Supporting Information

Simultaneous Detection of Cysteine Sulfenate, Sulfinate and Sulfonate during Cysteine Interfacial Ozonolysis

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Experimental Details

Our experiment simulates the chemical interaction of $O_3(g)$ with CyS on biosurfaces by spraying aqueous CyS solutions into dilute $O_3(g)/N_2(g)$ mixtures at atmospheric pressure. The composition of the interfacial layers of the resulting microdroplets is directly monitored by online mass spectrometry (ESMS, HP-1100) of the electrostatically ejected anions after sub-millisecond reaction times.[1-6] Aqueous solutions are pumped at 50 μl/min into the spraying chamber of the mass spectrometer through a grounded stainless steel needle surrounded by a coaxial sheath issuing nebulizer N_2 gas. The large difference between the exit velocities of the liquid jet and nebulizer gas forces the liquid to fragment into fine droplets.[7-9] After leaving the reaction zone, fast solvent evaporation leads to droplet shrinkage and concomitant surface charge crowding. Such droplets become mechanically unstable because Coulomb repulsion eventually overtakes liquid cohesion, triggering the spontaneous shedding of their interfacial films into nanometer size droplets.[10] This phenomenon repeats itself until ions are ultimately ejected from last-generation nanodroplets by the large electric fields created thereby.[6] These gas-phase ions can then be deflected into the mass spectrometer by applying a suitable electric bias to its inlet port. This analytical technique reports therefore the composition of nanodroplets created from the interfacial layers of microdroplets that had just reacted with $O_3(g)$. Direct evidences that the detected products are formed in reactions between $O_3(g)$ and reactants at the air/water interface, rather than in the gas or bulk liquid

phases, are described in elsewhere.[2, 3, 11] Further experimental details and validation tests can be found in previous reports from our laboratory.[1-3, 12, 13] Since a few ms elapse between droplet creation and product detection, the extent of secondary reactions involving the H_2O_2 and $O_2(^1\Delta_g)$ byproducts of O_3(aq) decomposition is minimized.[14] Typical instrumental parameters were as follows: drying gas temperature, 250 ^oC; nebulizer pressure, 2 atm; collector capillary voltage, +3.5 kV; fragmentor voltage, 17 V. Solutions were prepared with MilliQ water. CyS (L-cysteine, >97 %), $CySO_2H$ (L-cysteine sulfinic acid monohydrate, >99 %), CySSCy (L-cystine, > 99%) were obtained from Sigma-Aldrich. Solution pH was adjusted by adding NaOH and measured with a calibrated pH meter.

Appendix S1

Let us consider the following reactions:

$$CyS^- + O_3 \rightarrow CySO^- + O_2 \qquad R1$$

$$CySO^- + O_3 \rightarrow CySO_2^- + O_2 \qquad R2$$

$$CySO_2^- + O_3 \rightarrow CySO_3^- + O_2 \qquad R3$$

with rate constants k_n (n = 1-3). Under present pseudo first-order conditions, i.e., $[O_3] >> [CySO_m^-]$, and constant reaction times: $t = \tau \sim 1$ ms, we obtain:

$$[CyS^-] = [CyS^-]_0 \exp(-k_1'[O_3]) \qquad (I)$$

$$[CySO^-] = A\{\exp(-k_1'[O_3]) - \exp(-k_2'[O_3])\} \qquad (II)$$

$$[CySO_2^-] = P \exp(-k_1'[O_3]) + Q \exp(-k_2 [O_3]) + T \exp(-k_3'[O_3]) \qquad (III)$$

$$[CySO_3^-] = [CyS^-]_0\{1 - P'\exp(-k_1'[O_3]) + Q'\exp(-k_2'[O_3]) + T'\exp(-k_3'[O_3])\} \qquad (IV)$$

where A, P, Q, T, P', Q', T' are constants that depend on experimental and instrumental parameters, and $k_n' = k_n \tau$. The preceding scheme and attending assumptions, however, apply at low CyS conversions. It is apparent, for example, that the $[CyS^-]$ vs. $[O_3]$ data of Figure 2A (main text) do not adhere to a single exponential decay throughout, Equation (1), for reasons that have been explained in detail elsewhere,[13] and relate to the fact that fast interfacial reactions ultimately involve mass transport within the microdroplets. The reactant CyS^- is depleted according to Equation (1) at the interface proper only at low O_3(g) concentrations. At larger $[O_3]$s, the reaction reaches into deeper layers where CyS^- depletion via R1 is effectively attenuated by CyS^- diffusion from the microdroplets core. Since this phenomenon that does not apply to reaction products, we fitted the $[CyS^-]$ vs. $[O_3]$ data of Figure 2A using an empirical function: $[CyS^-] = Y_0 + Y_1 \exp(-k_1'[O_3]) + Y_2 [O_3]$, but analyzed the $[CySO^-]$ vs. $[O_3]$ data of Figures 2B and S6B according to Equation (II).

Table S1. MS/MS of CyS and its ozonolysis products

*Parent ion decrease by >90 % at threshold voltage

Parent anion Da	Threshold voltage V*	Fragment anion Da	Neutral loss Da
120 (CyS^-)	0.65 ± 0.15	N/A	N/A
136 ($CySO^-$)	0.50 ± 0.15	74 ($C_2H_4NO_2^-$)	62 (CH_2OS)
152 ($CySO_2^-$)	0.60 ± 0.15	88 ($C_3H_6NO_2^-$)	64 (SO_2)
168 ($CySO_3^-$)	0.85 ± 0.15	151 ($C_3H_3O_5S^-$ or $C_3H_5NO_4S^-$)	17 (NH_3 or OH)
135 (X)	0.65 ± 0.15	95	40
271 (Y)	0.85 ± 0.15	N/A	N/A

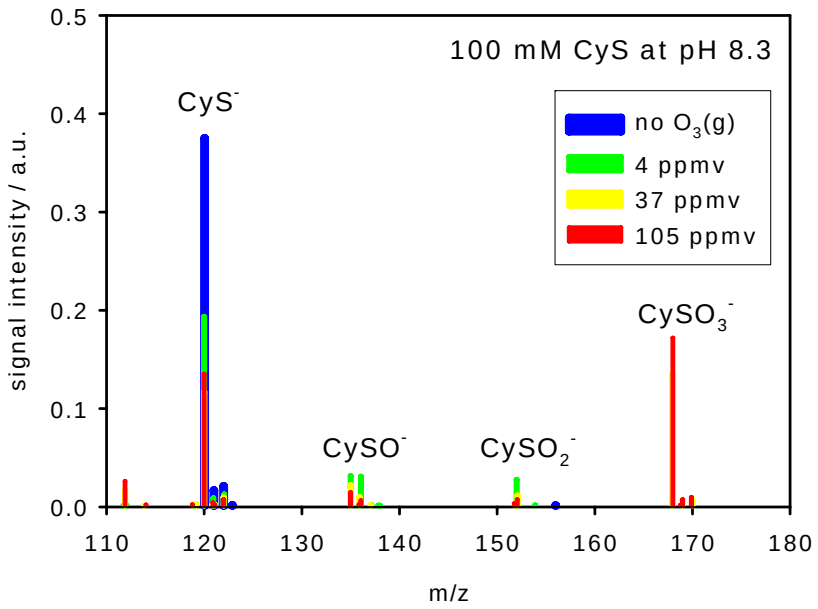

Figure S1. Negative ion mass spectra of aqueous 100 mM CyS at pH 8.3 in the absence/presence of O_3(g). Note that $CySO_n^-$ (n = 1-3) were observed even in high $[CyS]_0$.

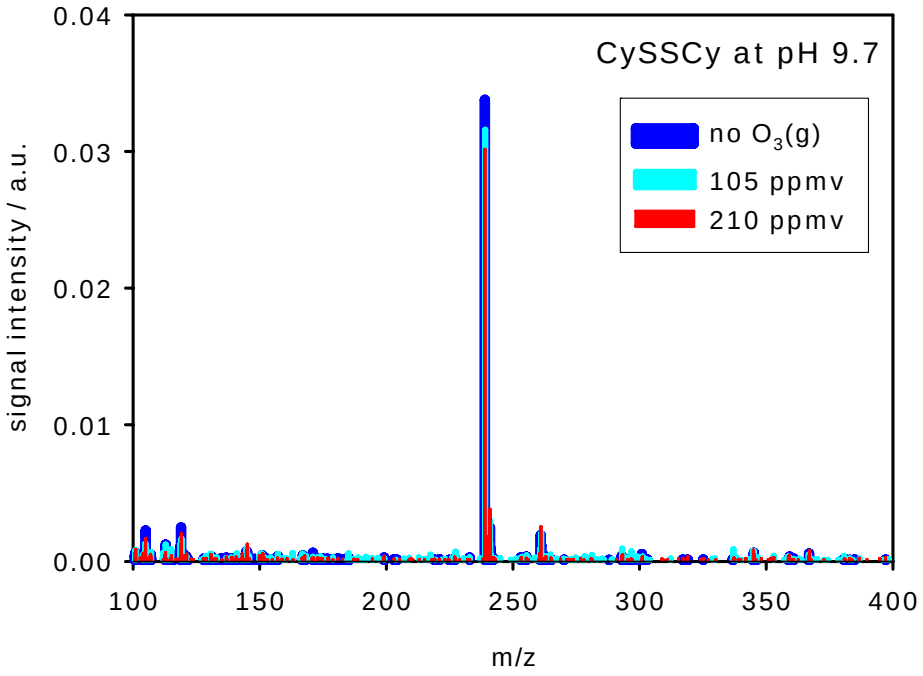

Figure S2. Negative ion mass spectra of aqueous 0.1 mM cystine, CySSCy at pH 9.7 in the absence(blue)/presence(light blue, red) of O_3(g). Note that no any anionic products are observed.

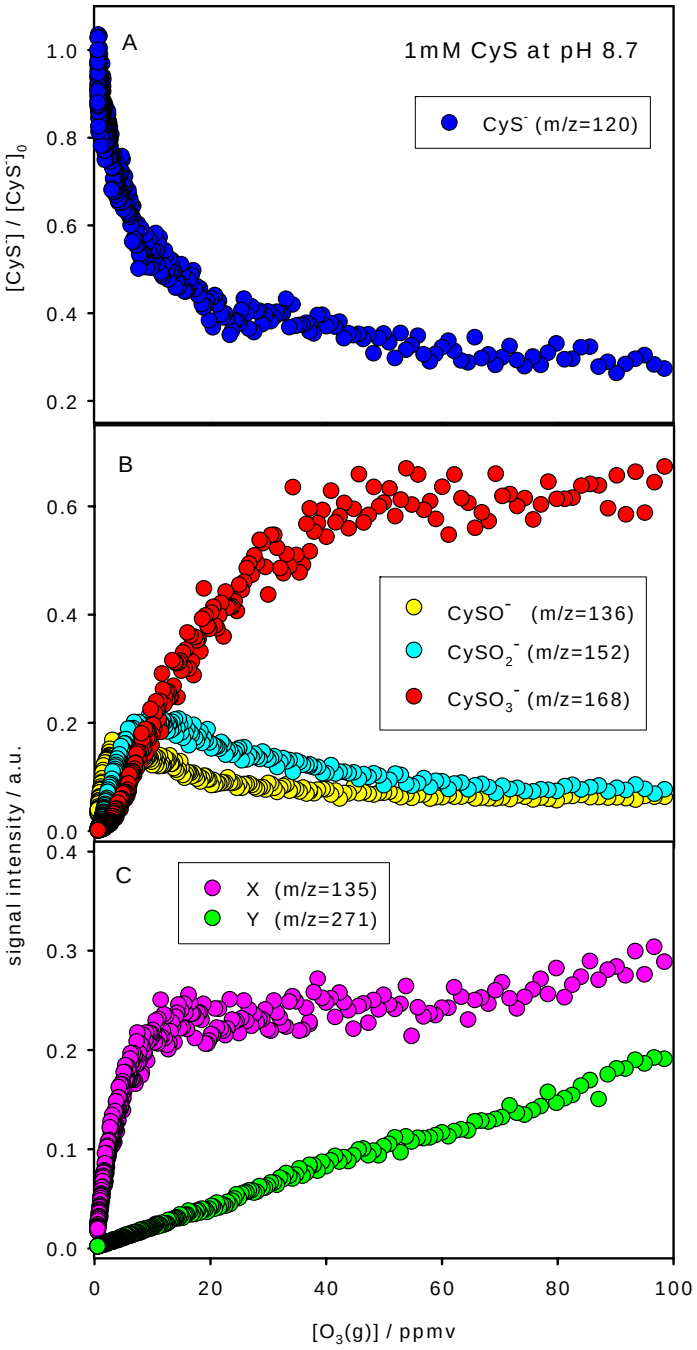

Figure S3. Mass spectral signals of aqueous CyS^- (A) and the ozonation products (B,C) as functions of $O_3(g)$ concentration where [CyS] = 1mM at pH 8.7 in the $[O_3(g)]$ range up to 100 ppmv.

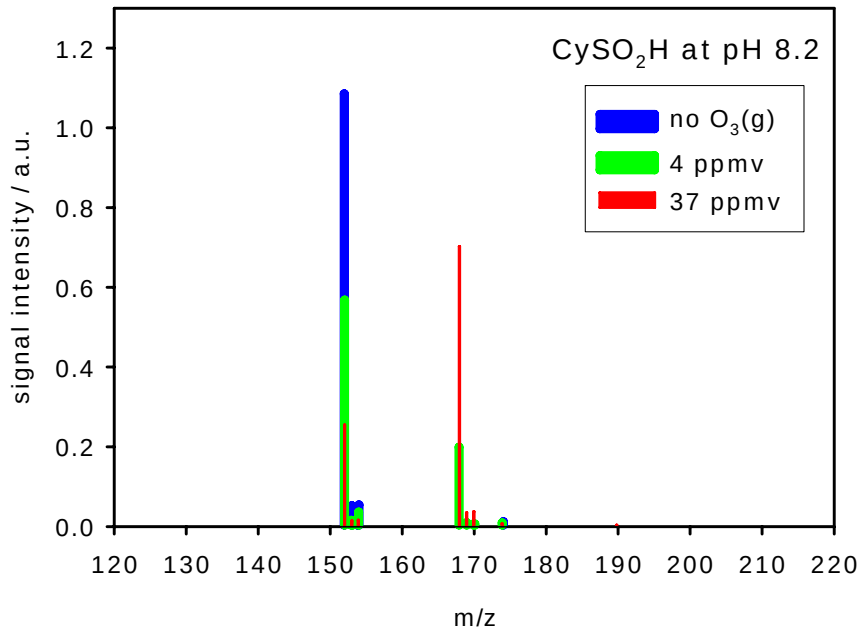

Figure S4. Negative ion mass spectra of aqueous 3 mM cysteine sulfinic acid, $CySO_2H$, at pH 8.2 in the absence(blue)/presence(green, red) of O_3(g). Note that $CySO_3^-$ is the only products.

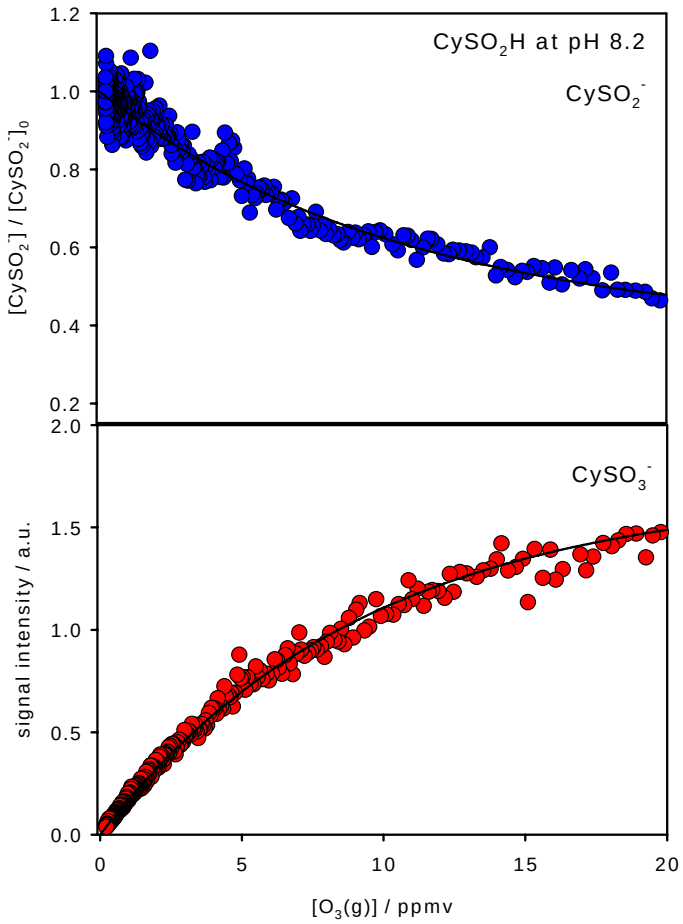

Figure S5. Mass spectral signals of reactant $CySO_2^-$ (blue) and the ozonation product $CySO_3^-$ (red) as functions of O_3(g) concentration where [$CySO_2H$] = 3 mM at pH 8.2. Decay of $CySO_2^-$ is independent of bulk pH between 3.7 and 8.3.

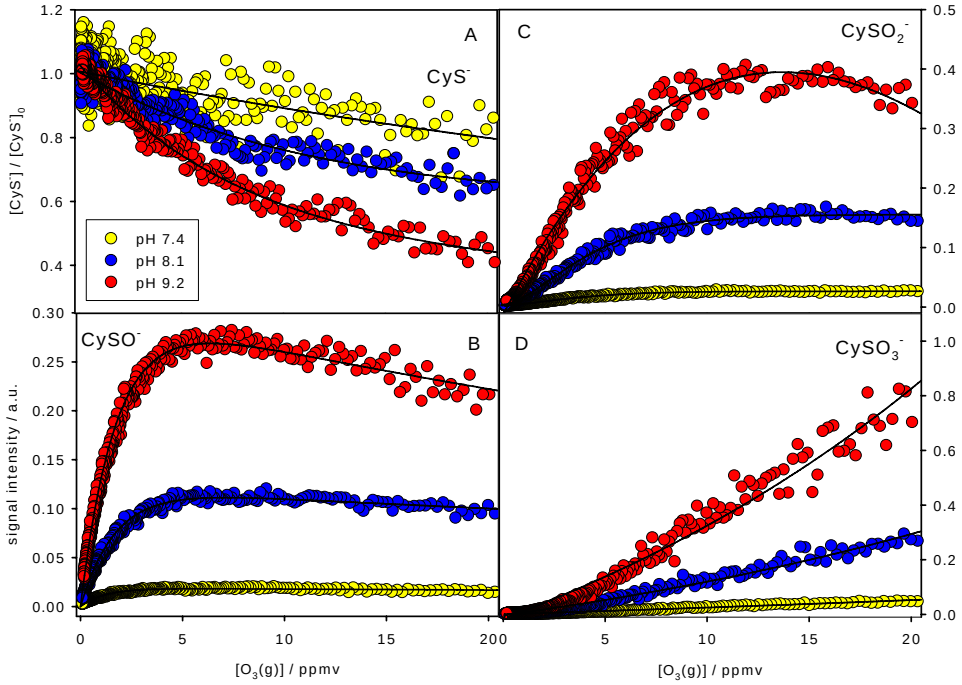

Figure S6. Normalized CyS^- (m/z = 120) signal intensities (A) and $CySO^-$(B), $CySO_2^-$ (C) and $CySO_3^-$ (D) signal intensities in the ozonolysis of 1mM CyS by O_3(g) at the air-water interface as functions of [O_3(g)] at various pH values: 7.4 (yellow), 8.1 (blue), 9.2 (red).

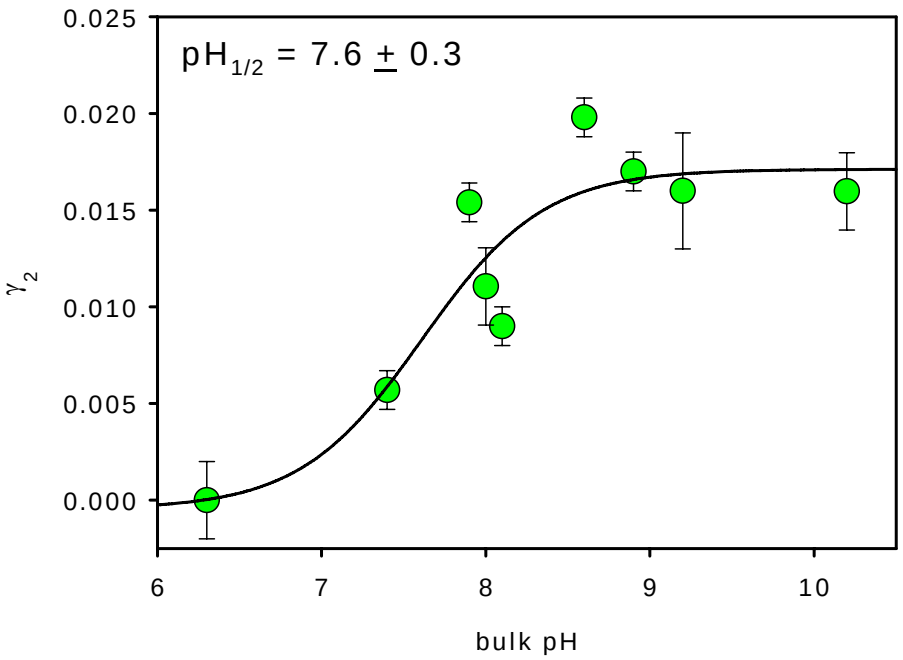

Figure S7. Rate of the reaction of $CySO^-$ with O_3 as a function of bulk pH. The rates are obtained from fits of eq. II to Fig. S6B. A sigmoid fitting yields $pH_{1/2}$ = 7.6 ± 0.3

References

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