

Advances in the field of bioelectrochemistry and surface enhanced IR spectroscopies for the study of the reaction of membrane proteins from bacterial respiratory chains

Laboratoire de Bioélectrochimie et Spectroscopie, UMR 7140, Chimie de la Matière Complexe, Université de Strasbourg – CNRS 4, rue Blaise Pascal, 67081 Strasbourg, France ; hellwig@unistra.fr

Although the architectures of several membrane proteins in respiration as well as the basic chemical reactions have been described, the interactions on molecular level, the diversity and efficiency of the reaction mechanisms in bacterial systems, are under debate. Electrochemical and spectroscopic experiments will be presented that have been developed to study coupled electron and proton reactions, identify the contribution of individual amino acids, study the reactivity towards small molecules and, importantly, correlate it with the microenvironment of the cofactors. The main projects will be presented:

1. Electrocatalytic studies on different membrane proteins from the respiratory chain, that demonstrate a different reactivity towards the substrate and the adaptation of the bacteria to the respective environment.^{1,2}
2. Surface enhanced IR spectroscopies (SEIRAS), that allow studies on the reactivity down to the picomolar level and on protein monolayers. Recent advances in the creation of ideal surfaces for SEIRAS will be given.³⁻⁵
3. Finally, the possibility to tailor nanostructure to enhance individual infrared signals will be demonstrated and the perspectives of these techniques for other fields of chemistry and biology discussed.⁶

Recent references

1. Safarian, S., Hahn, A., Mills, D.J., Radloff, M., Eisinger, M.L., Nikolaev, A., Meier-Credo, J., Melin, F., Miyoshi, H., Gennis, R.B., Sakamoto, J., Langer, J.D., Hellwig, P., Kühlbrandt, W., Michel, H., Active site rearrangement and structural divergence in prokaryotic respiratory oxidases (2019) *Science* 366(6461):100-104.
2. Nikolaev, A., Safarian, S., Thesseling, A., Wohlwend, D., Friedrich, T., Michel, H., Kusumoto, T., Sakamoto, J., Melin, F., Hellwig P. (2021) *Biochim Biophys Acta Bioenerg.* 1862 148436.
3. Grytsyk, N., Santos Seica, A.F., Sugihara, J., Kaback, H.R., Hellwig, P. 'Arg302 governs the pKa of Glu325 in LacY' (2019) *Proc. Natl. Acad. Sci. U.S.A.* 116, 4934-4939.
4. Santos Seica, A.F., Iqbal, M.H., Carvalho, A., Choe, J-Y., Boulmedais, F. and Hellwig, P. Study of Membrane Protein Monolayers Using Surface-Enhanced Infrared Absorption Spectroscopy (SEIRAS): Critical Dependence of Nanostructured Gold Surface Morphology (2021) *ACS Sensors*, doi.org/10.1021/acssensors.1c00238
5. Santos Seica, A.F., Schimpf, J., Friedrich, T., Hellwig, P. Visualizing the movement of the amphipathic helix in the respiratory complex I using a nitrile infrared probe and SEIRAS. (2020) *FEBS Lett.* 594(3):491-496.
6. Omeis, F., Santos Seica, A.F., Bernard, R., Javahiry, N., Majjad, H., Moss, D., Hellwig, P., Following the Chemical Immobilization of Membrane Proteins on Plasmonic Nanoantennas Using Infrared Spectroscopy (2020) *ACS sensors* 5 (7), 2191-2197.