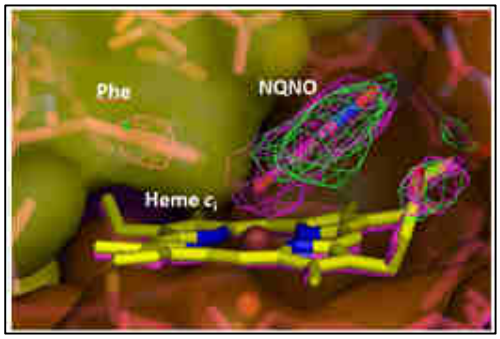
Revealing the ligand environment of heme c_i by FTIR spectroscopy thereby addressing one of the remaining mysteries of photosynthesis

Cytochrome b_6f complexes are integrated in the photosynthetic electron transfer chain where they shuttle electrons from plastoquinols to plastocyanin. Cytochrome bc_1 complexes fulfill the same role in the respiratory chain by oxidizing ubiquinols and reducing cytochrome *c*. Both enzymes transfer furthermore one electron from quinol oxidation to a quinone on the opposite site of the membrane and thus contribute to the build-up of a proton motif force. Their reaction mechanism is globally well characterized and highly similar between the two enzymes to the exception of their quinone reducing site. At variance with bc_1 complexes, b_6f complexes feature a heme in this site, named heme c_i. It may ligate the quinone, store an electron before reducing the quinone or accept electrons from the stroma, contributing to the ill-characterized cyclic electron transfer from ferredoxin into the quinone-pool. None of these putative roles has been experimentally proven so far. In this internship one of the last remaining big puzzles of photosynthesis will be addressed by spectroscopy and electrochemistry. The His-tagged b_6f complex from *Chamydomonas reinhardtii* will be purified and investigated by spectro-electrochemistry and FTIR spectroscopy: redox-induced FTIR-difference spectroscopy reflects changes in the immediate protein environment and the ligands of the heme during redox transitions and therefore holds information on the reaction mechanism of this enigmatic heme. It has never been applied to b_6f complexes before.



Quinone-reducing site of the b_6f complex from *Chlamydomonas reinhardtii* with heme c_i at the bottom of the cavity. This heme is unique in Nature having no axial protein ligand. A quinone has not yet been co-crystalized in the site but the inhibitor NQNO, a quinone analog, binds as a ligand to the heme iron.

The internship will take place in Marseille in the BIP laboratory at the Institute of Microbiology of the Mediterranean. Frauke Baymann will supervise enzyme purification and the spectro-electrochemical approach. FTIR spectra will be recorded at the nearby Campus of Luminy at the BIAM, supervised by Rainer Hienerwadel, an expert in the field of Infrared spectroscopy.

A basic knowledge in biochemistry and physico-chemistry and interest in bioenergetics and spectroscopy are requested.

Contact: baymann@imm.cnrs.fr

Relevant Literature

Baymann, F., Giusti, F., Picot, D., Nitschke, W. (2007) The c_i/b_H moiety in the b_6f complex studied by EPR: A pair of strongly interacting hemes. *PNAS* 104, 519-524.

Stroebel D, Choquet Y, Popot JL, Picot D. (2003) An atypical haem in the cytochrome b_6f complex. *Nature* 426, 413-418.

Zuccarello L, Berthomieu C, Boussac A, Brubach J-B, Díaz-Moreno I, Díaz Quintana AJ, Hienerwadel R (2020) Protonation of the Cysteine axial ligand investigated in His/Cys *c*-type cytochrome by UV-Vis, Mid- and Far-IR spectroscopy *J Phys Chem Lett*. 11, 4198-4205.