

**STATIC AND TIME-RESOLVED INFRARED DIFFERENCE SPECTROSCOPY.
HOW TO STUDY THE MECHANISM OF BIOCHEMICAL REACTIONS
IN MEMBRANE PROTEINS AT THE ATOMIC SCALE**

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Infrared difference spectroscopy (DS) makes it possible to investigate the molecular mechanism of biochemical reactions taking place in membranes and membrane proteins. Whereas other spectroscopic techniques usually carry information on specific cofactors, pigments, or chemical species, infrared DS make it possible to follow changes on all the constituents of the system of interest. When isolated membrane proteins are studied, events like proton transfer, electron transfer, cofactor displacement, protein rearrangement (localized or large-scale), redox changes, hydrogen bond formation, etc can be followed, usually in a time-resolved way. Little or no specific treatment of the sample is needed, and also large-scale systems (membranes, whole organisms...) can be studied. The obtained spectra do not usually need any specific data analysis procedure, and advanced physics (e.g. quantum mechanics) is generally not necessary to perform the experiments nor to interpret the results.

I will present some examples to illustrate the power of the technique. First, in the bacterial photosynthetic reaction center, ubiquinone reduction has been followed; key information about the mechanism of this reaction have been obtained [1-4 & refs. therein]. The release of ubiquinol in the membrane has also been followed; infrared DS has also shown that coenzyme Q, depending on its redox state (Q or QH₂), interacts differently with the phospholipids [1, 3, 4].

A similar approach has also been applied to understand the working mechanism of another membrane protein, cytochrome oxidase. In the second example, the technique has then been applied to the study of photoprotective mechanisms in cyanobacteria, dinoflagellates and diatoms [5-7]. In particular, the chemical modification of specific carotenoids, carried out in the membrane by specific enzymes, has been followed in real time in whole living diatoms.

Finally, the effect of the hydration of proteins has also been studied. It was shown that the modification of the hydration layer can strongly influence also reactions within the protein interior, also in detergent-isolated membrane proteins [8]. These results will be discussed in the framework of the synergic use of different spectroscopic techniques in the investigation of reaction mechanisms in membrane proteins.

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