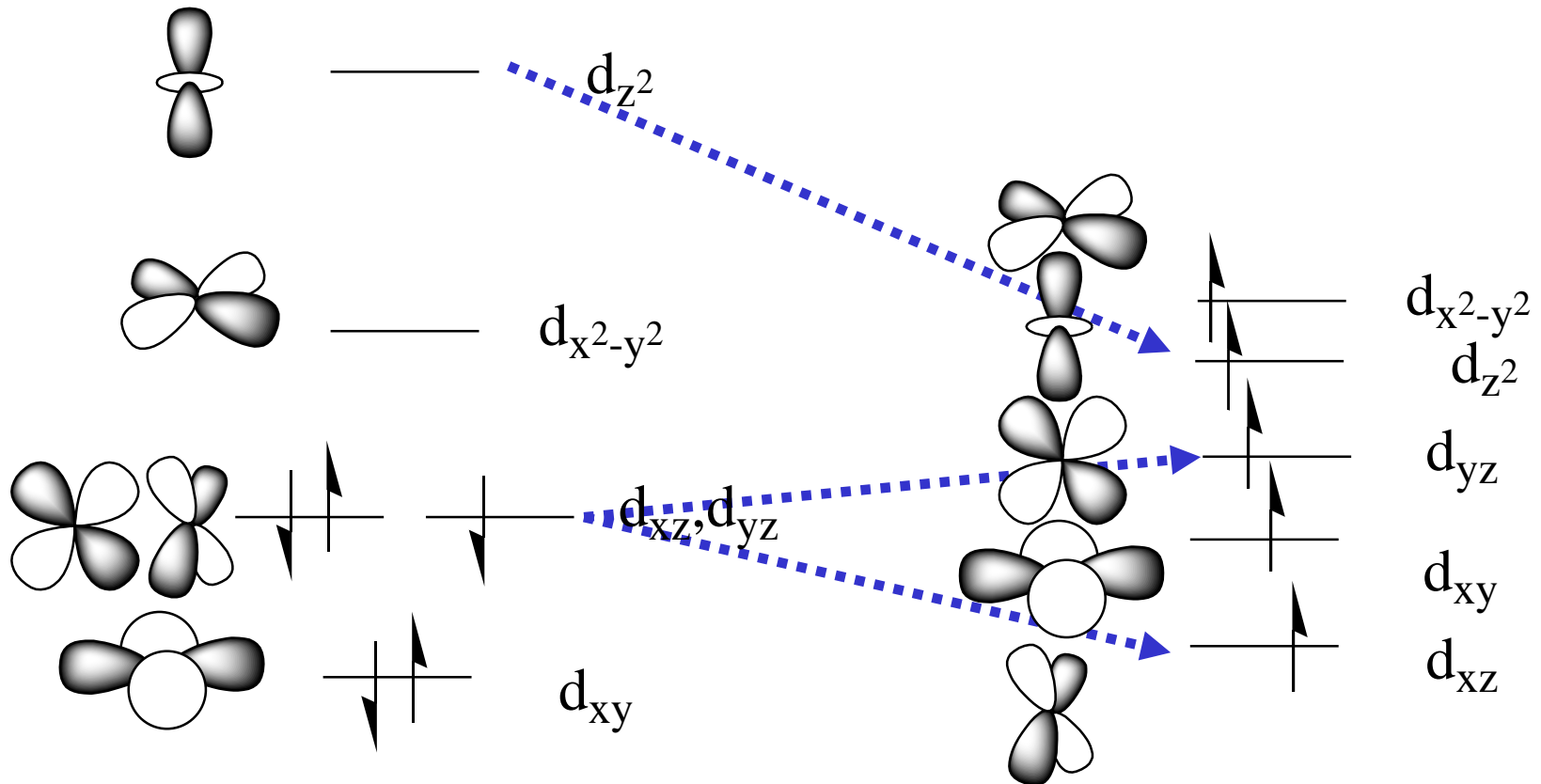


# The ligation at the sixth position changes the spin state of the heme iron

$S = 1/2$

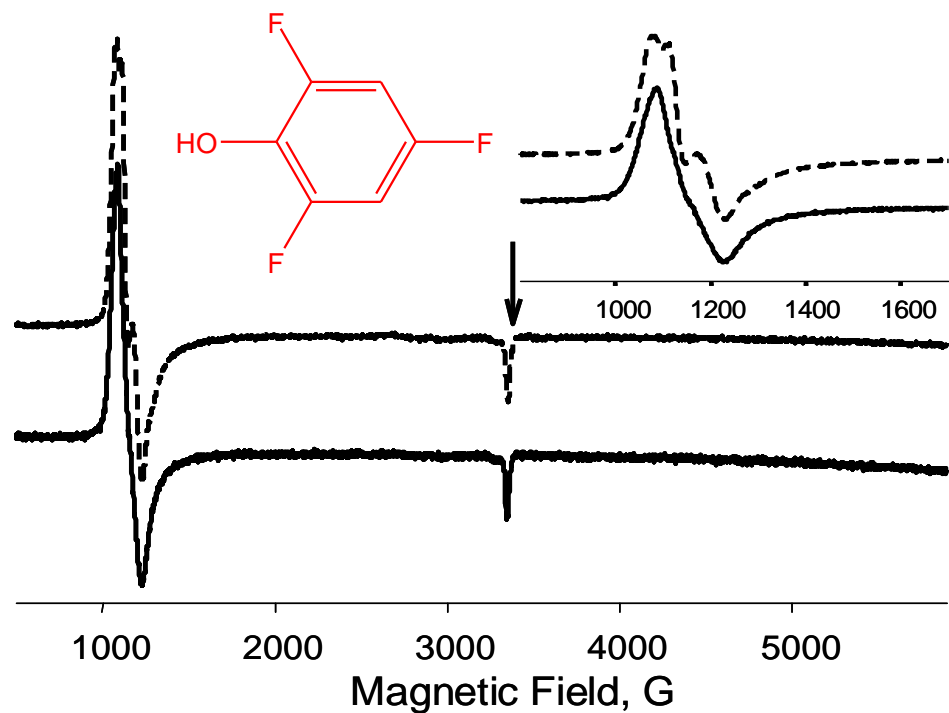
$S = 5/2$



Low spin Fe(III)

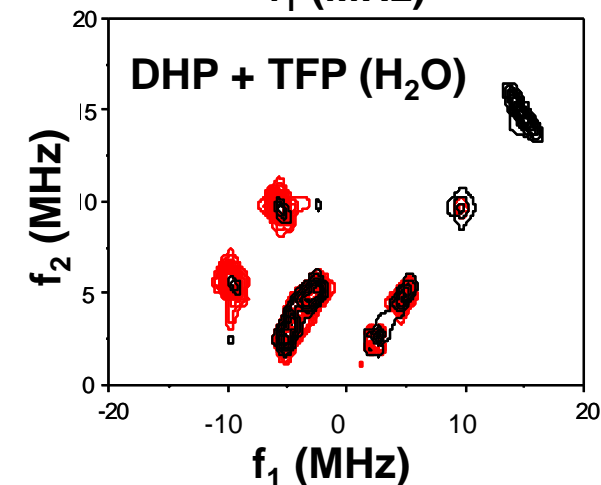
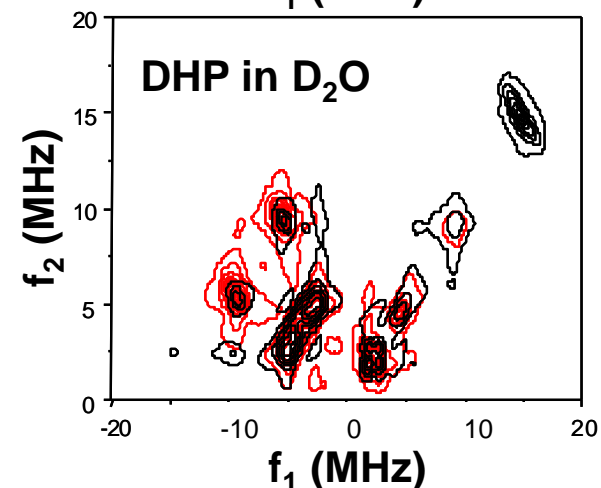
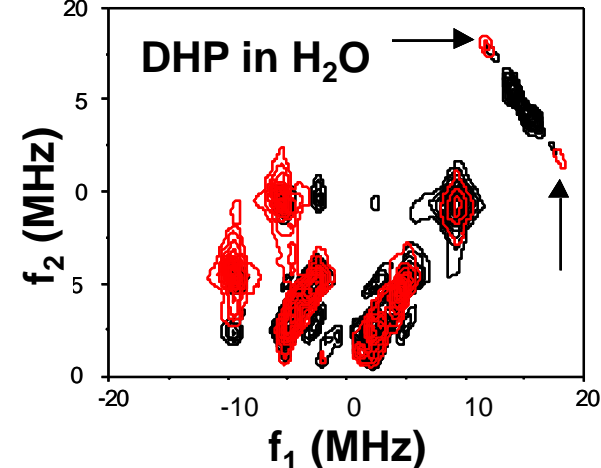
High spin Fe(III)

EPR and HYSCORE data show that H<sub>2</sub>O is displaced when 2,4,6-trifluorophenol binds



CW X-band (9.5 GHz) EPR spectra of DHP without substrate (dashed line) and with substrate (solid line). Data were obtained at pH 6.

HYSCORE spectra of DHP at pH 6.0 at 4.5 K. (Red = 128 ns, Black = 100 ns). Comparison of DHP in H<sub>2</sub>O and D<sub>2</sub>O shows that exchangeable protons are lost. These are waters on Fe-OH<sub>2</sub>. The same resonances are lost when TFP binds. →



# Paramagnetic NMR spectroscopy

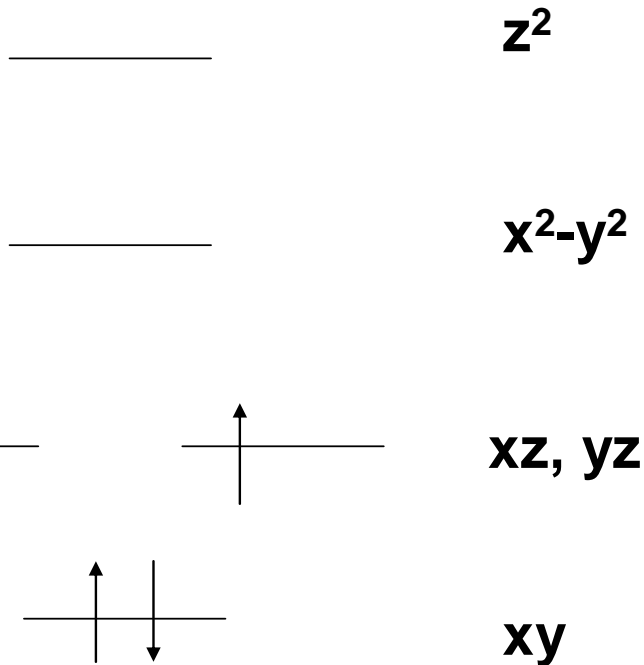
Fe(III)CN form of DHP

Binding of cyanide, to Fe(III) results in low spin  $S = \frac{1}{2}$

The unpaired electron gets delocalized throughout the heme group.

The magnetic moment of the unpaired electron is 660x greater than a proton (nucleus).

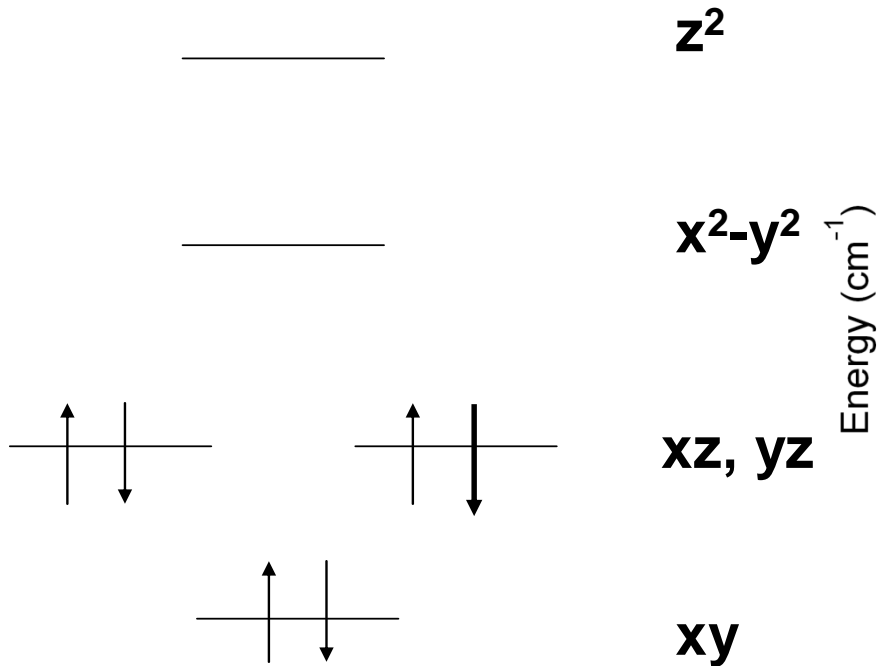
Hyperfine interaction is the electron-nuclear term.



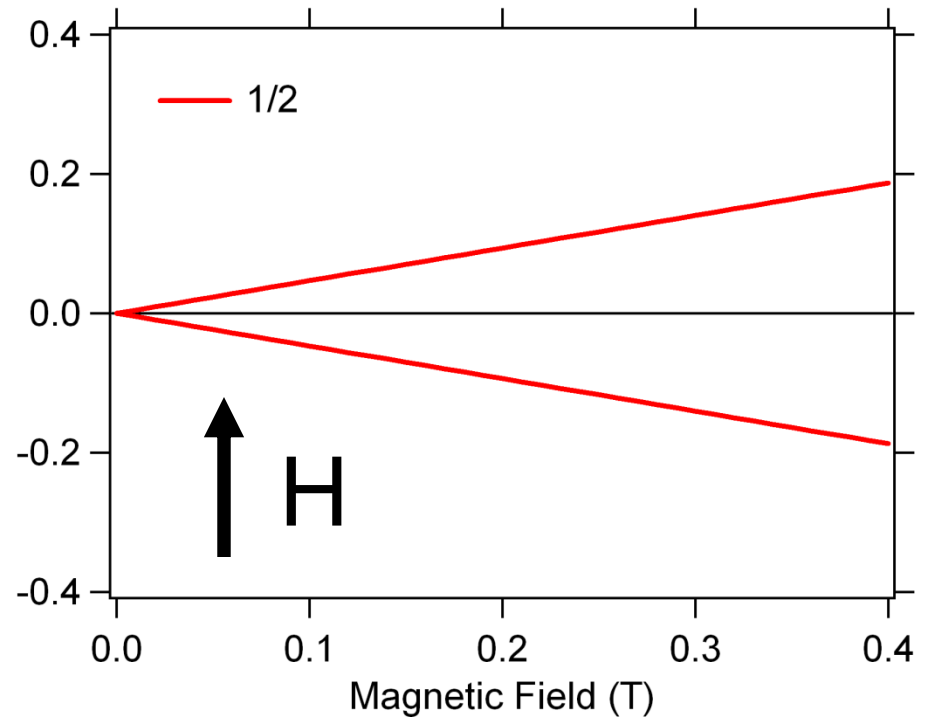
**Low Spin Fe(III),  $S = \frac{1}{2}$**

# NMR spectroscopy

Diamagnetic Iron Center



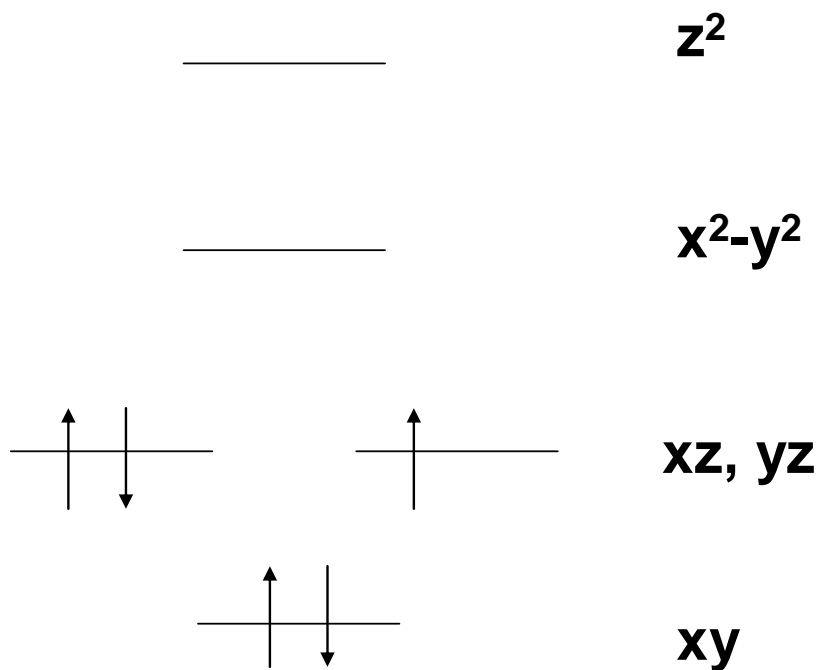
Nuclear Energy Splitting



Low Spin Fe(II),  $S=0$

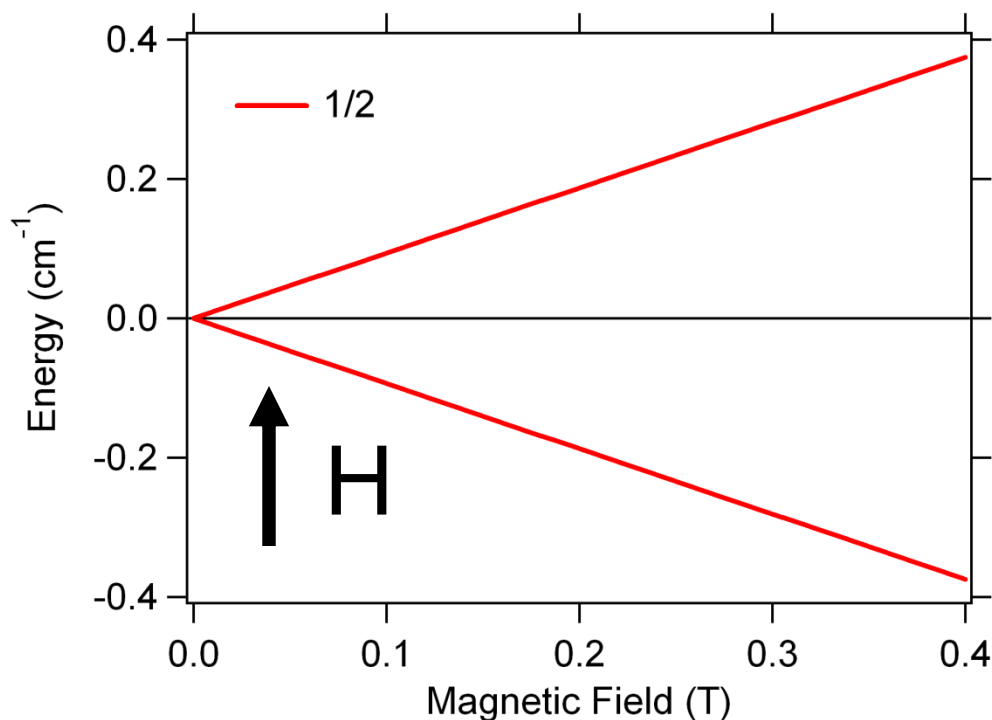
# Paramagnetic NMR spectroscopy

Paramagnetic Iron Center



**Low Spin Fe(III),  $S=1/2$**

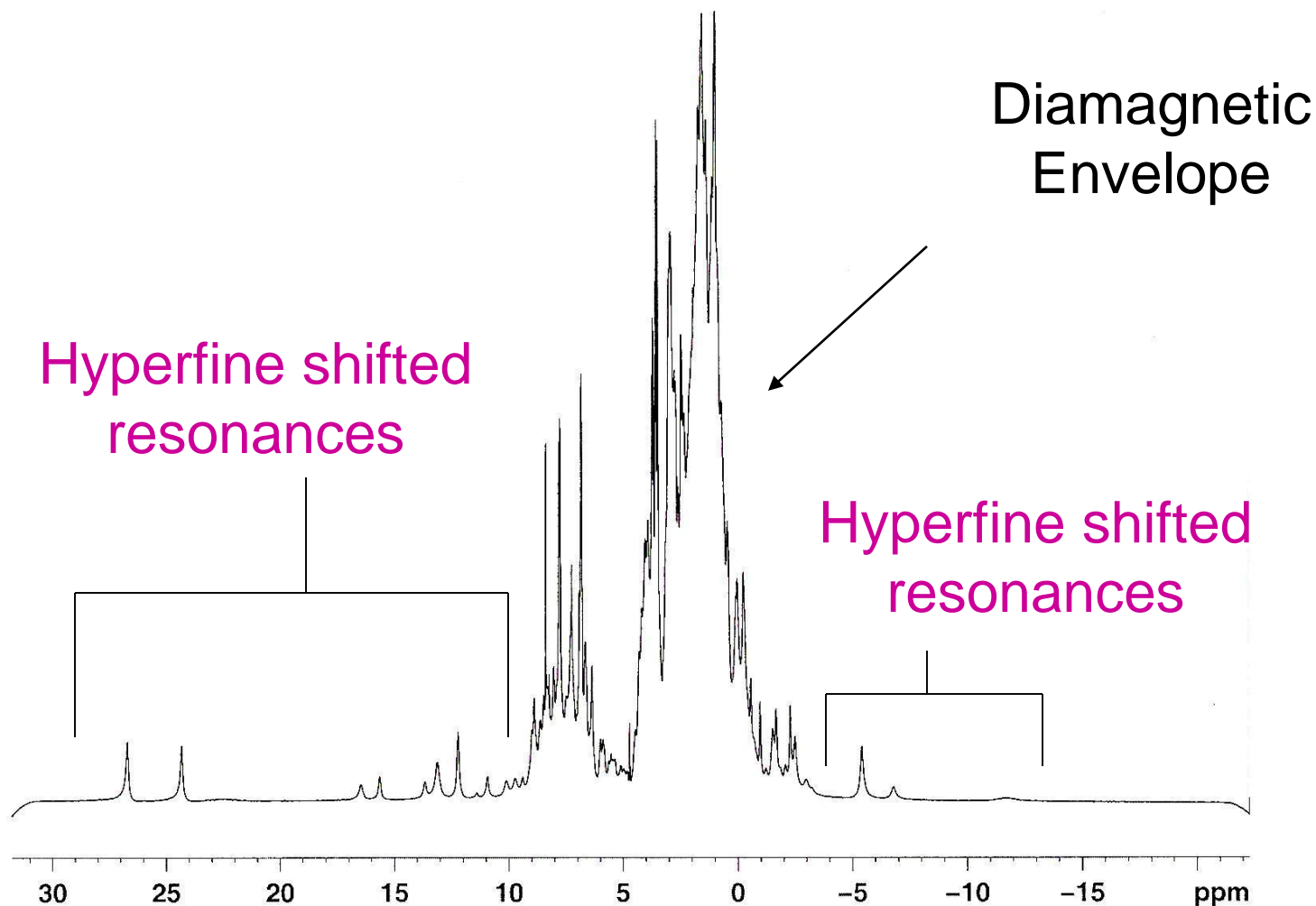
Nuclear Energy Splitting



Altered chemical shift due to hyperfine interaction

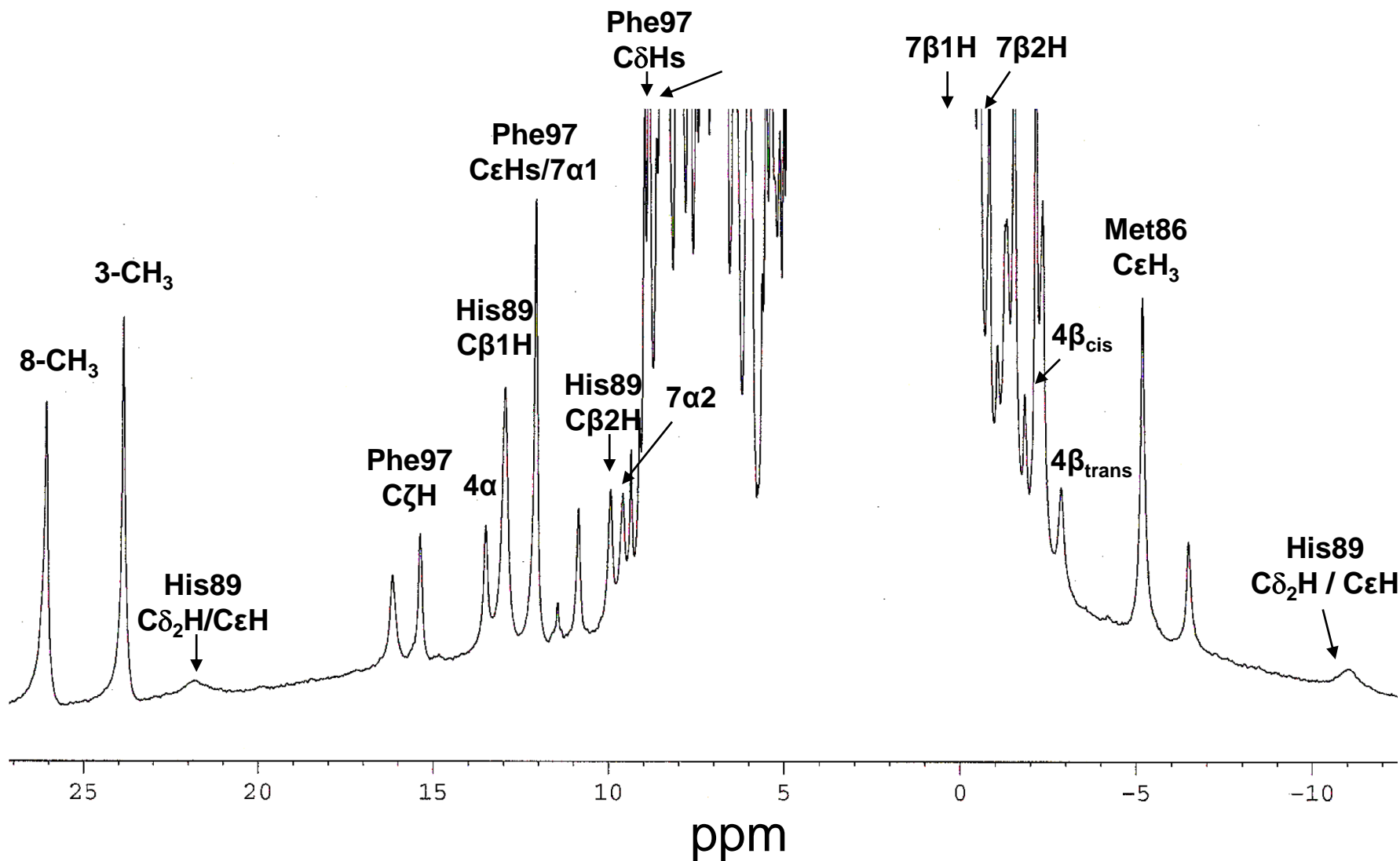
# $^1\text{H}$ NMR spectra of DHPCN

## Influence of a paramagnetic center

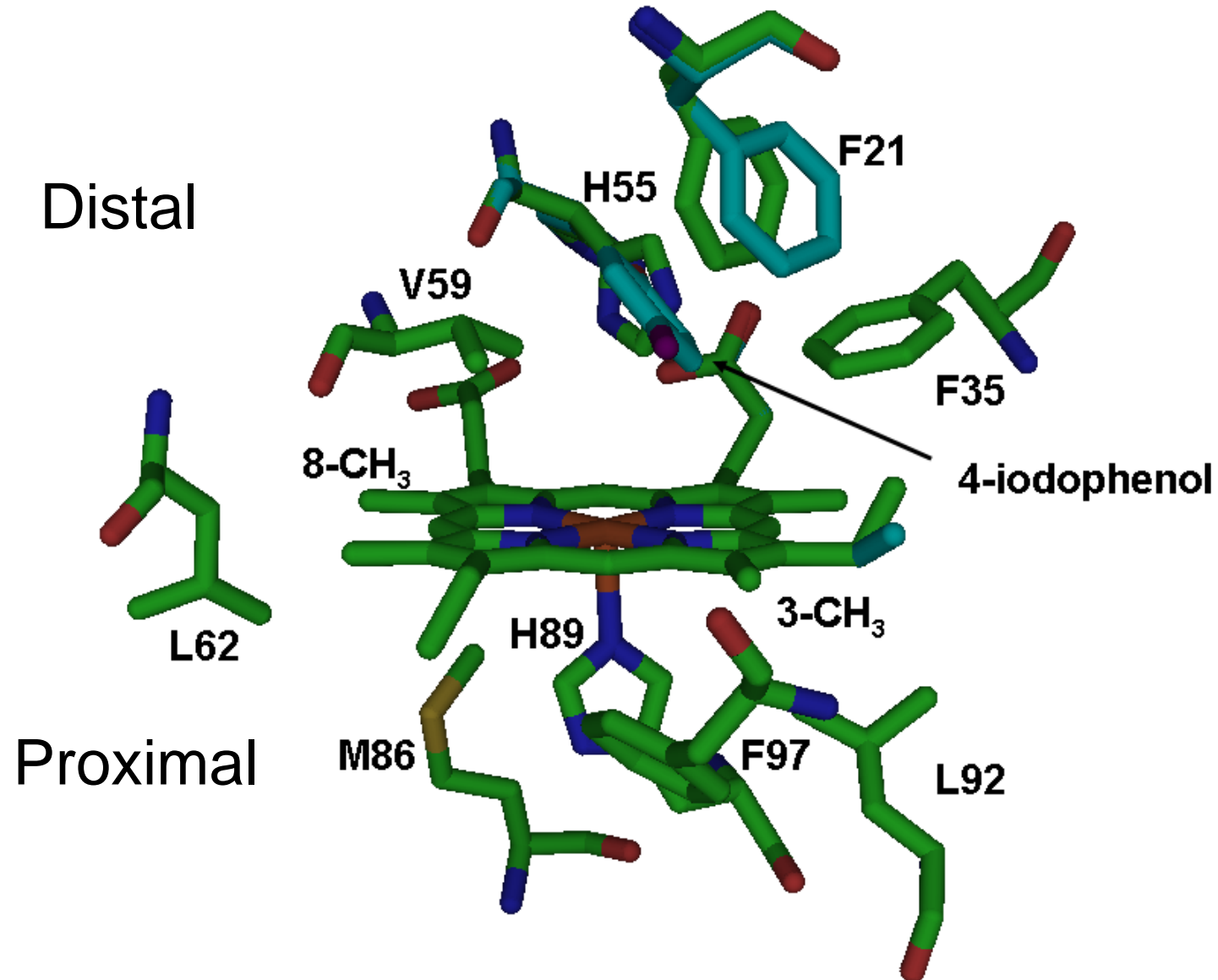


# Hyperfine resonance assignments

All amino acids observed are on the proximal side

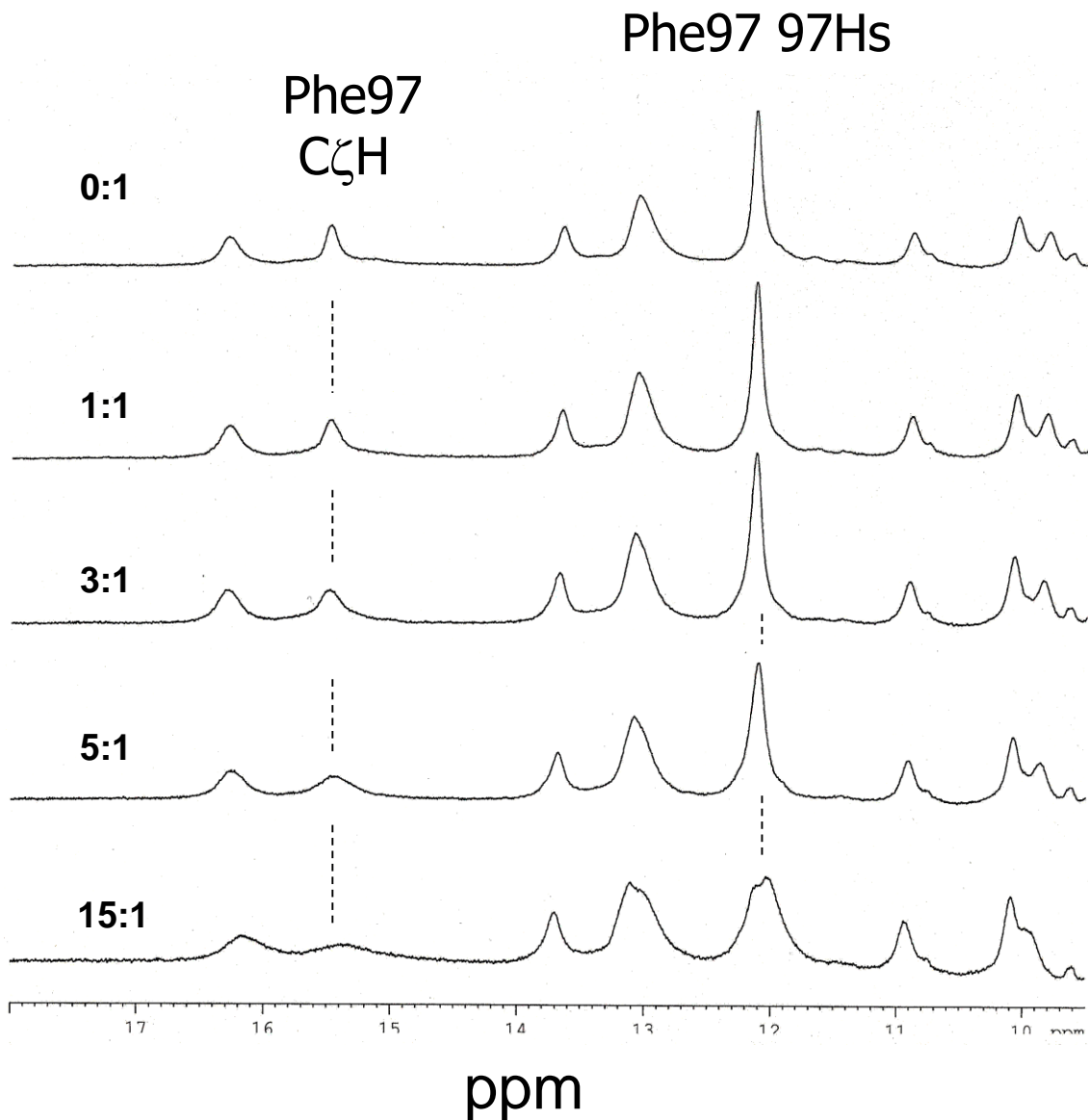


# Spectral changes in the NMR spectrum are on the proximal side





# Effect of substrate on $^1\text{H}$ NMR spectrum

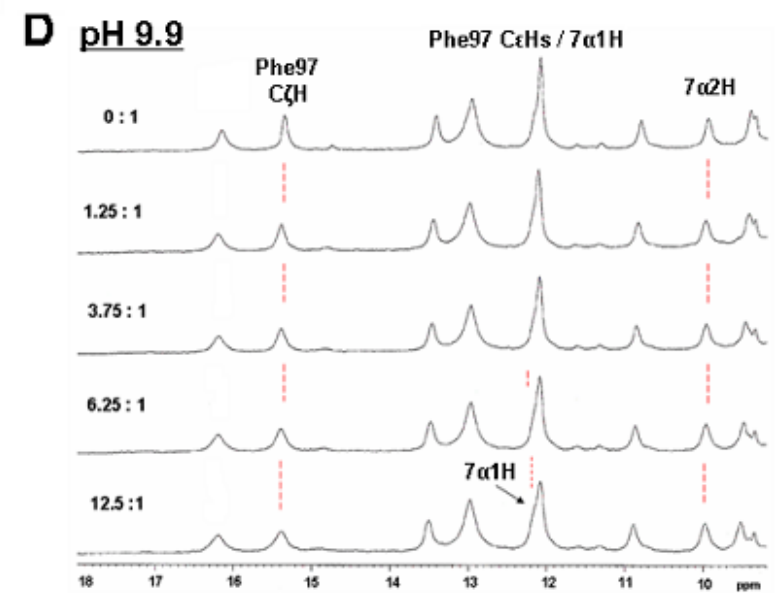
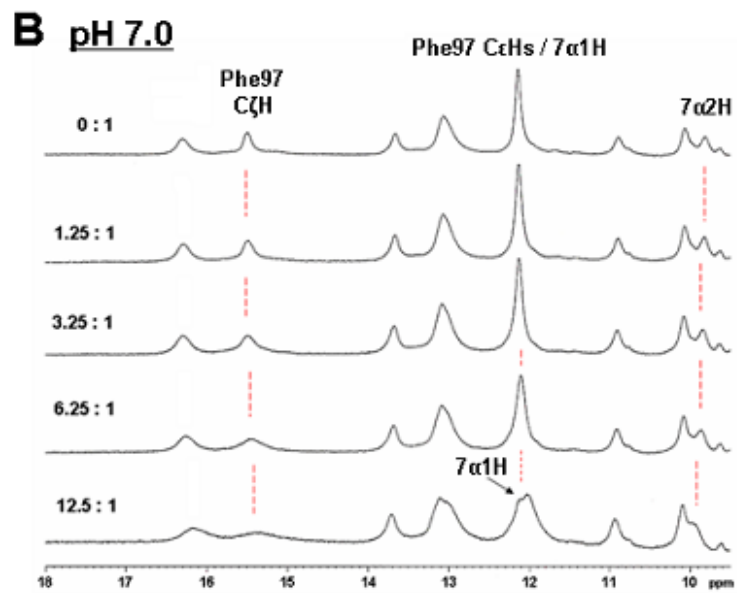
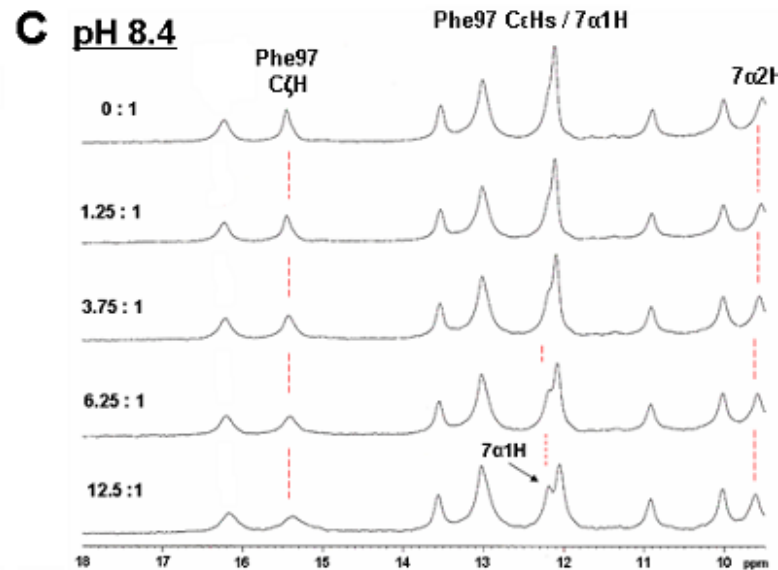
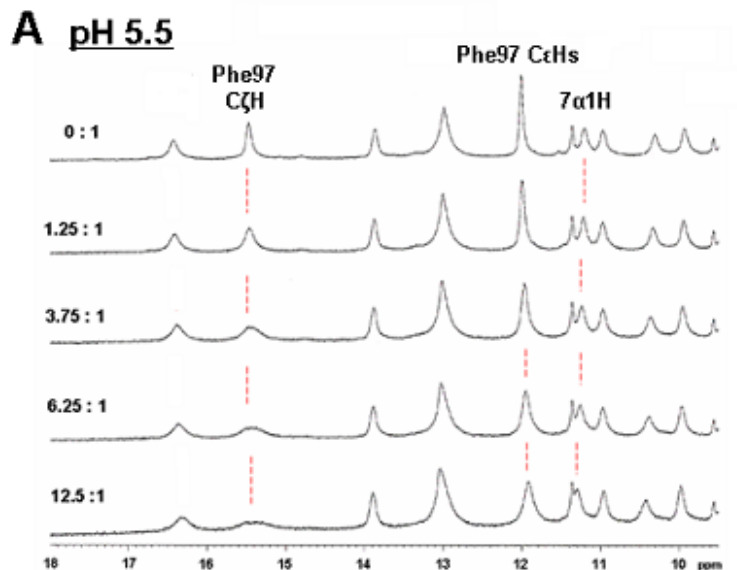


Titration of 2,4-dichlorophenol (DCP) to DHPCN causes both the F97 C $\zeta$ H and C $\epsilon$ Hs signals to decrease in intensity and broaden.

Data obtained at pH 6.0

Davis and Franzen  
JACS submitted

# Effect of substrate on $^1\text{H}$ NMR spectrum



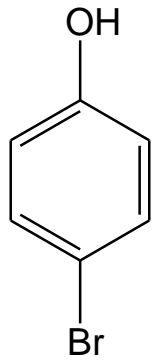
ppm

ppm

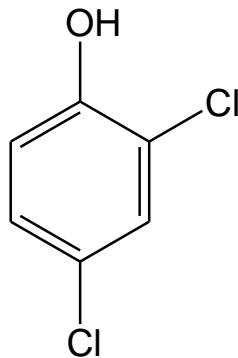
# Differential effect of substrate binding on the $^1\text{H}$ NMR spectrum

Both 4-bromo and 2,4,-dichloro phenol cause changes at the heme 3-CH<sub>3</sub> and Phe97.

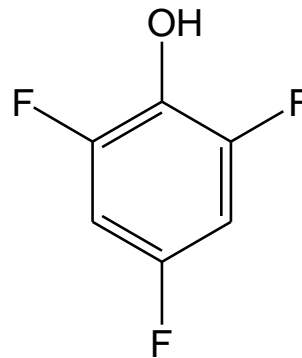
However, 2,4,6-trifluoro-phenol causes no apparent changes to the interior amino acids of DHP.



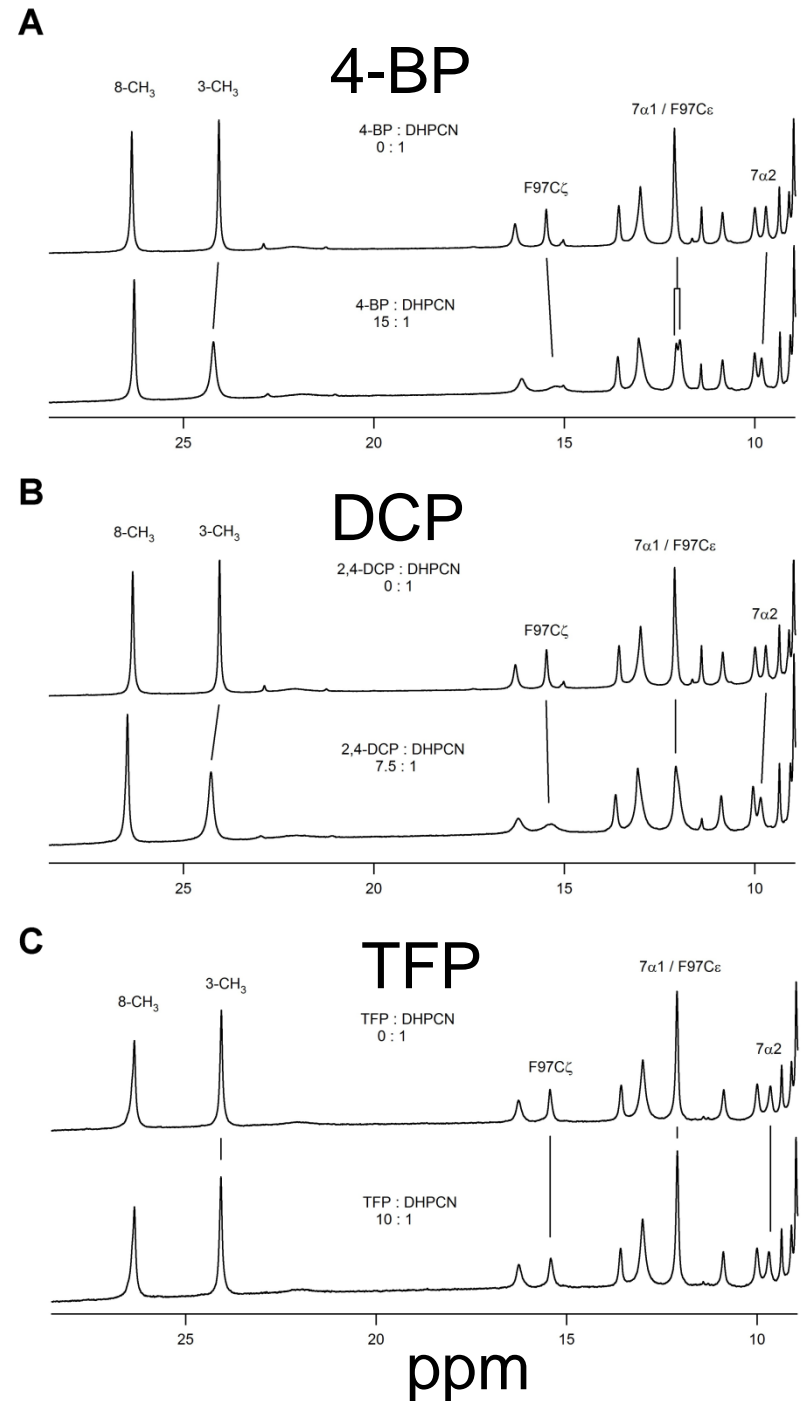
4-BP



DCP



TFP



# Effect of TFP binding on $^{19}\text{F}$ NMR spectrum

2,4,6-trifluorophenol (TFP) interaction results in changes in the  $^{19}\text{F}$  signal, which are indicative an interaction with the protein.

Binding presumably occurs on the surface rather than at the interior binding site.

