

Supporting Information

On the usage of dielectric constant and atomic charges: Though one might vary the dielectric constant for proteins and the atomic partial charges to adjust their values for different applications, our policy was and is to employ the same dielectric conditions for all computations. This allows to directly compare results without an unintended bias. Discussions about the appropriate choice of the dielectric constant for the protein interior can be found in refs. (1-4).

Error estimate: The error in redox potentials computed by solving the linearized Poisson Boltzmann equation and performing an ensemble average over all charge pattern has statistical, numerical and systematic contributions. The standard deviation in the protonation probability of a single titratable group estimated from the MC sampling procedure accounting for correlation between subsequent MC steps as described in ref. (5) was much smaller than 0.01 protons. The sum of standard deviations of protonation probabilities from all titratable groups was for each redox state of the cofactors that we considered about 0.02 protons similarly as in former applications (6, 7). The numerical error in evaluating electrostatic energies resulting from a grid resolution of 0.3 Å that we used was estimated to be considerably smaller than 10 meV (7). The resulting estimates of the combined numerical and statistical error of about 10 mV for the absolute values of the computed redox potentials are in the same range as typical uncertainties in experimental values for conventional molecular systems if measured under favorable conditions.

The computational method involves also systematic errors, which are generally difficult to estimate and which can have a major influence on the final value of the computed cofactor redox potential. These errors can be due to uncertainties in atomic partial charges, the value of the dielectric constant used inside the protein and the reliability of atomic coordinates in general but in particular for the hydrogen atoms and their suitability for the considered charge states. Regarding the coordinates, there are a number of error sources. In the present application, we used as in most of the past applications a single coordinate set taken from the crystal structure. A statistical average of different conformations would be

more appropriate but a suitable ensemble of conformations is not readily available. Conformations of crystal waters are generally uncertain with regard to hydrogen atom positions and are likely to involve a number of energetically equivalent conformations. Therefore, they were removed in most of our past treatments. The distance dependence of electrostatic interactions also is for inhomogeneous dielectrics roughly proportional to the inverse of the distance between charges, which is rather mild distance dependence. Therefore, the influence from different conformations is generally not so critical. But, this may no longer be the case in the presence of variably charged groups (titratable or redox-active) that are close to the considered redox-active group and are about to change their charge state. Under these circumstances, subtle changes in conformation may lead to shifts in the charge state of that group, which in turn changes the electrostatic interactions with the considered redox-active group and shifts its potential. In these cases, shifts of the redox potential by 100 mV or more can be observed.

We demonstrated in many different applications that our computational procedure is reliable with respect to the known experimental redox potentials. Here, those calculated redox potential values often agree with experimental results within 20 mV (8-12). For convenience, the computed redox potentials are given with a 1 mV accuracy, albeit this does not suggest that the last digit is significant.

Table S1: Atomic partial charges on Mn-cluster models. The total charge of the Mn-cluster was set to +6 unit charges for both crystal structures.

3.5 Å-structure

component	atomic charge	number	total charge
Mn	+3.25 ^a	4	+13 ^b
O	−2	4	−8
Ca	+2	1	+2
bicarbonate	−1	1	−1
Mn-cluster			+6

3.2 Å-structure

component	atomic charge	number	total charge
Mn	+1.5 ^c	4	+6
O	n.d. ^d	0 ^d	0
Ca	n.d. ^d	0 ^d	0
bicarbonate	n.d. ^d	0 ^d	0
Mn-cluster			+6 ^e

^a The total charge $[\text{Mn}_4]^{13+}$ was divided by 4 and assigned to each Mn atom.

^b The total charge of $[\text{Mn}_4]^{13+}$ is based on its oxidation state $[\text{Mn}_4]$ (II, III, IV₂) in state S_0 (13).

^c The total charge $[\text{Mn-cluster}]^{6+}$ ($=[\text{Mn}_4]^{6+}$) was divided by four and assigned to each Mn atom.

^d No explicit atoms are given in the crystal structure. However, the influence of these atomic partial charged were implicitly considered in the constraining of the total charge of $[\text{Mn-cluster}]^{6+}$ as was obtained in the 3.5 Å-structure.

^e The same total charge $[\text{Mn-cluster}]^{6+}$ of the 3.5 Å-structure was applied to the 3.2 Å-structure.

References to Supporting Information

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