Supporting information for:

Prominent Contribution of Hydrogen Peroxide to Intracellular Reactive Oxygen Species Generated upon Exposure to Naphthalene Secondary Organic Aerosols

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Section S1: Design and optimization of catalase assay

Determination of exposure time for catalase treatment of cells. Previous studies have shown that catalase treatment of cells can mitigate the effects of redox insults and effectively reduce ROS/RNS signals.[1-7] However, there are substantial variations in the employed methodologies depending on cell lines used, such as time of treatment with catalase, fluorescent dyes, and stimulants tested. In previous studies,[5-7] cells were pretreated with catalase for a range of 0.5 to 4 h before exposing them to stimulants. However, these studies used specific features to facilitate the intracellular access of catalase, a macromolecule, to cellular and subcellular regions.[7] In this study, to ensure that there was sufficient time for the catalase to interact with and/or be absorbed by the cells, it was added to naphthalene SOA extracts at a specific concentration and incubated for 24 h. Note that H_2O_2 in naphthalene SOA extracts can be decomposed immediately after adding catalase. The chosen time of exposure was based on the stability of catalase for 24 h at 37°C[8, 9] and on the exposure time used in the intracellular ROS/RNS assay.[10]

Optimization of catalase concentration. The optimized catalase concentration was determined by exposing macrophage cells to positive controls (1 μg/mL LPS and 200 μM H_2O_2) with the addition of 0, 50, and 200 U/mL catalase (Sigma-Aldrich, C-3515), which were concentrations generally used in prior studies.[1, 2] As shown in Fig. S1, LPS and H_2O_2 induced a response of 1.5-fold compared to control cells. The addition of 50 and 200 U/mL catalase decreased ($p < 0.1$) the ROS/RNS signal induced by LPS and H_2O_2 to control values. This confirmed that catalase removed the species that mediated the oxidation of the probe compound and that the protocol can be used to identify reactive species that drive ROS/RNS response. The difference in the response after treating the cells with 50 and 200 U/mL catalase was not significant ($p = 0.9$), therefore, the chosen concentration of catalase was 50 U/mL.

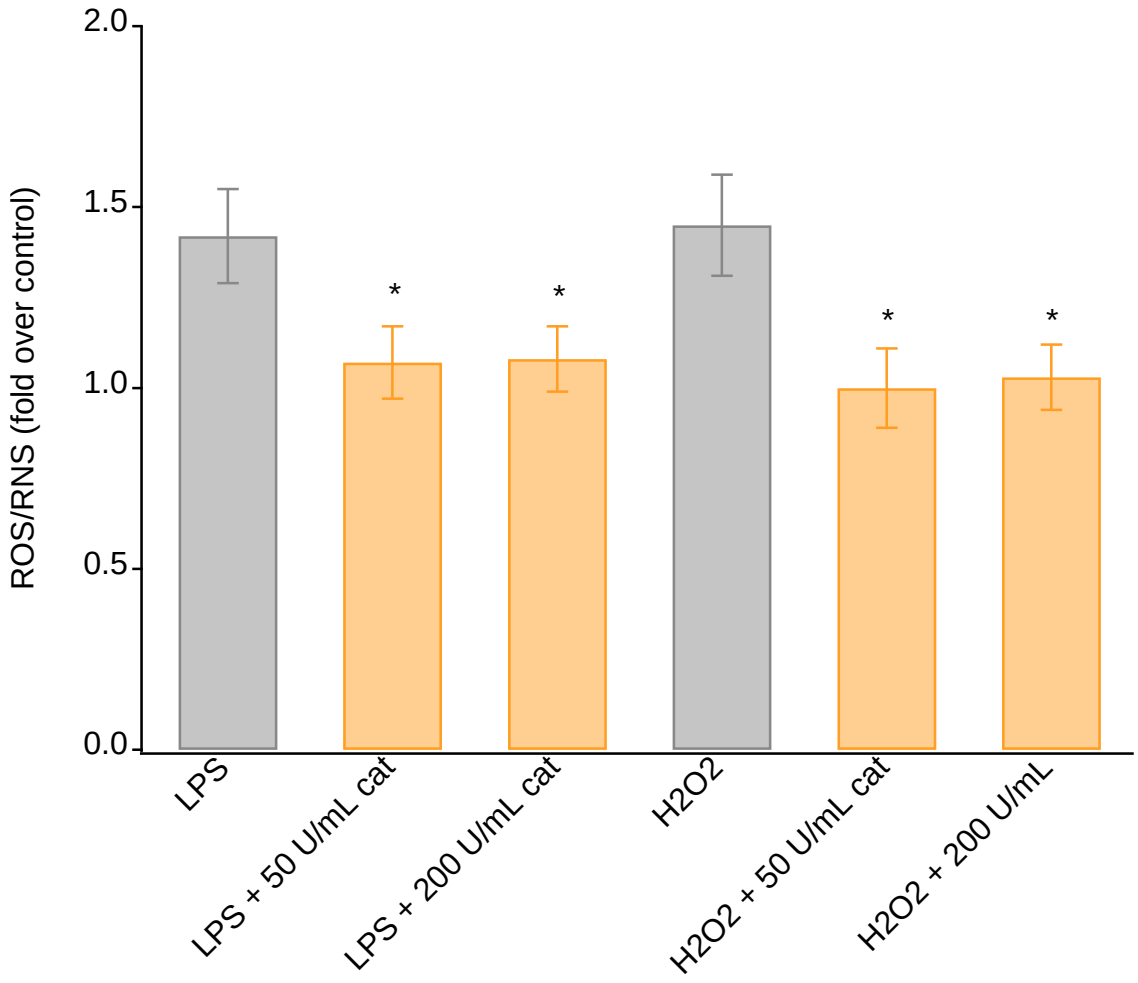

Figure S1. ROS/RNS response of cells exposed to positive controls (1 μg/mL LPS, 200 μM H_2O_2) without (grey) and with (orange) the addition of catalase (50 and 200 U/mL). ROS/RNS was measured using the fluorescent dye carboxy-H_2DCFDA. Values represent the fold of change over control cells. Data are presented as mean ± SE of measurements carried out in triplicate. Statistically significant differences were determined with the *t*-test using a 90% confidence interval. * ($p < 0.1$) indicates significance compared to LPS or H_2O_2 samples without the addition of catalase.

Established catalase assay protocol. The cells were plated and exposed to the probe following steps (1) to (3) of our intracellular ROS/RNS protocol (Fig. S2).[10] Step (4) involved replacing the ROS/RNS probe solution with naphthalene SOA containing 50 U/mL catalase. H_2O_2 (200 μM)

was used as a positive control and unstained cells exposed to probe solution were used as a negative control. Stained cells exposed to supplemented media or catalase (50 U/mL) only were used to correct for the background ROS/RNS signal. After 24 h of incubation, the medium was removed and replaced with phosphate buffer solution (PBS). Lastly, the plate was placed in a microplate reader (BioTek Synergy H4) to measure the fluorescence intensity at excitation of 485 nm and emission of 528 nm.

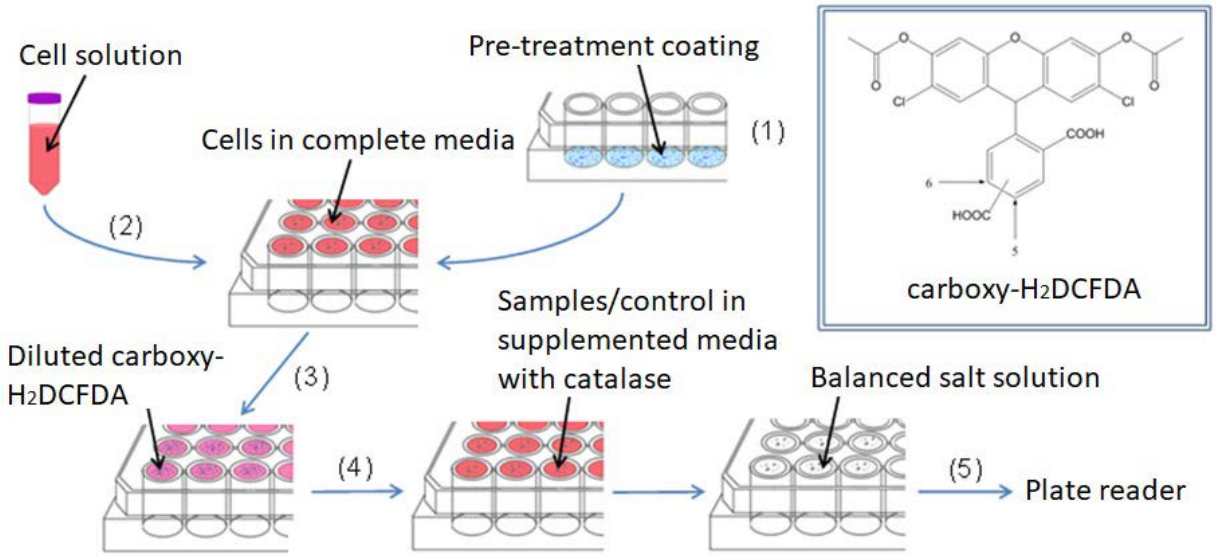

Figure S2. Optimized protocol for catalase assay. Cells were plated and exposed to naphthalene SOA containing 50 U/mL catalase following the methodology described in Tuet et al. [10]. Fluorescence intensity was measured after 24 h exposure with a microplate reader. Figure was modified from Tuet et al.[10]

Section S2: List of experimental conditions in aerosol generation and elemental composition of SOA

Table S1. Experimental Conditions and Elemental Composition of Naphthalene SOA Formed in the Presence of NO_x

Sample	*Initial naphthalene*	*Relative humidity*	*[SOA]* [a]	*Initial NO*	*Initial NO_2*	*O:C ratio*	*H:C ratio*	*N:C ratio*	*OSc*
	ppb	*%*	*μg m^{-3}*	*ppb*	*ppb*				
a	222	< 5%	213	229	392	0.30	0.96	0.01	-0.36
b	321	< 5%	430	245	471	0.28	0.97	0.01	-0.40
c	450	< 5%	596	225	333	0.29	0.98	0.01	-0.39
d	550	< 5%	492	236	401	0.25	0.98	0.01	-0.48

[a] Average SOA mass concentration in the chamber during filter collection; The SOA mass concentration was calculated by multiplying SMPS volume concentrations with a density of 1.48 g cm^{-3} based on prior experiments.[11] The total aerosol mass collected on filters was calculated by multiplying average SOA mass concentration with the total volume of air collected, as described in Tuet et al. [12].

Section S3: Results of intracellular ROS/RNS with and without catalase

Results of naphthalene SOA samples

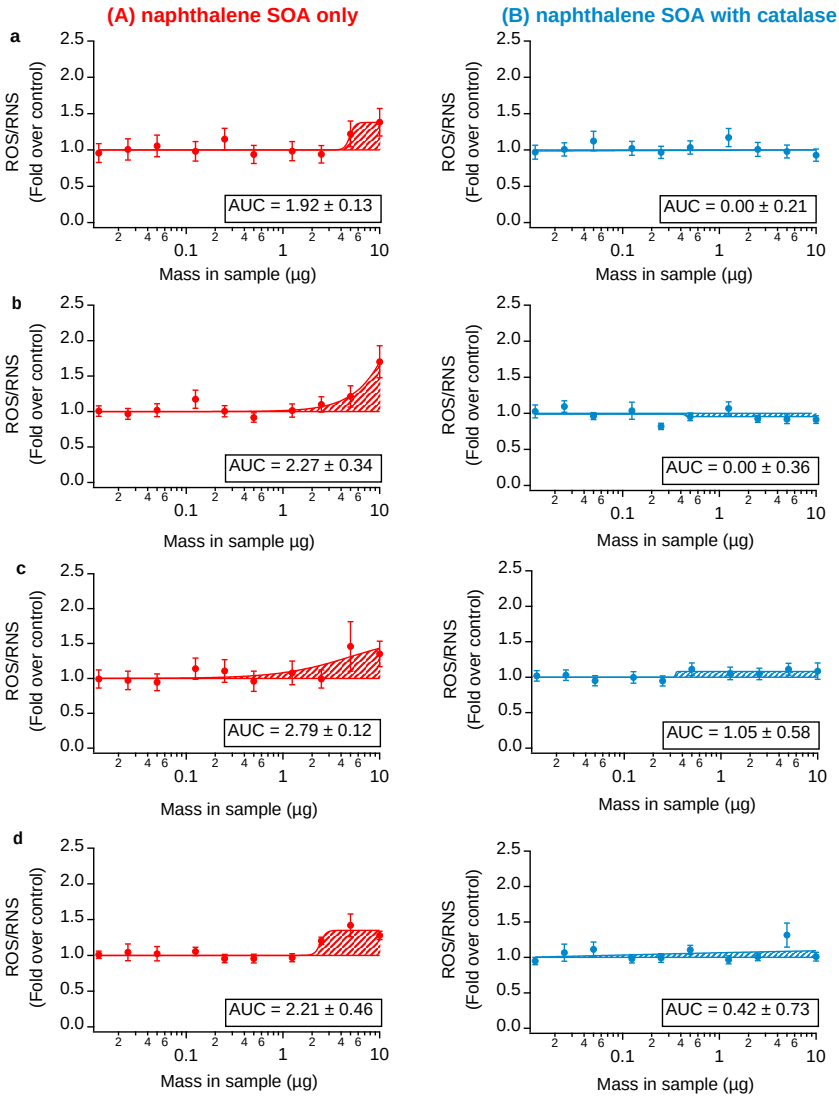

Figure S3. Dose-response curve of ROS/RNS produced by exposing to naphthalene SOA (samples a-d). Cells were exposed to (A) naphthalene SOA only (red curves) and (B) naphthalene SOA + 50 U mL^{-1} catalase (blue curves) for 24 h. ROS/RNS values represent the fold change in fluorescence over control cells. Data points are presented as mean ± SE of measurements carried out in triplicate, generating 3 sets of response for each naphthalene SOA sample. Every 10-dilution set of response was fitted with a dose-response curve and used to calculate the area under the curve (AUC) as described in Tuet et al.[10] The reported AUC represents the mean ± SE estimated from 3 AUCs for each measurement.

Results of laboratory-prepared tBOOH and H_2O_2 solutions

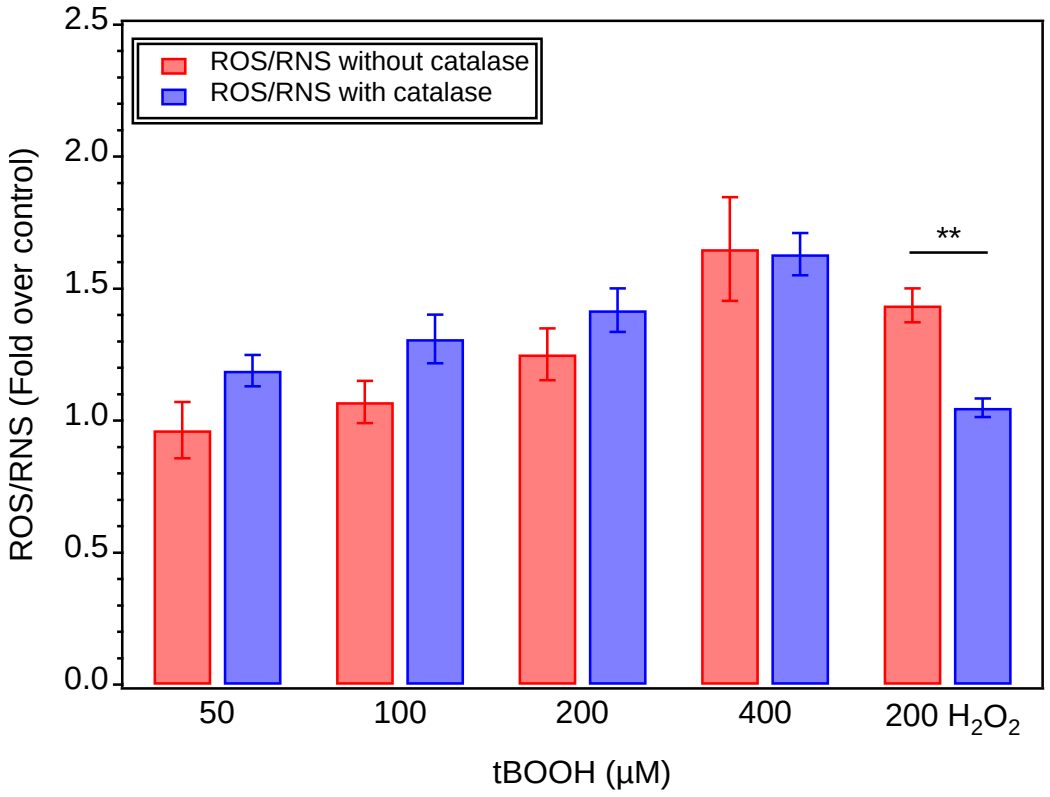

Figure S4. ROS/RNS response by cells exposed to 50, 100, 200, and 400 µM tBOOH solutions without (red) and with (blue) catalase. 200 µM H_2O_2 was used as a positive control. Values represent the fold change in fluorescence over control cells. Data are presented as mean ± SE of measurements carried out in triplicate. ** indicates $p < 0.01$.

Section S4: Results of H_2O_2 quantification using Amplex red

Calibration curves for H_2O_2 quantification

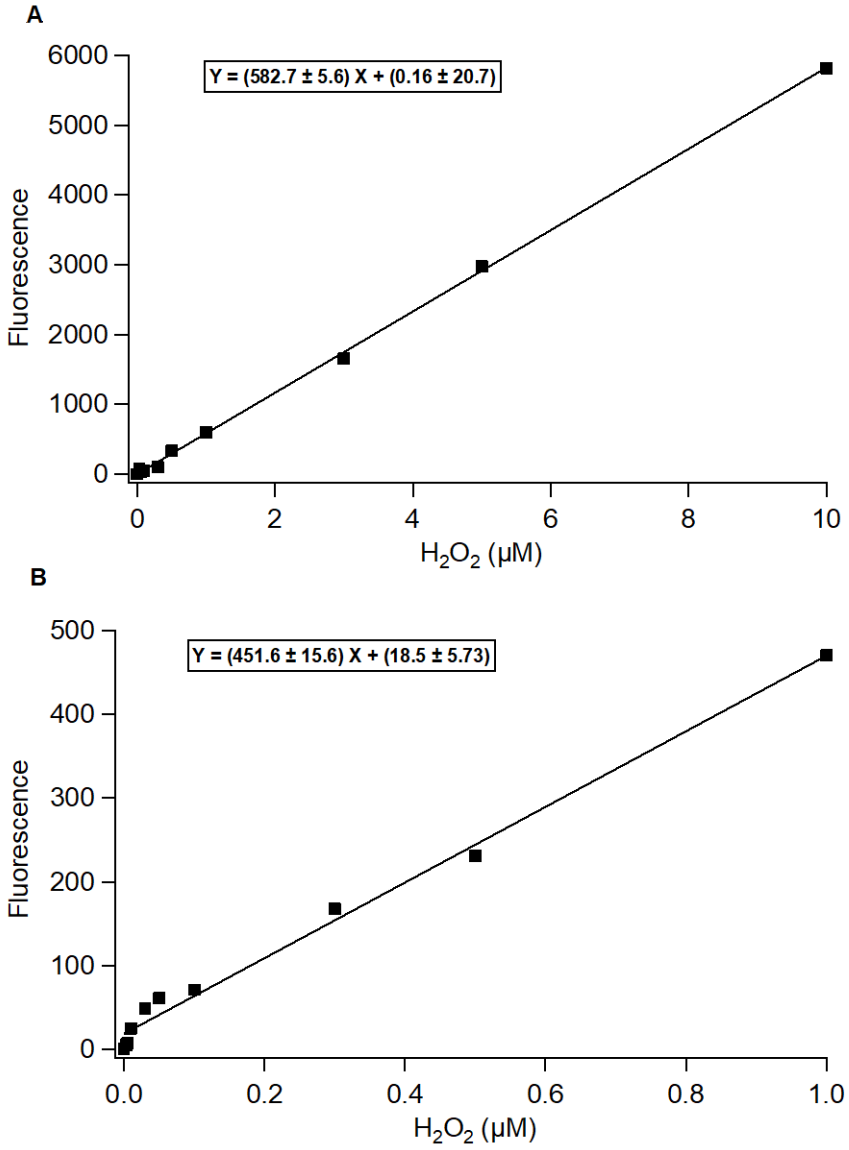

Figure S5. Calibration curve of H_2O_2 (in PBS) for the quantification of H_2O_2 (A) in naphthalene SOA extracts and diffusion of H_2O_2 into cells; and (B) produced by cells using Amplex red. An additional calibration curve was made for quantifying H_2O_2 produced by cells to account for the lower concentration range.

Determination of $H_2O_{2[SOA]}$ diffusion into cells. In order to investigate the diffusion of $H_2O_{2[SOA]}$ into cells, we separately determined $H_2O_{2[SOA]}$ without cells and $H_2O_{2[SOA]}$ in the presence of cells. The decrease in H_2O_2 in the presence of cells compared with that in the absence of cells was considered to be due to uptake into the cells.

As described in the main text, for each SOA sample (samples a-d), 50 µL of working solution (100 µM Amplex red reagent and 0.2 U/mL horseradish peroxidase (HRP) diluted in 1x reaction buffer) were added to 50 µL of naphthalene SOA extracted in PBS and incubated for 30 min at room temperature based on the Amplex red protocol (Invitrogen). Fluorescence intensity was measured after 30 min incubation. Here, for selected samples (samples b and d), we also determined $H_2O_{2[SOA]}$ at multiple time points (every 15 min over a period of 100 min after the 30 min incubation), as shown in Fig. S6 (blue circles).

To determine $H_2O_{2[SOA]}$ in the presence of cells, cells were plated and stained with the probe following steps (1) to (3) of our intracellular ROS/RNS protocol (Fig. S2).[10] Step (4) involved replacing the ROS/RNS probe solution with naphthalene SOA samples extracted in PBS. H_2O_2 in stained cells exposed to naphthalene SOA was quantified every 15 minutes over 1 h. At each time point, 50 µL of cellular medium (PBS) containing naphthalene SOA was transferred to a 96 well plate. Then, the reaction was started by adding 50 µL of working solution to each well with the samples. Fluorescence intensity was measured at multiple time points after 30 min incubation. Results are shown in Fig. S6 (green circles).

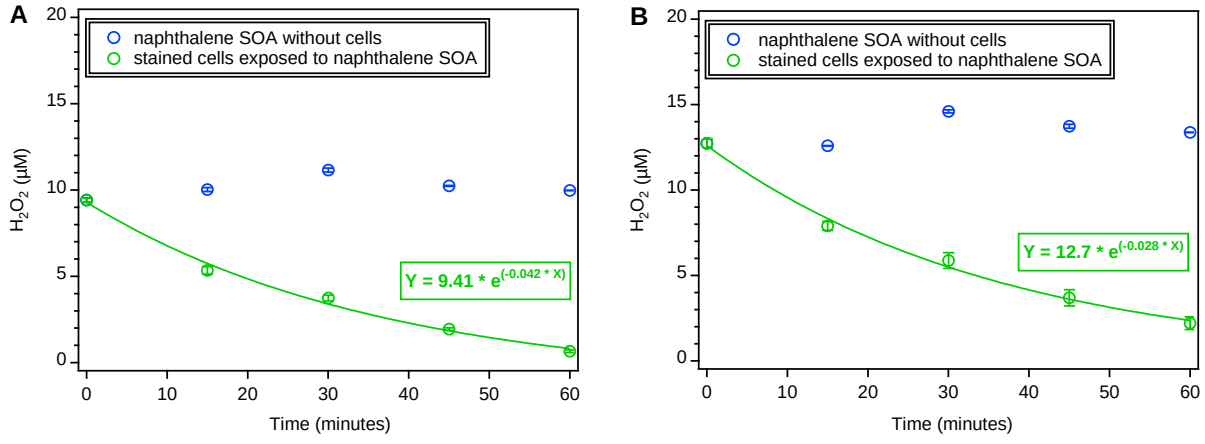

Figure S6. Time evolution of H_2O_2 concentration in naphthalene SOA extracts (without cells) and H_2O_2 concentration in cell medium containing naphthalene SOA (cells stained with ROS probe).

Panels (A) and (B) correspond to data from SOA samples b and d, respectively. H_2O_2 was quantified using Amplex red. Fluorescence signal was converted to H_2O_2 concentration based on calibration curves in Fig. S5-A. Data are presented as mean ± SE of measurements carried out in triplicate.

Quantification of H_2O_2 produced by cells

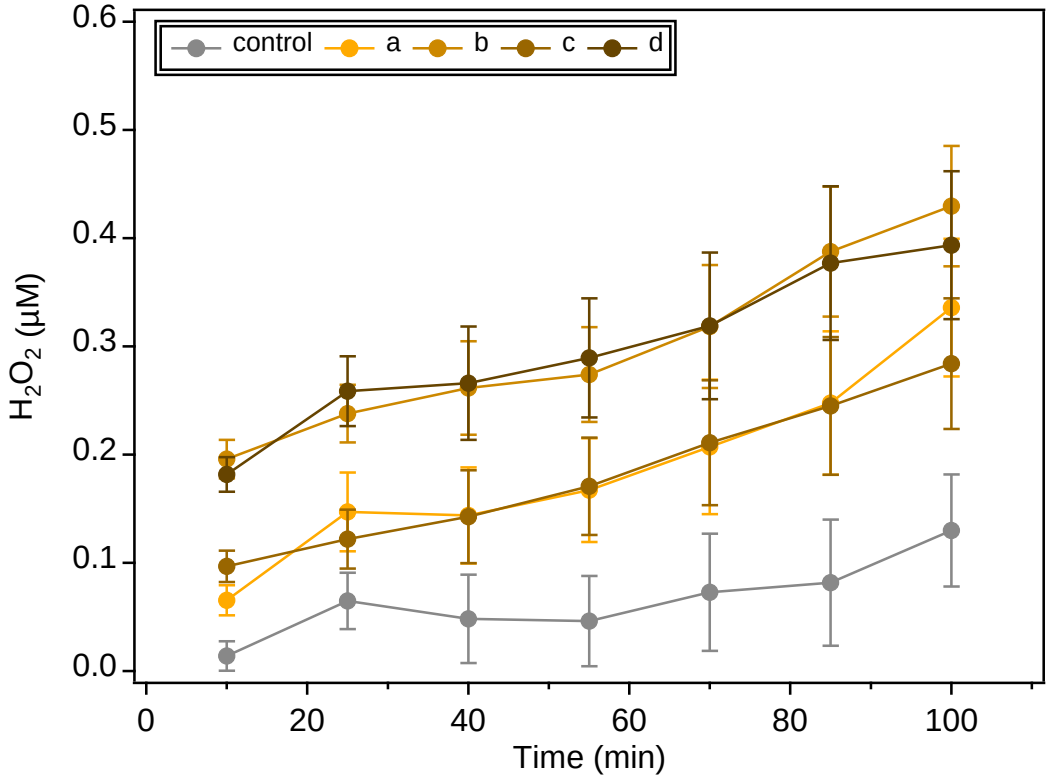

Figure S7. Quantification of H_2O_2 produced by cells. The cells were first exposed to naphthalene SOA samples a-d for 24 h (data in orange). Afterwards, the cell medium was replaced by 100 µL of working solution (100 µM Amplex red reagent and 0.2 U/mL HRP diluted in 1x reaction buffer) and incubated for 10 min, and then the fluorescence signal was measured every 15 minutes over a period of 100 min. Data in grey correspond to cells that were exposed to culture media only (no naphthalene SOA) for 24 h. Values are presented as mean ± SE of experiments carried out in triplicate. Fluorescence signal was converted to H_2O_2 concentration based on calibration curve in Fig. S5-B.

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