**[g007]**

# Calculation of partition coefficients of Fe–S/Se protein models

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**Abstract**

A method permits semiquantitative estimation of partitioning of solutes between pairs of media. The organic solvent-water partition coefficients $P$ are calculated. For $Fe_4S_4Cys_n$, the organic solvent–water partition coefficients for 1-octanol $P_o$, cyclohexane $P_{ch}$ and chloroform $P_{cf}$ decrease 4.46, 6.25 and 4.60 per Cys, respectively. $P_o$ are in line with CDHI calculations, and $P_{ch–cf}$, with calculations performed with a method by Leo–Hansch. Log$P_{o–ch–cf}$ mean relative errors are –17%, 25% and –17%, which represent mean and unsigned relative errors of –3% and 20%. On varying the number of Cys, the structures show hydrophobic moments indicative of amphipathic structures. For Se substitutions in $Fe_4Se_4Cys_n$ $P_{o–ch–cf}$ decrease 4.52, 6.30 and 4.66 per Cys. With the references $P_{o–ch–cf}$ decrease 4.03, 4.80 and 5.76 per Cys. The similar calculated partition coefficients and hydrophobic moments for $Fe_4S_{4–m}Se_mCys_4$ suggest a role of FeSe clusters in physiological processes.

*Keywords:* Solvation parameter model; Partition coefficient; Hydrophobic moment; Iron-sulphur protein; Iron-selenium protein; Mixed-valence non-integer oxidation number; Cysteine ligand.

## Introduction

Ferredoxin is an example for many redox enzymes present in living organisms containing Fe–S clusters, which mediate electron-transfer reactions. The enzyme nitrogenase, which catalyzes the reduction of $N_2$ to $NH_3$, contains such clusters as well, but in its structure some of the Fe atoms have been replaced by Mo. Some other enzyme systems, which contain Fe–S clusters, are rubredoxin, adrenodoxin and putidaredoxin. In general, Fe–S clusters in these types of enzymes possess two or four atoms of Fe and equal number of inorganic sulphide ions, which are called *labile sulphur* because, on treatment with acid, $H_2S$ gas is produced. However, those enzymes also contain organic S in the structures of ligands of Fe atom, which are cysteinyl (Cys)–

sulphydryl (–SH) groups of the protein. Ferredoxin is a non-heme Fe protein and stands for the best-known examples of Fe–S proteins found in bacteria and chloroplast. However, several different proteins are known by this name, *e.g*. soluble ferredoxins or bound ferredoxin ($FD_X$, $FD_A$ and $FD_B$), *etc*. Some of the soluble ferredoxins obtained from some bacteria contain two of the $[F_4S_4]$ units. However, the structures of some ferredoxins are still obscure. Some experimental and theoretical studies have been published on $[F_2S_2]$ and $[F_4S_4]$ type Fe–S clusters or ferredoxin/ferrodoxin from living organisms [1–10]. Partly, the experimental work was concentrated on structure elucidation of Fe–S containing natural products [11]. On the other hand, the theoretical studies were mainly on modelling of ferrodixin/ferredoxin-like systems. Due to the complexity of the biochemistry behind these Fe–S clusters present in living organisms, some sort of model studies are necessary to understand the basic behaviour of these systems or part of them.

High-potential Fe–S proteins (HiPIPs) are a class of small electron-transfer metalloproteins, predominantly found in purple S bacteria [12-19]. The $[Fe_4S_4]$ is widespread in nature, functioning in electron transfer, catalytic or other roles in various proteins including ferredoxins, hydrogenase, aconitase and gene regulatory proteins; $[Fe_4S_4]$ is an element of complex centres, *e.g*. the P-cluster of nitrogenase. The HiPIPs are studied by a variety of biophysical techniques and are important paradigms in elucidating the structural determinants of the physicochemical properties of both $[Fe_4S_4]$ and biological metallocentres. The HiPIPs have relatively high midpoint reduction potentials of 50–450mV. The cluster of the reduced HiPIP has a charge of +2 and spin of zero. Mössbauer spectra indicate that the Fe atoms are in equivalent, $Fe^{2.5+}$, oxidation states. The oxidized HiPIP cluster, with a charge of +3 and spin of 1/2, is made up of two antiferromagnetically coupled spin pairs. One spin pair consists of two $Fe^{3+}$ whereas the other, of two $Fe^{2.5+}$, which is consistent with the chemical shift of the $\beta CH_2$ Hs of the cluster Cys and their temperature dependence. Nuclear magnetic resonance (NMR) work demonstrates that the position of the spin pairs within the protein framework varies from one HiPIP to another and may be determined by the relative reduction potentials of the individual Fe atoms. A third oxidation state of $[Fe_4S_4]$ possesses a charge of +1, which corresponds to the reduced ferredoxin cluster, and has been observed in HiPIPs only when the protein is partially denatured. The cluster contains two $Fe^{3+}$ ions and a mixed-valence $Fe^{2.5+}$ pair [20,21]. The cluster of HiPIP II from *Ectothiorhodospira halophila* is the best understood system: its electron spin resonance spectrum at 4K is consistent with an $S = 1/2$ ground state [22], and magnetic Mössbauer data indicate that this state is the result of antiferromagnetic coupling between the mixed-valence pair and the $Fe^{3+}$ pair, the former having a larger subspin than the latter [23,24]. The interpretation of the electronic structure of the oxidized cluster of HiPIP II from *E. halophila* is consistent with NMR data [25,26]. The NMR spectrum of this protein possesses two clearly divided sets of four signals arising

from Cys $\beta CH_2$ H signals, one set being upfield and the other downfield [27]. The spectrum was interpreted by assigning a negative $<S_z>$ to the $Fe^{2.5+}$ pair, accounting for the downfield hyperfine shifts of the $\beta CH_2$ Hs of the Cys ligated to the $Fe^{2.5+}$ pair, and a positive $<S_z>$ to the $Fe^{3+}$ pair, accounting for the upfield hyperfine shifts of the $\beta CH_2$ Hs of the Cys ligated to the $Fe^{3+}$ pair [28,29]. A pseudo-Curie behaviour is observed because the excited states of the $Fe^{3+}$ ions have negative $<S_z>$ [30]. In all other characterized HiPIPs, only one of the two Cys bound to the $Fe^{3+}$ ions has upfield-shifted $\beta CH_2$ Hs, while the $\beta CH_2$ Hs of the other Cys are downfield shifted, albeit to a lesser extent than those of the Cys bound to the $Fe^{2.5+}$ ions [31-33]. The data were interpreted by assuming the existence of an equilibrium between two electronic distributions in the cluster [34]. It was performed the sequence-specific assignment of Cys Hs of oxidized, recombinant HiPIP I from *E. halophila* [35], investigated the unfolding properties of HiPIP from *Chromatium vinosum* [36] and compared them with those of other proteins representative of various classes of Fe–S proteins [37].

The present model is an extension of the solvent-dependent conformational analysis program (SCAP) 1-octanol–water model to organic solvents, using extended versions of the functional forms [38]. In earlier publications, the method was applied to the calculation of the organic solvent–water partition coefficients of porphyrins, phtahlocyanines, benzobisthiazoles, fullerenes, acetanilides, local anaesthetics [39], lysozyme [40], barbiturates, hydrocarbons [41], polystyrene [42] and $Fe_4S_4Cys_n$ of HiPIP [43]. In the present report $Fe_4S_{4-m}Se_mCys_n$ ($0 \le m \le 4$, $0 \le n \le 4$) models have been studied. Section 2 presents the improvements in the solvation model. Section 3 is devoted to the results. Section 4 summarizes the conclusions.

The hydrophobic moment calculation is based on the Eisenberg *et al*. formula (Equation 7), where the gyration angle $\delta_i$ is the successive angle between an atom and the next, around the $z$ axis; *e.g*., $\delta$ increases 97 degrees in the successive $C^{\alpha}$ atoms of an $\alpha$-helical structure (*cf*. Figure 1).

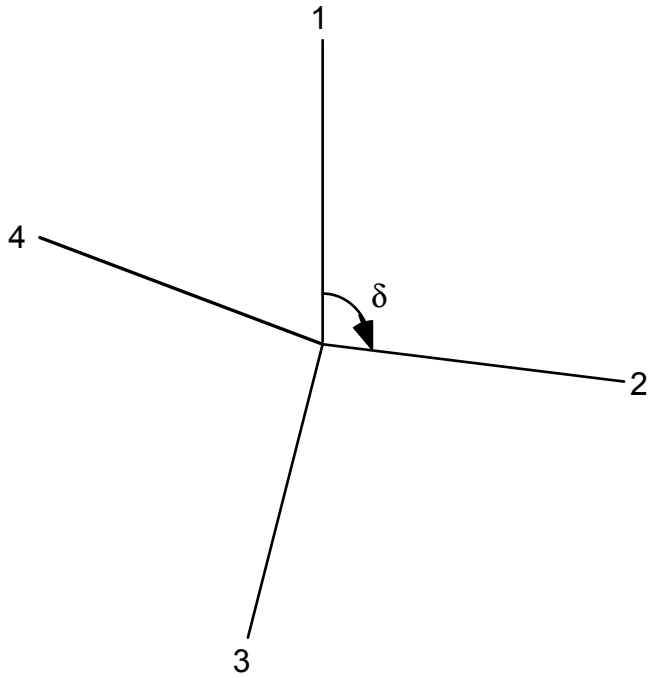


Figure 1. Top view of an $\alpha$-helix showing the gyration angle ($\delta = 97°$) in the successive $C^{\alpha}$ atoms of the first four amino acids.

**Results and discussion**

*Iron–sulphur clusters with cysteine ligands*

The free energies of solvation, partition coefficients and hydrophobic moment for $Fe_4S_4Cys_n$ ($0 \leq n \leq 4$) models of HiPIP (*cf.* Table 1) show that the organic solvent–water partition coefficients for 1-octanol $P_o$, cyclohexane $P_{ch}$ and chloroform $P_{cf}$ decrease monotonically 4.46, 6.25 and 4.60 log units per Cys ligand, respectively. Notice that for values of $\log P < 3$, more than 99.9% of the solute is in the aqueous phase. Therefore, some results predict a negligible quantity of solute in the organic phase. One minus $\log P_{ch}$ value is greater than the Avogadro number exponent 23 ($P_{ch} < 10^{-23}$). The corresponding interpretation is that no solute molecule would be present in the organic solvent, to allow experiments for validation. Furthermore, all the $\log P$ figures are kept with the only purpose of comparison along the series. The $P_o$ results are of the same order of magnitude as CDHI computations [55]. Both $P_{ch}$ and $P_{cf}$ results are of the same order of magnitude as calculations carried out with a method by Leo and Hansch [56]. With the reference methods, $P_o$, $P_{ch}$ and $P_{cf}$ decrease monotonically 4.12, 4.75 and 5.70 log units per Cys. The $\log P_o$, $\log P_{ch}$ and $\log P_{cf}$ mean relative errors (MREs) are –17%, 25% and –17%. These represent globally an MRE of –3%. However, this result should be taken with care because the mean unsigned relative error (URE) is 20%.

Table 1

Free energy of solvation, partition coefficient and hydrophobic moment results for iron–sulfur protein models

| Cluster | $\Delta G^{o}_{solv,w}$ [a] | $\Delta G^{o}_{solv,o}$ [b] | $\Delta G^{o}_{solv,ch}$ [c] | $\Delta G^{o}_{solv,cf}$ [d] | log $P_o$ (SCAP)[e] | log $P_o$ (CDHI)[f] |
|---|---|---|---|---|---|---|
| $Fe_4S_4$ | -2.6 | -2.8 | -1.4 | -2.0 | 0.03 | 0.28 |
| $Fe_4S_3Se$ | -1.4 | -3.0 | -1.6 | -2.4 | 0.29 | 0.59 |
| $Fe_4S_2Se_2$ | -0.1 | -3.2 | -1.8 | -2.8 | 0.55 | 0.90 |
| $Fe_4SSe_3$ | 1.1 | -3.5 | -2.0 | -3.2 | 0.81 | 1.21 |
| $Fe_4Se_4$ | 2.4 | -3.7 | -2.2 | -3.5 | 1.07 | 1.52 |
| $Fe_4S_4Cys$ | -53.6 | -28.2 | -16.6 | -26.6 | -4.47 | -5.60 |
| $Fe_4S_3SeCys$ | -52.3 | -28.4 | -16.8 | -27.0 | -4.19 | -5.08 |
| $Fe_4S_2Se_2Cys$ | -51.3 | -28.6 | -17.0 | -27.3 | -3.99 | -4.62 |
| $Fe_4SSe_3Cys$ | -50.1 | -28.8 | -17.1 | -27.7 | -3.75 | -4.10 |
| $Fe_4Se_4Cys$ | -48.8 | -29.0 | -17.3 | -28.1 | -3.47 | -3.76 |
| $Fe_4S_4Cys_2$ | -103.9 | -53.6 | -31.8 | -51.1 | -8.83 | -7.21 |
| $Fe_4S_3SeCys_2$ | -102.8 | -53.8 | -31.9 | -51.4 | -8.61 | -6.86 |
| $Fe_4S_2Se_2Cys_2$ | -101.7 | -54.0 | -32.1 | -51.8 | -8.39 | -6.54 |
| $Fe_4SSe_3Cys_2$ | -100.7 | -54.2 | -32.3 | -52.1 | -8.17 | -6.13 |
| $Fe_4Se_4Cys_2$ | -99.2 | -54.3 | -32.4 | -52.4 | -7.89 | -6.12 |
| $Fe_4S_4Cys_3$ | -154.3 | -79.0 | -46.9 | -75.6 | -13.2 | -14.3 |
| $Fe_4S_3SeCys_3$ | -153.2 | -79.1 | -47.1 | -75.9 | -13.0 | -13.8 |
| $Fe_4S_2Se_2Cys_3$ | -152.2 | -79.3 | -47.3 | -76.3 | -12.8 | -13.2 |
| $Fe_4SSe_3Cys_3$ | -151.2 | -79.4 | -47.4 | -76.6 | -12.6 | -12.7 |
| $Fe_4Se_4Cys_3$ | -149.9 | -79.5 | -47.5 | -76.8 | -12.4 | -12.4 |
| $Fe_4S_4Cys_4$ | -205.5 | -104.0 | -62.0 | -100.1 | -17.8 | -16.2 |
| $Fe_4S_3SeCys_4$ | -204.4 | -104.2 | -62.2 | -100.5 | -17.6 | -15.7 |
| $Fe_4S_2Se_2Cys_4$ | -203.1 | -104.2 | -62.3 | -100.7 | -17.4 | -15.0 |
| $Fe_4SSe_3Cys_4$ | -202.4 | -104.4 | -62.5 | -101.0 | -17.2 | -14.8 |
| $Fe_4Se_4Cys_4$ | -201.2 | -104.4 | -62.5 | -101.2 | -17.0 | -14.6 |

| Cluster | log $P_{ch}$ (SCAP)[g] | log $P_{ch}$[h] | log $P_{cf}$ (SCAP)[i] | log $P_{cf}$[h] | $\mu$[j] |
|---|---|---|---|---|---|
| $Fe_4S_4$ | -0.21 | -0.71 | -0.11 | 0.21 | 0.26 |
| $Fe_4S_3Se$ | 0.04 | -0.43 | 0.18 | 0.54 | 0.09 |
| $Fe_4S_2Se_2$ | 0.30 | -0.15 | 0.47 | 0.87 | 0.02 |
| $Fe_4SSe_3$ | 0.55 | 0.12 | 0.75 | 1.20 | 0.27 |
| $Fe_4Se_4$ | 0.80 | 0.40 | 1.04 | 1.54 | 0.21 |
| $Fe_4S_4Cys$ | -6.51 | -5.48 | -4.75 | -5.53 | 4.46 |
| $Fe_4S_3SeCys$ | -6.23 | -5.19 | -4.44 | -5.17 | 4.70 |
| $Fe_4S_2Se_2Cys$ | -6.04 | -4.98 | -4.22 | -4.92 | 4.73 |
| $Fe_4SSe_3Cys$ | -5.80 | -4.72 | -3.94 | -4.61 | 4.93 |
| $Fe_4Se_4Cys$ | -5.53 | -4.42 | -3.64 | -4.26 | 5.03 |
| $Fe_4S_4Cys_2$ | -12.7 | -10.1 | -9.28 | -11.1 | 3.52 |
| $Fe_4S_3SeCys_2$ | -12.5 | -9.89 | -9.02 | -10.8 | 3.81 |
| $Fe_4S_2Se_2Cys_2$ | -12.2 | -9.65 | -8.78 | -10.5 | 4.07 |
| $Fe_4SSe_3Cys_2$ | -12.0 | -9.42 | -8.53 | -10.3 | 4.40 |
| $Fe_4Se_4Cys_2$ | -11.7 | -9.12 | -8.23 | -9.90 | 4.11 |
| $Fe_4S_4Cys_3$ | -18.9 | -14.8 | -13.8 | -16.7 | 0.77 |
| $Fe_4S_3SeCys_3$ | -18.6 | -14.6 | -13.6 | -16.4 | 1.08 |
| $Fe_4S_2Se_2Cys_3$ | -18.4 | -14.4 | -13.3 | -16.2 | 0.77 |
| $Fe_4SSe_3Cys_3$ | -18.2 | -14.1 | -13.1 | -15.9 | 0.78 |
| $Fe_4Se_4Cys_3$ | -18.0 | -13.9 | -12.8 | -15.6 | 0.79 |
| $Fe_4S_4Cys_4$ | -25.2 | -19.7 | -18.5 | -22.6 | 2.64 |
| $Fe_4S_3SeCys_4$ | -25.0 | -19.5 | -18.3 | -22.3 | 2.84 |
| $Fe_4S_2Se_2Cys_4$ | -24.7 | -19.2 | -18.0 | -22.0 | 2.75 |
| $Fe_4SSe_3Cys_4$ | -24.6 | -19.0 | -17.8 | -21.8 | 2.97 |
| $Fe_4Se_4Cys_4$ | -24.4 | -18.8 | -17.6 | -21.5 | 2.82 |

[a] Gibbs free energy of solvation in water ($kJ \cdot mol^{-1}$).

[b] Gibbs free energy of solvation in 1-octanol ($kJ \cdot mol^{-1}$).

[c] Gibbs free energy of solvation in cyclohexane ($kJ \cdot mol^{-1}$).

[d] Gibbs free energy of solvation in chloroform ($kJ \cdot mol^{-1}$).

[e] $P_o$ is the 1-octanol–water partition coefficient.

[f] CDHI: calculations carried out with a method developed by Kantola *et al*.

[g] $P_{ch}$ is the cyclohexane–water partition coefficient.

[h] Calculations carried out with a method developed by Leo *et al*.

[i] $P_{cf}$ is the chloroform–water partition coefficient.

[j] $\mu$ is the hydrophobic moment.

On the other hand, the hydrophobic moment $\mu$ differentiates well the $Fe_4S_4Cys_n$ structures. On varying the number of Cys units, the structures show $\mu$ values indicative of particularly amphipathic structures as $Fe_4S_4Cys$.

*Iron–selenium clusters*

The free energies of solvation, partition coefficients and hydrophobic moment for substitutions of S by Se in $Fe_4S_{4-m}Se_m$ ($0 \le m \le 4$) models are included in Table 1. The organic solvent–water partition coefficients $P_{o-ch-cf}$ increase monotonically with the number of Se atoms. Again, $P_o$ and both $P_{ch-cf}$ results are of the same order of magnitude as CDHI calculations performed with a method by Leo and Hansch. Notice the variation in the three log$P$ values from $Fe_4S_4$ to $Fe_4Se_4$. In particular for $Fe_4S_4$ the three $P$s are *ca*. zero log unit and, for $Fe_4Se_4$, the three $P$s are *ca*. one log unit. The log$P_{o-ch-cf}$ absolute errors are –0.4, 0.5 and –0.4 log units, which represent globally an error of –0.1 and an unsigned error of 0.4 log units.

On the other hand, for $Fe_4S_{4-m}Se_m$ the similar hydrophobic moments $\mu$ (within 0.1 log unit) suggest once more the possible role of Fe–Se clusters in physiological processes.

*Iron–selenium clusters with cysteine ligands*

The free energies of solvation, partition coefficients and hydrophobic moment for substitution of S by Se in $Fe_4S_{4-m}Se_mCys_n$ ($0 \le m \le 4$, $0 \le n \le 4$) models are included in Table 1. The $P_{o-ch-cf}$ decrease for $Fe_4S_3SeCys_n$ 4.47, 6.26 and 4.62, for $Fe_4S_2Se_2Cys_n$ 4.49, 6.25 and 4.62, for $Fe_4SSe_3Cys_n$ 4.50, 6.29 and 4.64, as well as for $Fe_4Se_4Cys_n$ 4.52, 6.30 and 4.66 log units per Cys, respectively. With the reference methods $P_{o-ch-cf}$ decrease for $Fe_4S_3SeCys_n$ 4.07, 3.98 and 4.00, for $Fe_4S_2Se_2Cys_n$ 4.77, 4.76 and 4.78, for $Fe_4SSe_3Cys_n$ 5.71, 5.72 and 5.75, as well as for $Fe_4Se_4Cys_n$ 4.03, 4.80 and 5.76 log units per Cys. For $Fe_4S_{4-m}Se_mCys_n$ $P_{o-ch-cf}$ increase monotonically with the number of Se atoms. Again, $P_{o-ch-cf}$ results are of the same order of magnitude as CDHI calculations executed with a method by Leo and Hansch. The log$P_{o-ch-cf}$ MREs are for $Fe_4S_{4-m}Se_mCys$ –14%, 22% and –14%

(global MRE = –2%, URE = 16%), for $Fe_4S_{4-m}Se_mCys_2$ 28%, 27% and –17% (global MRE = 13%, URE = 24%), for $Fe_4S_{4-m}Se_mCys_3$ –3%, 28% and –18% (global MRE = 2%, URE = 16%), as well as for $Fe_4S_{4-m}Se_mCys_4$ 14%, 29% and –18% (global MRE = 8%, URE = 20%). Furthermore, for $Fe_4S_{4-m}Se_mCys_4$, the similar calculated partition coefficients (3% or 4% in the reference calculations) suggest that, possibly, Fe–Se clusters like Fe–S clusters may take role in important physiological processes.

On the other hand, for $Fe_4S_{4-m}Se_mCys_4$ the similar hydrophobic moments $\mu$ (8%) suggest, one more time, the possible role of Fe–Se clusters in physiological processes.

The relative permittivities and molecular volumes of the studied solvents at $T = 298$K (*cf.* Table 2) shows the greatest similarity in relative permittivity between chloroform and 1-octanol (4.806 and 10.34, respectively), when compared with cyclohexane (2.023) and 1-octanol. This is in concordance with the fact that for both Fe–S and –Se clusters with Cys ligands, the log$P$ absolute error for chloroform (27%) is smaller than that for cyclohexane (17%, Table 1).

Table 2

Relative permittivity and molecular volume of the solvents at $T = 298$K

| Solvent | Relative permittivity $\varepsilon$ | Molecular volume (Å$^3$) |
|---|---|---|
| water | 78.54 | 21.2 |
| 1-octanol | 10.34 | 155.0 |
| cyclohexane | 2.023 | 93.4 |
| chloroform | 4.806 | 72.1 |

The classification by *information entropy* and *equipartition conjecture* [57] is represented in Figure 2.

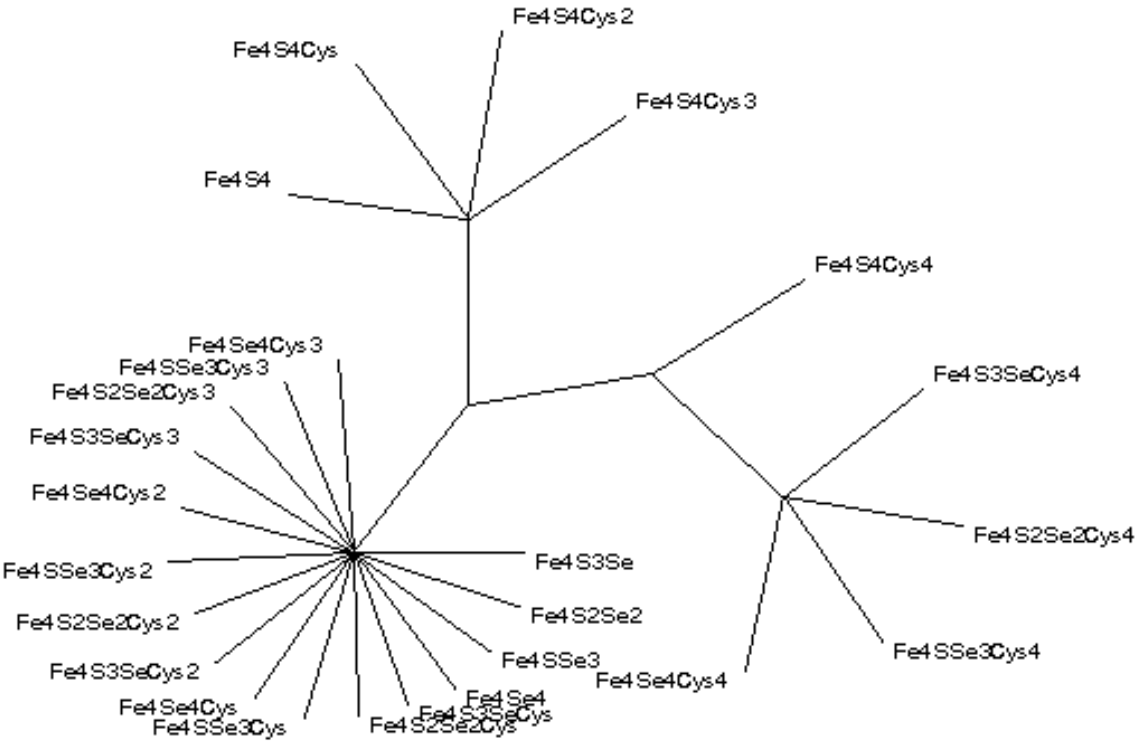


Figure 2. Radial tree for the $Fe_4S_{4-m}Se_mCys_n$ ($0 \le m \le 4$, $0 \le n \le 4$) models.

From the previous results the following conclusions can be drawn.

1. The three organic solvent–water *P*s for $Fe_4S_4Cys_n$ ($0 \leq n \leq 4$) models of HiPIP decrease *ca*. 5.1 log units per Cys ligand. With reference methods the three *P*s diminish 4.9 log units per Cys. The $\log P_{o-ch-cf}$ mean relative errors are –17%, 25% and –17%, respectively, representing mean and unsigned relative errors of –3% and 20%. For $Fe_4Se_4Cys_n$, the three log*P* drop 5.2 log units per Cys. With the references the three *P*s reduce 4.9 log units per Cys. The $\log P_{o-ch-cf}$ mean relative errors are 14%, 29% and –18%, expressing mean and unsigned relative errors of 8% and 20%. The organic solvents similar to 1-octanol in relative permittivity are those that are best modelled by SCAP. The relative permittivity results a physicochemical property more important than molecular volume in characterizing different solvents.

2. The hydrophobic moment differentiates well $Fe_4S_4Cys_n$ structures. On varying the number of Cys units, the structures show hydrophobic moments indicative of particularly amphipathic structures as $Fe_4S_4Cys$. An extension of the present study to other proteins would give an insight into a possible generality of this conclusion. Further work is in progress on the characterization of lysozyme.

3. The $P_o$ results are in line with CDHI calculations, as well as $P_{ch}$ and $P_{cf}$ results, with calculations carried out with a method by Leo and Hansch. The similar calculated partition coefficients and hydrophobic moments for $Fe_4S_{4-m}Se_mCys_4$ suggest that possibly Fe–Se clusters, like Fe–S clusters may take role in important physiological processes.

**Experimental Procedures**

SCAP is based on the model of Hopfinger parameterized for the 1-octanol–water solvent pair with solute molecules composed of H, C, N, O, F, S, Cl and Br, and containing a wide variety of functional groups [44,45]. SCAP was initially used to calculate the Gibbs free energy of solvation of molecules. From these data and with the equation

$$RT \ln P = \Delta G^{o}_{\mathrm{solv}}\ (\mathrm{water}) - \Delta G^{o}_{\mathrm{solv}}\ (1\text{-octanol}) \qquad (1)$$

one can calculate the logarithm log*P* at a given *T*, which is taken as 298K. *R* is the gas constant, and $\Delta G_{solv}$°(1-octanol) and $\Delta G_{solv}$°(water), in kJ·mol$^{-1}$, are the standard-state free energies of solvation of a given solute considered in 1-octanol and water, respectively. SCAP manages up to 4 fitting parameters for a given solvent: (1) $n$ = maximum number of solvent molecules allowed for filling the solvation sphere; (2) $\Delta g^{o}$ = variation of Gibbs free energy associated with the extraction of one solvent molecule out of the solvation sphere; (3) $R_v$ = radius of the solvation sphere and (4) $V_f$ = free volume available for a solvent molecule in the solvation sphere. In order to generalize SCAP for a different organic solvent, it was decided not to fit new

parameters, because of the lack of available experimental data for many solvents. Instead, the four parameters of 1-octanol have been used, but modified taking into account the effect of only the new permittivity and molecular volume on the original parameters of 1-octanol.

For a general organic solvent, *e.g.*, cyclohexane, the maximum number of solvent molecules allowed to fill the solvation sphere is related to the molecular volume of the solvent molecule as

$$n_s = n_o \left( \frac{V_s}{V_o} \right)^{\log \frac{n_o}{n_w} \Big/ \log \frac{V_o}{V_w}} \tag{2}$$

where $V_o$, $V_w$ and $V_s$ are the molecular volumes of 1-octanol, water and a general organic solvent, respectively. The $n_o$, $n_w$ and $n_s$ are the maximum numbers of molecules of 1-octanol, water and a general organic solvent, respectively, allowed to fill the solvation sphere. The variation in the standard Gibbs free energy associated with the extraction of one solvent molecule out of the solvation sphere $\Delta g_s^o$ is calculated using the generalized Born equation,

$$\Delta g_s^o = \Delta g_o^o \frac{1 - \frac{1}{\varepsilon_s}}{1 - \frac{1}{\varepsilon_o}} = \Delta g_o^o \frac{\varepsilon_o (\varepsilon_s - 1)}{\varepsilon_s (\varepsilon_o - 1)} \tag{3}$$

where $\varepsilon_o$ and $\varepsilon_s$ are the relative permittivities [46]. The radius of the solvation sphere is related to the molecular volume of the solvent molecule as

$$R_{v,s} = R_{v,o} \left( \frac{V_s}{V_o} \right)^{1/3} \tag{4}$$

Finally, the free volume available for a solvent molecule in the solvation sphere is related to the molecular volume of the solvent molecule as

$$V_{f,s} = V_{f,o} \frac{V_s}{V_o} \tag{5}$$

The only parameters needed are the relative permittivity $\varepsilon$ and molecular volume $V_s$ of the organic solvent. $V_s$ values have been calculated with our program TOPO [47]. In the present study, the following values have been used: $\varepsilon$ = 10.34 (1-octanol), 2.023 (cyclohexane) and 4.806 (chloroform); $V_S$ = 155.0Å$^3$ (1-octanol), 93.4Å$^3$ (cyclohexane) and 72.1Å$^3$ (chloroform) [48]. The 1-octanol is a linear molecule, very mobile thus it has different volumic properties than the cyclohexane and the chloroform.

The algorithm of Kyte and Doolittle has been used to calculate the hydropathy profile of the structures [49]. The hydrophobicity of each molecule is calculated from its atomic contributions as:

$$H = \sum_{i=1}^{M} h_i \tag{6}$$

where $h_i$ is the hydrophobicity of atom $i$ and the sum extends to the number of atoms in the molecule, $M$. The hydrophobic moment calculation is based on the Eisenberg *et al*. formula:

$$\mu = \left[ \left( \sum_{i=1}^{M} h_i \cos \delta_i \right)^2 + \left( \sum_{i=1}^{M} h_i \sin \delta_i \right)^2 \right]^{1/2} \tag{7}$$

where the gyration angle $\delta_i$ is the successive angle between an atom and the next, around the $z$ axis [50-52]. For instance, $\delta$ increases 97 degrees in the successive $C^{\alpha}$ atoms of an $\alpha$-helical structure (*cf*. Figure 1). This hydrophobic moment is a widely used method for determining amphipathic helices in a protein [53,54].

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**References**

[1] J. K. Shergill, M. P. Gollinelli, R. Cammack, J. Meyer, Biochemistry 39 (1996) 12842

[2] A. L. Delacey, M. T. Bes, C. Gomezmoreno, V. M. Fernandez, J. Electroanal. Chem. 390 (1995) 69.

[3] S. L. Davy, M. J. Osborne, J. Breton, G. R. Moore, A. J. Thomson, I. Bertini, C. Lunchinat, FEBS Lett. 363 (1995) 199.

[4] J. M. Mouesca, L. Noodleman, D. A. Case, Int. J. Quantum Chem. 22 (1995) 95.

[5] C. R. Staples, E. Gaymard, A. L. Stritt-Elter, Biochemistry 13 (1998) 4612.

[6] J. M. Mouesca, J. L. Chen, L. Noodleman, D. Bashford, D. A. Case, J. Am. Chem. Soc. 116 (1994) 11898.

[7] S. E. Harding, K. P. Williams, Int. J. Biol. Macromol. 10 (1988) 318.

[8] U. Gehring, D. I. Arnon, J. Biol. Chem. 246 (1971) 4518.

[9] J. M. Mouesca, L. Noodleman, D. A. Case, B. Lamotte, Inorg. Chem. 34 (1995) 4347.

[10] L. Noodleman, C. Y. Peng, D. A. Case, J. M. Mouesca, Coord. Chem. Rev. 144 (1995) 199.

[11] M. K. Chan, D. C. Rees, S. Mukund, Abstr. PAP Am. Chem. Soc. 208 (1994) 370-Inor. Part I.

[12] E. T. Adman, L. C. Sieker, L. H. Jensen, J. Biol. Chem. 251 (1976) 3801.

[13] M. M. Benning, T. E. Meyer, I. Rayment, H. M. Holden, Biochemistry 33 (1994) 2476.

[14] R. Langen, G. M. Jensen, U. Jacob, P. J. Stephen, A. Warshel, J. Biol. Chem. 267 (1992) 25625.

[15] C. W. J. Carter, J. Kraut, S. T. Freer, N.-H. Xuong, R. A. Alden, R. G. Bartsch, J. Biol. Chem. 249 (1974) 4212.

[16] D. R. Breiter, T. E. Meyer, I. Rayment, H. M. Holden, J. Biol. Chem. 266 (1991) 18660.

[17] C. T. Przysiecki, T. E. Meyer, M. A. Cusanovich, Biochemistry 24 (1985) 2542.

[18] G. Backes, Y. Mino, T. M. Loehr, T. E. Meyer, M. A. Cusanovich, W. V. Sweeney, E. T. Adman, J. Sanders-Loehr, J. Am. Chem. Soc. 113 (1991) 2055.

[19] J. Huang, R. L. Ostrander, A. L. Rheingold, Y. Leung, M. A. Walters, J. Am. Chem. Soc. 116 (1994) 6769.

[20] P. Middleton, D. P. E. Dickson, C. E. Johnson, J. D. Rush, Eur. J. Biochem. 104 (1980) 289.

[21] I. Bertini, A. P. Campos, C. Luchinat, M. Teixeira, J. Inorg. Biochem. 52 (1993) 227.

[22] H. Beinert, A. J. Thomson, Arch. Biochem. Biophys. 222 (1983) 333.

[23] T. H. Moss, A. J. Bearden, R. G. Bartsch, M. A. Cusanovich, Biochemistry 7 (1968) 1591.

[24] D. P. E. Dickson, C. E. Johnson, R. Cammack, M. C. W. Evans, D. O. Hall, K. K. Rao, Biochem. J. 139 (1974) 105.

[25] J. M. Mouesca, G. J. Rius, B. Lamotte, J. Am. Chem. Soc. 115 (1993) 4714.

[26] J. Gloux, P. Gloux, B. Lamotte, J. M. Mouesca, G. J. Rius, J. Am. Chem. Soc. 116 (1994) 1953.

[27] R. Krishnamoorthi, J. L. Markley, M. A. Cusanovich, C. T. Przysiecki, T. E. Meyer, Biochemistry 25 (1986) 60.

[28] L. Banci, I. Bertini, F. Briganti, A. Scozzafava, M. Vicens Oliver, C. Luchinat, Inorg. Chim. Acta 180 (1991) 171.

[29] L. Banci, I. Bertini, F. Capozzi, P. Carloni, S. Ciurli, C. Luchinat, M. Piccioli, J. Am. Chem. Soc. 115 (1993) 3431.

[30] I. Bertini, F. Briganti, C. Luchinat, A. Scozzafava, M. Solá, J. Am. Chem. Soc. 113 (1991) 1237.

[31] L. Banci, I. Bertini, F. Briganti, C. Luchinat, A. Scozzafava, M. Vicens Oliver, Inorg. Chem. 30 (1991) 4517.

[32] I. Bertini, F. Capozzi, C. Luchinat, M. Piccioli, Eur. J. Biochem. 212 (1993) 69.

[33] I. Bertini, A. Gaudemer, C. Luchinat, M. Piccioli, Biochemistry 32 (1993) 12887.

[34] L. Banci, I. Bertini, S. Ciurli, S. Ferretti, C. Luchinat, M. Piccioli, Biochemistry 32 (1993) 9387.

[35] I. Bertini, F. Capozzi, L. D. Eltis, I. C. Felli, C. Luchinat, M. Piccioli, Inorg. Chem. 34 (1995) 2516.

[36] I. Bertini, J. A. Cowan, C. Luchinat, K. Natarajan, M. Piccioli, Biochemistry 36 (1997) 9332.

[37] I. Bertini, C. Luchinat, M. Piccioli, A. Soriano, Inorg. Chim. Acta 283 (1998) 12.

[38] F. Torrens, J. Sánchez-Marín, I. Nebot-Gil, J. Chromatogr., A 827 (1998) 345.

[39] F. Torrens, J. Chem. Inf. Comput Sci. 40 (2000) 236.

[40] F. Torrens, J. Chromatogr., A 908 (2001) 215.

[41] F. Torrens, Chromatographia 53 (2001) S199.

[42] F. Torrens, V. Soria, Sep. Sci. Technol. 37 (2002) 1653.

[43] F. Torrens, Polyhedron 21 (2002) 1357.

[44] A. J. Hopfinger, Macromolecules 4 (1971) 731.

[45] A. J. Hopfinger, R. D. Battershell, J. Med. Chem. 19 (1976) 569.

[46] M. Born, Z. Phys. 1 (1920) 45.

[47] F. Torrens, E. Ortí, J. Sánchez-Marín, J. Chim. Phys. Phys.-Chim. Biol. 88 (1991) 2435.

[48] A. A. Maryott, E. R. Smith, Table of Dielectric Constants of Pure Liquids, National Bureau of Standards Circular No. 514, United States Department of Commerce-National Bureau of Standards, Washington, 1951.

[49] J. Kyte, R. F. Doolittle, J. Mol. Biol. 157 (1982) 105.

[50] D. Eisenberg, E. Schwarz, M. Komaromy, R. Wall, J. Mol. Biol. 179 (1984) 125.

[51] D. Eisenberg, Annu. Rev. Biochem. 53 (1984) 595.

[52] D. Eisenberg, R. M. Weiss, T. C. Terwilliger, Proc. Natl. Acad. Sci. USA 81 (1984) 140.

[53] J. L. Pellequer, E. Westhof, J. Mol. Graphics 11 (1993) 204.

[54] X. Gallet, F. Festy, P. Ducarme, R. Brasseur, A. Thomas-Soumarmon, J. Mol. Graphics Mod. 16 (1998) 72.

[55] A. Kantola, H. O. Villar, G. H. Loew, J. Comput. Chem. 12 (1991) 681.

[56] A. Leo, C. Hansch, J. Org. Chem. 36 (1971) 1539.

[57] F. Torrens, G. Castellano, Int. J. Mol. Sci. 7 (2006) 12.