

## Development of supramolecular metalloprotein mimics

M. C. Feiters, R. J. M. Klein Gebbink, A. P. H. J. Schenning, G. P. F. van Strijdonck, C. F. Martens, and R. J. M. Nolte

Department of Organic Chemistry, NSR Centre, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

**Abstract.** Supramolecular mimics of metalloproteins, viz. cytochrome P-450 and iron-sulfur proteins are described. The cytochrome P-450 mimic consists of a Mn porphyrin and a Rh complex which are incorporated within a vesicle membrane in water. This system catalyses the reductive activation of molecular oxygen in the epoxidation of alkenes at Mn, with reducing equivalents derived from the simultaneous Rh catalysed oxidation of formate to carbon dioxide. In the mimics for iron-sulfur proteins, iron-sulfur clusters are encapsulated in diphenylglycoluril and cyclotrimeratrylene cavitands. The encapsulation by the cavitands modifies the electrochemical parameters, e.g. the redox potential, of the iron-sulfur clusters, in a way that has certain analogies to the effects of encapsulation by the protein in ferredoxins and high-potential iron-sulfur proteins.

### INTRODUCTION

In metalloproteins,<sup>1</sup> the properties of one or more metal ions are tuned by a protein environment to produce efficient and selective catalysts for a large variety of reactions, e.g. the activation of small inorganic molecules like oxygen and nitrogen, group transfer, redox reactions, and solvolysis. Bioinorganic chemists study the characterization of the function and structure of metalloproteins and structural and functional models for them. Supramolecular chemists,<sup>2,3</sup> studying the chemistry of non-covalent bonds in areas like host-guest chemistry or the chemistry of self-assembling molecules, are showing an increasing interest in the development of metalloprotein mimics that combine a metal in a catalytic site with a hydrophobic binding pocket like a cavity or a membrane.<sup>4</sup>

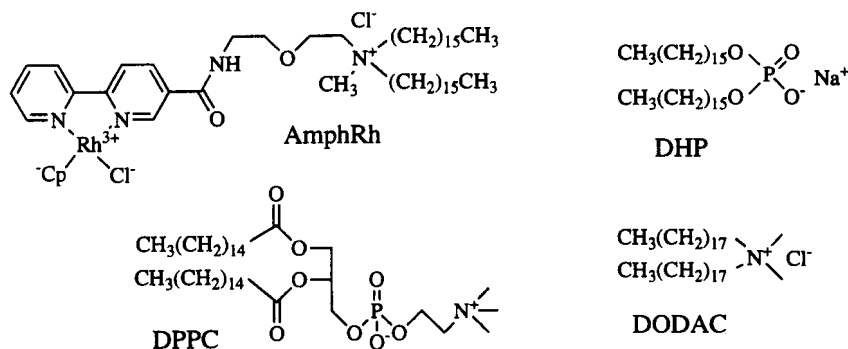
As part of our programme aimed at the design and synthesis of new supramolecular systems, in particular catalysts for the oxidation of hydrocarbons and sensor systems whose redox potentials depend on the concentration of alkali cations, we have designed and synthesized a number of self-assembling and cavitand systems containing metal clusters.

### CYTOCHROME P-450

Cytochrome P-450<sup>5</sup> is a monooxygenase, i.e. it catalyses the reductive activation of molecular oxygen. One oxygen atom is incorporated in an organic substrate, and the other is reduced to water. The electrons required for the reaction are obtained from the nicotinamide redox cofactor NADPH + H<sup>+</sup> via the flavoprotein NADPH-cytochrome P-450 reductase. In the proposed catalytic cycle,<sup>6</sup> the electrons are transferred separately, viz. after the binding of the hydrocarbon substrate and oxygen, respectively. The crystal structure<sup>7</sup> of cytochrome P-450 shows that there is a substrate binding cavity close to a catalytic iron porphyrin, which has a thiolate as an axial ligand. Cytochrome P-450 'mimics' possess Mn-porphyrins and Fe-porphyrins as metal complexes, and often catalyse oxygen transfer from a single oxygen donor like iodosobenzene instead of molecular oxygen.<sup>6,8,9</sup> An interesting aspect of cytochrome P-450 is that it is a membrane-bound enzyme, and that phospholipids stimulate the transfer of electrons to the isolated enzyme and enhance its affinity for the substrate.<sup>10</sup>

This feature has stimulated a number of groups<sup>11-13</sup> to develop cytochrome P-450 mimics in which a hydrophobic porphyrin is incorporated within the bilayer of a vesicle membrane. In our first model,<sup>12</sup> the electrons required for the partial reduction of oxygen were derived from the oxidation of molecular hydrogen by colloidal Pt.<sup>14</sup> This approach has the disadvantage, apart from the potentially hazardous combination of hydrogen and oxygen, that a non-productive reaction between these gases is possible, viz. water formation. In a later stage in the development of our system<sup>15</sup> we applied another reaction, viz. the Rh-catalysed oxidation of formate to carbon dioxide,<sup>16</sup> for the supply of the reducing equivalents.

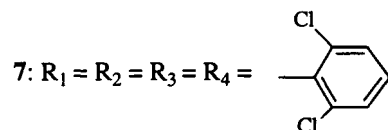
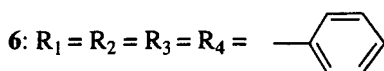
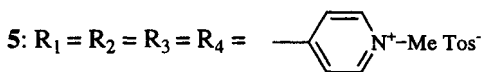
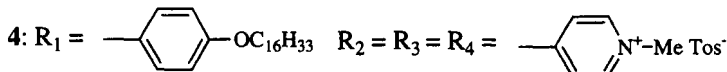
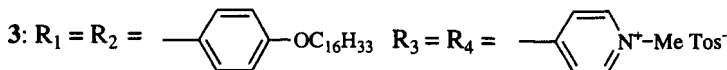
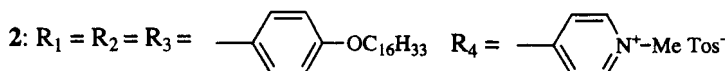
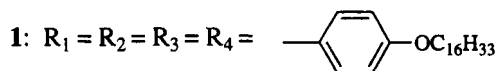
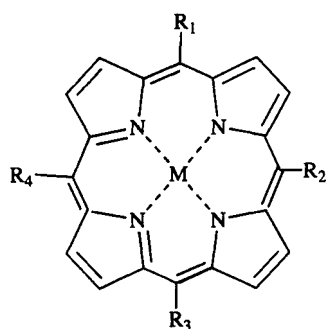
CHART 1



In order to determine which porphyrin would be most suited as a catalytic group in our cytochrome P-450 mimic, the location, aggregation and orientation of a number of tetraarylporphyrins in vesicle bilayers of both positively and negatively charged amphiphiles, viz. dioctadecyldimethylammonium chloride (DODAC) and sodium dihexylphosphate (DHP), respectively, were investigated in detail.<sup>17-19</sup> A study of the effects of hydrophobic and hydrophilic quenchers on the porphyrin fluorescence in DODAC bilayers revealed that the hydrophobic porphyrin, 21,23-2H-5,10,15,20-tetra(4-hexadecyloxyphenyl)porphyrin (THPP, **1**) is located in the centre of the bilayer, whereas the positively charged porphyrin 21,23-2H-5-(1-methyl-4-pyridyl)-10,15,20-tri(4-hexadecyloxyphenyl)porphyrin monotosylate (**2**) is close to the surface of the vesicles.<sup>17</sup> The aggregation behaviour of the porphyrins was investigated by looking at the self-quenching of their fluorescence as a function of the [amphiphile]/[porphyrin] ratio. Following the series **1-5**, the aggregation in DHP vesicles decreases as the number of positive charges on the porphyrin increases, which can be explained by an increasing electrostatic repulsion between the porphyrin molecules.<sup>19</sup> In contrast, the aggregation of the porphyrins in the series **2-4** in DODAC bilayers was found to increase markedly with the number of positive charges, and 21,23-2H-5,10,15,20-tetra(1-methyl-4-pyridyl)-porphyrin tetrasylate (**5**) could not be incorporated in DODAC bilayers. These results indicate that in this case the electrostatic repulsion between porphyrin and amphiphile is the dominant factor. Steric effects are probably even more important than electrostatic effects, since the association constant of *o*-dichlorosubstituted tetraphenylporphyrin (TDCPP, **7**) in DODAC bilayers was found to be lower ( $K_{\text{ass}} = 6.5 \times 10^3 \text{ M}^{-1}$ ) than that of the other hydrophobic porphyrins studied, viz. tetraphenylporphyrin (TPP, **6**) and THPP (**1**), as well as the charged porphyrins **2-5** (typical  $K_{\text{ass}} = 1 \text{ to } 4 \times 10^4 \text{ M}^{-1}$ ). The aggregation-induced shifts in the UV-vis spectra of the porphyrins could be interpreted according to the exciton model.<sup>20</sup> We concluded that in DODAC bilayers, the charged porphyrins **2-4** form face-to-face aggregates, whereas the hydrophobic porphyrin **6** aggregates in an edge-to-edge fashion.<sup>19</sup> The orientation of the porphyrins in the bilayers was investigated by measuring the EPR spectra of the porphyrins in dried bilayers at various orientations.<sup>18,19</sup> It was established that monomers of **7** have an orientation perpendicular to the normal of the DODAC bilayer.<sup>19</sup> We decided to use porphyrin **7** as the catalytic group in our cytochrome P-450 model (see below),<sup>21</sup> because its aggregation appears to be prohibited by the bulky *o*-substituents and therefore its catalytic efficiency should not be impaired.

CHART 2

M = 2 H, Mn(III)Cl, Fe(III)Cl



The efficiency of the reduction of the amphiphilic Rh complex (AmphRh, (N-(2-(2-(2,2'-bipyridyl-5-carbosamido)-ethoxy)ethyl)-N,N-dihexadecyl-N-methylammonium)rhodium trichloride) by formate in bilayers of various types of amphiphiles, viz. cationic (DODAC), anionic (DHP), and zwitterionic (DPPC), and at various pH values was investigated. The pH determines whether the reduced Rh complex occurs as Rh(III)H<sup>-</sup> or Rh(I). The intrinsic pK<sub>a</sub> of AmphRh is altered upon incorporation in the bilayers, because protons are electrostatically attracted and repelled at anionic and cationic vesicle surfaces, respectively.<sup>22</sup> As expected, the pK<sub>a</sub><sup>obs</sup> value of reduced AmphRh in the bilayers was found to decrease in the series DHP>DPPC>DODAC (Table 1).<sup>23</sup> At pH values where reduced AmphRh is expected to occur only as Rh(I), the rate of its reduction by formate increased in the series DHP<DPPC<DODAC (Table 1), in line with the higher concentration of formate ions expected at the surface of the cationic vesicles. The reduction rates of porphyrins incorporated in bilayers together with AmphRh increased in the same order, because in the sequence of reactions leading to the reduction of Mn(III) porphyrin by formate, the formation of the AmphRh-formate complex is the rate-determining step.

Table 1. Apparent pK<sub>a</sub> (pK<sub>a</sub><sup>obs</sup>) of AmphRh, rate of its reduction by formate, rate of reduction of 7.Mn(III)Cl by AmphRh and formate, and turnover number in the epoxidation of styrene, in various bilayers at various temperatures just above the phase transitions.

Surfactant	AmphRh		7.Mn(III)Cl	
	pK <sub>a</sub> <sup>obs</sup>	k <sub>1</sub> (ms <sup>-1</sup> ) <sup>a</sup>	k <sub>0</sub> (nmol.l <sup>-1</sup> s <sup>-1</sup> ) <sup>b</sup>	turnover no. (h <sup>-1</sup> ) <sup>c</sup>
DHP (72-75 °C)	8.6 <sup>d</sup>	10 <sup>d,e</sup>	60 <sup>f</sup>	55 <sup>f,g</sup>
DPPC (47-48 °C)	5.7 <sup>h</sup>	3.2 <sup>h,i</sup>	12.4 <sup>j</sup>	4 <sup>j,k</sup>
DODAC (47-48 °C)	4.9 <sup>h</sup>	13 <sup>h,l</sup>	5.9 <sup>i</sup>	
DODAC (72-75 °C)	5.0 <sup>d</sup>	25 <sup>d,l</sup>	>100 <sup>f</sup>	0 <sup>f,g</sup>

<sup>a</sup>) Reduction rate of AmphRh, [surfactant] = 926 μM, [AmphRh] = 72 μM; <sup>b</sup>) Reduction rate of

7.Mn(III)Cl by AmphRh in ethylmorpholine (50 mM) - sodium formate (250 mM) buffer, pH 7.0;

<sup>c</sup>) Turnover number of the epoxidation by the system in Fig. 1, [Mn-7] = 2.4 μM, [AmphRh] = 2.4 \*n μM (n = AmphRh/Mn-7 ratio), [N-methylimidazole] = 3.5 μM, [surfactant] = 910 μM, ethylmorpholine (50 mM) - sodium formate (250 mM) buffer, pH 7.0, [styrene] = 200 μM; <sup>d</sup>) at 72 °C; <sup>e</sup>) at pH 10.5; <sup>f</sup>) at 75 °C; <sup>g</sup>) [AmphRh]/[Mn-7] = 10; <sup>h</sup>) at 47 °C; <sup>i</sup>) at pH 7.0; <sup>j</sup>) at 48 °C; <sup>k</sup>) [AmphRh]/[Mn-7] = 5; <sup>l</sup>) at pH 5.9.

When the rates of epoxidation of styrene in the reaction sequence of our cytochrome P-450 mimic (Fig. 1) were studied at pH 7, the relative rates were found to be reversed, with total absence of product formation in the case of DODAC. For epoxidation to occur, an efficient supply of protons to the vesicle surface appears to be important, probably for the step in which the Mn(II)-O<sub>2</sub> complex breaks down into the active epoxidizing Mn(V)=O species and water. With  $\alpha$ -pinene as the substrate, a turnover number of 360 was observed in DHP vesicles above the phase transition temperature. This is comparable to the turnover numbers of cytochrome P-450 itself at room temperature.

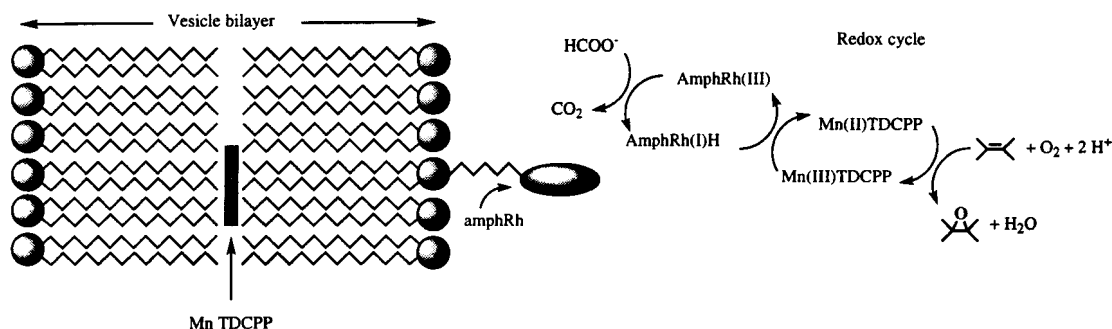


Figure 1. Membrane-bound bimetallic Cytochrome P-450 mimic.

### IRON-SULFUR CLUSTERS

[4Fe4S] or cubane clusters are coordinated by cysteine thiol ligands in proteins, and can occur in three redox states:<sup>24</sup>



In different classes of electron transfer proteins, nature has modulated the protein environment of these clusters in such a way that one of the redox transitions is selected and the potential is tuned to the required value. Most [4Fe4S] ferredoxins cycle between the 2- and 3- states (2+ and 1+ if only the cluster core is considered) at midpoint potentials between -700 and +10 mV (vs. NHE at pH 7.0), but in one specific subclass, the so-called high potential iron-sulfur proteins (HiPIP), the [4Fe4S] centers cycle between the 1- and 2- states (3+ and 2+ for the core) at potentials between +100 and +450 mV.<sup>25</sup> From crystal structures, it emerges that one difference between most ferredoxins and the HiPIPs is the number of N-H...S bonds to the cluster (8 and 5, respectively).<sup>26</sup> Another important feature that stabilizes the 1- state in HiPIPs is the conserved hydrophobic environment around the [4Fe4S] cluster.<sup>27</sup> There must, however, be other factors that tune the midpoint potentials within each (sub)class of [4Fe4S] proteins,<sup>28</sup> and the study of relevant model compounds can help elucidate these factors. Furthermore, there is an increasing number of enzymes, e.g. cis-aconitase,<sup>29</sup> that have been found to contain [4Fe4S] clusters which have one coordination site open. In the reactions catalysed by these enzymes, the [4Fe4S] cluster is more likely to act as a Lewis acid than as a redox-active group. Numerous examples of models with ligands that bring about site-differentiation in [4Fe4S] clusters are known.<sup>30</sup> Nature also uses subsite-differentiation in order to couple the cluster to other cofactors, e.g. to a siroheme by a sulfide bridge as in the proposed structure for the cofactor arrangement in assimilatory sulfite reductase,<sup>31</sup> which has been shown to be feasible in models.<sup>32</sup> It should be noted that this arrangement is still under dispute,<sup>33</sup> and that it has been proposed that the spectroscopic complexity of this and other biological systems can be explained by assuming that they contain 'superclusters',<sup>34</sup> for example the so-called prismane ([6Fe6S])<sup>35</sup> cluster.

Following the cavitant concept,<sup>36</sup> various groups<sup>37,38</sup> have designed ligands that have thiol functions preorganized for the encapsulation of a [4Fe4S] cluster. We have taken the diphenylglycoluril clip,<sup>39</sup> and extended it with 4 polyethyleneglycol arms terminated with thiols. An exchange reaction of the chlorines in (Bu<sub>4</sub>N)<sub>2</sub>[Fe<sub>4</sub>S<sub>4</sub>Cl<sub>4</sub>] with the 4 thiolates of the diphenylglycoluril derivative gives a cluster complex 8.[4Fe4S], in which the cluster is semi-encapsulated (Fig. 2).<sup>40</sup> During this reaction, the gradual disappearance of the 2-/3- wave of the starting cluster at -1.34 V (vs. Fe<sup>+/0</sup>) and the concomitant appearance of the 2-/3- wave of the product at -1.70 V were observed with cyclic voltammetry (CV) and

differential pulse polarography (DPP). The product showed interesting electrochemical behaviour since metal ions like  $\text{Ba}^{2+}$  and  $\text{Na}^+$  gave a considerable increase in the current response (Table 2).<sup>40</sup> This promoting effect of cations has also been observed in electrochemical studies on a number of proteins.<sup>41</sup> We established by scanning tunneling microscopy (STM) and microanalysis by X-rays that  $\text{Ba}^{2+}$  ions are absorbed on the electrode.<sup>42</sup> A mechanism for the modulation has been proposed in which the  $\text{Ba}^{2+}$  ions help orient the clusters on the electrode in a position that is favourable for electron transfer.

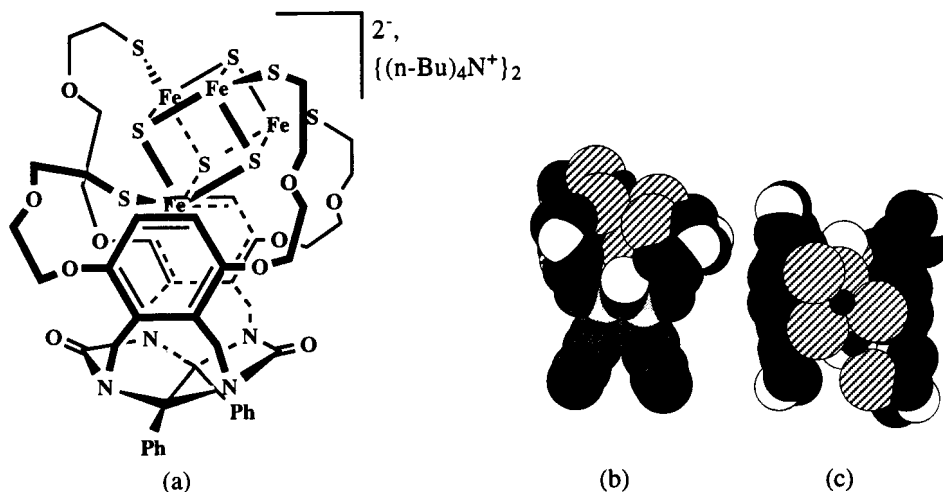


Figure 2. (a) Schematic drawing of the cluster complex, **8**. $[\text{4Fe4S}]$ . (b) and (c), side and top view, respectively, of space filling models of the cluster complex.

Cyclotrimeratrylenes (CTVs, **9**) are cavitands that can be prepared by trimerisation of veratryl alcohol derivatives. Their chemistry has been explored in detail<sup>43</sup> and appeared to be an ideal starting point for the development of a tripodal ligand for  $[\text{4Fe4S}]$  clusters in which subtle variations can be made in order to probe electronic effects and the effects of parameters mentioned above, viz. hydrogen bonding and hydrophobicity. We established that cluster complexes could be obtained from derivatives of **9** both with aliphatic and aromatic spacers between the thiol ligands and the cyclotrimeratrylene core, for example by exchange reactions of  $[\text{Fe}_4\text{S}_4\text{Cl}_4](\text{NBu}_4)_2$  or  $[\text{Fe}_4\text{S}_4\text{Cl}_4](\text{PPh}_4)_2$  with **9a** and **9d**, respectively. The cluster complexes were characterised by  $^1\text{H-NMR}$ , Mössbauer, and electrochemistry.<sup>44-46</sup> The addition of  $\text{Ba}^{2+}$  ions was found both to promote and to modulate the electrochemistry, resulting in an increased current response as well as a shift in the midpoint potential (Table 2). With  $\text{Fe}_4\text{S}_4\text{Cl}_4$  as the starting material, a cluster complex was obtained in which the unique Fe and its coordinating Cl were pointing into the cavity (Cl(in)isomer, Fig.3). This cluster complex showed reactivity only with small reagents like  $\text{OH}^-$ . Starting with the more bulky  $[\text{Fe}_4\text{S}_4\text{S}^t\text{Bu}_4]^{2-}$ , the unique iron pointed outwards and was susceptible to substitution reactions, e.g. with acyl chlorides yielding the Cl(out) isomer (Fig.3).<sup>45</sup> Starting from this isomer, ligand exchange reactions could be carried out exclusively at the unique iron site of the subsite-differentiated cyclotrimeratrylene  $[\text{4Fe4S}]$  cluster complex.<sup>47</sup> The effects of a variety of thiolate, phenolate, bidentate, and bridging ligands on the redox potential of the subsite-differentiated  $[\text{Fe-4S}]$  cluster complex were studied. The redox potential could be modulated within the range of  $-1.60$  to  $-1.80$  V (vs.  $\text{Fc}^{0/+}$ ) by varying the ligand, with the value of the redox potential depending on the electron donating and electron withdrawing properties of the ligands. Comparison with the effects of such ligands on a  $[\text{Fe}_4\text{S}_4\text{Cl}_4]^{2-}$  cluster showed that a linear relationship exists between the number of sites that are substituted and the effects on the reduction potential of the cluster. In an attempt to model the putative arrangement of cofactors in the active site of sulfite reductase,<sup>31</sup> **9d**. $[\text{4Fe4S}]^t\text{S}^t\text{Bu}$  was reacted with  $\text{Fe(III)TPP}\cdot\text{Cl}$  ( $6\cdot\text{Fe(III)Cl}$ ).<sup>43,47</sup> The spectroscopy (UV-vis, EPR, NMR) and electrochemistry of the product implicated an interaction between the porphyrin and the cluster, but it could not be established whether  $\text{S}^t\text{Bu}$  was retained as the bridging ligand.

CHART 3

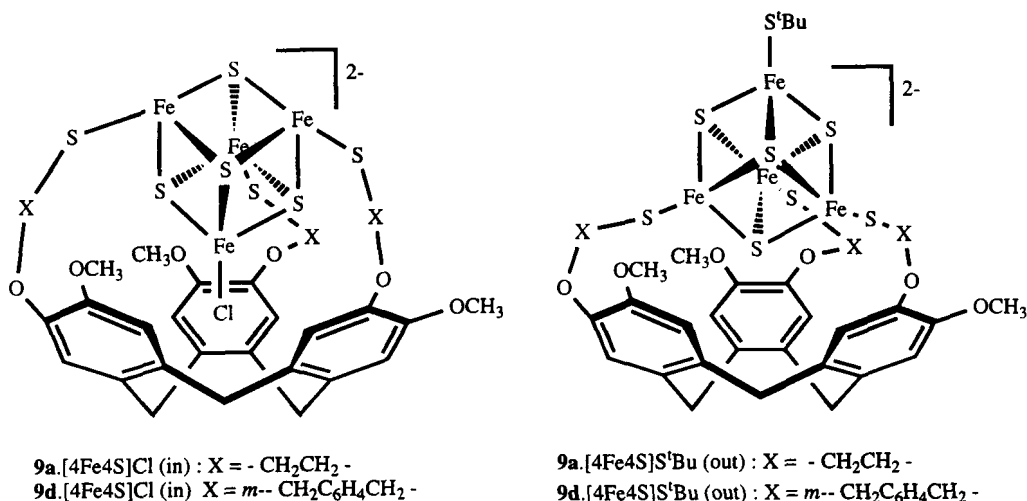
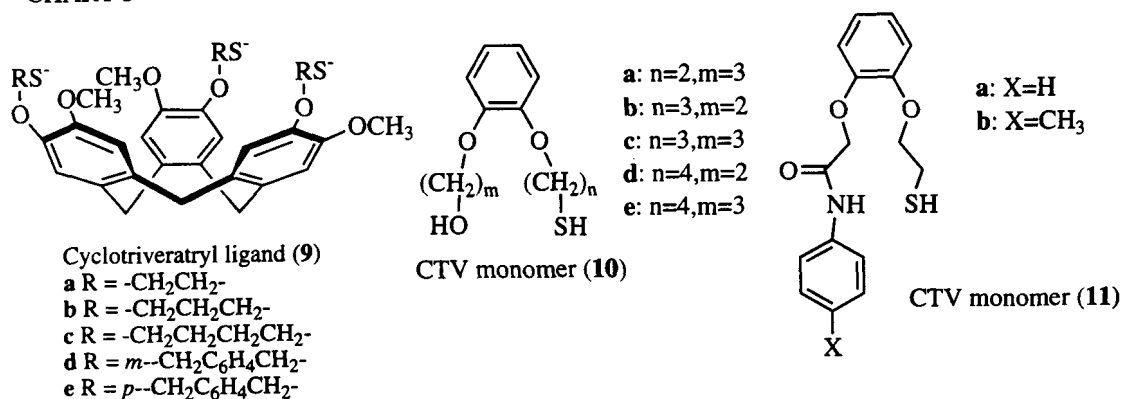
Figure 3. In/out isomerism of CTV-[4Fe<sub>4</sub>S]-Cl complexes

Table 2. Electrochemical properties of the cluster compounds of **8** and **9** at approx. 25 °C in dimethylformamide (DMF) using a Pt auxiliary electrode, a Ag/AgCl reference electrode, and 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte.

Compound	without modulator		with Ba(ClO <sub>4</sub> ) <sub>2</sub>	
	E <sub>1/2</sub> (V) <sup>a</sup>	i <sub>pa</sub> /i <sub>pc</sub>	E <sub>1/2</sub> (V) <sup>a</sup>	i <sub>pa</sub> /i <sub>pc</sub>
<b>8</b> . [4Fe <sub>4</sub> S] <sup>b</sup>	-1.70	-	-1.67 <sup>c</sup>	0.85
<b>9a</b> . [4Fe <sub>4</sub> S]Cl (in) <sup>e</sup>	-1.68	1.0	-1.64 <sup>d</sup>	0.79
<b>9b</b> . [4Fe <sub>4</sub> S]Cl (in) <sup>e</sup>	-1.74	-	-1.65 <sup>f</sup>	-
<b>9c</b> . [4Fe <sub>4</sub> S]Cl (in) <sup>e</sup>	-1.78	-	-1.70 <sup>f</sup>	-
<b>9a</b> . [4Fe <sub>4</sub> S]S <sup>t</sup> Bu (out) <sup>e</sup>	-1.80	0.9	-1.72 <sup>f</sup>	1.0
<b>9d</b> . [4Fe <sub>4</sub> S]Cl (in) <sup>e</sup>	-1.69	1.1	-1.62 <sup>f</sup>	1.0
<b>9d</b> . [4Fe <sub>4</sub> S]S <sup>t</sup> Bu (out) <sup>e</sup>	-1.78	1.0	-1.70 <sup>f</sup>	1.0
<b>9e</b> . [4Fe <sub>4</sub> S]Cl (out) <sup>e</sup>	-	-	-1.63 <sup>f</sup>	-
<b>9e</b> . [4Fe <sub>4</sub> S]Cl (in) <sup>e</sup>	-1.69	-	-1.62 <sup>f</sup>	-

a) Potentials vs. Fe<sup>+/0</sup> in DMF; b) From ref.40; c) 5 mM Ba(ClO<sub>4</sub>)<sub>2</sub>; d) 25 mM Ba(ClO<sub>4</sub>)<sub>2</sub>; e) From ref.45; f) 20 mM Ba(ClO<sub>4</sub>)<sub>2</sub>

In our studies of the functionalization of the CTV-building block we have investigated the effects of hydrophobic environment, hydrogen bonding, and electron density on the redox potential of the cluster complex.<sup>46</sup> The hydrophobicity was varied by changing the length of the spacer between the CTV and the cluster core. The redox potential was found to become more negative with increasing chain length (Table 2), which is explained by the longer distance between the cluster and the electron withdrawing phenoxy moiety of the CTV. Attempts to investigate hydrogen bonding effects were made by the synthesis of the 'CTV monomers' **10a-e** with one thiol and one alcohol spacer per phenyl unit, and comparison with corresponding derivatives where no H-bonding was possible. With CH<sub>2</sub>Cl<sub>2</sub> as the solvent, no significant differences were found, however, in DMF, which is expected to disrupt weak hydrogen bonds, a small negative shift was observed, opposite to what was anticipated. It was concluded that H-bonds do not play a significant role in either solvent. Finally, the effects of electron withdrawing substituents on an aromatic amide group, that could possibly hydrogen bond to the thiol coordinating the [4Fe4S] cluster, were investigated by synthesizing **11a** and **b** and its cluster complexes, and measuring the redox potential. A weak effect was found upon substitution of methyl for H, viz. a decrease, as expected.

In principle, it should be possible to fully encapsulate a prismane or [6Fe6S] cluster with 2 CTV ligand molecules. Preliminary attempts to achieve this with ligand **9c** have yielded black precipitates which defied detailed characterization. The EPR spectra<sup>44</sup> showed some of the features expected for 'superclusters'.<sup>34</sup> These are probably due to traces of the expected product, as the elemental analysis indicated that the cluster in the bulk of the precipitate was decomposed.

## CONCLUDING REMARKS

It can be concluded from the results presented here, and from other studies in our group on diphenylglycoluril baskets with Cu<sup>48,49</sup> and Rh<sup>50</sup> ligands which was not included in this overview, that the chemistry of both self-assembling systems and cavitands yield interesting supramolecular mimics for metalloproteins.

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## REFERENCES

- 1) Bio-inorganic Chemistry, I. Bertini, H.B. Gray, S.J. Lippard, and J.S. Valentine, editors, University Science Books, Mill Valley, California (1994)
- 2) J.M. Lehn, *Angew. Chem., Int. Ed. Engl.* 27 (1988) 89
- 3) F. Vögtle, *Supramolecular Chemistry*, John Wiley and Sons, Chichester (1991)
- 4) M.C. Feiters, in 'Comprehensive Supramolecular Chemistry' (J.M. Lehn, editor), Vol. 10: 'Supramolecular Technology and Applications' (D.N. Reinhoudt, volume editor), to appear in 1996
- 5) F.P. Guengerich and T.L. Macdonald, *Acc. Chem. Res.* 17 (1984) 9
- 6) D. Mansuy, *Pure Appl. Chem.* 59 (1987) 759
- 7) T. Poulos, B.C. Finzel, I.C. Gonsalus, G.C. Wagner, and J. Kraut, *J. Biol. Chem.* 260 (1985) 16122
- 8) M.J. Gunter and P. Turner, *Coord. Chem. Rev.* 108 (1991) 115
- 9) J.P. Collman, X. Zhang, V.J. Lee, E.S. Uffelman, and J.I. Brauman, *Science* 261 (1993) 1404
- 10) M.J. Coon and R.E. White, in 'Metal ions in Biology' (T.G. Spiro, editor), Vol. 5: 'Metal Ion Activation of Dioxygen', p. 73, John Wiley and Sons, New York (1980)
- 11) A.B. Sorokin, A.M. Khenkin, S.A. Marakushev, A.E. Shilov, and A.A. Shteinman, *Dokl. Akad. Nauk SSSR* 29 91984) 1101
- 12) J. van Esch, M.F.M. Roks, and R.J.M. Nolte, *J. Am. Chem. Soc.* 108 (1986) 6093
- 13) J.T. Groves and R. Neumann, *J. Am. Chem. Soc.* 109 (1987) 5045
- 14) I. Tabushi and A. Yazaki, *J. Am. Chem. Soc.* 103 (1981) 7371

- 15) P.A.Gosling, M.A.M.Hoffmann, J.H. van Esch, and R.J.M.Nolte, *J.Chem.Soc., Chem.Commun.* (1993) 472
- 16) R.Ruppert, S.Herrmann, E.Steckhan, *J.Chem.Soc., Chem.Commun.* (1988) 1150
- 17) J.H. van Esch, A.-M.P.Peters, and R.J.M.Nolte, *J.Chem.Soc., Chem.Commun.* (1990) 638
- 18) J.H. van Esch, M.C.Feiters, A.M.Peters, and R.J.M.Nolte, *J.Phys.Chem.* 98 (1994) 5541
- 19) A.P.H.J.Schenning, D.H.W.Hubert, M.C.Feiters, and R.J.M.Nolte, submitted to *Langmuir*
- 20) R.Kasha, H.R.Rawls, M.Asraf El-Bayoumi, *Pure Appl. Chem.* 11 (1965) 371
- 21) A.P.H.J.Schenning, D.H.W.Hubert, J.H. van Esch, M.C.Feiters, and R.J.M.Nolte, *Angew.Chem. Int.Ed.Engl.* 33 (1994) 2468
- 22) M.S.Fernandez and P.Fromherz, *J.Phys.Chem.* 81 (1977) 1755
- 23) A.P.H.J.Schenning, J.H.Lutje Spelberg, D.H.W.Hubert, M.C.Feiters, and R.J.M.Nolte, submitted to *J.Am.Chem.Soc.*
- 24) C.W.Carter, jr., J.Kraut, S.T.Freer, R.A.Alden, L.C.Sieker, E.Adman, L.H.Jensen, *Proc.Natl.Acad.Sci.U.S.A.* 69 (1972) 3526
- 25) Iron-sulfur Protein Research, H.Matsubara, Y.Katsube, K.Wada, eds. (1986) Springer-Verlag, Berlin
- 26) E.Adman, K.D.Watenpaugh, and L.H.Jensen, *Proc.Natl.Acad.Sci.U.S.A.* (1975) 4854
- 27) I.Rayment, G.Wesenberg, T.E.Meyer, M.A.Cusanovich, H.M.Holden, *J.Mol.Biol.* 228 (1992) 686
- 28) G.Backes, Y.Mino, T.M.Loehr, T.E.Meyer, M.A.Cusanovich, W.V.Sweeney, E.T.Adman, and J.Sanders-Loehr, *J.Am.Chem.Soc.* 113 (1991) 2055
- 29) H.Lauble, M.C.Kennedy, H.Beinert, and C.D.Stout, *Biochemistry* 31 (1992) 2735
- 30) R.H.Holm, S.Ciurli, and J.A.Weigel, *Prog.Inorg.Chem.* ed. S.J.Lippard, 38 (1990) 1
- 31) D.E.McRee, D.C.Richardson, J.S.Richardson, and L.M.Siegel, *J.Biol.Chem.* 261 (1986) 10277
- 32) L.Cai, J.A.Weigel, and R.H.Holm, *J.Am.Chem.Soc.* 115 (1993) 9289
- 33) A.J.Pierik and W.R.Hagen, *Eur.J.Biochem.* 195 (1991) 505
- 34) W.R.Hagen, A.J.Pierik, and C.Veege, *J.Chem.Soc., Faraday Trans.I.* 85 (1989) 4083
- 35) M.G.Katzinidis, W.R.Hagen, W.R.Dunham, R.K.Lester, and D.Coucovanis, *J.Am.Chem.Soc.* 107 (1985) 953
- 36) D.J.Cram, *Science* 219 (1983) 1177
- 37) T.D.P.Stack and R.H.Holm, *J.Am.Chem.Soc.* 109 (1987) 2546
- 38) D.J.Evans, G.Garcia, G.J.Leigh, M.S.Newton, M.D.Santana, *J.Chem.Soc., Dalton Trans.* (1992) 3229
- 39) J.W.H.Smeets, R.P.Sybesma, F.G.M.Niele, A.L.Spek, W.J.J.Smeets, and R.J.M.Nolte, *J.Am.Chem.Soc.* 109 (1987) 928
- 40) C.F.Martens, H.L.Blonk, T.Bongers, J.G.M.van der Linden, G.Beurskens, P.T.Beurskens, J.M.M.Smits, and R.J.M.Nolte, *J.Chem.Soc., Chem.Commun.* (1991) 1623
- 41) F.A.Armstrong, H.A.O.Hill, and N.J.Walton, *Acc.Chem.Res.* 21 (1988) 407
- 42) C.F.Martens, Ph.D.Thesis (1993) University of Nijmegen, C.F.Martens, P.J.A.Kenis, M.M.G.Bongers, M.C.Feiters, R.Czajka, J.G.M. van der Linden, and R.J.M.Nolte, *Inorg.Chim.Acta*, manuscript in preparation
- 43) A.Collet, *Tetrahedron* 43 (1987) 5725
- 44) G.P.F. van Strijdonck, PhD Thesis (1994), University of Nijmegen
- 45) G.P.F. van Strijdonck, J.A.E.H. van Haare, J.G.M. van der Linden, J.J.Steggerda, and R.J.M.Nolte, *Inorg.Chem.* 33 (1994) 999
- 46) G.P.F. van Strijdonck, J.A.E.H. van Haare, P.Hönen, R.G.C.M. van den Schoor, M.C.Feiters, J.G.M. van der Linden, J.J.Steggerda, and R.J.M.Nolte, *J.Chem.Soc., Dalton Trans.*, manuscript in preparation
- 47) G.P.F. van Strijdonck, P.T.J.H. ten Have, A.J.Nijdam, R.J.M.Klein Gebbink, M.C.Feiters, J.G.M. van der Linden, J.J.Steggerda, and R.J.M.Nolte, *Inorg.Chem.*, manuscript in preparation
- 48) C.F.Martens, R.J.M.Klein Gebbink, P.J.A.Kenis, A.P.H.J.Schenning, M.C.Feiters, J.L.Ward, K.D.Karlin, and R.J.M.Nolte, in *Bioinorganic Chemistry of Copper* (K.D.Karlin and Z.Tyeklár, eds.) Chapman & Hall, New York, London (1993) p. 374
- 49) C.F.Martens, R.J.M.Klein Gebbink, M.C.Feiters, and R.J.M.Nolte, *J.Am.Chem.Soc.* 116 (1994) 5667
- 50) H.K.A.C.Coolen, P.W.N.M. van Leeuwen, and R.J.M.Nolte, *Angew.Chem., Int.Ed.Eng.* 31 (1992) 905