Supporting Electronic Information

Structural and functional relevance of the conserved residue V13 in the triheme cytochrome PpcA from *Geobacter sulfurreducens*

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Figure S1. Diagram of a heme *c* numbered according to the IUPAC-IUB nomenclature [1]. The arrows correspond to the connectivities that are used to assign the heme substituent signals. The solid arrows correspond to the connectivities that are observable in the 2D ^{1}H-TOCSY and constitute the starting point for the assignment. The dashed arrows correspond to the additional connectivities identified in the 2D ^{1}H-NOESY spectrum.

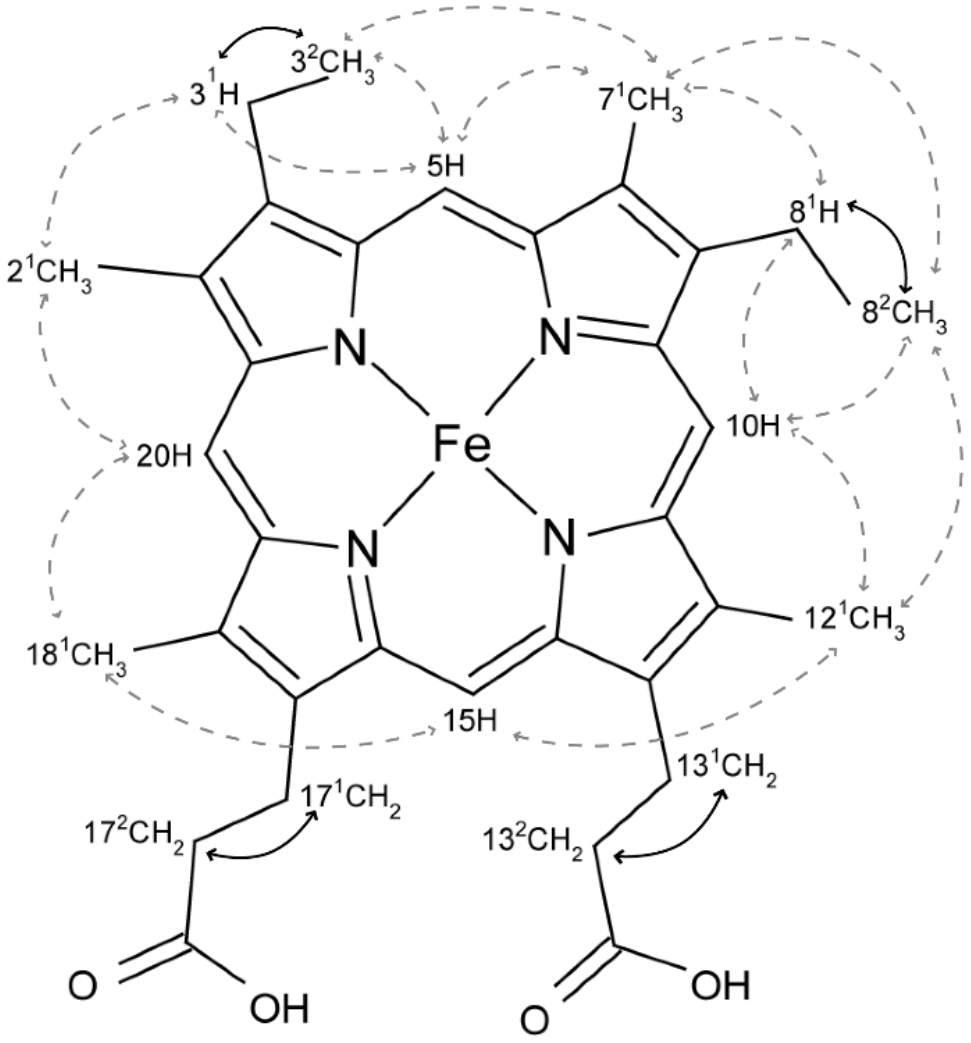

Figure S2. Expansions of 2D ^{1}H-EXSY NMR spectra obtained for PpcA and V13 mutants at different levels of oxidation (288 K and pH 6). Cross-peaks resulting from intermolecular electron transfer between the oxidation stages *1-3* are indicated for the heme methyls $12^{1}CH_{3}^{I}$ (green dashed lines), $7^{1}CH_{3}^{III}$ (orange dashed lines) and $12^{1}CH_{3}^{IV}$ (blue dashed lines). Roman (I, III and IV) and Arabic (*1*, *2* and *3*) numbers indicate the hemes and the oxidation stages, respectively. In order not to overcrowd the figure, the cross-peaks to oxidation stage *0* are not shown. The chemical shift values for each heme methyl in the oxidation stage *0* are listed in Table 2. The squares indicate the position of the signals not visible at the spectra level represented in the figures.

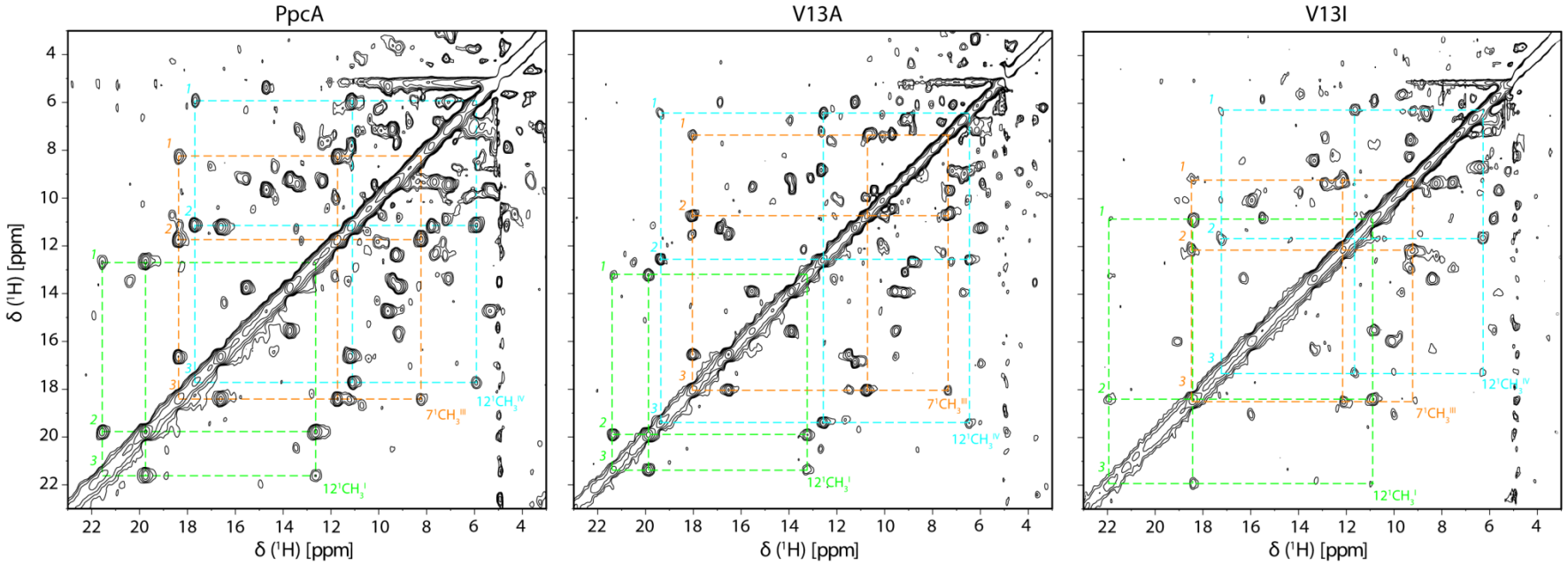

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Figure S2 *(continued)*

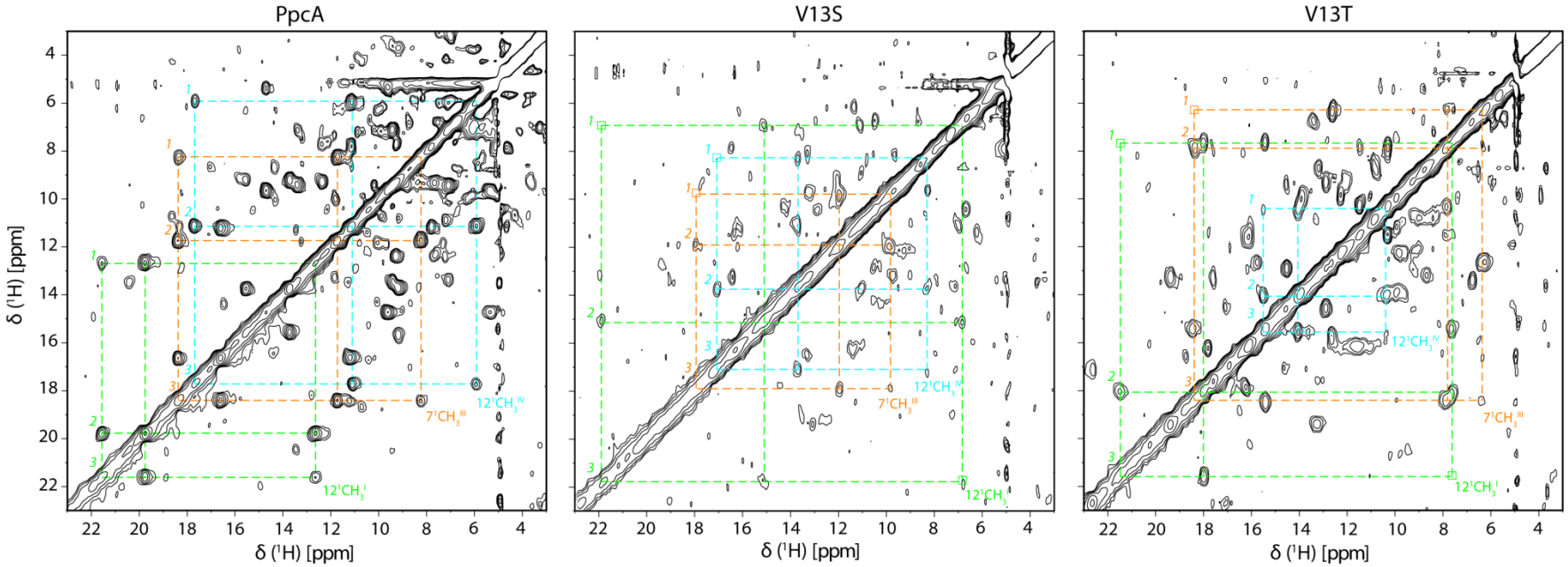

Figure S3. The C_α tracings derived from the crystal structures of the V13A (PDB code: 4HBF) and V13T (PDB code: 4HC3) mutants overlaid on the native PpcA crystal structure (PDB code: 1OS6). C_α and heme carbons are shown in green for PpcA, cyan for V13A and magenta for V13T. The heme nitrogen atoms are shown in blue while iron and oxygen atoms are shown in red in all cases. The spatial orientation of the hemes is identical to the represented in Figure 1. From left to right: Heme IV, III, and I.

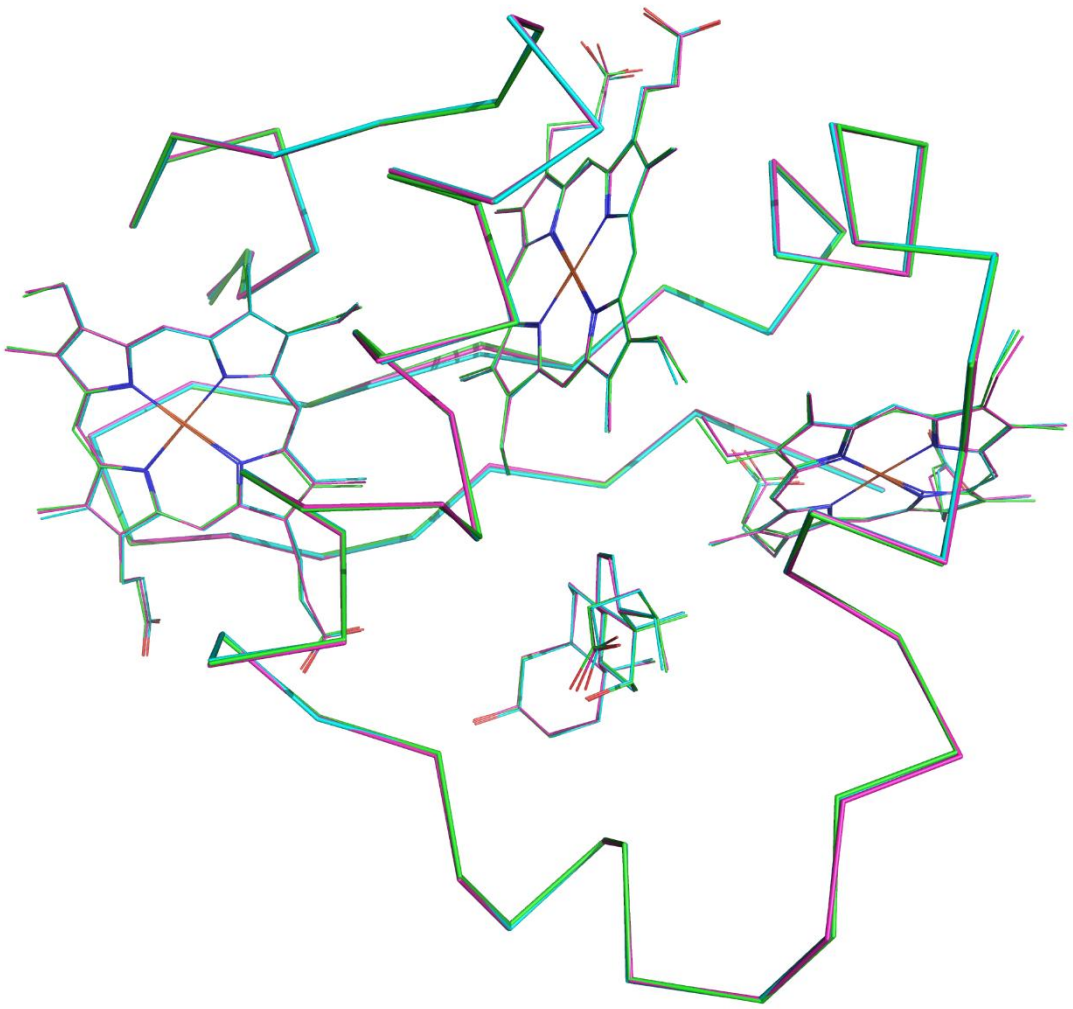

Figure S4. The normalized CD signal at the heme peak in the visible region (approximately 402-404 nm) for each protein is plotted against temperature. The inset shows the variation of the CD signal between 20 and 90 ºC for each mutant. Protein samples at a concentration of 0.6 mg/ml in 20 mM sodium phosphate pH 7.8 buffer were used for measurements. Spectra were collected in 0.1 cm path length quartz cells on a Jasco J-810 Spectropolarimeter. Spectra in the wavelength range of 185 – 260 nm and 360 – 425 nm were collected using the following acquisition parameters: 0.1 nm steps, 1 nm band-width, 4 s response, 100-millidegree sensitivity, 50 nm/min scanning speed with an accumulation of 3. Spectra were collected every 5 ºC in the temperature range of 20 – 90 ºC. Jasco peltier PTC-424S was used for temperature control.

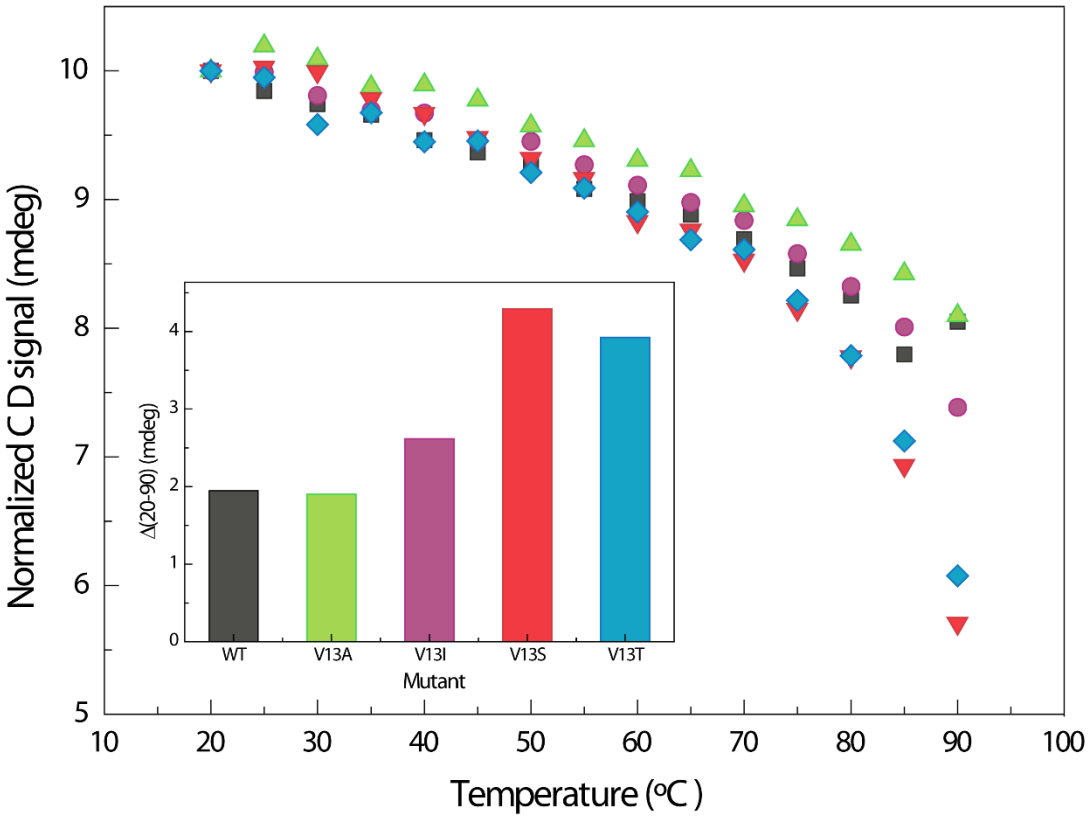

Table S1 - Chemical shifts (ppm) of the heme protons of PpcA V13 mutants in the reduced state at pH 8 and 288 K. The chemical shifts correspondent to the second conformation observed for mutants V13I, V13S and V13T are given in parenthesis. The values for PpcA were previously determined [2] and are listed for comparison.

Heme substituent	Mutant	Heme I	Heme III	Heme IV
5H	V13A	9.64	10.59	9.03
	V13I	9.65 (9.62)	10.56 (10.51)	9.04 (9.04)
	V13S	9.65 (9.60)	10.54 (10.46)	9.05 (9.09)
	V13T	9.65 (9.57)	10.58 (10.45)	9.00 (9.11)
	PpcA	9.65	10.58	9.02
10H	V13A	9.10	9.85	9.32
	V13I	9.11 (9.02)	9.86 (9.86)	9.35 (9.26)
	V13S	9.00 (8.86)	9.85 (9.83)	9.43 (9.54)
	V13T	9.10 (8.84)	9.85 (9.85)	9.35 (9.54)
	PpcA	9.12	9.86	9.33
15H	V13A	9.27	9.47	9.54
	V13I	9.28 (9.28)	9.44 (9.44)	9.52 (9.52)
	V13S	9.31 (9.31)	9.43 (9.43)	9.52 (9.52)
	V13T	9.28 (9.28)	9.48 (9.48)	9.54 (9.54)
	PpcA	9.26	9.45	9.51
20H	V13A	9.52	10.12	9.40
	V13I	9.50 (9.50)	10.29 (10.34)	9.42 (9.42)
	V13S	9.49 (9.49)	10.32 (10.33)	9.46 (9.46)
	V13T	9.50 (9.50)	10.17 (10.38)	9.41 (9.41)
	PpcA	9.50	10.14	9.39
2^1CH_3	V13A	3.56	4.36	3.61
	V13I	3.56 (3.56)	4.41 (4.49)	3.64 (3.64)
	V13S	3.58 (3.58)	4.45 (4.60)	3.66 (3.66)
	V13T	3.58 (3.58)	4.39 (4.63)	3.64 (3.64)
	PpcA	3.56	4.35	3.61
7^1CH_3	V13A	3.57	4.14	3.03
	V13I	3.58 (3.56)	4.14 (4.14)	3.03 (3.03)
	V13S	3.56 (3.52)	4.14 (4.14)	3.04 (3.06)
	V13T	3.58 (3.50)	4.14 (4.14)	3.01 (3.07)
	PpcA	3.58	4.14	3.02
12^1CH_3	V13A	2.55	3.50	3.94
	V13I	2.55 (2.52)	3.51 (3.51)	3.95 (3.93)
	V13S	2.53 (2.50)	3.51 (3.51)	4.02 (4.04)
	V13T	2.57 (2.50)	3.51 (3.51)	3.99 (4.01)
	PpcA	2.55	3.50	3.95
18^1CH_3	V13A	3.35	3.86	3.36
	V13I	3.34 (3.34)	3.85 (3.84)	3.37 (3.37)
	V13S	3.33 (3.33)	3.84 (3.84)	3.41 (3.41)
	V13T	3.33 (3.33)	3.88 (3.79)	3.37 (3.37)
	PpcA	3.34	3.86	3.34

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Supplementary Table S1*(continued)*

Heme substituent	Mutant	Heme I	Heme III	Heme IV
3^1H	V13A	6.28	6.94	6.03
	V13I	6.30 (6.27)	6.90 (6.89)	6.05 (6.05)
	V13S	6.28 (6.24)	6.91 (6.90)	6.05 (6.02)
	V13T	6.29 (6.19)	6.93 (6.89)	6.02 (6.06)
	PpcA	6.30	6.91	6.04
8^1H	V13A	6.27	6.59	6.30
	V13I	6.28 (6.19)	6.61 (6.61)	6.29 (6.25)
	V13S	6.18 (6.04)	6.61 (6.60)	6.33 (6.39)
	V13T	6.28 (6.00)	6.60 (6.60)	6.32 (6.43)
	PpcA	6.29	6.60	6.28
3^2CH_3	V13A	2.13	1.88	2.07
	V13I	2.14 (2.16)	1.73 (1.82)	2.06 (2.06)
	V13S	2.16 (2.19)	1.85 (2.00)	2.06 (2.04)
	V13T	2.14 (2.22)	1.82 (2.07)	2.06 (2.05)
	PpcA	2.14	1.73	2.06
8^2CH_3	V13A	1.81	2.97	1.55
	V13I	1.76 (1.70)	2.99 (2.99)	1.55 (1.55)
	V13S	1.69 (1.57)	2.98 (2.97)	1.58 (1.58)
	V13T	1.81 (1.55)	2.97 (2.97)	1.58 (1.60)
	PpcA	1.79	2.98	1.55

REFERENCES

1 Moss, G. P. (1988) Nomenclature of tetrapyrroles. Recommendations 1986 IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Eur J Biochem 178, 277-328.

2 Morgado, L., Bruix, M., Orshonsky, V., Londer, Y. Y., Duke, N. E., Yang, X., Pokkuluri, P. R., Schiffer, M. and Salgueiro, C. A. (2008) Structural insights into the modulation of the redox properties of two *Geobacter sulfurreducens* homologous triheme cytochromes. Biochim Biophys Acta 1777, 1157-1165.