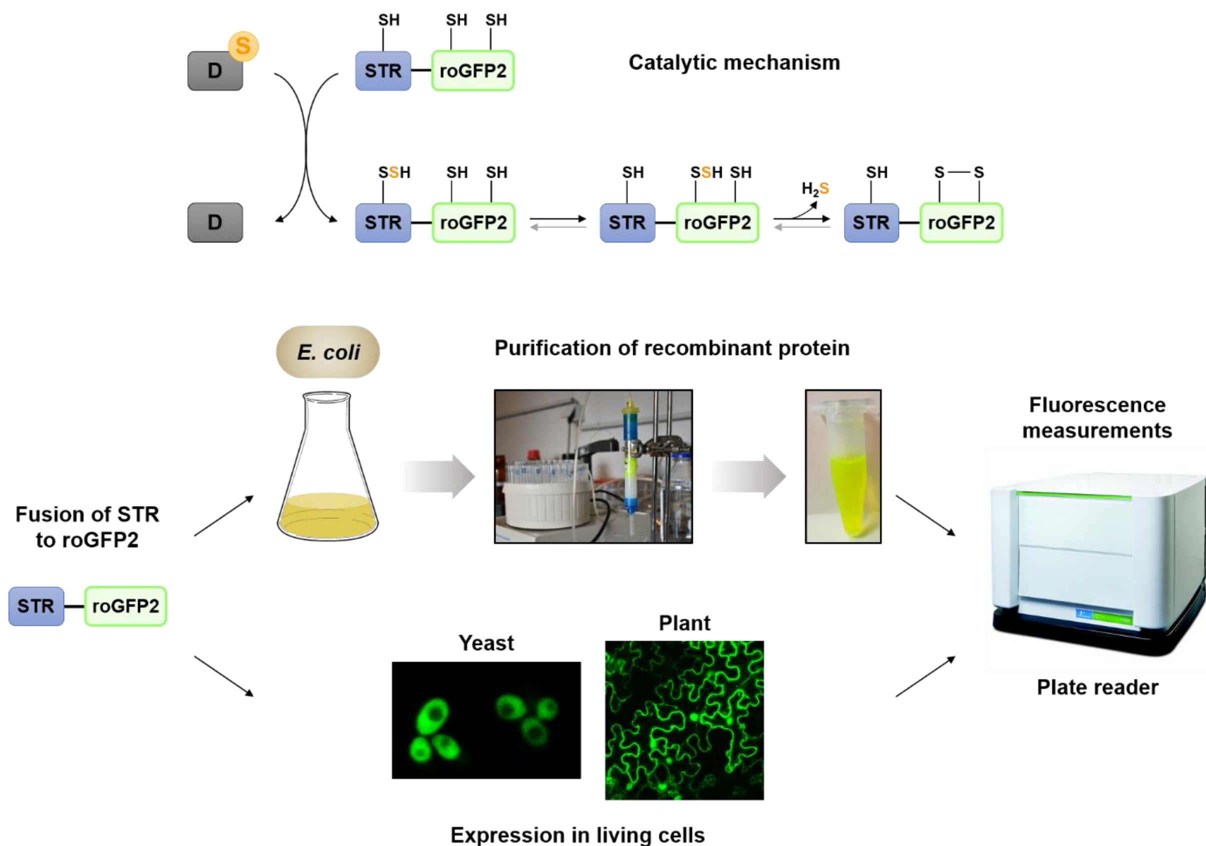
SULPRO



Developing new optogenetic redox biosensors for sulfur compounds

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Context — In recent years, the development of fluorescent probes has revolutionized our experimental access to physiological parameters in live cells. To further our understanding of cellular physiology, there is an increasing demand to develop a variety of new sensors and/or to optimize or further validate existing ones for real-time live-cell imaging. Intracellular concentrations of cysteine are highly controlled to conciliate its potential toxic effect and its role as a major sulfur donor molecule in cells. For instance, cysteine degradation in mitochondria results in the production of 3-mercaptopyruvate (3-MP) and thiosulfate. Sulfurtransferases (STRs) constitute a large and complex protein family characterized by the presence of a rhodanese domain and implicated in diverse molecular and signalling processes as sulfur carriers. They are capable of transferring a sulfur atom in form of a persulfide (-SSH) from suitable sulfur donors to nucleophilic sulfur acceptors. From a biochemical point of view, two STR groups referred to as thiosulfate-STRs (TSTs) and 3-MP-STRs (MSTs), can be distinguished according to their ability to use these compounds as sulfur donor in *in vitro* assays. Sulfur compounds are essential for life. Their metabolic fluxes are so far only understood in part and development of probes for detecting sulfur compounds is of tremendous importance for further progress in this field.

Objectives — This project aimed at engineering new-redox-based GFP probes specific to 3MP and thiosulfate and at assessing the functionality of newly created fusion proteins in cells.

Approach — This project has been conceived based on preliminary experiments demonstrating that some STR isoforms catalyze the oxidation of roGFP2 in the presence of 3-MP and thiosulfate, thus opening up the possibility to engineer new fluorescent biosensors to study the cysteine metabolism in living cells. The theoretical reaction mechanism implies that after addition of the sulfur donor the STR is firstly persulfidated. In the second step the persulfide group is transferred to roGFP2 (persulfidation of roGFP2) and in the third step a disulfide bond is formed with the concomitant release of H_2S. Arabidopsis MST isoform, STR1, and both TST isoforms, STR16 and STR18, were fused to the N-terminus of roGFP2, expressed in *E. coli* and purified. Changes in fluorescence of the STR:roGFP2 fusion proteins were analysed with a plate reader. Furthermore, STR:roGFP2 fusion proteins have been tested *in vivo* by expressing them in yeast and plant cells.

Key results —

- The fusion of the STRs to roGFP2 does not influence the biochemical properties of roGFP2.
- STR:roGFP2 fusion proteins are functional *in vitro* and the oxidation of roGFP2 is reversible.
- STR:roGFP2 fusion proteins are specific to their respective substrate.
- *B*oth glutathione (GSH) and the thioredoxin (TRX) systems can affect the persulfidation of STR:roGFP2 *in vitro*.
- Only GSH system affects the persulfidation of STR-roGFP2 *in vivo* in yeast cells.
- Cytosolic expressed STR:roGFP2 in yeast are not sensitive to external application of respective substrate.

Main conclusions including key points of discussion — The STR1, STR16 and STR18:roGFP2 fusion proteins are functional and can use specifically their respective substrate (3-MP and thiosulfate) to oxidize roGFP2 *in vitro*. In yeast cells, no changes in fluorescence of STR:roGFP2 fusion proteins have been observed when the probes were expressed in cytosol and the cells cultivated in standard conditions. Only, the addition of digitonin, a mild detergent for solubilising the yeast membrane, led to an oxidation of the STR:roGFP2 sensor. This lack of response might due to the absence of respective 3-MP and thiosulfate in the cytosol of yeast and/or the cross-reactions with reducing systems as suggested by our *in vitro* experiments in the presence of both GSH and TRX systems. *In vivo* the competition between other reducing systems such as the TRX system or other sulfur acceptors like GSH might prevent the efficient transpersulfidation reaction between STR and roGFP2 domains and thus the changes of fluorescence associated to the oxidation of roGFP2.

Future perspectives —

- To express the biosensors in other subcellular compartments like mitochondria and chloroplasts.
- To optimize the biosensors to be less sensitive to the reducing systems and other sulfur acceptors.
- To optimize the biosensors to be more sensitive to the respective substrate (3-MP and thiosulfate).
- To analyze Arabidopsis mutants for genes encoding components of the cysteine metabolism, GSH- and thioredoxin-reducing pathways, or by specifically applying stress conditions or varying the source of nutrients.

Valorization —

Ces travaux ont fait l'objet d'une présentation sous la forme d'un poster, lors du congrès « SPP1710 Conference Thiol-based switches and redox regulation – from microbes to men» qui s'est déroulé à Sant Feliu du 15 au 20 septembre 2019.

Moseler A, Morgan B, Rouhier N, **Couturier J**. Developing redox biosensors for sulfur compounds. SPP1710 Conference Thiol-based switches and redox regulation – from microbes to men Sant Feliu de Guixols, Espagne, 15-20 Septembre 2019).