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We were prompted to develop this book by our experiences in teaching at the United Kingdom/European Union Circular Dichroism (CD) Summer Schools run at Warwick University in the U.K. for the past seven years, and at the BioCD Workshops run at the National Synchrotron Light Source (NSLS) in the United States since 2005. The Warwick schools were organised by Professor Alison Rodger, and the NSLS ones by Professor John Sutherland (of Brookhaven National Lab and East Carolina University). One of us (BAW) was co-director of both of these courses, and both of us (BAW RWJ) have lectured, demonstrated, and organised experimental exercises for both sets of courses. Indeed, all of the authors of this volume have participated in teaching at one or both of these. The one-week intensive courses were initially aimed at PhD students and postdocs working in the general area of biophysics, to give them a background for undertaking, analysing and interpreting both the established technique of CD spectroscopy, and the newer related methods of Synchrotron Radiation Circular Dichroism (SRCD) and Linear Dichroism (LD) spectroscopies. However, as the courses evolved, both industrial users and senior academics also became "students". It is on the basis of all of our experiences at these courses, and requests for a permanent record from the students who participated in them – and from other members of their labs who didn't attend the workshops – that we decided to compile this volume which deals with the practical issues and state-of-the-art methods for analyses involved in CD, SRCD, and LD spectroscopic research.

This is also a particularly timely endeavour given the emergence of SRCD as an important new tool for structural biology. This is evidenced by the Second International SRCD Meeting held in Beijing, China in 2009 (which followed the First International SRCD Meeting held at Daresbury, U.K. in 2001 that was organized by

the two editors of this volume and supported by a grant from the U.K. Biotechnology and Biological Sciences Research Council (BBSRC)).

The U.K. versions of the CD course were supported in their first three years by a grant from the U.K. Engineering and Physical Sciences Research Council (EPSRC) to Alison Rodger and BAW; later they were sustained by support from Warwick University and the MOAC Centre (of which Professor Rodger is director) and the E.U. Marie Curie BIOCONTROL network (of which BAW is a partner). The NSLS course was supported by Brookhaven National Lab, through the U.S. Department of Energy. All lecturers freely gave of their time to enable the courses to take place. We thank them and the members of our labs who helped in running of the courses (especially Dr. Andy Miles and Dr. Lee Whitmore) for their contributions to this volume. The research on CD and SRCD in our labs was supported by grants from the BBSRC.

It is hoped that this volume will be a valuable resource for past and future course participants, and especially for other researchers who plan to, and use, CD and SRCD as part of their structural biology studies.

BAW, RWJ

London, January 2009

An Introduction to Circular Dichroism and Synchrotron Radiation Circular Dichroism Spectroscopy

Authors
Robert W. Janes, B.A. Wallace

Abstract

The aim of this chapter is to introduce the techniques of circular dichroism (CD) and synchrotron radiation circular dichroism (SRCD) spectroscopies. A brief account is given of how the spectrum of a molecule is generated as a consequence of excitations resulting in electronic transitions, which for chiral molecules produce differential absorbances for left– and right– handed circularly polarised light. There follows an overview of the important basic principles of CD and of good practice protocols for collecting data, areas which are further developed in subsequent

chapters of this book. In addition there are sections describing potential applications of CD for studies of proteins and nucleic acids. The final section illustrates the enhanced capacity inherent in the technique of SRCD spectroscopy for applications in structural biology, again discussed in detail in other chapters.

Measurement of Circular Dichroism and Related Spectroscopies with Conventional and Synchrotron Light Sources: Theory and Instrumentation

Authors
John C. Sutherland

Abstract

A linearly polarized optical beam that passes through a photoelastic modulator can be used to measure circular dichroism, linear dichroism, optical rotary dispersion, fluorescence detected circular dichroism and fluorescence polarization anisotropy. These parameters, along with the simultaneous measurement of absorption and fluorescence are possible with a conceptually simple single beam spectrometer, with only minor adaptations. Practical spectrometers and the components needed to build them using both conventional (Xenon arc) and synchrotron light sources are reviewed. The need to match the components throughout the instrument is discussed and methods of calibrating critical components are described. Potential artifacts associated with sample inhomogenity are discussed. An explanation for the observation that a linear dichroism signal can appear at the modulation frequency expected for circular dichroism is presented. Spectra demonstrating the ability of a synchrotron source spectrometer to extend the range of wavelengths for the circular dichroism of proteins are presented. Three general classes of practical spectrometers are described based on the components used and the performance achievable. Vendors of polarization–modulation spectrometers and specialized components required to build them are listed, as are existing and planned synchrotron based user facilities.

Calibration Techniques for Circular Dichroism and Synchrotron Radiation Circular Dichroism Spectroscopy

Authors
Andrew J. Miles, B.A. Wallace

Abstract

Good practice in basic research and industrial applications necessitates the accurate and regular calibration of circular dichroism instruments and synchrotron radiation circular dichroism beamlines for magnitude, optical rotation and wavelength. If spectra obtained in different laboratories are to be comparable and usable with standard reference datasets for secondary structure analyses, then instruments must be standardized, enabling cross-validation of spectra produced. In this chapter calibration methods are discussed along with techniques for measuring other relevant parameters such as sample cell pathlengths and protein concentrations. Accurate knowledge of the values of these parameters is important for producing correct spectral magnitudes, that are, in turn, essential for correct secondary structural analyses.

Sample Preparation and Good Practice in Circular Dichroism Spectroscopy

Authors
Sharon M. Kelly, Nicholas C. Price

Abstract

This chapter aims to set out the guidelines for obtaining reliable CD data when studying molecules of biological interest. The main focus will be on the study of protein samples, but the points about sample purity and characterisation, attention to the solvent system employed, the proper use of the instrument and appropriate methods of data handling apply to all samples, whether they are small molecules, such as drugs or other ligands or macromolecules such as nucleic acids or their fragments. More details on most of these aspects are given in the review by Kelly et al. [1].

Sample Preparation and Good Practice in Synchrotron Radiation Circular Dichroism Spectroscopy

Authors
Andrew J. Miles, B.A. Wallace

Abstract

The development of synchrotron radiation circular dichroism (SRCD) spectroscopy, which uses the intense light of a synchrotron beam, has greatly expanded the utility of circular dichroism (CD), enabling the measurement of lower wavelength data

containing more electronic transitions and hence more structural information. Furthermore the higher signal–to–noise and the ability to do faster measurements facilitate high–throughput data collection using smaller samples. In general the good practice protocols required for conventional CD studies also apply to SRCD studies; however there are additional good practice issues unique to SRCD spectroscopy and these are covered in this chapter.

Reproducible Circular Dichroism Measurements for Biopharmaceutical Applications

Authors
Jascindra Ravi, Anna E. Hills, Alex E. Knight

Abstract

Circular Dichroism (CD) spectroscopy is an important technique for measuring the structure of protein biopharmaceutical products, which are increasingly important to the pharmaceutical industry. Its application tends to be rather different to pure research, in that it is more important to compare spectra of different samples or batches of material. However, this requires that spectra are obtained that are comparable, and that there exist objective methods for comparing spectra. We review the issues involved in obtaining good quality spectra, including instrument calibration, reference materials, cell pathlength, protein concentration and instrument maintenance, and the progress that is being made in these areas. We also discuss the available approaches for the objective comparison of CD spectra.

Synchrotron Radiation Circular Dichroism Spectroscopy: Applications in the Biosciences

Authors
Andrew J. Miles, B.A. Wallace

Abstract

Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy is enabling a number of new applications in biology because of its ability to measure lower wavelength data, lower requirement for sample quantities, higher signal–to–noise levels and ability to examine proteins under a wide range of conditions. Advances in SRCD instrumentation, data collection methodologies, data processing, reference datasets and methods of analysis have lead to new applications of SRCD for examining interesting biological questions. Although most of the SRCD studies to

date have focused on proteins, the technique has been successfully applied in the study of other macromolecules including DNA and carbohydrates. This chapter briefly discusses a number of examples of studies that have been undertaken with this new methodology.

Linear Dichroism Spectroscopy: Techniques and Applications

Authors
Alison Rodger

Abstract
Linear dichroism is the difference in absorbance of light polarized perpendicular and parallel to an orientation direction. It is ideal for determining the relative orientations of transitions within molecules and hence of the subunits of biomacromolecular systems.

Methods of Analysis for Circular Dichroism Spectroscopy of Proteins and the DichroWeb Server

Authors
Lee Whitmore, B.A. Wallace

Abstract
Circular dichroism spectroscopy is a very useful method for the analysis of the secondary structures of proteins. This chapter summarises the current (and past) algorithms, methodologies and reference datasets developed for such analyses, including the online analysis server DichroWeb. The chapter also includes sections on practical approaches to such analyses with existing software, and potential sources of errors in analyses and how they can be avoided.

Reference Datasets for Protein Circular Dichroism and Synchrotron Radiation Circular Dichroism Spectroscopic Analyses

Authors
Robert W. Janes

Abstract
In this chapter the contents and compositions of existing reference datasets used for the analysis of protein secondary structures from circular dichroism (CD) data

are described. It includes discussions of the quality of the CD data and the crystal structures that are used. Early reference datasets are compared with a new dataset, SP175, which was specifically created based on bioinformatics assessments of protein secondary structure and fold space. Finally, the issues that need to be considered when creating a new reference dataset are discussed.

Ab Initio Calculations for Circular Dichroism and Synchrotron Radiation Circular Dichroism Spectroscopy of Proteins

Authors
Benjamin M. Bulheller, Jonathan D. Hirst

Abstract

Circular dichroism (CD) and synchrotron radiation circular dichroism (SRCD) spectroscopy are widely used techniques for secondary structure determination of proteins. Using the matrix method, protein CD can be calculated from first principles, with an accuracy which is almost quantitative for helical proteins. Thus, CD calculations and experimental data can be used in conjunction to aid structure analysis.

The Protein Circular Dichroism Data Bank (PCDDB): A Resource for Data Archiving, Sharing, Validation and Analysis

Authors
B.A. Wallace, Lee Whitmore, Robert W. Janes

Abstract

The Protein Circular Dichroism Data Bank is a deposition and user-friendly archive of circular dichroism (CD) and synchrotron radiation circular dichroism (SRCD) spectra, with associated software for validation, analysis and searching. It aims to provide a publicly-accessible resource for structural biology, biotechnology and bioinformatics that enables data sharing and data mining of validated CD data. It contains both spectral data and its associated metadata describing the sample and experimental conditions, with links to sequence and crystallographic data bases. It is located online at: http://pcddb.cryst.bbk.ac.uk. Its functions, contents, structure and features are described in detail in Wallace et al., 2006 [1] and Whitmore et al., 2006 [2].