Second coordination sphere effects in [FeFe]-Hydrogenase mimics

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Hydrogenase mimics immobilized on FTO electrodes; a strategy towards solar fuel devices
Introduction

The development of devices for direct conversion of solar energy into fuels is attracting much attention as solution for sustainable energy storage and supply.\textsuperscript{1,2} Of particular interest is the ‘electrolysis’ of water into hydrogen and oxygen using efficient catalysts, driven by solar energy.\textsuperscript{3} Regarding the generation of hydrogen from protons and electrons, platinum is still the most efficient catalyst as it can work at high rates without significant overpotential.\textsuperscript{4} On the other hand, its high cost and low abundance make application of Pt-based systems unattractive on large scale. Nature, however, provides superb examples of hydrogen evolution catalyst based on non-noble metals. Hydrogenases, metalloenzymes that utilize iron, di-iron or iron-nickel as metallic components, are able to perform the reversible proton reduction reaction at ambient temperature and pressure; furthermore their activity, efficiency and overpotential are comparable to platinum.\textsuperscript{5,6} It is thus not surprising that chemists take inspiration from nature to mimic the active site of these metalloenzymes. Hydrogenases are well-studied classes of proton reduction catalysts.\textsuperscript{7,8} Although literature contains several hundreds of examples of molecular hydrogenases models, the majority of them are studied as homogeneous catalysts in organic solvents.\textsuperscript{9} Studies about their immobilization, key for the development of devices, are relatively limited. The potential and advantages of immobilizing molecular catalysts has already been illustrated mainly for cobalt-\textsuperscript{10-18} and nickel-based catalysts\textsuperscript{19-21} immobilized onto various surfaces via their organic ligand structure.\textsuperscript{22}

The immobilization of molecular di-iron hydrogenase models onto substrates is however less studied. Ott and coworkers showed that incorporation of a di-iron catalyst into a metal-organic framework has a beneficial effect on photochemical hydrogen evolution, improving both the activity and stability of the catalyst in pH 5 aqueous solution.\textsuperscript{23} Li et al. reported on the absorption of a di-iron hydrogenase mimic onto high surface area MCM-41 K\textsuperscript{+} exchange molecular sieves, used for photocatalytic proton reduction in pure water.\textsuperscript{24} The same group also reported another photocatalytic system using a hydrogel material as scaffold for the hydrogenase catalyst.\textsuperscript{25}

Effective immobilization of di-iron hydrogenase mimics on electrodes has been reported, although hydrogen evolution studies on the modified electrodes had very limited success. In 2005 the Brest group reported the immobilization of a hydrogenase mimic containing a succinimide ester that was attached onto a glassy carbon electrode functionalized either with amines or aminophenyl groups.\textsuperscript{26} Although the prepared electrodes were exhaustively characterized by voltammetric measurements in organic solvents, they showed featureless cyclic voltammograms in the presence of acid. A few years later Darensbourg et al. reported a similar system based on a propane dithiolate bridged di-iron complex featuring a carboxylic acid moiety used to graft the complex on an aniline functionalized, highly ordered pyrolytic
graphite electrode.\textsuperscript{27} Although the immobilization was successful, the reductive electrochemical response did not persist over repeated scans, the reason being the irreversible nature of the reduction of the complex employed. Artero et al. reported a di-iron complex containing a modified propane dithiolate bridge featuring a succinimide ester anchored on amine functionalized carbon and gold electrodes.\textsuperscript{28} They could not observe hydrogen evolution from their electrodes, due to deactivation of the iron catalyst in the presence of strong acids, but they nicely showed the stability of the amide bond under the highly reductive and acidic conditions trough immobilization of ferrocene moieties, achieved with the same strategy.

In this contribution, we present a di-iron benzenedithiolate complex featuring a short linker terminated with a chelating bis-carboxylic acid moiety. Direct immobilization on metal oxides is achieved by submerging the substrate into a solution of the catalyst. The molecular catalyst is firstly studied in homogeneous solution, then on (nano)FTO (fluorine doped tin oxide) functionalized electrodes. Finally, the electrodes are evaluated for catalytic proton reduction carried out in 0.05 M NaHSO\textsubscript{4} solution at pH 3.5. A bias potential of -0.9 V vs. Ag/AgCl is applied to afford a current density of 1.6 mAcm\textsuperscript{-2} at about 500 mV overpotential. Hydrogen formation was confirmed and quantified by GC analysis.

\textbf{Strategy}

Benzenedithiolates installed on the di-iron platform give some interesting properties to the hydrogenase models. In particular, such complexes display a mild and reversible two-electron reduction potential.\textsuperscript{29} The catalytic proton reduction potential is known to be dependent on the acid strength. It has been reported that proton reduction catalysis in acidic aqueous environment occurs at the first reduction wave of the complexes.\textsuperscript{30-32} For these various reasons we employed a di-iron benzenedithiolate derivative in these immobilization studies.

Carboxylic acids are well-known functional groups for immobilization to metal oxides, providing an effective strategy to decorate electrodes with molecular catalysts. The readily accessible synthon Fe\textsubscript{2}(mcbdt)(CO)\textsubscript{6}, \textbf{4}, featuring a carboxylic acid on the benzene ring, enables modification of the di-iron catalyst without dramatically altering its electronic properties. On the other hand, mono-carboxylic acids may bind too weakly to metal oxides, especially under acidic conditions. Direct immobilization of \textbf{4} was not investigated, instead a suitable linker featuring a bis-carboxylic acid functional group that was anticipated to more strongly bind to metal oxides, was attached to \textbf{4}.

The electrode of choice for this investigation is glass-coated FTO, as this material offers a con-
ductive surface and relatively large potential window, even in aqueous environment. A drawback is the small surface area of such electrodes due to their relatively flat surface. To surpass this limitation, FTO nano-crystals were prepared and deposited onto the flat FTO electrodes by spin-coating, in order to obtain high surface area electrodes of the same material, Figure 1.

Figure 1. Schematic representation of the envisioned high surface area, catalyst modified electrodes.

Results and discussion

Synthesis and characterization

Compound 4 has been isolated before by Ott and coworkers as by-product during the synthesis of the di-carboxylic acid derivative Fe₂(dcbdt)(CO)₆.²³ So far, neither detailed synthesis nor characterization of 4 has been described. A convenient and scalable synthesis route was therefore developed. Preparation of 4 started with nucleophilic aromatic substitution of 1,2-dichlorobenzene with an isopropylthiolate salt that cleanly and quantitatively afforded di-isopropylbenzene thioester (Scheme 1). The long reaction time 5 days at 90 °C ensures quantitative di-substitution to avoid inconvenient chromatography or vacuum distillation steps. Selective mono-ortho-lithiation with nBuLi and subsequent quenching of the lithio-derivative of 1 with CO₂ afforded the carboxylic acid derivative 2. Deprotection of both thioether groups was achieved by treatment with sodium naphthalenide; addition of concentrated HCl to the crude reaction mixture gave access to benzenedithiol monocarboxylic acid 3. Finally, complex 4 was prepared in about 40% yield from an equimolar reaction of the iron precursor Fe₃(CO)₁₂ and 3.
Linker 6 was obtained according to the reaction sequence outlined in Scheme 2. Intermediate 5 was isolated in quantitative yield from the HATU-mediated coupling reaction of di-methyl iminodiacetate and N-BOC protected β-alanine. The BOC-protecting group was easily removed by reaction with a 20% (v/v) solution of trifluoroacetic acid in dichloromethane to provide 6.

Linker 6 was used directly for HATU-assisted coupling to di-iron compound 4. This coupling reaction was performed in THF-MeCN at room temperature in the presence of 5 equivalents of DIPEA. The reaction was considered finished after 30 minutes, according to TLC and work-up afforded compound 7 in 80% yield (Scheme 3). Methyl ester deprotection employing common saponification methods proved to be problematic as it resulted in decomposition of the di-iron core, likely due to irreversible binding of the generated carboxylates to the iron centers. The ester hydrolysis was successfully achieved by treating 7 with an excess of the very mild deprotecting agent trimethyltin hydroxide (Me₃SnOH), affording the desired complex 8 in 70% yield.³³
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Single crystals suitable for X-ray diffraction were grown by layering a THF solution of 8 with hexanes at 4 °C. Complex 8 crystallizes with one water molecule. Its solid state structure is shown in Figure 2; the relevant distances and angles are in agreement with literature values for similar complexes.34

Electrode preparation and catalyst immobilization

The high surface area FTO electrodes were prepared by attachment of a layer of FTO nano-crystals to flat commercial glass-coated FTO. A known amount of FTO nano-crystals, prepared according to a modified literature procedure,35 were suspended in formic acid and grinded with a mortar and pestle for 15 minutes. After this, the solids were used to prepare a 30% (m/m) suspension in neat formic acid. This suspension was sonicated for 30 minutes where after it was used to spin-coat clean FTO electrodes. The slides were then placed into a furnace oven, the temperature was increased to 500 °C over 3 hours. The samples were kept at this temperature for 30 minutes and then allowed to cool to room temperature.
SEM measurements of the high surface area FTO electrodes display a homogeneous distribution of the particle size of the FTO nano-crystals, Figure 3. The particle size is in the order of 10-30 nm, consistent with the value calculated from the XRD data by using the Scherrer equation (see Figure 20, appendix section).

**Figure 3.** SEM pictures of glass-coated FTO after spin-coating with FTO nano-crystals, at 500 nm scale (left) and 50 nm scale (right). The bigger crystals (right picture, lower half) are FTO crystals from the commercial glass-coated FTO electrodes.

Immobilization of compound 8 on glass coated FTO electrodes and on FTO nano-crystals coated electrodes is achieved by submerging the electrodes (after heat treatment at 120 °C for 30 minutes) into a 0.5 mM catalyst solution in THF overnight. Subsequently, the electrodes are thoroughly washed with THF to remove any unbound catalyst, dried with a stream of argon and used directly for the measurements. ATR FT-IR spectroscopy of the functionalized electrodes reveals the characteristic signature of the di-iron CO ligands and the other carbonyl peaks at 2076, 2040, 2004, 1739 and 1641 cm⁻¹ (Figure 4) and only minor shifts with respect to the parent compound 8 in THF solution are observed.
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The SEM pictures of the electrodes with and without catalysts do not show evident differences, indicating that the immobilization did not change the FTO profile. EDX measurements on the catalyst-functionalized electrodes demonstrate homogeneous distribution of iron and sulfur in a 1:1 ratio. Such elements are absent in the unfunctionalized clean electrodes.

Cyclic voltammetry studies in homogeneous solution

Complex 7

Cyclic voltammograms of complex 7 in THF display one Nerstian reversible reduction at -0.88 V vs. Ag/AgCl, as shown in Figure 5 (left). Application of the iso-point method, developed by Paul and Leddy, suggests a two-electron process. Bulk electrolysis at constant potential (-1.3 V vs. Ag/AgCl) confirms the two-electron reduction process (Figure 16, appendix section). Although benzenedithiolate based di-iron complexes typically display potential inversion, modeling of the cyclic voltammograms indicates two consecutive one electron reductions, about 70 mV apart from each other. In contrast, preliminary cyclic voltammograms of 7 in acetonitrile or dichloromethane point to potential inversion, indicating structural rearrangement of the complex upon reduction and a large solvent reorganization. This finding is in line with literature. Interestingly, 7 displays a redox potential that is about 75 mV milder than the parent unfunctionalized benzenedithiolate complex. This effect is attributed to the electron-withdrawing character of the carbonyl substituent on the bridge.
The electrochemical behavior of 7 in THF in the presence of acid was investigated by adding various acids with different strength to its solution while the reduction peak was monitored. When acetic acid ($pK_a = 23.5$ in MeCN) or benzoic acid ($pK_a = 21.5$ in MeCN) were added, the shape and position of the reductive peak did not change. In contrast, when increasing amounts of chloroacetic acid ($pK_a = 18.8$ in MeCN) were added, the forward peak potential shifts anodically while the current of the backwards oxidative peak progressively decreases (Figure 6). This is consistent with protonation at the iron-iron bond. In agreement with this, the newly formed bridging hydride undergoes re-oxidation around 0 V vs. Ag/AgCl.

Figure 5. Left: cyclic voltammograms of a THF solution of 7 at different scan speeds. Right: plot of peak currents vs. square root of the scan speed, displaying Nernstian behavior.

Figure 6. Voltammetric response of a THF solution of 7 in the presence of increasing amounts of chloroacetic acid.
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The progressive anodic shifting of the peak potential indicates a process involving proton transfer as part of the electrochemical event. Peak potential analysis was applied to obtain information about the number of protons involved in this step, having already established that the reduction event is a two-electron process. This analysis relates the variation of the peak potential with the acid concentration. The shift in potential is a function of the number of electrons (n) and protons (m) involved in the electrochemical event, according to Equation 1. From the data collected, a 10.5 mV slope is obtained (Figure 19, appendix section) in agreement with a two-electron, one-proton process (theoretical value 12.5 mV).

\[
\frac{\partial E_p}{\partial \ln[H^+]} = \frac{m}{n} \cdot \frac{RT}{F}
\]

**Equation 1.** Expression for the variation of peak potential \( E_p \) as function of the acid concentration in solution

Increasing the potential window to more reductive potentials results in the appearance of a catalytic proton reduction peak around -1.6 V vs. Ag/AgCl as shown in Figure 7. This event corresponds to the reduction of the previously generated bridging hydride and its subsequent protonation that is associated with hydrogen release. Using all the information gained by voltammetry, a proposed mechanism for proton reduction is shown in Scheme 4. This mechanism is the same as for previously reported benzenedithiolate di-iron hexacarbonyl complexes, indicating that modification of the bridge does not significantly affect the electronic properties of the complex.

![Figure 7. Voltammetric catalytic response for a THF solution of 7 in the presence of increasing amounts of chloroacetic acid.](image)
Although 7 has a milder first reduction potential compared to the benzenedithiolate derivative, it shows catalysis at potentials that are very similar to the unfunctionalized complex. The milder reduction potential could be relevant in aqueous media where the complex is expected to show catalysis at its first reduction potential, based on previous observations on electrocatalysis in water with benzenedithiolate-based di-iron systems.\textsuperscript{30-32}

**Complex 8**

The redox behavior of 8 in THF is different compared to that of 7, rather peculiar and unique. When a small potential window is investigated in the absence of external chloroacetic acid, the reductive scan displays a non-reversible reduction wave at -0.86 V vs. Ag/AgCl. Interestingly, a re-oxidation wave is present around 0 V vs. Ag/AgCl, consistent with re-oxidation of the bridging hydride, as observed for 7 in the presence of acid, Figure 8. The electrochemical process can be summarized as a two-electron reduction followed by intramolecular protonation of the iron-iron bond by a carboxylic acid group (Scheme 5, top half). This protonation step is relatively fast as the reduction wave is fully irreversible at all scan speeds. In comparison, about 16 equivalents of chloroacetic acid are needed to achieve full protonation of complex 7 in its doubly reduced state (Figure 6).

\textbf{Scheme 4.} Proposed catalytic cycle of complex 7, according to an (E)ECEC mechanism.
When the scan window is increased to -1.8 V, a second irreversible wave is observed at -1.6 V, similarly to where 7 displays proton reduction. Following the back-oxidation scan, the first reduction wave is quasi-reversible under these conditions, showing approximately half of the intensity of the forward first reduction of the complex, Figure 9. After the two-electron reduction event and intramolecular proton transfer, the bridging hydride that is formed is reduced at -1.6 V and a second proton from the linker is transferred to the iron-iron core, resulting in hydrogen evolution. The second proton transfer seems considerably slower than the first one, as the feature assigned to the re-oxidation of the di-reduced bridging hydride is still evident around 0 V. After hydrogen evolution, a mono-reduced di-iron species is generated with dicarboxylate functional side-arms $\left[\text{8(COO)}_2\right]^{3-}$. This species, overall tri-anionic, can be mono-oxidized at -0.85 V vs. Ag/AgCl, resulting in a di-anionic Fe$^1$-Fe$^1$ species. The processes described are depicted in Scheme 5.

**Figure 8.** Voltammetric response for a THF solution of 8 at different scan speeds, in the absence of external chloroacetic acid, including only the first reduction.
Next we studied the electrochemistry of 8 in the presence of acid. Addition of increasing aliquots of chloroacetic acid confirms the presence of the catalytic wave at 1,6 V, consistent with hydrogen evolution as shown in Figure 10. This is at the same potential as observed for 7.

The carboxylic acid groups present on the linker of complex 8 act as proton relays to preorganize the substrates for proton reduction. Proton preorganization around the catalyst is expected to have a clear effect on the catalytic performance of the catalyst, although at first
sight it appears that does not have an effect on the catalytic reaction. The next paragraph contains a discussion on the catalytic rates for the proton reduction reaction for complexes 7 and 8 under identical conditions.

**Foot-of-the-wave analysis in organic solvent**

Plateau currents, necessary to determine catalytic rates, were not experimentally reached. Foot-of-the-wave analysis\(^{37}\) (FotW) was therefore performed to access the catalytic rate constants for 7 and 8. This analysis provides curves which slopes can be used to obtain the catalyst rate constants \((k_{\text{cat}})\) under defined conditions.\(^{37}\) Comparison of the FotW curves obtained for 7 and 8 with 64 mM of acid reveals an interesting effect, as shown in Figure 11. The curve for 7 presents a constant slope, indicating that the catalyst operates at the same rate from the catalytic onset potential to the catalytic half-wave potential \((\xi=0.5)\) without substrate depletion or catalyst decomposition. The corresponding FotW curve for catalyst 8 instead shows a steep rise, followed by a second regime that is similar to that of 7. This accelerating effect is attributed to the presence of the internal carboxylic acids, which are associated with rapid intramolecular proton transfer relative to the intermolecular protonation events involving the external acid. The difference in \(pK_a\) between the external acid (chloroacetic acid) and the internal carboxylic acid could also be of influence, although the two \(pK_a\)'s are expected to be rather similar. Furthermore, this apparent acceleration effect only occurs for a couple of turnovers (as indicated by \(I_p/I_p^0 \sim 5\), according to the proposed mechanism one electron activation step and two electrons per catalytic cycle); when the internal acidic protons are depleted, 8 operates at similar catalytic rates as 7. The FotW curve for 8 also gives some insights concerning catalyst decomposition, likely arising from the irreversible coordination of the

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**Figure 10.** Voltammetric catalytic response for a THF solution of 8 in the presence of increasing amounts of chloroacetic acid.
carboxylate groups to the di-iron core of the complex, as indicated by the decrease in the second slope for complex 8, observed around $\xi=0.35$.

![Graph showing foot-of-the-wave analysis for complexes 7 and 8](image)

**Figure 11.** Foot-of-the-wave analysis for 7 and 8 for voltammograms recorded at 0.1 V/s in the presence of 64 equivalents of chloroacetic acid. FotW analysis is showed only for one scan speed for clarity reasons, nevertheless the slopes indicated are obtained from datasets with six different scan speeds.

For each of the three slopes noted in Figure 11, a value of $k_{\text{cat}}$ can be calculated. The calculated values and related parameters for the two complexes are summarized in Table 1. The catalytic performances of the two complexes are nearly identical. The main difference is the proton preorganization around 8 that is responsible for the initial acceleration effect. The data suggest that during a catalytic cycle, only the first protonation happens intramolecularly from the acidic protons on the linker. The second one, associated with hydrogen evolution likely involves the external acid in solution. This is indicated by the studies in the absence of acid, which suggest that the second protonation by the internal carboxylic acid is slow compared to the first proton transfer event. After the second catalytic cycle and depletion of the acidic protons on the linker, which cannot be replenished by chloroacetic acid because it is not a strong enough acid, 8 operates at similar rates as 7. This would suggest that there would be a great advantage in having proton relays on the catalyst that can be re-protonated by the external acid, which could result in increased catalytic activity whilst keeping the over-potential unaffected.
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Table 1. Summary of the relevant catalytic parameters for complexes 7 and 8. TOF<sub>max</sub> is calculated for 1 M concentration of substrate. The overpotential reported is valid under the assumption that the thermodynamic proton reduction potential of the acid used (chloroacetic acid) is equal in acetonitrile and THF.

<table>
<thead>
<tr>
<th></th>
<th>Complex 7</th>
<th>Complex 8</th>
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<tbody>
<tr>
<td></td>
<td>Main domain</td>
<td>First acceleration</td>
</tr>
<tr>
<td>( k_{cat} ) (M&lt;sup&gt;-1&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2.25 ( \times ) 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.25 ( \times ) 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \text{TOF}_{\text{max}} ) (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>4.5 ( \times ) 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.5 ( \times ) 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catalytic E&lt;sub&gt;1/2&lt;/sub&gt; Potential (V vs. Ag/AgCl)</td>
<td>-1.48</td>
<td>-1.50</td>
</tr>
<tr>
<td>Overpotential (V)</td>
<td>0.66</td>
<td>0.68</td>
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Electrochemical measurements of modified electrodes

It is anticipated that the redox behavior of the immobilized 8 will be comparable to the redox behavior of 7 in THF solution, as the acidic protons of the linker are absent when the carboxylates are bound to the metal oxide substrate (electrode). Two sets of data will be presented for catalyst 8 immobilized on electrodes; one on flat FTO, the other for electrodes coated with FTO nano-crystals.

**Figure 12.** Voltammetric response of catalyst functionalized electrodes measured in THF with N(Bu)<sub>4</sub>PF<sub>6</sub> as supporting electrolyte. Left: for complex 8 immobilized on flat FTO. Right: for complex 8 immobilized on nano-crystal FTO modified electrodes.
Hydrogenase mimics immobilized on FTO electrodes; a strategy towards solar fuel devices

The voltammograms feature a wave at -0.90 V vs. Ag/AgCl consistent with the reversible two-electron reduction of the di-iron complex, Figure 12.

Although peak potential separation is larger than what would be expected for a species immobilized on the electrode (around 0 V for a well-behaved system with relatively high heterogeneous rate of electron transfer \( k^0 \)), a plot of the peak current vs. scan rate shows a linear trend, which confirms the immobilization of 8, Figure 13. The slopes of the lines in Figure 13 are used to estimate the catalyst loading for the different types of electrodes, according to Equation 2. The catalyst loading is found to be in the order of \( 10^{-12} \) mol·cm\(^{-2} \) for the flat FTO electrodes, in accordance with monolayer coverage of the electrode. For the high surface area FTO nano-crystals coated electrodes the catalyst loading is about \( 10^{-9} \) mol·cm\(^{-2} \).

![Figure 13. Current response as function of the scan speed for catalyst functionalized electrodes. Left: complex 8 immobilized on flat FTO. Right: complex 8 immobilized on nano-crystal FTO coated electrodes. The slopes reported are used to calculate the catalyst loading according to Equation 2.](image)

\[
\Gamma = \frac{i_p}{v} \cdot \frac{4RT}{n^2F^2A}
\]

**Equation 2.** Expression to estimate the catalyst loading (\( \Gamma \)), wherein \( i_p/v \) represents the slope as seen in Figure 13, \( A \) is the flat geometrical area of the electrode and \( n, F, R \) and \( T \) have their usual meaning.

The shape of the voltammetric waves and the large peak potential separation suggest that the system is under electron diffusion control, meaning that the electrons tunnel from the electrode to the di-iron core with some difficulties. Direct electron transfer, although possible due to the flexibility of the linker, which would in principle allow the di-iron center to be in
the proximity of the electrode surface, can be excluded on the basis of the observed large peak potential separation. This phenomenon is believed to be minimized by the presence of THF, which is able to properly solvate both the organic and inorganic parts of the catalyst. The data collected for the flat FTO electrodes allow for the direct determination of the heterogeneous electron transfer constant $k^0$ through the graphical Laviron method based on peak potential separation. The calculated value of $k^0$ was found to be in the order of $6 \text{ s}^{-1}$. This value is considered to be on the lower side of the fast regime, in agreement with the observations described. On the contrary, with the nano-crystal FTO electrodes, the heterogeneous electron transfer constant is believed to be considerably slower, as indicated by the different slopes of their respective trumpet plots (Figure 21, appendix section).

**Electrolysis at constant potential in aqueous media**

The catalyst modified electrodes were examined for hydrogen evolution in a 50 mM NaHSO$_4$ acidic aqueous buffer solution adjusted to pH 3.5. This pH value is a compromise between activity of the catalyst and limited background reaction from the FTO electrodes.

The bias potential applied for the electrolysis was -0.9 V vs. Ag/AgCl, sufficient to access the first reduction of the complex. The electrolysis cell employed was a two compartment cell; one containing the modified working electrode and a reference electrode, the other containing a platinum coil counter electrode, separated from the main compartment by a glass frit. The cell was connected to a gas chromatography set-up to monitor hydrogen evolution in time. Visual inspection of the electrode, under catalytic conditions, revealed the formation of small hydrogen bubbles trapped at the electrode surface, especially for the nano-sized material. Therefore, the bias potential was applied for 5 minutes, subsequently the electrodes were gently shaken to liberate the small bubbles, thereafter the headspace was allowed to equilibrate for 30 minutes before H$_2$ was quantified. The results for the two types of electrodes are summarized in Table 2 together with their respective blank measurements.
Table 2. Summary of the results from GC of the headspace coupled with electrolysis experiments (50 mM NaHSO$_4$ at pH 3.5 and 0.9 V bias potential vs. Ag/AgCl) of complex 8 immobilized on modified FTO electrodes.

<table>
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<tr>
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<th>8 on FTO</th>
<th>8 on nano-crystals FTO</th>
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<tbody>
<tr>
<td>Blank electrode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$ evolved (µL)</td>
<td>2.7</td>
<td>5.9</td>
</tr>
<tr>
<td>Catalyst loading</td>
<td>3.36·10$^{-12}$</td>
<td>1.01·10$^{-9}$</td>
</tr>
<tr>
<td>(mol·cm$^{-2}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current density</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>(mA·cm$^{-2}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$ evolved (µL)</td>
<td>11.1</td>
<td>29.2</td>
</tr>
<tr>
<td>Faradaic efficiency (%)</td>
<td>95</td>
<td>86</td>
</tr>
<tr>
<td>TON</td>
<td>1.35·10$^5$</td>
<td>1.19·10$^3$</td>
</tr>
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The nano-crystal FTO coated electrodes, having a higher surface area, accommodate about 300 times more catalyst than the flat FTO electrodes. Interestingly, they show about two orders of magnitude lower turnover numbers (TON). This is tentatively attributed to fast depletion of protons around the catalyst. Slow diffusion of electrolyte due to the intrinsic properties of the mesoporous substrate, associated with fast depletion of the protons, would increase the local pH around the catalyst, hampering catalyst stability and activity. As observed during the deprotection of methyl esters groups of complex 7 via traditional saponification methods, basic pHs are incompatible with 8.

Although the prepared electrodes proved to produce hydrogen, their stability under catalytic conditions still remains an issue. As can be seen by the current density plot of the experiment using complex 8 immobilized on nano-crystal FTO coated electrodes, Figure 14, the catalyst is only stable for a couple of minutes.
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A possible explanation for the limited stability is hydrolysis of the chelating carboxylates from the metal oxide surface due to the acidic nature of the medium. This would result in loss of active catalyst from the surface. Alternatively, it is conceivable that the di-iron catalyst is quickly inactivated, possibly by the metal oxide surface. As indicated by the lower Faradaic efficiency for the nano-crystal FTO coated electrodes, side reactions at the metal oxide could be relevant for catalyst inactivation. Although this does not appear to be an issue in organic solvents, this phenomenon could be relevant in aqueous media. Water could also assist the decomposition of the metal complex, especially when in its reduced state.

Foot-of-the-wave analysis was employed to deduce the catalytic rate for proton reduction in acidic aqueous environment for the immobilized catalyst on FTO electrodes. It is noted that the catalytic response does not persist for several voltammetric scans. Nevertheless, this analysis for complex 8 immobilized on flat FTO with a catalyst loading of 3.36·10^{-12} mol·cm^{-2} reveals a catalytic constant $k_{cat}$ of 5.95·10^{3} M^{-1}s^{-1}. The calculated constant is in agreement with the one calculated for the same catalyst dissolved in organic solvents with weak acid, corroborating the presence of the molecular catalyst on the FTO surface. The information from the electrocatalytic data in organic solvent and acidic aqueous media allow for the construction of a Tafel plot, Figure 15. The Tafel plot clearly evidences the advantage of the immobilization. Going from organic solvents to aqueous media has the effect of lowering the overpotential by about 160 mV, while the activity of the catalyst is also improved four-fold.

Figure 14. Current density plot for complex 8 immobilized on nano-crystal FTO coated electrode.
Conclusions

In this work we have shown the successful immobilization of benzenedithiolate di-iron complexes on conductive FTO electrodes. The activity of the catalyst has been evaluated both in the homogeneous phase and for the heterogenized system. From the studies in organic media it is evidenced that proton relays around the catalyst could greatly improve the catalytic rates of the catalyst. The studies of the immobilized catalyst reveal on the other hand that the same catalyst can operate at lower overpotentials yet with similar rates in acidic aqueous media. These observations could pave the road for the development of a second-generation catalyst equipped with proton relays. Such functional groups could induce important (side) effects, such as better solvation of the immobilized catalyst, more favorable proton transfer from the ‘bulk’ medium to the catalyst and faster electrolyte refreshing rates, functioning as a buffer. Improvement of the anchoring group strategy is also needed and essential to improve the stability of the catalyst-functionalized electrodes. Nevertheless, this first generation of electrodes displays hydrogen evolution at mild overpotential.

Acknowledgments

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Garnett for SEM and EDX data collection. Remko Detz is acknowledged for help with nano-
crystal FTO preparation.

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 re-
ceived without further purification. Solvents used for synthesis were dried via the most suit-
able 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-STAT302N potentiostat at
glassy carbon disk electrode (2 mm diameter). A platinum coil was used as auxiliary elec-
trode and a leak free silver electrode (inner compartment 3 M KCl/Ag).

Bulk electrolysis in organic solvents was carried out in a two-compartment cell using Du-
ocel® 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).
The platinum auxiliary electrode was separated from the main solution by a P4 glass frit.
Both compartments contained 0.2 M tetrabutylammonium hexafluorophosphate as support-
ing electrolyte.

Bulk electrolysis in aqueous media was carried in the same two-compartment cell, but using
0.05 M NaHSO₄ adjusted at pH 3.5 as electrolyte.

Hydrogen detection: The bulk electrolysis setup was connected to an in-line gas chromatog-
rapher (Interscience CompactGC equipped with molecular sieve column and TCD detector,
argon was used as carrier gas). 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 ($\lambda = 0.71073$ 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 SADABS. The structures were solved using intrinsic phasing with the program SHELXT. Least-squares refinement was performed with SHELXL-2013 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 $U$ equiv. of the attached C atoms.

Preparation of compounds

1,2-Bis(isopropylmercapto)benzene, 1 and 2,3-Bis(isopropylmercapto)benzoic acid, 2 were prepared according to a modified literature procedure.

1,2-bis(isopropylmercapto)benzene, 1. To an oven-dried, argon-flushed round bottom Schlenk flask equipped with a reflux condenser and a dropping funnel were added sodium (12.38 g, 0.538 mol, 1 equiv.) and 500 mL of dry diethyl ether. This suspension was cooled with an ice bath before isopropylthiol (55 mL, 0.592 mol, 1.1 equiv.) was added dropwise over the course of six hours, being careful to keep the reaction temperature below the diethyl ether boiling point. A white precipitate appeared over time. This suspension was stirred overnight, whereafter it was heated to reflux for two hours. After cooling the suspension to room temperature, most of the solvent was removed by cannula filtration and the white solid was dried under vacuum. The dried solid was cooled to -10 °C with an ice/salt bath and dry DMF (200 mL) was slowly added. The suspension was allowed to warm to room temperature before 1,2-dichlorobenzene (15.2 mL, 0.134 mmol, 0.25 equiv.) was added by syringe. The mixture was stirred at 90 °C for 5 days, then cooled to room temperature, poured into water and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with water (2 × 100 mL) and brine (100 mL), dried over MgSO$_4$ and the solvents removed under reduced pressure to afford a colorless liquid in quantitative yield (30.2 g). The product was used without further purification. $^1$H NMR (CD$_2$Cl$_2$, ppm) $\delta$ 7.40-7.33 (m, 2H), 7.23-7.15 (m, 2H), 3.51 (hept, $J = 6.7$Hz, 2H), 1.34 (d, $J = 6.7$Hz, 12H).

2,3-bis(isopropylmercapto)benzoic acid, 2. An oven-dried, argon-flushed round bottom Schlenk flask was charged with 1,2-bis(isopropylmercapto)benzene (1) (20 g, 88.34 mmol, 1 equiv.) and dry hexane (350 mL). The flask was cooled to 0 °C before TMEDA (13.2 mL, 88.34 mmol, 1 equiv.) and nBuLi (2.5 M solution in hexane, 35.33 mL, 88.34 mmol, 1 equiv.)
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were added dropwise over the course of one hour. The reaction mixture was then allowed to warm up to room temperature and stirred for two hours before being re-cooled to 0 °C and dry CO₂ passed through a column containing concentrated sulfuric acid, was bubbled through the suspension for one hour. The hexane layer was extracted with water until the pH of the water phase was neutral (~800 mL). The hexane layer was discarded and concentrated HCl was added to the aqueous phase until a pH of 2 was reached. The mixture was extracted with diethyl ether (3 × 100 mL) which was then dried over MgSO₄, filtered and the solvent removed under reduced pressure to afford a pale yellow liquid that crystallizes over time. The solid material was triturated in hexanes (100 mL), filtered and dried under vacuum (19.65 g, 82.3% yield).

Fe₂(bdtCOOH)(CO)₆, 4. 2,3-Bis(isopropylmercapto)benzoic acid (2) (4 g, 14.79 mmol, 1 equiv.) and naphthalene (4.73 g, 36.98 mmol, 2.5 equiv.) were added to an argon flushed round bottom Schlenk flask and dissolved in 400 mL of dry THF. Sodium (2.04 g, 88.65 mmol, 6 equiv.) was added against a constant stream of argon. The mixture was stirred overnight at room temperature. Then, 20 mL of degassed methanol were added dropwise over the course of 10 minutes. After stirring for further 10 minutes, the volatiles were removed under vacuum and the residue dissolved in degassed water. The aqueous phase was washed with toluene (3 × 30 mL) in anaerobic conditions, to remove naphthalene. To the aqueous phase, concentrated HCl was added, causing precipitation of an off-white compound that was extracted with ethyl acetate. The organic layer was dried in aerobic conditions over MgSO₄ and the solvent removed under vacuum to afford 2,3-bis(mercapto)benzoic acid as a yellow solid that was used directly in the next step without further purification.

To the same flask containing 2,3-bis(mercapto)benzoic acid, Fe₃(CO)₁₂ (7.46 g, 14.80 mmol, 1 equiv) was added under a positive argon pressure together with 200 mL of dry toluene. The mixture was stirred overnight at 80 °C causing the color to change from dark green to dark red. The mixture was allowed to cool to room temperature and filtered over Celite to remove a black precipitate. The solvent was removed under vacuum and the residue was chromatographed over silica gel using gradient DCM:MeOH eluent. Product is obtained from the red band that elutes with 8:2 DCM:MeOH. After collecting the right fraction the solvent was removed under reduced pressure and the residue is dissolved again in DCM and washed 0.5 M HCl (3× 50 mL) and brine (50 mL). The organic phase is dried with MgSO₄ and solvent removed under vacuum to afford 4 as red powder (2.68 g, 39.4% yield). ³¹H NMR (CD₂Cl₂, ppm) δ 7.37 (br s, 2H), 6.83 (bs s, 1H). FT-IR (THF, ν) 2077, 2041, 2001, 1668 cm⁻¹.

Compound 5. To a round bottom Schlenk flask containing dimethyl iminodiacetate hydrochloride (1.5 g, 7.9 mmol, 1 equiv.), N-Boc-β-alanine (1.72 g, 8.7 mmol, 1.1 equiv.) and HATU
coupling agent (3.31 g, 8.7 mmol, 1.1 equiv.) dry DMF (50 mL) and DIPEA (6.9 mL, 39.6 mmol, 5 equiv) were added and the mixture stirred at room temperature overnight. The volatiles were removed under vacuum and the residue dissolved in ethyl acetate. The organic phase was washed with saturated NaHCO$_3$ (3 × 50 mL), 0.5 M HCl (3 × 50 mL) and brine (50 mL). The organic layer was dried with MgSO$_4$ and solvent removed under vacuum to afford a red-orange oil that was chromatographed on silica gel with ethyl acetate. The product can be spotted on TLC using ninhydrin stain solution. After the solvent was evaporated a clear oil was obtained (2.51 g, 95.5%). $^1$H NMR (CD$_2$Cl$_2$, ppm) δ 5.22 (br s, 1H), 4.18 (s, 2H), 4.17 (s, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.41 (q, $J = 6$ Hz, 2H), 2.52 (t, $J = 6$ Hz, 2H), 1.44 (s, 9H).

Compound 6. To a 20 mL DCM solution of 5 (2 g, 6.0 mmol), trifluoroacetic acid (TFA, 5 mL) was slowly added. The solution was stirred at room temperature for two hours whereafter the volatiles were removed under vacuum to afford a pale yellow liquid. The residue was dissolved in a minimum amount of DCM and 150 mL of toluene was added. All volatiles were removed under reduced pressure to afford a very hygroscopic off-white solid in quantitative yield (2.0 g), which was used without further purification. $^1$H NMR (CD$_3$CN, ppm) δ 7.40 (bs s, 1H), 4.21 (s, 2H), 4.15 (s, 2H), 3.74(s, 3H), 3.70 (s, 3H), 3.21 (t, $J = 6$ Hz, 2H), 3.19 (t, $J = 6$ Hz, 2H).

Compound 7. To an argon-flushed round bottom Schlenk flask containing 4 (250 mg, 0.54 mmol, 1 equiv.), 6 (242.5 mg, 0.70 mmol, 1.3 equiv.) and HATU coupling agent (266.3 mg, 0.7 mmol, 1.3 equiv.) a 1:1 mixture of dry THF and dry acetonitrile (total 20 mL) and DIPEA (469 µL, 2.69 mmol, 5 equiv.) were added and the mixture stirred at room temperature. The reaction was monitored in time by TLC and considered finished after 30 minutes. The solvents were removed in vacuum and the residue dissolved in ethyl acetate (50 mL). The organic layer was washed with water (3 × 50 mL), saturated NaHCO$_3$ (50 mL), 0.5 M HCl (50 mL) and brine (50 mL). The organic layer was dried with MgSO$_4$, filtered and the solvent removed under vacuum to afford a red solid which was chromatographed on silica gel with ethylacetate:NEt$_3$ 99:1 to afford a red solid (312 mg, 83.3% yield). $^1$H NMR (CD$_2$Cl$_2$, ppm) δ 7.26 (d, $J = 7.8$ Hz, 1H), 6.93 (d, $J = 7.8$ Hz, 1H), 6.90 (br t, $J = 6$ Hz, 1H), 6.74 (t, $J = 7.4$ Hz, 1H), 4.19 (s, 2H), 4.17 (s, 2H), 3.77 (s, 3H), 3.74 (s, 3H), 3.70 (q, $J = 6.0$ Hz, 2H), 2.64 (t, $J = 6.0$ Hz, 2H). FT-IR (THF, ν) 2075, 2038, 2003 cm$^{-1}$. HR CSI(pos.)-MS (m/z) (m + H$^+$) found: 678.9086 expected: 678.9077, C$_{22}$H$_{19}$Fe$_2$N$_2$O$_{12}$S$_2$; (m + Na$^+$) found: 700.8902 expected: 700.8899, C$_{22}$H$_{19}$Fe$_2$N$_2$NaO$_{12}$S$_2$.

Compound 8. To an argon-flushed round bottom Schlenk flask containing compound 7 (260 mg, 0.37 mmol, 1 equiv.) and trimethyltin hydroxide (676 mg, 3.7 mmol, 10 equiv.), 1,2-dichloroethane was added (10 mL) and the solution was heated at 80 °C for three hours. After cooling the solution to room temperature, ethyl acetate was added and the organic phase
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washed with 1 M HCl (3 × 50 mL) and brine (50 mL), dried with MgSO₄ and the solvent removed under vacuum to afford an orange solid. The solid is dissolved in DCM and precipitated by addition of hexane (187 mg, 75% yield). $^1$H NMR (CD$_2$Cl$_2$, ppm) δ 7.28 (d, $J = 7.8$ Hz, 1H), 7.02 (br t, $J = 6$ Hz, 1H), 6.91 (d, $J = 7.8$ Hz, 1H), 6.76 (t, $J = 7.4$ Hz, 1H), 4.23 (s, 2H), 4.16 (s, 2H), 3.71 (q, $J = 6.0$ Hz, 2H), 2.67 (t, $J = 6.0$ Hz, 2H). FT-IR (THF, ν) 2076, 2040, 2004, 1739, 1641 cm$^{-1}$. HR CSI(neg.)-MS (m/z) (m - H)+ found: 648.8893 expected: 648.8610, C$_{20}$H$_{13}$Fe$_2$N$_2$O$_{12}$S$_2$.

Crystallographic details

Complex 8: C$_{20}$H$_{16}$Fe$_2$N$_2$O$_{12}$S$_2$. Fw = 668.17, red/orange plate, 0.37 × 0.19 × 0.03 mm, Monoclinic, P2$_1$/c (No: 14), a = 18.4321 (10), b = 7.8043 (4), c = 18.4076 (9) Å, β = 95.837 (3)°, V = 2634.2 (2) Å$^3$, Z = 4, D$_x$ = 1.685 g/cm$^3$, μ = 1.33 mm$^{-1}$. 25732 reflections were measured up to a resolution of (sin θ/λ)$_{max}$ = 0.644 Å$^{-1}$. 5857 reflections were unique (R$_{int}$ = 0.063), of which 4173 were observed [I>2σ(I)]. 362 parameters were refined with 3 restraints. R1/wR2 [I > 2σ(I)]: 0.0471/0.1012 R1/wR2 [all refl.]: 0.0820/0.1171. S = 1.05. Residual electron density between -0.74 and 0.46 e/Å$^3$.

FTO nano-crystals. FTO nano-crystals were prepared according to a modified literature procedure. In a teflon beaker containing 30 mL of water, tin tetrachloride (1.5 mL) was slowly added. After stirring this solution for 5 minutes, HF (50% solution in water, 0.5 mL) was added. Concentrated ammonia was added to the solution dropwise until a sol-gel was obtained and the pH of the liquid became basic. The sol-gel was dried at 100 °C in an oil-bath overnight. The resulting solid was triturated in a mortar and calcined at 500 °C for 30 minutes. The powder obtained was further grinded in a mortar. XRD powder diffraction, 2θ: 26.5, 33.8, 37.8, 51.7, 54.6, 55.5. A lower indication of the crystallites particle size can be obtained from the Scherrer equation by measuring the width of the peaks at half-height. The mean particle size is estimated to be around 6 nm.

Glass coated FTO cleaning: Glass coated fluorine doped tin oxide electrodes were cut in 2.5 × 1 cm pieces, washed with ethanol and then died. Prior to use, the FTO slides were immersed in a 3:1 piranha acid solution for 10 minutes, rinsed with Milli-Q water, acetone then dried in an oven at 120 °C for 30 minutes.
Appendix

Figure 16. Bulk electrolysis experiment for complex 7 in THF solution confirming the two-electron process.

Figure 17. Trumpet plot for complex 7 in THF solution.
Figure 18. Blank experiments for a THF solution containing increasing amounts of chloroacetic acid (blue lines) compared to a THF solution containing catalyst 7 showing no direct proton reduction from the glassy carbon electrode at the catalytic potential of the catalyst. During an electrolysis experiment of a THF solution of 7 in the presence of 250 equivalents of chloroacetic acid at -1.75 V vs. Ag/AgCl hydrogen was detected by gas GC.

Figure 19. Peak potential analysis for complex 7 in the presence of chloroacetic acid.

\[
E_p = c - \frac{RT}{nF} \ln \frac{[H^+]^m}{v} = c - \frac{RTm}{nF} \ln[H^+] + \frac{RT}{nF} \ln(v)
\]

\[
\frac{\partial E_p}{\partial \ln[H^+]} = \frac{mRT}{nF}
\]
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**Figure 20.** Left: powder XRD spectrum for FTO nano-crystals. Right: linearization of the Scherrer equation.

**Figure 21.** Left: trumpet plot for 8 immobilized on flat FTO. This plot was used to determine heterogeneous electron transfer rate constant through the graphical Laviron method. Right: trumpet plot for 8 immobilized on FTO nano-crystal functionalized electrode.
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References

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