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Data Article

Fluorescence spectroscopy of roGFP2-based redox probes responding to various physiologically relevant oxidant species *in vitro*



Alexandra Müller^{a,*}, Jannis F. Schneider^a, Adriana Degrossoli^a,
Nataliya Lupilova^a, Tobias P. Dick^b, Lars I. Leichert^a

^a Institute of Biochemistry and Pathobiochemistry – Microbial Biochemistry, Ruhr-University Bochum, 44780 Bochum, Germany

^b Division of Redox Regulation, DKFZ-ZMBH Alliance, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

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ABSTRACT

This article contains representative fluorescence excitation spectra of roGFP2-based probes used for ratiometric analysis of redox changes as presented in the article "Systematic *in vitro* assessment of responses of roGFP2-based probes to physiologically relevant oxidant species" [1]. The recombinant probes roGFP2, roGFP2-Orp1, and Grx1-roGFP2 were exposed to various oxidative and nitrosative species, including hydrogen peroxide (H₂O₂), aldrithiol-2 (AT-2), glutathione disulfide (GSSG), hypochlorous acid (HOCl), S-nitrosoglutathione (GSNO), peroxynitrite (ONOO⁻), potassium polysulfide (K₂S_x), spermine NONOate (SperNO), and diethyl amino NONOate (DeaNO) at different molar ratios. Fluorescence excitation spectra of the probes were recorded in the excitation wavelength range between 350 and 500 nm and for a total of 60 min. Analysis and interpretation of the data is presented in an associated article [1].

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* Corresponding author.

E-mail address: alexandra.mueller-2@ruhr-uni-bochum.de (A. Müller).

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Specifications Table

Subject area	Biology
More specific subject area	Redox Biology
Type of data	figure, raw data file (.csv)
How data was acquired	JASCO FP-8500 fluorescence spectrometer equipped with a Peltier thermo-holder 'EHC-813' (JASCO, Darmstadt, Germany)
Data format	Raw
Experimental factors	Reduction of the probes with DTT
Experimental features	Assessment of changes in the fluorescence excitation characteristics of roGFP2-based probes upon oxidant treatment
Data source location	Bochum, Germany, Latitude 51.4445974, Longitude 7.258836
Data accessibility	Spectral data are displayed in Figs. 1–10. Associated raw data can be accessed as .csv text files in the supplementary data section

Value of the data

- The influence of diverse oxidative and nitrosative species on roGFP2-based probes is compared side-by-side.
- Spectral responses of roGFP2, roGFP2-Orp1 and Grx1-roGFP2 are compared side-by-side
- Full spectra are recorded every minute for 60 min.
- The data delineate probe redox responses under strictly controlled *in vitro* conditions.

1. Data

Each experiment represents a time series of 60 fluorescence excitation spectra with one spectrum recorded per minute. The first spectrum shows the fluorescence excitation spectrum prior to treatment. Following the addition of oxidative or nitrosative species, spectral changes of roGFP2, roGFP2-Orp1, and Grx1-roGFP2 were recorded for a total of 60 min. Autoxidation of roGFP2-Orp1 under aerobic conditions is shown in Fig. 1. Experiments involving roGFP2-Orp1 were thus performed under anaerobic conditions, while measurements with roGFP2 and Grx1-roGFP2 were performed under aerobic conditions. Reference spectra were recorded using 2 μ M of control reductant (DTT or GSH), or 2 μ M of control oxidant (AT-2) (Fig. 2). The specificity of the two fusion probes roGFP2-Orp1 and Grx1-roGFP2 was tested using 2 μ M H₂O₂ and GSSG (Fig. 3). Spectral responses to 2 μ M of various other oxidative and nitrosative species were recorded (Fig. 4). Unfused roGFP2 was treated with increasing concentrations of H₂O₂, HOCl, and ONOO⁻ to determine the minimal oxidant concentration eliciting a spectral response (Figs. 5–7). The response of the three probes to treatment with 100 μ M of the above tested oxidants was recorded (Figs. 8, 9). To rule out artifacts, non-redox-sensitive eGFP was treated with all compounds used in this study (Fig. 10). The raw data for all figures (excitation wavelength vs. fluorescence intensity values) is made available as comma separated value (.csv) text files.

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