



# Toward Diiron Dithiolato Biomimetics with Rotated Conformation of the [FeFe]-Hydrogenase Active Site: A DFT Case Study on Electron-Rich, Isocyanide-Based Scaffolds

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The electron rich  $\text{Fe}_2(\text{pdt})(\text{RNC})_6$  ( $\text{pdt} = \text{CH}_2(\text{CH}_2\text{S}^-)_2$ ) has been used as starting point for a DFT multi-functional study to assess the feasibility of designing a stable inverted (or rotated) disposition of the two  $\text{FeL}_3$  pyramidal moieties in the dimetallic core, a key feature of [FeFe]-hydrogenase cofactor. The choice of **1** was motivated by the presence of a rotated form in solution, slightly less stable than the unrotated stereoisomer. Aimed to find an upgraded version of **1**, featuring the rotated

isomer as ground state, various combinations of factors have been tested, for their effect on the relative stability of rotated vs unrotated isomers. The general result is that combining coordination asymmetry, electron donor presence and isocyanides R substituents able to establish intramolecular interactions is effective in stabilizing the rotated isomer. Our DFT study may inspire the design of synthetic biomimetics, with improved resemblance to the natural system.

## Introduction

In Nature,  $\text{H}_2$  is produced and oxidized with extraordinary efficiency by two classes of enzymes, [FeFe]-hydrogenases and [NiFe]-hydrogenases, named after the metal content of their respective active site.<sup>[1]</sup> While [NiFe]-hydrogenases are biased towards  $\text{H}_2$  oxidation, [FeFe]-hydrogenases operate in both directions with high turnovers, being capable of producing up to 7500 molecules of  $\text{H}_2$  per second.<sup>[2]</sup> This makes [FeFe]-hydrogenases particularly promising in the context of carbon-free energy storage/production, avoiding, at the same time, the costly and non-sustainable exploitation of rare metals.<sup>[3,4]</sup> Such enzymes use a unique organometallic cofactor, called H-cluster, composed of a canonical 4Fe–4S cluster ( $[\text{4Fe–4S}]_{\text{H}}$ ) covalently linked to a diiron subsite  $[\text{2Fe}]_{\text{H}}$ , via a bridging cysteine (Scheme 1A). In  $[\text{2Fe}]_{\text{H}}$ , the coordination sphere of iron atoms consists of CO,  $\text{CN}^-$  ligands and of an azadithiolato ( $\text{adt} = \text{NH}(\text{CH}_2\text{S}^-)_2$ ) group.<sup>[5–8]</sup> This diiron dithiolato scaffold has been a source of inspiration for the synthesis and investigation of a huge number of biomimetic and bioinspired systems, grown

explosively over the past few decades.<sup>[9–16]</sup> All these efforts aimed at developing a synthetic proficient catalyst for processing  $\text{H}_2$ , circumventing the technological issues related to the utilization, on a large scale, of the full enzyme.<sup>[11]</sup> In this context, the continuous research of structural and functional details to complete information on the enzyme functioning is fundamental.<sup>[17]</sup>

One of the crucial requirements for effective catalysis found in the enzyme, is the proximity of the adt pendant base to the Fe referred to as “distal” ( $\text{Fe}_d$ ), to distinguish it from the “proximal” one ( $\text{Fe}_p$ ), directly bound to  $[\text{4Fe–4S}]_{\text{H}}$ .<sup>[1]</sup> Such a structural arrangement makes the active site a frustrated Lewis pair system, prompted for efficient and reversible heterolytic  $\text{H}^+/\text{H}^-$  coupling. However, this is possible *only* if  $\text{Fe}_d$  exposes *stably* and *persistently* a vacant coordination site, i.e.  $\text{Fe}_d$  should be in what is called a “rotated conformation”, characterized by an inverted  $\text{Fe}_d$  square pyramidal coordination geometry with respect to  $\text{Fe}_p$  one (Scheme 1B). The vacant site at the apical position of  $\text{Fe}_d$  is available for protonation to form preferentially a *reactive* terminal hydride (t-H).<sup>[18,19]</sup> Recent investigations suggest that the formation of a bridging hydride ( $\mu\text{-H}$ ) in the natural system is not compatible with a fast catalytic process, which should keep the rotated conformation along turnover to achieve high rates.<sup>[14,20,21]</sup> This can be enabled by an efficient interplay between the protein matrix and the H-cluster,  $\text{CN}^-$  ligands in particular (Scheme 1A).<sup>[22–24]</sup> The major implication of this, is that *outside* the protective environment of the catalytic pocket, i.e. when dealing with diiron dithiolato biomimetic systems, the rotated conformation is not stable and the two Fe square pyramids tend to be eclipsed (Scheme 1C).

In an eclipsed (also referred to as *unrotated*) conformation, protonation is driven at the Fe–Fe bond, to form preferentially a *highly stable* bridging hydride ( $\mu\text{-H}$ ), which is a species reported to be less reactive in hydrogen evolving reactions.<sup>[25,26]</sup>

While the enzyme-like conformation with rotated geometry has been characterized and reviewed in various  $\text{Fe}(\text{I})\text{Fe}(\text{II})$

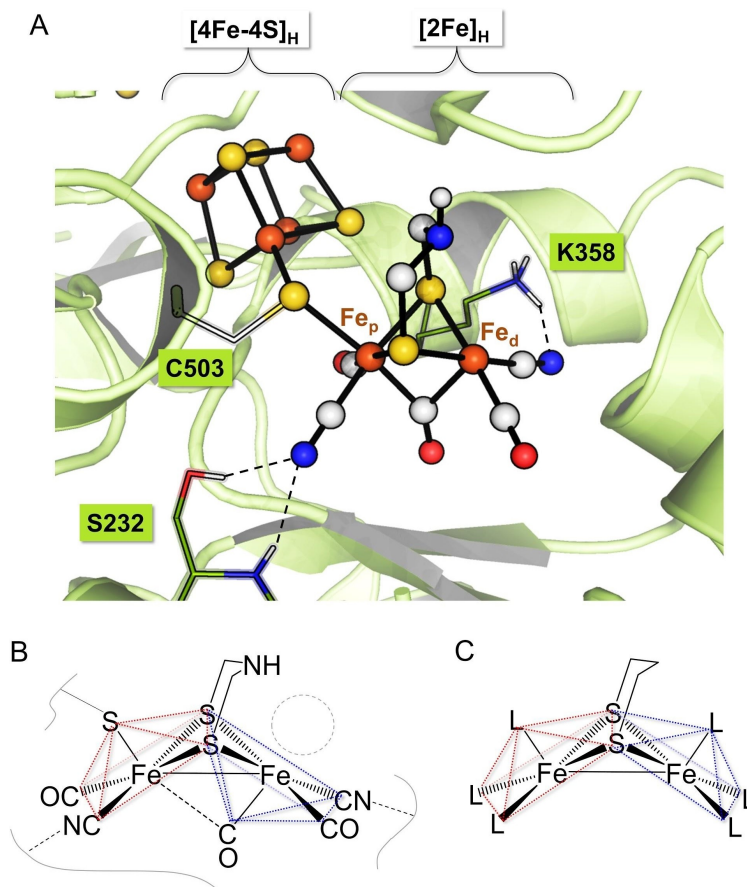
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**Scheme 1.** A: Focus on the H-cluster of *Clostridium pasteurianum* [FeFe]-hydrogenase (PDB ID: 6 N59). CN<sup>-</sup> H-bonds with the protein are put in evidence as dashed black lines. Atom coloring: C = grey for H-cluster, green for selected residues; O = red; N = blue; S = yellow; Fe = orange. B: Schematic representation of the H-cluster rotated conformation, showing the two inverted Fe coordination geometries highlighted in red and blue square pyramids. The vacant coordination site is indicated by a grey circle, while the protein matrix interacting with CN<sup>-</sup> ligands is sketched as grey curves. C: The general structure of a diiron dithiolato biomimetic complex featuring eclipsed square pyramids at the two Fe(I) sites.

synthetic systems,<sup>[11,15,27–31]</sup> its reproduction in more reduced states is still challenging. Indeed, some attempts have been made to stabilize in biomimetics a rotated conformation at the Fe(II)Fe(II) redox level, competent for H<sup>+</sup> reduction, both theoretically and experimentally. For instance, synthetic steric “tricks”, such as the introduction of very bulky dithiolato bridges, helped destabilize the unrotated conformation of one adjacent Fe unit and stabilized the rotated isomer through weak remote (or *pseudo*-) agostic interaction.<sup>[32–35]</sup> Very recently, it was also suggested to take advantage of intramolecular ligation, to restrict rotation at a single Fe and kinetically stabilize the t-H formation occurring after protonation.<sup>[36]</sup> Besides the use of steric congestion, other strategies based both on synthetic and computational approaches, have been put forward. In a previous combined experimental-theoretical investigation it was proposed a novel highly electron-rich prototype, with the general formula Fe<sub>2</sub>(pdt)(RNC)<sub>6</sub> (pdt = CH<sub>2</sub>(CH<sub>2</sub>S<sup>-</sup>)<sub>2</sub>; RNC = isocyanide ligand). In particular, Fe<sub>2</sub>(pdt)(MeNC)<sub>6</sub> (**1**) revealed a relatively low energy rotated Fe(II)Fe(II) isomer (although slightly less stable than the unrotated one),<sup>[37]</sup> that represents the first example of [2Fe]<sub>H</sub> model featuring a rather stable rotated conformation, achieved

exclusively by using a set of strong  $\sigma$ -donor ligands with streamlined shape, such as isocyanides. Besides resembling the same tapered architecture of metal coordination employed by Nature, this approach also avoids the introduction of any steric hindrance at the vacant Fe site that may interfere with substrates binding. Furthermore, it is known that *breaking the symmetry* in iron atoms coordination sphere would trigger rotation, at least at the computational level.<sup>[34,38,39]</sup> This matches with a computational investigation by Tye et al. revealing that rotation causes a charge transfer (CT) from the unit that does not rotate to the rotating one, which stabilizes the vacant apical site exposed by the Fe with rotated geometry.<sup>[40]</sup> For this reason, rotation is expected to occur at the more electron-poor metal center. In the same investigation, it was shown that a dithiolato ligand with a Lewis base installed in the bridgehead, such as the azadithiolato ligand, can compete with the above described CT in stabilizing the vacant site at Fe.

In the present investigation we have explored all the hints described above to rationally design a [2Fe]<sub>H</sub> model in the Fe(II)Fe(II) oxidation state with a rotated *ground state structure*, taking **1** as the starting point. As mentioned before, **1** represents a promising starting point, because of a rotated

conformation that is very close in energy to this last, although less stable than the unrotated one.

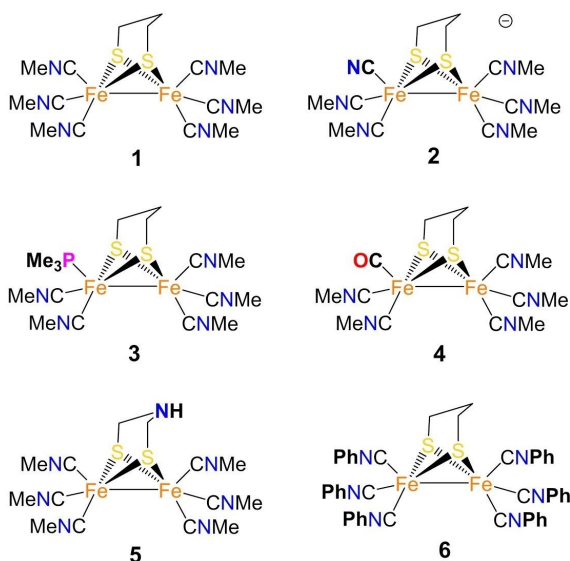
Our aim was therefore to identify which modifications of **1** could be suggested to experimental chemists to synthesize a diiron dithiolate featuring a stable and persistent “enzyme-like structure”.

In particular, we have modified both the symmetry and the electron richness of the diiron scaffold by replacing one MeNC with a different electron-rich ligand, such as  $\text{CN}^-$  or  $\text{PMe}_3$ , or with an electron-withdrawing ligand, such as CO, to give complexes  $[\text{Fe}_2(\text{pdt})(\text{MeNC})_5\text{CN}]^-$  (**2**),  $\text{Fe}_2(\text{pdt})(\text{MeNC})_5\text{PMe}_3$  (**3**) and  $\text{Fe}_2(\text{pdt})(\text{MeNC})_5\text{CO}$  (**4**), respectively.

We have also tested a phosphine ligand as an alternative for  $\text{CN}^-$  since it is known that the latter could be involved in undesired processes along catalysis, mainly due to its high proton affinity.<sup>[41]</sup> Subsequently, the effect of pdt replacement by adt has been investigated, by designing the complex  $\text{Fe}_2(\text{adt})(\text{MeNC})_6$  (**5**). Eventually, we have also changed the nature of R in RNC, by considering  $\text{R}=\text{Ph}$  to give  $\text{Fe}_2(\text{pdt})(\text{PhNC})_6$  (**6**). All investigated complexes are displayed in Scheme 2.

## Methods

All calculations were performed with the TURBOMOLE 7.4 suite of programs.<sup>[42]</sup> The BP86 functional<sup>[43,44]</sup> has been used in conjunction with a TZVP basis set,<sup>[45]</sup> plus D3 correction to account for dispersive forces,<sup>[46]</sup> a protocol that has been intensively validated in the investigation of transition metal-containing complexes, also in relation to hydrogenase modelling.<sup>[47–51]</sup> In order to corroborate the calculated energy trend, we also performed calculations with both TPSS and TPSSh functionals,<sup>[52,53]</sup> since they provided very satisfactory performances in previous investigations on FeS clusters.<sup>[54–56]</sup>



Scheme 2.  $\text{Fe}_2\text{S}_2$  systems investigated in the present work.

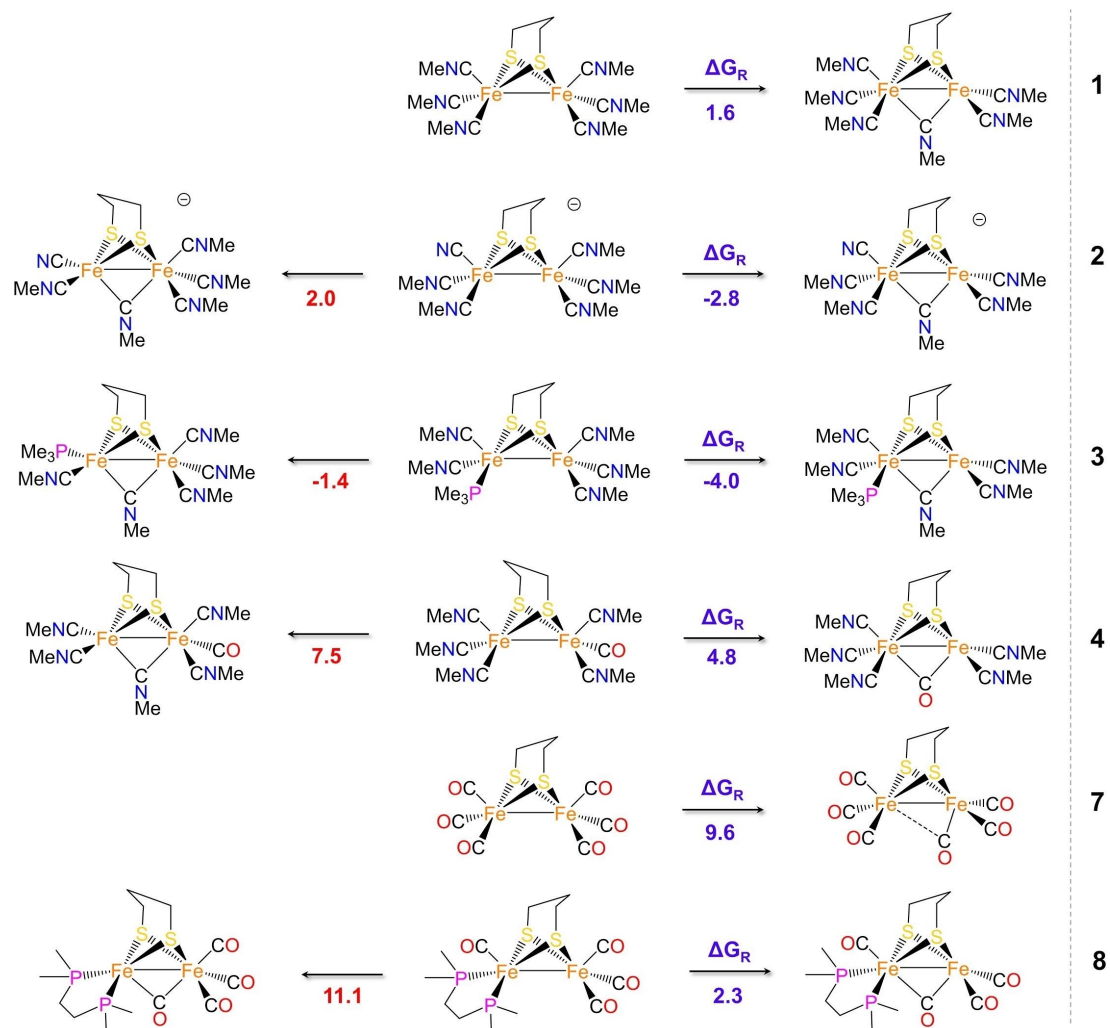
The COSMO approach allowed us to implicitly model the solvation effects.<sup>[57,58]</sup> We set a  $\epsilon=8.9$  since  $\text{CH}_2\text{Cl}_2$  is a common solvent in experimental investigations of hydrogenases biomimetic systems. A full vibrational analysis was performed in order to characterize the nature of each stationary point, looking for zero imaginary frequencies for pure energy minima. Free energies were calculated from the electronic SCF energy considering three contributions to the total partition function (Q), q-translational, q-rotational, q-vibrational, assuming that Q can be written as the product of them. To evaluate enthalpy and entropy contributions, the values for temperature and pressure have been set to 298.15 K and 1 bar, respectively. The scaling factor for the SCF wavenumbers was set either to 0.9914 (default value in TURBOMOLE). The resolution-of-identity (RI) technique has been adopted to speed up calculations.<sup>[59]</sup> The softwares IboView<sup>[60]</sup> and PyMol<sup>[61]</sup> were used to generate pictures of molecular orbitals and structures. For each investigated compound, a complete thermodynamic speciation has been carried out, considering the combination of i) rotation at each  $\text{FeL}_3$  unit, ii) apical vs basal disposition of ligands in asymmetrically-substituted systems, iii) dithiolato (pdt or adt) flipping and iv) different respective orientation of R groups in RNC units. The most significant structures are reported in the SI.

## Results and Discussion

### The effect of the asymmetry of metal coordination on rotation (1–4)

The lowest energy processes associated with rotation at each  $\text{Fe}(\text{L})_3$  unit, in the 1–4 series are summarized in Scheme 3 (see SI for a complete thermodynamic speciation for each complex). The disposition of  $\text{CN}^-$ ,  $\text{PMe}_3$  and CO at basal or apical sites has a negligible effect on the energy of the complexes. Indeed, the free energy difference between apical and basal isomers is 1.0 kcal/mol for **2** (in favor of the apical disposition), 1.8 kcal/mol for **3** (in favor of the basal disposition) and 1.4 kcal/mol for **4** (in favor of the basal isomer). Similar considerations can be made on their rotated counterparts.

Installing a donor ligand in a single Fe unit of **2** and **3** induces the preferential rotation of the adjacent  $\text{Fe}(\text{MeNC})_3$  moiety (Scheme 3). Rotation of the more electron-poor Fe unit is also preferred in **4**, since the CO ligand, replacing one MeNC, switches to the FeFe bridging position in the rotated isomer. The analysis of rotated vs unrotated equilibrium has been extended to two well-characterized  $\text{Fe}_2\text{S}_2$  hydrogenase mimics, namely  $\text{Fe}_2(\text{pdt})\text{CO}_6$  (**7**) and  $\text{Fe}_2(\text{pdt})(\text{CO})_4\text{dmpe}$  (**8**). These systems have been taken as “reference” since their different tendency towards rotation is well known (vide infra). Again, in **8** rotation preferentially occurs at the  $\text{Fe}(\text{CO})_3$  unit, i.e. at the more electron-poor Fe site. The different tendency towards rotation of the two different  $\text{Fe}(\text{L})_3$  units correlates with HOMO distribution in the unrotated form (Figure 1). i.e. the larger



**Scheme 3.** Lowest free energy variations for rotation processes occurring at each Fe unit in systems 1–4, 7 and 8.  $\Delta G_R$  (in blue, right arrows, kcal/mol, BP86-D3/TZVP) is associated with the rotation at the Fe with the higher tendency to rotate, giving the lowest energy rotated isomer of each complex. In asymmetric systems, rotation at the other Fe unit (red values, left arrows) is also reported.

participation of a Fe center to the HOMO, the more hindered its rotation in favor of rotation of the other Fe.

For what concerns rotation free energies ( $\Delta G_R$ , calculated as  $\Delta G_R = G_{\text{rot}} - G_{\text{unrot}}$ , where “rot” refers to the most stable rotated form and “unrot” to the most stable unrotated one), we observed that the substitution of one MeNC ligand with a different electron donor significantly decreases the value of  $\Delta G_R$  (Scheme 3). In particular,  $\Delta G_R$  goes from 1.6 to  $-2.8$  and  $-4.0$  kcal/mol by replacing one MeNC with a  $\text{CN}^-$  or a  $\text{PMe}_3$ , respectively. (The value of  $\Delta G_R$  in ref<sup>37</sup>) was predicted to be 2.2 kcal/mol at the BP86/TZVP/COSMO level without D3 corrections, so the addition of an explicit treatment of dispersive forces lowers only slightly the calculated  $\Delta G_R$ .) It is noteworthy that rotation at a single Fe center is favored independently of the total charge of the donor, as both negatively charged cyanide and neutral phosphine lead to the same effect: the  $\sigma$ -donation appears to be determinant regardless of the general amount of electron density contributed by the donor.

In Figure 1 unrotated isomers of all the compounds already presented in Scheme 3 are shown: both 2 and 3 are asymmetric and feature only electron donors in the metals’ coordination environment. Considering the subset 2–4, only asymmetric and “all-donor-ligand” compounds (2 and 3) feature almost all RNC ligands heavily distorted, clear evidence of strong  $\pi$  back-donation of electron density from iron atoms to ligands. Such distortion from linear arrangement of RNC ligands appears to push R groups of these ligands to establish intramolecular dispersive interactions, whose number turns out to be maximized in the rotated isomers (vide infra for discussion of this crucial factor).

In contrast to 2 and 3, in 4 the asymmetry of the iron atoms coordination sphere is attained by replacing MeNC with an electron acceptor ligand (e.g. CO). The result is a significant increase of  $\Delta G_R$  (4.8 kcal/mol in 4) that implies a destabilization of the rotated isomer. The DFT prediction on the instability of this last in case 4 is supported by the comparison of the related  $\Delta G_R$  value to that computed for known references, such as 7

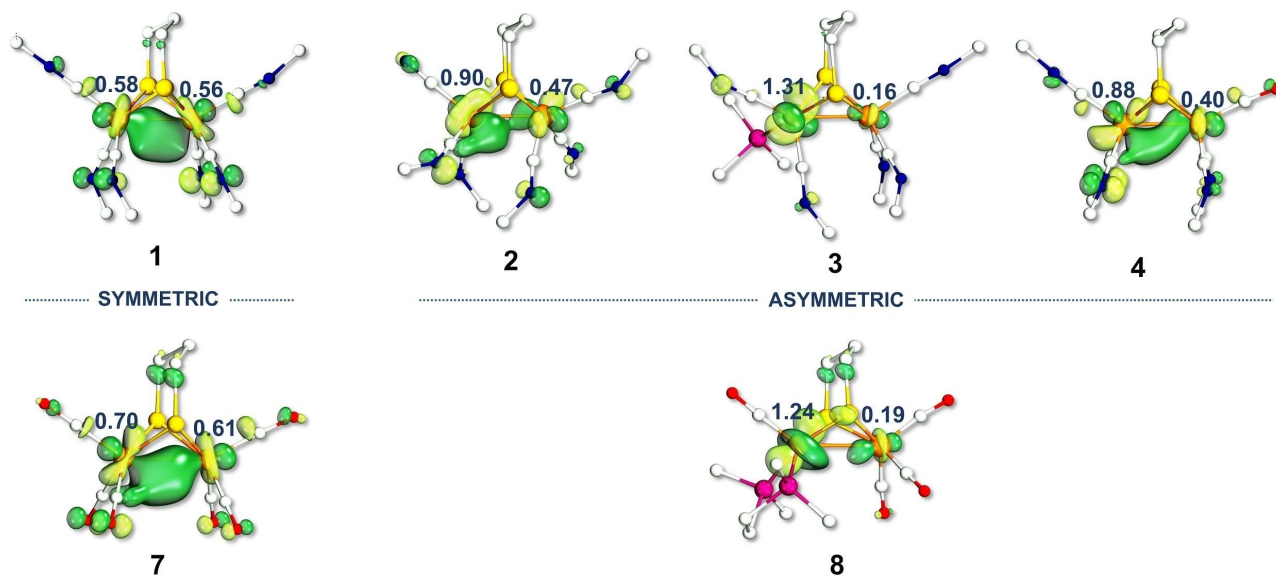


Figure 1. HOMO (cutoff 0.05 a.u.) of complexes 1–4, 7 and 8. The contribution of iron atoms (in electron) to the HOMO (BP86-D3/TZVP) is also reported.

and 8:  $\Delta G_R$  for 4 lies in between the respective values obtained for 7 and 8. These last have an unrotated ground state, although rotation in 7 is much more disfavored (9.6 kcal/mol) than in 8 (2.3 kcal/mol), as a result of symmetry loss in the latter.

#### Charge transfer between Fe centers correlates with rotated vs unrotated stability

In order to rationalize the different behavior of 1–4, we performed a Natural Bond Orbitals (NBO) analysis, aimed to quantify the charge redistribution occurring upon rotation. Interestingly, we found that  $\Delta G_R$  values correlate with the extent of CT from the unrotated molecular fragment to the rotated one (Figure 2): the higher the CT, the lower  $\Delta G_R$  (the more stable is

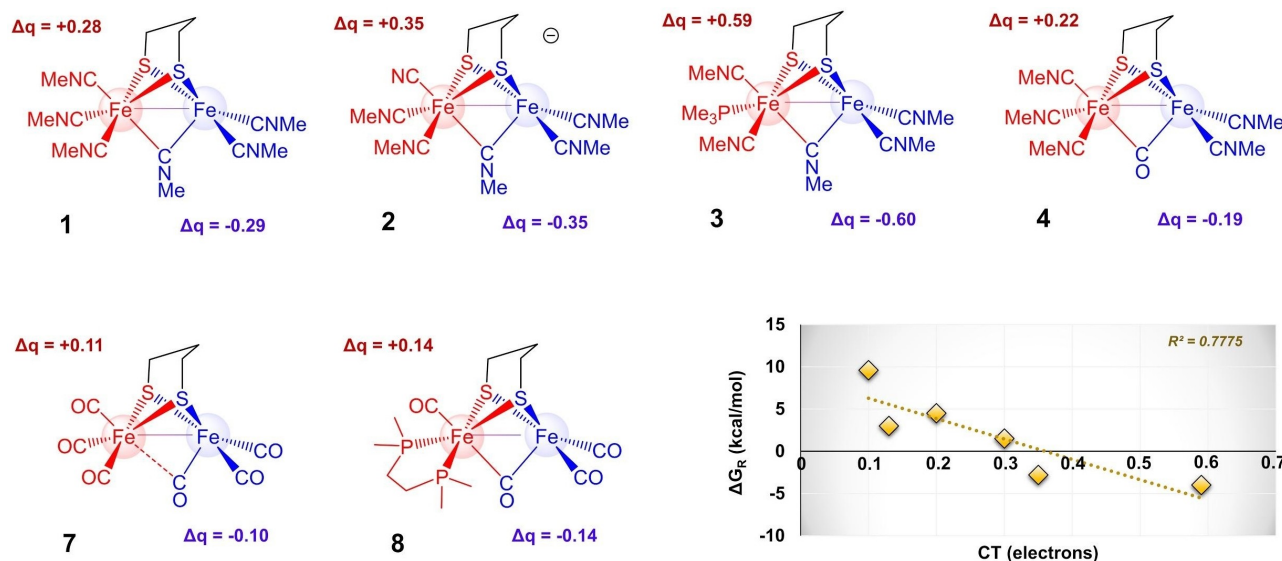


Figure 2. CT calculated (according to NBO analysis at the BP86-D3/TZVP level) upon rotation. In particular,  $\Delta q$  refers to the charge variation of each molecular fragment going from unrotated to rotated isomers. The negative sign of  $\Delta q$ , associated with the blue unit, corresponds to an increase of its negative charge, while the positive sign of  $\Delta q$  at the red unit indicates a decrease of its negative charge. The fragment associated with the Fe unit that does not rotate is shown in red, the other one in blue. Correlation between CT value and  $\Delta G_R$  is also reported. CTs are calculated as the average of the two  $|\Delta q|$  values (red and blue values) obtained for each complex.

the rotated isomer). Analogous considerations can be made on the basis of other population analysis, such as Mulliken and Löwdin charges calculation (Table S1).

Upon rotation, the unit that rotates receives an amount of negative charge ( $\Delta q$ ) from the one that does not rotate, which implies an increase of positive charge of the latter by about  $-\Delta q$ . We compared CT values calculated from NBO analysis for the series 1–4 to the ones obtained for the reference systems 7 and 8. The degree of correlation ( $R^2 = 0.78$ ) between  $\Delta G_R$  and CT values is graphically shown in Figure 2. This result suggests that a possible strategy to stabilize a rotated conformation in  $\text{Fe}_2\text{S}_2$  systems is to maximize the charge transfer between the two  $\text{Fe}(L)_3$  units. If a strong CT between the two units is viable, then the Fe(I) that donates negative charge tends to approximate to a formal Fe(II), while the other Fe accepting electron density tends to be reduced to a formal Fe(0). In order to fulfill the 18  $e^-$  rule, the non-rotating Fe is expected to compensate for the loss of electron density by forming one additional bond (with the bridging MeNC ligand, in the kind of derivatives herein considered). In this context, the rotating Fe unit acts as an electronic redistribution center, operating first the uptake (through the Fe–Fe bond) and then the reallocation of electrons (in the C–Fe bond newly formed in the rotated isomers).

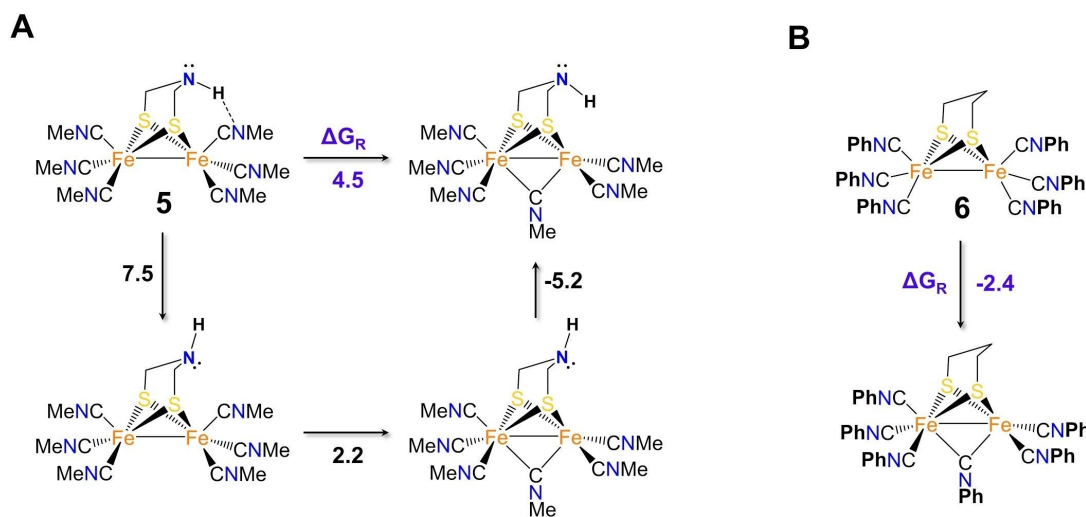
#### Dithiolato and isocyanide ligands – modification (5, 6) and combination of strategies

The most relevant rotated and unrotated isomers of 5 are reported in Scheme 4A. In 5, rotation free energies are strongly affected by the orientation of the adt N–H bond. Indeed, N–H can be axial or equatorial, according to its disposition with respect to the six-membered ring  $\text{Fe}(\text{S})_2(\text{CH}_2)_2\text{N}$ . Previous

computational studies suggested that the presence of an amine (–NH) as dithiolato bridgehead should trigger rotation, since the nitrogen lone pair in the equatorial N–H disposition could stabilize the rotated Fe.<sup>[40]</sup> Our calculations show, however, that the axial N–H disposition is preferred in the rotated form (by 5.2 kcal/mol). It is worth highlighting that the axial N–H disposition points the N–H bond toward the vacant apical site at Fe. This observation is reasonable in electron-rich systems where a strong CT from the unrotated unit to the rotated one occurs: indeed the rotated Fe receiving electron density from the unrotated one is preferentially stabilized by a long range interaction with a relatively acidic  $\text{H}^{\delta+}$  rather than with the nitrogen lone pair. This result is in line with recent theoretical predictions on  $\text{Fe}_2\text{S}_2$  biomimics, revealing a strong influence of the polarization of the dithiolato bridgehead in stabilizing the rotated Fe upon reduction i.e. upon increasing electron density in the system.<sup>[62]</sup> However, this intramolecular stabilizing interaction is presumably too weak to compensate for the rotation energy cost, and the predicted  $\Delta G_R$  is positive (4.5 kcal/mol). Another factor contributing to destabilizing the rotated form is the presence of a H-bond between the axial N–H group and MeNC nitrogen, which in fact stabilized the unrotated isomer.

In view of the above, the replacement of *pdt* with *adt*, does not appear to be a promising strategy to favor rotation, at least in the class of systems under this investigation. Instead, the switch from R=Me to R=Ph in RNC ligands has a beneficial impact on rotation ( $\Delta G_R = -2.4$  kcal/mol, Scheme 3B). The amount of CT upon rotation is essentially the same in 1 and 6 ( $\Delta q = 0.27e^-$  in the latter), suggesting that other factors come into play.

Indeed, in the rotated conformation, R groups are mutually closer than in the unrotated one, as a result of the shift of one RNC ligand from equatorial position to the Fe–Fe region. When R=Ph the rotated form is thus stabilized thanks



**Scheme 4.** Relevant rotation processes occurring at each Fe unit in systems 5 (A) and 6 (B).  $\Delta G_R$  (in blue, kcal/mol, BP86-D3/TZVP) is associated with the lowest energy rotation for the two complexes, occurring at the Fe with the higher tendency to rotate and involving most stable rotated and unrotated species.

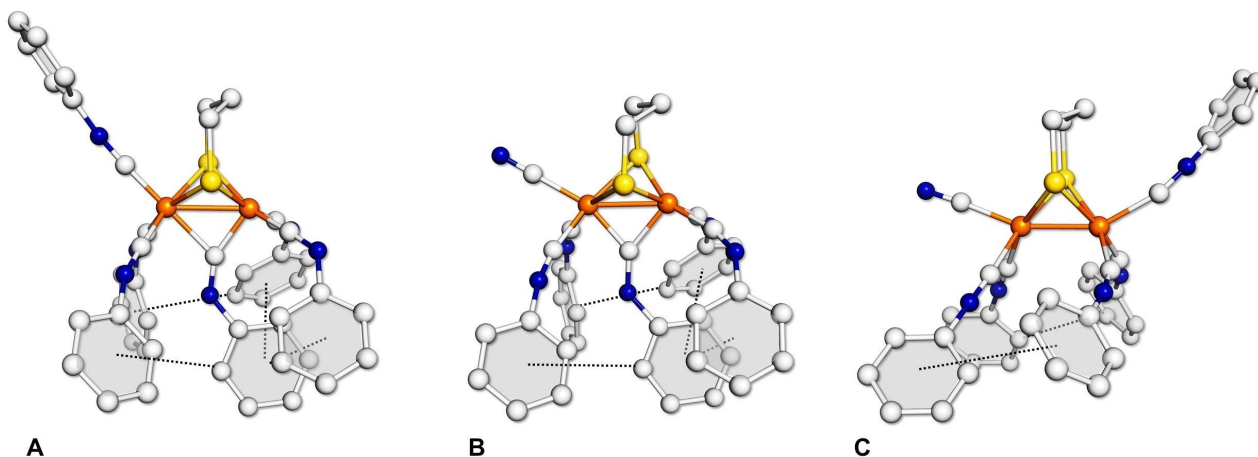
to the formation of relatively stronger dispersive interactions, compared to the R=Me case. The stacking interactions formed between phenyl rings upon rotation in **6** (displayed in Figure 3) fairly provide a more efficient allocation of intramolecular groups than the feeble and purely dispersive interactions among Me groups (in **1**). Irrespective of the R nature of the RNC ligand, it is worth noting (see Figure 3) that *the rotated isomer features a higher number of favorable interactions than the unrotated isomer*.

Consequently, maximizing the number and selecting the proper kind of stabilizing interactions among ligands proves to be a good alternative to trigger rotation in electron rich Fe<sub>2</sub>S<sub>2</sub> mimics, at least according to DFT. The term “alternative” refers to other synthetic or computational strategies proposed in the literature to date.<sup>[32,35,38–40]</sup>

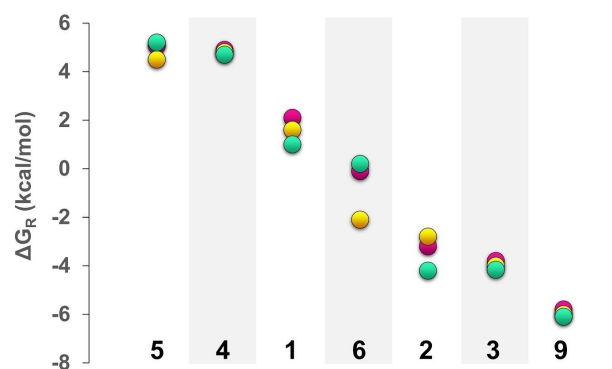
Complexes with aromatic R groups of RNC ligands may thus represent a promising synthetic scaffold to tailor in the design of diiron dithiolato systems that can more closely mimic the dimetallic subunit of the [FeFe]-hydrogenase active site.

The results described so far have prompted us to verify the effect of the combination of various factors on the rotation free energy. In particular, the most logical strategy consists in the combination of desymmetrization of the coordination environment of the Fe<sub>2</sub> core, with the RNC ligands replacement by a different strong donor, and setting R=Ph. A compound with the formula [Fe<sub>2</sub>(pdt)(PhNC)<sub>5</sub>(CN)]<sup>−</sup> (**9**) has therefore been designed: in this case, we obtained  $\Delta G_R = -6.0$  kcal/mol, which clearly indicates that the aforementioned factors have a synergistic effect. The optimized structure of the most stable rotated isomer of **9** is reported in Figure 3.

In order to corroborate our results and to exclude functional dependency, we re-calculated  $\Delta G_R$  values by using other functionals, namely TPSS–D3 and TPSSh–D3. We obtained exactly the same trend and very close  $\Delta G_R$  values independently from the functional used (Figure 4), which allows us to draw general conclusions, on the basis of the DFT perspective.



**Figure 3.** Optimized structures (BP86-D3/TZVP) of the most stable isomers of **6** (A) and **9** (B), that both feature a rotated conformation. The increase in the number of stacking interactions (highlighted with black dotted lines) among aromatic R substituents upon rotation, can be assessed by comparing the rotated isomer **B** to the unrotated form of **9** (C).



**Figure 4.**  $\Delta G_R$  (kcal/mol) calculated with BP86-D3 (yellow), TPSS–D3 (pink) and TPSSh–D3 (green) functionals for complexes 1–6 and 9, from highest (5 or 4 according to the functional used) to lowest (9).

## Conclusions

The present study provides a DFT recipe aimed to inspire the organometallic synthesis toward 2Fe(I) compounds that are more and more resembling a peculiar structural feature of the Fe<sub>2</sub> unit of the [FeFe]-hydrogenase active site: the so called inverted or rotated disposition of the two Fe(I) square pyramidal coordinations. The rotated stereoisomer is thought to be retained by the enzyme cofactor throughout the entire catalytic cycle and thus in both the more relevant oxidation states: Fe(I)Fe(I) and Fe(I)Fe(II). Although synthetic models of such rotated form have been isolated in the mixed valence Fe(I)Fe(II) state,<sup>[28,29,63]</sup> only recently a fully rotated geometry has been achieved in synthetic biomimicry.<sup>[32,35]</sup>

The electron rich scaffold of the (MeNC)<sub>6</sub> diiron dithiolato system (**1**)<sup>[37]</sup> has been herein considered a reasonable prototype to start from and improve in this sense, because of three main characteristics:

- (i) a rotated/inverted disposition that was only slightly less stable compared to the ground state isomer with eclipsed geometry.
- (ii) The isocyanide ligands provide the dimetallic core with electron richness, an essential feature of the proton reduction catalysis or HER in general.
- (iii) RNC ligands shape is streamlined and generally less bulky compared to phosphines of different denticity ( $-\text{PR}_3$  or  $-\text{P}(\text{R})\text{P}$ - chelates) or carbon-based ligands of various hapticity (e.g. *N*-heterocyclic carbenes such as  $\text{IMes} = 1,3\text{-Bis}(2,4,6\text{-trimethylphenyl})\text{-}1,3\text{-dihydro-}2\text{H-imidazol-}2\text{-ylidene}$  or cyclopentadienes).

About the last point, we should mention that synthetic strategies to stabilize *fully* rotated isomers of diiron dithiolates by steric congestion have been successfully adopted by Rauchfuss<sup>[35]</sup> and Schollhammer.<sup>[32]</sup> Also noteworthy in this context are some contributions by Weigand<sup>[33]</sup> et al. and Darensbourg<sup>[31,63]</sup> on semi-rotated forms. In contrast, MeNC and more in general RNC ligands considered herein, allow not to clutter the metal sites where substrates ( $\text{H}^+$  or  $\text{H}_2$ ) have to be hosted during HER.

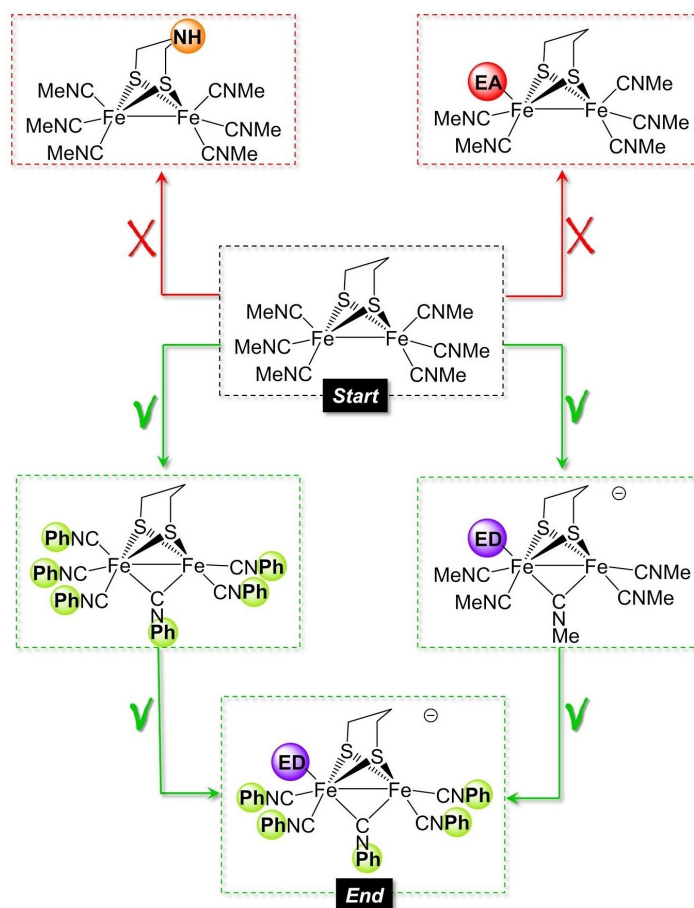
A significant correlation between  $\Delta q$  (amount of electron density transferred from one Fe unit to the other) and  $\Delta G_{\text{R}}$  (the relative stability between unrotated and rotated isomers) has

been found. The role of the charge transfer between the two metal units was elucidated by Tye and Hall.<sup>[40]</sup> If on one side our results confirm the known necessity of providing the dimetallic cluster with electron richness coming from isocyanides, along with its asymmetric distribution within the diiron system, on the other side they reveal that the desymmetrization should not entail the use of strong electron acceptors, like CO.

The most relevant point of the present study is, however, that the R nature of the RNC ligands can be in itself decisive to observe a rotated isomer as ground state. Indeed, by switching from the original symmetric  $(\text{MeNC})_6$  to the strictly analogue  $(\text{PhNC})_6$  derivative, the rotated isomer goes from being slightly less stable than the unrotated isomer in the former to becoming the ground state isomer, in the latter.

In summary (see Scheme 5 for a visual perspective), the emerging recipe for stabilizing rotated geometries in diiron biomimetics exploiting the versatility of RNC ligands can be summarized as:

- (A) use of R groups that provide the RNC ligands with phenyl rings or in general chemical functionalities able to establish stabilizing intramolecular interactions, because these are more numerous within rotated isomers, compared to unrotated ones.



**Scheme 5.** The guideline used in the present work to rationally design an isocyanide-based diiron system featuring a highly stable, ground state rotated conformation.



- (B) Desymmetrization of the dimetallic coordination environment may be beneficial.
- (C) If asymmetrically substituted derivatives are considered, then the use of a different electron donor in place of RNC ligands appears mandatory. This implies that the electron richness introduced by RNC ligands in the diiron dithiolato system should not be reduced.
- (D) The pdt replacement by the nitrogen-containing adt is not beneficial in the class of compound considered, probably because of electron richness of the diiron core. Since the central N of adt is crucial during the enzyme turnover, as it works as proton relay from solution to the vacant Fe site, one might conclude that the absence of this kind of dithiolato could be detrimental also for catalysis. However, it has been reported,<sup>[37,64,65]</sup> that RNC and CN<sup>-</sup> ligands can circumvent the problem, as RNC ligands themselves can afford the proton relay function.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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