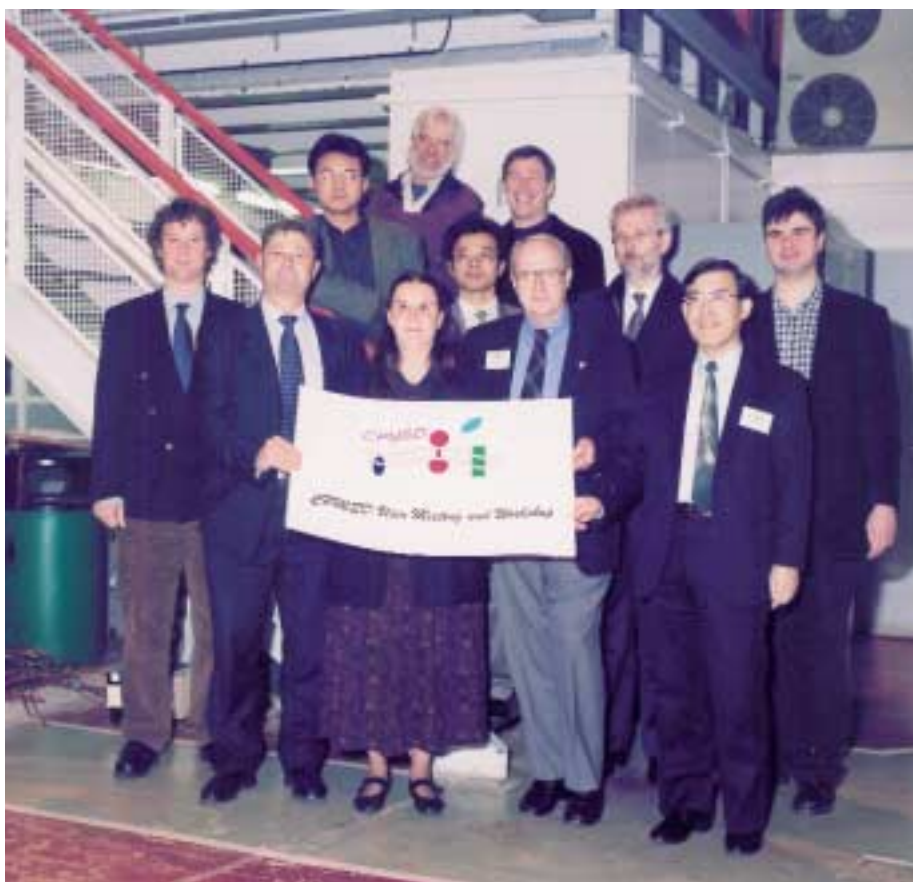


1st International Workshop on SRCD Spectroscopy



Participants in the First International Workshop on Synchrotron Radiation Circular Dichroism Spectroscopy standing in front of the new CD12 station for SRCD spectroscopy at the SRS, Daresbury. Front row: Robert Janes, Gareth Jones, Bonnie Wallace (organizer), John Sutherland, Kunihiko Gekko. Middle Row: Ye Tao, Zhang Guobin, Alberto Spisni, David Clarke. Back Row: John Kenney, Markus Sauerborn.

The First International Workshop on Synchrotron Radiation Circular Dichroism (SRCD) Spectroscopy was held on November 9, 2001, at the Daresbury Laboratory, Warrington, UK, under the sponsorship of the UK Biotechnology and Biological Sciences Research Council. The

meeting was chaired by Bonnie Ann Wallace, Director of the Centre for Protein and Membrane Structure and Dynamics (CPMSD, the UK Structural Biology Centre of Excellence in CD Spectroscopy) at Daresbury and Professor of Molecular Biophysics at Birkbeck College, University

of London. Representatives from all existing biological SRCD facilities, as well as those from many sites developing or planning SRCD beamlines, were present.

A series of interesting reports were presented on the work being undertaken at the existing stations worldwide, and the designs being planned for future stations. Professor John Sutherland (Brookhaven National Laboratory, USA), Dr. Gareth Jones (Daresbury, UK), and Dr. John Kenney (ASTRID, Denmark) described the characteristics of their respective existing beamlines. Professor Sutherland, who designed and operates the longest running SRCD station, discussed work that has been done on the Brookhaven Vacuum Ultraviolet (VUV) stations U9B and U11 for the past 20 years. Dr. Jones discussed the new station CD12 just being commissioned at Daresbury (which will replace stations 3.1 and 13.1) and how it would improve data collection and enable time-resolved studies. Dr. Kenney discussed work from the ASTRID UV1 beamline, which has been used by both chemists and biochemists and has been operational for about a year. Drs. Kunihiko Gekko (HiSOR, Japan) and Ye Tao (BSRF, China) discussed the status and plans for their new stations on beamlines BL15 and 3B1B, respectively, which are currently being commissioned and expect to be available for users in 2002, and Dr. Markus Sauerborn (BESSY2, Germany), Professor Alberto Spisni (LNLS, Brazil) and Dr. Zhang Guobin (NSRL, China) discussed beamlines that were in the design stage and are planned for operation in mid-2003. The discussions following the presentations focused on the various design characteristics of the SRCD beamlines and stations, including mirror types, gratings, window materials, monochromators, VUV versus nitrogen-purged chambers, and the merits of continuous scan versus scan and dwell measurements.

Probably the most important aspects of the workshop were the roundtable discussions. The first of these centered on the

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establishment of common standards and methods for calibration. Professor Sutherland presented his research on several alternative methods for calibrations of photoelectric modulators and Dr. Kenney discussed the calibration of sample cell pathlengths by interferometry. Professor Wallace stressed the importance of accurate determinations of protein concentrations in order to obtain reliable ellipticity measurements; she indicated the merits of quantitative amino acid analyses for determining protein concentrations. Alternative methods for quantitation were discussed. It was generally felt that calculated extinction coefficients based on tryptophan contents, as well as colorimetric assays were too inaccurate for these purposes. Dr. Gekko indicated that gravimetric assays were highly accurate; however, there was some feeling from amongst the participants that this is a difficult method for users to do properly in a routine manner. It was concluded that accurate protein quantitation is very important for SRCD measurements, that comparisons of the various methods should be considered at future meetings, and that station managers should discuss this issue with their users.

Participants from all the operating beamlines and as well as those from all the beamlines currently being commissioned brought sample spectra of the commonly-used standard camphor sulphonic acid (CSA) in order to enable comparisons between instruments. In addition, Dr. Robert Janes (University of London, UK) compared spectra obtained for the proteins myoglobin and concanavalin A on the Daresbury and ASTRID beamlines with those obtained on a commercial instrument and present in existing CD reference databases. It was clear from this work that it would be important in the future to establish more than one standard, and a considerable amount of the discussion focused on what samples would be most appropriate as standards, sources of, and experimental conditions for the standards to be used for calibration of wavelengths, optical rotations and

ellipticities. It was decided that at a minimum, all sites should obtain CSA, myoglobin and concanavalin A spectra and post them on their websites in the future. It was also agreed that a common sample (which had been characterized for purity by mass spectrometry) for each of these proteins would be made available to all sites. It was also recognized that these could also be useful as standards for conventional CD machine calibrations. Dr. Jones then emphasized the need to recalibrate and validate a station on a regular (he suggested weekly) basis, or at least each time a change was made to any part of the setup.

Sample cells were also a topic of intense discussion in this roundtable session, as high-transmittance, short pathlength cells are needed in order to obtain the lowest wavelength data possible in SRCD. Standard, commercially available cells that can be routinely used are 10- or 5-micron Suprasil cells (either demountable or closed). Demountable cells were found to be easier for users to clean and fill, but some concern was raised as to the precise reproducibility of pathlengths between loadings. Professor Sutherland described the special short pathlength (Gray) cells developed at Brookhaven and Dr. Gekko presented data from the variable pathlength cells specially designed at Hiroshima, made with MgF₂ windows to improve light transmission, with pathlengths as low as 2 microns. A potential problem identified for all cells used in SRCD (as opposed to CD) was that because of the small focal spot size of the SR beam, determination of the exact pathlength of the part of the cell that was being illuminated was important. The pathlength could vary slightly across the body of the cell, but this would not be detected if the pathlength were calibrated offsite (i.e. on a conventional CD machine) with a larger beam that bathed more of the cell. This then pointed to a clear advantage in being able to measure simultaneous absorption and CD measurements in the SRCD, as is possible with the Brookhaven beamlines.

A second roundtable discussion focused on the future for SRCD and its applications in biology. One topic that excited a lot of interest was the idea of establishing a PCDDB (Protein CD Data Bank), as proposed by Professor Wallace. This would be similar to the Protein Data Bank (PDB) for crystal structures and crystallographic data, and would be for depositing *validated* CD spectra of proteins. It would be linked with Swiss-Prot sequence and PDB structural databases. It would include a variety of information on the protein and experimental conditions as well as the CD and dynode voltage spectra. Validation tools would be developed for checking the data prior to deposition, and would be available on line so that those who access the Data Base could evaluate the quality of the data. Initial feasibility studies and discussions with the organizers of the PDB are to be coordinated by Prof. Wallace and Dr. Janes, with all other participants expressing interest at ultimately establishing this as an international project in which they would participate.

This last roundtable session also examined the advantages of SRCD over conventional CD and potential new uses for SRCD in the context of post-genomics and proteomics programs. Professor Wallace presented some of her work on fold recognition, which is being made possible by the extra low wavelength data available in SRCD, and she discussed its possible role in the context of a Structural Genomics target screening program. Dr. Jones discussed the potential for high throughput binding studies of ligands for pharmaceutical screening, and their potential role in Proteomics and Functional Genomics and Metabolomics programs. Professor Wallace finished by describing the Membrane Protein Reference Data Base her group is creating using SRCD data obtained at Daresbury for the improved analyses membrane protein structures by both CD and SRCD spectroscopy. It will include CD spectra of membrane proteins whose crystal structures have been determined, obtained under conditions (i.e., con-

centration, detergents, pH, salt) used for crystallization. The samples have been generously provided by crystallographers from around the world. Plans are to make this new database accessible to interested users via the Dichroweb secondary structure calculation website (<http://www.cryst.bbk.ac.uk/cdweb>).

Finally, a decision was made to continue the dialogues begun at this meeting via a

Second International Workshop on SRCD Spectroscopy in two years' time, and through the establishment of an SRCD electronics bulletin board to be hosted at the CPMSD website (<http://www.srs.dl.ac.uk/VUV/CD/cpmsd.html>) and coordinated by Dr. David Clarke at Daresbury. Dr. Sauerborn proposed the creation of the SRCD Inter-national Network. It was agreed that future meetings and the electronic bulletin board, along with

the standardization websites, would become the initial projects of this network, and that other projects such as the PCCDB would be added in the future. Initial membership in the Network will consist of all the participants from the First Workshop; membership will later be open to other developers of SRCD beamlines. Anyone interested in joining this Network should contact b.wallace@mail.cryst.bbk.ac.uk. ■