Characterization of Biological Molecules using Circular Dichroism, Fluorescence, and Single Molecule Spectroscopy

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X-ray crystallography techniques provide atomic resolution structural information, and the resolution of structures from cryo-electron microscopy is improving steadily. However, both of these methods obtain snapshots of structure frozen in time, and require extensive and potentially disruptive sample preparation. There is therefore a requirement to complement these high resolution structural techniques with other methods that are able to obtain structural information in real time, in the real-world environment of solutions and cells. Circular dichroism spectroscopy uses circularly polarised ultraviolet light to probe the structure of biological macromolecules in solution. It can be used in time-resolved mode to monitor changes in structure on the millisecond time scale, for the study of processes such as protein folding. Fluorescence spectroscopy allows the specific labelling of molecules of interest in complex systems, providing structural information on, for example, macromolecular complexes. Single molecule spectroscopy techniques are becoming increasingly popular and have the advantage of avoiding ensemble averaging, enabling the characterization of different structural populations within samples. In this lecture I will describe the principles of these methods, and give examples of their use on large scale facilities for the study of biological systems and processes.