Replies and answers to reviews regarding manuscript "Measuring molecular singlet oxygen ($^1O_2^*$) from atmospheric photosensitizers: Intercomparison of techniques, irradiation setups, data analysis and protocol recommendations"

Reply Legend:
Black: reviewer comment
Blue: author response
Blue + italics: text modified

Reviewer 1:

Gemmell et al. compared singlet oxygen ($^1O_2^*$) measurements from four photosensitizers across four photoreactor setups at three institutions. Using their results, they determined the factors influencing the rates of light absorbance, $^1O_2^*$ steady-state concentrations, and quantum yields. Consequently, they made five recommendations to improve the accuracy and reproducibility of $^1O_2^*$ measurements, which include considering wavelength-dependent quantum yields, avoiding suppression of $^1O_2^*$, controlling and reporting photoreactor temperature, considering light scattering from nanoparticles, and conducting control experiments. These recommendations will help standardize $^1O_2^*$ measurements across different laboratories in studying photochemical processing of atmospheric aerosols and droplets. In general, this is a thorough and valuable intercomparison study. The authors have conducted rigorous control experiments and thoughtfully addressed potential artifacts. This manuscript makes a valuable contribution to standardizing $^1O_2^*$ measurements. The recommendations are practical and well-supported by the intercomparison data. However, there are some points that need clarification before acceptance for publication:

We thank the reviewer for this support and feedback.

Major Comments

- Scattering artifacts: The supplement's Section S8 and Figures S15-S17 address a previously underappreciated artifact. The sensitivity analysis showing up to 48.8% error in Rabs for lignin is compelling. However, I notice that the lignin absorbance before and after 0.22 μm filtration (Figure S15) shows negligible difference, yet the Mie scattering modeling (Figure S16) suggests significant scattering contributions. This apparent contradiction needs explanation in the main text. I suggest that the authors clarify that scattering can occur from particles smaller than the filter pore size, and that the absence of filtration effect does not rule out scattering artifacts. This nuance is important for readers who might incorrectly conclude that filtration alone solves the problem.

We thank the reviewer for highlighting this point. What the reviewer has stated is exactly the point we intended to convey, so we've clarified the text which now reads:

"Light scattering should be considered a source of uncertainty in UV–Vis absorbance measurements, even in filtered samples, as particles smaller than filter pore size (0.22 µm here) can still contribute to scattering and filtration alone will not necessarily eliminate this effect. Dissolved organic matter can form colloidal structures or aggregates smaller than the filter pore size, which remain in solution and can scatter light. The formation and optical properties of these colloids depend on the composition and source of the organic matter, and thus filtration does not guarantee removal of all scattering effects (Sec. S8, Fig. S15, S16) (Bieber et al., 2024)."

- UCD photon flux calibration (Section S11): This section reveals a significant methodological challenge that deserves more attention in the main text: The observation that "the ratio of the peak in photon flux at 548 nm to the peak at 347 nm varies from 2.0 to 5.2" across 60 measurements, and that they had to use perinaphthenone to constrain the long-wavelength flux. I suggest adding a brief discussion in Section 3.2.2 or 3.2.3 about the importance of validating spectrophotometer measurements with chemical actinometry or reference sensitizers, especially when internal reflections or positioning variability may affect spectral shape. This is a practical recommendation that would be useful for home- built photoreactors.

  Good point. We modified Section 3.2.2 to explicitly discuss this issue and indicate how we optimized the photon flux by combining measurements from the spectrophotometer, 2NB actinometry, and the perinaphthenone quantum yield for singlet oxygen. The entire section has been edited for clarity and flow, and to address the reviewers comment the following text was added:

  "Calculating absolute irradiance required combining the spectrophotometer measurements of relative photon fluxes as a function of wavelength with chemical actinometry. UCD found significant variability in the relative photon fluxes for measurements made on the same day but with different optical probe positions in the sample chamber (see SI Section S11). We believe that this variability was due to internal reflections within the UCD illumination chamber. To determine the most correct relative photon fluxes, we used experiments to determine the singlet oxygen quantum yield from perinaphthenone to constrain the 548 nm/347 nm intensity ratio, which we used as a marker of the photon fluxes at long and short wavelengths (SI Section S11 and Fig. S21). The combination of actinometry (to get the short-wavelength region) and a reference photosensitizer (to characterize the long-wavelength region) allowed us to constrain the UCD photon flux by identifying the influence of internal reflections on the spectral shape. By testing if experiments with two actinometers yielded equivalent photon fluxes, and by

ensuring that experiments with reference photosensitizers yielded published values, this tested the photon flux across a wide range of wavelengths. This highlights the utility of actinometry and model photosensitizers as robust tools to constrain the photon flux in an experimental illumination system."

- Temperature dependence: Figure S19 is particularly striking: the 30°C vs. 22°C experiments show dramatically different $[^1O_2^*]_{ss}$ even after applying temperature-corrected rate constants. This suggests that temperature affects not just the probe kinetics, but also potentially the sensitizer photophysics and/or $^1O_2^*$ production efficiency. The recommendation to control temperature within 20 to 25°C seems sound, but the manuscript should acknowledge that the underlying causes of this temperature sensitivity are still not fully understood and warrant further investigation. Also, the recommendation to control "within a minimum range of 20 to 25°C" is vague. What is the acceptable variability within an experiment? Across experiments? I suggest the authors consider providing quantitative guidance (e.g., ±1 °C, ±2 °C).

  Great points. The range of 20 to 25°C comes from the UBC photoreactor's design, which uses liquid N2 cooling through a copper coil, and makes it harder to control the temperature precisely. The temperature in the UBC photoreactor is also susceptible to environmental temperature in the laboratory. On the other hand, the cooling systems at UCD and Ircelyon use water cooling and are able to control within ±2 °C. However since temperature influences steady state concentrations of $^1O_2^*$ beyond what is captured in temperature corrected rate constants, we can only recommend temperature control that is practically viable for each experimental set up. To make this point more clear, the text has been updated to say:

  "We recommend that photoreactor setups be temperature controlled within a minimum range of 20–25 °C. This recommendation is based on practically achievable temperature control for photoreactor set ups depending on the cooling system. For example, UBC's liquid N2 through the copper coil system is less precise than UCD and Ircelyon's water cooling system. In addition, there are temperature effects beyond furfuryl alcohol's rate constant, likely involving impacts on the photophysics of the excitation (Fig. S18, S19)."

- Line 580: "[FFA]0 < 145 μM": I understand that this threshold comes from Ossola et al., but is it universally applicable? I think the appropriate concentration may depend on the photosensitizer's $^1O_2^*$ production rate and the light source intensity? I suggest the authors consider adding guidance on how to verify that probe scavenging is negligible for a given system.

We thank the reviewer for highlighting this point. Indeed, this concentration of FFA is not universally applicable and depends on the sinks of $^1O_2$* in the system. The main text has been updated to read:

*"2. **Chemical probe concentration:** We recommend using concentrations of furfuryl alcohol such that the reaction of $^1O_2$* with the probe accounts for less than 1% of the total $^1O_2$* loss, ensuring that the probe does not perturb steady-state $^1O_2$* concentrations. This condition can be evaluated by calculating the fraction of $^1O_2$* lost to FFA relative to other sinks (Sec. S12), which corresponds to [FFA]0 < 27 µM (at 25 °C)."*

The following text was added to the SI to clarify: "*When using the chemical probe FFA to quantify $^1O_2$* concentrations, it is essential to determine if the probe perturbs the steady-state $^1O_2$* concentration. This can be done by calculating the fraction of $^1O_2$* lost to FFA in a given experiment. The main $^1O_2$* sinks in dilute photosensitizing solutions include $^1O_2$* quenching by water ($k'_{^1O_2^*,H_2O} = 2.76(0.02)\times10^5\ M^{-1}\ s^{-1}$, Appiani et al. 2017), $^1O_2$* quenching by dissolved organic carbon (DOC, $k_{^1O_2^*+DOC} = 1\times10^5\ M^{-1}\ s^{-1}$, Ma et al., 2023), and $^1O_2$* reactions with FFA. The fraction of $^1O_2$* lost to FFA ($f_{^1O_2^*,FFA}$) can be calculated with*

$$f_{^1O_2^*,FFA} = \frac{k_{^1O_2^*+FFA}[FFA]}{k_{^1O_2^*+FFA}[FFA] + k'_{^1O_2^*,H2O} + k_{^1O_2^*+DOC}[DOC]}$$

where $k_{^1O_2^*+FFA}$ is the rate constant for the reaction of $^1O_2$ with FFA, [FFA] is the initial FFA concentration, and [DOC] is the concentration of dissolved organic carbon. In this work, DOC concentrations were small and therefore DOC is a negligible sink, though this is not always the case in concentrated particulate matter extracts. The system is considered unperturbed by the addition of FFA when the fraction of $^1O_2$* lost to FFA is less than 1% of the overall $^1O_2$* loss, which corresponds to an FFA concentration of less than 27 µM (at 25 °C). Ossola et al. (2021) have a similar, though less strict threshold of ensuring FFA incurs less than a 5% decrease in the steady-state $^1O_2$* concentration, corresponding to an FFA concentration of 145 µM (at 25 °C). If the probe concentration is greater than this threshold, then quenching will need to be accounted for (De Laurentiis et al., 2013; Frimmel et al., 1987)."*

- Line 225: The statement that "the triplet state of perinaphthenone does not react with furfuryl alcohol" is supported by Schmidt et al., but is this universally true across all conditions (pH, concentration ranges)? I suggest the authors consider adding a brief note about the conditions under which these holds.

The triplet state one-electron reduction potential of perinaphthenone is 1.03 V SHE, making it a weakly oxidizing triplet. Additionally, the triplet energy of perinaphthenone is 1.70 eV, which is also relatively low for oxidizing triplets (Mcneill & Canonica, *Environ. Sci.: Processes Impacts, 2016*). Finally, perinaphthenone has no acid base functionality so its triplet reactivity is not expected to exhibit a pH dependence. The text has been updated:

"Additionally, the triplet state of perinaphthenone does not react with furfuryl alcohol under typical experimental conditions (concentrations in the μM range) meaning that furfuryl alcohol loss reflects only reaction with singlet oxygen. This greatly simplifies steady-state and quantum yield calculations by enabling relative rate comparisons between singlet oxygen production and furfuryl alcohol consumption (Schmidt et al., 1994; Ossola et al., 2021). Finally, perinaphthenone possesses no acid-base functionality; therefore, its triplet reactivity is not expected to exhibit a pH dependence, further supporting its use as a robust reference photosensitizer."

- Relative vs. Absolute quantum yield methods: This paper presents both methods but it could more clearly guide readers on when to use each approach. The statement that values were "consistently 15% within each other" (line 458) is helpful, but what is the threshold for "acceptable" agreement? Providing more specific guidance will be useful.

  Since this is the first study to compare the relative and absolute quantum yields of singlet oxygen the only guideline we can provide is the agreement we observed for each individual photoreactor set up, which is 15%. Text was added to make this point more clear:
  "*... were consistently within 15% of each other for all sensitizers, and we thereby suggest 15% as a reasonable metric for acceptable agreement between quantification methods (Tables S1 - S4).*"

Specific Technical Comments

- Units' consistency: Table S1 reports R_{abs} for perinaphthenone in units of $\times 10^{-6}$ mol_{photon} L^{-1} s^{-1}, which is consistent with the main text Figure 4a. However, equation 1 in the main text gives units of $mol_{photons}$ cm^{-2} s^{-1}. This discrepancy should be resolved. There should be consistency between the equations, text, and figures.

  We thank the reviewer for noting this discrepancy. To resolve this discrepancy, equation 1 has been changed to give units of mol_{photon} L^{-1} s^{-1}. Now the text reads:

"$R_{abs} = \sum_{\lambda} (I_{\lambda,0} \cdot \alpha_{\lambda} \cdot \Delta\lambda \cdot 2.303 \cdot 10^3)$
where α_{λ} is light absorption coefficient of the sample (cm^{-1}}, baseline corrected), $I_{\lambda,0}$ is the spectral irradiance of the light source ($mol_{photons}\, cm^{-2}\, s^{-1} nm^{-1}$), $\Delta\lambda$ is the interval between adjacent wavelengths, and the values 2.303 and 10^3 are conversions for base and units, respectively."

- Blank controls (Section S1, Figure S1): All three labs show negligible FFA decay in blank controls, which is reassuring. However, the Ircelyon blank appears to show a slight downward trend. Is this within experimental uncertainty? Also, to avoid any confusion, I suggest renaming "Lyon Blank" in the figure's legend to "Ircelyon Blank".

  The reviewer is correct that the Ircelyon blank data show a slight downward trend (k_{obs} = 2.48 x 10^{-6} s^{-1}). However, this trend falls within experimental uncertainty. The slope of the blank measurement is a factor of 2 smaller than the smallest decay slope observed for a photosensitizer (Ircelyon lignin, k_{obs} = 5.08 x 10^{-6} s^{-1}), indicating that the trend is negligible relative to the measured photochemical signals.

  The figure legend has also been updated to read "Ircelyon Blank" to avoid any confusion.

Minor Comments

- Figure 5 caption: "Normalized to peak (i.e., peak value = 1)": The authors should consider adding "for each photoreactor setup individually" to clarify that normalization is per setup, not global.

  Thank you for flagging this unclear wording. The figure caption has been updated to read: *"Normalized to peak for each photoreactor setup individually (i.e., peak value = 1)"*

- Atmospheric implications section: This section is somewhat general. The authors should consider adding a more concrete example of how the recommendations would improve model parameterization.

  This section points explicitly to Zhang et al.'s modeling study of SOA processing by oxidants including $^1O_2$* and we highlight here how a key uncertainty in this modeling is the concentration of $^1O_2$* . We also point to Manfrin et al.'s lifetime calculations of key functional groups. We state that, "this work enables more robust inter-study comparisons and facilitates integration of $^1O_2$* chemistry into multiphase chemical models." and our

goal is here is to help constant the *concentration* of $^1O_2$* to expect from the photochemistry of BrC. Nevertheless, to help clarify the focus on concentration, we've added the following sentence:

"Ultimately, we need to better constrain the concentrations of $^1O_2$ and $^3C^*$ generated from different types of BrC to better predict aerosol photochemical aging."*

- Building a new photoreactor Section: This is interesting but feels disconnected from the rest of the main text. The authors should consider integrating it into the recommendations or moving to SI.

  We thank the reviewer for this valuable comment. We think it is a fair point, and have removed this section and incorporated it into the recommendations section. We've also added a reference to Niedek's et al.'s recent photochemical setup:

  "Short pathlength photoreactors have also recently been developed (Niedek et al., 2026), which reduce light attenuation in strongly absorbing solutions by minimizing optical pathlength while maintaining temperature control. "

- Recommendation 5 (Control experiments): The authors should consider stating that results from deoxygenated experiments should be interpreted cautiously since removing O_2 changes the system fundamentally (e.g., $^3C^*$ lifetime increases, other pathways may emerge). The absence of FFA decay in N_2-purged samples confirms no $^3C^*$ + FFA reaction, but I don't think it proves that $^3C^* + O_2 \rightarrow {}^1O_2^*$ is the only pathway in oxygenated conditions.

  We agree that results from deoxygenated experiments should be interpreted with caution, as the removal of O_2 alters the photochemical system, including increasing ^3C* lifetimes and potentially enabling alternative reaction pathways. We also agree that the absence of FFA decay under N_2 does not demonstrate that the $^3C^* + O_2 \rightarrow {}^1O_2^*$ pathway is the only mechanism contributing to FFA loss under oxygenated conditions. Rather, these control experiments primarily indicate that direct reaction between ^3C* and FFA is negligible in our system. To clarify this point, we have revised the manuscript to acknowledge that, while $^1O_2$* is expected to be the dominant oxidant under oxygenated conditions, additional pathways such as reactions involving ^3C*, •OH (even we added a quencher), or other reactive oxygen species, cannot be fully excluded.

  These changes have been incorporated in the revised manuscript as: *"Although it is not possible to completely isolate the reaction of $^1O_2$* with furfuryl alcohol, incorporating these control experiments ensures that measured probe decay can be primarily attributed*

to the intended reactive species. However, results from deoxygenated experiments must be interpreted with caution, as the removal of oxygen may fundamentally alter the system, for example by increasing $^3C^*$ lifetimes and allowing other reaction pathways to emerge. Nevertheless, these controls enhance the reliability and intercomparability of $^1O_2^*$ quantification."

- Section S8.2: The sentence "Using a particle concentration of 1.18×10^{13} m^{-3} and a mean particle diameter of 142 nm (values scaled from Bieber et al. (2024))". The term "scaled" implies modification. Please clarify whether these are directly from Bieber et al. or adjusted for this study. If adjusted, the authors should explain the scaling rationale.

  The term "scaled" was used to indicate that the particle concentration used for the scattering analysis was adjusted to account for dilution and to match the concentration of lignin used for our study (20 mg/L). The mean particle diameter is directly from Bieber et al. The text has been updated to read:

  "Using a particle concentration of 1.18×10^{13} m^{-3} (accounting for dilution to 20 mg/L of Lignin) and a mean particle diameter of 142 nm (Bieber et al., 2024) …"

- Section S11, line 86: "... perinaphthenone 1O2* quantum yield" There is a typo here (missing superscripts and subscripts). Should be "$^1O_2^*$".

  We thank the reviewer for flagging this typo. The superscripts and subscripts have been updated to the correct formatting.