Supplemental Information

A Semi-Automated Multi-Endpoint Reactive Oxygen Species Activity Analyzer (SAMERA) for measuring the Oxidative Potential of Ambient $PM_{2.5}$ Aqueous Extracts

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Supporting Information	
Number of pages	7
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S1. Fluorescence intensity of GS-OPA at different excitation/emission wavelengths

Although the peak of the fluorescence intensity for GS-OPA occurs at the excitation/emission wavelength of 340 nm/420 nm (Roušar et al. 2012), we chose 310 nm/427 nm to allow the measurement of both GSH and •OH. Here, we show the contour plot of the fluorescence spectra of GS-OPA at different wavelengths. 400 µL of 100 µM GSH was diluted 10 times and mixed with 1.6 mL of 2 mM OPA. Although lower than at 340/420 nm, the fluorescent intensity at 310/427 nm is still substantially high (>150000 CPS/µA), indicating the suitability of this wavelength setting for measuring GS-OPA. We also conducted the calibration of GSH at both wavelength settings (Figure S1b). Although, the calibration equation is different at 310/427 nm than at 340/420 nm, it is highly linear for both cases (R_2 > 0.99), which allows the measurement of GSH at any of these wavelength settings.

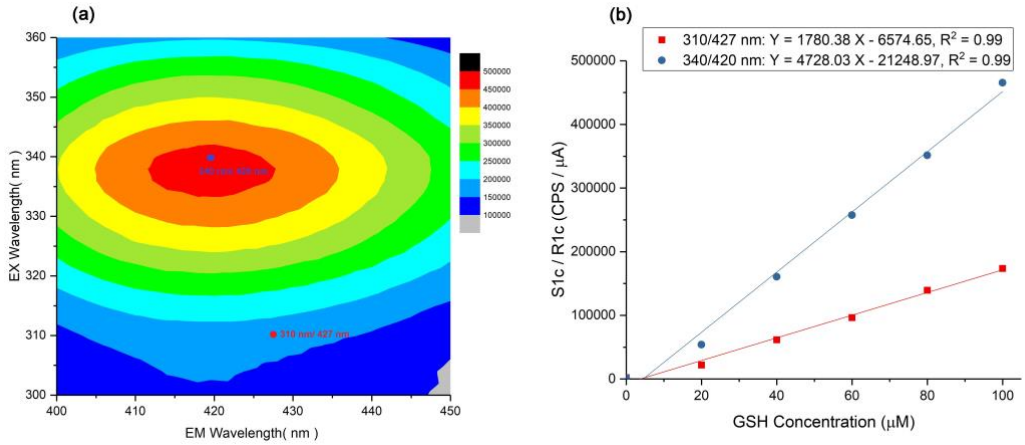

Figure S1. The effect of excitation/emission wavelength settings on the measurement of GSH, (a) the contour plot of fluorescence intensity of GS-OPA; (b) the calibration curve of the fluorescence intensity under two wavelength settings (i.e. 310/427 nm and 340/420 nm) versus GSH concentration.

S2. Effect of TPT on DTT consumption using PQ as the positive control

In SAMERA, OP_{DTT} and $OP_{OH\text{-}DTT}$ are measured in the same reaction vial (RV) and the latter requires adding TPT as the •OH probe in RV. To check if there is any interference of TPT on DTT consumption, we performed the DTT assay by using both K-PB (pH = 7.4) and TPT (prepared in K-PB; pH = 7.4) in separate reaction vials. Five different concentrations of PQ within the range of 0.05 – 0.25 μM (in RV) were tested. Figure S2 shows the orthogonal fit regression of DTT activity measured with and without TPT. As apparent, both slope and coefficient of determination (R_2) for the regression are close to 1. A 2-tailed paired *t*-test showed no significant difference in two sets of measurement ($p = 0.59$). Based on these results, we can conclude that TPT does not have any significant effect on the consumption rate of DTT, which allows us to conduct both DTT consumption and •OH measurement in the same vial.

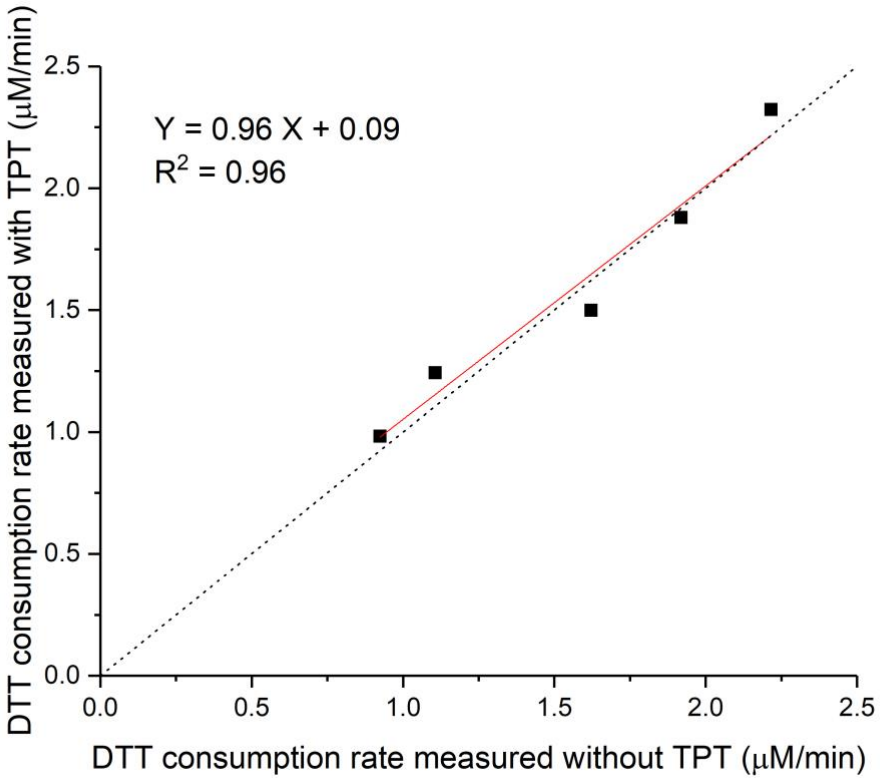

Figure S2. Comparison of DTT consumption rate measured with and without TPT in the reaction mixture using 0.05 μM – 0.25 μM PQ as positive control. The dotted line denotes identity line.

S3. OP determination from absorbance or fluorescence data

Figure S3 shows the data analysis procedure from the raw absorbance/fluorescence intensity data, using GSH as an example. The absorbance at 265 nm, 412 nm, 600 nm and the fluorescence intensity at excitation/emission wavelength of 310 nm/427 nm are measured continuously (resolution time = 2 seconds for absorbance and 3 seconds for fluorescence) during the operation of SAMERA. At each measurement step, the pump pushes the diluted mixture from MV into the flow cells of spectrophotometer or spectrofluorometer, causing an increase in the absorbance or fluorescence intensity at specific wavelengths under measurement. The self-cleaning program at the end of each measuring step drives the pump to push DI through the flow cells, which restores the absorbance and fluorescence intensity to zero, and thus generating five columns on the strip chart for both absorbance and fluorescence intensity plots (Figure S3a).

Figure S3b shows an example of the calibration curve for different concentrations of the indicator compound (here GS-OPA for GSH measurement). The calibration equation is then used to convert the fluorescence to GSH concentration versus time plot (Figure S3c). A simple linear regression is used to calculate the slope of this plot, which represents raw activity of the sample. Based on numerous tests with positive controls and ambient PM sample extracts, the coefficient of determination (R^2) for the regression is always above 0.98 for all the endpoints, indicating high accuracy of the slope. A negative control, i.e. DI for chemical standards and blank filter extract for ambient $PM_{2.5}$ extracts was always analyzed along a batch of the samples, for the respective blank correction. For the calculation of ambient OP activities, these blank corrected slopes were further normalized by either mass of collected PM or volume of the sampled air. Mass normalized OP (OPm, nmol·min^{-1}·μg^{-1}) indicates an intrinsic OP property, which is driven by the specific chemical composition of PM. Volume normalized OP (OPv, nmol·min^{-1}·m^{-3}) represents the total oxidative load caused by the exposure to the ambient aerosols, and is driven by both chemical composition and ambient concentration of PM. The calculation of normalized GSH activity is shown as an example:

$$\mathrm{OP}_{\mathrm{sample}}^{\mathrm{GSH-SLF}} = \mathrm{OP}_{\mathrm{sample,raw}}^{\mathrm{GSH-SLF}} - \mathrm{OP}_{\mathrm{Blank}}^{\mathrm{GSH-SLF}} \quad \text{(S1)}$$

$$\mathrm{OPm}_{\mathrm{sample}}^{\mathrm{GSH-SLF}} = \frac{\mathrm{OP}_{\mathrm{sample}}^{\mathrm{GSH-SLF}}}{\frac{\mathrm{m}_{\mathrm{sample}}}{\mathrm{V}_{\mathrm{DI}}} \times 0.7} \quad \text{(S2)}$$

where: m_{sample} is the total mass of PM on fraction of the filter used for extraction (μg), V_{DI} is the volume of DI used for extracting the filter, and 0.7 is the ratio of the sample extract volume to the total reaction volume in RV.

OPm and OPv are related by the following equation:

$$\mathrm{OPv}_{\mathrm{sample}}^{\mathrm{GSH-SLF}} = \dot{\mathrm{m}}_{\mathrm{sample}} \mathrm{OPm}_{\mathrm{sample}}^{\mathrm{GSH-SLF}} \quad \text{(S3)}$$

where: $\dot{m}_{sample}$ is the mass concentration of PM in ambient air (μg/m3).

The calculation of OP activities for other four endpoints is similar to $OP_{GSH\text{-}SLF}$ and therefore not discussed here.

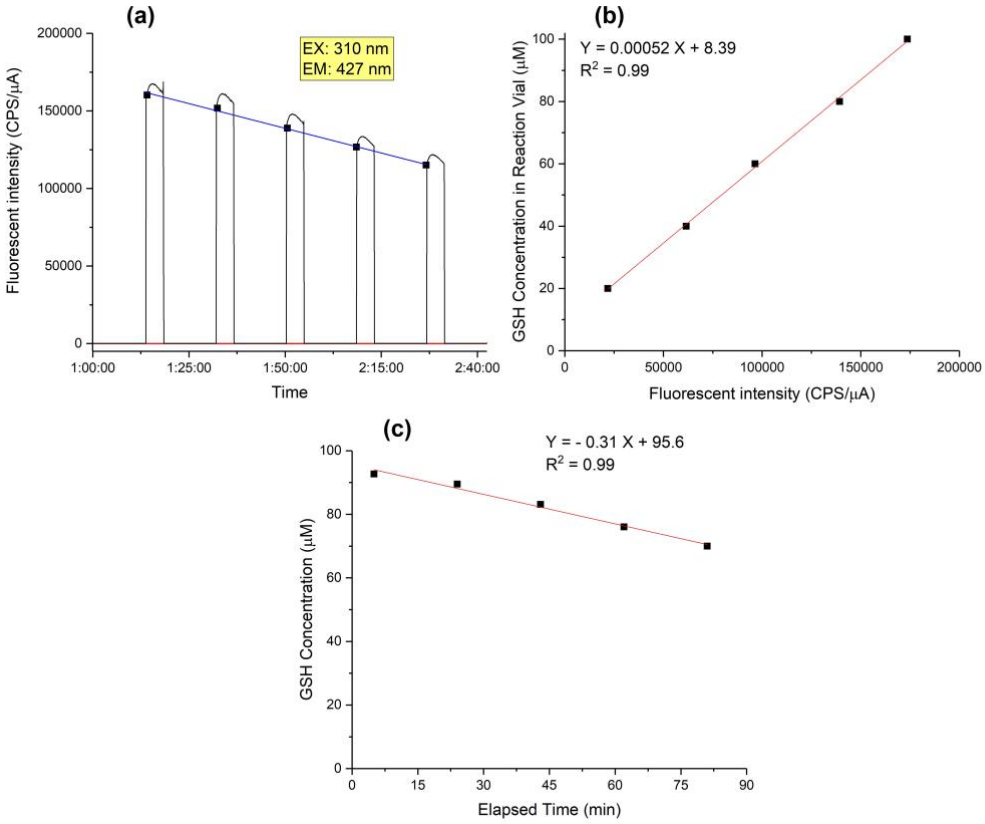

Figure S3. The illustration of OP determination using $OP_{GSH\text{-}SLF}$ as an example; (a) the fluorescence intensity vs. time plot at excitation/emission wavelength of 310/427 nm for a sample; (b) the calibration curve of GSH; (c) GSH consumption rate derived from (a) and (b).

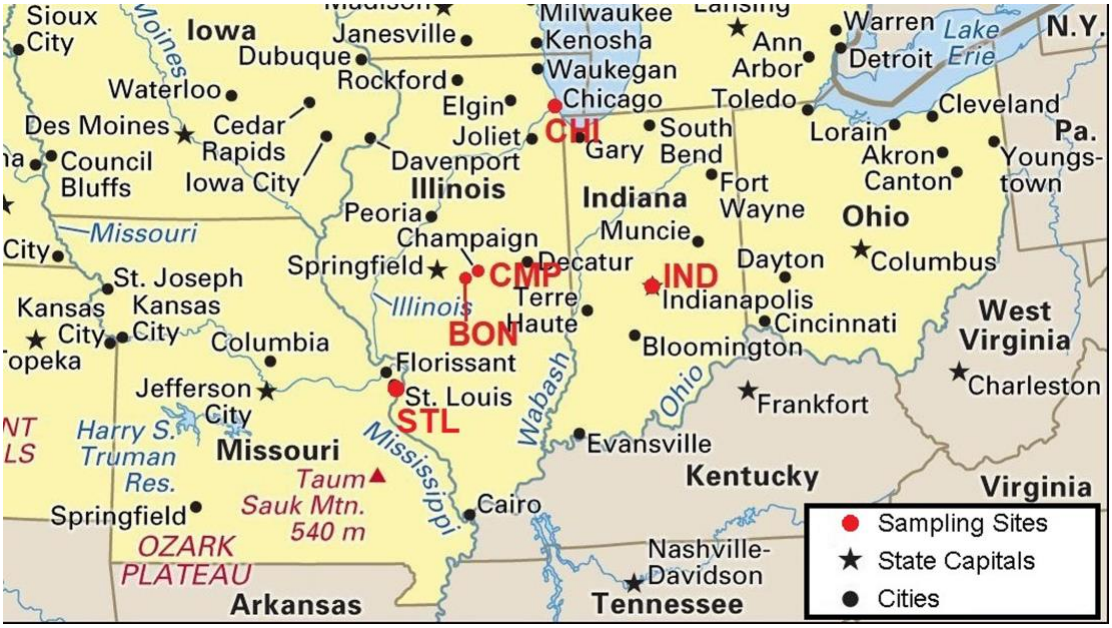

Figure S4. The map of sampling sites.

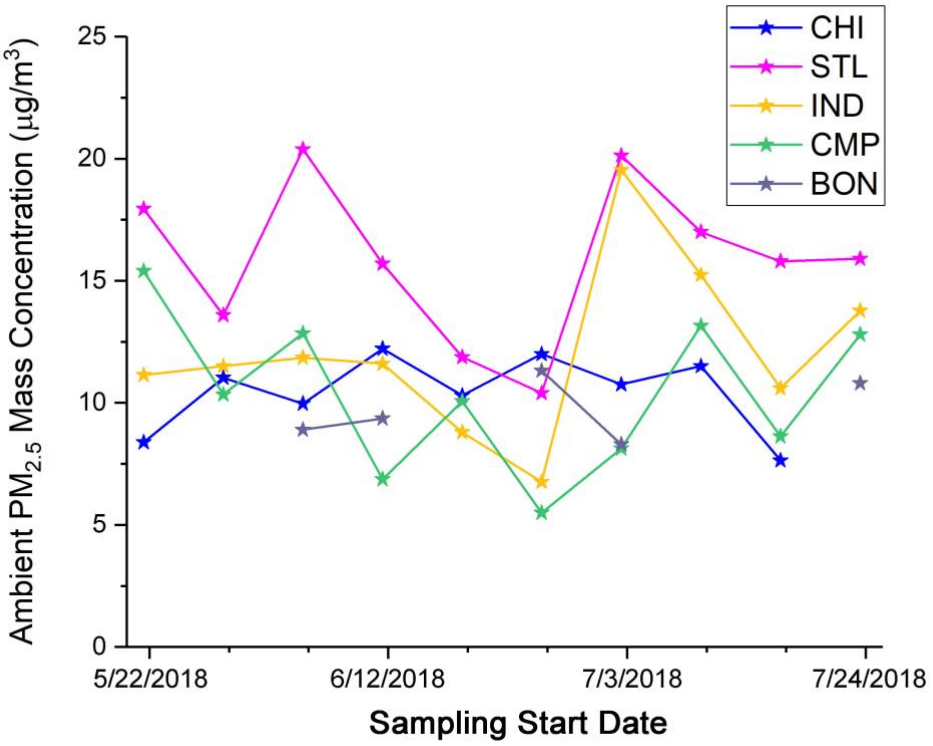

Figure S5. The mass concentrations of ambient $PM_{2.5}$ at five sampling sites in the Midwest US.

Table S1. The dates of sampling for the ambient $PM_{2.5}$ samples collected from five sites in the Midwest US (N = 54). All the samples were collected in the year 2018.

Sampling site	CHI	STL	IND	CMP	BON
Sampling dates (Start-End date)	5/22-5/25	5/22-5/25	5/22-5/25	1/30-2/2*	6/5-6/8
	5/29-6/1	5/29-6/1	5/29-6/1	2/9-2/12*	6/12-6/15
	6/5-6/8	6/5-6/8	6/5-6/8	2/21-2/24*	6/19-6/22
	6/12-6/15	6/12-6/15	6/12-6/15	2/26-3/1*	7/3-7/6
	6/19-6/22	6/19-6/22	6/19-6/22	3/11-3/14*	7/24-7/27
	6/26-6/29	6/26-6/29	6/26-6/29	3/26-3/29*	
	7/3-7/6	7/3-7/6	7/3-7/6	5/7-5/10*	
	7/10-7/13	7/10-7/13	7/10-7/13	5/14-5/17*	
	7/17-7/20	7/17-7/20	7/17-7/20	5/22-5/25	
		7/24-7/27	7/24-7/27	5/29-6/1	
				6/5-6/8	
				6/12-6/15	
				6/19-6/22	
				6/26-6/29	
				7/3-7/6	
				7/10-7/13	
				7/17-7/20	
				7/24-7/27	
				11/16-11/19*	
				11/23-11/26*	

* These samples were used for the precision and accuracy test of SAMERA.

Reference

Roušar, T., O. Kučera, H. Lotková, Z. Červinková. 2012. Assessment of reduced glutathione: comparison of an optimized fluorometric assay with enzymatic recycling method. *Analytical biochemistry* 423(2):236-240. doi: 10.1016/j.ab.2012.01.030.