

## Monitoring of Hydrogen-Deuterium Exchange in Membrane Proteins using Attenuated Total Reflection Infrared (ATR-IR) Spectroscopy and a Microdialysis Perfusion-Cell

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Protein structure and flexibility was studied by probing water accessibility in  $^1\text{H}/^2\text{H}$  exchange experiments using ATR-IR spectroscopy and a microdialysis perfusion-cell. The perfusion-cell was developed using a diamond ATR unit with 7 reflections. The solution or suspension of target protein in  $\text{H}_2\text{O}$  buffer contained in a chamber with sample volumes of below  $5\ \mu\text{l}$  is in contact with the ATR crystal and separated from the flowing  $^2\text{H}_2\text{O}$  by a dialysis membrane (molecular weight cut-off value of 25 kDa). The perfusion ATR unit is characterised by small volumes for the target protein and the buffer solution used for perfusion, by high stability and fast response, and by high sensitivity for the detection of binding-induced conformational changes. Its design is shown in Fig. 1.

As an example, the amide proton exchange of  $\text{Na}^+/\text{H}^+$  antiporters was followed, NhaA [1] from *Escherichia coli* and MjNhaP1 [2] from *Methanococcus jannaschii*. They are involved in cell energetics, regulation of cytoplasmic  $\text{Na}^+$ , alkaline pH homeostasis and cellular volume [3, 4]. Fundamental for cytoplasmic pH regulation is their activity which is strongly regulated by pH. NhaA is inactive at pH 7 and below but highly active at alkaline pH value from above pH 8. The active state of MjNhaP1 is between pH 6 and 6.5, which is identical to the human homologue NHE1, but opposite to NhaA. The analysis of  $^1\text{H}/^2\text{H}$  exchange using amide II mode has been carried out for inactive and active state of the protein, respectively. This provides information about accessible fraction of polypeptide chain between these two states. With further study of the exchange amide protons with respect to time, it was possible to distinguish between exchanging rate of amide protons in different regions of the protein.  $^1\text{H}/^2\text{H}$  exchange experiments allowed to investigate the flexibility and structural changes between inactive and active state of the protein.

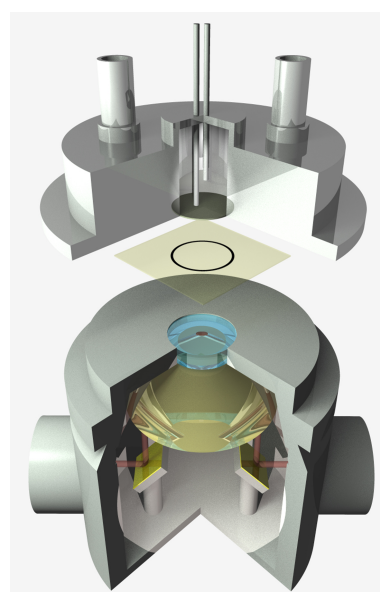


Fig. 1: ATR-IR Microdialysis Perfusion-Cell

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