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Calculating Standard Reduction Potentials of [4Fe-4S] Proteins

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Abstract

The oxidation-reduction potentials of electron transfer proteins determine the driving forces for their electron transfer reactions. While the type of redox site determines the intrinsic energy required to add or remove an electron, the electrostatic interaction energy between the redox site and its surrounding environment can greatly shift the redox potentials. Here, a method for calculating the reduction potential versus the standard hydrogen electrode, E° , of a metalloprotein using a combination of density functional theory and continuum electrostatics is presented. This work focuses on the methodology for the continuum electrostatics calculations, including various factors that may affect the accuracy. The calculations are demonstrated using crystal structures of six homologous HiPIPs, which give E° that are in excellent agreement with experimental results.

Keywords

Density functional theory; Poisson-Boltzmann continuum electrostatics; reduction potentials; iron-sulfur proteins; metalloproteins

Introduction

Electron transfer reactions play an important role in many bioenergetic processes.¹ Most biological electron carriers are proteins, which have redox sites that undergo oxidation-reduction reactions. The reduction potentials of these proteins are essential functional characteristics because they determine the driving forces for electron transfers, with the favorable direction toward the redox site of higher reduction potential. At the lower end of biological range is the splitting of water into H^+ and O_2 at an oxidation potential of -0.8 V, which occurs in photosynthesis, while the higher end is formation of water at a reduction potential of $+0.8$ V, which occurs in respiration¹. However, proteins contain only a limited number of different types of redox sites to cover this range, so electron transfer pathways rely on the protein environment to tune the reduction potential to the appropriate value. For instance, multiple [4Fe-4S] redox sites are used in the electron transport chains in mitochondrial respiratory complex I² and chloroplastic photosystem I³; the former has six [4Fe-4S] redox sites with reduction potentials spanning a range between -0.3 and -0.1 V⁴ and the latter has three [4Fe-4S] redox sites with reduction potentials spanning a range between -0.55 and -0.59 V^{5,6}.

The effects of the protein environment on the reduction potential can be understood by considering electron transfer as the reduction reaction for a redox site A

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where m is the net charge of the redox site in the oxidized state. The standard reduction potential against the standard hydrogen electrode, E° , for A can be related to the free energy ΔG° of the above reduction reaction using the Nernst equation

$$-nFE^\circ = \Delta G^\circ \approx \Delta G_{\text{in}} + \Delta G_{\text{out}} + \Delta G_{\text{SHE}} \quad (2)$$

where n is the number of electrons being transferred and F is the Faraday constant. In the second equality, ΔG_{in} is the change in the inner sphere energy upon reducing the redox site, ΔG_{out} is the change in the interaction energy of the redox site upon reduction with the outer sphere environment, and ΔG_{SHE} is the free energy for the removal of an electron from the standard hydrogen electrode (SHE). For a simple ion in solution, the ion is the inner sphere and the solvent is the outer sphere while for a small molecule in solution, the redox active part of the molecule may be defined as the inner sphere and the outer sphere may include parts of the ligands not directly bonded to the metals in addition to the solvent. For a redox site in a metalloprotein, the inner sphere may be defined as the metal and its ligands so that the outer sphere consists of the protein and surrounding solvent. Thus, to the extent that the protein does not affect the electronic structure of the redox site, the outer sphere contribution is mainly responsible for differences in reduction potentials between different proteins with the same redox site.

Computational studies have played an important role in understanding reduction potentials of metalloproteins. Many earlier studies of proteins have assumed that ΔG_{in} for a given redox site is constant and thus have focused on understanding *relative* values of E° between different proteins with the same redox site rather than E° . For instance, Warshel and co-workers have calculated relative E° for cytochrome c and iron-sulfur proteins via the protein-dipoles-Langevin dipoles (PDL) method^{7,8} and Gunner and Honig have calculated relative E° of heme proteins using Poisson-Boltzmann (PB) continuum electrostatics^{9,10}. In addition, Ichiye and coworkers have calculated differences in E° of as little as 0.05 V due to specific residues referred to as sequence determinants in iron-sulfur proteins^{11–15} using molecular mechanics combined with bioinformatics analysis. Although these earlier studies have provided much insight into reduction potentials of metalloproteins, it is difficult to evaluate different methods for calculating the protein contribution since deviations from experiment can be attributed to the missing inner sphere contribution. Thus, calculations of reduction potentials versus the SHE for [4Fe-4S] proteins by Noodleman, Case, Bashford and co-workers using broken-symmetry (BS) density functional theory (DFT) with the BP86 functional to calculate ΔG_{in} and PB electrostatics to calculate ΔG_{out} are an important step forward¹⁶. However, more recent studies¹⁷ indicate that inner sphere redox energies calculated with the BP86 functional differ considerably from electrospray ionization - photoelectron spectroscopy (EI-PES) detachment energies of [4Fe-4S] cluster analogs measured by Wang and co-workers^{18–20}.

Recently, our group has predicted E° vs. SHE for [4Fe-4S] proteins that are in excellent agreement with experiment using a simple additive approach we refer to as DFT+PB in which ΔG_{in} is obtained by BS-DFT calculations of gas phase redox site analogs and ΔG_{out} is obtained by PB calculations of the redox site in the protein using partial charges from the BS-DFT calculation²¹. This may be considered a first order approximation since the interaction between the perturbation of the electronic structure of the redox site is ignored; however, surprisingly good results are obtained. A key factor has been our calibration of the functionals and basis sets for the BS-DFT calculations by comparing the calculated detachment energies of redox site analogs in the gas phase against the EI-PES data of Wang and co-workers, with the best agreement using B3LYP and double- ζ basis sets with the appropriate polarization and diffuse functions¹⁷. Our BS-DFT calculations also show that the value of ΔG_{in} for the iron-sulfur clusters is relatively independent of environment¹⁷,

since environmental factors (such as hydrogen bonds to the redox site) affect the electron detachment energy mainly by electrostatic contributions rather than perturbations of the electronic structure, thus making the DFT+PB approach possible²². Our BS-DFT calculations further show that the ligand dihedral conformations may tune the energies by ~100 mV, but this falls within ΔG_{in} ²³. However, the protein environment may tend to polarize the partial charges of the redox site so a strategy similar to that of the CHARMM force field is adapted by using the 6-31G** basis set, which tends to overestimate polarization.²⁴ Another key factor in the DFT+PB method is the accuracy of the calculation of ΔG_{out} by PB electrostatic methods; for instance, the deviation in the calculated E° from experiment due to the resolution of the crystal structure used in the calculation has been previously estimated as $< \sim -0.04R$ to $0.09R$ eV, where R is the resolution²¹. Finally, the value of ΔG_{SHE} comes from the half-cell potential for the SHE. IUPAC recognizes ΔG_{SHE} as 4.44 eV, which is determined as summation of the free energy for the atomization of hydrogen, the ionization free energy of a hydrogen atom, and the solvation free energy of H^{+25} . Another commonly accepted value is 4.43 eV determined by Reiss and Heller²⁶ using a thermodynamic cycle for the removal of an electron from a saturated Pt electrode, although the determination of the solvation free energy of a proton remains an issue^{27,28}.

The DFT+PB methods are demonstrated here for the [4Fe-4S] proteins; however, the methodology is being generalized to other metalloproteins for implementation of user-friendly redox potential calculations that have a reasonable estimate for both the inner and outer sphere contributions. To this end, standard parameters for partial charges and atomic radii of the protein including liganding side chains and standard values for the dielectric permittivity of the protein and ΔG_{SHE} , are used so that the PB calculations can be made consistent. In addition, since the stumbling block for non-computational users is generally in the DFT calculations of the metal sites, which are CPU intensive and can be off by 1 eV in energy if the wrong functional is used,¹⁷ a library of ΔG_{in} , partial charges, and atomic radii of the redox sites is being developed, in which the DFT approach has been calibrated against ES-PES data for analogs.

Here, the focus is on the methods used in the calculation of ΔG_{out} for [4Fe-4S] proteins by PB continuum electrostatic methods. First, the procedure for obtaining ΔG_{out} from quantities reported by typical PB equation solvers is described, including the proper reference states, treatment of atoms at the boundary, and techniques to reduce errors from the grid approximations. Next, the effects of the grid and factors concerning the protein structure are assessed. The effects of the assignment of the redox layers for the [4Fe-4S] sites, the oxidation state of the protein crystal, and the assignment of the nitrogen and oxygen of the carboxamides of asparagines and glutamines are examined. Finally, the effects of different basis sets on the calculation of E° and the overall accuracy with respect to experiment are examined. The procedure is demonstrated using six HiPIPs, but has shown to be accurate for two other types of ferredoxins and the nitrogenase iron-proteins as well²¹. Since these three types of [4Fe-4S] proteins have different folds with different exposures of the redox site, the methods are expected to be general for the [4Fe-4S] proteins. A discussion of generalizing the methods to other redox sites is included.

Methods

All protein crystal structures were obtained from the Protein Data Bank (PDB)²⁹. Crystal structures of six HiPIP species were utilized: *Rhodocyclus tenuis* (*Rt*) [1ISU] at 1.50 Å resolution³⁰, *Rhodoferrax fermentans* (*Rf*) [1HLQ] at 1.45 Å resolution³¹, *Ectothiorhodospira vacuolata* (*Ev*) [1HPI] at 1.80 Å resolution³², *Ectothiorhodospira halophila* (*Eh*) [2HIP] at 2.50 Å resolution³³, *Thermochromatium tepidum* (*Tt*) [3A39] at 0.72 Å³⁴, [1IUA] at 0.80 Å³⁵, [2FLA] at 0.95 Å (reduced), and [2AMS] at 1.40 Å

resolution, and *Chromatium vinosum* (Cv) [1CKU] at 1.20 Å resolution³⁶. For crystal structures containing more than one molecule in the asymmetric unit, the calculated energies were averaged over all structures with the exception of *Eh*; chain B from the *Eh* structures contained unusual Fe-S bond lengths so it was not utilized. Hydrogen atoms were added to the crystal structures with the HBUILD facility in CHARMM 35b1.³⁷ Since inconsistencies in naming atoms in the PDB files can lead to large errors, the naming for the redox cluster, histidine residues, and terminal residues were corrected to match the parameter sets.

The ΔG_{in} and partial charges of $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^n$ ($n = 1-, 2-, 3-$) are from BS-DFT calculations using the NWChem program package³⁸ with the B3LYP exchange-correlation functional^{39,40} as previously described by Niu and Ichiye¹⁷. The $\text{C}_\beta\text{-S}_\gamma\text{-Fe-S}_i$ (where S_i is the inorganic sulfur on the opposite plane from the iron) dihedral angles, χ_3 , were $\sim 60^\circ$ in one redox layer and $\sim -60^\circ$ in the other redox layer. Briefly, the geometries were optimized using the 6-31G**⁴¹ and DZVP2⁴² basis sets with a fine integration grid of 140 radial shells and 974 angular points per shell (140,974) for Fe, (123,770) for S, (70,590) for C, and (60,590) for H. The vibrational analysis for the free energies and CHELPG⁴³ electrostatic potential (ESP) charges were calculated at the level of the geometry optimization. Single point energies with added *sp*-type diffuse functions on the sulfurs of 6-31G**^{41,42} were calculated from the 6-31G** geometries, and are referred to as 6-31(++)sG**//6-31G** basis set calculations. Most of the calculations here used $\Delta G_{in} = -0.232$ (3.453) eV for the 1-/2- (2-/3-) redox couple at the B3LYP/6-31(++)sG**//B3LYP/6-31G** level of theory/basis set and partial charges at B3LYP/6-31G** level of theory/basis set. In addition, calculations using $\Delta G_{in} = -0.371$ (3.369) eV for the 1-/2- (2-/3-) couple and partial charges both at the B3LYP/DZVP2 level/basis set were examined. $\Delta G_{SHE} = 4.43$ eV²⁶ was utilized. All partial charges were averaged such that equivalent atoms on each redox layer had the same charge. In addition, to account for the difficulty in assigning charges for buried atoms in the methyl groups, all hydrogen atoms were assigned a charge of 0.09 e and the charge on the carbon to which they were attached was assigned a charge such that the net charge on the entire methyl group was maintained. The resulting partial charges are reported in Table S1 and S2 of the supplementary material.

The Poisson continuum electrostatic calculations were performed using APBS,⁴⁴ a program for solving the PB equation. In addition, these calculations can now be performed using our implementation for redox calculations in CHARMMing,⁴⁵ a web-based interface for the program CHARMM that now includes the APBS program³⁷. Although the ionic concentration here is zero, the calculations are referred to as “PB” following common practice. Unless otherwise noted, calculations were performed in a $51.2 \text{ Å} \times 51.2 \text{ Å} \times 51.2 \text{ Å}$ cubic grid with a grid spacing of $0.2 \text{ Å} \times 0.2 \text{ Å} \times 0.2 \text{ Å}$. Charges were mapped onto the dielectric grid with a cubic B-spline discretization and a multiple Debye-Hückel boundary condition was applied to all calculations. The protein in solution has three dielectric regions representing the redox site, the protein, and the solvent, with permittivities $\epsilon_c = 1$, $\epsilon_p = 4$, and $\epsilon_s = 78$, respectively. The dielectric maps were constructed using the Connolly surfaces⁴⁶ of the redox site and the protein with a probe radius of 1.4 Å and the boundary grid points were smoothed as the average of the original values of the grid point and its eight nearest neighbors.⁴⁷ The three-region dielectric maps were constructed from two maps: one in which points within the redox site surface were assigned $\epsilon_c = 1$ while those outside were assigned $\epsilon_p = 4$ and another in which all points within the protein surface were assigned $\epsilon_p = 4$ while those outside were assigned $\epsilon_s = 78$. Using the DXMATH program, which installs with APBS, the final map was constructed by adding the dielectric maps together and subtracting $\epsilon_p = 4$ from each point for the over-counting. The partial charges for the protein and the atomic radii for all protein atoms are taken from the CHARMM22 parameter set.²⁴ For the redox site, the radius for iron is 1.07 Å⁴⁸, the same radii for the cysteinyl ligands are used as equivalent protein cysteine atoms, and the radius for inorganic sulfur is chosen to be

equivalent to the cysteinyl sulfur; i.e., 2.00 Å. The assignments of the mixed-valance layers for all HiPIPs were based on NMR experiments for CvHiPIP in which the first two Cys in the sequence bind the “top” layer and the second two Cys bind the “bottom” layer, with the top layer being the more oxidized layer⁴⁹.

The absolute free energy ΔG for the reduction reaction of a protein (Eqn. 1) can be calculated using a thermodynamic cycle (Figure 1). Since the overall reduction can be separated into the reduction of the redox site in vacuum, and the solvation of the redox site in both the oxidized and reduced states, ΔG_{out} is the difference in $\Delta_{\text{sol}}G(A)$, the solvation energy of the *redox site* A, between the oxidized (m) and reduced ($m-1$) states

$$\Delta G_{\text{out}} = \Delta_{\text{sol}}G(A^{m-1}) - \Delta_{\text{sol}}G(A^m) \quad (3)$$

where $\Delta_{\text{sol}}G(A)$ is the interaction energy of the redox site with both the surrounding protein and the solvent; i.e.,

$$\Delta_{\text{sol}}G(A^m) = G(A^m \text{ in protein and solvent}) - G(A^m \text{ in vacuo}) - G(\text{protein and solvent only}) \quad (4)$$

If the same structure is used for the protein to calculate $\Delta_{\text{sol}}G(A^m)$ for the oxidized and reduced states, then the last term in Eqn. 4 cancels in Eqn. 3. In addition, for comparisons between different proteins, all structures are aligned by the redox site to reduce errors arising from extrapolation of the partial charges onto the grid. Redox site atoms are identical in the solvated (i.e. in the protein and solvent) and in the reference state (i.e. *in vacuo*); thus, here the thiol sulfurs in the reference state are terminated by methylene groups.

Results

The calculation of E° vs. SHE using our DFT+PB method is demonstrated here. First, the effects of the grid and various factors of the protein crystal structures are examined. Next, the effects of the basis set used for calculation of the partial charges and ΔG_{out} for the calculations of E° are examined. The state of redox site is referred to by the net charge n of $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^n$.

Grid Dependence

The dependence of ΔG_{out} on grid spacing and the orientation of the molecule is demonstrated for 1-/2- couple of THiPIP in Figure 2. Calculations were performed at grid spacings between 0.6 and 0.1 Å, and, at each grid spacing, the protein and dielectric maps were rotated at 10° steps around the z-axis for 90°. All other parameters, such as the size of the calculation grid, were kept constant. The dependence on the grid spacing and the variation due to orientation began to decrease at grid spacings below 0.3 Å. Below this point, the continued decrease is most likely due to the method used to smooth the boundary between regions, which continues to become more sharp depending on the grid spacing. A single calculation of HiPIP using a 0.2 Å spacing took 17 minutes of CPU time and required 4 GB of memory, while at 0.1 Å spacing took 17.5 hours and required over 30 GB of memory (memory requirements are greater than 200 B per grid point). While the computational time at a 0.1 Å grid spacing is feasible for small proteins, the 0.2 Å grid spacing is deemed sufficient since the E° are only 2 to 3 mV larger, which is well within the error of the DFT+PB approximation, with much greater computational efficiency. All further calculations described are at the 0.2 Å spacing.

Assignment of Redox Layers

The cuboidal [4Fe-4S] cores are characterized by two redox layers, with each layer consisting of two irons and two sulfurs (Figure 3)⁵⁰. The assignment of the layers may affect

ΔG_{out} because while the layers are equivalent in the 2- state, the oxidation or reduction from the 2- state occurs mainly in one layer, resulting in different partial charges in each layer. Each layer will be referred to as an n - m layer, where n and m are the formal charges on the two irons in the layers. In addition, when the irons should be formally 2+ and 3+, a minority spin becomes delocalized in the layer and each iron can be said to have a formal charge of 2.5+. Thus, in all states of the [4Fe-4S] redox site, each layer has the same partial charges for both irons, etc. (See supplementary material for partial charges). For the HiPIPs in the 1- state, the 3-3 layer can be assigned as having Cys43 and Cys46 ligands while the 2.5-2.5 layer can be assigned as having Cys61 and Cys75 ligands, according to 2D NMR studies of *Cv*HiPIP,⁴⁹ which is also consistent with the distortion of the [4Fe-4S] cubane found in our DFT studies.⁵¹ However, although it is reasonable to assume that the layer assignment is the same within a homologous set of proteins (i.e., the rest of the HiPIPs), the assignment may not be clear in other [4Fe-4S] proteins.

Therefore, the effects of different assignments of the redox layers were tested in *T*HiPIP for the 1-/2- couple, which leads to a ~0.03 V range in E° (Table 1). The largest deviations in E° occur when the irons bound to Cys43 and Cys75 are assigned to one layer and the irons bound to Cys46 and Cys61 are assigned to the other, since the Cys75 backbone amide interacts with the inorganic sulfur bridging the irons bound to Cys43 and Cys75, which leads to greater stabilization of negative charge on layers containing either of these two irons. Thus $\Delta_{\text{sol}}G([\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{1-})$ is more negative when the 2.5-2.5 layer is assigned as Cys43/75 and more positive when the 3-3 layer is assigned Cys43/75. However, as a whole, even incorrect assignment of layers will lead to relatively small errors since the range is only 30 mV.

Oxidation State of the Crystal Structure

The oxidation state of the protein crystal structure used for the calculation will also affect the calculated E° . Due to the difficulty of preventing oxidation of the crystal structure before the diffraction data is collected, most of the HiPIP structures have been solved at the 1- oxidation state. In the case of *T*HiPIP, many crystal structures have been solved at different resolutions, with one structure solved in the 2- oxidation state (Table 2). The root mean-square deviation (RMSD) of the backbone atoms between the oxidized structures is relatively small and E° from the oxidized structures shows a range of less than 20 mV for the three structures from different resolutions. (Note: the 1IUA has a slightly larger RMSD due to differences remote from the redox site.) While the reduced structure has a similar RMSD, E° from the reduced crystal structure is ~40 mV higher than the oxidized structures in both redox couples, which indicates that the reduced structure is more capable of stabilizing a negative charge at the redox site due to an increase in polarization of the protein around the redox site. For the biologically relevant 1-/2- redox couple of the HiPIPs, the E° from the oxidized structures are in better agreement with experiment. Although the 2-/3- couple cannot be seen in many HiPIPs, E° for this couple has been estimated as -0.64 V for *Rhodospila globiformis* HiPIP,⁵² and the E° from the reduced structure is in better agreement with this value. Thus, the correct oxidation state of the crystal appears to have a more significant effect on the calculated E° , although a reasonable estimate can be obtained even if the oxidation state is inconsistent with the redox couple of E° .

Nitrogen and Oxygen Assignment in Amides Groups

The effect on the calculated E° of the assignments of nitrogen and oxygen atoms in amide groups of Asn and Gln within 8 Å of the redox site was also examined for all of the HiPIPs (Table 3). Errors in assignment has been estimated as 15%^{53,54} in x-ray crystallographic studies due to their similar electron density. Of the six structures, only *R*HiPIP has no change in E° when the N and O are interchanged since the amine and carbonyl dipoles in

Gln45 are perpendicular to the redox site and there is essentially no change in interaction energy, ΔE_{int} , with the rest of the protein. The calculated E° for *Cv*, *Tt*, *Ev* and *HiPIP* are slightly higher by 10 to 60 mV for the interchanged N and O while the calculated E° of *Rt* HiPIP is nearly 50 mV lower because the interchange breaks a hydrogen bond with the redox site in chain B of the asymmetric unit although it has little effect in chain A because Gln27 is oriented away from the redox site (Figure 4). However, the ΔE_{int} is also considerably more favorable for the crystal assignment. so the in other cases, Asn or Gln side chains may be rotating as was seen previously in molecular dynamics simulations of rubrerythrin¹⁴ so that molecular dynamics simulations may be useful in examining calculated E° that disagree with experiment.

Basis Set Dependence

The dependence on the basis set used for calculation of the partial charges and ΔG_{in} was examined by calculating E° for the 1-/2- couple of the HiPIPs and comparing with experiment (Figure 5). The partial charges at the B3LYP/6-31(++)_SG**//B3LYP/6-31G** (i.e., the same level at which ΔG_{in} was calculated) were not tested since they are only very slightly more polarized than those at B3LYP/6-31G** (i.e, the level of the geometry optimization, differing by only 0.03e on the Fe in the 2- state. E° using ΔG_{in} calculated at B3LYP/6-31(++)_SG**//B3LYP/6-31G** level/basis set combined with ΔG_{out} using partial charges calculated at B3LYP/6-31G** level/basis set agree well with experiment. Since the DZVP2 charges are slight less polarized than the 6-31G** charges, differing by 0.15 e on the Fe in the 2- state, the calculated values of ΔG_{out} for the six HiPIPs differ by only 0.015 $\pm$ 0.004 V. While this leads to slightly better agreement with experiment with ΔG_{in} calculated at B3LYP/6-31(++)_SG**//B3LYP/6-31G**, using ΔG_{in} calculated at B3LYP/DZVP2 leads to much worse agreement with experiment. Moreover, other preliminary studies indicate that the slight overestimation by ΔG_{in} at B3LYP/6-31(++)_SG**//B3LYP/6-31G** level/basis set combined with ΔG_{out} using partial charges at B3LYP/6-31G** level/basis set comes from the ligand dihedral angle dependence in the calculation of ΔG_{in} .

Discussion

While the methods are shown here for the [4Fe-4S] proteins, they will be generalized to other metalloproteins for implementation into user-friendly redox potential calculations in CHARMMing, as mentioned in the introduction. Of course, the PB calculations are highly dependent on the radii of the redox site atoms (although relatively insensitive to the metal radii since they are buried by their ligands) or alternatively to the choice of dielectric for the redox site so that the choices here may account for errors due to choice of ΔG_{SHE} ,²⁸ polarization effects, etc. However, since the main goal is to provide two reasonable reference points, namely in the inner and outer sphere contributions, a best practices for each type of redox site can be established. Thus, the methods for each type of redox site are being tested in multiple proteins and in solvated analogs.

Conclusions

The DFT+PB method for calculating reduction potentials relative to the standard hydrogen electrode is presented in which the inner-sphere contribution is calculated by DFT as the change in energy of the relevant gas phase analog upon reduction while the outer sphere contribution is calculated by PB continuum electrostatics as the change in solvation energy of the redox site upon reduction. The calculated reduction potentials for [4Fe-4S] proteins are in excellent agreement with experimental values. Although the perturbations by the protein to the electronic structure of the redox site are neglected, previous calculations have shown that these are small and that the use of hybrid functionals and full-core basis sets are much more important in the redox energetics of the redox site. The influence of the protein

is mostly electrostatic, and so using ESP charges for the different redox states to calculate the outer sphere contribution is sufficient. Moreover, this method allows deviations from experiment to be explored separately, as both perturbations to the electronic structure of the redox site and classical effects within the protein.

Several factors can affect the accuracy of the outer sphere continuum calculation. For instance, a grid spacing of 0.2 Å gives good results with reasonable computational times and memory requirements but larger spacing is not recommended. Although the results were relatively insensitive to the assignment of the redox layers, specific interactions may cause greater sensitivity. Also, the assignment of the nitrogen and oxygen atom of Asn and Gln that are close to the redox site may affect the E° ; however, the assignments found in the crystal structures are reasonable in most cases. In addition, the oxidation state of the protein in the crystal structure appears to be important, possibly leading to errors of ~40 mV. Importantly, the results are relatively insensitive to the partial charges, while they are much more sensitive to the proper value of the inner sphere contribution. Thus, using ΔG_{in} at the B3LYP/6-31(++)_SG**//B3LYP/6-31G** level/basis set and ΔG_{out} from partial charges at the B3LYP/6-31G** level/basis set is considered the most accurate and consistent combination for the [4Fe-4S] proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference

1. Lehninger, A.; Nelson, DL.; Cox, MM. *Lehninger Principles of Biochemistry*. 4th ed.. New York, NY: W.H. Freeman & Co.; 2003.
2. Sazanov LA. *Biochemistry*. 2007; 46
3. Romberger S, Golbeck J. *Photosynth. Res.* 2010; 104:333. [PubMed: 20405215]
4. Reda T, Barker CD, Hirst J. *Biochemistry*. 2008; 47:8885. [PubMed: 18651753]
5. Ke B, Hansen RE, Beinert H. *Proc. Natl. Acad. Sci. U. S. A.* 1973; 70:2941. [PubMed: 16592113]
6. Heathcote P, Williams-Smith DL, Sihra CK, Evans MCW. *Biochim. Biophys. Acta Bioenerg.* 1978; 503:333.
7. Churg AK, Warshel A. *Biochemistry*. 1986; 25:1675. [PubMed: 3011070]
8. Stephens PJ, Jollie DR, Warshel A. *Chem. Rev. (Washington, DC, U. S.)*. 1996; 96:2491.
9. Gunner MR, Honig B. *Proc. Natl. Acad. Sci. U. S. A.* 1991; 88:9151. [PubMed: 1924378]
10. Mao J, Hauser K, Gunner M. *Biochemistry*. 2003; 42:9829. [PubMed: 12924932]
11. Swartz P, Beck B, Ichiye T. *Biophys. J.* 1996; 71:2958. [PubMed: 8968568]
12. Ergenekan CE, Thomas D, Fischer JT, Tan ML, Eidsness MK, Kang CH, Ichiye T. *Biophys. J.* 2003; 85:2818. [PubMed: 14581187]
13. Eidsness MK, Burden AE, Richie KA, Kurtz DMJ, Scott RA, Smith ET, Ichiye T, Beard B, Min T, Kang C. *Biochemistry*. 1999; 38:14803. [PubMed: 10555962]

14. Luo Y, Ergenekan C, Fischer J, Tan M, Ichiye T. *Biophys. J.* 2010; 98:560. [PubMed: 20159152]
15. Beck BW, Xie Q, Ichiye T. *Biophys. J.* 2001; 81:601. [PubMed: 11463610]
16. Torres RA, Lovell T, Noodleman L, Case DA. *J. Am. Chem. Soc.* 2003; 125:1923. [PubMed: 12580620]
17. Niu S, Ichiye T. *Mol. Simul.* 2011; 37:572.
18. Zhai H, Yang X, Fu Y, Wang X, Wang L. *J. Am. Chem. Soc.* 2004; 126:8413. [PubMed: 15237997]
19. Fu Y-J, Yang X, Wang X, Wang LS. *Inorg. Chem.* 2004; 43:3647. [PubMed: 15180419]
20. Wang XB, Wang LS. *J. Phys. Chem.* 2000; 112:6959.
21. Perrin BS Jr, Ichiye T. *Proteins: Struct. Funct. Bioinf.* 2010; 78:2798.
22. Yang X, Niu S, Ichiye T, Wang L-S. *J. Am. Chem. Soc.* 2004; 126:15790. [PubMed: 15571403]
23. Niu S, Ichiye T. *J. Am. Chem. Soc.* 2009; 131:5724. [PubMed: 19341280]
24. MacKerell AD Jr, Bashford D, Bellott M, Dunbrack RL Jr, Evanseck JD, Field MJ, Fischer S, Gao J, Guo H, Ha S, Joseph-McCarthy D, Kuchnir L, Kuczera K, Lau FTK, Mattos C, Michnick S, Ngo T, Nguyen DT, Prodhom B, Reiher WE, Roux B, Schlenkrich M, Smith JC, Stote R, Straub J, Watanabe M, Wiorkiewicz-Kuczera J, Yin D, Karplus M. *J. Phys. Chem. B.* 1998; 102:3586.
25. Trasatti S. *Pure Appl. Chem.* 1986; 58:955.
26. Reiss H, Heller A. *J. Phys. Chem.* 1985; 89:4207.
27. Kelly CP, Cramer CJ, Truhlar DG. *J. Phys. Chem. B.* 2007; 111:408. [PubMed: 17214493]
28. Lamoureux G, Roux B. *J. Phys. Chem. B.* 2006; 110:3308. [PubMed: 16494345]
29. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. *Nucleic Acids Res.* 2000; 28:235. [PubMed: 10592235]
30. Rayment I, Wesenberg G, Meyer TE, Cusanovich MA, Holden HM. *J. Mol. Biol.* 1992; 228:672. [PubMed: 1453470]
31. Gonzalez A, Benini S, Ciurli S. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* 2003; 59:1582. [PubMed: 12925788]
32. Benning MM, Meyer TE, Rayment I, Holden HM. *Biochemistry.* 1994; 33:2476. [PubMed: 8117708]
33. Breiter DR, Meyer TE, Rayment I, Holden HM. *J. Biol. Chem.* 1991; 266:18660. [PubMed: 1917989]
34. Takeda K, Kusumoto K, Hirano Y, Miki K. *J. Struct. Biol.* 2010; 169:135. [PubMed: 19782139]
35. Liu L, Nogi T, Kobayashi M, Nozawa T, Miki K. *Acta Crystallogr. Sect. D: Biol. Crystallogr.* 2002; D58:1085. [PubMed: 12077426]
36. Carter CW, Kraut J, Freer ST, Alden RA. *J. Biol. Chem.* 1974; 249:6339. [PubMed: 4417854]
37. Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. *J. Comput. Chem.* 1983; 4:187.
38. Valiev M, Bylaska EJ, Govind N, Kowalski K, Straatsma TP, Van Dam HJJ, Wang D, Nieplocha J, Apra E, Windus TL, de Jong W. *Comput. Phys. Commun.* 2010; 181:1477.
39. Becke AD. *J. Chem. Phys.* 1993; 98:5648.
40. Lee C, Yang W, Parr RG. *Phys. Rev. B.* 1988; 37:785.
41. Hehre, WJ.; Radam, L.; Schleyer, PvR; Pople, J. *Ab initio molecular orbital theory.* New York: Wiley-Interscience; 1986.
42. Godbout N, Salahub DR, Andzelm J, Wimmer E. *Can. J. Chem.* 1992; 70:560.
43. Breneman CM, Wiberg KB. *Journal of Comput. Chem.* 1990; 11:361.
44. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. *Proc. Natl. Acad. Sci. U. S. A.* 2001; 98:10037. [PubMed: 11517324]
45. Miller BT, Singh RP, Klauda JB, Hodoscek M, Brooks BR, Woodcock HL III. *J. Chem. Inf. Model.* 2008; 48:1920. [PubMed: 18698840]
46. Connolly ML. *Science.* 1983; 221:709. [PubMed: 6879170]
47. Bruccoleri RE, Novotny JR, Davis ME, Sharp K. *J. Comput. Chem.* 1997; 18:268.
48. Jafri JA, Logan J, Newton M. *Isr. J. Chem.* 1980; 19:340.

49. Bertini I, Capozzi F, Ciurli S, Luchina C, Messori L, Piccioli M. *J. Am. Chem. Soc.* 1992; 114:3332.
50. Mouesca JM, Chen JL, Noodleman L, Bashford D, Case DA. *J. Am. Chem. Soc.* 1994; 116:11898.
51. Niu S, Ichiye T. *J. Phys. Chem. A.* 2009; 113:5710. [PubMed: 19378967]
52. Heering HA, Bultink YBM, Hagen WR, Meyer TE. *Eur. J. Biochem.* 1995; 232:811. [PubMed: 7588720]
53. Donald IKM, Thornton JM. *Protein Eng.* 1994; 8:217.
54. Word JM, Lovell SC, Richardson JS, Richardson DC. *J. Mol. Biol.* 1999; 285:1735. [PubMed: 9917408]
55. Moulis J, Scherrer N, Gagnon J, Forest E, Petillot Y, Garcia D. *Arch. Biochem. Biophys.* 1993; 305:186. [PubMed: 8393645]
56. Luchinat C, Capozzi F, Borsari M, Battistuzzi G, Sola M. *Biochem. Biophys. Res. Commun.* 1994; 203:436. [PubMed: 8074688]
57. Hochkoeppler A, Ciurli S, Venturoli G, Zannoni D. *FEBS Lett.* 1995; 357:70. [PubMed: 8001683]
58. Heering HA, Bultink YBM, Hagen WR, Mayer TE. *Biochemistry.* 1995; 34:14675. [PubMed: 7578075]

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Table 1

Calculated $\Delta_{\text{sol}}G[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{1-}$ and E° for different redox layer assignments for *T*HiPIP. First line corresponds to the experimentally determined layer assignments.

Top Layer	Bottom Layer	$\Delta_{\text{sol}}G$ (V)	E° (V)
Cys 43, Cys 46*	Cys 61, Cys 75*	-2.428	0.334
Cys 43, Cys 75	Cys 46, Cys 61	-2.444	0.318
Cys 46, Cys 75	Cys 43, Cys 61	-2.428	0.335
Cys 61, Cys 75	Cys 43, Cys 46	-2.429	0.334
Cys 46, Cys 61	Cys 43, Cys 75	-2.411	0.351
Cys 43, Cys 61	Cys 46, Cys 75	-2.428	0.335
Exp		N/A	0.32 ^I

^I_{Ref.}⁵³

Table 2

Calculated E° for TtHiPIP from crystal structures at different resolutions and oxidation states. The RMSD of the backbone atoms is calculated relative to the 0.72 Å oxidized crystal structure.

PDB ID	Oxidation State	Resolution (Å)	E° (1-/2-) (V)	E° (2-/3-) (V)	RMSD (Å)
3A39	Oxidized	0.72	0.342	-0.712	0.0
1IUA	Oxidized	0.80	0.334	-0.728	0.474
2AMS	Oxidized	1.40	0.337	-0.711	0.141
2FLA	Reduced	0.95	0.377	-0.681	0.114
	Exp		0.32 ¹	-0.64 ²	

¹ Ref. 53

² Estimated for *Rhodospila globiformis* HiPIP. 50

Table 3

Calculated E° for the HiPIPs with the nitrogen and oxygen atoms of Asn or Gln in the crystal structure assignment and with nitrogen and oxygen exchanged. ΔE_{int} , the change in interaction energy of the Asn or Gln with the rest of the proteins when the nitrogen and oxygen are interchanged, is also given.

Species	Residue	ΔE_{int} (kcal/mol)	E° (V)	
			Crystal Assignment	N/O Exchanged
<i>Cv</i>	Asn 45	28.4	0.384	0.394
				0.36 ¹
<i>Tt</i>	Asn 45	29.8	0.334	0.390
				0.32 ²
<i>Elh</i>	Asn 33	25.2	0.104	0.138
				0.11 ¹
<i>Ev</i>	Asn 36	11.8	0.222	0.272
				0.15 ¹
<i>Rf</i>	Gln 45	-1.5	0.350	0.349
				0.33 ³
<i>Rt</i>	Gln 27	23.3	0.418	0.366
				0.35 ⁴
<i>Rt(a)</i>	Gln 27	20.9	0.414	0.433
<i>Rt(b)</i>	Gln 27	25.9	0.423	0.299

¹Ref. 54

²Ref. 53

³Ref. 55

⁴Ref. 56