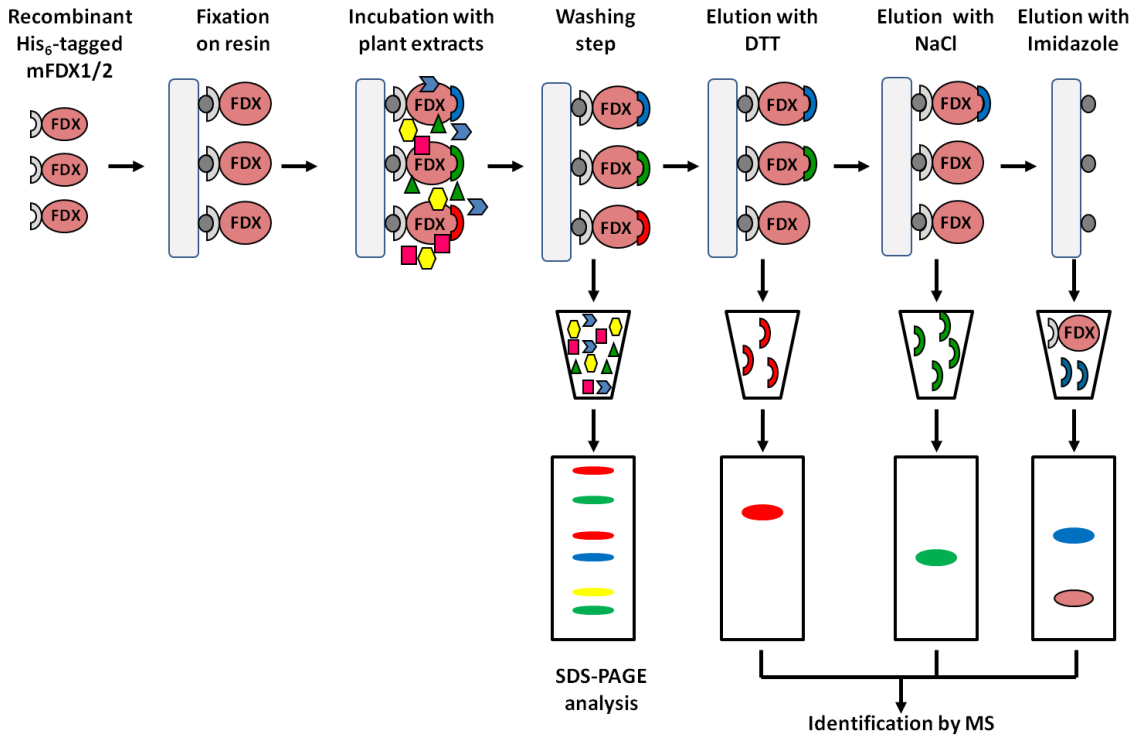
# FERPAR




## Identification of Plant Ferredoxin Protein Partners

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**Context** — Ferredoxins (FDXs) are iron-sulfur (Fe-S) proteins located at a branch point of organellar metabolism. In chloroplasts, they distribute electrons to metabolic processes as carbon, sulfur and nitrogen assimilation. In mitochondria, FDXs participate to the maturation of Fe-S proteins and to coenzyme Q or steroid biosynthesis. FDXs recognize their partners by forming electrostastic interactions. This property was exploited for fishing FDX partners by affinity chromatography. However, these experiments have been performed at a time where proteomic facilities were not as developed as now. As FDXs are hubs for many aspects of plant physiology and development, understanding their functions is a pre-requisite before thinking to engineer plants with better agronomical yield or improved stress tolerance.

**Objectives** — This project aimed at identifying new protein partners of the two mitochondrial FDXs (mFDXs) for which such study has never been done.

**Approach** — This project proposed to combine the FDX trapping approach with more sensitive proteomic technologies that will allow to identify protein partners present in minor amounts and that were not previously identified. FDX-target interactions are mainly based on electrostatic contacts, which property may be used to isolate and identify their protein partners, before further studying protein-protein interactions in detail. Potential interacting partners have been isolated by pull down approaches from soluble protein extracts of *Arabidopsis thaliana* leaves (enriched or not in mitochondria) and of *Solanum tuberosum* tuber using recombinant his-tagged mFDX1 and mFDX2 immobilized on IMAC (Immobilized Metal Affinity Chromatography) resin. For some proteins of

interest, yeast two hybrid assays were performed to confirm their *in vivo* interaction with mFDX1 and mFDX2.

**Key results** —

- Whatever the protein extract used for pull-down experiments, the protein profiles obtained after elution differed according to mFDX isoform, indicating that our approach was adequate for identifying potential specific partners. It has been then confirmed by mass spectrometry analysis revealing 513 common putative partners and 334 and 266 specific partners for mFDX1 and mFDX2, respectively.
- For both mFDX isoforms, using mitochondria-enriched extracts allowed to reduce the number of non-mitochondrial targets, to increase the number and proportion of mitochondrial ones and to identify additional partners.
- Proteins known to be involved in Fe-S protein maturation in mitochondria were isolated as mFDX partners.
- In yeast two hybrid assays, when expressed alone, mFDXs show strong auto-activation preventing the observation of their interaction.

**Main conclusions including key points of discussion** — Besides the presence of 513 common putative partners and despite the relative high sequence identity (86% between mFDX1 and mFDX2), it is interesting to note that our approach has identified 334 and 266 specific partners for mFDX1 and mFDX2, respectively. These results suggest that mFDX1 and FDX2 may have non-redundant functions. In addition, protocol aiming at obtaining protein extracts enriched in mitochondrial proteins was successful despite the presence of residual chloroplastic proteins.

**Future perspectives** — The number of isolated putative partners of mFDXs remains quite high. Hence an in depth analysis is now necessary to identify specific partners for either mFDX1 or mFDX2. A particular attention will be paid on proteins possessing the ability to transfer electrons or necessitating them for their function. In addition, the presence of plastidial proteins as putative partners of mitochondrial FDXs is not physiologically relevant but it may represent putative partners for chloroplastic FDXs. In this case the comparison between our analysis and those performed with plastidial FDXs may enable to identify new partners of FDXs in chloroplasts.
From this project, we have isolated some proteins known to be involved in Fe-S protein maturation in mitochondria. As yeast two hybrid assays method seems unappropriate, these interactions will be validated by other *in vivo* approaches such as BiFC or co-immunoprecipitation. This method necessitates specific antibodies that could be generated from recombinant proteins during this project. Furthermore, producing recombinant proteins corresponding to FDX partners will enable to explore the molecular details of protein interactions. Indeed, given the essential role of Fe-S proteins in living cells, understanding the mechanisms underlying their biogenesis remains an important topic in plant biology and includes the study of FDX roles.

**Valorisation** —

Hego E, Przybyla-Toscano J, Roret T, Couturier J, Rouhier N. Identification of mitochondrial ferredoxin partners from *Arabidospis thaliana* leaves. 18th International Symposium on Iron Nutrition and Interaction in Plants (Madrid, Espagne, 30 mai-3 juin 2016).