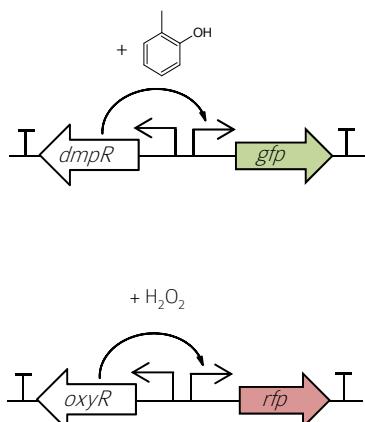


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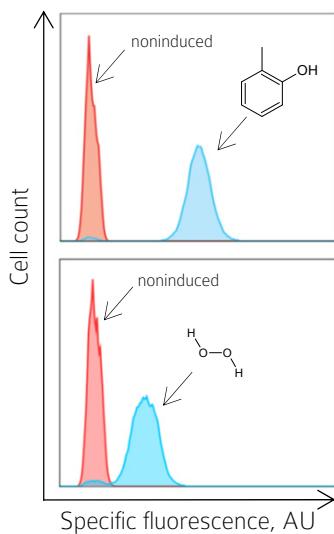
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GENETIC CIRCUITS FOR DETECTION AND QUANTIFICATION OF PHENOLS AND H₂O₂

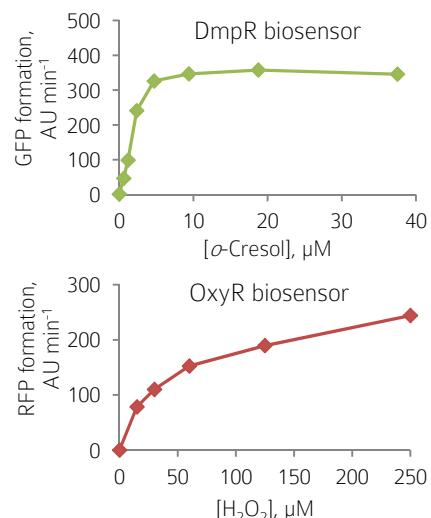
Circuit architecture



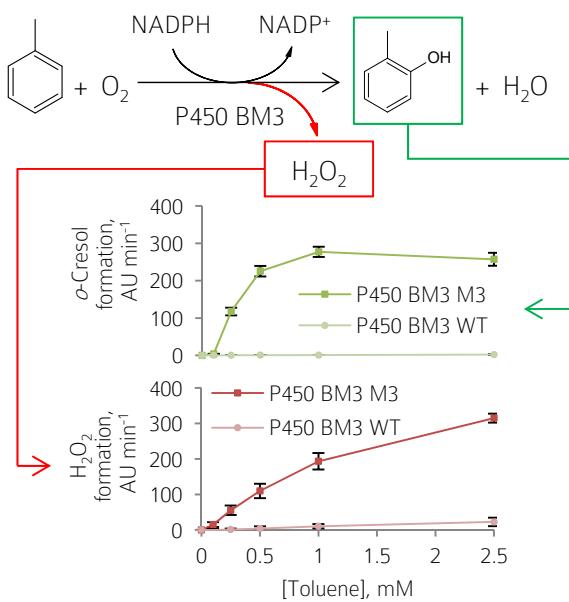
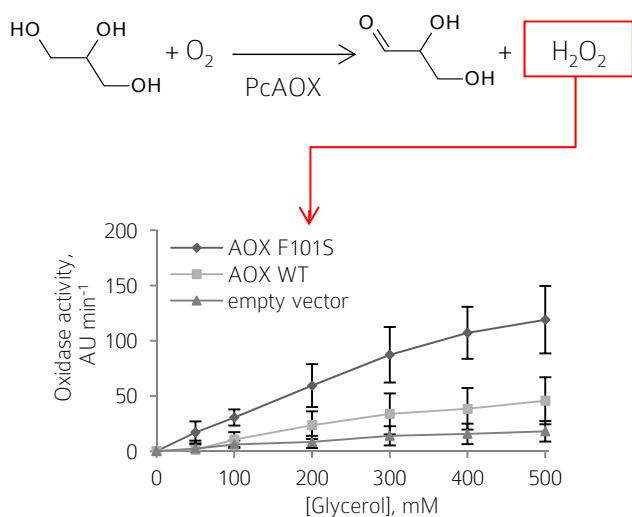
Flow cytometric analysis



Dose response curves



QUANTIFICATION OF OXIDATIVE ENZYMATICAL REACTIONS INSIDE LIVING CELLS

Simultaneous detection of the hydroxylation product and H₂O₂ generated by a P450 monooxygenaseMonitoring of oxidase activity using OxyR biosensor for H₂O₂*In vitro* characterization of toluene hydroxylases

P450	NADPH Ox. ¹ (μmol _{cat} μmol _{P450} ⁻¹ min ⁻¹)	iTOF ² (μmol _{prod} μmol _{P450} ⁻¹ min ⁻¹)	Coupling (%)
WT	24 ± 1	n.d.	10 %
M3	255 ± 89	140	50 %

¹ NADPH depletion monitored via absorbance at 340 nm² Product formation via gas chromatographySteady-state kinetic parameters^a for Pcaox

Pcaox	K _M (mM)	k _{cat} (s ⁻¹)	K _M /k _{cat} (M ⁻¹ s ⁻¹)
WT	n.d.	0.2 ^b	n.d.
F101S	580	3	5

^a Values obtained using the HRP-coupled assay in 50 mM potassium phosphate, pH 7.5^b k_{obs} at 2 M substrate

REFERENCE:

- Shingler, V. & Moore, T., (1994) J. Bacteriol. 176, 1555–1560.
 Jones, R.M. & Williams, P.A., (2001) J. Bacteriol. 183, 405–409.
 González-Flecha, B. & Demple, B., (1999) J. Bacteriol. 181, 3833–3836.
 Dennig, A. et al., (2013) Angew. Chem. Int. Ed. 52, 8459–8462.
 Wong, T.S., et al., (2005) J. Biol. Screen., 10(3).
 Nguyen Q.T. et al., (2018) Biochemistry 57(43), 6209–6218

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 More information about H2020 ROBOX @ <https://h2020robox.eu/>