Title	A Novel Fluorescent Sensor Protein for Visualization of Redox States in the Cytoplasm and in Peroxisomes.
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Citation	Molecular and cellular biology (2010), 30(15): 3758-3766
Issue Date	2010-08
URL	http://hdl.handle.net/2433/120332
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Type	Journal Article
Textversion	author

Online Supplemental materials

Fig. S1. The redox response of Redoxfluor in vitro. Effect of pH upon the FRET ratio of Redoxfluor. Circles, reduced probes; squares, oxidized probes; red, C-probe; blue, A-probe.

Fig. S2. Visualization of the redox state in *P. pastoris*. The C-probe responds to various oxidants in *P. pastoris*. Bar, 2 μm.

Fig. S3. Redoxfluor response in the presence of cycloheximide. The wild-type CHO-K1 strain used in Fig. 3B was pre-treated with 20 μg/ml cycloheximide for 1 h, incubated in medium containing 200 μM H_2O_2 and the same concentration of cycloheximide for 20 min, and transferred to cycloheximide-containing medium without H_2O_2 (H_2O_2-washout) for the indicated periods. The cycloheximide treatment alone exerted an oxidizing effect upon the redox state, but the H_2O_2-washout increased the FRET ratio showing the reversibility of Redoxfluor response. Bar, 10 μm.

Fig. S4. Biochemical assessment of the redox state in *pex5* cells using mPEG-maleimide. Cell lysate from wild-type (CHO-K1) or *pex5* (ZP105) cells expressing cytosolic Redoxfluor (C-probe or A-probe) was incubated with mPEG-maleimide, and subjected to immunoblot analysis. The molecular-weight distributions of the probe proteins are slightly greater in the lysate from the *pex* cells. The arrow indicates non-modified probe proteins and the asterisks show the modified forms of the protein.

Fig. S5. Detection of accumulated ROS by 2', 7'-dichlorodihydrofluorescein diacetate (DCF). Wild-type CHO-K1 cells exhibited greater levels of intracellularly accumulated ROS at 37°C than ZP105 cells mutant for peroxisome assembly. The values represent the fluorescent intensities of DCF in arbitrary units.

Table S1. Primers used for qRT-PCR

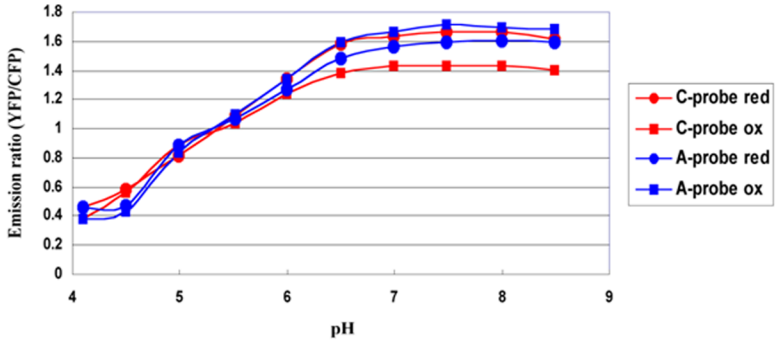
Video 1. The H_2O_2-induced FRET response in CHO-K1 cells expressing the C-probe. Time series speed was 1 frame per minute, and the images were taken for 20 minutes. Using our conventional FRET microscope, an increase in background fluorescence with both A- and C-probes was observed due to the increase of the medium volume of the object upon reagent addition.

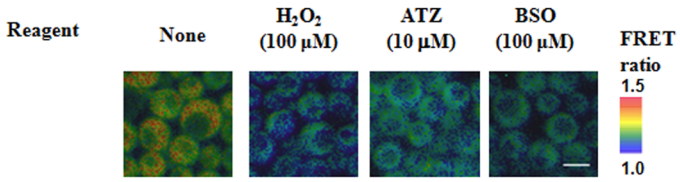
Video 2. The ATZ-induced FRET response in CHO-K1 cells expressing the C-probe. Time series speed was 1 frame per minute, and images were taken for 40 minutes. ATZ was added at 20 minutes.

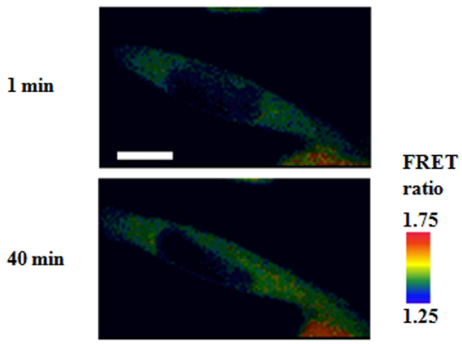
Video 3. The H_2O_2-induced FRET response in CHO-K1 cells expressing the C-probe-PTS1. Time series speed was 1 frame per minute, and images were taken for 40 minutes. H_2O_2 was added at 20 minutes.

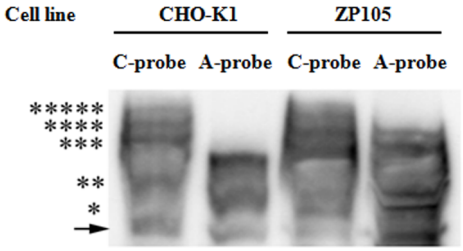
TABLE SI. Primers used in the qRT-PCR

Gene	Primers
GST	5′-TGGAAGGAGGAGGTGGTTACTGTAG-3′
	5′-CCCATCATTCACCATATCCACCAGG-3′
PpCTA1	5′-CGAGTATCCTTCATGGACTTGTTAC-3′
	5′-TCCTCAATGGGAAGTCTTTGTGTGG-3′
PpGPX1	5′-ACCAGTTTGGTCATCAGGAACCAGG-3′
	5′-ACCTTTGAATCCGAGGAGACCAGAC-3′
PpSOD2	5′-AACACACCTAAGGTGATCGAGCTAC-3′
	5′-ACCTGCCAACTTAGAGTTGGTAAGG-3′
PpTSA1	5′-CATTGTTGGCTGACACCAACCACAC-3′
	5′-TCCGACTGGCAGATCGTTGATAGTG-3′


Emission ratio (YFP/CFP)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4
5
6
7
8
9
pH
C-probe red
C-probe ox
A-probe red
A-probe ox


Reagent
None
H2O2
(100 µM)
ATZ
(10 µM)
BSO
(100 µM)
FRET
ratio
1.5
1.0


1 min
40 min
FRET
ratio
1.75
1.25


Cell line
CHO-K1
ZP105
C-probe
A-probe
C-probe
A-probe
*****
****
***
**
*

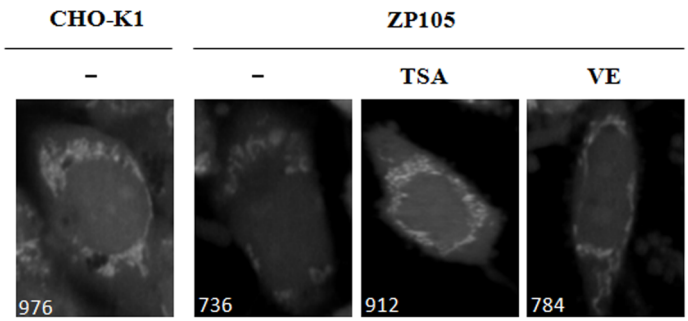

CHO-K1
ZP105
–
–
TSA
VE
976
736
912
784

TABLE SI. Primers used in the qRT-PCR

Gene	Primers
GST	5′-TGGAAGGAGGAGGTGGTTACTGTAG-3′
	5′-CCCATCATTCACCATATCCACCAGG-3′
PpCTA1	5′-CGAGTATCCTTCATGGACTTGTTAC-3′
	5′-TCCTCAATGGGAAGTCTTTGTGTGG-3′
PpGPX1	5′-ACCAGTTTGGTCATCAGGAACCAGG-3′
	5′-ACCTTTGAATCCGAGGAGACCAGAC-3′
PpSOD2	5′-AACACACCTAAGGTGATCGAGCTAC-3′
	5′-ACCTGCCAACTTAGAGTTGGTAAGG-3′
PpTSA1	5′-CATTGTTGGCTGACACCAACCACAC-3′
	5′-TCCGACTGGCAGATCGTTGATAGTG-3′