Navigation links:

1) EPR spectra of native dSiR (from *E. coli*):

A) Reference 1

Link to paper (journal site):
https://doi.org/10.1021/bi00271a038

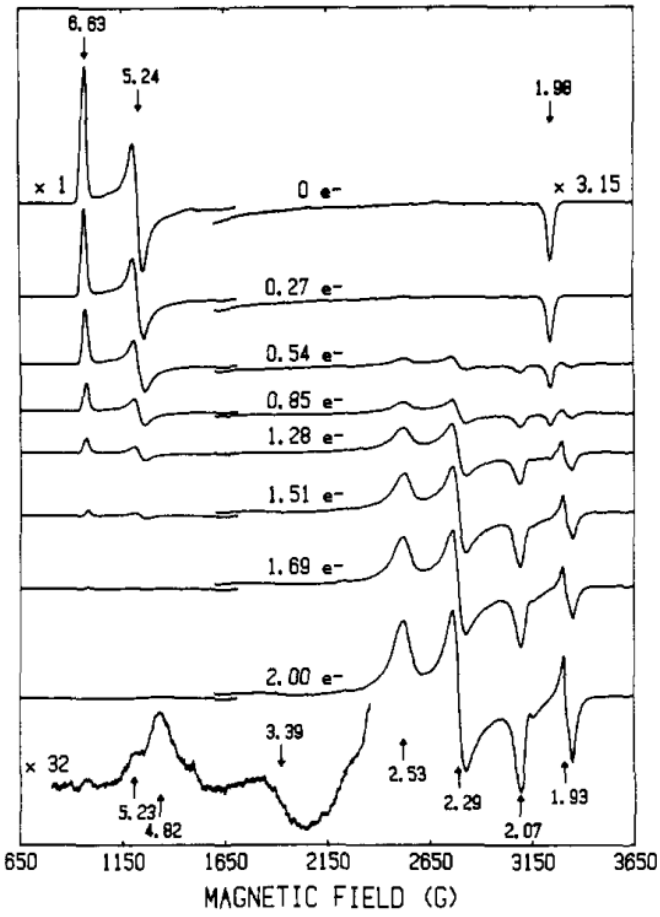


FIGURE 3: EPR spectra at 20 K of photoreduced SiR-HP. Anaerobic solutions containing 210 μM SiR-HP, 40 μM Dfl, and 10 mM EDTA in standard buffer were illuminated in anaerobic EPR tubes for times between 0 and 100 min. The extent of reduction in each tube was determined from the optical spectrum as described in the text. EPR spectra were recorded at microwave frequency 9.12 GHz, 50-mW microwave power, and at a temperature of 20 K. The high-field portion of the spectra was recorded by using an instrument gain 3.15 times that used for the low-field portion. The high-field portion of the spectra for the 1.51-, 1.69-, and 2.00-electron-reduced samples has been displaced slightly upward for the sake of clarity. The bottom magnified spectrum of the low-field region in the 2.00 electron reduced enzyme sample was run at 200-mW power at a relative gain of 32.

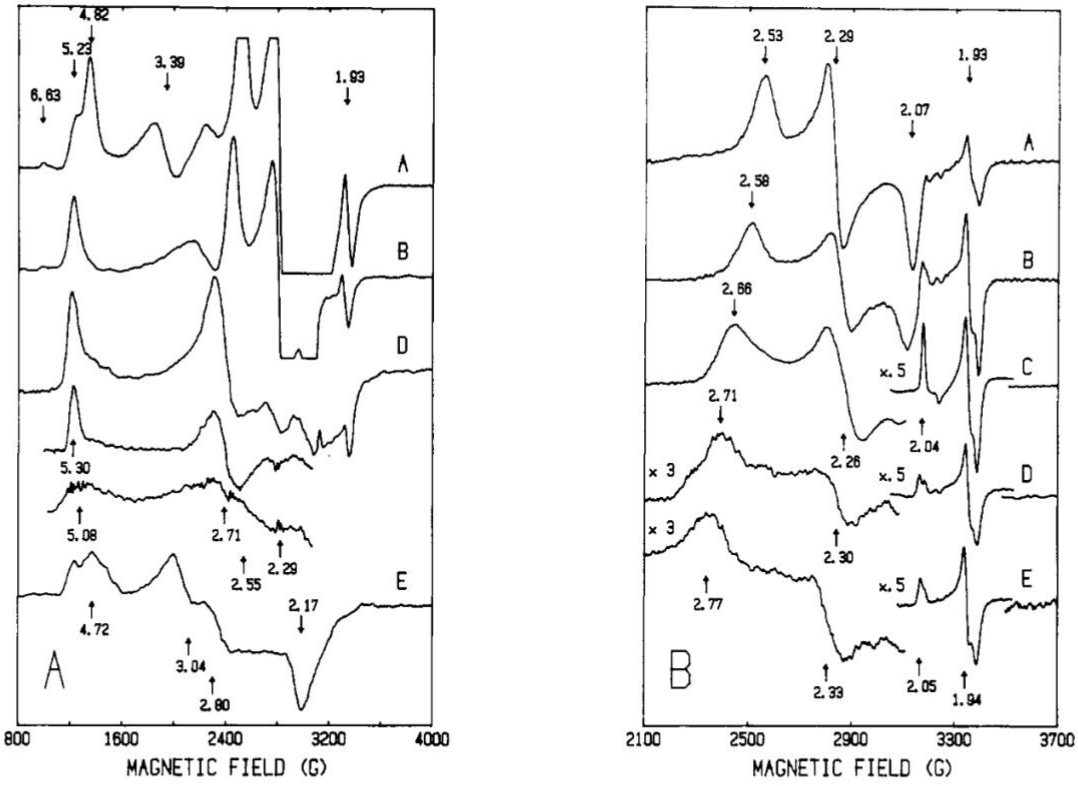


FIGURE 10: EPR spectra of SiR-HP fully reduced in the presence of potential weak field heme ligands. Anaerobic solutions in standard buffer containing 10 mM EDTA and either 210 μM SiR-HP and 40 μM Dfl (spectrum A), 72 μM SiR-HP, 25 μM Dfl, and 5 mM KF (spectrum B), 72 μM SiR-HP, 25 μM Dfl, and 40% (v/v) dimethyl sulfoxide (spectrum C), 72 μM SiR-HP, 25 μM Dfl, and 5 mM KCl (spectrum D), or 100 μM SiR-HP, 25 μM Dfl, and 100 mM sodium formate (spectrum E) were prepared in EPR tubes, and the solutions were photoreduced until no further changes in optical spectrum could be detected. The solutions were frozen in liquid N_2 and EPR spectra recorded at 9.12-GHz microwave frequency. (Panel A) $S = {}^3/_2$ type species: Spectra A, B, and D were recorded at 8 K temperature and 100-mW microwave power, conditions at which the $S = {}^1/_2$ and $g = 1.94$ type species are highly saturated, whereas the $S = {}^3/_2$ type species are not saturated. The features of the $S = {}^1/_2$ type species were permitted to go off scale. The two spectra shown immediately below spectrum D are those derived for the $g = 5.30$ (upper) and $g = 5.08$ (lower) species by the procedure indicated in footnote 6 of the text. Spectrum E was recorded at 5 K and 50-mW microwave power in order to better show the position of the high-field features of the $S = {}^3/_2$ type spectrum (by more fully saturating the overlapping $S = {}^1/_2$ type species). (Panel B) $S = {}^1/_2$ and $g = 1.94$ type species: Spectra were recorded at 20 K temperature and 50-mW microwave power. Under these conditions the spectra of the $S = {}^3/_2$ species are not observed. The spectra have been normalized to constant enzyme concentration in panel B but not in panel A.

B) Reference 2

Full text link (.pdf):
https://www.jbc.org/article/S0021-9258(20)65147-X/pdf

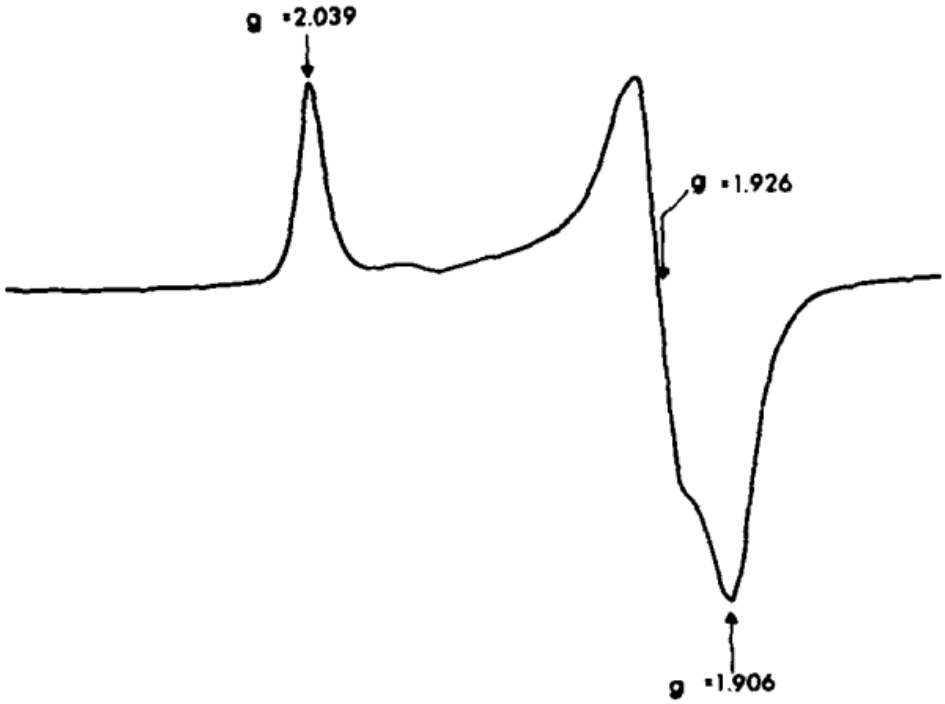


FIG. 4. **EPR spectrum of reduced iron-sulfur center in hemoprotein-CO complex.** A 0.10 mM solution of HP, 1 mM MV^+, and 0.4 mM CO in standard buffer, in a total volume of 0.5 ml, was incubated for 1 h at 23 °C under CO. The sample was then quickly subjected to gel filtration at 4 °C to remove excess CO and methyl viologen. The eluted fraction containing maximum amount of enzyme (0.026 mM HP) was quickly made anaerobic under Ar, 1 mM $Na_2S_2O_4$ added, and the solution quickly frozen in liquid N_2. The EPR spectrum was recorded at microwave frequency 9.14 GHz, temperature 20 K, 50-milliwatt microwave power, and 1 millitesla modulation amplitude. The integrated signal intensity in this sample was 0.86 reduced Fe_4S_4 spin/HP.

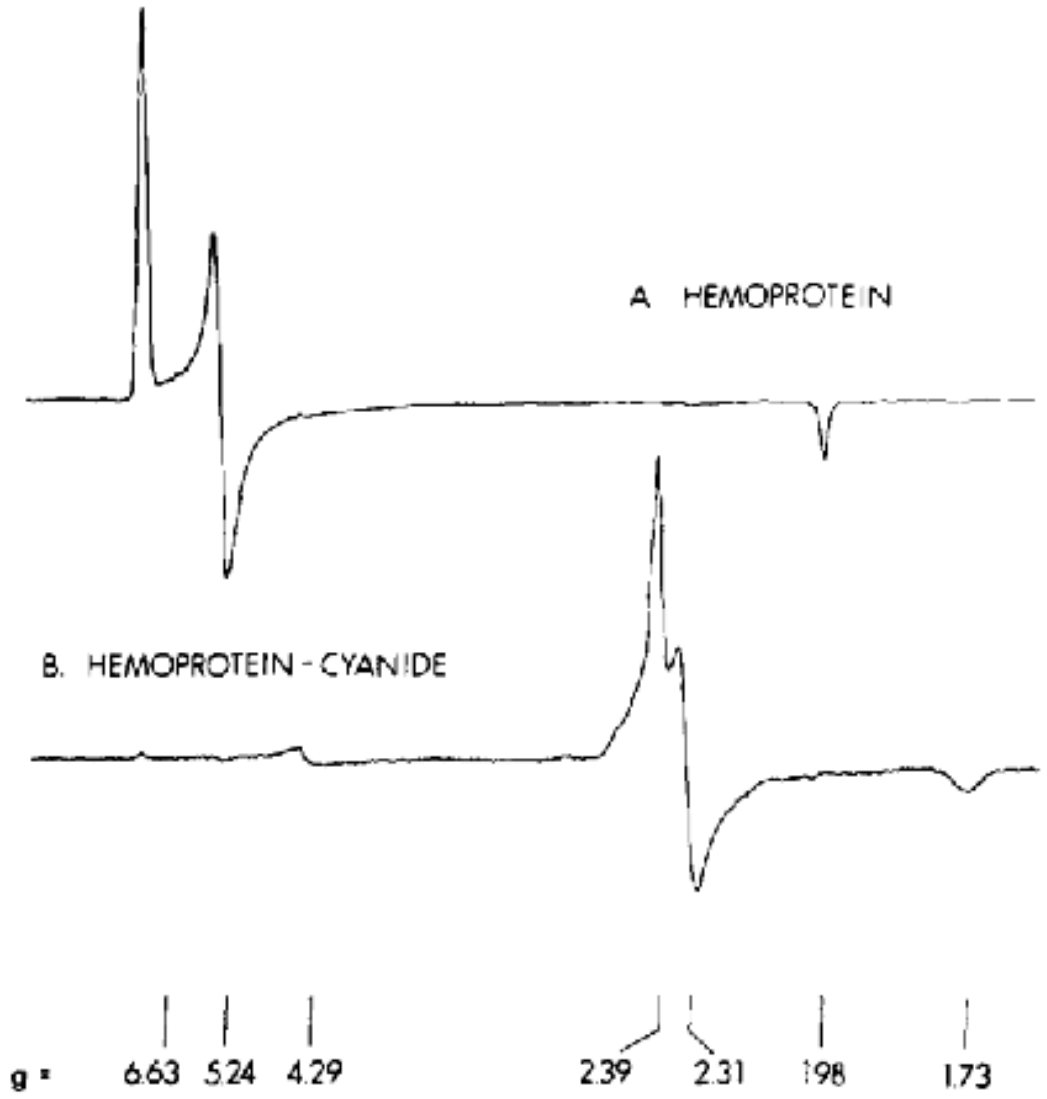
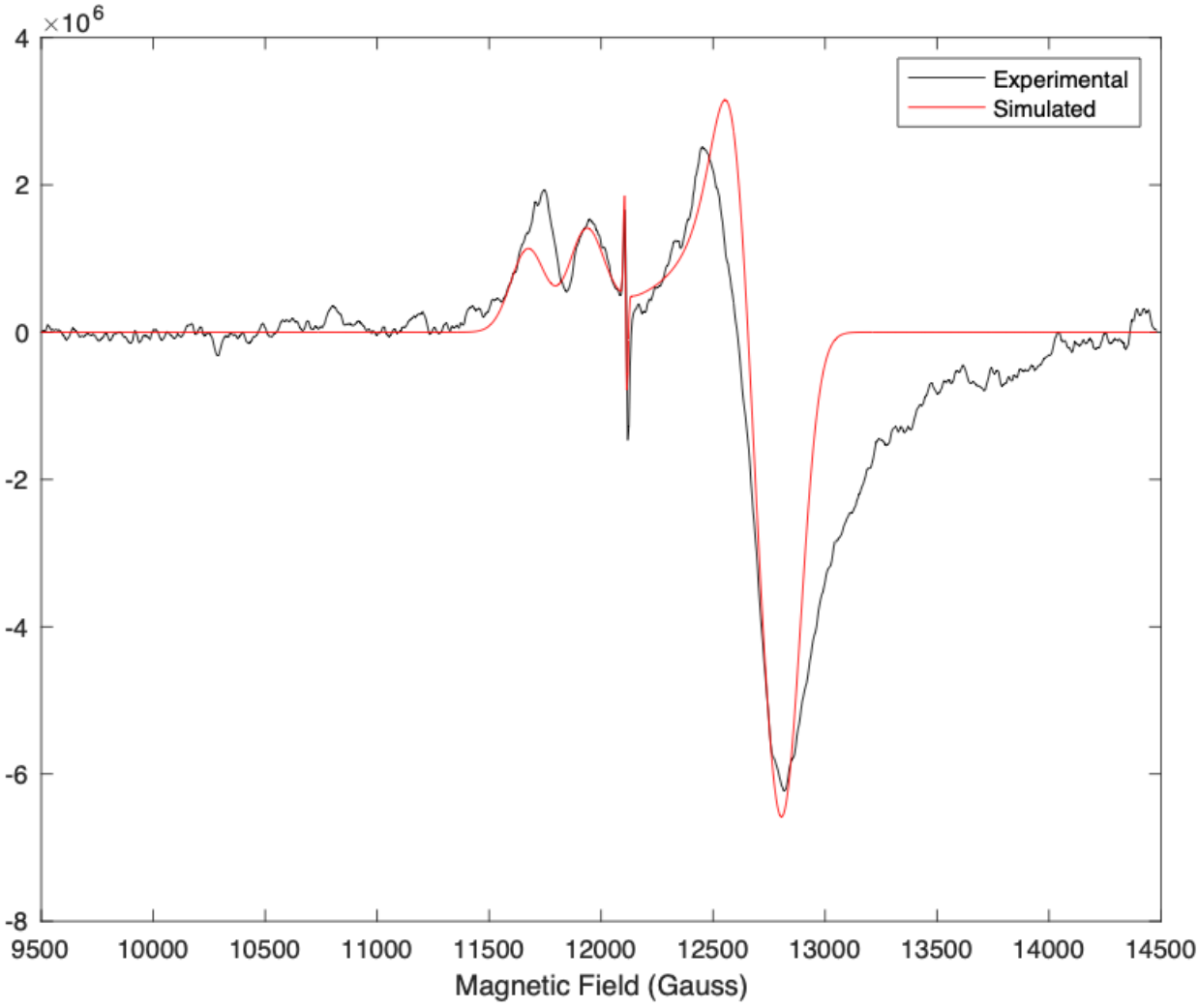


FIG. 2. **EPR spectra of oxidized hemoprotein and its cyanide complex.** *A*, HP, 0.22 mM in standard buffer. *B*, HP-CN^- complex, 0.10 mM in standard buffer. The complex was formed by incubating an anaerobic solution of 0.05 mM HP in standard buffer with 0.5 mM MV^+ and 1 mM KCN for 5 min at 23 °C. The solution was then opened to air, and the oxidized HP-CN complex freed of excess methyl viologen and KCN by chromatography on a column of Sephadex G-25. The eluted protein was concentrated by ultrafiltration with a Schleicher and Schuell colloidon membrane apparatus. Conditions of spectroscopy: temperature, 13 K; microwave frequency, 9.14 GHz; microwave power, 3 milliwatts; modulation amplitude, 1 millitesla. The receiver gain in *spectrum B* was 5 times that in *spectrum A*.

2) Additional spectra of dSiR-APX models:

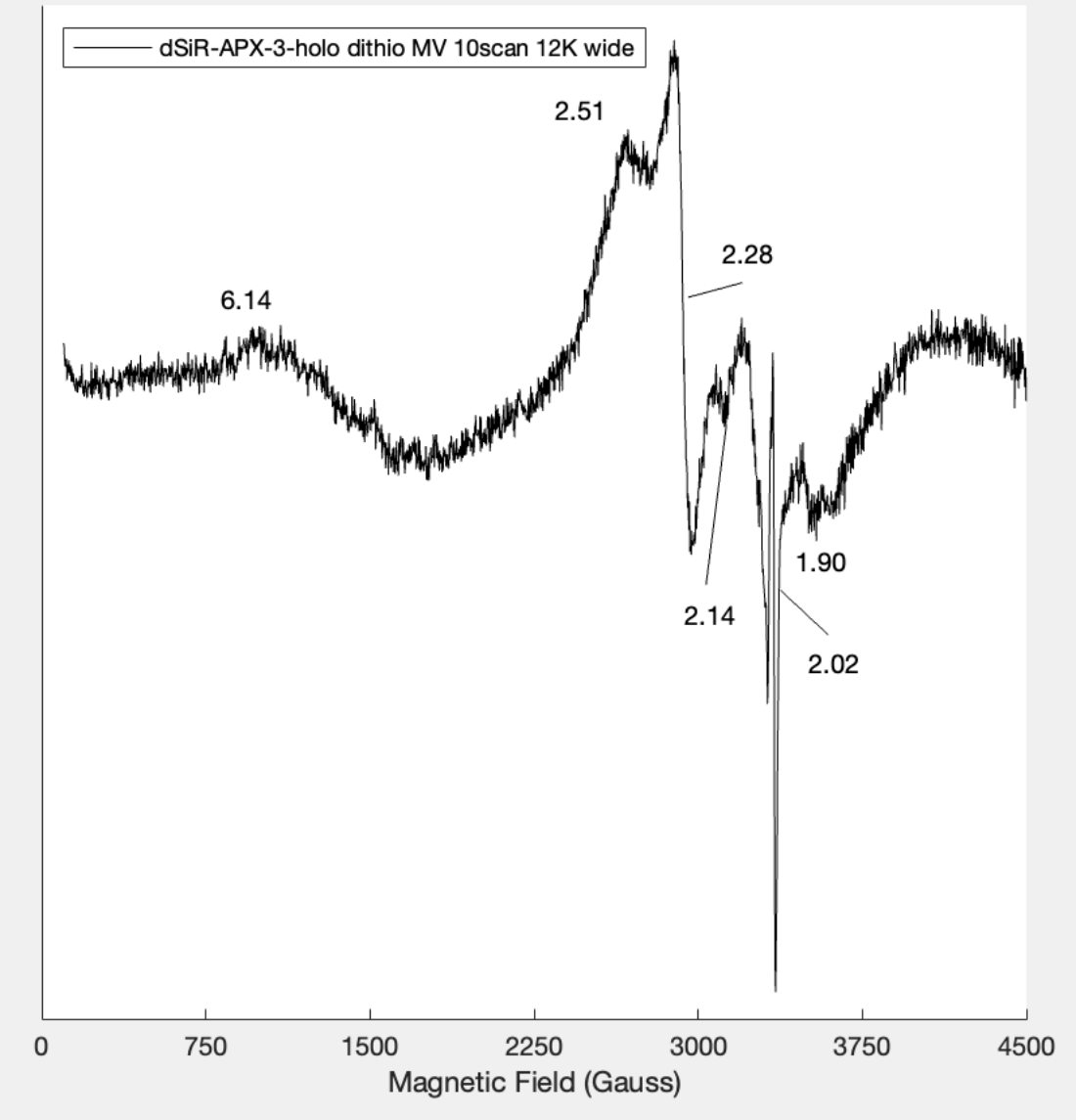
A) dSiR-APX.2-KRK – 4Fe4S only (Q-band)

Fitting the two features:



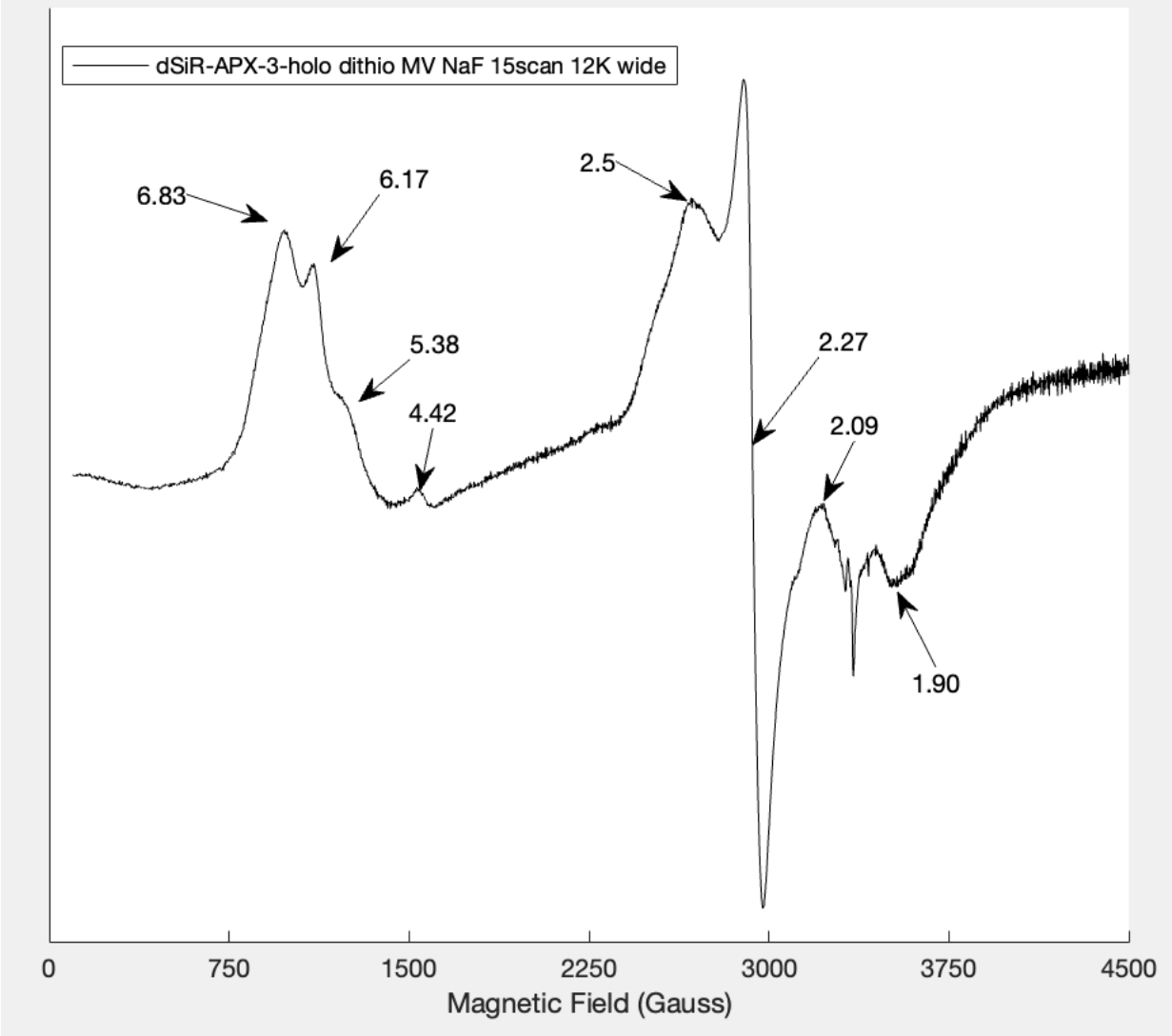
B) dSiR-APX.3 – holo (X-band) (+ dithionite + MV)

Even without NaF, coupled signal observed:



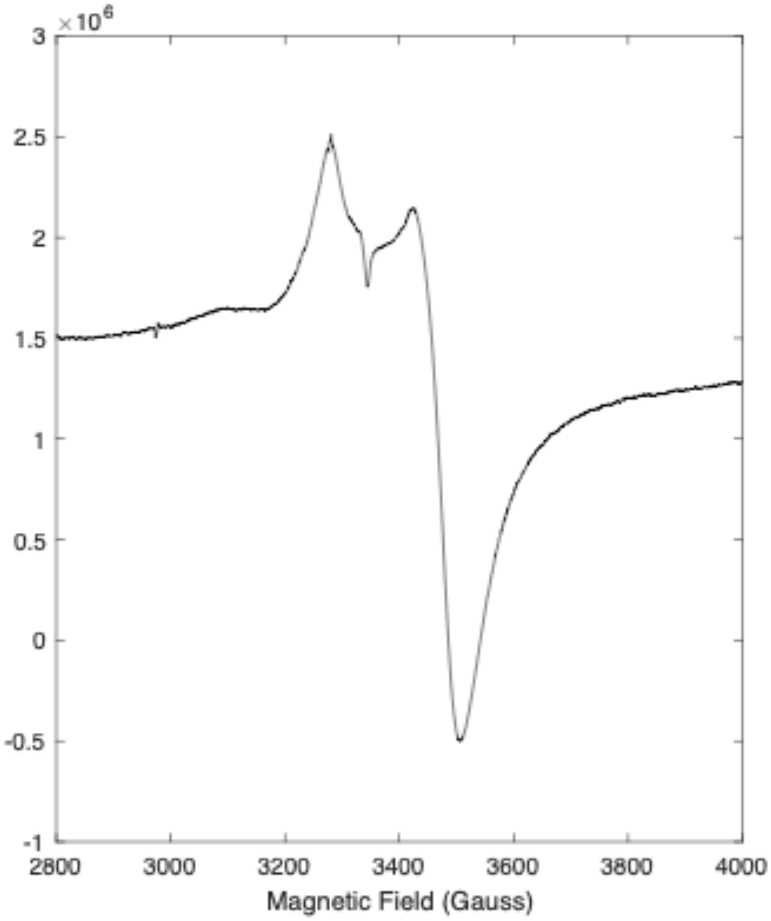
C) dSiR-APX.3 – holo (X-band) (+ dithionite + MV + NaF)

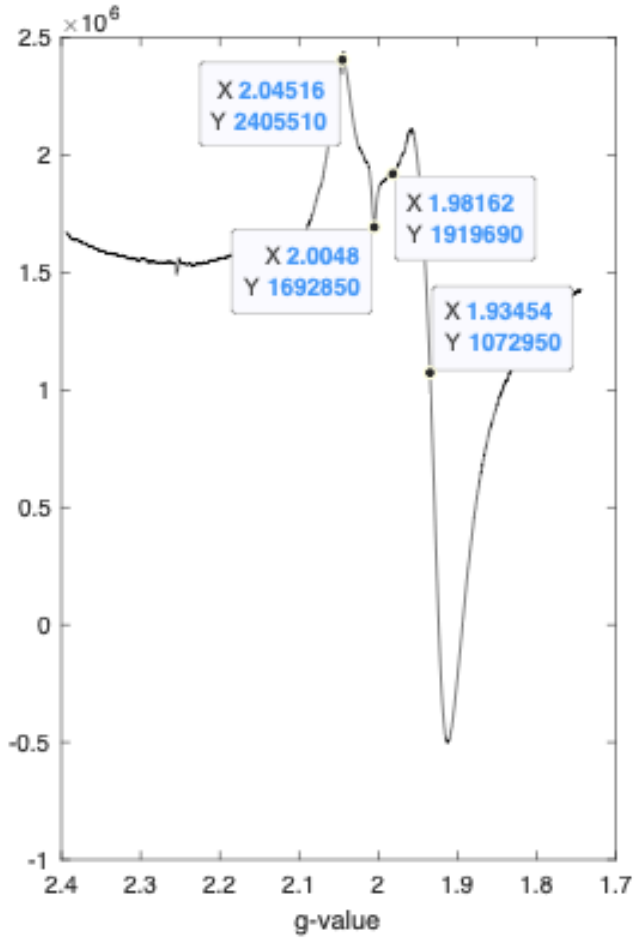
Addition of NaF means more high-spin character?



D) dSiR-APX.2-KRK – 4Fe4S only (X-band) (+ dithionite)

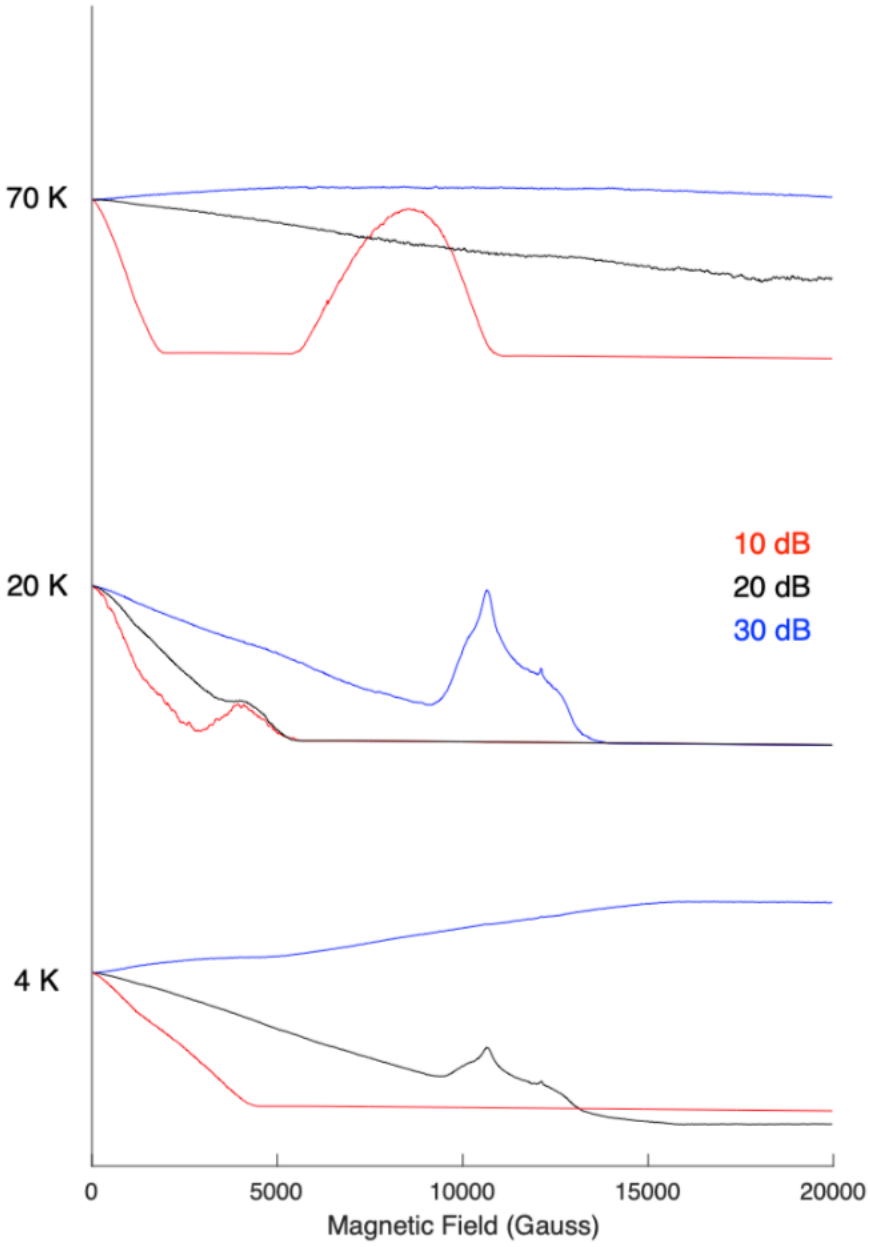
In-house X-band again of the series-characterized system .2-KRK:





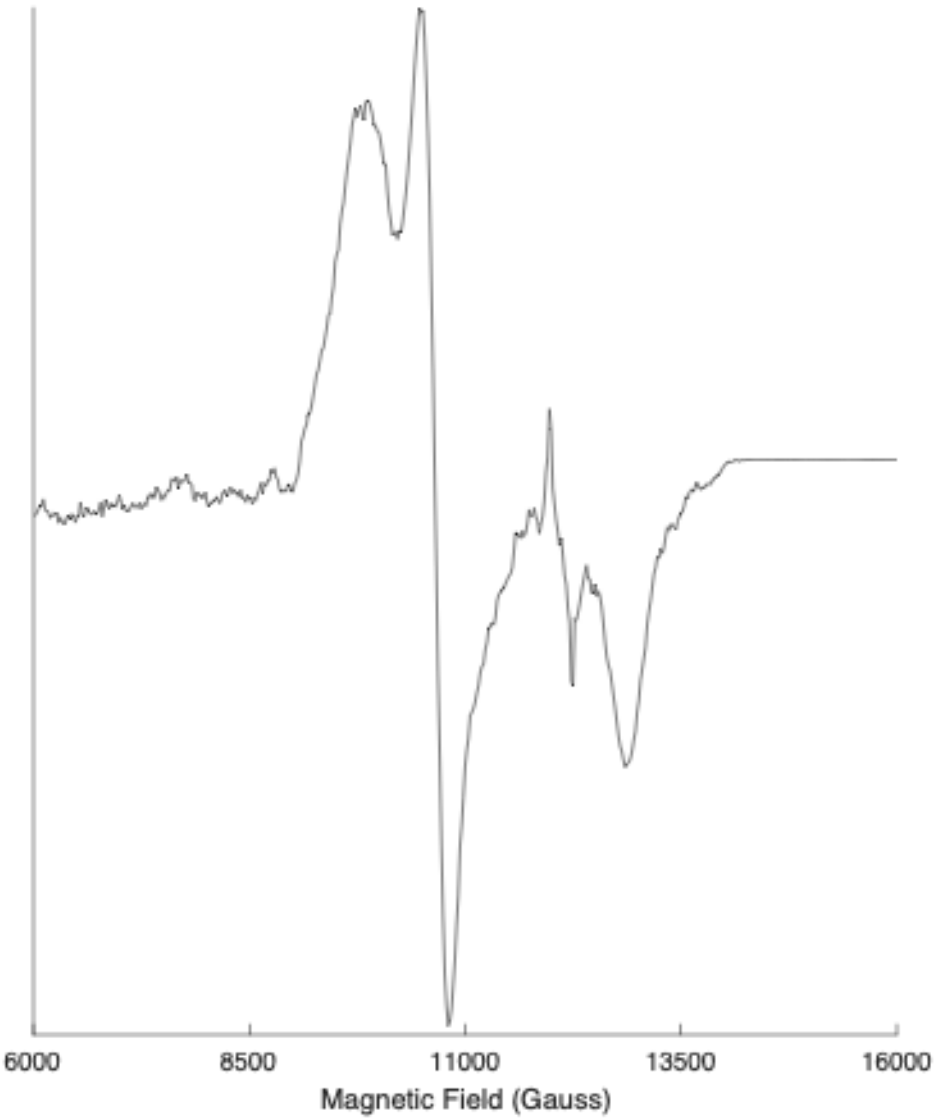
E) dSiR-APX.2-KRK – holo (Q-band) (+ dithionite)

Exploring Temperature and Power:



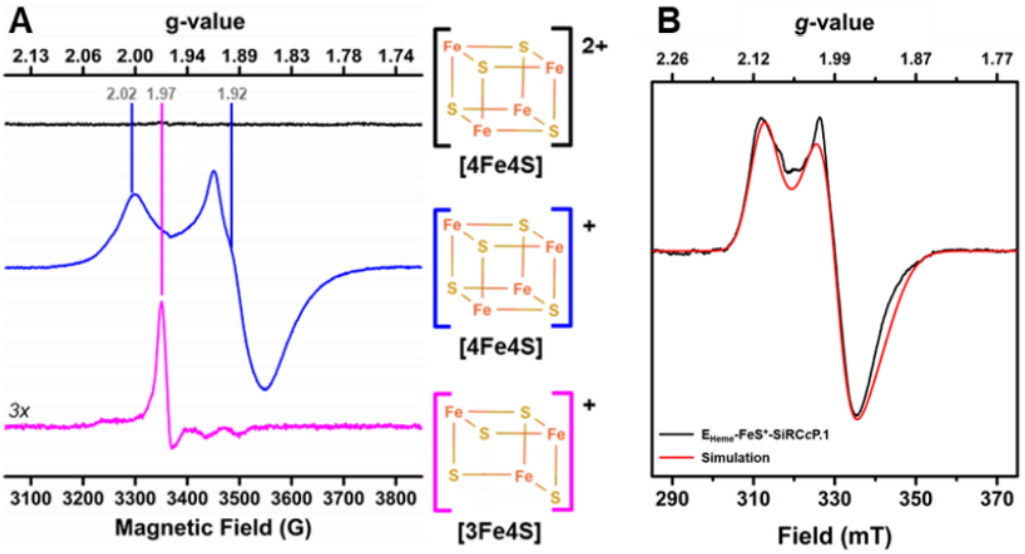
F) dSiR-APX.2-KRK – holo (Q-band) (+ dithionite)

taking the derivative of that signal (which already was a derivative of the detector's abs-based signal???)



3) Comparative spectra – previous work, dSiR-CcP model

Link to paper (journal site):
A designed heme-[4Fe-4S] metalloenzyme catalyzes sulfite reduction like the native enzyme (2018)



X-band EPR spectra of E-FeS-SirCcP.1 corresponding to (A) the freshly reconstituted species (black) is EPR-silent but reducing with excess sodium dithionite gives a g-parallel 1.94-type species (blue). Addition of potassium ferricyanide (~5 equivalents) produces an isotropic species indicative of a 3Fe-species resulting from the oxidative loss of one iron atom. The structures for each presumed 4Fe-4S cluster are drawn to the right. EPR spectra were measured at 18 K with 10 mW at 9.24 GHz. (B) Typical EPR spectrum of a 4Fe4S reconstituted E-FeS-SiRCcP reduced with excess dithionite (black) and simulated (red). The g ~ 2 feature is attributed to the quartz cuvette. Simulations in Simpow6 give the following g-values: g_x = 1.8907, g_y =1.9185, g_z = 2.0354, linewidths (MHz) A_x = 117.09, A_y = 77.01, A_z = 69.77. Spectra collected at 9.17 GHz and at 15 K.