A novel red fluorescent redox sensor with a highly negative midpoint potential for live cell imaging

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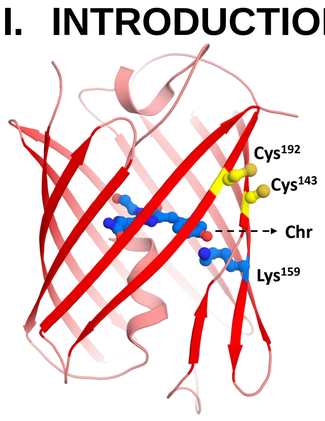
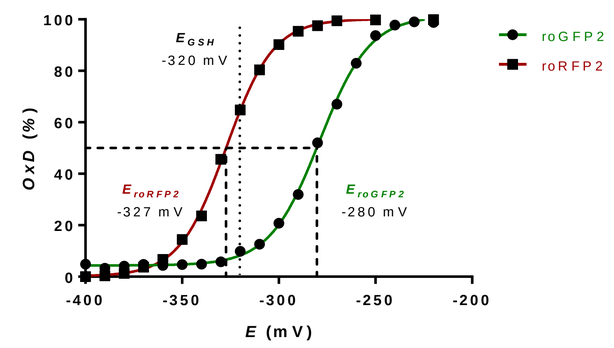
I. INTRODUCTION

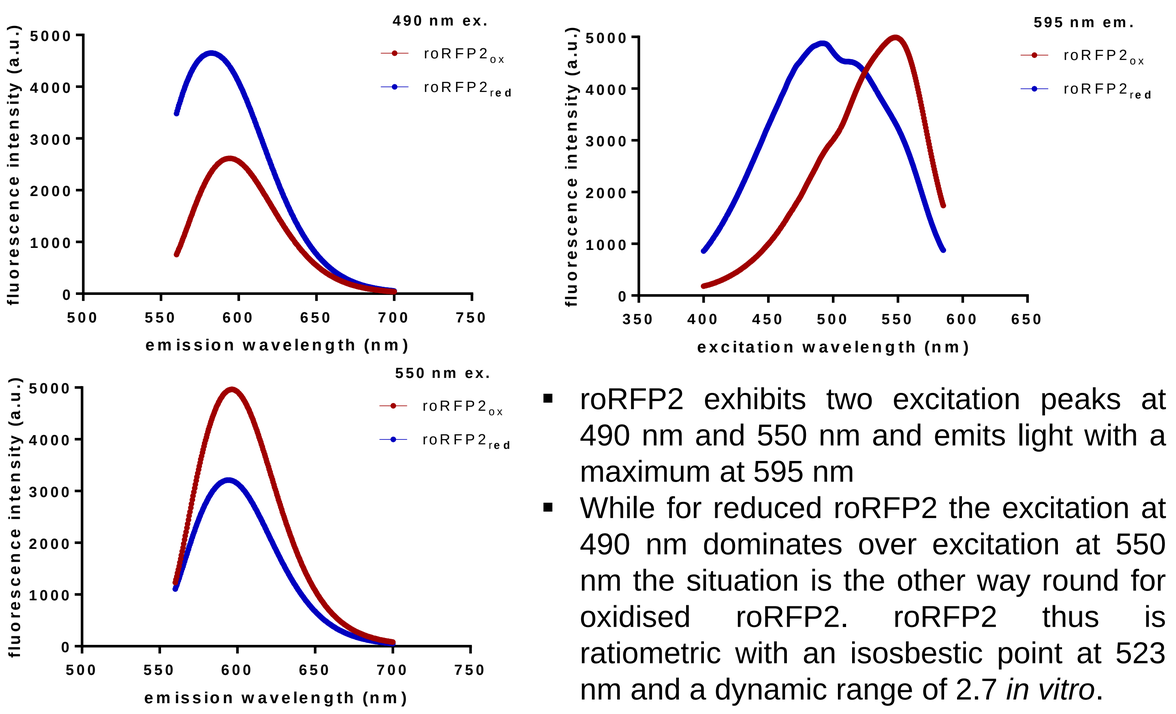


Redox-sensitive fluorescent proteins are excellent tools for monitoring cellular redox changes *in vivo* (Schwarzländer *et al*., 2015). The well-characterized sensor roGFP2 allows the reliable read-out of oxidations in the cytosolic glutathione redox potential (E_{GSH}) (Meyer *et al*., 2007). However, with a midpoint potential of -280 mV, the sensor is very limited at resolving changes in E_{GSH} to a more reduced state, since roGFP2 is almost fully reduced under normal conditions. Thus, alternative sensors with more negative midpoint potentials are required. The redox sensor roRFP2, derived from the red fluorescent protein mKeima originally discovered in the stony coral *Montipora* sp., obtains its redox sensitivity through two genetically engineered cysteine residues close to its chromophore (Koon *et al*., 2011). With a redox potential of -327 mV, roRFP2 might be a suitable candidate for dynamic measurements of the cytosolic E_{GSH} *in vivo*.



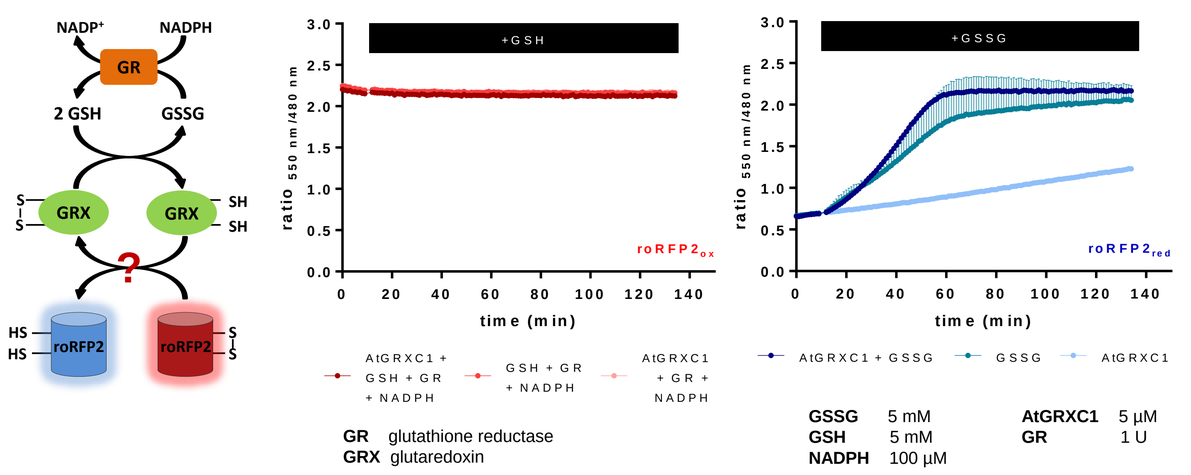
II. RESULTS

1. roRFP2 is a ratiometric biosensor



- roRFP2 exhibits two excitation peaks at 490 nm and 550 nm and emits light with a maximum at 595 nm
- While for reduced roRFP2 the excitation at 490 nm dominates over excitation at 550 nm the situation is the other way round for oxidised roRFP2. roRFP2 thus is ratiometric with an isosbestic point at 523 nm and a dynamic range of 2.7 *in vitro*.

2. *In vitro* roRFP2 interacts with the GR/GSH/GRX system

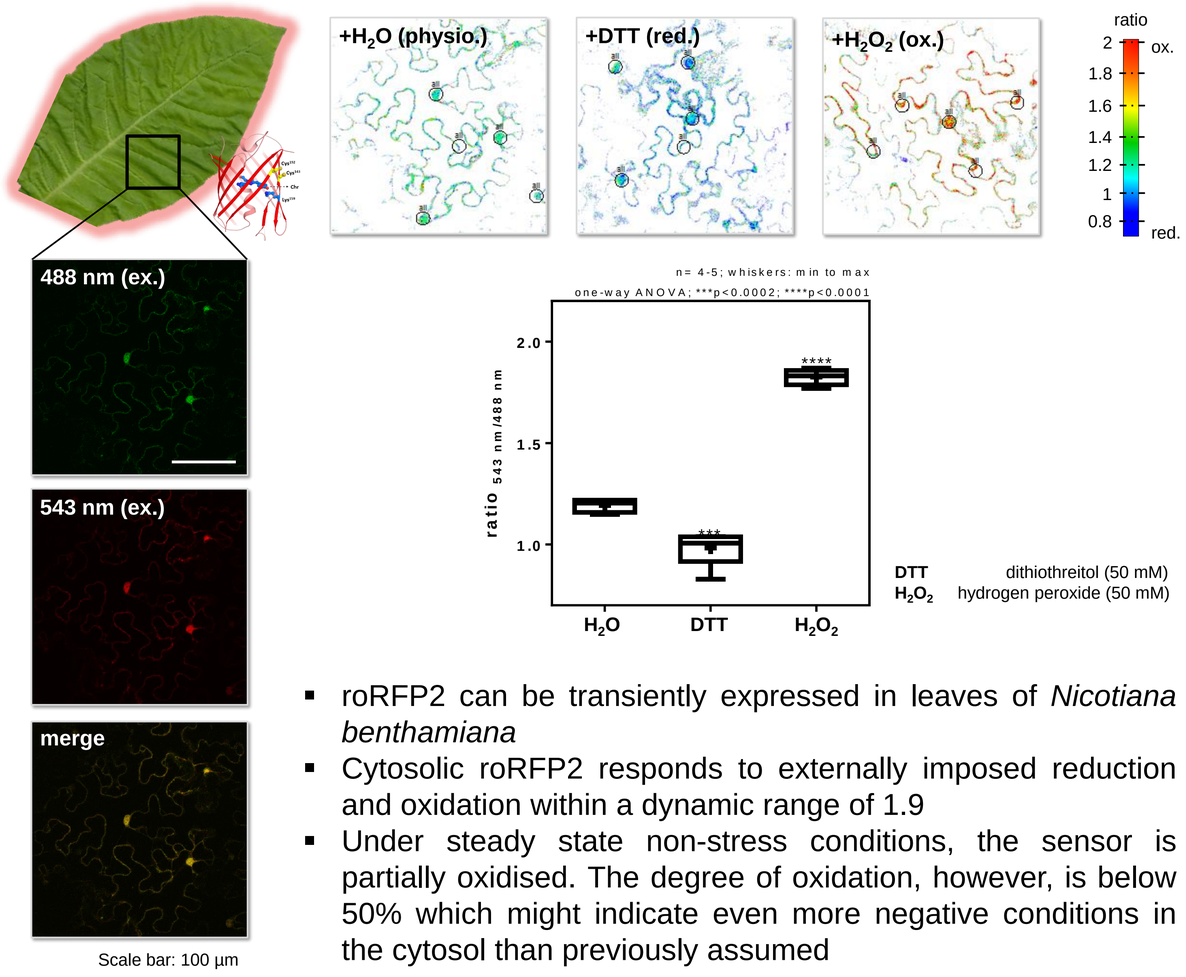


GR glutathione reductase
GRX glutaredoxin

GSSG	5 mM	**AtGRXC1**	5 µM
GSH	5 mM	**GR**	1 U
NADPH	100 µM		

- Oxidized roRFP2 can not be reduced by GSH even in the presence of glutathione reductase and NADPH for reduction of residual amounts of GSSG. Lack of reduction might be due to insufficient reducing power of GSH for the highly negative roGFP2in a GRXC1-mediated reaction with GSH
- However, pre-reduced roRFP2 can be easily oxidized by GSSG in a reaction that is further accelerated through the addition of GRXC1

3. roRFP2 transiently expressed in tobacco leaves responds ratiometrically to externally imposed reduction and oxidation

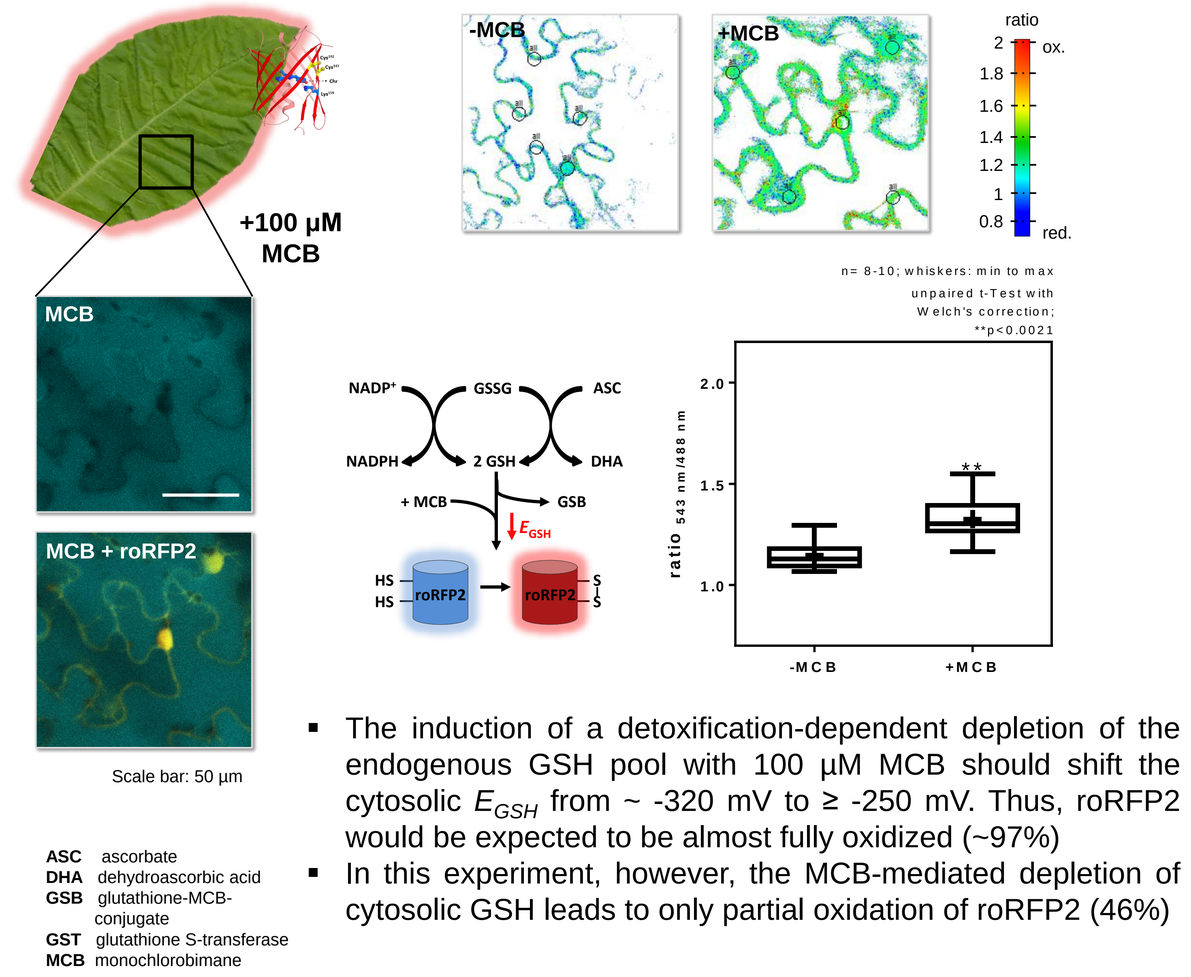


Scale bar: 100 µm

DTT dithiothreitol (50 mM)
H_2O_2 hydrogen peroxide (50 mM)

- roRFP2 can be transiently expressed in leaves of *Nicotiana benthamiana*
- Cytosolic roRFP2 responds to externally imposed reduction and oxidation within a dynamic range of 1.9
- Under steady state non-stress conditions, the sensor is partially oxidised. The degree of oxidation, however, is below 50% which might indicate even more negative conditions in the cytosol than previously assumed

4. Depletion assay reveals interaction of roRFP2 with GSH *in planta*



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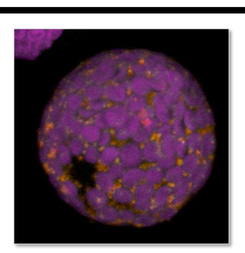
ASC ascorbate
DHA dehydroascorbic acid
GSB glutathione-MCB-conjugate
GST glutathione S-transferase
MCB monochlorobimane

- The induction of a detoxification-dependent depletion of the endogenous GSH pool with 100 µM MCB should shift the cytosolic E_{GSH} from ~ -320 mV to ≥ -250 mV. Thus, roRFP2 would be expected to be almost fully oxidized (~97%)
- In this experiment, however, the MCB-mediated depletion of cytosolic GSH leads to only partial oxidation of roRFP2 (46%)

III. CONCLUSIONS & OUTLOOK

- roRFP2 has the potential to be applied as alternative biosensor for dynamic measurements of the E_{GSH} in living cells
- roRFP2 provides the opportunity to perform multichromatic imaging experiments in combination with green fluorescent probes
- The applicability in plants needs to be further verified since the generation of stable Arabidopsis transformants was so far not successful

Maximum projection of *A. thaliana* protoplast transfected with roRFP2. Magenta: chloroplasts, orange: merged fluorescent signal derived from cytosolic roRFP2 excited at 488 and 543 nm.



IV. REFERENCES

Koon, N., Yei, S.M., Risenmay, A.J., Kallio, K., Remington, S.J. & Magpiong, I. (2011) Developing a redox-sensitive red fluorescent protein biosensor. *Journal of Biomolecular Techniques,* **22** (Suppl), S52.

Meyer, A.J., Brach, T., Marty, L., Kreye, S., Rouhier, N., Jacquot, J.P. & Hell, R. (2007) Redox-sensitive GFP in Arabidopsis thaliana is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. *Plant Journal*, **52**(5), 973-986.

Schwarzländer, M., Dick, T.P., Meyer, A.J. & Morgan, B. (2015) Dissecting redox biology using fluorescent protein sensors. *Antioxidants & Redox Signaling,* **24** (13), 680-712.

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