Chemical and cellular oxidant production induced by naphthalene secondary organic aerosol (SOA): effect of redox-active metals and photochemical aging

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Exposure to air pollution is a leading global health risk. Secondary organic aerosol (SOA) constitute a large portion of ambient particulate matter (PM). In this study, the water-soluble oxidative potential (OP) determined by dithiothreitol (DTT) consumption and intracellular reactive oxygen and nitrogen species (ROS/RNS) production was measured for SOA generated from the photooxidation of naphthalene in the presence of iron sulfate and ammonium sulfate seed particles. The measured intrinsic OP varied for aerosol formed using different initial naphthalene concentrations, however, no trends were observed between OP and bulk aerosol composition or seed type. For all experiments, aerosol generated in the presence of iron-containing seed induced higher ROS/RNS production compared to that formed in the presence of inorganic seed. This effect was primarily attributed to differences in aerosol carbon oxidation state \( \Delta \Omega_{\text{Sc}} \). In the presence of iron, radical concentrations are elevated via iron redox cycling, resulting in more oxidized species. An exponential trend was also observed between ROS/RNS and \( \Delta \Omega_{\text{Sc}} \) for all naphthalene SOA, regardless of seed type or aerosol formation condition. This may have important implications as aerosol have an atmospheric lifetime of a week, over which \( \Delta \Omega_{\text{Sc}} \) increases due to continued photochemical aging, potentially resulting in more toxic aerosol.

Air pollution exposure ranks among the top ten global human health risks¹ with multiple epidemiological studies reporting associations between various cardiopulmonary health effects, elevated particulate matter (PM) concentrations¹–⁸, and particle oxidative potential (OP)⁹–¹². Toxicological studies suggest PM-induced oxidant production as a possible mechanism linking PM exposure and observed health effects¹³–¹⁶. Multiple chemical and cellular assays have been developed and utilized to measure PM-induced oxidant production. For instance, cell-free chemical assays that utilize an antioxidant to simulate biologically relevant redox reactions and ultimately measure the redox potential of PM¹⁷,¹⁸ and cellular assays that employ a probe capable of reacting with reactive oxygen and nitrogen species (ROS/RNS) produced as a result of PM exposure¹⁹,²⁰ have been developed. Both types of assay have been used in prior studies to elucidate chemical species associated with oxidant production²⁰,²⁶–³¹. Despite these efforts, the specific constituents responsible for the overall health effects induced by PM exposure remain unclear as ambient mixtures are complex.

Organic aerosol constitute a significant portion of ambient PM³²,³³, and multiple field studies have repeatedly shown that secondary organic aerosol (SOA, formed from the oxidation of volatile organic compounds in the atmosphere) often dominate over aerosol of primary origin (e.g., aerosol emitted directly from combustion engines), even in urban centers³³–³⁵. While there have been several recent studies regarding the health effects of
SOA36–45, there are still important gaps in knowledge that have not been addressed. For instance, organic aerosol have a lifetime of approximately one week46; continued photochemical aging can alter the chemical and physical properties of aerosol, which may have implications on resulting health effects. These potential effects have not been fully explored as the majority of current studies have focused on freshly formed SOA36,47–49. In addition, the presence of redox-active metals on SOA health effects have not been considered even though laboratory studies have shown that the presence of metal-containing seeds influences SOA formation and chemical composition50–53, and these metals are readily emitted via various processes (e.g., traffic, mechanical processes, combustion)12,24. Furthermore, redox-active metals such as iron may participate in redox cycling, as well as Fenton-like reactions35,36. These reactions produce radicals capable of enhancing the degree of oxidation of organic aerosol when internally mixed with organic aerosol, resulting in stronger oxidizing agents that may induce more ROS/RNS production upon cellular exposure37. Depending on the source, iron may exist in either coarse or fine mode, with a majority in the coarse mode and a small fraction in the fine mode37–39. As such, there exists some overlap between the size distributions of iron and submicron organic aerosol, which is sufficient for iron to serve as a catalyst in Fenton-like reactions in some fraction of the organic aerosol.

In the present study, naphthalene photooxidation SOA were generated in the presence of metal-containing (iron (II) sulfate, FS) and inorganic (ammonium sulfate, AS) seed. For both seed types, a series of laboratory chamber experiments with different initial naphthalene concentrations was conducted to produce aerosol of various degrees of oxidation. Multiple samples were also collected from a single experiment to obtain aerosol of different photochemical age. Oxidant production was measured using chemical and cellular assays (i.e., water-soluble OP as determined by dithiothreitol (DTT) consumption41 and intracellular ROS/RNS production as detected using carboxy-H,DCFDA36). Tuet et al.36,46 recently investigated the water-soluble oxidative potential and cellular ROS/RNS production for SOA formed from common biogenic and anthropogenic precursors. Here, we choose to focus on naphthalene SOA as it was shown to have the highest response among different SOA systems previously studied in Tuet et al.35,36.

Results and Discussion

Laboratory-generated aerosol. Experiments were conducted in the Georgia Tech Environmental Chamber (GTEC) facility. Typical time series for NO, NO₂, O₃, gas-phase naphthalene concentrations, and aerosol mass concentrations are shown in Fig. S1 for the two seed particles investigated. In both cases, NO decreased due to reaction with peroxy radicals (RO₂), which are important radical intermediates formed from hydrocarbon oxidation, and whose fates affect the oxidation products and SOA formation60,61. Aerosol growth was observed shortly following the initiation of photooxidation (i.e., turning on the lights). Most of the hydrocarbon was consumed in two hours and peak aerosol mass was reached. In general, FS seeded experiments (Fig. S1B) yielded less aerosol mass compared to AS seeded experiments (Fig. S1A). Previous studies exploring the effect of iron sulfate seed on aerosol formation (e.g., α-pinene and toluene photooxidation SOA in the presence and absence of iron sulfate seed) have also reported on the decreasing effect of iron sulfate seed on SOA yield, that is less aerosol mass was formed in the presence of iron sulfate seed62,63.

Aerosol chemical composition was monitored using a high resolution time-of-flight aerosol mass spectrometer (HR-ToF-AMS, Aerodyne; henceforth referred to as the AMS) for all chamber experiments. The average, normalized AMS mass spectra (Fig. S2) are consistent with those reported in previous studies62,63. A fragmentation pattern characterized by distinct ions at m/z 77, 91, 105, 119, 133, 147, and 160, was observed, which is likely representative of phenylalkyl fragments64. Differences in AMS mass spectra between aerosol formed in the presence of AS and FS seed were observed as well (Figs S3 and S4). Elemental ratios (O:C, H:C, and N:C) of SOA were also determined using the AMS, and average aerosol carbon oxidation states (OSₐ = 2 O:C–H:C)30 of SOA were calculated. O:C ratios and OSₐ were higher for all FS seeded SOA compared to AS seeded SOA (Table S1). This is consistent with previous laboratory studies, where the presence of iron sulfate seed resulted in the generation of more oxidized aerosol (higher O:C and OSₐ) due to Fenton-type reactions63. Additionally, for both AS and FS seeded SOA, OSₐ, followed a decreasing trend with the mass of organic aerosol formed (∆Mₐ), which is consistent with semi-volatile partitioning66–68 (Fig. S5). Specifically, more SOA was formed in experiments with a higher initial naphthalene concentration. With a higher aerosol mass loading, more volatile species (with a lower O:C and OSₐ) will also partition into the particle phase; thus lowering the overall OSₐ of the aerosol.

Effect of iron seed on cellular ROS/RNS production. To investigate whether the presence of metal-containing seed particles affected SOA toxicity, chemical and cellular oxidant production was measured for naphthalene SOA formed in the presence of iron-containing seed vs. inorganic seed (denoted OPseed=SOA or ROS/RNSseed=SOA, where seed = FS or AS, where applicable). ROS/RNS production, expressed as the area under the dose-response curve (AUC) per mass of SOA (μg) in the filter extract, is shown in Fig. 1, colored by seed type. AUC was used as previous drug and aerosol studies have shown that it is the most robust dose-response metric, whose informativeness does not rely on the presence of a baseline or maximum response20,68. It should be noted that for all experiments, FS seeded SOA exposure resulted in higher ROS/RNS levels compared to AS seeded SOA. This observed difference can potentially be attributed to both the seed itself (FS vs. AS) and organic aerosol formed in the presence of difference seeds.

The seed effect was explored by exposing cells to pure iron sulfate seed. Exposure to both aerosolized (injected into the chamber, collected onto a filter, and extracted into media; see methods section and SI for details on filter collection and extraction) and aqueous (seed solution diluted in media) iron sulfate resulted in ROS/RNS levels that fall along the same dose-response curve (Fig. S6). This suggests that the aerosolization, collection, and extraction process does not alter the iron sulfate in a way which changes its ROS/RNS inducing ability. We then use this dose-response curve to estimate the ROS/RNS response attributable to the presence of iron sulfate alone (ROS/
RNS in SOA experiments. For each FS seeded SOA experiment, the seed mass collected onto the filter was approximated by fitting a double exponential to the seed concentration time series (in the absence of chemical reactions, prior to aerosol formation) and integrating the fitted function over the filter collection period (Fig. S7). The corresponding ROS/RNS FS response as a result of exposure to this seed mass was then calculated using the iron sulfate dose-response curve (Fig. S6). These calculations were only performed for FS seeded SOA as exposure to ammonium sulfate seed has previously been shown to induce negligible ROS/RNS response at similar seed mass concentrations. The ROS/RNS FS response based on the determined iron sulfate seed mass accounted for about 2–12% of the measured ROS/RNS SOA response. It should be noted that these estimated contributions are only simple approximations to provide perspective as concentration addition may not apply for cellular responses. Nevertheless, these results are interesting as pure iron sulfate seed induced relatively low ROS/RNS production compared to that induced by the collected samples (i.e., ROS/RNS SOA). This suggests that the measured ROS/RNS SOA response may be predominantly attributed to organic components. These results confirm the importance of organic species to aerosol health effects, and previous studies on ROS/RNS produced as a result of aerosol exposure have also found significant correlations between the concentration of water soluble organic carbon (WSOC) and ROS/RNS response.

The degree of oxidation is a parameter of interest for organic aerosol, as atmospheric photochemical aging occurs over an aerosol’s lifetime, yielding more oxidized species and aerosol with a higher OSc. The observed difference in ROS/RNS levels between AS and FS seeded SOA is likely an effect of the degree of oxidation, where the presence of iron serves to increase the oxidation of species via Fenton-like reactions (Table S1). In fact, a positive exponentially decreasing trend was observed between ROS/RNS levels and OSc of aerosol for all experiments (Fig. 2). These results are consistent with our previous study on the ROS/RNS levels of SOA generated from various precursors, where a significant positive correlation was observed between ROS/RNS and OSc. Results from this study therefore further support the idea that more oxidized products are likely better oxidizing agents which can induce higher levels of ROS/RNS. In addition, the observed trend suggests that different seed types do not affect the ROS/RNS response as both AS and FS seeded SOA fall on the same ROS/RNS vs OSc curve.

It is also interesting to note that the ROS/RNS levels for filter samples collected over the course of a single experiment (Expt. 5) roughly follow the time series for aromatic phenyl and benzyl ions measured by the AMS (m/z 77 and 91, respectively, Fig. 3). Previous studies comparing cellular inflammatory responses from naphthalene and m-xylene SOA have suggested that aromatic-retaining products may have significant health implications. While results from this study are not sufficient to conclude causation, these observations along with findings from previous studies on the importance of humic-like substances (HULIS) should inspire future studies to focus on assessing the health implications of aromatic SOA and determine whether the presence of aromaticity directly induces adverse outcomes.

The ROS/RNS levels induced by naphthalene SOA generated under different formation conditions (e.g., RH, peroxy radical fate, OH source) have been measured in our previous study and are also shown in Fig. 1 for comparison. In both the previous and this study, the same cellular assay and analysis method was utilized. However, comparing ROS/RNS levels directly between these two studies may not be applicable as there are several differences between SOA formation condition (e.g., different initial naphthalene concentrations, different relative humidities, and different OH radical precursors). It is interesting to note that the exponential relationship between ROS/RNS and OSc holds for all naphthalene SOA generated under different formation conditions (Fig. 2).
Effect of iron seed on OP. Intrinsic OP values (per µg) for naphthalene SOA (OPseed+SOA) and pure iron sulfate seed (OPFS) are shown in Fig. 4, colored by seed type. For each FS seeded SOA experiment, the contribution of seed alone to the overall OPFS+SOA level is relatively low (<20%), which parallels that observed for the ROS/RNS response. It should be noted that DTT does not respond significantly to iron, and the low OPFS is consistent with previous studies, where a low DTT reactivity by iron was observed 54. Previous studies have shown that AS alone is not redox active, that is OPAS is equivalent to the response of a blank filter within experimental error 45. It is therefore also interesting to note that OPFS+SOA is not always higher than OPFS, suggesting that the presence of iron seed does not always induce an additive effect. Further studies should explore various effect models for OP to investigate additivity.

Overall, there are no apparent trends for the OP values obtained for SOA generated using different initial naphthalene concentrations (hence different organic aerosol mass loadings and \( \bar{O}\delta_C \)) or in the presence of different seed types. Furthermore, there was no observable relationship between OP and \( \bar{O}\delta_C \) (Fig. S8). While these results are in contrast to trends observed for ROS/RNS levels, they are consistent with previous studies on the DTT activities of different SOA systems and various ambient PM subtypes 23,45,73,74. Tuet et al. 45 previously measured the intrinsic OP of different SOA systems (including naphthalene SOA) and found that while different SOA precursors and formation conditions produced SOA of differing \( \bar{O}\delta_C \), there was no apparent relation between OP and \( \bar{O}\delta_C \). The study also showed that for both laboratory-generated SOA and different organic aerosol sub-

Figure 2. Exponential trend between ROS/RNS levels and average carbon oxidation state (\( \bar{O}\delta_C \)) for naphthalene photooxidation SOA generated in the presence of different seed particles (ammonium sulfate or iron sulfate), OH radical precursor (H\(_2\)O\(_2\)), and NO. ROS/RNS production are expressed as the area under the dose-response curve (AUC). Error bars were determined using the methodology outlined in Tuet et al. 20. Data from previous studies were included for comparison 45. \( \bar{O}\delta_C \) ranges for less oxidized oxygenated organic aerosol (LO-OOA) and more oxidized OOA (MO-OOA) are shaded for context 65.

Figure 3. Intrinsic OP and ROS/RNS levels for naphthalene photooxidation SOA collected over the course of a single experiment (Expt. 5). Time series for AMS \( m/z \) 77 and 91, which are likely phenyl and benzyl ions, are also shown. SOA was generated in a humid chamber in the presence of ammonium sulfate, OH radical precursor (H\(_2\)O\(_2\)), and NO. Error bars represent a 15% coefficient of variation for OP 21. ROS/RNS levels are expressed as the area under the dose-response curve (AUC) with error bars calculated following the methodology described in Tuet et al. 20.
OSc hydrocarbon concentrations. This suggests that the proxy for aging (anti-oxidants and less sensitive to the oxidants themselves (exogenous oxidants, e.g., H_2O_2). The cellular ROS/RNS response and RNS probe is removed after the probe incubation time. However, while the cellular assay may not directly measure endogenous oxidants, these species can interact with cells and induce pathways that may produce ROS/RNS.

Relationship between photochemical aging of aerosol and oxidant production. As the laboratory experiment progressed, OH exposure of aerosol and O_S increased as a result of increased photochemical aging. To investigate whether the effects of photochemical aging are comparable to those observed for SOA of different O_S (a proxy for aging), multiple filter samples were collected over the course of a single experiment (Table 1, repeat of Expt. 5). It should be noted that this aging experiment is an exact repeat of the previous experiment (Expt. 5), with the exception of a longer experimental time and multiple filter sample collections to explore changes in O_S associated with photochemical aging. The ROS/RNS levels and OP for these samples are shown in Fig. 3. The OP for these three samples are the same within uncertainty, consistent with the hypothesis that the DTT assay may only be sensitive to larger differences (such as precursor identity). On the other hand, the ROS/RNS response followed the same trend as that of O_S. The ROS/RNS response induced by these samples and the O_S calculated for each collection period are also shown in Fig. 2 (opened markers) for comparison. These values fall within the exponential trend observed between ROS/RNS and O_S for SOA generated from different initial hydrocarbon concentrations. This suggests that the proxy for aging (O_S) investigated in this study may be used to understand the potential health implications of aged particles for SOA from a single pure compound.

These observations have significant implications for future health studies as atmospheric aging leads to increases in aerosol oxidation, which may affect cellular responses. This is important as aerosol have an atmospheric lifetime of about a week, over which these aging processes can occur. If the observed relationship between cellular ROS/RNS response and O_S holds for other SOA systems, as well as ambient mixtures, these results may lead to ROS/RNS predictions based on more accessible bulk aerosol properties that are readily measured by the AMS. These approximations would not require the additional processing (e.g., filter collection and extraction) that actual ROS/RNS measurements entail. As an example, the O_S ranges for various organic aerosol subtypes resolved from ambient data world-wide, specifically less-oxidized oxygenated organic aerosol (LO-OOA) and more-oxidized OOA (MO-OOA), have been measured previously and are shaded in Fig. 2 to provide...
OSc RNS produced as a result of SOA exposure. The aerosol formed in all experiments fall on the same ROS/RNS vs. tions arising from this trend. For one, the trend implies that there is negligible seed effect with respect to ROS/
course of a single experiment (different degrees of photochemical aging). There are several important implica-
formation conditions (dry vs. humid, RO 2 vs. NO), and those collected at different times over the course of a single experiment (different degrees of photochemical aging). There are several important implica-
An exponential trend was also observed between ROS/RNS levels and OSc for all naphthalene photooxidation SOA, including those formed in the presence of different seed particles (AS and FS), those formed under different formation conditions (dry vs. humid, RO 2 + HO 2 vs. RO 2 + NO), and those collected at different times over the course of a single experiment (different degrees of photochemical aging). There are several important implica-
Table 1. Experimental conditions. 4Ammonium sulfate seed (15 mM (NH 4 ) 2 SO 4 ); 4Iron sulfate seed (15 mM FeSO 4 ); 4Average SOA concentration in the chamber during filter collection; 4Experiment was repeated and multiple filters were collected over the course of the experiment to investigate the effects of photochemical aging.

| Experiment | Hydrocarbon | Seed  | Relative humidity (%) | [HC] 0 (ppb) | [NO] 0 (ppb) | [SOA] c (µg m$^{-1}$) |
|------------|-------------|-------|----------------------|-------------|-------------|----------------------|
| 1          | naphthalene | AS a  | 51%                  | 32          | 315         | 11.7                 |
| 2          | naphthalene | FS b  | 50%                  | 32          | 303         | 12.8                 |
| 3          | naphthalene | AS a  | 49%                  | 32          | 368         | 66.7                 |
| 4          | naphthalene | FS b  | 48%                  | 32          | 214         | 24.0                 |
| 5          | naphthalene | AS a  | 54%                  | 32          | 344         | 187                  |
| 6          | naphthalene | FS b  | 52%                  | 32          | 321         | 149                  |
| 7          | naphthalene | AS a  | 53%                  | 32          | 342         | 348                  |
| 8          | naphthalene | FS b  | 51%                  | 32          | 295         | 369                  |

Implications. The intracellular ROS/RNS production and water-soluble OP were measured for naphthalene photooxidation SOA formed under humid conditions in the presence of metal-containing and inorganic seed. Experiments were conducted using different initial hydrocarbon concentrations to generate aerosol of differing mass loadings and degrees of oxidation. Multiple filters were also collected from a single experiment to obtain aerosol of different photochemical age. Cellular assay results show that exposure to FS seeded aerosol resulted in higher levels of ROS/RNS production compared to AS seeded aerosol. Furthermore, the ROS/RNS response may be largely attributed to the organic components rather than the metals portion. This has important implications for future studies as organic aerosol constitute a large fraction of ambient fine PM32,33. However, it should be noted that possible synergistic and/or antagonistic metal-organic interactions were not explored and only one metal species and volatile organic compound (VOC) were investigated in this study. Further studies are necessary to determine how metals and organics interact with each other and in the context of biologically-relevant species (e.g. proteins, sugars, and lipids present in the alveolar fluid). These interactions between co-exposed species may increase or decrease the overall cellular response77–79, and a thorough understanding of these dynamics are necessary to evaluate the health implications of ambient aerosol. Results from this study also highlight the differences between chemical and cellular assays. There were no obvious trends between OP values and aerosol bulk composition measured by the AMS, suggesting that the DTT assay may only be sensitive to large differences, such as that arising from different SOA precursors. The lack of correlation between OP and OSc is consistent with previous DTT studies, where a higher OSc did not correspond to a higher OP32,33,74.

An exponential trend was also observed between ROS/RNS levels and OSc for all naphthalene photooxidation SOA, including those formed in the presence of different seed particles (AS and FS), those formed under different formation conditions (dry vs. humid, RO 2 + HO 2 vs. RO 2 + NO), and those collected at different times over the course of a single experiment (different degrees of photochemical aging). There are several important implica-

Methods
Naphthalene aerosol generation. Naphthalene photooxidation SOA (naphthalene + hydroxyl (OH) radical) was generated under humid conditions in the presence of NO in the Georgia Tech Environmental Chamber.
tal conditions were also collected to account for potential H2O2 uptake onto seed particles since this may affect cellular exposure described in Tuet et al.22 with modifications for aerosol collection and extraction. Aerosol samples were collected at peak growth onto 47 mm Teflon™ filters (0.45 µm pore size, Pall Laboratory) for 1.6 hrs at a flow rate of 29 L min−1. The total mass collected on each filter was determined by integrating time-dependent SMPS volume concentrations over the filter collection period and multiplying the integrated value by the total volume of air collected. A density of 1 g cm−3 was assumed to facilitate comparison between studies, as SOA density varies with precursor identity and formation condition83–88. Background filters containing only seed (AS or FS), OH precursor (H2O2), and NO at experimental conditions were also collected to account for potential H2O2 uptake onto seed particles since this may affect oxidative potential and ROS/RNS measurements. After collection, filters were placed in sterile petri dishes, sealed with Parafilm M®, and stored at −20 °C until extraction and analysis23.

Collected filter samples were extracted following the procedure outlined in Fang et al.22 with modifications for integral water soluble oxidative potential as measured by DTT (OP) of naphthalene aerosol, method blanks, and positive controls (9,10-phenanthraquinone) were determined using a semi-automated DTT system, described in detail in Fang et al.22. Briefly, the method consisted of three major steps: (1) oxidation of DTT by redox-active species in the extract, (2) reaction of remaining DTT with 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) to form 2-nitro-5-mercaptobenzoic acid (TNB), and (3) measurement of TNB at 412 nm.

Intracellular ROS/RNS measurement. Murine alveolar macrophages (MH-S, ATCC®CRL-2019™) were cultured in RPMI-1640 media supplemented with 10% FBS, 1% penicillin-streptomycin, and 50 µM β-mercaptoethanol (BME) at 37 °C and 5% CO2. ROS/RNS were detected using the assay described in Tuet et al.20. The assay consisted of five steps: pre-treatment of 96-well plates with 10% FBS in phosphate buffered saline (PBS), (2) seeding of cells at 2 × 104 cells well−1, (3) incubation of cells with ROS/RNS probe (10 µM, carboxy-H,DCFDA, Molecular Probes C-400), (4) exposure of cells to samples and controls for 24 hrs, and (5) detection of ROS/RNS using a microplate reader (BioTek Synergy H4; ex: 485 nm, em: 525 nm). Positive controls included bacterial cell wall component, lipopolysaccharide (LPS, 1 µg mL−1), H2O2 (100 µM), and reference filter extract (10 filter punches mL−1, 1 per filter sample, from various ambient filters collected at the Georgia Tech site23; negative controls included blank filter extract and control cells (probe exposed cells to media only, no stimulants).

For each filter sample, intracellular ROS/RNS production was measured over ten doses to fully capture dose-response relationships (Fig. S9). At each dose, ROS/RNS levels were normalized to basal ROS/RNS production9 (i.e. ROS/RNS produced from probe-treated control cells) and corrected for changes in relative cellular metabolic activity96 (measured using MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, assay) (Biotium) prior to fitting dose-response curves. Area under the dose-response curve (AUC) was then used to represent ROS/RNS for comparison to chemical oxidative potential as AUC is the most robust metric for comparing different PM samples97.
Cellular metabolic activity. MTT was used to assess cellular metabolic activity post-exposure. Sample extracts were removed after the exposure period (24 hrs), replaced with media containing MTT, and returned to the incubator for 4 hrs. Dimethyl sulfoxide was then added to solubilize the insoluble purple salt formed from the reduction of the tetrazolium dye and the absorbance at 570 nm was measured using a microplate reader (BioTek Synergy H4).

Data availability. Data are available upon request to the corresponding author (ng@chbe.gatech.edu).

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Author Contributions
W.T., Y.C., and N.N. designed experiments. W.T., Y.C., S.F., and D.G. conducted experiments. W.T., Y.C., R.W., J.C., and N.N. analyzed and interpreted data. W.T. and N.N. wrote the manuscript. All authors reviewed the manuscript.

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