Raman optical activity (ROA) provides vibrational optical activity spectra of chiral molecules, and molecules in magnetic fields, by means of a circular polarization-dependence of Raman scattering [1,2]. Natural ROA probes molecular chirality; magnetic ROA probes magnetic structure. ROA can be applied to a vast range of molecular structures, from paramagnetic inorganic molecules and small chiral organics, to supramolecular structures and large biomolecules. Whereas conventional UV-visible circular dichroism (CD) measures optical activity in electronic transitions, ROA, like its sister technique of vibrational circular dichroism (VCD), measures optical activity in vibrational transitions and hence provides much more structural and stereochemical information. Since water is a favourable solvent for Raman studies, ROA is ideal for studying biomolecules in their natural aqueous environments. This talk will present a review of ROA from first observations to current applications.

ROA may be measured as a small difference in the intensity of vibrational Raman scattering in right- and left-circularly polarized incident light or, equivalently, the intensity of a small circularly polarized component in the scattered light using incident light of fixed (linear or unpolarized) polarization. The first and second experiments are called *incident circular polarization* (ICP) and *scattered circular polarization* (SCP) ROA, respectively. Measurement of SCP ROA has significant advantages over ICP ROA for routine measurements.

Figure 1 LDB with the first Cambridge ROA instrument (ICP) in 1972, showing the chart
recorder traces of the first observations on the enantiomers of 1-phenylethylamine.

Using the ICP strategy with visible excitation, the first observations of genuine natural ROA (Figure 1), published in 1973 amid much controversy [3], constituted the first observations of vibrational optical activity of molecular origin. These measurements were made in depolarized 90° scattering, this being the optimum strategy at the time for suppressing the large polarization artefacts that plagued the early attempts. Although the same configuration was used very recently for an ICP ROA instrument with deep UV excitation constructed in my Glasgow laboratory [4], nowadays ROA spectra are usually recorded in backscattering, which is the optimum strategy for maximizing the signal-to-noise ratio [1,2]. Thanks to the introduction of a new generation of instrument based on SCP in backscattering, designed by Werner Hug and commercialized by BioTools, Inc., that incorporates a sophisticated artefact suppression protocol, ROA measurements may now be made easily and routinely on a wide range of samples.

Natural ROA originates in interference between light waves scattered via the electric dipole–electric dipole polarizability tensor and the electric dipole–magnetic dipole and electric dipole–electric quadrupole optical activity tensors, respectively; whereas magnetic ROA originates in interference between the electric dipole–electric dipole polarizability and the same tensor perturbed to first order in the static magnetic field [1]. Although the electric dipole–electric quadrupole tensor only contributes to optical rotation and circular dichroism in oriented systems [1], it contributes to the polarizability–optical activity ROA cross-terms even in isotropic samples.

Simulation of the observed ROA spectrum of a chiral molecule can provide the complete solution structure (conformation, absolute configuration, conformational populations). Calculations, which are usually based on the Placzek approximation, can proceed in several ways. Models of ROA such as the bond-polarizability model [1] can provide valuable physical insight into the generation of ROA by various chiral structural elements, but the associated calculations do not reproduce experimental data at all well. Quantum-chemical ab initio calculations are now the method of choice. For example, the Gaussian software package implements an analytic time-dependent protocol for the calculation of the ROA property-tensor derivatives, both HF and DFT. Comparison of experimental with simulated vibrational optical activity spectra (ROA and VCD) is now a powerful technique for determining absolute configuration: it is even more reliable than anomalous X-ray scattering and more widely applicable since crystals are not required. Large chiral molecules such as oligosaccharides, oligopeptides and small proteins are now accessible to ab initio simulations. An example is presented in Figure 2. This is taken from a study in my Glasgow laboratory which revisited the ‘old friends’ used for the first observations of ROA, namely both enantiomers of 1-phenylethanol and 1-phenylethylamine. The top panel in Figure 3 displays the experimental backscattered SCP Raman and ROA spectra of the (+)-sample of 1-phenylethanol, with the calculated spectra for the (R) absolute configuration shown below. The very close agreement in sign and magnitude between the two means that one would ‘stake one’s life’ on the (+)-enantiomer having the (R)
absolute configuration! The computed normal modes finally provided an understanding of the vibrational origins of our first observations of ROA in 1972.

Dramatic improvements in instrumentation in the late 1980s [2] in the form of backscattering, backthinned CCD detectors, high efficiency notch filters and spectrographs with single gratings based on volume holographic technology rendered biomolecules in aqueous solution accessible to ROA for the first time. It was not long before studies on amino acids, peptides, proteins, carbohydrates, glycoproteins, nucleic acids and even viruses were being made in my Glasgow laboratory. It quickly became apparent that ROA is much more incisive than conventional vibrational spectroscopy for large biomolecules due to the fact that vibrations which sample the skeletal chirality most directly generate the largest ROA intensity. For example, whereas the conventional Raman spectra of proteins are dominated by bands from the amino acid side chains, protein ROA spectra are dominated by the peptide backbone bands and so provide direct information about secondary and tertiary structure, with ROA from side chains often being weaker due to some degree of conformational freedom. The example of hen lysozyme is shown in Figure 3. The largest features, associated with secondary structure, appear in the amide I and extended amide III regions; but every feature, including the weaker side-chain bands, is real and reproducible and is reporting on some element of the three-dimensional structure. Shown beneath the ~1200-1450 cm\(^{-1}\) region is our very first protein ROA spectrum,

![Figure 2 Experimental and calculated backscattered SCP Raman and ROA spectra of (+)-(R)-1-phenylethanol.](image)
measured on hen lysozyme in 1990: it may be seen that it was indeed the ‘real thing’ and represented a milestone in the spectroscopy of biomolecules.

Figure 3 The backscattered SCP Raman and ROA spectra of hen lysozyme in aqueous solution, together with our first protein ROA spectrum below recorded with an ICP instrument in 1990.

A recent review of the development of biomolecular ROA describes the large range of current applications [5]. The plethora of structure-sensitive bands in protein ROA spectra makes the application of multivariate analysis ideal for extracting structural information, including the tertiary fold in addition to secondary structure of proteins. Remarkably, the fold type of the major coat proteins of intact viruses can be simply ‘read off’ visually from the characteristic band patterns. ROA can even provide information about polypeptide and carbohydrate structure of intact glycoproteins, and protein and nucleic acid structure of intact viruses. Aided by the special sensitivity of ROA to residual structure, especially the poly(L-proline II) conformation, in unfolded sequences, ROA is especially valuable in studies of aberrant proteins involved in misfolding diseases such as Parkinson’s, Alzheimer’s and the prion encephalopathies.

ROA may also be induced in achiral molecules by means of a static magnetic field [1]. A ‘Raman EPR’ version involving spin-flip resonance Raman transitions via antisymmetric scattering in paramagnetic molecules provides new information about the magnetic structure of ground and excited vibrational–electronic states [1].

Experiments based on 2D correlation methods, resonance Raman, SERS, CARS, etc., will further enhance ROA’s ability to address many current problems at the forefront of physics, chemistry and biomolecular science.

REFERENCES