

This Week's Citation Classic

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Greenfield N & Fasman G D. Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* 8:4108-16, 1969.
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The circular dichroism (CD) spectra of several proteins, whose secondary structures were known from X-ray diffraction studies, were fitted by a linear combination of the CD spectra of poly-L-lysine in the α helical, β pleated sheet, and random coil forms. The agreement between the structures predicted by CD and those found by X-ray crystallography was good. [The SCI® indicates that this paper has been cited over 850 times since 1969.]

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"This paper is an extension of work that was done with Betty Davidson when I had just started graduate research in Gerald Fasman's lab. My thesis project was to study the spectral transitions of model amides to help understand the optical activity of proteins. Davidson, a postdoctoral fellow in the lab, was studying factors affecting the conformational transitions of poly-L-lysine, as monitored by optical rotatory dispersion (ORD). It was felt that this basic polypeptide might ultimately serve as a model for the histones. She expected to observe a helix-coil transition when the peptide was heated and was surprised when instead it aggregated to give a spectrum which she had not seen before. This new spectrum was identified as that of the anti-parallel pleated sheet, or β form.

"We realized with some excitement that we inadvertently had obtained the first good model reference ORD spectra for the β pleated sheet conformation of proteins in solution. We therefore tried to see if we could predict the secondary structure of a protein by fitting its ORD spectrum with a linear combination of the spectra of the α helix, β pleated sheet, and random coil. I had just finished taking a two-week course in how to program in FORTRAN so the job

of curve fitting was given to me.¹ The results using ORD unfortunately were not in good agreement with those determined from X-ray crystallography.

"When we obtained a CD attachment for our spectropolarimeter we repeated our study because CD gives better resolution of spectral transitions than ORD. This time our fits were good; in retrospect they were actually better than we reported. When we did our studies there were only preliminary reports on the X-ray crystallography of chymotrypsin. Our predicted values turned out to agree very well with what was later found.

"I think that the paper has been cited as often as it has for several reasons. 1. It contained useful methods to calculate protein conformation whether or not one had a computer. 2. It gave precise reference data for the CD spectra of poly-L-lysine in the three reference conformations with clear illustrations. 3. When it was written very little was known about the secondary structure of globular proteins since only six proteins had been examined by X-ray crystallography. CD proved to be an excellent method to show that β structure was as ubiquitous in globular proteins as the well-characterized α helix.

"Today, calculations of protein conformation from CD spectra usually employ reference data obtained from the analysis of the CD spectra of proteins whose structure has been determined by X-ray crystallography.^{2,3} These methods give somewhat improved correlation between CD spectroscopic and X-ray crystallographic estimations of secondary structure.

"It is amazing to me that I have on my desk an inexpensive computer which is more powerful and easier to use than the one at Brandeis University which took up a large room and cost over 100 times as much; and the statistical and mathematical techniques that I once found so complicated I now apply routinely in areas ranging from enzyme kinetics to difference spectral measurements of cholesterol binding to cytochrome P-450. It was so difficult. Now it is so simple."

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2. Saxena V P & Wedauner D P. A new basis for interpreting the circular dichroism spectra of proteins. *Proc. Nat. Acad. Sci. US* 68:969-72, 1971.
3. Chen Y H, Yang J T & Chan K H. Determination of the helix and form of proteins in aqueous solution by circular dichroism. *Biochemistry* 13:3350-9, 1974.
4. Provencher S W & Glockner J. Estimation of globular protein secondary structure from circular dichroism. *Biochemistry* 20:33-7, 1981.
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