Fluorescence correlation spectroscopy as a tool to investigate the protonation dynamics of cytochrome c oxidase

A. Wolf, *C. Schneider, K. Kirchberg, T.- Y. Kim, H. Michel, U. Alexiev[1]

Freie Universität, Physics, Berlin, Germany [2]Max-Planck-Institut für Biophysik, Frankfurt am Main, Germany

Fluorescence correlation spectroscopy (FCS) is a single-molecule based technique to temporally resolve rate-dependent processes by correlating the fluorescence fluctuations of individual molecules traversing through a confocal volume. These fluctuations can arise from diffusion or chemical reactions such as protonation.
FCS therefore provides an excellent tool for protonation dynamics investigations of the redox-coupled proton pump cytochrome c oxidase (CcO) (1). To achieve this, the pH-dependent fluorescent dye fluorescein was used as a protonation sensor and covalently attached to the surface of a K299C CcO mutant via site-specific labeling (2). The labeling site is located close to the entrance of the K pathway. Local proton association and dissociation rates can therefore yield information about protein environmental effects on the transport properties of proton transfer channels. The analysis of the FCS data of CS-K299C-AF at different pH showed an increase in the apparent second order rate constant of the reporter group at pH values above 7.5 indicating a “proton collecting antenna” like behavior.
For a better understanding of the experimental data, a random-walk based single-particle simulations program was developed in the group that is able to reproduce the experimental FCS data. It also provided information about the overlap of different rates due to chemical reactions (e.g. protonation, photooxidation), photochemical destruction, intersystem crossing and can further be used to improve the accuracy of future data analytics.

References:

[1] Kirchberg K, Michel H, Alexiev U. Net proton uptake is preceded by multiple proton transfer steps upon electron injection into cytochrome c oxidase. J Biol Chem. 2012;287(11):8187-93
[2] Kirchberg K, Michel H, Alexiev U. Exploring the entrance of proton pathways in cytochrome c oxidase from Paracoccus denitrificans: surface charge, buffer capacity and redox-dependent polarity changes at the internal surface. Biochim Biophys Acta. 2013;1827(3):276-84