Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Charge transfer through a fragment of the respiratory complex I and its regulation: an atomistic simulation approach

Supporting Information

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1. Sample raw $\partial H/\partial \Lambda$ data

2. Sample $\partial H/\partial \Lambda$ distributions

3. Sample molecular dynamics data - equilibration

4. Sample molecular dynamics data - production

5. Protonation pattern check

6. Thermodynamic integration data, no NAD^+

7. Thermodynamic integration data, NAD^+

8. Thermodynamic cycle analysis in the presence of NAD^+

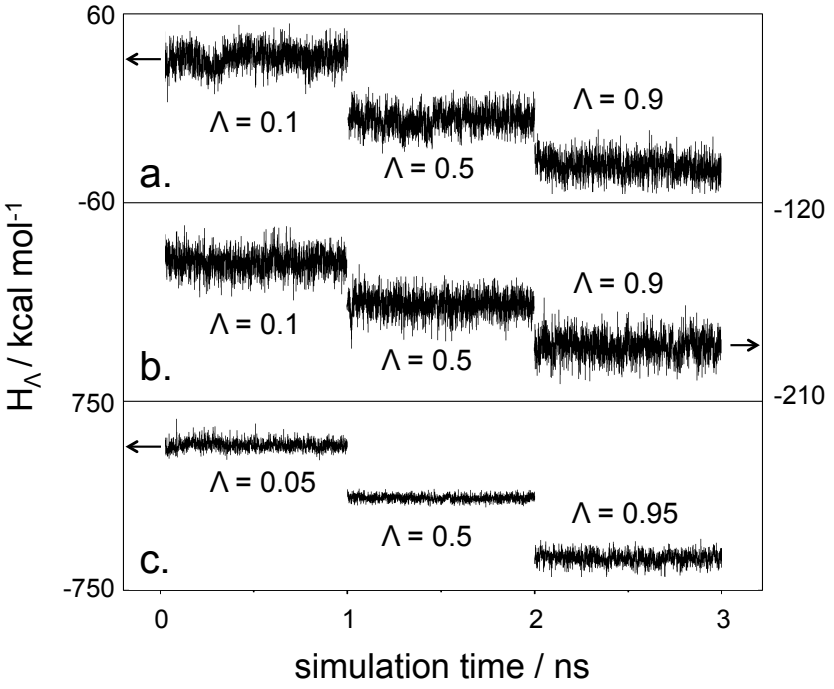
9. Thermodynamic cycle analysis in the absence of NAD^+

10. Interacting model analysis, no NAD^+

11. Interacting model analysis, NAD^+

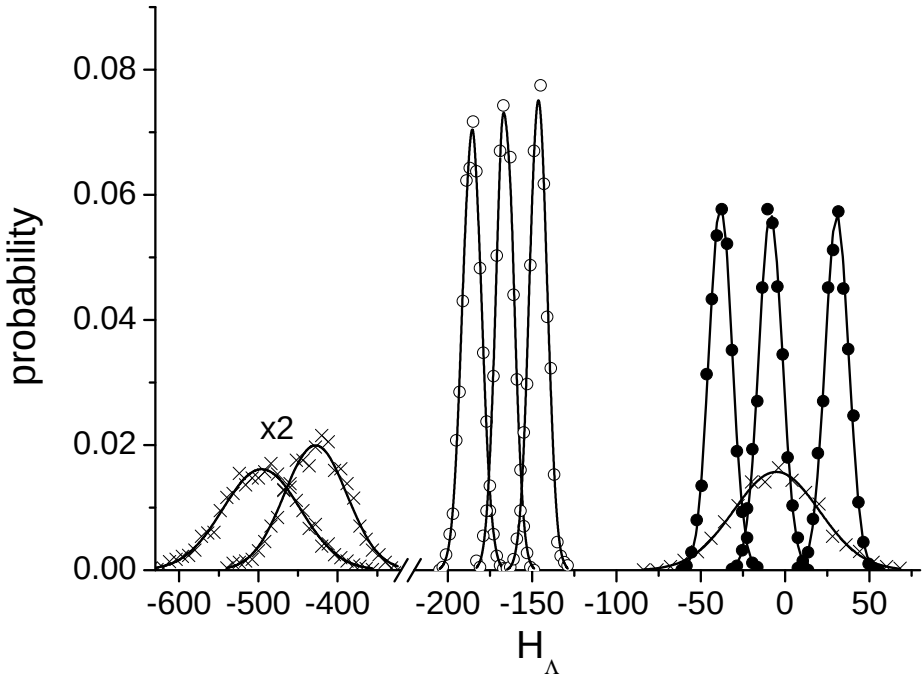
12. Force field parameters for the iron sulfur clusters FES and SF4

1. Sample raw $\partial H/\partial\Lambda$ data



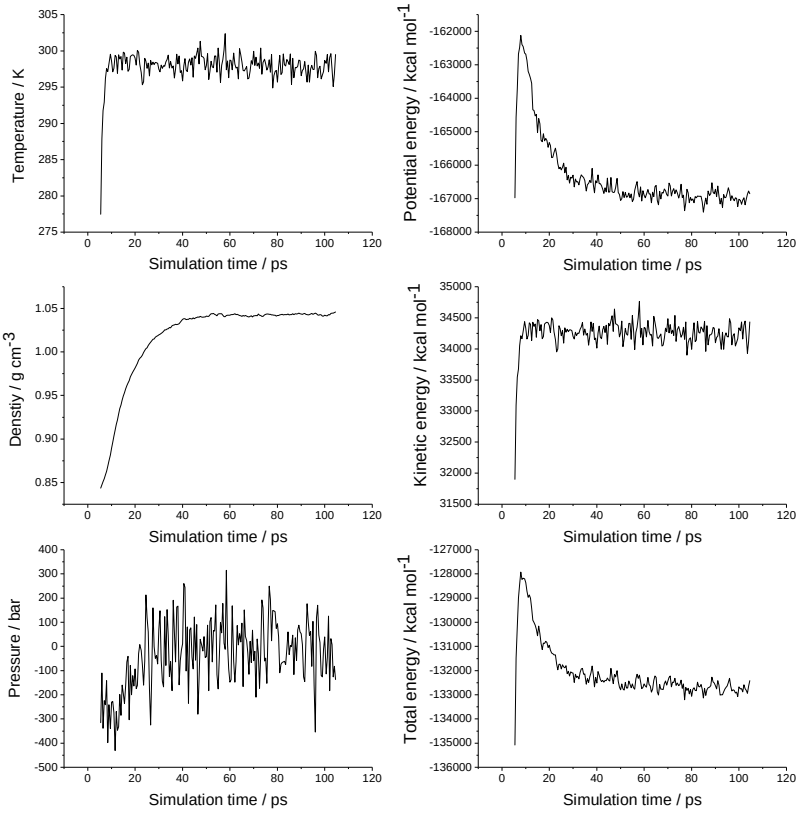
Sample raw $H_\Lambda = \partial H/\partial\Lambda$ data as a function of the simulation time for the three types of reactions described in the article. We show data for the smallest and the largest noninteger Λ values of the thermodynamic integration process, and for Λ=1/2. The enumeration refers to fig. 3 of the main article. a. electron transfer, 7→2, b. charging, 3→4, c. FMN reduction, 1→5.

2. Sample $\partial H/\partial\Lambda$ distributions



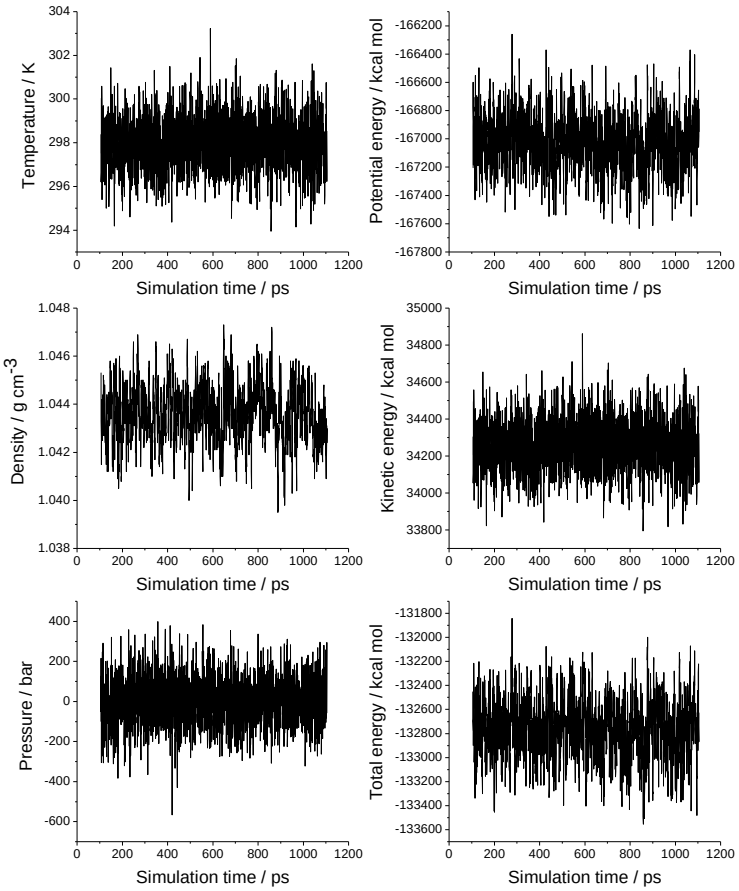
Sample $H_\Lambda = \partial H/\partial\Lambda$ probability distributions corresponding to the data shown in the previous section. Symbols denote histogram bins, solid lines a Gaussian fit to the raw data. Data are given for the processes 7→2 (• symbol, from left to right: Λ=0.9, Λ=0.5, Λ=0.1), 3→4 (∘ symbol, from left to right: Λ=0.9, Λ=0.5, Λ=0.1), and 1→5 (× symbol, from left to right: Λ=0.95, Λ=0.05, Λ=0.5). The enumeration refers to figure 3 of the main article. Note the disruption of the H_Λ scale and the multiplication of the H_Λ values for the process 1→5 by a factor of minus one for Λ=0.05. H_Λ is given in kcal/mol.

3. Sample molecular dynamics data - equilibration



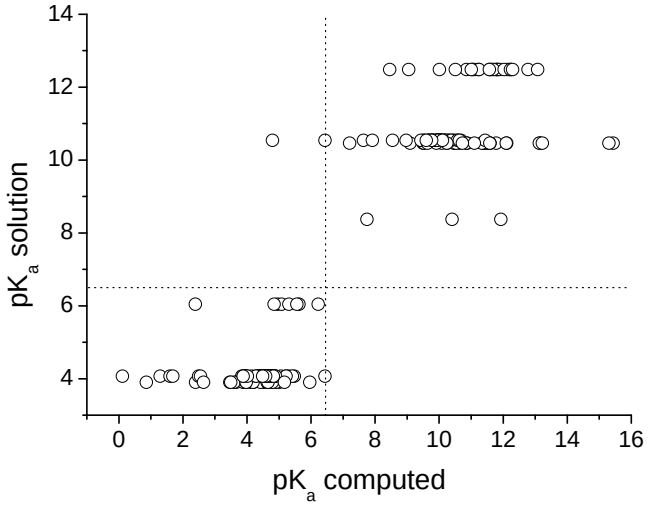
Equilibration phase of a sample molecular dynamics simulation, reaction 3→4 (charging of the N3 iron sulphur cluster at $\lambda = 0.1$). Characteristic quantities, such as temperature, density, pressure, kinetic energy, potential energy and total energy reach average values after a time of ca. 20-30 ps.

4. Sample molecular dynamics data - production

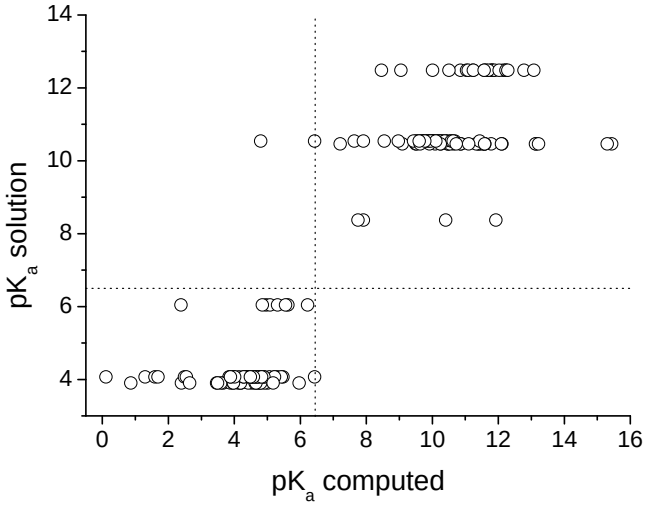


Production phase of a sample molecular dynamics simulation, reaction 3→4 (charging of the N3 iron sulphur cluster at $\Lambda = 0.1$). Characteristic quantities, such as temperature, density, pressure, kinetic energy, potential energy and total energy exhibit fluctuations, but no drift during a 1 ns simulation.

5. Protonation pattern check



Solution pK_a values as a function of their computed counterparts in the absence of NAD^+. Amino acids located in the lower left or in the upper right rectangle of the plot – as divided by the dotted lines – show the same protonation in solution and within the computation.



Solution pK_a values as a function of their computed counterparts in the presence of NAD^+. Amino acids located in the lower left or in the upper right rectangle of the plot – as divided by the dotted lines – show the same protonation in solution and within the computation.

6. Thermodynamic integration data, no NAD^+

Uncalibrated thermodynamic integration data for the cycle shown in figure 3 in the absence of NAD^+, prior to a thermodynamic cycle least squares analysis. Errors in ΔG stem from a linear regression analysis, which is not appropriate to the FMN reductions 1→2 and 4→8. All energy values in kcal/mol.

ΔG_{12} =	-166.3	±	1.2
ΔG_{15} =	7.1		
ΔG_{34} =	-164.9	±	2.2
ΔG_{56} =	-166.4	±	2.3
ΔG_{78} =	-167.1	±	1.9
ΔG_{72} =	1.9	±	4.3
ΔG_{63} =	-1.0	±	2.4
ΔG_{84} =	9.8		

7. Thermodynamic integration data, NAD^+

Uncalibrated thermodynamic integration data for the cycle shown in figure 3 in the presence of NAD^+, prior to a thermodynamic cycle least squares analysis. Errors in ΔG stem from a linear regression analysis, which is not appropriate to the FMN reductions 1→2 and 4→8. All energy values are in kcal/mol.

ΔG_{12} =	-168.1	±	3.5
ΔG_{15} =	0.8		
ΔG_{34} =	-165.2	±	2.5
ΔG_{56} =	-165.7	±	3.6
ΔG_{78} =	-168.0	±	1.5
ΔG_{72} =	-1.5	±	3.6
ΔG_{63} =	-2.7	±	3.2
ΔG_{84} =	2.7		

8. Thermodynamic cycle analysis in the presence of NAD^+

Data analysis corresponding to the thermodynamic cycle, the enumeration of redox states corresponds to figure 3. The extended coefficient matrix gives the initial and final states (indicated by -1 and +1, respectively) of a reaction, and the ΔG values obtained from the thermodynamic integration as the last column. It is followed by the solution of eq. 8, and the residual vector $\mathbf{A}^T\mathbf{A}\vec{G} - \mathbf{A}^T\Delta\vec{G}_{sim}$ and the measures of error, cf. section 2.3, main article.

```
least squares thermodynamic network analysis

         8  species, 9  equations

extended coefficient matrix
-1   1   0   0   0   0   0   0 -166.3
-1   0   0   0   1   0   0   0    7.1
 0   0  -1   1   0   0   0   0 -164.9
 0   0   0   0  -1   1   0   0 -166.4
 0   0   0   0   0   0  -1   1 -167.1
 0   1   0   0   0   0  -1   0    1.9
 0   0   1   0   0  -1   0   0   -1.0
 0   0   0   1   0   0   0  -1    9.8
 1   0   0   0   0   0   0   0    0

solution
i, residue, Delta G (kcal/mol), potential (meV)
   1     1      0.000       0.0
   2     2   -166.262   -7209.7
   3     3   -160.412   -6956.0
```

```
   4     4   -325.350  -14108.3
   5     5      7.063     306.3
   6     6   -159.375   -6911.0
   7     7   -168.125   -7290.5
   8     8   -335.187  -14534.9

eq. no., residual (kcal/mol)
   1    0.037
   2    0.037
   3    0.037
   4    0.038
   5    0.038
   6    0.038
   7    0.037
   8    0.037
   9    0.000

 3.8E-002   = maximum residual (kcal/mol)
 0.11       = rms error (kcal/mol)
```

9. Thermodynamic cycle analysis in the absence of NAD^+

Data analysis corresponding to the thermodynamic cycle, the enumeration of redox states corresponds to figure 3. The extended coefficient matrix gives the initial and final states (indicated by -1 and +1, respectively) of a reaction, and the ΔG values obtained from the thermodynamic integration as the last column. It is followed by the solution of eq. 8, and the residual vector $\mathbf{A}^T\mathbf{A}\vec{G} - \mathbf{A}^T\Delta\vec{G}_{sim}$ and the measures of error, cf. section 2.3, main article.

```
least squares thermodynamic network analysis

         8  species, 9  equations

extended coefficient matrix
-1   1   0   0   0   0   0   0 -168.1
-1   0   0   0   1   0   0   0    0.8
 0   0  -1   1   0   0   0   0 -165.2
 0   0   0   0  -1   1   0   0 -165.7
 0   0   0   0   0   0  -1   1 -168.0
 0   1   0   0   0   0  -1   0   -1.5
 0   0   1   0   0  -1   0   0   -2.7
 0   0   0   1   0   0   0  -1    2.7
 1   0   0   0   0   0   0   0    0

solution
i, residue, Delta G (kcal/mol), potential (meV)
   1     1      0.000       0.0
   2     2   -168.213   -7294.3
```

```
   3     3   -167.262   -7253.1
   4     4   -332.350  -14411.8
   5     5      0.913      39.6
   6     6   -164.675   -7140.9
   7     7   -166.825   -7234.1
   8     8   -334.937  -14524.0

eq. no., residual (kcal/mol)
   1    0.113
   2    0.113
   3    0.112
   4    0.112
   5    0.112
   6    0.112
   7    0.113
   8    0.113
   9    0.000

0.11       = maximum residual (kcal/mol)
0.32       = rms error (kcal/mol)
```

10.1. Interacting model analysis, no NAD^+, input

Input to the least-squares analysis of the interaction model, eq. 8, using the ΔG values computed from the thermodynamic cycle analysis, table 1. The extended coefficient matrix encodes the linear combination of thermodynamic state free energies, figure 3, that sum up to a reaction within the thermodynamic cycle, the corresponding ΔG value is given in the last column. The first three unknowns correspond to the site energies, the last three to the interactions.

```
8 6  equations, variables
  1  0  0  0  0  0    6.0
  0  0  1  0  1  0    5.0
  0  1 -1  1 -1  0    0.7
  0  0  1  0  1  1    5.5
  1  0  0  1  1  0    7.7
  0  1  0  0  0  1    5.8
  0  1 -1  0  0  0    1.9
  0  1  0  0  0  0    5.7

list:
1  1   VA      FMN
2  2   VB      N1A
3  3   VC      N3
4  4   VAB     FMN,N1A
5  5   VAC     FMN,N3
6  6   VBC     N1A,N3
```

10.2. Interacting model analysis, no NAD^+, output

Output of the least-squares analysis of the interaction model, eq. 8, using the ΔG values computed from the thermodynamic cycle analysis, table 1. The extended coefficient matrix encodes the linear combination of thermodynamic state free energies, figure 3, that sum up to a reaction within the thermodynamic cycle, the corresponding ΔG value is given in the last column. Solution and residual vector are followed by measures of error.

```
least squares network analysis

         6  species
         8  equations

extended coefficient matrix:
 1   0   0   0   0   0   6.00
 0   0   1   0   1   0   5.00
 0   1  -1   1  -1   0   0.70
 0   0   1   0   1   1   5.50
 1   0   0   1   1   0   7.70
 0   1   0   0   0   1   5.80
 0   1  -1   0   0   0   1.90
 0   1   0   0   0   0   5.70

solution
i, V (kcal/mol)
   1    6.012
   2    5.588
   3    3.675
```

```
   4    0.237
   5    1.438
   6    0.300

eq. no., residual (kcal/mol)
   1    0.012
   2    0.113
   3    0.012
   4    0.088
   5    0.013
   6    0.087
   7    0.013
   8    0.112

0.11       = maximum residual (kcal/mol)
0.14       = rms error (kcal/mol)
```

11.1. Interacting model analysis, NAD^+, input

Input to the least-squares analysis of the interaction model, eq. 9, using the ΔG values computed from the thermodynamic cycle analysis, table 1. The extended coefficient matrix encodes the linear combination of thermodynamic state free energies, figure 3, that sum up to a reaction within the thermodynamic cycle, the corresponding ΔG value is given in the tenth column. The 11th and 12th column refer to the intial and the final state of the reaction to be modelled. The first three unknowns correspond to the site energies, the last six to the interactions.

```
13 9
  1  0  0  0  0  0  0  0  0   6.0  1 5    no NAD+
  0  0  1  0  1  0  0  0  0   5.0  5 6    no NAD+
  0  1 -1  1 -1  0  0  0  0   0.7  6 3    no NAD+
  0  0  1  0  1  1  0  0  0   5.5  3 4    no NAD+
  1  0  0  1  1  0  0  0  0   7.7  8 4    no NAD+
  0  1  0  0  0  1  0  0  0   5.8  7 8    no NAD+
  0  1 -1  0  0  0  0  0  0   1.9  7 2    no NAD+
  0  1  0  0  0  0  0  0  0   5.7  2 1    no NAD+
  1  0  0  0  0  0  1  0  0  12.2  1 5    NAD+
  0  1  0  0  0  0  0  1  0   7.6  1 2    NAD+
  0  0  1  0  1  0  0  0  1   4.2  5 6    NAD+
  0  1 -1  0  0  0  0  1 -1   5.2  7 2    NAD+
  1  0  0  1  1  0  1  0  0  14.9  8 4    NAD+

list
1  1   VA
2  2   VB
```

```
3  3   VC
4  4   VAB
5  5   VAC
6  6   VBC
7  7   VAD
8  8   VBD
9  9   VCD

A=FMN, B=N1A, C=N3, D=NAD+
```

11.2. Interacting model analysis, NAD^+, output

Output of the least-squares analysis of the interaction model, eq. 9, using the ΔG values computed from the thermodynamic cycle analysis, table 1. The extended coefficient matrix encodes the linear combination of thermodynamic state free energies, figure 3, that sum up to a reaction within the thermodynamic cycle, the corresponding ΔG value is given in the last column. Solution and residual vector are followed by measures of error.

```
least squares network analysis

         9  species
        13  equations

extended coefficient matrix
  1    0    0    0    0    0    0    0    0    6.00
  0    0    1    0    1    0    0    0    0    5.00
  0    1   -1    1   -1    0    0    0    0    0.70
  0    0    1    0    1    1    0    0    0    5.50
  1    0    0    1    1    0    0    0    0    7.70
  0    1    0    0    0    1    0    0    0    5.80
  0    1   -1    0    0    0    0    0    0    1.90
  0    1    0    0    0    0    0    0    0    5.70
  1    0    0    0    0    0    1    0    0   12.20
  0    1    0    0    0    0    0    1    0    7.60
  0    0    1    0    1    0    0    0    1    4.20
  0    1   -1    0    0    0    0    1   -1    5.20
  1    0    0    1    1    0    1    0    0   14.90
```

```
solution
i, V (kcal/mol)
   1     5.785
   2     5.512
   3     3.504
   4     0.446
   5     1.685
   6     0.000
   7     6.000
   8     2.127
   9    -1.027

eq. no., residual (kcal/mol)
   1    0.2154
   2    0.1885
   3    0.0692
   4    0.0115
   5    0.2154
   6    0.0115
   7    0.1077
   8    0.1885
   9    0.2846
  10    0.0385
  11    0.0385
  12    0.0385
  13    0.2846

0.28       = maximum residual (kcal/mol)
0.29       = rms error (kcal/mol)
```

12. Force field parameters of iron sulfur clusters FES and SF4

Force field parameters are given in the Amber notation. Units are kcal/mol for energies, Å for bond lengths, degrees for bond and dihedral angles. Masses are in Dalton, charges as multiples of the (positive) elementary charge.

12.1. FES, oxidated

MASS	
Atom	Mass
Fe	55.00
S	32.06

BOND		
Bond	K_r	r_{eq}
Fe - S	88.20	2.21

ANGLE		
Angle	K_θ	θ_{eq}
Fe - S - Fe	20.3	75.2
S - Fe - S	20.2	104.8

CHARGE	
Atom	Charge
S	-0.4127625
Fe	0.4438375
S	-0.4103625
Fe	0.4444375

12.1.1. FES-coordinating cysteine, oxidated

MASS		BOND		
Atom	Mass	Bond	K_r	r_{eq}
S_{cys}	32.06	S_{cys} - Fe	63.3	2.32

ANGLE		
Angle	K_θ	θ_{eq}
S_{cys} - Fe - S	14.6	110.50
S_{cys} - Fe - S_{cys}	10.1	109.05
C - S_{cys} - Fe	10.9	109.60

CHARGE	
Atom	Charge
N	-0.415700
C	0.042900
C	-0.079000
S_{cys}	-0.516288
C	0.597300
O	-0.567900
H	0.271900
H	0.076600
H	0.091000
H	0.091000

12.2. FES, reduced

MASS

Atom	Mass
Fe	55.00
S	32.06

BOND

Bond	K_r	r_{eq}
Fe - S	71.35	2.25

ANGLE

Angle	K_θ	θ_{eq}
S - Fe - S	16.6	104.45
Fe - S - Fe	17.5	75.60

CHARGE

Atom	Charge
S	-0.6515625
Fe	0.4802375
S	-0.6523625
Fe	0.4953375

12.2.1. FES-coordinating cysteine, reduced

MASS		BOND		
Atom	Mass	Bond	K_r	r_{eq}
S_{cys}	32.06	S_{cys} - Fe	42.7	2.36

ANGLE		
Angle	K_θ	θ_{eq}
S - Fe - S_{cys}	10.85	108.80
Fe - S_{cys} - C	13.35	107.00
S_{cys} - Fe - S_{cys}	8.25	106.90

CHARGE	
Atom	Charge
N	-0.4157000
C	0.0429000
C	-0.0790000
S_{cys}	-0.6679125
C	0.5973000
O	-0.5679000
H	0.2719000
H	0.0766000
H	0.0910000
H	0.0910000

12.3. SF4, oxidated

MASS

Atom	Mass
Fe	55.00
S	32.06

BOND

Bond	K_r	r_{eq}
Fe - S	67.65	2.285

ANGLE

Angle	K_θ	θ_{eq}
S - Fe - S	10.0	105.75
Fe - S - Fe	9.5	72.15

CHARGE

Atom	Charge
Fe	0.2836833333
Fe	0.2820833333
Fe	0.2842833333
Fe	0.2834833333
S	-0.2901166667
S	-0.2863166667
S	-0.2889166667
S	-0.2871166667

12.3.1. SF4-coordinating cysteine, oxidated

MASS		BOND		
Atom	Mass	Bond	K_r	r_{eq}
S_{cys}	32.06	Fe - S_{cys}	82.8	2.28

ANGLE		
Angle	K_θ	θ_{eq}
S - Fe - S_{cys}	12.45	115.00
C - S_{cys} - Fe	18.2	104.90

CHARGE	
Atom	Charge
N	-0.4157000000
C	0.0429000000
C	-0.0790000000
S_{cys}	-0.4952666667
C	0.5973000000
O	-0.5679000000
H	0.2719000000
H	0.0766000000
H	0.0910000000
H	0.0910000000

12.4. SF4, reduced

MASS

Atom	Mass
Fe	55.00
S	32.06

BOND

Bond	K_r	r_{eq}
Fe - S	55.2	2.31

ANGLE

Angle	K_θ	θ_{eq}
S - Fe - S	8.2	105.40
Fe - S - Fe	8.8	71.50

CHARGE

Atom	Charge
Fe	0.289516667
Fe	0.337416667
Fe	0.297016667
Fe	0.330316667
S	-0.471283330
S	-0.453783330
S	-0.419883330
S	-0.419883330

12.4.1. SF4-coordinating cysteine, reduced

MASS		BOND		
Atom	Mass	Bond	K_r	r_{eq}
S_{cys}	32.06	Fe - S_{cys}	60.9	2.31
		S_{cys} - C	154.2	1.82

ANGLE		
S - Fe - S_{cys}	11.0	113.20
Fe - S_{cys} - C	15.6	105.60

CHARGE	
Atom	Charge
N	-0.415700
C	0.042900
C	-0.079000
S_{cys}	-0.622358
C	0.597300
O	-0.567900
H	0.271900
H	0.076600
H	0.091000
H	0.091000