Interactive comment on

"Light-induced protein nitration and degradation with HONO emission"

by Hannah Meusel et al.

Anonymous Referee #3

This MS reports on HONO formation resulting (mostly) from the interaction of NO2 with a particular protein under visible illumination in a flow tube reactor. The HONO released to the gas phase is formed both by photolysis of nitrated tyrosine and a Langmuir-Hinshelwood surface reaction involving NO2 uptake; this latter process forms HONO even in the dark. For bot dark and illuminated channels, there is a positive dependence on RH which suggests that water is involved somehow, although this may be by changing the protein surface morphology rather than as a chemical promoter. The experiments are well constructed and the results are of some interest. I do have a few comments for the authors' consideration, however:

Comment:
page 5, lines 28-29: I am not convinced that you have demonstrated nitration with the very small signal reported.

Response:
The nitration degree was determined by HPLC-DAD as described elsewhere (Selzle et al., 2013). This technique is sensitive and well established (detection limit < 1%). The difference of the nitration degree of native BSA (ND = 0%) and BSA treated with NO_2/light (ND = 1%) is significant. Yes, it is a small nitration degree, but still nitration was detected!
Now modified in the main text (page 5 lines 25-26): "…nitration degree…by means of HPLC-DAD was (1.0±0.1)%., significantly higher than the ND of untreated BSA (0%)"

Comment:
page 6, lines 3-5: Again, this is one possible inference, but is certainly not conclusively shown!

Response:
We tune down the tone and it now reads, "…possibly *suggesting the deficiency…"*

Comment:
page 6, section 3.2.1: this experiment is very poorly described - please explain exactly what was done.

Response:
The method part (2.1 and 2.2) describes the procedure of the experiments and gives an overview on conditions… (table 1). In this experiment previously nitrated OVA (method part) was coated on a tube and irradiated with light (0,1,3,7 lights) while flushing with either zero air or 20 ppb NO_2. HONO emissions

were detected at the outlet. After the trace gas exchange measurements the protein was extracted by pure H2O and nitration degree was determined via HPLC-DAD.
Now modified in the main text: "To study HONO emission from nitrated proteins, OVA was nitrated with TNM (see section 2.1) in liquid phase. The nitrated OVA (2 mg; ND = 12.5%) was coated onto the reaction tube and exposed to VIS lights under either pure nitrogen flow or 20 ppb NO_2 gas. Strong HONO emissions were found…"

Comment:
Page 6, line 32-33: Could this be related to the photodecomposition of the protein, reposted above?

Response:
Yes indeed, as it was already stated in the main text: "…which is in line with the observed decomposition of the native protein presented above."

Comment:
Sections 3.2.2 and 3.2.3: Brigante et al (J. Phys. Chem. A 2008, 112, 9503–9508) made these same observations.

Response:
Brigante et al., (2008) observed a linear dependency of NO_2 loss (ln c0/c) to light intensity (number of photons) for the NO_2 uptake on pyrene. Furthermore, they plotted NO_2 uptake coefficient as function of NO_2 concentration and shows an exponential (decay) dependence. They found that roughly 15% of the NO_2 loss on pyrene accounts for HONO production. Both cannot be directly compared to our results ("saturation" of HONO formation at high light intensities and very high NO_2 concentration).
However, Brigante is now additionally cited when discussing similarities to other studies.

Comment:
Page 8, line 19-20: On what basis do you claim that nitration / reaction takes place below the surface layer?

Response:
Indeed, the dependency of layer thickness on the HONO formation is a complex matter. Light penetrates into the bulk (according to the set-up illumination is from outside - light will first pass the protein layer at the inner glass surface and then the layer in contact with the carrier gas) and hence activation of the aromatic residues of the protein and photolysis of nitrated proteins can occur in the bulk. Also NO_2 might diffuse into the bulk (depending on humidity and therefore viscosity/solid or semi-solid state), and the formed HONO would also be able to diffuse out of the bulk. But we didn't mean to say that the reaction takes place only below the surface. Our point is that the observed dependence on the coating thickness suggests the Indeed, the dependency of layer thickness on the HONO formation is a complex matter. Light penetrates into the bulk (according to the set-up illumination is from outside - light will first pass the protein layer at the inner glass surface and then the layer in contact with the carrier gas) and hence activation of the aromatic residues of the protein involvement of bulk reactions and the reactions can happen in both, surface and bulk phase.
We added one more conclusively sentence to the manuscript: "The observed dependence on the coating thickness suggests the involvement of the bulk reactions, but the reactions can happen in both, surface and bulk phase."

Comment:
page 8, line 28, ff: Brigante et al (2008) also saw no RH dependence for NO2/HONO on solid pyrene.

Response:
Now additionally cited in the manuscript: "No impact of humidity on NO_2 uptake coefficients on pyrene was detected (Brigante et al., 2008)"

Comment:
page 10, Eq. 1 and kinetic arguments: Why is the desorption reaction not included here? The implication of the L-H mechanism, suggested in Fig 5, is that this should be important. The kinetic scheme should reflect this, I think.

Response:
To simplify the calculations, the reversible processes were neglected. In addition, the adsorption of HONO to the protein surface is supposed to be very small in relation to the desorption as proteins are slightly acidic (please see respective comments/reply of referee #1)
Modifications in the manuscript accordingly to referee #1: the equations of the single processes (eq.1-5) were removed to a new supplement and only the final equation is shown.

Comment:
page 11, lines 17-23: This paragraph seems out of place here; perhaps in the Conclusions? In its place - can the authors in any way (semi)quantify their suggestion that HONO production via NO2/protein interactions could be atmospherically important?

Response:
Paragraph moved to section 3.2.1 (page 7, lines 3-9)
See also referee 2 conclusion section (page 13, lines 10-17)

Comment:
The figure captions are not very descriptive. They should be rewritten, to explain what is displayed in the figures.

Response:
The figure captions were modified:
The term "normalized HONO" (several y-axes) was changed to "scaled HONO".

Fig. 1: Overview on possible reaction mechanisms of atmospheric BSA nitration and subsequent HONO emission. The tyrosine phenoxyl radical intermediate is either formed by the reaction of tyrosine with a) NO_2, b) light or c) ozone. A second reaction with NO_2 forms 3-nitrotyrosine (was adapted from Houée-Levin et al. (2015) and Shiraiwa et al. (2012)) Subsequent intramolecular H-transfer initiated by irradiation decompose the protein and HONO is emitted (adapted from Bejan et al., 2006).

Fig. 2: Flow system and set-up: thin blue lines show the flow of the gas mixture, which direction is indicated by the grey triangles of the mass flow controllers (MFC). Nitrogen passes a heated water bath to humidify the gas and a HONO scrubber to eliminate any HONO impurities of the NO_2 supply. The overflow provides a stable pressure through the reaction tube and the detection unit. The dotted boxes (blue, green, orange) indicate the three different parts, the gas supply, reaction unit and detection unit.

Fig. 3: Light enhanced HONO formation from TNM-nitrated proteins (n-OVA: ND 12.5%, coating 21.5 µg cm^{-2}). Black squares indicate HONO formation via decomposition from nitrated proteins (without NO_2) while red squares indicate additional HONO formation via heterogeneous NO_2 conversion (20 ppb NO_2) at 50% RH (HONO is scaled to the HONO concentration measured without NO_2 and no light ($[HONO]_{lights;\ NO2}/[HONO]_{dark;\ NO2=0}$)) .

Fig. 4: Light induced HONO formation on BSA. a) HONO formation under alternating dark and light conditions on BSA surface (22.5 µg cm^{-2}), yellow shaded areas indicate periods in which 7 VIS lamps were switched on (RH = 50%, NO_2 = 20 ppb); b) Dependency of HONO formation on radiation intensity at 20 ppb NO_2 and 50% RH (BSA = 31.4 µg cm^{-2}). The experiment started with 7 VIS lights switched on, sequentially decreasing the number of lights (red symbols, nominated 1-4), prior to apply the initial irradiance again (blue symbol, 5). HONO was scaled to the HONO concentration in darkness ($[HONO]_{lights}/[HONO]_{dark}$). Error bars indicate standard deviation of 20-30 min measurements, standard deviation of point 5 covers 2.75 h measurement.

Fig. 5: Comparison of HONO formation dependency on NO_2 at different organic surfaces. HONO concentrations are scaled to the HONO concentration at 20 ppb NO_2 ($[HONO]_{NO2}/[HONO]_{NO2=20ppb}$). Red square = BSA coating (16 µg cm^{-2}) at 161 W m^{-2} and 50% RH (this study), blue triangles pointing up = humic acid coating (8 µg cm^{-2}) at 162 W m^{-2} and 20% RH (Stemmler et al., 2006), dark blue triangles pointing down = humic acid aerosol with 100 nm diameter and a surface of 0.151 m^2 m^{-3} at 26% RH and $1x10^{17}$ photons cm^{-2} s^{-1} (Stemmler et al., 2007), black circles = gentisic acid coating (160-200 µg cm^{-2}) at 40-45% RH and light intensity similar as in the humic acid aerosol case (Sosedova et al., 2011), green diamonds = ortho-nitrophenol in gas phase (ppm level) illuminated with UV/VIS light. Dotted lines are exponential fittings of the measured data points and are guiding the eyes.

Fig. 6: HONO formation on three different BSA coating thicknesses, exposed to 20 ppb of NO_2 under illuminated conditions (7 VIS lamps). The HONO concentrations were scaled to reaction tube coverage (black: 100% of reaction tube was covered with BSA, blueish: 70% of tube was covered and red: 50% of tube was covered with BSA). The middle thick coating (22.46 µg cm^{-2}) was replicated and studied with different reaction times (cyan and blue triangle). Solid lines (with circles or triangles) present continuous measurements, when those are interrupted other conditions (e.g. light intensity, NO_2 concentration) prevailed. Dotted lines show interpolations and are for guiding the eyes. Arrows indicate the intervals in which the shown decay rates were determined. Error bars indicates standard deviations from 10-20 measuring points (5-10 min).

Fig. 7: Dependency of relative humidity on HONO formation. 25 ppb NO_2 was applied on BSA surface (17.5 µg cm-2) either in darkness (blue triangle) orat 7 VIS lights (red star). HONO was scaled to HONO concentrations in darkness under dry conditions ($[HONO]_{lights\ on\text{-}off;\ RH}/[HONO]_{dark;\ RH=0}$). Dotted lines are for guiding the eyes.

Fig. 8: Extended measurements (20 h) of light-enhanced HONO formation on BSA (three coatings of 17.5 µg cm^{-2}) at 80% RH, 100 ppb NO_2. HONO formation under VIS light is shown in red and orange, under UV/Vis light in blue. HONO decay rates [ppt h^{-1}] are shown with time periods (in brackets) in which they were calculated, suggesting a stable HONO formation after 4 hours. Right: zoom in on the first 2 hours. Straight lines (black, grey, light and dark blue) show the slopes of which d[HONO]/dt were used in the kinetic studies.

References:

Brigante, M., Cazoir, D., D'Anna, B., George, C., and Donaldson, D. J.: Photoenhanced uptake of NO2 by pyrene solid films, The Journal of Physical Chemistry A, 112, 9503-9508, 10.1021/jp802324g, 2008.

Selzle, K., Ackaert, C., Kampf, C. J., Kunert, A. T., Duschl, A., Oostingh, G. J., and Poschl, U.: Determination of nitration degrees for the birch pollen allergen Bet v 1, Analytical and Bioanalytical Chemistry, 405, 8945-8949, 10.1007/s00216-013-7324-0, 2013.