Synchrotron IR microspectroscopy of biomolecules in microfluidic systems

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FTIR spectroscopy of proteins has the unusual disadvantage of providing too much information. Thousands of individual bands contribute to the spectrum, leading to an overlap so extensive that essentially all detail is obscured. FTIR difference spectroscopy is a perturbation approach designed to overcome this problem: instead of the complete FTIR spectrum, only the changes in the spectrum in response to a biologically interesting perturbation of the sample are recorded [1]. The resulting difference spectra are far simpler than complete infrared spectra, and thus can be interpreted at the level of individual molecular bonds. But at the same time, they retain all the information pertaining to the structural dynamics related to the protein's catalytic cycle, and are thus of direct relevance to the study of molecular mechanisms in protein reactions.

An essential ingredient of an FTIR difference spectroscopy experiment is a method for triggering the desired reaction while avoiding any unwanted perturbation of the sample. Here, rapid mixing techniques have the advantage over standard approaches such as photochemistry [2-4] or electrochemistry [5], because the former are universally applicable: perturbations such as ligand, substrate or inhibitor binding, changes in pH or ionic strength, enzymatic degradation, etc. can be applied to essentially any protein. Achieving rapid fluid mixing in a 10 µm cell pathlength suitable for FTIR spectroscopy of aqueous solutions is quite a challenge, but several elegant solutions based on microsystems technology have been described [6-11].

The brilliance advantage of synchrotron light sources can be exploited to implement rapid mixing FTIR spectroscopy in microstructured fluidic systems much smaller than those presented to date [12]. In addition to the obvious advantages of drastically decreased sample material requirements, this can be expected to improve the performance of such experiments by decreasing the distances to be covered in diffusional mixing.

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