# 5 Protein infrared spectroscopy

Infrared spectroscopy is based on the infrared absorption of molecules and is, compared with crystallography, a relatively simple and inexpensive tool for the global characterization of molecular conformations and conformational changes of proteins and other biomolecules. Depending on the measurement technique, scanning infrared (IR) spectrometers, Fourier transform infrared (FTIR) spectrometers, and single wavelength infrared apparatuses are distinguished (see Sect. 5.1). Typically the most interesting spectral region for biomolecules is $\nu = 400 - 4000\ \mathrm{cm}^{-1}$, where the wavenumber, $\nu$, is defined as $\nu \equiv 1/\text{wavelength}$. Infrared activity requires a change of dipole moment upon excitation (Fig. 5.1). For proteins the amide chromophore absorption in the region of $1500\ \mathrm{cm}^{-1} - 1700\ \mathrm{cm}^{-1}$ ($\approx 6\,\mu\mathrm{m}$ wavelength) is particularly important for the assessment of secondary structure content and structural changes. Regarding the resolution of protein secondary structure, the information content of IR and FTIR spectroscopy is comparable with that of circular dichroism (see, e.g., Nölting et al., 1997b; Nölting, 2005), and regarding the resolution of features of the tertiary structure of proteins, IR and FTIR are often inferior, and yet IR is much easier to apply on a fast time scale and for remote sensing (see, e.g., LIDAR in Sect. 5.1.3).

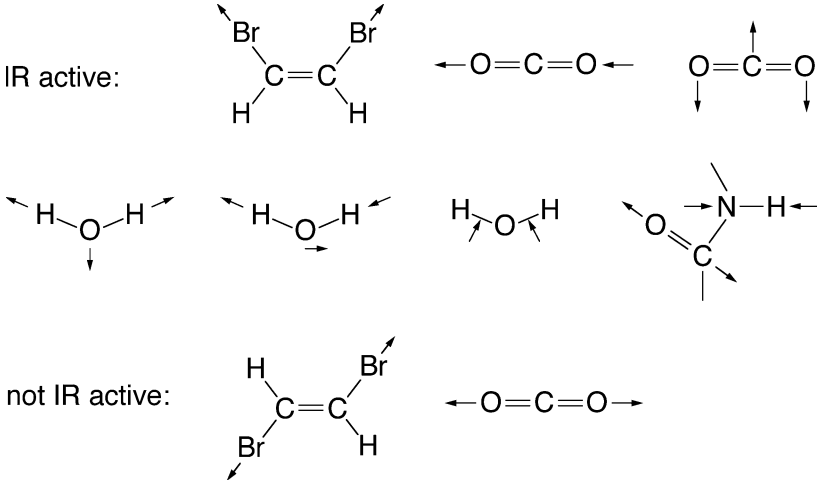


**Fig. 5.1** Example of infra-red active and non-active vibrations. Note that infra-red activity requires a change of dipole moment

## 5.1 Spectrometers and devices

### 5.1.1 Scanning infrared spectrometers

Early IR spectrometers (Fig. 5.2) were constructed similarly to scanning UV/VIS absorption spectrometers. The emission of the source, e.g., a thermal source operated at 1000 °C, is passed through a monochromator selecting a single wavelength. The monochromatic beam is split into two beams – one having the sample in the path. A shutter passes through only one of the two beams at a time. Both beams are alternatingly detected by an IR detector, e.g., a pyroelectric detector, and compared which each other. The optical density of the sample is calculated from the logarithm of the intensity quotient. The use of light modulation is quite indispensable since the problem of background radiation is much more severe than in UV/VIS spectrometers. Spectra are recorded by scanning the wavelength region of interest. This scanning principle of operation is still widely used in IR spectrometers with time resolutions in the femtosecond to nanosecond region, where infrared lasers serve as IR source (see Nölting, 2005).

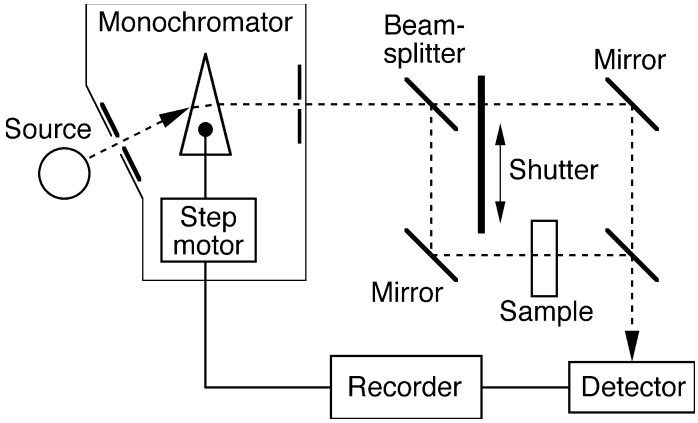


**Fig. 5.2** Example of a scanning infrared (IR) spectrometer. The monochromator separates the radiation of the IR source into its different wavelengths and selects one wavelength at a time. A beam splitter separates the monochromatic beam into sample beam and reference beam. The absorption coefficient, according to the chemical and structural properties of the sample molecules, is calculated using the detected intensity quotient between both beams, the pathlength, and the sample concentration

### 5.1.2 Fourier transform infrared (FTIR) spectrometers

FTIR spectrometers (Figs. 5.3–5.7) use the technique of Michelson interferometry and have the advantage of using a larger part of the emission of the IR source during the measurement of a spectrum, compared with scanning IR spectrometers