Orthogonal translation in *Paracoccus denitrificans* yielding the membrane protein cytochrome c oxidase with noncanonical amino acids and application to the investigation of respiratory energy production

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The amino-acyl-tRNA synthetase from *Methanocaldococcus jannaschii* together with a corresponding suppressor tRNA with the amber-anticodon (MjAARS/MjtRNACUA) has become a successful tool for the site-specific incorporation of non-canonical amino acids (ncAAs). While this method is mainly used in E. coli we transferred it to soil bacterium *P. denitrificans* to investigate the protonation dynamics of cytochrome c oxidase (CcO), i.e. complex IV of the respiratory chain exhibiting great homology to human mitochondrial complex IV, and which cannot be expressed heterologeously in E. coli. By employing ncAAs, we aim in a first step to expand our site-specific fluorescence tools with the means to attach probes by click chemistry. In the past, we have successfully used mutagenesis together with site-directed labeling to investigate the protonation rates of the enzyme at the surface of the K-channel using simulation-guided fluorescence correlation spectroscopy [1,2,3]. As a next step, we intend to create functional ncAA containing mutants to directly manipulate the protonation dynamics of CcO with halogenated analogues and by this the enzymatic turnover rate of the enzyme. This will allow us to investigate the electroncoupled proton transport and its determinants in CcO with minimal changes to the membrane protein.

[1] Kirchberg K., Michel H., Alexiev U. (2012) J. Biol. Chem. 287, 8187-93 [2] Kirchberg K., Michel H., Alexiev U. (2013) Biochim. Biophys. Acta 1827, 276-84 [3] Wolf A., et al. (2016) Phys. Chem. Chem. Phys. 18, 12877-85