Second coordination sphere effects in [FeFe]-Hydrogenase mimics

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Proton preorganization effect in $\text{M}_{12}\text{L}_{24}$ nano-spheres containing hydrogenase mimics
Introduction

Hydrogenases are fascinating metalloenzymes, because they can reversibly convert protons into molecular hydrogen with virtually no overpotential, yet their active site only contains abundant metals such as iron and nickel. This reversible interconversion is of great interest in view of the transition from our current fossil fuel-based society to one that is powered by renewable energy sources. In particular, fuel cells need catalysts for hydrogen oxidation, while water-splitting devices require proton reduction catalysts for the formation of dihydrogen in a sustainable fashion. For both reactions the best synthetic catalysts are based on scarce metals such as platinum and iridium, but the hydrogenase enzymes outperform even those synthetic catalysts. The natural systems, but in particular the iron-iron hydrogenases, have thus been subject of intense studies to discover the detailed operational mechanism and the key features that render these enzymes superb catalysts. Mechanistic studies on hydrogenase enzymes revealed an important function for the internal proton relay, i.e. the amine moiety in the azadithiolate bridge, and for the Fe₄S₄ cluster ligated to the proximal iron of the H-cluster, which functions as electron reservoir. In parallel, many groups around the world made synthetic analogues of the active site at which the actual proton reduction takes place. Many different compounds have been prepared, based on readily accessible di-iron di-sulfur hexacarbonyl complexes, and variations can be prepared by exchange of one or more carbonyls for different ligands. Installment of proton relay moieties has been successfully achieved and demonstrated to improve the catalytic function of the synthetic models. Less attention has been given to the redox-active Fe₄S₄ cluster; nevertheless recent work on synthetic models with appended electron reservoirs demonstrated that also such function changes the catalytic properties. Interestingly, despite all efforts, up to now there are no synthetic mimics that can perform the proton reduction reaction at low overpotential. This suggests that the protein environment, i.e. the second coordination sphere around the active site, may play a more important role than initially anticipated. Recent experiments, in which synthetic mimics of the active site are installed in the inactive apo-hydrogenase enzyme, show full competence enzymatic activity, hinting at the importance of the protein matrix or second coordination sphere around the H-cluster. In this work we investigated the influence of the second coordination sphere by mimicking the protein matrix using a synthetic nano-sphere. A self-assembly strategy was used to install mimics of the hydrogenase active site into a specific nano-environment by generating M₁₂L₂₄ nano-spheres based on mixtures of different ditopic bis(pyridyl) building blocks. The hydrogenase models are still electrocatalytically active for proton reduction, and importantly, we demonstrate for the first time that by changing the second coordination sphere around the synthetic catalyst, proton reduction catalysis takes place at lower overpotential.
Proton preorganization effect in $M_{12}L_{24}$ nano-spheres containing hydrogenase mimics

**Strategy**

In order to mimic the protein matrix around the catalyst and create a nano-environment able to effectively preorganize protons around di-iron hydrogenase models, modified $M_{12}L_{24}$ Fujita-type cages are employed. Such cages provide sufficiently large space within their cavity that can be easily decorated with various customized functional groups.

Two main different approaches of introducing hydrogenase models into the nano-cages have been explored, i) the catalyst is encapsulated into the pre-assembled cage through supramolecular interaction of complementary functional groups and ii) the catalyst is covalently connected to the cage building block prior to cage formation. Self-assembly of mixtures of catalyst functionalized building block and unfunctionalized building block affords cages with encapsulated catalyst.

Common to both strategies is a building block functionalized with a group carrying acidic protons, which are the substrate for the proton reduction reaction. Figure 1 schematically represents the strategies envisioned.

![Figure 1](image.png)

*Figure 1. Schematic representation of the two different strategies envisioned to introduce a second coordination sphere around synthetic models of the iron-iron hydrogenases.*

The synthon for the acid functionalized building block features a short aliphatic chain terminated with a tertiary amine moiety. This base is about five orders of magnitude stronger than the pyridyl moieties present on the building block, therefore its selective protonation should be achieved rather easily. As the tertiary ammonium moiety is a weaker acid than the pyridinium, the presence of the acidic proton should not interfere with the self-assembly process. A sulfonated di-iron catalyst is then encapsulated by electrostatic interaction of the two complementary functional groups; ammonium moieties and sulfonate group.
Regarding the covalently encapsulated di-iron model, a short aliphatic linker connects the building block to a monocarboxylic acid benzenedithiolate di-iron complex through an amide bond coupling.

In both described approaches, the hydrogenase model would be effectively encapsulated into a nano-confined space. Furthermore, protons are effectively preorganized around the catalyst within the nano-environment defined by the cage structure. Electrons, the other substrate the catalyst needs to evolve hydrogen, are provided by electrochemical means. Figure 2 provides a graphical representation of the two model systems.
Results and discussion

Synthesis and characterization of building blocks

The acid functionalized building block, BBNH+ was prepared in three steps according to Scheme 1. A Williamson reaction between 2,6-bibromophenol and 3-chloro-N,N-dimethylpropan-1-amine afforded 1 in quantitative yield. A palladium catalyzed Sonogashira reaction was carried out to introduce the ethynylpyridine moieties. The resulting building block 2 was treated with sub-stoichiometric amounts of pyridinium hexafluorophosphate in acetonitrile. As pyridinium has a pKa value about five times higher that ammonium, itselectively transfers the proton to the di-methyl amine fragment. Precipitation of BBNH+ by addition of diethyl ether afforded the clean desired building block in qualitative yield.

Crystals suitable for X-ray diffraction were grown by slow diffusion of diethyl ether layered over an acetonitrile solution of BBNH+. The solid state structure confirms the selective protonation of the tertiary amine, leaving the pyridines free for coordination with metal ions during cage self-assembly. The presence of the acidic proton bound to the amine group was established by the clear presence of residual electron density in the difference Fourier map around the amino nitrogen. Such residual electron density was not observed in the proximity of the pyridine nitrogen atoms.

Figure 3. X-ray crystal structure for BBNH+. Ellipsoids are set at 50% probability.
Preparation of the di-iron hydrogenase functionalized building block, \textbf{Fe2BB} was achieved in two steps according to Scheme 2. The di-iron model featuring a mono-carboxylic acid benzenedithiolate bridge, \textbf{4} was prepared according to the synthesis route described in Chapter 2. Complex \textbf{4} was reacted with excess HATU in the presence of base and the resulting HATU activated di-iron ester was isolated by chromatography. Although this time-consuming step is typically avoided as the HATU intermediate is reacted \textit{in situ} with a primary amine, for the current reaction it is essential. When this step is carried out, cleaner crude reaction mixtures and higher yields are obtained in the following step. The pure HATU-activated carboxylic acid \textbf{5} is thus reacted in DCM with the building block precursor \textbf{3}, which was treated with excess DIPEA prior to the reaction. Preparation of building block \textbf{3} has been described in Chapter 4. The reaction affords the desired di-iron containing building block in moderate yield after chromatography purification.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme2.png}
\caption{Synthetic route for the preparation of the di-iron functionalized building block, \textbf{Fe2BB}.}
\end{scheme}

A third building block, \textbf{BBH}, is also prepared featuring a non-acidic aromatic proton at the endohedral position of the ditopic bis(pyridyl) building block. The preparation of \textbf{BBH} is straightforward, involving a palladium catalyzed Sonogashira reaction between 1,3-dibromoobenzene and 4-ethynylpyridine to afford the desired product, Scheme 3.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme3.png}
\caption{Synthetic route for the preparation of the unfunctionalized building block \textbf{BBH}.}
\end{scheme}
The di-iron hydrogenase model compound featuring a sulfonate group was prepared by reaction of the dichlorobenzenedithiolate parent hexacarbonyl complex $^{10}$ and 3-(diphenylphosphino)benzenesulfonate tetrabutylammonium salt, $^{19}$ both prepared according to literature procedures. The reaction is performed in dichloromethane in the presence of the decarbonylating agent Me$_3$NO, dissolved in minimal amount of acetonitrile before the addition to the reaction mixture. Addition of hexanes to the crude mixture caused precipitation of complex 7, which is obtained after filtration and washing with hexanes in 78% yield, according to Scheme 4.

Scheme 4. Synthetic route for the preparation of the sulfonate containing di-iron complex 7.

The building blocks prepared and the di-iron complex 7 have been characterized by $^1$H, $^{13}$C and DOSY NMR spectroscopy. NMR data are in agreement with the expected signals and values of the diffusion coefficients. High resolution mass data to confirm the molecular weight and the elemental composition were also obtained, in all cases within 5 ppm error range.

Preparation and characterization of $M_{12}L_{24}$ cages

Supramolecular catalyst encapsulation

The cage containing 24 endohedral acidic moieties (ammonium cage) was prepared similarly as reported in literature for other $M_{12}L_{24}$ cages, 24 equivalents of BBNH$^+$ were mixed with 12 equivalents of a palladium source in MeCN-$d_3$, and the mixture was stirred overnight at 45 °C. $^{16}$H NMR of the resulting solution suggests quantitative formation of the expected $M_{12}L_{24}$ supramolecular assembly as indicated by the shift of the pyridyl protons by roughly 0.4 ppm to lower fields and by the symmetry of the spectrum. $^1$H DOSY NMR, shown in Figure 4, confirms the formation of a singular diffusing species with a logD value of -9.5 m$^2$s$^{-1}$, which corresponds to a structure that is considerably larger (around 5 nm diameter) than the free diffusing building block BBNH$^+$, which displays a logD value of -8.8 m$^2$s$^{-1}$.
Second coordination sphere effects in [FeFe]-Hydrogenase mimics

After cage self-assembly, the sulfonated di-iron catalyst 7 is conveniently encapsulated into the cavity of the cage by simply dissolving it in the same cage solution. The encapsulation is driven by supramolecular interactions between the sulfonate groups and the ammonium moieties present within the cavity of the cage, schematically represented in Figure 5.

![Figure 4.](image)

**Figure 4.** $^1$H DOSY NMR overlap for BBNH+, showing a single diffusing species with a logD of -8.8 m$^2$s$^{-1}$ and a palladium cage [Pd$_{12}$(BBNH+)$_{24}$]$^{48+}$, showing a single diffusing species with a logD of -9.5 m$^2$s$^{-1}$, in MeCN-$d_3$ at 25 °C.

Catalyst encapsulation can be monitored through $^1$H NMR titration experiments, shown in Figure 6. Encapsulation is suggested by the broadening of the cage peaks with increasing equivalents of catalyst in solution. Encapsulation is further indicated by the shifting of the signals of the aliphatic chain that connects the ammonium moiety to the rest to the building

![Figure 5.](image)

**Figure 5.** Schematic representation of ammonium cage self-assembly and catalyst 7 encapsulation by supramolecular interactions between the sulfonate and ammonium groups.
Proton preorganization effect in M\(_{12}\)L\(_{24}\) nano-spheres containing hydrogenase mimics

block. In addition, very small shifts are observed for the pyridyl moieties (indicated by black lines). In particular, the signal attributed to the acidic proton present on the quaternary nitrogen moiety shifts from about 7.6 to almost 9.5 ppm, which is in line with the expected interaction between the two complementary functional groups. Appearance of a new set of signals is observed (red lines), consistent with the signals expected for complex 7. These signals only shift to a small extend compared to the free compound. It is noted that after the addition of about 20 equivalents of di-iron complex 7, some precipitate appears rendering further analysis problematic.

![Figure 6.](image)

**Figure 6.** \(^1\)H NMR titration of complex 7 into a solution of ammonium cage [Pd\(_{12}\)(BBNH\(^+\))\(_{24}\)]\(^{48+}\). The black lines indicate shifting of signals belonging to the cage while the red lines indicate the appearance of the encapsulated complex 7.

A \(^1\)H DOSY experiment of an ammonium cage sample, [Pd\(_{12}\)(BBNH\(^+\))\(_{24}\)]\(^{48+}\), in the presence of ten equivalents of complex 7 shows a single diffusing species with logD value of about -9.4 m\(^2\)s\(^{-1}\), as shown in Figure 7. The signals belonging to the catalyst, in particular the one at 6.2 ppm, have the same diffusion coefficient as those that belong to the cage, confirming encapsulation of the hydrogenase mimic. As this DOSY signal is not significantly broadened over a large rage of D values, the encapsulation equilibrium is likely to be shifted towards the encapsulated species. Exchange, if present, is slow on the NMR timescale. A second set of signals is present in the \(^1\)H DOSY spectrum with a lower diffusion coefficient, logD value about -8.8 m\(^2\)s\(^{-1}\). This species is consistent with tetrabutylammonium ions liberated after complex 7 is encapsulated.
Second coordination sphere effects in [FeFe]-Hydrogenase mimics

A first striking advantage of catalyst encapsulation is showcased by the gained stability of the di-iron hydrogenase model. While the catalyst decomposes rather fast (within one hour) when dissolved in aerobic acetonitrile solutions, its stability is drastically increased when the ammonium cage is present in solution (several days without any apparent decomposition under daylight and atmospheric conditions), further confirming the successful encapsulation. Preventing oxygen intolerance is a major advantage for proton reduction catalysts, especially for practical applications.²⁰

Figure 7. $^1$H DOSY NMR in MeCN-$d_3$ at 25 °C for an ammonium cage $[\text{Pd}_{12}(\text{BBNH}^+)]^{40+}$ containing ten equivalents of complex 7, showing a single diffusing species with logD of -9.4 m²s⁻¹. This diffusing species contains the signals for both the ammonium cage and complex 7 (signal at 6.2 ppm while the other signal for this complex overlap to the cage signals). A second diffusing species is present with a logD of -8.8 m²s⁻¹ consistent with tetrabutylammonium ions liberated upon complex 7 encapsulation.
Proton preorganization effect in \( M_{12}L_{24} \) nano-spheres containing hydrogenase mimics

**Covalent catalyst encapsulation**

Preparation of the covalently encapsulated di-iron model complex, surrounded by acidic moieties, is achieved by mixing \( \text{BBNH}^+ \) with \( \text{Fe2BB} \) in the desired ratio, in the presence of a palladium source, as shown schematically in Figure 8. After stirring the mixture in MeCN-\( d_3 \) overnight at 45 °C a clear solution of the mixed cage is obtained.

![Schematic representation of the formation of \([\text{Pd}_{12}(\text{Fe2BB})_n(\text{BBNH}^+)]_{24-n}\)^{(48-n)+} cages containing both di-iron functionalized building blocks and ammonium functionalized building blocks. The di-iron di-sulfur cores of the hydrogenase mimics are represented in orange and yellow CPK-style.](image)

Figure 8. Schematic representation of the formation of \([\text{Pd}_{12}(\text{Fe2BB})_n(\text{BBNH}^+)]_{24-n}\)^{(48-n)+} cages containing both di-iron functionalized building blocks and ammonium functionalized building blocks. The di-iron di-sulfur cores of the hydrogenase mimics are represented in orange and yellow CPK-style.

The formation of the large supramolecular assembly is again supported by the pyridyl shifts in \(^1H\) NMR and by \(^1H\) DOSY NMR, shown in Figure 9. DOSY NMR displays a single diffusing species which presents the signals belonging to both building blocks used. The logD value of about -9.3 m\(^2\)s\(^{-1}\) indicates the formation the desired species. Figure 9 shows a DOSY overlay for mixed cage sample together with \( \text{Fe2BB} \) and \( \text{BBNH}^+ \) DOSY experiments measured under identical conditions.

*It is noted the when two different types of building blocks (generically \( L \)) are mixed together for the self-assembly process, a statistical distribution of different cage assemblies is obtained; the major cage species being the one resembling the ratio of building block chosen. In this chapter, a maximum of five equivalents of \( \text{Fe2BB} \) are used for the cage self-assembly. This ratio affords quantitatively cage samples. From here, the notation \([\text{Pd}_{12}([\text{L1}]_n[\text{L2}])_{24-n}]^{m+}\) will be used referring to a solution that contains a mixture of different cage assemblies in which the major species in solution is the one that resembles the ratio of the building blocks chosen.*
Second coordination sphere effects in [FeFe]-Hydrogenase mimics

A different cage sample containing the iron-iron model complex was also prepared by mixing Fe2BB and BBH building blocks to obtain the assembly [Pd12(Fe2BB)5(BBH)19]24+. Also in this case both 1H NMR and DOSY NMR, shown in Figure 10, indicate the formation of the desired large assembly.

Figure 9. Overlay of 1H DOSY NMR in MeCN-d₃ at 25 °C for a mixed cage of the type [Pd₁₂(Fe₂BB)₅(BBH⁺)]¹⁹⁺ with logD of -9.3 m²s⁻¹ (top). This diffusing species contains the signals for both the ammonium functionalized building block and for the di-iron functionalized building block. The Fe₂BB shows a logD value of -8.9 m²s⁻¹ (middle) and BBNH⁺ shows a logD value of -8.8 m²s⁻¹.

Figure 10. 1H DOSY NMR in MeCN-d₃ at 25 °C for a mixed cage of the type [Pd₁₂(Fe₂BB)₅(BBH)]²⁴⁺ with logD of -9.3 m²s⁻¹.
Electrochemistry

Ammonium cage

The di-iron complex 7 was subjected to electrochemical investigations in acetonitrile solution in the presence of tetrabutylammonium hexafluorophosphate as electrolyte, to elucidate its redox behavior and catalytic properties, the typical voltammograms are plotted in Figure 11. Complex 7 displays a non-reversible one-electron reduction event at -1.1 V as indicated by semi-integrative convolution analysis in the presence of stoichiometric amounts of ferrocene. Following this first wave, a second event is detected at -1.4 V, consistent with the reduction of the bis-phosphine substituted di-iron complex originating from a disproportionation reaction of the mono-reduced complex. Similar behavior has been observed and described in Chapter 2 of this thesis for related mono-phosphine substituted benzenedithiolate di-iron complexes. Semiderivative convolution analysis of the voltammogram indicates the extent of disproportionation is minor, in the order of about 20%, Figure 11. Spectroelectrochemistry coupled with IR confirms the disproportionation event upon mono-reduction of 7, Figure 12.

Figure 11. Cyclic voltammogram for complex 7 (black line) and its semiderivative convolution plot (red line).
Sequential addition of an external acid (HNEt$_3$PF$_6$) causes the appearance of a new catalytic peak around -1.65 V that is associated with hydrogen evolution, Figure 13. This set of experiments briefly shows that complex 7, although displaying minor side reactivity upon mono-reduction, can perform proton reduction in the presence of external weak acids. The redox and catalytic behavior are in agreement with similar complexes described in Chapter 2 of this thesis, following an ECEC catalytic mechanism.

Figure 12. Spectroelectrochemistry coupled with IR for complex 7 showing disproportionation. Red circles signals are assigned to the bis-phosphine neutral di-iron complex while the blue squares are assigned to the doubly reduced species [Fe$_2$(Cl$_2$bdt)(CO)$_4$]$_2^{-}$.

Figure 13. Voltammetric response for complex 7 in the absence (black line) and presence of increasing amounts of the weak acid with acid HNEt$_3$PF$_6$. 
Proton preorganization effect in \( \mathcal{M}_{12} \mathcal{L}_{24} \) nano-spheres containing hydrogenase mimics

Next, a solution of ammonium cage, \([\text{Pd}_{12}(\text{BBNH}^+))_{24}]^{48+}\) is subjected to electrochemical measurement in the presence of one equivalent of di-iron complex 7. The voltammogram features two sharp peaks at -1.6 and -1.7 V followed a third broad reduction around -2.0 V, Figure 14 (left). The first sharp peak at -1.6 V is not present when the ammonium cage alone is subjected to reductive potentials. The shape of the waves would immediately suggest multi-electron processes typically associated with electro-deposition of material at the surface of the electrode. Nevertheless, consecutive voltammograms perfectly overlap to one another suggesting that the surface of the electrode remains rather clean. Regardless of this observation, decomposition of this cage system at the surface of the electrode cannot be excluded.

Interestingly, a comparison of the voltammograms obtained for the free diffusing catalyst 7 in the presence of external acid and the voltammogram obtained for the encapsulated species at the cavity of the ammonium cage, shows that the first sharp peak at -1.6 V has a similar redox potential to that of the proton reduction catalytic wave displayed by complex 7, Figure 14 (right). This peak, indicating a multi-electron event, is in principle compatible with a catalytic process where diffusion of substrates to the catalyst does not play any significant role, with substrates being quickly and completely consumed. As for the system under study, the di-iron catalyst is surrounded by 24 equivalents of substrates (acidic ammonium ions) that can be promptly converted into hydrogen by the di-iron catalyst, thereby quickly consuming 24 electrons. Therefore the sharp peak is consistent with actual fast catalysis operated by the encapsulated catalyst.

![Figure 14](image-url)

**Figure 14.** Left, voltammetric comparison for a solution of ammonium cage \([\text{Pd}_{12}(\text{BBNH}^+))_{24}]^{48+}\) and for the same sample where one equivalent of catalyst 7 is added in solution. Right: voltammetric comparison between the ammonium cage with one equivalent of complex 7 and the catalytic response observed for complex 7 in the presence of external acid.
In a different experiment, a solution of the ammonium cage, in the presence of one equivalent of catalyst 7 but with no additional external acid added, was subjected to electrolysis at constant potential (-1.75 V vs. Ag/AgCl) in a two-compartment cell equipped with a carbon sponge working electrode and a platinum coil counter electrode separated from the main solution by a glass frit. The electrochemical response was monitored in time, showing a typical logarithmic decay indicating no apparent deposition of material at the electrode. Hydrogen evolution was monitored by in-line GC analysis. After about 20 minutes, white precipitate started to appear. At this point, integration of the gas chromatograms revealed that about 40% of the protons originally present within the cavity of the cage had been converted into hydrogen. Furthermore integration of the amperometric plot indicates the consumption of about 6.25 C (only 5.55 C were necessary to evolve the hydrogen detected) which accounts for a faradaic efficiency of around 88%, indicating that most of the electrons are used for hydrogen evolution, Figure 15.

![Amperometric plot](image)

**Figure 15.** Amperometric plot for the ammonium cage $[\text{Pd}_{12}(\text{BBNH}^+)_{24}]^{48+}$ in the presence of one equivalent of catalyst 7 (black line) and its integral. No additional external acid was added. At time 1500 s, about 40% of the acidic protons present at the cavity of the cage were converted into hydrogen with a faradaic efficiency of about 88%.

The set of experiments described here suggests that the di-iron catalyst is still active for proton reduction when encapsulated by the supramolecular assembly, as suggested by the combination of voltammetry and electrolysis. The precipitate observed during the electrolysis experiment was not analyzed. Addition of external acid to the solution of ammonium cage containing catalyst 7 afforded unclear voltammograms that were difficult to interpret.

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Second coordination sphere effects in [FeFe]-Hydrogenase mimics

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Covalent hydrogenase encapsulation

First, the electrochemical behavior of the di-iron functionalized building block Fe2BB was investigated to gain insights into its redox properties. In acetonitrile solution, Fe2BB displays a single reversible reduction event at -0.91 V, as shown in Figure 16 (left), which is consistent with a two-electron process as suggested by semi-integral convolution plot in the presence of stoichiometric amounts of ferrocene and by the iso-point method.\(^{21}\) A plot of the peak current against the square root of the scan rate shows a linear trend, indicating that the complex does not deposit onto the electrode during the voltammetric scan, Figure 16 (right). Nevertheless, the oxidative scan displays a slight deviation from linearity that could be associated with a small deviation of the electron transfer coefficient \(\alpha\). Importantly, the experiment suggests no apparent decomposition nor irreversible modification of the di-iron core upon the reductive event. Furthermore, it shows that the ditopic bis(pyridine) building block does not feature any redox process in the electrochemical window measured which coincides to the window where proton reduction would be expected. Spectroelectrochemical measurements coupled with IR, shown in Figure 17, confirm formation of the di-reduced di-iron complex, suggesting that the pyridyl moieties do not interfere with the reduced metal ions.

**Figure 16.** Left: voltammetric response for a solution of Fe2BB at different scan speeds. Right: plot of the peak current against the square root of the scan speed indicating no significant decomposition at the electrode surface.
Sequential addition of the weak acid HNEt$_{3}$PF$_{6}$, which is not sufficiently strong to protonate the pyridyl groups or the non-reduced iron-iron bond, causes the appearance of a new catalytic peak at potentials around -1.6 V vs. Ag/AgCl, Figure 18. The back-oxidation trace displays decreased reversibility for the peak around -0.9 V, indicating fast protonation of the di-reduced Fe$_{2}$BB, even when low equivalents of acid are present. The behavior displayed is consistent with that observed for benzenedithiolate di-iron hexacarbonyl species. The experiment suggests that the di-iron functionalized building block is stable under reductive conditions in the presence of weak acid within the electrochemical window of interest. The di-iron fragment, although it is covalently connected to the building block scaffold, is still able to perform the proton reduction reaction.

**Figure 17.** IR coupled spectroelectrochemical measurement for Fe$_{2}$BB showing the bleaching of carbonyl peaks of the neutral complex and the appearance of the doubly reduced species.

**Figure 18.** Voltammetric response for a solution of Fe$_{2}$BB in the absence (black line) and presence of external weak acid.
Next, \textbf{Fe2BB} is introduced in the large supramolecular cage assemblies. The first cage to be analyzed is of the type [Pd\textsubscript{12}(Fe\textsubscript{2}BB)\textsubscript{5}(BBH)\textsubscript{19}]\textsuperscript{24+}, where BBH is the standard ditopic bis(pyridine) cage building block featuring a non-acidic aromatic proton at the \textit{endo} position. The voltammogram for this cage sample shows a reduction event around -0.9 V consistent with the reduction of the di-iron moiety, as shown in Figure 19 (left). Contrary to the free diffusing Fe\textsubscript{2}BB this peak is not reversible, suggesting a follow-up decomposition of the encapsulated di-iron core. Nevertheless, spectroelectrochemical investigation coupled to IR, where the reductive sweep was stopped around -1.0 V vs. Ag/AgCl - a potential just sufficient to reduce the di-iron core - revealed bleaching of the neutral species together with the appearance of the doubly reduced di-iron core, Figure 19 (right). This suggests that the non-reversibility observed during the voltammetric scan is induced by a later redox event, likely involving decomposition of the cage. Indeed, if the potential range -1.3 to -1.8 V is entered, deposition of material at the surface of the electrode is observed and polishing of the latter is essential to restore the voltammetric response.

\textbf{Figure 19.} Left: voltammetric comparison for a solution of cage [Pd\textsubscript{12}(Fe\textsubscript{2}BB)\textsubscript{5}(BBH)\textsubscript{19}]\textsuperscript{24+} (black line) and for the free diffusing Fe\textsubscript{2}BB (red line). Right: IR coupled spectroelectrochemical measurement for the cage sample [Pd\textsubscript{12}(Fe\textsubscript{2}BB)\textsubscript{5}(BBH)\textsubscript{19}]\textsuperscript{24+} showing the appearance of the doubly reduced di-iron species.

Addition of external acid to this cage solution causes the appearance of a new peak at -1.35 V. This peak slightly increases in intensity when additional equivalents of acid are added, suggesting a proton reduction event. The increase in the intensity of this peak would suggest that the external acid can enter the cage void but this process appears to be rather slow as indicated by the very modest peak current increase and by the diffusion limited shape of the catalytic wave, Figure 20.
Second coordination sphere effects in [FeFe]-Hydrogenase mimics

Interestingly, comparison of this catalytic peak potential to the catalytic peak potential obtained for the free diffusing Fe2BB reveals a potential shift of about 200 mV towards anodic values. The local environment around the catalyst apparently lowers the overpotential for the catalytic proton reduction reaction. Nevertheless the encapsulated di-iron complex is only capable of limited turnover frequency (estimated $k_{\text{cat}}$ roughly 1 s$^{-1}$). This is attributed to the slow diffusion of protons to the cavity of the cage. The unfavorable encapsulation of external acid could be due to electrostatic repulsive interactions as the cage is highly positively charged.

The second di-iron containing cage analyzed features BBNH+ building blocks with acidic quaternary ammonium moieties that should effectively preorganize proton substrates around the di-iron catalysts, circumventing the slow diffusion of external acid to the encapsulated catalytic centers. The [Pd$_{12}$(Fe$_2$BB)$_5$(BBH)$_{19}$]$^{24+}$ cage can be prepared in a 0.2 mM concentration but it is noted that when electrolyte (TBAPF$_6$) is added to this solution, copious amount of precipitate appears. Therefore, to avoid such undesired event, the cage solution is diluted about ten times, thereby yielding a concentration of the encapsulated di-iron moiety of about 0.1 mM. When this cage solution is subjected to electrochemical analysis, the voltammogram displays a broad reduction event with a first peak at -1.28 V and a second peak at -1.56 V vs. Ag/AgCl, as shown in Figure 21 (left). The expected peak current for the reduction of the di-iron complex, predicted by Randles–Sevcik equation, is only roughly -1.6 µA, given the di-iron concentration and cage diffusion coefficient obtained from $^1$H DOSY NMR. Such small current cannot be clearly distinguished in the voltammogram obtained. As the di-iron complex is present in such low concentration, the broad reduction event observed around -1.28 V with a peak current of -25 µA would suggest a catalytic reduction event. Comparison

Figure 20. Voltammetric response for the cage sample [Pd$_{12}$(Fe$_2$BB)$_5$(BBH)$_{19}$]$^{24+}$ in the absence (black line) and presence of external acid, HNEt$_3$PF$_6$. 
of this peak to the catalytic wave obtained for the $[\text{Pd}_{12}(\text{Fe}2\text{BB})_5(\text{BBH})_{19}]^{24+}$ cage indicates that the two reduction events are rather similar in terms of potential. Furthermore, addition of increasing equivalents of external acid to this same cage solution reveals a current increase of the peak at -1.28 V, suggesting that the process involves proton reduction, Figure 21 (right).

When about 30 equivalents of external acid are present in solution the voltammogram displays and S-shaped catalytic wave, indicating pure kinetic conditions with negligible substrate consumption. From such voltammogram, the apparent catalytic rate constant can be obtained utilizing the DuBois method shown in Equation 1. The catalytic rate constant observed for the encapsulated di-iron catalyst is found to be in the order of 170 s$^{-1}$, about ten times slower than the free diffusing Fe$2\text{BB}$ species, for which the rate constant was extrapolated from foot-of-the-wave analysis (FofW) in the presence of an equal acid concentration. Most importantly, the rate constant for this system, which features preorganization of proton substrates around the catalyst within the nano-confined space defined by the cage, is about 200 times higher than the cage system $[\text{Pd}_{12}(\text{Fe}2\text{BB})_5(\text{BBNH}^+)_{19}]^{24+}$ where the protons have to diffuse to the catalyst. This underlines the importance of substrate preorganization and proton relays in general, for proton reduction catalysis.

$$\frac{i_{pl}}{i_p} = \frac{2}{0.446} \left(\frac{RTk_c}{FV}\right)^{1/2}$$

Equation 1. DuBois method to determine $k_{cat}$ under pure kinetic conditions
Comparing the voltammograms obtained for the free Fe2BB in the presence of acid with those obtained for the cage sample [Pd_{12}(Fe2BB)_{5}(BBNH+)_{19}]^{43+}, either in the absence or presence of external acid, reveals that the catalytic half wave potential ($E_{1/2\text{cat}}$) is shifted from about -1.43 V to roughly -1.22 V, Figure 22 (left). Encapsulation of the di-iron catalyst within the larger cage assembly leads to a reduction of the overpotential required for proton reduction catalysis by about 200 mV. The catalyst in the supramolecular assembly has an overpotential for the proton reduction reaction of about 400 mV, clearly visualized in the Tafel plots, shown in Figure 22 right.

![Figure 22. Left: comparison for the cage [Pd_{12}(Fe2BB)_{5}(BBNH+)_{19}]^{43+} in the absence (black line) and presence of increasing amounts of external acid, HNEt_{3}PF_{6}, and free diffusing Fe2BB in the presence of ten equivalents of external acid (red line). Right: comparative Tafel plot for the free diffusing Fe2BB and for the cage sample [Pd_{12}(Fe2BB)_{5}(BBNH+)_{19}]^{43+}.](image)

Interestingly, the encapsulation of the di-iron catalyst leads to a reduction of the overpotential, for both cages investigated, [Pd_{12}(Fe2BB)_{5}(BBNH+)_{19}]^{43+} and [Pd_{12}(Fe2BB)_{5}(BBH)_{19}]^{24+}. This suggests that the effect is unrelated to the presence of the acidic moieties within the cage assembly or to the total charge of the cage assembly. Nevertheless, preorganization of proton substrates within the cavity of the supramolecular assembly proved to have a beneficial effect as it allows for higher turnover frequencies, underlining the importance of proton relays around the di-iron moiety.
Conclusions

In this work we have shown two general methods to encapsulate synthetic di-iron hydrogenase models into supramolecular self-assembled $M_{12}L_{24}$ cages, thereby controlling the second coordination sphere around such proton reduction catalysts. Encapsulation of the proton reduction catalysts into such confined environment can lead to interesting effects such as higher catalytic rates due to proton preorganization. Most importantly, we have shown for the first time that changing the local environment around the catalysts has a significant impact on the catalytic overpotential, suggesting that the second coordination sphere around hydrogenase mimics plays an important role in their catalytic function. The strategy presented in this work allows for closely mimicking the essential amino acid residues found around the structure of the natural H-cluster. Introduction of synthetic mimics of such residues into the nano-environment provided by the $M_{12}L_{24}$ cages could be a key factor to further lower the operational overpotential of the synthetic models, finally approaching enzymatic rates and efficiencies.

Acknowledgments

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Experimental

Materials and methods

General procedures: all synthetic procedures were carried out under an argon atmosphere using standard Schlenk techniques. All commercially available chemicals were used as received without further purification. Solvents used for synthesis were dried via the most suitable method, distilled and degassed. Column chromatography was performed open to air using solvents as received.

Electrochemistry: Cyclic voltammetry was performed on 1 mM solution of analyte (unless otherwise stated) containing 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. The voltammograms were recorded using a PG-STAT302 N potentiostat at glassy carbon disk electrode (2 mm diameter). A platinum coil was used as auxiliary electrode and a leak free silver electrode (inner compartment 3 M KCl/Ag).

Electrolysis experiment were carried in a two compartment cell using Duocel® reticulated vitreous carbon foam as working electrode (used as received from ERG Aerospace Corporation) and a leak free silver electrode (inner compartment 3 M KCl/Ag). Platinum auxiliary electrode was separated from the main solution by a P4 glass frit. Both compartment contained 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte.

Spectroelectrochemistry was performed in an optically transparent thin layer Ottle cell with platinum working electrode, platinum auxiliary electrode and silver wire as reference electrode, containing 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte.

Hydrogen detection: The bulk electrolysis setup was connected to an in-line GC (Inter-science CompactGC equipped with molecular sieve column and TCD detector, argon gas was used as carrier). The head-space of the cell was sampled every 2 minutes. The integrals for hydrogen peaks were compared to a calibration curve obtained using the same setup and buffer volume.

Mass analysis: mass spectra for all compounds were collected on an AccuTOF GC v 4g, JMS-T100G-CV mass spectrometer (JEOL, Japan).

X-ray crystal structure determination: X-ray intensities were measured on a Bruker D8 Quest Eco diffractometer equipped with a Triumph monochromator (\(\lambda = 0.71073 \text{ Å}\)) and a CMOS Photon 50 detector at a temperature of 150(2) K. Intensity data were integrated with the Bruker APEX2 software. Absorption correction and scaling was performed with
Proton preorganization effect in $M_{12}L_{24}$ nano-spheres containing hydrogenase mimics

SADABS.\textsuperscript{25} The structures were solved using intrinsic phasing with the program SHELXT.\textsuperscript{24} Least-squares refinement was performed with SHELXL-2013\textsuperscript{26} against $F^2$ of all reflections. Non-hydrogen atoms were refined with anisotropic displacement parameters. The H atoms were placed at calculated positions using the instructions AFIX 13, AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 times $U_{eq}$ of the attached C atoms.

Synthesis of compounds

Synthesis of compound 1: A round bottom Schlenk flask was charged with 4.03 g of 2,6-dibromophenol (1 equiv, 16 mmol) and 11 g of $K_2CO_3$ (5 equiv, 80 mmol). To this mixture was added 100 mL of dry and degassed DMF, followed by slow addition of 2.53 g of 3-chloro-$N,N$-dimethylpropan-1-amine hydrochloride (1 equiv, 16 mmol). The mixture was stirred overnight at 90 °C before being cooled to room temperature and the volatiles removed under reduced pressure. To the residue, 100 mL of water were added and the suspension extracted with dichloromethane (4x50 mL) then dried over MgSO$_4$ and the volatiles removed under vacuum. The crude mixture, whether needed can be purified by silica column chromatography with ethylacetate:triethylamine 99:1 to afford 5.38 g of 1 in quantitative yield. $^1$H NMR (CD$_2$Cl$_2$, ppm) $\delta$ 7.55 (d, $J = 8.0$ Hz, 2H), 6.91 (t, $J = 8.0$ Hz, 1H), 4.08 (t, $J = 6.6$ Hz, 2H), 2.53 (t, $J = 7.2$ Hz, 2H), 2.26 (s, 6H), 2.05 (p, $J = 6.8$ Hz, 2H).

Synthesis of compound 2: A round bottom Schlenk flask was charged with 1, 1.85 g (1 equiv, 5.50 mmol), 1.9 g of 4-ethynlypyridine hydrochloride (2.6 equiv, 14.3 mmol) and 42 mg of Cul (0.22 mmol). The flask was flushed with argon before a degassed mixture of 40 mL of dioxane and 10 mL of triethylamine was added. A separate Schlenk flask was charged with 126.6 mg of Pd(PhCN)$_2$Cl$_2$ (0.33 mmol) and 191.5 mg of P(tBu)$_3$HBF$_4$ (0.66 mmol). To this
mixture, 5 mL of triethylamine and 10 mL of dioxane were added. This mixture was stirred for five minutes before it was transferred to the first flask by syringe. The mixture was stirred at 45 °C overnight then cooled to room temperature, quenched with water and extracted with ethyl acetate (4x50 mL). The organics were washed with water (2x50 mL) and brine (2x50 mL) then dried over MgSO$_4$ and the volatiles removed under vacuum. The residue was purified by silica column chromatography with ethyl acetate:trimethylamine 75:25. After the first impurities came out of the column 10% methanol was added to the eluent to elute the desired compound obtained as white solid, 2.1 g, 86% yield. $^1$H NMR (CD$_3$CN, ppm) δ 8.77 – 8.54 (m, 4H), 7.64 (d, $J = 7.7$ Hz, 2H), 7.61 – 7.45 (m, 4H), 7.22 (t, $J = 7.7$ Hz, 1H), 4.43 (t, $J = 6.3$ Hz, 2H), 2.53 (t, $J = 7.2$ Hz, 2H), 2.14 (s, 6H), 2.03 (p, $J = 6.6$ Hz, 2H).

Synthesis of compound BBNH+: A round bottom flask was charged with 2, 1.0 g (1 equiv, 2.6 mmol) and 531 mg of pyridinium hexafluorophosphate (0.9 equiv, 2.34 mmol) The solids were dissolved in 20 mL of acetonitrile and stirred for 10 minutes before diethyl 50 mL of diethyl ether were added causing the precipitation of with solids. The solids were collected by filtration washed with hexanes and dried under vacuum to afford BBNH+ in quantitative yield, 1.38 g. $^1$H NMR (CD$_3$CN, ppm) δ 8.80 – 8.60 (m, 4H), 7.69 (d, $J = 7.7$ Hz, 2H), 7.61 – 7.43 (m, 4H), 7.29 (t, $J = 7.7$ Hz, 1H), 7.00 (br s, 1H), 4.44 (t, $J = 5.7$ Hz, 2H), 3.41 (t, $J = 7.6$ Hz, 2H), 2.81 (s, 6H), 2.27 (q, $J = 7.1, 5.8$ Hz, 2H). $^{13}$C NMR (CD$_3$CN, ppm) δ 163.6, 153.4, 153.4, 138.4, 133.8, 128.7, 128.7, 128.2, 120.0, 94.5, 92.1, 75.0, 59.7, 46.6, 28.5. HR ESI(pos.)-MS (m/z) found: 382.1925 expected: 382.1919, C$_{25}$H$_{24}$N$_3$O.
Preparation of the building block synthon 3 is described in Chapter 4 while preparation of di-iron precursor 4 is described in Chapter 2.

**Synthesis of compound 5:** A round bottom Schlenk flask was charged with 250 mg of 4 (1 equiv, 0.59 mmol) and 249 mg of HATU (1.1 equiv, 0.65 mmol). The flask was flushed with argon before a degassed mixture of 20 mL of dry THF, 10 mL of dry acetonitrile and 0.5 mL of DIPEA was added. This mixture was stirred at room temperature for 2 hours before the volatiles were removed under vacuum. The residue was dissolved in dichloromethane and chromatographed over a DCM silica plug to afford the HATU-activated di-iron complex 5, 295 mg, 85% yield, which was used immediately afterward.

**Synthesis of Fe2BB:** A Schlenk flask was charged with compound 5 295 mg (1 equiv, 0.51 mmol) and dissolved into 10 mL of DCM. A separate Schlenk flask was charged with building block 3 274 mg (0.85 equiv, 0.43 mmol) and dissolved in a degassed mixture of 10 mL of DCM and 2 mL of DIPEA. The content of the first flask is added dropwise to the second flask and the mixture stirred at room temperature for an additional hour. The volatiles were removed under vacuum and the residue chromatographed over a silica column eluted with ethyl acetate:methanol 94:6 to afford Fe2BB as mustard colored powder, 219 mg, 65% yield. 

\[ ^{1}H \text{ NMR (CD}_{3}\text{CN, ppm)} \delta 8.65 - 8.55 \text{ (m, 4H), 7.66 (d, } J = 7.8 \text{ Hz, 2H), 7.57 - 7.40 \text{ (m, 4H), 7.25 (d, } J = 7.7 \text{ Hz, 1H), 7.24 (t, } J = 7.8 \text{ Hz, 1H) 7.05 (br t, } J = 5.0 \text{ Hz, 1H), 6.75 (d, } J = 7.7 \text{ Hz, 1H), 6.60 (t, } J = 7.6 \text{ Hz, 1H), 4.56 (t, } J = 5.7 \text{ Hz, 2H), 3.82 (m, 2H) \].

\[ ^{13}C \text{ NMR (CD}_{2}\text{Cl, ppm)} \delta 207.3, 149.9, 134.9, 130.4, 129.4, 126.7, 126.0, 125.1, 124.1, 116.4, 89.0, 73.2, 40.3. \]

\[ \text{FT-IR (MeCN, } \nu \text{) } 2080, 2044, 2005 \text{ cm}^{-1}. \]

\[ \text{HR ESI(pos.)-MS (m/z) found: 785.9419 expected } (m + H^+): 785.9392, (m + H^+) C_{35}H_{20}Fe_{2}N_{3}O_{8}S_{2} \].
Second coordination sphere effects in [FeFe]-Hydrogenase mimics

**Synthesis of BBH:** A round bottom Schlenk flask was charged with 1.62 g of 1,3-dibromo-benzene (1 equiv, 6.9 mmol), 2.5 g of 4-ethynylpyridine hydrochloride (2.6 equiv, 17.9 mmol) and 52 mg of Cul (0.27 mmol). The flask was flushed with argon before a degassed mixture of 40 mL of toluene and 10 mL of triethylamine was added. A separate Schlenk flask was charged with 158 mg of \( \text{Pd(PhCN)}_2\text{Cl}_2 \) (0.41 mmol) and 240 mg of \( \text{P(tBu)}_3\text{HBF}_4 \) (0.82 mmol). To this mixture, 5 mL of triethylamine and 10 mL of toluene were added. This mixture was stirred for five minutes before it was transferred to the first flask by syringe. The mixture was stirred at 75 °C overnight then cooled to room temperature and filtered through a Celite plug. The organics were removed under vacuum and the residue dissolved in diethyl ether. The insoluble material was removed by filtration before diethyl ether was removed under vacuum. The residue is filtered through a plug of silica with a mixture of chloroform:methanol 96:4 to afford a mixture of mono-substituted derivative and BBH. The solid material was suspended in 20 mL of ethyl acetate which solubilizes the mono-substituted compound along with some BBH. Clean BBH was obtained by filtration, 1.05 g, 54% yield. Nevertheless the mother liquors still contain high amounts of BBH for which second crystallization could be attempted.

\[ ^1H\text{ NMR (CD}_2\text{Cl}_2, \text{ppm}) \delta 8.72 – 8.58 (m, 4H), 7.81 (s, 1H), 7.63 (dd, J = 7.8, 1.6 Hz, 2H), 7.48 (t, J = 7.8 Hz, 1H), 7.48 – 7.37 (m, 4H). \]

\[ ^13C\text{ NMR (CD}_2\text{Cl}, \text{ppm}) \delta 151.8, 136.8, 134.3, 132.6, 130.8, 127.3, 124.6, 94.2, 89.3. \]

**HR FD-MS (m/z) found:** 280.0996 expected: 280.1000, \( \text{C}_{20}\text{H}_{12}\text{N}_2 \).
Proton preorganization effect in $M_{12}L_{24}$ nano-spheres containing hydrogenase mimics

Synthesis of compound 7: An oven-dried, argon-flushed round bottom Schlenk flask was charged with 93 mg of hexacarbonyl complex 6 (1 equiv. 0.19 mmol) prepared according to literature procedure, 59 mg of 3-(diphenylphosphino)benzenesulfonate tetrabutyrammonium salt (0.9 equiv. 0.17 mmol), prepared according to literature procedure and Me$_3$NO (1.2 equiv. 0.23 mmol). The solids were dissolved in a degassed mixture of 2 mL of acetonitrile 20 mL of DCM. After stirring the reaction mixture for 30 minutes at room temperature the solvents were removed under reduced pressure. The residue was dissolved in minimal amount of DCM hexanes added to cause the precipitation of a red/purple crystalline material which was isolated by filtration and washed with hexanes before it was dried under reduced pressure, 106 mg, 78% yield. $^1$H NMR (CD$_2$Cl$_2$, ppm) δ 8.12 – 7.24 (m, 14H), 6.27 (s, 2H), 3.22 (m, 8H), 1.64 (m, 8H), 1.45 (m, 8H), 1.01 (t, $J = 7.5$ Hz, 12H). $^{31}$P NMR (CD$_2$Cl$_2$, ppm) δ 62.21. $^{13}$C NMR (CD$_2$Cl$_2$, ppm) δ 213.4, 208.9, 149.0, 134.8, 134.5, 134.2, 133.5, 132.9, 132.9, 131.7, 130.1, 129.7, 128.6, 128.3, 58.9, 23.9, 19.7, 13.4. FT-IR (MeCN, ν) 2056, 1998, 1980 sh, 1942 cm$^{-1}$. HR ESI(neg.-MS) (m/z) found: 800.7790 expected: 800.7823, C$_{29}$H$_{16}$Cl$_2$Fe$_2$O$_8$PS$_3$.

Crystallographic details

BBNH+: C$_{27}$H$_{27}$F$_6$N$_4$OP, Fw = 568.49, yellow plate, 0.37 × 0.26 × 0.16 mm, Monoclinic, P2$_1$/n (No: 14), a = 13.6743 (7), b = 14.1034 (8), c = 14.7769 (8) Å, $\beta = 104.352$ (3) $^\circ$, V = 2760.8 (3) Å$^3$, Z = 4, D$_x$ = 1.368 g/cm$^3$, $\mu = 0.17$ mm$^{-1}$. 24875 reflections were measured up to a resolution of (sin $\theta$/$\lambda$)$_{\text{max}}$ = 0.595 Å$^{-1}$. 4870 reflections were unique (R$_\text{int} = 0.055$), of which 3721 were observed [I>2σ(I)]. 355 parameters were refined with 0 restraints. R1/wR2 [I > 2σ(I)]: 0.0613/0.1492 R1/wR2 [all refl.]: 0.0845/0.1694. S = 1.03. Residual electron density between −0.89 and 0.80 e/Å$^3$. 

Second coordination sphere effects in [FeFe]-Hydrogenase mimics

References

Proton preorganization effect in $M_{12}L_{24}$ nano-spheres containing hydrogenase mimics

11235.

(24) *Bruker, APEX2 software, Madison WI, USA, 2014.*
