Femtosecond redox-induced 2D-IR difference spectroscopy of proteins and biomolecules

Youssef El Khoury†*, Luuk J. G. W. van Wilderen, Julian Schmidt-Engler and Jens Bredenbeck

Institut für Biophysik, Johann-Wolfgang-Goethe-Universität, Max-von-Laue-Str. 1, 60438, Frankfurt am Main, Germany. †Current address: Institut de Physique et Chimie des Matériaux de Strasbourg, 23 Rue de Loess, 67034, Strasbourg, France. *elkhoury@ipcms.unistra.fr

In analogy to redox-induced FTIR difference spectroscopy, we developed redox-induced 2D-IR difference spectroscopy to investigate ultrafast dynamics of redox-sensitive vibrational modes. Lifetimes, couplings, vibrational energy transfer and spectral diffusion can be investigated under redox control. The methodology and first applications including the protein cytochrome c are presented.

Electron transfer is an elementary reaction in chemistry and biology, where it is involved in cellular respiration, photosynthesis and catalysis.[1] Spectroelectrochemistry in the infrared can be used to probe redox-sensitive structural changes in chemical and biological samples.[2,3] Here we use a novel reflection mode spectroelectrochemical cell (see Fig. 1a) in combination with 2D-IR spectroscopy to probe ultrafast redox-dependent processes.[4] We measure distinct redox-dependent vibrational lifetimes and anharmonicities in flavin mononucleotide and ferricyanide/ferrocyanide (Fig. 1b),[4,5] as well as different anharmonic couplings and ultrafast vibrational dynamics in the protein cytochrome c.

Fig.1 (a) Schematic drawing of the reflection mode spectroelectrochemical cell. The sample is contained between two windows, separated by a spacer (6). One window features gold coated areas (5) that serve as electrode and mirror at the same time. The other is a 2 mm CaF₂ window (7). A reference electrode (9) and a thermometer (3) are connected. The figure is generated using Solid Edge (http://www.solidedge.co.za/). (b) Reduced-minus-oxidized FTIR spectrum of ferricyanide/ferrocyanide in the νC≡N region (top), and the corresponding reduced-minus-oxidized-2D-IR difference spectrum (bottom; asinh(signal) is plotted in order to better visualize the small signals of the oxidized form). Reprinted with permission from [4]. Copyright 2015, AIP Publishing LLC.