text{C}(\text{O})_d^{\text{ax}}$	0.117	0.124	0.122	0.145	0.152	0.153
$L_{\text{donation}}$	0.584	0.737	0.450	0.660	0.833	0.508
$L_{\text{back-donation}}$	0.358	0.112	0.030	0.054	-0.011	-0.068

<sup>a</sup>  $L_{\text{donation}}$  is defined as  $L_{\text{donation}} = \sum_i (p_i^{\text{L-free}} - p_i^{\text{L-complex}})$  where  $p_i$  values are populations of occupied orbitals of ligand L in free and in complexed form.  $L_{\text{back-donation}}$  is defined as  $L_{\text{back-donation}} = \sum_i (p_i^{\text{L-complex}} - p_i^{\text{L-free}})$  when  $i$  runs over antibonding orbitals of L. Lp stands for lone pair. The subscripts p and d stand for proximal and distal position with respect to the cysteine residue that in the enzyme bridges the  $[\text{Fe}_4\text{S}_4]$  and the  $[\text{2Fe}]_{\text{H}}$  clusters, respectively. The superscripts br and ax are used as labels of CO groups that have bridged/semibridged and axial positions, respectively.

species such as  $\text{H}_2$  and promoting their cleavage, or disadvantageous, leading to very stable species that are generally avoided in catalytic cycles.

The analysis of CO orbital populations computed for bridged isomers shows, as expected, that also for partially oxidized species the back-donation to the bridging CO is increased moving from soft to hard L ligands. Notably, also the back-donation to the CO groups coordinated to  $\text{Fe}_d$  in open isomers increases moving from soft to hard L ligands, indicating that  $\text{Fe}_p$  ligands can influence the structural and electronic properties of groups coordinated to the distal iron center even when a CO group does not bridge the metal centers.



The marked influence on Fe(I)Fe(I) and Fe(II)Fe(I) species of subtle differences in the electronic properties of L, which corresponds to the cysteine residue bridging the [Fe<sub>4</sub>S<sub>4</sub>] cluster and the [2Fe]<sub>H</sub> subcluster in the enzyme, may also suggest that the oxidation state of the [Fe<sub>4</sub>S<sub>4</sub>] cluster could affect significantly the structural and electronic properties of the binuclear subcluster and therefore modulate the relative stability of open and bridged forms. In fact, the relative stabilities of open and bridged Fe(I)Fe(II) isomers are very similar. In particular, even if the bridged forms are always slightly more stable, in agreement with the presence of a  $\mu$ -CO group in the partially oxidized form of the enzyme,<sup>1,2</sup> the substitution of soft ligands with relatively hard species systematically decreases the energy gap. In light of the small computed energy differences, it is not unconceivable that, even if still not characterized experimentally, short-lived intermediate species related to the open isomers might be formed in the catalytic cycle of the enzyme. In this context, it is worth noting that when L is a relatively hard ligand, the conversion of bridged to open forms implies only the concerted movement of the two CO ligands coordinated to Fe<sub>d</sub>, whereas the position of the CN<sup>-</sup> group, which in the enzyme is strongly anchored to the polypeptide chain due to hydrogen bonding interactions,<sup>1</sup> does not change in the two isomers (Figure 2).

Also, the structure of the fully oxidized models, and, consequently, the localization of the vacant coordination site, is extremely dependent on the nature of the L ligand. In addition, the nature of L has also some influence on the relative electrophilicity of the two iron centers. These results suggest that the relative hardness of Fe<sub>p</sub> ligands may exert some control on the localization of the binding site for  $\sigma$ -donor species, such as H<sub>2</sub>, leading either to Fe<sub>p</sub> or Fe<sub>d</sub> coordination.

In summary, the systematic DFT investigations of a series of model complexes related to the active site of Fe-hydrogenases has allowed the disclosure of some key structural and electronic characteristics of the bimetallic cluster. The properties of these models have been shown to be strongly dependent on the redox state and on the electronic characteristic of the ligand L, which corresponds to the cysteine residue bridging the [2Fe]<sub>H</sub> and the [Fe<sub>4</sub>S<sub>4</sub>] cluster in the enzyme. These observations are expected to be relevant for the design of new synthetic models of the [2Fe]<sub>H</sub> cluster and may help in better understanding the enzymatic system.

**Supporting Information Available:** Vibrational frequencies (cm<sup>-1</sup>) corresponding to the CO and CN stretching modes of the models. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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