CHAPTER

6

Circular Dichroism Spectroscopy for Protein Characterization: Biopharmaceutical Applications

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6.1 INTRODUCTION

Circular dichroism (CD) is an optical spectroscopic method that exploits the differential absorption of left- and right-handed circularly polarized light by optically active molecules to determine their absolute configurations. When applied to proteins, CD in the far-UV wavelength range of the electromagnetic spectrum (260–170 nm) can be used to characterize and quantify the secondary structural content in terms of α-helical, β-strand, and unordered structure. It follows that any resolvable changes that occur due to interactions with ligands or other moieties or environmental factors such as changes in pH or ionic strength can be monitored. The CD signal from the aromatic residues tryptophan, tyrosine, and phenylalanine are detectable in the near-UV range between 260 and 300 nm and can be used to monitor changes in the environment of these moieties, which may be related to protein tertiary structure.

6.1.1 Theory

6.1.1.1 The Physical Origins of CD Signals

Electromagnetic (EM) radiation comprises an electric field and a magnetic field that oscillate in perpendicular planes, which are, in turn, perpendicular to the direction of a light propagation (Figure 6.1).

A light source generally gives rise to wave trains with the fields oriented isotropically; linear polarization selects light with the electric field oscillating in a single plane. In circularly

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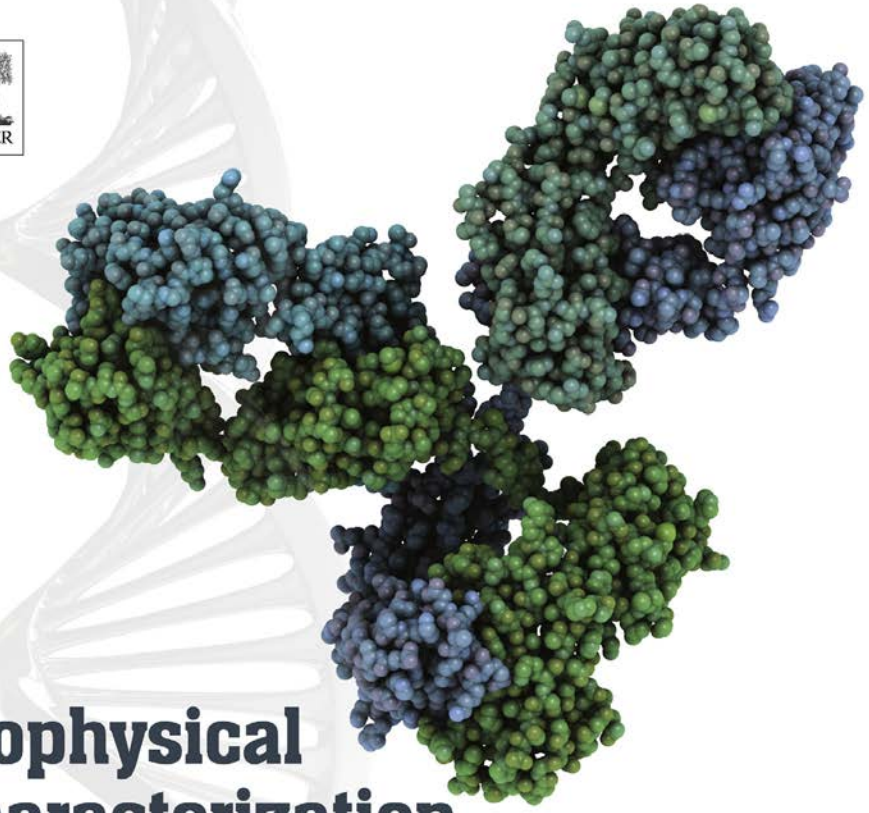

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