Vibrational spectroscopy approach to photosensitive membrane proteins

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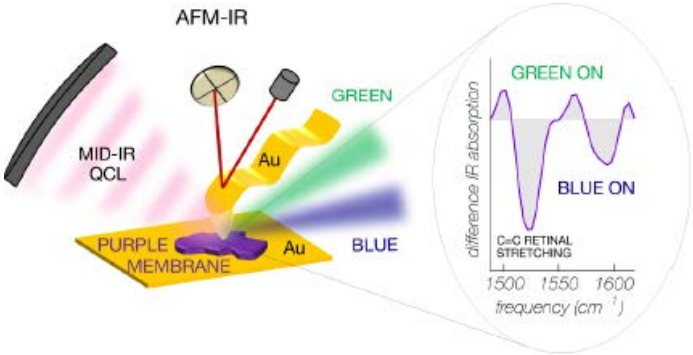

The structure of rhodopsins consists of a seven-helix protein and a non-proteic cofactor called retinal that crucially absorbs visible light. Following light absorption, rhodopsins can move ions or molecules against the membrane potential (or vice versa). This "photocycle" mechanism is based on very fast retinal photo-isomerization (hundreds of fs) and on a cyclic sequence of conformational changes of the protein backbone ranging from few milliseconds to several seconds. At the end of the photocycle, the protein ideally returns to the initial state after having moved an ion across the membrane: the protein then works as a pump (if the ion is moved against the membrane potential, for example in Bacteriorhodopsin), or as a channel (if the concentration gradient determines the direction of movement, for example in the optogenetic gate Channelrhodopsin).

The most important label-free investigation methods for protein conformational changes is **IR difference absorption spectroscopy** (wavenumber 500 – 2000 cm^{-1}, wavelength 20 – 5 □ m), usually performed with Fourier-Transform (FT) IR spectrometers**.** Raman spectroscopy, instead, has been found to be sensitive mostly to conformational changes of the retinal. The advantage of vibrational spectroscopies resides in their sensitivity to dynamic conformational changes in physiologic environments, but they are not fully sufficient to determine the unknown protein secondary structure (folding). Therefore, it is of high interest to bring vibrational spectroscopy from the purified protein solution environment to the real biological interaction environment, i.e. moving steps from the statistically significant ensemble towards the single-molecule limit.

In this talk we will report on novel instrumental developments for the vibrational spectroscopy analysis of photosensitive proteins in their native membrane environment. A first line of research is the IR nanospectroscopy (nano-IR) of few oriented proteins in a single 5-nm thick membrane flake, employing atomic-force-microscopy-assisted photothermal expansion spectroscopy. As an ancillary technique to nano-IR, we are also developing IR time-resolved confocal microspectroscopy with quantum cascade lasers that allows single-photocycle studies with millisecond resolution. A second line of research is the development of Surface Enhanced Raman Spectroscopy (SERS) for FT-Raman with laser excitation at 1064 nm, i.e. out of the absorption range of the retinal.