Metal homeostasis in Chlamydomonas chloroplasts

Anna Caccamo^{a,b,c,d}, Félix Vega de Luna^a, Khadija Wahni^{b,c,d}, Jonathan Przybyla-Toscano^{a,c}, Sébastien Pyr dit Ruys^{f,g}, Agnieszka E. Misztak^a, Alexandre Kriznik^h, Alexander N. Volkov^{b,i}, Pierre Cardol^a, Nicolas Rouhier^j, Didier Vertommen^f, Joris Messens^{b,c,d}, <u>Claire Remacle^a</u>

^a: Botanical Institute, Genetics and physiology of microalgae, InBios/Phytosystems Research Unit, University of Liege, 4000, Liège, Belgium.

b: VIB-VUB Center for Structural Biology, 1050 Brussels, Belgium c: Brussels Center for Redox Biology, 1050 Brussels, Belgium

d: Structural Biology Brussels, Vrije Universiteit Brussel, 1050 Brussels, Belgium

e: present address: Univ. Grenoble Alpes, CEA, INRAE, CNRS, IRIG, LPCV, 38000 Grenoble, France

f: SSS/DDUV/PHOS, de Duve Institute and MASSPROT platform, UCLouvain, 1200 Brussels, Belgium

g: SSS/LDRI/PMGK, Faculty of Pharmacy and Louvain Drug Research Institute, UCLouvain, 1200 Brussels, Belgium
h: Université de Lorraine, CNRS, IMoPA and IBSLor, F-54000 Nancy, France

i: Jean Jeener NMR Centre, Vrije Universiteit Brussel (VUB), 1050 Brussels, Belgium

^j: Université de Lorraine, INRAE, IAM, F-54000 Nancy, France

The function of ascorbate peroxidase-related (APX-R) proteins, found in all green photosynthetic eukaryotes, remains unknown. This research focuses on Chlamydomonas ascorbate peroxidase 2 (APX2) protein, which belongs to the APX-R protein family. We found that this enzyme does not rely on ascorbate as electron donor for peroxidase activity and resides in the chloroplasts, featuring a TAT-motif signal for translocation to the lumen. *apx2* mutants displayed a low plastocyanin abundance which resulted in a faster P700 oxidation of photosystem I. *In vitro* experiments showed that recombinant APX2 binds copper in addition to heme. We also demonstrated that APX2 interacts with the Cu-binding site of plastocyanin using ¹H-NMR experiments and AlphaFold2 structural prediction. In conclusion, we propose a dual role for APX2: participating in the transfer of copper to plastocyanin and regulating the intracellular copper distribution.