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Fine-Tuning of Ferrocene Redox Potentials Towards Multiplex DNA Detection.

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Electron-withdrawing or –donating groups are known to directly affect the Fe(III/II) formal potential of ferrocene derivatives by affecting their energy levels relative to the vacuum level. However, perhaps surprisingly, also more subtle indirect "tuning" of the formal potential is possible by changing the "dielectric environment". This is demonstrated here by systematically changing the chain length of alkyl-chain derivatives. The resulting formal potentials are shown to be correlated to the hydrophobicity of the ferrocene molecule which can be used to predict the redox potential of a ferrocene.

Libraries of ferrocenes with defined redox properties are important in many areas of redox-chemistry,[1] including electro-analytical glucose sensing,[2] biofuel cells,[3] as bioelectrochemical mediators[4], and in DNA sensing. The development of a fast and accurate detection of DNA is an important sensing challenge, particular for the on-site detection of pathogenic DNA. This technology has grown from the need for rapid parallel screening primarily using technologies using fluorescene based probe systems.[5,6] With rapid screening of DNA now possible, this has opened the door for sensors for various viral and bacterial pathogens to be developed also based on electrochemical mediator methods, e.g. ferrocenes.[7,8] With an appropriate library of ferrocenes multiplex detection (detecting several DNA targets simultaneously) could be realised. The systematic "tuning" of formal potentials of ferrocenes is therefore important.

The use of organic and organometallic compounds as mediators in sensing applications towards use in the medical industry is an ever expanding field in chemistry. Of particular interest has been the development of ferrocene-based probes for use in electrochemical sensing methodologies. The detection of specific gene fragments using electrochemistry has some advantages and some challenges compared with the widely used fluorescence assays. The principle advantages are: (i) optical sample transparency is not necessary; (ii) direct signal read out; (iii) potential for ease of miniaturisation and device manufacture. The current challenge is to synthesise electrochemically active molecules such as ferrocenes to use as labels in biosensor systems to allow for the rapid detection of various specific gene fragments particularly in the point of care medical field.

In our previous research we have developed an electrochemical based biomedical assay,[8,9,10] which allows for rapid detection of a single specific bacterial target. To achieve this, the assay incorporates an extraction of the target cells from which DNA is purified and extracted (see Figure 1). The extracted DNA is then amplified via the polymerase chain reaction, these single stranded DNA fragments then hybridise with a target specific DNA sequence tagged with a ferrocene probe. This double stranded DNA is then digested with an exonuclease which releases the ferrocene probe and is then detected electrochemically (Figure 1).

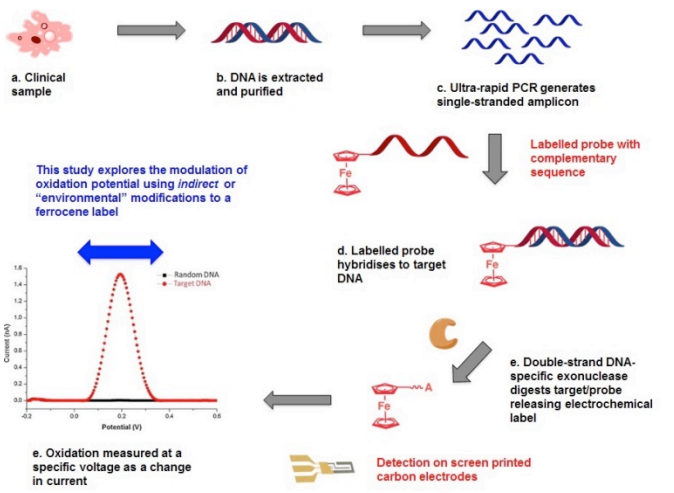
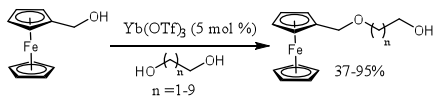


Figure 1. Schematic drawing of the ferrocene-based redox-assay for DNA.

We are interested in the use of this ferrocene based technology as a probe for the detection of a broad scope gene specific DNA panel. In particular, our research has been focussed on the development of a library of ferrocene compounds that could be utilised in a multiplex assay for the detection of multiple DNA analytes in a single assay using this proprietary technology. Early stage library synthesis was focused on the modulation of the ferrocene oxidation potential *via* incorporation of functionality directly onto the ferrocene core using standard methodologies[11],[12,13,14] to modify the oxidation potential of the ferrocene (direct redox potential tuning *via* electronic effects). However, it is desirable to minimise synthetic efforts and chemical diversity in redox labels, and therefore a strategy based on a simple "ferrocene core" with linker and side chain is suggested here. By systematically varying the length of the alkyl side chain well-defined label "tuning" is achieved, which could allow for multiplex DNA detection when applied to a DNA sensing assay.

Ferrocenemethanol was chosen as the model system for fast access to a range of ferrocene species. A range of ferrocenes were designed based upon this core with an increasing tether length between the ferrocene and alcohol head group that could be used to attach the ferrocene probe to the target specific DNA. These compounds were prepared via modification of the procedure of Jiang *et al.*[15] The synthesis is based on the etherification of ferrocene methanol with a range of diols in the presence of a catalytic amount of ytterbium (III) triflate. Generally, these compounds were prepared in good yield (Scheme 1, see SI for specifics) after stirring overnight in a solution of the desired diol. In the cases where the diol is a solid a small amount of 1,4-dioxane was used to solubilise the diol prior to adding it to ferrocene methanol.

Electrochemical analysis was performed using differential pulse voltammetry (DPV, with 50 mV modulation amplitude, see experimental) in pH 9.0 Tris buffer with a ferrocene concentration of 3.8 μM to mimic conditions used in DNA detection experiments. Detection was achieved on screen printed electrochemical cells, utilising a carbon working electrodes with a carbon counter electrode and a silver pseudo reference electrode (potentials are here referenced to ferrocene$^{+/0}$ at 0.316 V vs. pseudo-Ag). The inset in Figure 2A shows a typical DPV trace.



Scheme 1. Ytterbium (III) triflate catalysed etherification of ferrocene methanol.

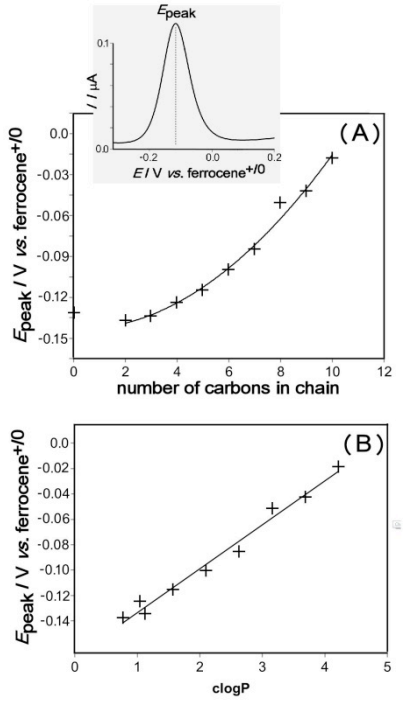
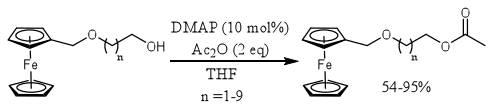


Figure 2. (A) Correlation between DPV oxidation peak potential and the carbon chain length in linker (see SI). Inset shows DPV of ferrocene 4 (see SI). (B) Correlation between clogP and oxidation potential of the ferrocene alcohols.

From DPV data, it quickly became apparent that there was an increase in ferrocene oxidation potential once the carbon chain length had increased past five carbons in length. The results are particularly pronounced for the 1,10-deacandiol derivative, which has an almost 100 mV difference in electrode potential in comparison to its ethylene diol counterpart (see Figure 2A). With the structure of the ferrocene core identical throughout this series of compounds and the alcohol functionality too remote to play a direct role in altering the reversible potential of the ferrocene core, the working hypothesis for this change in peak potential can be based on the "dielectric environment" (i.e the solvent environment around the ferrocenium cation). The side chain can replace polar water molecules and thereby destabilise the cationic oxidation product. Based on this hypothesis the longer the carbon chain on the linker will have an increased destabilising effect on the "dielectric environment" of the ferrocenium cation therefore increasing the oxidation potential of the ferrocene.

This hypothesis was further confirmed by comparing the oxidation of these ferrocene compounds and their predicted octanol-water partition coefficent (clogP[17]) values (see Figure 2B). This analysis shows a strong positive correlation between the increasing hydrophobicity and the observed oxidation potential of the compounds. These data show that this correlation equated to a 34 mV increase in the oxidation potential of the ferrocene with every unit increase in the clogP.



Scheme 2. Acylation of the ferrocene ethers.

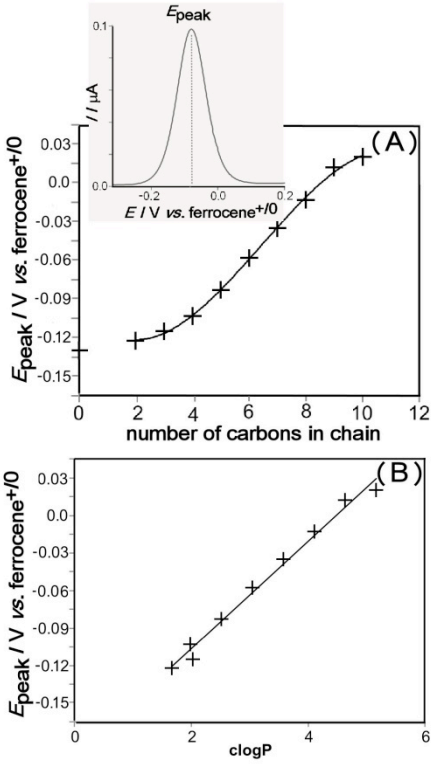


Figure 3. (A) Correlation between chain length of ferrocene esters and DPV oxidation peak potentials. Inset shows DPV of ferrocene 4 (see SI). (B) Correlation between clogP and the oxidation potential of the ferrocene esters.

As this correlation between the hydrophobicity of the ferrocenes and the corresponding clogP was quite striking, we were curious to see if the correlation was restricted to these specific compounds or more general. To this end the alcohols prepared previously were esterified via treatment with acetic anhydride (Scheme 2) and analysed by DPV. This ester series already has a higher starting oxidation potential than the corresponding alcohol (e.g. increased hydrophobicity due to acylation, see Figure 3A). However, the difference between the corresponding alcohols and esters (when comparing oxidation potentials of molecules with similar carbon lengths) is not constant throughout the series. The difference is as low as 12 mV in the case of the ethylene derivative but this increases as the carbon chain is lengthened to give a maxima of 69 mV with the 1,8-octandiol derivative. The difference then begins to plateau past this point. In general, the characteristic reversible potential shift with increase in carbon chain length appears similar for both sets of materials. That is, a similar change in "dielectric environment" of the ferrocene core is induced by substitution. The supposition for these results is again that by altering the hydrophobicity of the molecule the electrode potential can be indirectly fine-tuned. In the case of the esters, this effect is observed already for shorter chains. Further analysis was performed by comparing the clogP of these esters with their corresponding DPV peak potentials. There is a positive correlation between the clogP and the oxidation potential (see Figure 3B). The correlation was similar to that observed for the alcohol series. The increase in electrode potential per clogP unit was approximately 34 mV in the case for the alcohols and for the ester series it was approximately 43 mV per unit change in clogP. These sets of results are indicative that this kind of correlation is not restricted to one particular class of ferrocene but can be applied across a particular series (given the same ferrocene core), indicating that the ferrocene formal potential can be tuned and predicted via changes in "dielectric environment". The difference in oxidation potential between the ferrocenes described is significant enough to allow for possible multiplex detection when applied to a DNA sensing assay.

It has been shown that the formal potential of ferrocene derivatives can be varied by changing the "dielectric environment" of the ferrocene core through modulation of an alkyl tether attached to the ferrocene. This approach is chemically simple and with the redox potential of a ferrocene predictable *via* 35–40 mV increase in redox potential per unit of clogP increase from ferrocenemethanol parent structure. Further work is currently ongoing to further exploit this effect for multiplex detection in DNA sensing applications.

Experimental

Ferrocene Ether Synthesis. Ferrocene methanol (1 eq) was dissolved in the appropriate diol (5 ml/mmol or 10 eq) and then treated with ytterbium triflate (5 mol %). The reaction was stirred at room temperature until TLC analysis showed full conversion. The reaction mixture was then diluted with ethyl acetate (20 mL) and the organics then washed with water (20 mL) and brine (sat.) (20 mL). The organic layer was then dried over $MgSO_4$, then filtered and concentrated *in vacuo*. Purification was then carried out by silica-gel chromatography eluting with hexane 1:1 ethyl acetate to give the desired product.

Acylation of Ferrocene Ethers. The ferrocene ether (1 eq) was dissolved in THF (5 ml/mmol) and then treated with DMAP (10 mol%) and acetic anhydride (2 eq) sequentially. The orange solution was then stirred at room temperature for 15 minutes. After this time the reaction was diluted with EtOAc (10 cm^3) and water (10 cm^3). The orange organic layer was separated, washed with brine (sat) (10 cm^3) and dried over $MgSO_4$. The suspension was filtered and concentrated *in vacuo* to give the desired ester without need for further purification.

Electrochemical Analysis. 3.8 μM solution of ferrocene prepared using pH 8.5 Tris buffer. Electrochemical potential recorded on screen printed electrochemical cell (12.5 μl cell volume), with carbon working electrodes and silver counter electrodes relative to Ag/AgCl using a potentiostat in a DPV mode (Modulation: 0.04 s, Interval: 0.1 s, Initial voltage: -0.2 V, End Voltage: 0.7 V, Step: 0.003 V, Modulation amplitude: 0.04995 V) (microAutolab III, Metrohm).

Partition coefficient analysis (clogP for octanol-water) was performed with prediction software (ChemBio Draw 13.0), which takes an average using three predictive methods.[18,19,20]

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Notes and references

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