

Holzwarth G & Doty P. The ultraviolet circular dichroism of polypeptides.  
*J. Amer. Chem. Soc.* 87:218-28, 1965.  
[Department of Chemistry, Harvard University, Cambridge, MA]

Synthetic  $\alpha$ -helical polypeptides were shown to exhibit three circular dichroism (CD) bands that were assigned to known electronic transitions of the peptide group. Metmyoglobin revealed the same three peptide bands. This demonstrated that ultraviolet CD could be used to monitor protein conformation in solution. [The SCI<sup>®</sup> indicates that this paper has been cited in over 500 publications since 1965.]

G. Holzwarth  
Department of Physics  
Wake Forest University  
Winston-Salem, NC 27109

February 8, 1985

This paper was written while I was a graduate student in the laboratory of Paul Doty at Harvard University. The solution conformation of proteins was an important problem at that time; the first crystal structure of a protein, myoglobin, was solved by Kendrew in 1960,<sup>1</sup> during my first year as a graduate student.

Optical rotatory dispersion (ORD) had been used exclusively to measure protein and polypeptide conformation in solution. Its major weakness was that it could not readily be tied to specific electronic transitions. Moffitt had made theoretical calculations for the rotational and dipole strengths of the

$\alpha$ -helix,<sup>2</sup> and we had already tested his theory by polarized absorption spectroscopy. After reading the papers of Kuhn<sup>3</sup> and Moffitt and Moscovitz,<sup>4</sup> I realized that circular dichroism (CD) would be more informative than ORD and calculated how big the experimental CD effect should be; it was surprisingly large. We therefore decided to measure ultraviolet CD rather than ultraviolet ORD. Our first attempt used a quartz multi-wave plate to generate left- and right-circularly polarized light.<sup>5</sup> It worked, but the precision was low. Later, we used the electro-optic CD method of Grosjean and Legrand<sup>6</sup> and obtained good data to about 188 nm for the synthetic polypeptides and, with Peter Urnes's help, for metmyoglobin as well. The  $n_1 \rightarrow \pi^+$ , parallel  $\pi^0 \rightarrow \pi^-$ , and perpendicular  $\pi^0 \rightarrow \pi^-$  exciton bands were clearly seen. The rotational strengths of the  $\pi \rightarrow \pi^+$  bands had the sign and, approximately, the magnitudes predicted by Moffitt for the right-handed  $\alpha$ -helix, which was a satisfying result. And numerical Kronig-Kramers transforms of our CD curve agreed nicely with the ORD curve.

The paper has probably been cited for two reasons. First, it was the earliest paper with high-resolution far-ultraviolet CD data for  $\alpha$ -helical polypeptides and proteins. It showed the striking advantages of CD over ORD in connecting observations to a quantum-mechanical theory and in assigning bands to particular electronic transitions. Second, CD instruments became commercially available, so that others could carry out similar measurements on many different proteins.

1. Kendrew J C, Dickerson R E, Strandberg B E, Hart R G, Davies D R, Phillips D C & Shore V C. Structure of myoglobin: a three-dimensional Fourier synthesis of 2Å. resolution. *Nature* 185:422-7, 1960. (Cited 460 times.)
2. Moffitt W. The optical rotatory dispersion of simple polypeptides. II. *Proc. Nat. Acad. Sci. US* 42:736-46, 1956. (Cited 225 times.)
3. Kuhn W. Optical rotatory power. *Annu. Rev. Phys. Chem.* 9:41-38, 1958. (Cited 105 times.)
4. Moffitt W & Moscovitz W. Optical activity in absorbing media. *J. Chem. Phys.* 30:648-60, 1959. (Cited 265 times.)
5. Holzwarth G, Gratzner W B & Doty P. The optical activity of polypeptides in the far ultraviolet. *J. Amer. Chem. Soc.* 84:3194-5, 1962. (Cited 80 times.)
6. Grosjean M & Legrand M. Appareil de mesure du dichroïsme circulaire dans le visible et l'ultraviolet. *CR Acad. Sci.* 251:2150-2, 1960. (Cited 70 times.)