Supporting Information

REACTIVITY OF INORGANIC SULFIDE SPECIES TOWARD A HEME PROTEIN MODEL.

Silvina A. Bieza,[†] Fernando Boubeta,[†] Alessandro Feis, [§] Giulietta Smulevich,[§] Darío A. Estrin,[†] Leonardo Boechi,[*,‡] and Sara E. Bari[*,†]

[†]Departamento de Química Inorgánica, Analítica y Química Física/INQUIMAE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Ciudad Universitaria, Buenos Aires C1428EHA, Argentina

[‡]Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Ciudad Universitaria, Buenos Aires
C1428EHA, Argentina

[§]Dipartimento di Chimica "Ugo Schiff", Universita' di Firenze, Via della Lastruccia 3-13, 50019 Sesto Fiorentino, Firenze, Italy

Figure S1 - QM optimization at PBE 6-31G** DFT level were performed on a ferric low-spin hexacoordinated core-porphyrinate, with both an imidazole ring and SH$^-$ as 5th and 6th ligand. An N-methyl acetamide molecule was used to provide a counterpart for hydrogen bonding to the imidazole ligand. Also, a control calculation (full-optimization at the same level of theory) was performed in the absence of N-methyl acetamide. Mülliken charge populations were computed for the optimized structures.
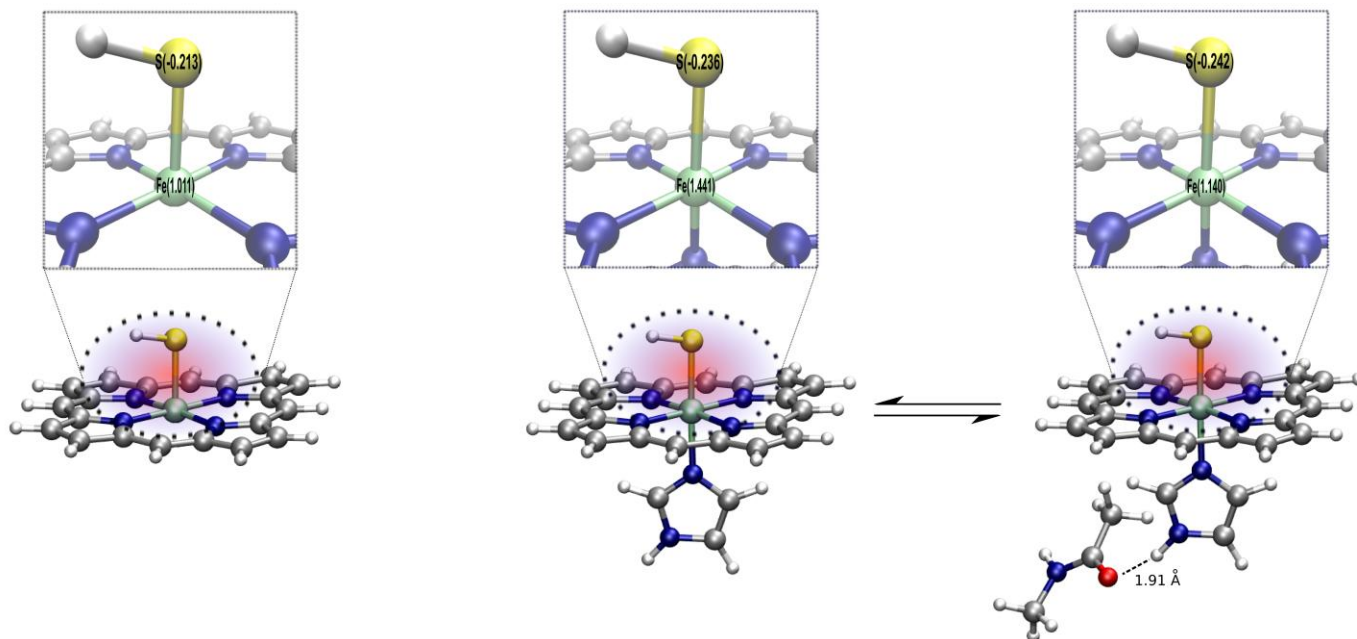

Figure S2 – Absorption spectra of 2.6 x 10^{-6} M Fe^{III}NAcMP11 in the absence (dashed line) and in the presence (continuous line) of 4.5 mM cysteine. The optical path was 1 mm.
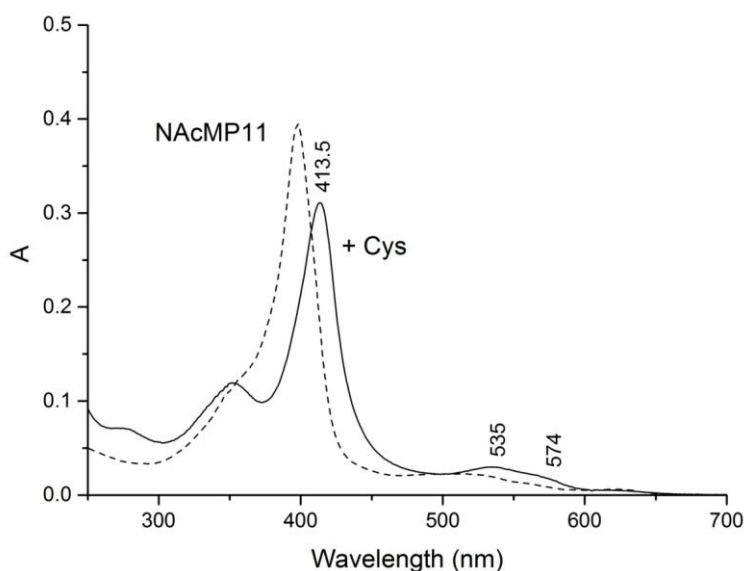
Figure S3A - Reactivity of the NacMP11Fe^{III}-S(sulfide) toward 1-methylimidazole. <u>A</u>: 1) [Fe^{III}NAcMP11]= 5x10^{-6}M, pH 6.8 (PO$_4^{3-}$, 0.1M, 2.5mL, black line), 2) 10 μL 1-methylimidazole (red line), 3) 100μL H$_2$S (g) (blue line). The reaction of Fe^{III}NAcMP11 with 1-methylimidazole led to the formation of a [NAcMP11Fe^{III} (Imid)(His)] complex, with λ$_{max}$= 406 nm, at pH 6.8. This complex was indifferent to the addition of H$_2$S (g). <u>B</u>: 1) [Fe^{III}NAcMP11]= 5x10^{-6}M, pH 6.8 (PO$_4^{3-}$, 0.1M, 2.5mL, black line), 2) 100μL H$_2$S (g) (red line), 3) 10 μL 1-methylimidazole (blue line). The samples were deaerated under stream of argon, in quartz cuvettes (1 cm) with silicon septa prior to the addition of the reagent. The addition of 1-methylimidazole after the addition of H$_2$S shows the conversion of the 414 nm complex to the [NAcMP11Fe^{III} (1-MeIm)(His)], without modification of the Fe^{III} redox state in the process. Reagents are numbered according the order of addition to the solution in each experiment.
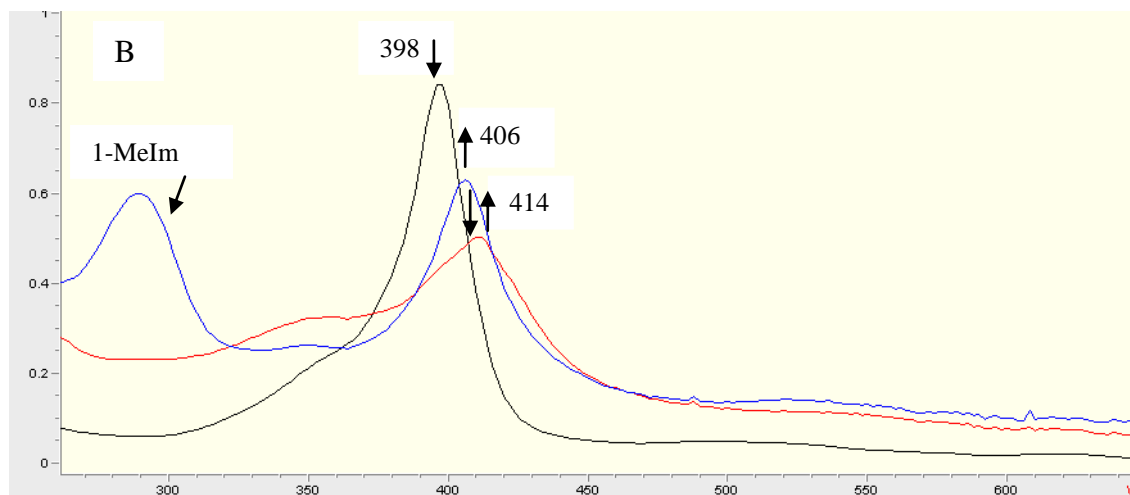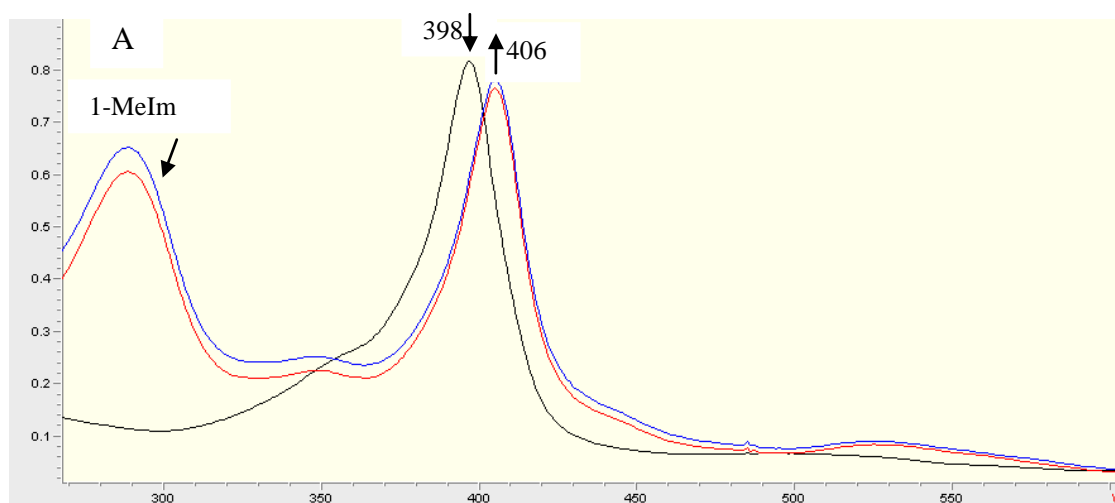
Figure S3B - Reactivity of the NAcMP11Fe^{III}-S(sulfide) toward the addition of dithionite. <u>A</u>: 1) [Fe^{III}NAcMP11]= 7x10^{-6}M, pH 6.8 (PO$_4^{3-}$, 0.1M, 2.5mL) (black line), 2) [S$_2$O$_4^{2-}$]$_{final\ conc}$= 0.15mM (red line), 3) 200µL H$_2$S (g) (blue line). The Fe^{II}NacMP11 is indifferent to the addition of H$_2$S (g). <u>B</u>: 1) [Fe^{III}NAcMP11]= 7x10^{-6}M, pH 6.8 (PO$_4^{3-}$, 0.1M, 2.5mL) (black line), 2) 200µL H$_2$S (g) (red line), 3) [S$_2$O$_4^{2-}$]$_{final\ conc}$= 0.15M (blue line). The samples were deaerated under stream of argon, in quartz cuvettes (1 cm) with silicon septa prior to the addition of the reagent. The NacMP11Fe^{III}-S(sulfide) reacts with sodium dithionite and yields the Fe^{II}NacMP11 form. Reagents are numbered according the order of addition to the solution in each experiment.
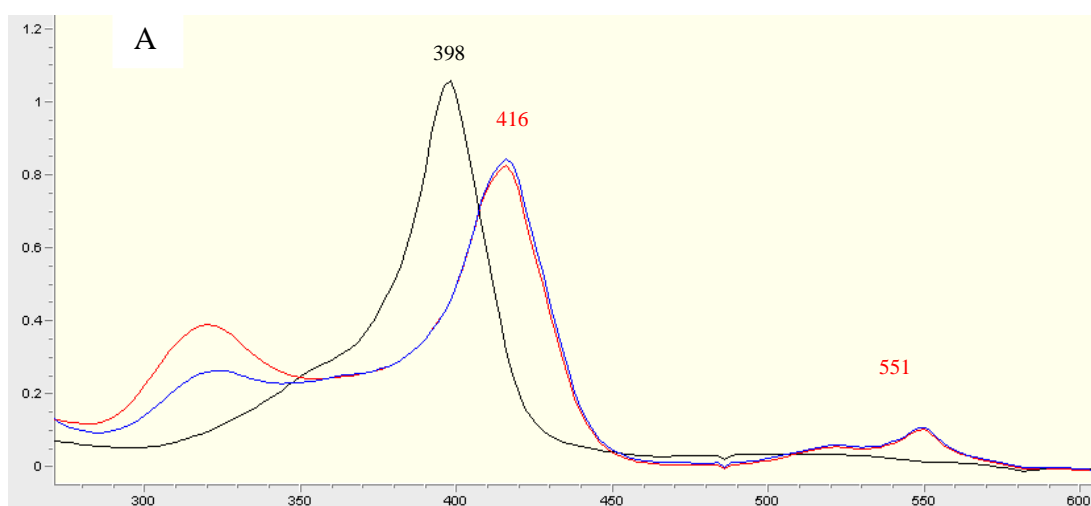

1) [Fe^{III}NAcMP11]= 7x10^{-6}M, pH 6.8 (PO$_4^{3-}$ buffer, 0.1M, 2.5 mL) , 2) [S$_2$O$_4^{2-}$]$_{final\ conc}$= 0.15M, 3) 200µL H$_2$S(g)
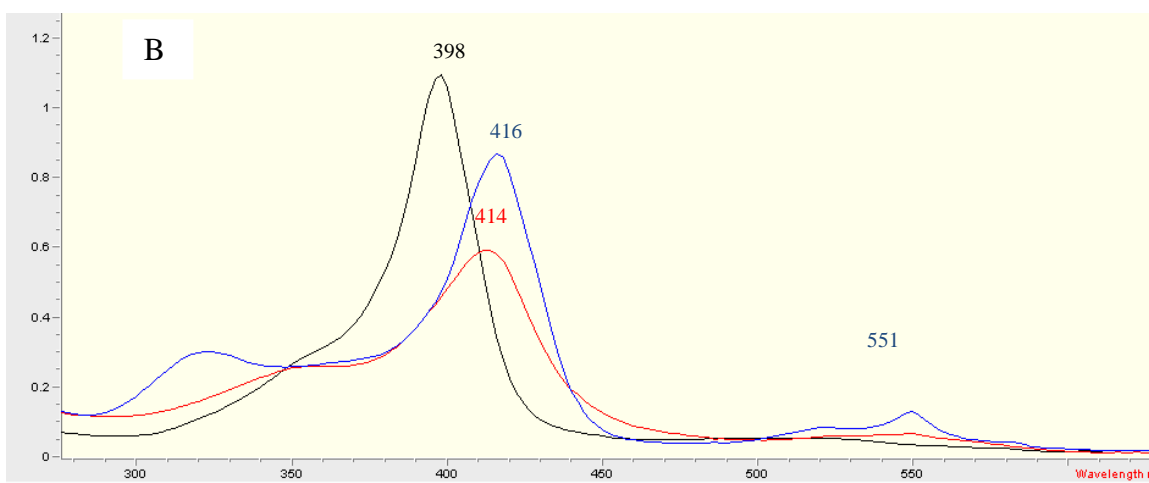
1) [Fe^{III}NAcMP11]= 7x10^{-6}M, pH 6.8 (PO$_4^{3-}$ buffer, 0.1M, 2.5 mL), 2) 200µL H$_2$S(g), 3) [S$_2$O$_4^{2-}$]$_{final\ conc}$= 0.15M

Figure S4 - (middle trace) RR spectra of 2.6 x 10^{-5} M NAcMP11 with 4.5 mM cysteine, in comparison with the RR spectra of unbound and sulfide-bound ferric NAcMP11. The intensities are normalized to the intensity of the v_4 band at 1372-1373 cm^{-1}. The low-frequency region of the cysteine complex is expanded to allow for a better visualization. Experimental conditions: 413.1 nm excitation wavelength, 3 mW power, 1.2 cm^{-1} spectral resolution; low-frequency range: 30 min integration time; high-frequency range: 30 min (NAcMP11), 10 min (+ cysteine), 6 min (+H$_2$S) integration time.
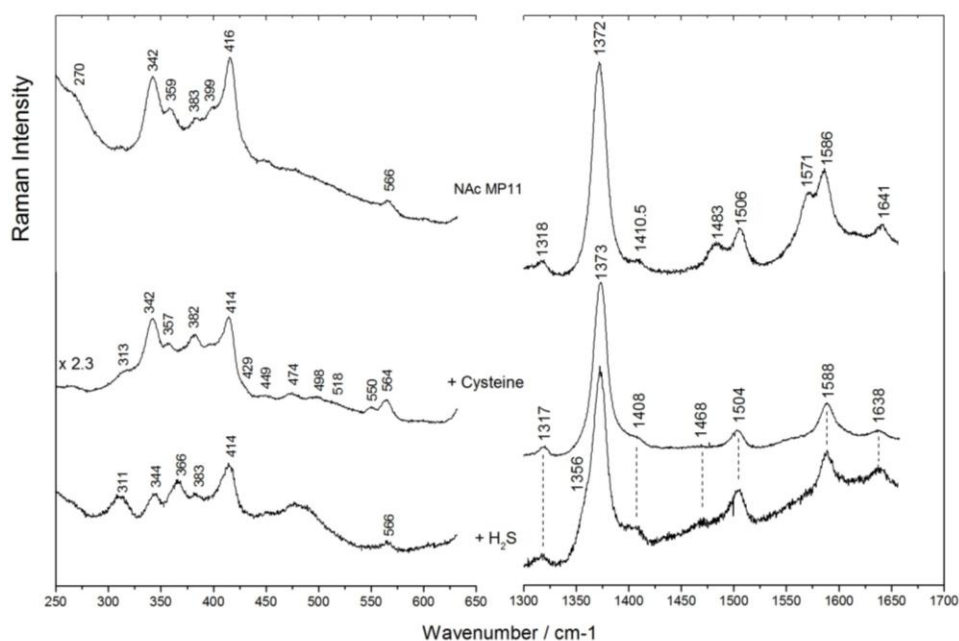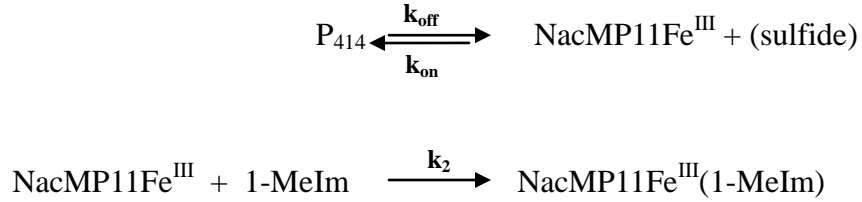

Figure S5. UV-Vis spectra of the Fe^{III}NAcMP11 toward Na$_2$S(anhydrous) solution. A) [Fe^{III}NAcMP11] = [Na$_2$S(anhydrous)]= 1.2x10^{-5}M; B) [Fe^{III}NAcMP11] = 8x10^{-6}M, [Na$_2$S(anhydrous)]= 8x10^{-4}M. Both reactions at pH 6.8; buffer PO$_4^{3-}$, 0.1M, 25ºC.
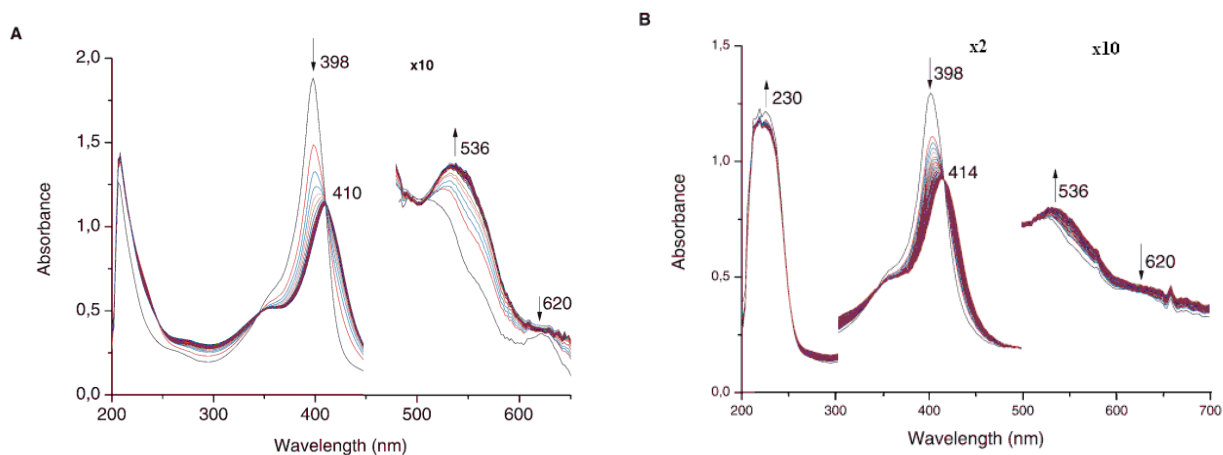
S6. Dissociation kinetic constant

By assuming the following reaction scheme

$$P_{414} \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} NacMP11Fe^{III} + (sulfide)$$

$$NacMP11Fe^{III} + 1\text{-MeIm} \xrightarrow{k_2} NacMP11Fe^{III}(1\text{-MeIm})$$

Expressing the reaction rate as

$$v = v_2 = k_2 \cdot [NacMP11Fe^{III}] \cdot [1-MeIm] = \frac{\partial [NacMP11Fe^{III}(1-MeIm)]}{\partial t}$$

and assuming steady-state for $[NacMP11Fe^{III}]$ the following relationship is obtained:

$$k_{off} \cdot [P_{414}] = k_{on} \cdot [NacMP11Fe^{III}] \cdot [sulfide] + k_2 \cdot [NacMP11Fe^{III}] \cdot [1-MeIm]$$

Thus, the reaction rate can be expressed as a function of the reactants

$$v = \frac{\partial [NacMP11Fe^{III}(1-MeIm)]}{\partial t} = \frac{k_2 \cdot k_{off} \cdot [P_{414}] \cdot [1-MeIm]}{k_{on} \cdot [sulfide] + k_2 \cdot [1-MeIm]}$$

The previous expression can be simplified as follows:

$$v = \frac{\partial [NacMP11Fe^{III}(1-MeIm)]}{\partial t} = k_{obs} \cdot [P_{414}]$$

$$\text{with} \quad k_{obs} = \frac{k_2 \cdot k_{off} \cdot [1-MeIm]}{k_{on} \cdot [sulfide] + k_2 \cdot [1-MeIm]}$$

At high concentration of [1-MeIm], k_{obs} becomes independent of [1-MeIm], and the following equivalence is derived:

$$k_{obs} = k_{off}$$