

# This Week's Citation Classic®

Wüthrich K. Structural studies of hemes and hemoproteins by nuclear magnetic resonance spectroscopy. *Struct. Bond.* 8:53-121, 1970.

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The proton nuclear magnetic resonance (NMR) spectra of diamagnetic and paramagnetic metalloporphyrins and hemoproteins in solution were presented, and the contributions from ring current fields, electron-proton contact coupling, and electron-proton pseudocontact coupling to the observed chemical shifts were analyzed. The results obtained provided a basis for NMR investigations of the polypeptide conformation near the heme groups and the electronic heme structure in hemoproteins, such as cytochromes, myoglobins, and hemoglobins. [The *SCI*® indicates that this paper has been cited in over 205 publications.]

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The paper reviews research pursued in 1968-1969 at the Biophysics Department, Bell Telephone Laboratories, Murray Hill, New Jersey, which was headed by R.G. Shulman. A starting point for this project was the first publication on <sup>1</sup>H nuclear magnetic resonance (NMR) spectra of proteins recorded with a superconducting 220 MHz spectrometer, which included a description of NMR spectral differences between the reduced, diamagnetic state and the oxidized, paramagnetic form of cytochrome c.<sup>1</sup>

During postdoctoral work with R.E. Connick at the University of California, Berkeley, I had received thorough training on spin relaxation in metal complexes, and from this background, the NMR spectral variations reported for cytochrome c seemed to present novel perspec-

tives. I was fortunate to be able to follow these up, since we had obtained our own 220 MHz spectrometer at Bell Labs. J. Peisach and E. Margoliash kindly provided samples of myoglobin and cytochrome c, and T. Yamane prepared hemoglobin KW, using blood sampled from my arm in the first-aid station. Within a few months, numerous well-separated resonance lines arising from electron-proton contact and pseudocontact coupling, and from interactions with the porphyrin ring current field, were identified in the <sup>1</sup>H NMR spectra of all these proteins. This opened new avenues to information on the molecular structure, e.g., in cytochromes c, on conformation changes during the oxygenation of myoglobin and hemoglobin, and on the electronic structure of the heme groups.

The well-separated resonance lines in hemoproteins represent "windows" in the complex <sup>1</sup>H NMR spectra, through which the spectroscopist can pinpoint molecular details. These natural <sup>1</sup>H NMR probes made hemoproteins accessible for investigations with the equipment available in the early 1970s, and they are still in use today.<sup>2,3</sup> In the late 1970s the observation of the well-resolved resonance lines in diamagnetic hemoproteins with one-dimensional NMR experiments enabled the development of sequential resonance assignment techniques,<sup>4</sup> as well as novel studies on the spin physics in macromolecules in solution, such as measurements of the build-up rates of the nuclear Overhauser effects.<sup>5</sup> When corresponding two-dimensional NMR experiments were ready for use with proteins in 1980, the techniques that had been developed around the heme groups in hemoproteins were adapted for studies of complete polypeptide chains. This eventually resulted in a method for determination of complete three-dimensional protein structures in solution.<sup>6</sup>

This article was accepted as my *Habilitationschrift* by the Eidgenössische Technische Hochschule (ETH) Zürich and was thus of importance for the formal start of my academic career. That it is now considered to be a *Citation Classic* is an unexpected but all the more welcome bonus.

1. McDonald C C & Phillips W D. Manifestations of the tertiary structures of proteins in high-frequency nuclear magnetic resonance. *J. Amer. Chem. Soc.* 89:6332-41, 1967. (Cited 135 times.)
2. Shulman R G, Hopfield J J & Ogawa S. Allosteric interpretation of hemoglobin properties. *Quart. Rev. Biophys.* 8:325-420, 1975. (Cited 110 times.)
3. La Mar G N. Model compounds as aids in interpreting NMR spectra of hemoproteins. (Shulman R G, ed.) *Biological applications of magnetic resonance*. New York: Academic Press, 1979. p. 305-43. (Cited 110 times.)
4. Keller R M & Wüthrich K. Assignment of the heme c resonances in the 360 MHz <sup>1</sup>H NMR spectra of cytochrome c. *Biochim. Biophys. Acta* 533:195-208, 1978. (Cited 95 times.)
5. Gordon S L & Wüthrich K. Transient proton-proton Overhauser effects in horse ferrocyclochrome c. *J. Amer. Chem. Soc.* 100:7094-6, 1978. (Cited 80 times.)
6. Wüthrich K. *NMR of proteins and nucleic acids*. New York: Wiley, 1986. 292 p.