**General Comments**

This study by Enarsson et al. investigates the interference and suppression of fluorescence from carbonaceous particles in reactive oxygen species (ROS) when analyzed using the BPEAnit probe in an online instrument.

The topic of this manuscript is of interest and relevance to AMT and is useful for improving the performance of online ROS measurements by reducing the effects of experimental artefacts. The intention and objective of the study are unique.

However, I have several concerns regarding the methodological approach the authors have employed to make their inferences. Some of the authors' interpretations are not directly derived from the results and need more clarity before they can be verified. I think the manuscript needs to be revised to address these concerns and shortcomings before I can recommend it for publication.

Dear Reviewer,

We would like to thank you for your valuable comments. Based on your suggestions and comments we have conducted series of additional measurements, which have led to revised results and modified conclusions. We present first a summary for quick overview, while below we have addressed all of your comments one by one.

Summary:

- We have performed new series of experiments investigating the probe-particle interactions using the same molar concentration of BPEAnit and BPEAnitMe. The trends and observed mechanisms remained the same, however the differences between BPEAnit-Me and BPEAnit (ROS attributed fluorescence intensity) have different values
- Additional filtration measurements (with and without particles) in BPEAniMe confirmed that absorption of light by particles present in solutions is not the main mechanism for the decrease in fluorescence intensity
- Due to particle-probe suppression, ROS generated by Pintex90 must be assessed after allowing the time for the reaction (1-3h), which is not captured by online methods.
- We suggest in parallel to online measurements, a collection of the particles on filters for subsequent offline ROS/OP assessment with correction for observed artefacts.
- A method (first approach) to correct for the carbonaceous particle-induced fluorescent decrease for offline ROS measurements with BPEAnit, namely by using a stable derivative of BPEAnit (i.e. BPEAnit-Me) is suggested
- Our results indicate that using BPEAnit as a probe for offline ROS and OP determinations is possible and should be explored

Once again thank you your valuable comments and insights. We have done our best to address them all and hope that you find that the manuscript improved. We have done additional experiments, included more references, and clarified our hypotheses and conclusions. Below, we present answers to all your comments point by point.

**Specific Comments**

- Page 2: Lines 84 – 86: Please cite a couple of papers that mention this interference or have quantified it while measuring OP or ROS. I don't think the four papers cited

by the authors in the earlier sentence give interference of insoluble particles as a reason for choosing water-soluble ROS/OP measurement approaches. To my knowledge, at least two of these papers are using an online instrument with microfluidic connections (Wragg et al. 2016 and Puthussery et al. 2018) or narrow flow cells (Utinger et al. 2023). Insoluble particles in their methods can, in turn, affect the results, likely by being deposited onto the microfluidic tubing, leading to clogging, or being difficult to clean when using a narrow flow cell, which may be possible reasons for those studies to not use insoluble PM. The authors have cited studies that have shown interference for non-ROS or non-OP measurements, but this still does not provide a justification as to whether insoluble particles interfere with OP measurements.

By mentioning instruments that remove insoluble particles, we wanted to emphasize that removing insolubles is common, despite the risk of underestimation of the results. However, the reason for the exclusion of such particles in ROS/OP is not stated clearly in the original papers. To keep the focus on the subject of the manuscript that sentence has now been removed. Further down in the introduction, we mention two studies where BC affects the measurements, in the following way:

*" The decrease in measured fluorescence when BC is present is in line with suggestions from other studies with different assays (Boyles et al., 2022; Utinger et al., 2025)."*

- Page 3: Lines 93 – 95; Authors are requested to substantiate the claim that BC concentrations are elevated in indoor environments due to candle burning. I don't fully agree with their assessments as several studies have reported high BC emissions from candles only when the candles are sooting and not burning cleanly. (Fine et al., 1999; Pagels et al., 2009; Stabile et al., 2012; Subramanian et al., 2025) The authors are expected to make this distinction as well, as clean combustion of a candle releases only a very small fraction of BC (Fine et al., 1999; Pagels et al., 2009; Stabile et al., 2012; Subramanian et al., 2025).

  This is a valid point, undisturbed (steady state) burning of candle results in emissions of a very small fraction of BC. However, steady state conditions of burning candles (undisturbed flame to ensure minimal emissions of BC) rarely occur in indoor environments (with air movements due to drafts, ventilation air movements, people movements). We have now clarified the sentence with the addition of the word sooting burn (*flickering flame)* and have added a couple of references, including Subramanian, 2025): It now reads:

  " *It is a ubiquitous component of atmospheric particulate matter and can also be found at elevated concentrations in indoor environments (e.g. from cooking or sooting burn (flickering flame) of candles) (Sankhyan et al., 2021; Subramanian et al., 2025)."*

- Line 139: Have the authors investigated if the insoluble particles are deposited in the tube, as it has a pretty small diameter? This may also lead to a reduction in fluorescence. The authors are requested to provide more details validating this.

Yes, at certain parts of the PINQ set up particles can deposit over time. It is, however, rare. To avoid this, the tubing is rinsed with solvent before and after each experiment, as well as the flow-through cell is manually cleaned at regular intervals, and the Teflon tubing is replaced. Moreover, all the results are adjusted to the fluorescence of particle-free probe solution, which is run before and after each experiment, and the average is used for the calculations. This allows us to identify issues that could emerge during the experiment (including deposition). It has been added to the manuscript as an additional explanation. In the section PINQ, we have added the sentence:

*" To prevent particle deposition, the tubing and the measurement cell are rinsed with solvent (DMSO) before and after each experiment. Additionally, the flow-through cell is manually cleaned, and the Teflon tubing is replaced at regular intervals."*

And in the section data analysis it now reads:

*" The particle-free references, $FI_{(BPEAnit)no\ particles}$ and $FI_{(BPEAnitMe)no\ particles}$ , were calculated as the average of measurements performed before and after each set of suspension measurements. These repeated measurements were also used to identify potential changes in instrument response, for example due to particle deposition in the measurement cell. "*

- Lines 186 - 191: This is not a strong enough analogy for selecting these concentrations without providing more details on the manuscript in preparation. 150 µg/m$^3$ of BC is very high and is likely a concentration obtained from chamber experiments, I believe. These concentrations are environmentally relevant except on rare occasions such as maybe during wildfires, or during highly polluted wintertime PM in China and India.

  Moreover, I am not sure how the authors converted the mass concentrations in the air to liquid concentrations. It seems to be that 1 mL of liquid corresponds to 16.67 L of Air according to this statement. How did the authors come about this number? BC only constitutes a small fraction of ambient PM (5 - 20%). Moreover, the study the authors cite is not a study using PM in real-world DCFH-DA, albeit one using nanoparticles.

  Concentrations quoted are from previous chamber experiments assessing ROS in emissions from indoor sources, that formed the basis of this study. We now provide additional details about the study in the appendix, including air sampling flow, which explains the conversion between liquid concentrations and mass concentrations in the air. During PINQ measurements, we sample the air at 16.7 L/min, and the continuous liquid sample flow is 1 mL/min, meaning that the particles sampled during one minute are collected in 1 mL sample liquid.

  We have also improved the initial introduction of the previous chamber study, and improved consistency in referencing the study throughout the revised manuscript.

Our study focuses on the interaction between Printex 90 and the probe used in PINQ for ROS measurements, to explain the artefact observed during chamber studies in candle smoke. The concentration used during our experiments was chosen to: match the concentration during the chamber studies, match the concentrations during offline DCFH assay, and be high enough to allow for a clear effect to emerge. For the revised manuscript, we have also included new measurements with lower particle concentrations (down to 0.25 µg/mL, corresponding to 16 µg/m3), in order to investigate a potential detection threshold of the artefact. We still saw suppression at this concentration. Therefore, we claim that our study is valuable, as the concentrations even if in a higher range, can still be encountered during laboratory emission studies, laboratory and field assessment of combustion sources, extreme pollution events, occupational exposure assessment in workplaces, or measurements in indoor environments. Moreover, the described effect itself has potential to be relevant for environmental monitoring. One example, that is mentioned in the Discussion, is the study by Stavanovic et al. (2019), that found non-linear effects at BC concentration above 3 µg/mL. For offline DCFH studies that does not follow the standard operating procedure described by Boyles (2022), the particle concentration could vary, but we do claim that it is likely that the artefact we describe could occur, since particle concentrations from 1 to 10 µg/mL are commonly used (Foucaud et al. 2007).

- The authors are requested to cite relevant studies of ambient PM using DCFH-DA or other assays and requested to verify if the concentrations of BC they used are environmentally relevant. I think 25 µg/mL of Printex 90 is a very high concentration that may not be environmentally relevant.

  In the discussion section, we are discussing the relevance for ambient measurements. We claim that interference could be highly relevant for lab and chamber experiments, occupational exposure, and that caution should be used for ambient studies. However, this study is focused on the effect of BC on fluorescence, and even though 25 µg/mL is higher than what is expected in chamber measurements, it is relevant when comparing to the offline DCFH assay (where such concentrations are used). We have also focused on lower concentrations (going down to 0.5 µg/mL and 0.25 µg/mL). We would like to emphasize that it was an exploratory study to understand observed artefacts. The higher concentration allows us to more clearly see the effects from the particle-probe interaction. However, we do not suggest using concentrations as high as 25 µg/mL in our correction method, since the depletion of the probe could elicit unreliable results.

- Lines 229 – 235: Please be more specific about the chamber experiments and add more details about the methodology in the SI. What were the concentrations of SOA and soot in the final liquid that was analyzed using fluorescence? In Figure 1, the intensity in the first peak with SOA is only 20% of the background. Were these experiments performed in triplicate, and the LoD of the measurement ascertained? What was the sampling time point for this measurement: 5 mins, 30 mins, 1h, 2 h, 3h, or 24 h?

We have added in the revised manuscript additional information about the previous chamber experiments assessing ROS in emissions from indoor sources, that formed the basis of this study. We improved initial introduction of the previous study, added details in Appendix and improved consistency in referencing the study throughout the manuscript. While Figure 1 (Figure 2 in the revised manuscript) shows results from the real-time PINQ measurements during the chamber experiment, it is only meant to illustrate the fluorescence suppression (observed artefact) during actual chamber ROS measurements, which were the starting point for our investigation. It is based on two-hour measurements, where data were obtained every 30 seconds. The results shown are average of the measurement period. Additional information on the chamber study was added to SI as suggested. In the revised manuscript, we have now added this additional information as the first paragraph in the Appendix. We have also added an extra panel to Fig. 2, showing the particle-bound ROS attributed response, where the negative values of candle smoke are highlighted.

The experiments were performed in triplicate. The reason for the relatively low increase from the control is the combination of the high concentration and the quantum yield of BPEAnit. By design, the BPEAnit probe is in excess (hence the high concentration) during PINQ operation, and despite having a low quantum yield in its pro-fluorescent state, the total fluorescence becomes significant. Any reaction between a fraction of the available BPEAnit and ROS increases the fluorescence. The illustrated response is significantly higher than the LoD (approximately 500 counts). In PINQ measurements, the reading is done continuously within a minute after the aerosol is mixed with the probe solution.

- Lines 243 – 244: DMSO is not fluorescent. Printex 90 particles are likely not fluorescent in the presence of only DMSO, as they need a reducing agent to oxidize and release ROS. Therefore, I am not sure about the inference the authors are trying to make for this statement.

  We understand the questioning of this addition. We have now removed this figure and focus on describing experiments on the filtered suspensions instead. We performed tests with three different concentrations of Printex90 in BPEAnit-Me (Table A1 and Fig A1). The results show very little difference in fluorescence between filtered and unfiltered suspensions.

- Lines 245 – 250: Why were these measurements only performed at 5 µg/mL Printex concentration and not at all three concentrations? In table A2, the relative decreases (1 – observation) would be 45 and 42%, respectively, instead of 55 and 58%. And are the authors referring to Printex 90 while mentioning particles or different particles?

  We have now clarified that the particles were Printex 90. Thank you for the correction regarding the table, it has now been changed. We have performed additional tests and added the results from 1 µg/mL and 25 µg/mL (in triplicates) as Figure A1 and Table A1 in the revised manuscript.

- Line 251: The authors are requested to be specific that they are referring to Printex 90 particles and not SOA or candle emissions here.

We have now improved the sentence by exchanging “particles” with “Printex 90”.

- Lines 270 – 275 and figure 3: Have the authors statistically substantiated their claim that the fluorescence of BPEAnit increases with sampling time? To me, the error bars are overlapping, and the replicate size (n =3) is too small to make this inference statistically significant. Please validate if this statement is statistically significant.

  Thank you for the suggestion of including significance. We will check statistical significance as pointed out and add it in the revised manuscript

- Why are these changes in lines 275-280 for 5 µg/mL of Printex 90 very different from those in Appendix 2?

  The filtration experiment was not done within 5 minutes after mixing Printex 90 with the BPEAnit-Me solution. Longer residence time gives a lower fluorescent response (increased suppression). In the revised manuscript, we have changed the concentration of BPEAnit-Me to 4 µM for comaprisons, and 130 nM of BPEAnitME has been used for the filter experiments.

- Lines 280 – 287: Have the authors validated whether the normalization at no particles holds true in the presence of particles? The authors mention that BPEAnit-Me is non-reactive in line 25, so it is likely that their quantum yields may not be the same at no particle vs. particle conditions.

  We do not think that the quantum yields would change at these conditions. Previously, we have tried similar experiments with a completely different type of particles (PSL) and saw no change in fluorescence at all.

- Lines 313 – 318: I am not sure if the authors can make the statement that the ab/adsorption are similar between the two probes without validation.

  It is an assumption made from the similarities between the molecules (only one bond changed) and experience based on earlier work when the probe was developed and assessed. We have expanded description of the chemical structure of the probes in introduction and added Fig 1 that shows the structural similarity of the probes.

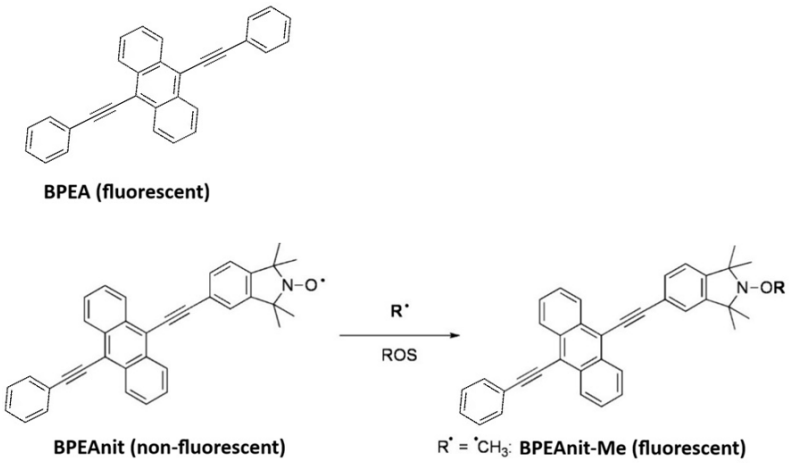


**Figure 1.** Adapted from Elkaee et al., 2025: Chemical structure and fluorescence description of BPEA, BPEAnit, and BPEAnit-R, including the reaction with ROS that elicits BPEAnit-R (primarily BPEAnit-Me).

Additional experiments have been performed (Fig A3 in the revised manuscript) that strengthens this assumption. In these experiments, we show clear similarities between BPEAnit-Me and BPEA (the aromatic, fluorophore part of BPEAnit), indicating that it is the fluorophore part of the molecule that is responsible for the interactions with the particle surface~~.~~ We emphasize that this is an assumption when applying the equations in the revised manuscript. The consistent lower fluorescence from BPEAnit, compared to BPEAnit-Me, in presence of particles, need to be validated and explored further.

- Line 335: I am not sure if a 5% decrease makes the fluorescence decrease evident. Are these differences statistically significant?

  We have now included more experiments at lower particle concentrations, to examine if there is a threshold were we cannot see any effect from the particles. The differences are even smaller, and statistical significance will be checked and included in the manuscript.

- Lines 358 – 362: I think the correction factor should not be 0.63, but rather [0.63/0.83 = 0.76] as only 84% of the BPEAnit fluorescence remains at 5 mins.

The explanation for not including the removal of BPEAnit in the equation is that, by design, BPEAnit should always be in excess when reacting with particle-bound ROS. This is to ensure that the fluorescent response is not related to ROS concentration in the measured sample.

- Conclusions: Adsorption/Absorption were not measured to conclude those as reasons for the reduction in fluorescence.

  This is correct, thank you for this comment. Ad/absorption is just one proposed mechanism of reduction of fluorescence. We had no possibility to measure adsorption, therefore, we have changed the wording throughout the manuscript from *ad/absorption* to *fluorescent suppression* or *interaction*. We explain that adsorption is one example of a possible reduction mechanism.