

Practical session of NMR spectroscopy
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The objectives of this practical session are to observe the paramagnetic effect of the heme iron on horse cytochrome c protons using 1D NMR spectroscopy see figure 1.

The NMR machine is a 600MHz, and the sample is 1mM horse cytochrome c in phosphate buffer pH 7.0

1/ **Curie's law:** a series of ^1H -1D NMR spectra will be recorded on the oxidized form of the cytochrome at 6 different temperatures (285, 290, 295, 300, 305 and 310K)

The chemical shifts of the heme Me3 and Me8 lines and axial ligand Methionine Methyl line will be measured for each temperature (see Table 1). A plot of chemical shifts (δ ppm) versus ($1/T$) will allow to observe a linear behavior according to Curie's law $X_m = C/T$ where X_m is the magnetic susceptibility.

2/ **Observation of the diamagnetic state:** the horse cytochrome c will be reduced by solid dithionite

The heme methyl lines disappear and the axial methionine methyl line shift (see table 1). The same set of temperatures will be used recording 1D spectra, and the same chemical shift ($1/T$) representation will be plotted for the axial methionine methyl line.

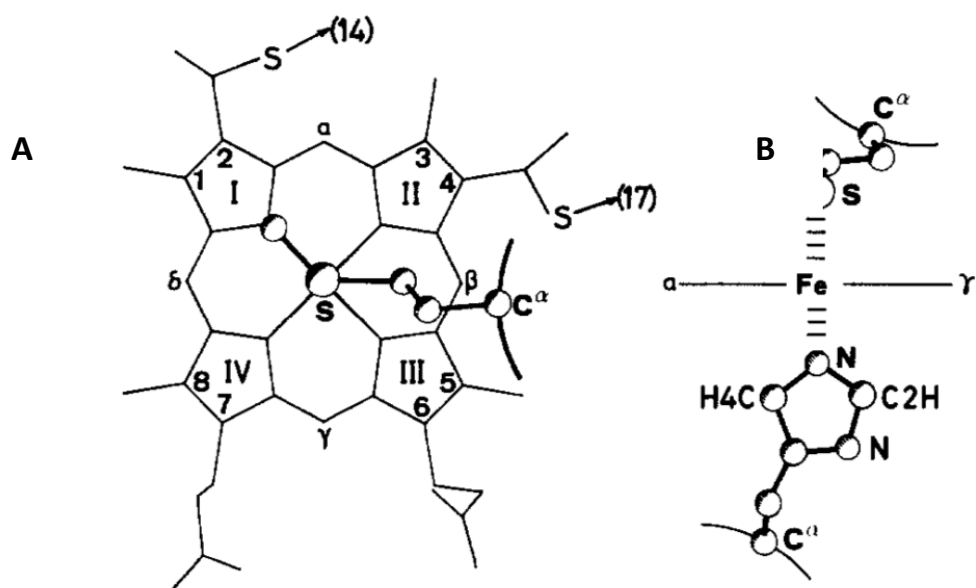


Fig1: A/ Heme c in cytochrome c. B/ the axial ligands of the Heme iron (Histidine and Methionine)

atom name	residue name	chemical shift	
		oxidized species (ppm)	reduced species (ppm)
			hh cyt c ^b
H α	Met	2.77	3.09
ϵ -CH ₃	Met	-24.7	-3.30
H α	His	9.10	3.50
H δ 2	His	24.6	0.14
H ϵ 1	His	-25.7	0.51
8-CH ₃	heme	35.7	2.16
<i>meso</i> - δ H	heme	2.11	9.06
1-CH ₃	heme	6.81	3.46
2-H α	heme	-1.33	5.20
2-CH ₃	heme	-2.63	1.46
<i>meso</i> - α H	heme	1.40	9.30
3-CH ₃	heme	32.8	3.84
4-H α	heme	2.09	6.30
4-CH ₃	heme	3.05	2.57
<i>meso</i> - β H	heme	-0.92	9.61
5-CH ₃	heme	9.72	3.58
<i>meso</i> - γ H	heme	7.50	9.64

Table 1: Chemical shifts of heme methyl lines and axial methionine line.