Interactive comment on

“Light-induced protein nitration and degradation with HONO emission”

by Hannah Meusel et al.

Anonymous Referee #1

General Comments:
This manuscript reports results of a study aimed at investigating photochemical formation of HONO from proteins exposed to NO2. The study employs coated wall flow tube techniques with LOPAP detection of HONO and chemiluminescence detection of NO2. The methods are appropriate for such a study and the results appear to meet the standards required by ACP. The topic is important as it addresses the byproducts associated with light-induced nitration of protein aerosols (e.g., pollen and other biological aerosols); it is novel in that it attempts to address the photochemical fate of the nitrated proteins.

The relevance of protein nitration to the potency of allergens has been discussed in several publications, so that is clear. However, it is not so clear that nitrated proteins will be an important component of the daytime HONO budget since proteinaceous aerosols would constitute only a minor fraction of the total aerosol surface area in the atmosphere. Furthermore, strong evidence has recently surfaced showing that the daytime HONO source is not linked to NO2 (see Pusede et al. Environ. Sci. Technol. 2016). In addition, there are limited situations where the aerosol phase has proved to have an impact on atmospheric HONO concentrations. Perhaps the authors could add a more extensive discussion of settings where they predict this chemistry to be important? Regardless, it is my opinion that the chemistry presented is interesting enough to warrant publication after these issues are addressed.

Response:
Pusede et al., 2015 (Environ. Sci. Technol., 49, 12774-12781, 2015) observed no significant weekday-weekend difference in HONO levels during daytime, while NO2 levels changed significantly during weekday-weekend and concluded that HONO didn’t derive from NO2. Several studies didn’t find correlations with NO2, but much more publications see a correlation with NO2 and an enhanced correlation with NO2 J (e.g. Costabile et al., 2010; Spataro et al., 2013; Sörgel et al., 2011 + 2015; Su et al., 2008; Lee et al., 2016), both being in line with our observations presented in here. The absence of NO2-HONO correlation does not exclude the involvement of NO2 conversion in HONO. Whatever the detailed mechanism is, there are many complex processes involved in aerosol particles and on the ground surface that could lead to a highly non-linear dependence on NO2 in both concentration and time domains (HONO precursors may be stored in reservoirs, both in the physical and chemical senses). Besides heterogeneous photochemistry on aerosols also heterogeneous photoenhanced NO2 conversion on ground surfaces has been proposed (Ren et al., 2011; Laufs et al., 2017). As proteins are found in both aerosol particles (coarse and fine mode) as well as on most ground surfaces (soil, leaf etc.), we think that their widespread occurrence provides reasonable justification to have a closer look into the characteristics of their HONO emissions. Indeed, we agree with the referee that pinning down their impact in individual settings is crucial, but for the time being, too uncertain to make a strong statement in here.

Comment:
Page 1, line 20: The authors write that “nitration degrees of about 1% were derived applying NO2 concentrations...” How was the nitration degree determined?

Response:
The nitration degree is defined as the concentration of nitrated tyrosine divided by the concentration of all tyrosine residues. As it is written in the method part (2.1.), the nitration degrees were determined by HPLC-DAD analysis.
Nitrated tyrosine residues were detected at 357 nm (and 280 nm) while tyrosine residues were detected at 280 nm only.
The respective section in the method part of the manuscript (page 4 lines 13-15) was slightly modified:
“Absorbance was monitored at wavelengths of 280 (tyrosine) and 357 nm (nitrotyrosine). The sample injection volume was 10-30 μL. Each chromatographic run was repeated three times. The protein nitration degree, which is defined as the ratio of nitrated tyrosine to all tyrosine residues, was determined by the method of Selzle et al. (2013). Native and un-treated BSA did not show any degree of nitration. “

Comment:
1, 21: The term “Gas exchange measurements of TNM-nitrated proteins” is ambiguous.
Response:
Now corrected in the manuscript (page 1, lines 21-22) to: “Measurements of gas exchange on TNM-nitrated proteins…”

Comment:
1, 23: The term “fumigation” is not appropriate here. Please replace.
Response:
Now corrected to “NO₂ exposure…”

Comment:
3, 22-24: I note that nitrated ovalbumin (OVA) was used in only one experiment in this study (section 3.2.1) while bovine serum albumin (BSA) was used for everything else. Ideally, one would use one protein for all the studies to facilitate comparison of results. Please explain why one protein was not used for everything.
Response:
Unfortunately, only nitrated OVA but no nitrated BSA was available from our partner.

Comment:
3, 32: The methods section indicates that tetranitromethane is used to nitrate the OVA samples. This is a highly toxic and explosive reagent. Appropriate warnings should be included in this section to bring awareness of the dangers of using this reagent to anyone wishing to repeat these experiments.
Response:
Although in most other publications safety notes/warnings of toxic chemicals are not mentioned, we acknowledge the advice and now added a respective note in the manuscript (page 3, line 34-35):
“Please note that TNM is toxic if swallowed, can cause skin, eye and respiration irritation, is suspected to cause cancer and causes fire or explosion.”

Comment:
9, 33 (and other places in the text, e.g. 10, 14): The term “catalytic converter” is an engineering term and is not appropriate in this context. I would replace with “catalytic surface”.
Response:
Now corrected in the text according to the referee’s suggestion.

Comment:
10, 6: It is not clear what ND refers to in this line. Please clarify.
Response:
ND refers for nitration degree as in the whole manuscript. This abbreviation was introduced when first mentioned in the manuscript on page 5 lines 19/20 (original manuscript). The nitration degree is defined as the concentration of nitrated tyrosine divided by the concentration of all tyrosine residues (see also comment above).

Comment:
10, 27: It seems to me the term \([\text{HONO}]1 + [\text{HONO}]2\) is incorrect. Instead of indicating concentrations, should one not be using rates (i.e., \(d[\text{HONO}]1/dt + d[\text{HONO}]2/dt\))?

Response:
Thank you very much for noting, indeed that’s the case. Now corrected in the text as suggested by the referee and moved to a new supplement (see comment below).

Comment:
Kinetics studies section: The derivation of some of the indicated terms is not so clear. I question the need to go into the level of detail displayed in eq. 1-5. Please check over the derivation of \(k_{\text{eff}}\). Also, perhaps I missed this explanation, but why are the reversible reactions in Figure 9 not included?

Response:
To simplify the calculations, the reversible processes of \(\text{NO}_2\) were neglected (\(k_1\) would be the effective rate constant for the adsorption; including adsorption and desorption). 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 (similar to \(k_1, k_3\) would be the effective rate constant including desorption and adsorption).
Details are now displayed and discussed in a new supplement.

Comment:
Figure 1: Ozone is included above the arrow in the first step. However, there is no indication that ozone was used in this study. Please clarify or correct.

Response:
True, in our study only \(\text{N}_2\) was applied as a carrier gas (no \(\text{O}_3\)). Fig. 1 is meant to give a complete overview of possible nitration mechanisms and refers to another study on nitration of proteins with \(\text{O}_3\) and \(\text{NO}_2\) (Shiraiwa et al., 2012 as indicated in the caption).
Figure 1 caption was modified: “Overview on possible reaction mechanism…”

Reference:


Anonymous Referee #2

Overview

In this paper, titled “Light-induced protein nitration and degradation with HONO emission” by Meusel et al., the authors present an interesting dataset focused on the uptake of NO2 and subsequent emission of HONO by protein surfaces. HONO is an important reservoir for OH radicals and NOx, but very little is known about its formation and subsequent photochemistry on the surface of aerosol particles, which represent a significant amount of reactive surface area in the atmosphere. Therefore, the topic is very much atmospherically relevant. Based on a series of flow tube experiments, the authors find a dependence of NO2 uptake and subsequent emission of HONO on light intensity, relative humidity, NO2 concentration, and flow tube coating thickness. The authors argue that surface-enhanced NO2 conversion to HONO follows a Langmuir-Hinshelwood reaction mechanism. While I find the topic to be of general interest to the community, I have several concerns regarding the experimental approach and interpretation, and therefore request that the authors make significant revisions to their manuscript before publication in ACP after considering my comments listed below.

Comment:

Section 3.1 (lines 22-23): The authors indicate that additional continuous exposure of the protein surface by light fully decomposed the protein so that no intact protein could be detected. However, the authors should clarify if only the nitrated protein residues decompose or all (nitrated and non-nitrated), and how that might affect ND.

Response:

The nitration degree in this study was detected with HPLC-DAD which only can detect the intact protein and the nitrated protein, but no possible degradation products like peptides, single amino acids and their nitrated forms, as those compounds are “filtered out” by the chromatography. According to our HPLC-DAD results also the non-nitrated proteins decomposed, i.e., the peak was below detection limit. If only nitrated-proteins decompose, the results would indicate that all proteins were firstly nitrated prior of being decomposed. Amino acids and peptides might still be present, either nitrated or not.
Now better specified in the main text of the manuscript (page 5, line 27): “Note that no intact protein (nitrated and non-nitrated) could be detected by HPLC-DAD after another 20 hours of irradiation without NO₂, indicating light induced decomposition of proteins.”

Comment:
Could the authors discuss the atmospheric implications of the irradiance intensity applied in this study compared to the solar irradiance intensity? They mention that their irradiance was 40% of clear sky conditions, similar to cloudy days, so does that imply that this chemistry could be more relevant in the atmosphere than the results suggest? Please elaborate.

Response:
There are two different processes to discuss: 1) the degradation of nitrated proteins with HONO formation and 2) the heterogeneous NO₂ conversion. As shown in Fig.3 the light dependency of HONO formation from previously nitrated proteins is almost linear in the range of applied light intensity. So here, yes during sunny days, when irradiance is higher than our applied light intensity, an even higher HONO formation can be anticipated, accompanied by a faster degradation of the nitrated proteins. However, as the dependency for higher light intensities was not investigated, we cannot make a firm statement here. The observed light dependency of the heterogeneous conversion of NO₂ on BSA was not linear as shown in Fig. 4b. Here our results rather indicate an upper limit for the HONO formation, as was reported similarly by Stemmler et al. (2006, 2007).

Now added in the conclusion (page 13, line 4-7): “While heterogeneous HONO formation of BSA exposed to NO₂ revealed light saturation at intensities higher than 161 W m⁻², the HONO formation from previously nitrated OVA was linearly increasing over the whole light intensity range investigated. The latter let assume even higher HONO formation under sunny (clear sky) ambient atmospheric conditions.”

Comment:
In the VIS light wavelength range of the lamps used in this study (between 400 nm and 700 nm), NO₂ photolysis could be significant and play an important role in the degree of protein nitration and HONO production. Was NO₂ photolysis a concern and how might it affect the results?

Response:
Direct NO₂ photolysis (<400 nm) won’t occur under conditions applied in this study (Gardner et al., 1987; Roehl et al., 1994). There might be some electronic excitation of NO₂, which disproportionate to NO and NO₃. Stemmler et al., 2007, determined a photolysis frequency of NO₂ of up to 5 x 10⁴ s⁻¹ for very similar light conditions as we used, which is much lower than in the atmosphere (e.g. up to 1 x 10⁻² during the CYPHEX campaign 2014, Meusel et al., 2016). But NO₃ will probably directly deplete under this irradiation back to NO₂ or NO (Johnston et al., 1996). NO₂ + hv \rightarrow NO + O(3P) (< 400 nm)
NO₂ + hv \rightarrow NO₂⁺ (+NO₂) \rightarrow NO₃ + NO (>410 nm)
NO₃ + hv \rightarrow NO₂ + O(3P) (~580 nm)
NO₃ + hv \rightarrow NO + O₂ (~ 600 nm)

Overall we assume the effect to be negligible. Furthermore, Shiraiwa et al. (2012) could exclude the importance of NO₃ (in their case formed by reaction of NO₂ + O₃) uptake on BSA.
Now added in section 3.1 (page 6, lines 16-23): “Shiraiwa et al. (2012) performed kinetic modelling and found that maximum 30% (conservative upper limit) of N-uptake on BSA could be explained by NO₃ or N₂O₅, which are generated by the reaction of NO₂ and O₃, while overall nitration was governed by an indirect mechanism in which a radical intermediate was formed by the reaction of BSA with ozone, which then reacted with NO₂. On NaCl surface N-uptake was dominated by NO₂ and N₂O₅. Furthermore, NO₃ radicals, which in this study could be formed by photolysis of NO₂ (>410 nm, disproportionation of excited NO₂), are not stable under the light condition applied (400-700 nm) (Johnston et al., 1996). Therefore, in the present study reactions with NO₃ were neglected. Photolysis of NO₂ forming NO (< 400 nm) can also be neglected (Gardner et al., 1987; Roehl et al., 1994). A photolysis

\begin{align*}
\text{NO}_2 + \text{hv} & \rightarrow \text{NO} + \text{O}(3\text{P}) (< 400 \text{ nm}) \\
\text{NO}_2 + \text{hv} & \rightarrow \text{NO}_2^+ (+\text{NO}_2) \rightarrow \text{NO}_3 + \text{NO} (>410 \text{ nm}) \\
\text{NO}_3 + \text{hv} & \rightarrow \text{NO}_2 + \text{O}(3\text{P}) (~580 \text{ nm}) \\
\text{NO}_3 + \text{hv} & \rightarrow \text{NO} + \text{O}_2 (~ 600 \text{ nm})
\end{align*}
frequency for NO$_2$ of up to 5 x 10$^4$ s$^{-1}$ under similar experimental light conditions was determined by Stemmler et al., 2007.”

Comment:
In the last paragraph of the results section 3.1, the authors compare their results, which were conducted in the presence of NO$_2$, with other nitration studies conducted in the presence of both NO$_2$ and O$_3$. How are these comparable, since NO$_2$ and O$_3$ combine to make N$_2$O$_5$ and NO$_3$, which is a much more effective nitrating agent? The authors argue that their low ND may be due to light exposure, whereas the studies with larger ND that they compare to were conducted in the dark in the presence of NO$_3$, so wouldn’t the authors expect more ND in the other studies anyway because of the higher reactivity of NO$_3$?

Response:
Shiraiwa et al. (2012) estimated that maximum 30% of the N-uptake is due to NO$_3$ and N$_2$O$_5$ uptake on BSA, while overall nitration was governed by an indirect mechanism in which a radical intermediate was formed by the reaction of BSA with ozone, which then reacted with NO$_2$. On NaCl surfaces, on the other hand, NO$_3$ and N$_2$O$_5$ uptake dominate. Therefore, the higher ND of BSA exposed to O$_3$ and NO$_2$ is mainly due to higher activation by O$_3$ and due to BSA decomposition by light. Please also see the comment above.

Comment:
Section 3.2.4: The authors conclude that HONO production is greater for larger protein coating thicknesses. However, the coatings also covered different surface area of the flow tube. Do you expect surface area to be important in the context of this study? My concern is that by shortening the coated length of the flow tube for the thicker coating experiments, the authors potentially introduce bias in their measurement since both NO$_2$ and HONO are exposed to different coated surface areas of the flow tube. Following NO$_2$ uptake by the shorter coated length flow tube, the HONO that is emitted is subsequently exposed to less coated surface area for the remaining length of the flow tube. If a fraction of the HONO is taken up by the protein surface, less protein surface area implies more of the HONO is present in the gas phase. A better approach would have been to either maintain the same length of coated flow tube between experiments or to maintain the same surface concentration of protein between experiments for different coated lengths. The authors should at least discuss potential caveats for changing the coated surface area of the flow tube between experiments.

Response:
As we manually coated the reaction tube, it was difficult to obtain equally/consistent surfaces. Therefore, each coating was different and also the covered surface area could only be roughly estimated. So, we agree with the referee that the coating thickness/surface is the biggest uncertainty in the experiment. And yes, there might be a bias based on NO$_2$/HONO uptake/emission on/from different coating surface areas. But we expect that HONO uptake coefficients on both proteins (as slightly acidic) as well as on glass surfaces were small (Syomin and Finlayson-Pitts, 203), so that the difference of HONO uptake due to different surface areas and covered tube length is low. Also NO$_2$ uptake on glass is supposed to be significantly lower than on proteins. We don’t expect a difference in tube coverage of 20% would increase HONO concentrations about three times.
According to the referee’s suggestion, we now added in the main text (page 8, lines 19-24): “Exposing (20%) different coated surface areas in the flow tube, potentially introduced bias comparing different data sets. Emitted HONO might be re-adsorbed differently by proteins and glass surface. However, as the protein is slightly acidic, a low uptake efficiency of HONO by BSA can be anticipated, which should not differ too much from the un-covered glass tube surface (Syomin and Finlayson-Pitts, 2003). Accordingly, NO$_2$ uptake on glass is assumed to be significantly lower than on proteins.”
Comment:
The rate of HONO emission decay as a function of exposure time as presented in Fig. 6 is also a bit confusing; the authors report emission decay rates in the range of 10-20 ppt hr⁻¹, but it is difficult to tell from the y-axis since [HONO] is reported in ppb. It would help if the y-axis and reported rates had the same concentration units. The authors might also consider changing their y-axis to a log scale or plotting the red data points on a separate y axis, so the reader can better observe the decay for different time periods. However, it appears the rate is more on the order of 160 ppt hr⁻¹ (linearly interpolated between 0 and 3 hrs). Why were the HONO emission decay rates only reported near the end of the exposure period (assuming the reported rates cover the exposure periods indicated by the arrows in Fig. 6)?

Response:
Fig. 6 does not show emission rates as a function of time, but normalized (to reaction tube coverage) HONO concentrations vs time! The numbers in the diagram represent the slope (decay rates) at the end (time period indicated by arrows), and indicate a stable HONO formation (as also seen in Fig.8). In figure 8 also several decay rates are shown for earlier exposure times, so that in the respective figure 6 the decay rates are only shown in the end when the concentrations are stable. The unit of the y axis was changed from ppb to ppt.

Comment:
Given the apparent strong dependence on coating thickness, how relevant are the thicknesses of the coatings applied to the flow tube (>200 nm) compared to typical atmospheric aerosol? The authors should at least discuss the implications of coating thickness and HONO formation in the context of atmospheric aerosol particles.

Response:
Typical aerosol concentrations of bacteria, fungal spores and pollen are 0.1, 0.1-1 and 1 µg m⁻³, respectively (Despres et al., 2012). Aerosol particles may contain up to 5% proteins. But it is not known how much proteins cover the aerosol surface nor how thick this coating would be. This was already mentioned in the conclusion part of the manuscript; and which is why it is hard to make a firm statement here. However, to address this important issue we now added in the text (page 13, lines10-17): “Typical aerosol surface concentrations in rural regions are about 100 µm² cm⁻³. Stemmler et al. (2007) estimated a HONO formation of 1.2 ppt h⁻¹ on pure humic acid aerosols in environmental conditions. As NO₂ uptake coefficients and HONO formation rates on proteins are similar to humic acid but only about 5% of the aerosol mass can be assumed to consist of proteins, it can be anticipated that HONO formation on aerosol is not a significant HONO source in ambient environmental settings. However, proteins on ground surfaces (soil, plants etc.) might play a more important role. Accordingly, Stemmler et al. (2006 and 2007) suggested that NO₂ conversion on soil covered with humic acid would be sufficient to explain missing HONO sources up to 700 ppt h⁻¹.”

Comment:
Section 3.2.6: Have the authors considered to what extent photolysis of HONO (in the case of the UV/VIS experiment) plays in the temporal evolution of the HONO concentration? The authors argue that the plateau in the HONO concentration in Fig. 8, followed by continuous and relatively stable emission of HONO from the protein surface is consistent with a Langmuir-Hinshelwood reaction mechanism. However, HONO photolyses under UV conditions (300 nm < λ < 400 nm), so might there be a point when the temporal HONO emission profile becomes limited by photolysis? The authors might consider including a photolysis term in their kinetics calculation (for both NO₂ and HONO), e.g. d[NO₂]s/dt = k₁[NO₂]g – j(NO₂)[NO₂]g and d[HONO]g/dt = k₃[HONO]s – j(HONO)[HONO]g.

Response:
HONO photolysis was not considered. The overlap of UV light spectrum and HONO absorption/photolysis spectrum is quiet low about 340-400 nm. The quartz glass tube has a transmission of 90% at these wavelengths. The applied light intensity (with 7 lights on) is about 40% of a clear sky irradiance for a solar zenith of 48°. In clear sky HONO
photolysis frequencies are in the range of $1.2\text{-}1.5 \times 10^3\text{ s}^{-1}$ (e.g., on Cyprus in summer 2014; Meusel et al. 2016). In the reaction tube the photolysis frequency would therefore decrease down to $0.4\text{-}0.5 \times 10^3\text{ s}^{-1}$. When only irradiated with VIS lights (exclusion of HONO photolysis, emission profile not limited) the pattern is the same as with UV (only a smaller absolute concentration) indicating a stable formation.

Now revised in the manuscript in the kinetic section (page 11, line 34 – page 12 line 3): “In this study, neither HONO nor NO$_2$ photolysis is considered, as the overlap of the applied UV/VIS or VIS range (340-700 nm or 400-700 nm) and the HONO and NO$_2$ photolysis spectrum (<400 nm) is low. Furthermore, the applied light intensity is lower compared to clear sky irradiance and the respective UV light is partly absorbed by the reaction tube although quartz glass was used (transmission ~ 90%) and the photolysis frequency would decrease down to $10^4\text{ s}^{-1}$. Hence, the photolysis is assumed to be not significant.”

Comment:
Section 3.3 and Fig. 8: a) Here, it appears the authors apply a series of kinetic equations to describe the temporal HONO emission profile shown in Fig. 8 based on Langmuir-Hinshelwood reaction kinetics. First, it is unclear if the lines plotted on top of the “UV/VIS” blue line in Fig. 8 are actually based on the kinetic equations described in section 3.3 or if they are simply linear fits with no theoretical basis, because in the figure description it states, “Straight lines: : :show the regressions: : :” If they are simply linear fits and then the kinetic terms were derived from the linear regression, my concern is this introduces significant ambiguity in the derived kinetics terms, because then the choice for each modeled section is entirely dependent on the user and not based on a sound theoretical description. Please clarify in both the Fig. 8 description and in sec. 3.3 whether these are simply linear fits or modeled based on the kinetic equations described in sec. 3.3. Furthermore, the authors must clarify what values were used (or derived from the linear fit) for k$_1$, k$_2$, k$_3$, k$_4$, k$_5$, and k$'$.

b) As a sensitivity test and validation of their model, I encourage the authors to apply their derived kinetic terms to model [HONO] as a function of [NO$_2$], as shown in Fig. 5. Can [HONO] as a function of [NO$_2$] be reproduced from the Langmuir–Hinshelwood terms described in sec. 3.3? Regarding Fig. 5, what do the dashed lines represent, are they fits to the data or just there to guide the eye? Please clarify in the figure description.

c) Alternatively, the authors could plot their derived uptake coefficients (instead of [HONO]) as a function of time, and apply the Langmuir–Hinshelwood framework, e.g., as described in Ammann et al. [2003]. This would also enable derivation of key kinetic terms describing NO$_2$ uptake by proteinaceous aerosol surfaces, including the Langmuir equilibrium constant, surface accommodation coefficient and second-order surface reaction rate constant, which the community might find useful.

Response:
a) The lines in fig 8 are linear fits (no theoretical basis). The slopes of those were taken to calculate k$_{\text{eff}}$. Other rate constants (k$_1$, k$_2$, k$_3$, k$_4$, k$_5$, k$'$) were not calculated. Single equations were moved to a new supplement.

b) In fig 5, the dotted lines are regressions of the measured data points (exponential fittings, e.g., $y = y_0 + A * e^{-x/t}$) only to guide the eye (now better described in the figure captions).

In our kinetic study we calculated an effective rate constant for the NO$_2$ conversion on BSA. In a range of 0-100 ppb NO$_2$ the HONO formation is almost linear (fig 5), which would be also indicated by the Langmuir-Hinshelwood mechanism (here first rate: $d[\text{HONO}] / dt = k*[\text{NO}_2]$). 

c) It is not possible to extract a full set of parameters for a LH model based on the present data. As pointed out in Bartels-Rausch et al. (2010) and to some degree also in the Stemmler et al. studies, the saturating behavior of photochemical HONO production may be due to either the adsorbed precursor on the surface or due to a photochemical competition process, which also leads to a Lindemann-Hinshelwood type kinetic expression (Minero, 1999). Therefore, mathematically, the rate expressions get a comparable NO$_2$ pressure dependence. Therefore, measurements of the NO$_2$ dependence at different light intensities would be required to disentangle the two. The nearly single exponential (linear in the log-log plot) decay of gamma vs time in the figure below (fig. R1) indicates that the system is governed by degradation and not by reaction steady state, so that modelling the system explicitly in terms of all the kinetic parameters would be ambiguous.
Added to the manuscript (page 12, lines 24-28): “It was not possible to extract a set of parameters for a Langmuir Hinshelwood mechanism (like Langmuir equilibrium constant, surface accommodation coefficient or second order rate constant) from the presented data. The saturating behavior of photochemical HONO production may be due to either the adsorbed precursor on the surface or due to a photochemical competition process, which also leads to a Lindemann-Hinshelwood type kinetic expression (Minero, 1999).”

Comment:
Have the authors considered the impact of photolysis of adsorbed HNO₃ on the production of HONO in this study? HNO₃(ads) + h_ν → HONO + O
Given the high relative humidity and [NO₂], HNO₃ adsorption or formation on the surface of the flow tube could be substantial. While there was some mention in the introduction that HONO production from the photolysis of HNO₃ may be important on organic substrates and soot, it was not discussed in the context of this study. The authors might consider estimating the contribution of adsorbed HNO₃ photolysis to HONO produced in their flow tube experiments. Adsorbed HNO₃ could be estimated based on the applied relative humidity and [NO₂] (and assuming some reasonable surface coverage of HNO₃), and the photolysis rate of HNO₃, e.g., as determined in a very recent study by Laufs and Kleffmann [2016].

Response:
Gas phase reaction doesn’t produce HNO₃, because N₂, but not synthetic air was used as the carrier gas. Usually HNO₃ photolysis happens at < 350 nm. Photolysis of adsorbed HNO₃ might be shifted to slightly higher wavelength. In the publication of Laufs and Kleffmann (2016), J values (HNO₃→HONO) as low as 10⁻⁷ s⁻¹ were obtained. Our UV lamps had a spectral range of 340-400 nm. As a conclusion, HNO₃ photolysis was negligible in this study.
Added to the section 3.2.6(page 10, lines 18-23), as only here UV light was applied: “HONO formation by photolysis of (adsorbed) HNO₃ is assumed to be insignificant in this study. With N₂ as carrier gas, gas phase reactions of NO₂ do not produce HNO₃. Even when small amounts of HNO₃ would be formed by unknown heterogeneous reactions, photolysis of HNO₃ is only significant at wavelengths < 350 nm, which is close to the lowest limit of the UV wavelength applied in this study. Likewise, the respective photolysis frequency recently proposed by Laufs and Kleffmann (2016) of about 2.4 x 10⁻⁷ s⁻¹ is very low.”

Minor Comment:
Page 6, lines 8-9: It’s not clear what the authors mean by “condensing condition” at a relative humidity (RH) of 98%, but not at 92%? Does this mean that the protein undergoes deliquescence at RH=98% and not 92%?
Response:
At 98% RH water vapor condensed (visible water layer), but not at lower RH of 92% (Reinmuth-Selzle et al., 2013). Deliquescence of BSA already occurs at 35% (see section 3.2.4).

Minor Comment:
*Figure 6:* Along with the surface concentration of the coating (in units of \( g \, cm^{-2} \)), please include the calculated thickness of the coating in units of \( nm \).

Response:
According to the referee’s advice, we now added the layer thickness in the plot:

![Figure R2](image)

Fig. R2 = new Fig. 6: HONO concentration vs exposure time for different coating thicknesses.

Minor Comment:
Summary and conclusions section, page 11 line 34: What is the significance of 1m² of BSA surface or how was that surface area chosen?

Response:
HONO formation per m² [ppt h⁻¹ m⁻²]

Now the main text of the manuscript(page 13, lines 2-4) is rephrased to: “At 20 ppb NO₂ HONO formation of 19.8 ppb h⁻¹ m⁻² could be estimated”

References


**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 both 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 NO2/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…

Comme
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\textsubscript{2} loss (ln c0/c) to light intensity (number of photons) for the NO\textsubscript{2} uptake on pyrene. Furthermore, they plotted NO\textsubscript{2} uptake coefficient as function of NO\textsubscript{2} concentration and shows an exponential (decay) dependence. They found that roughly 15% of the NO\textsubscript{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\textsubscript{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\textsubscript{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:

Response:
Now additionally cited in the manuscript: “No impact of humidity on NO2 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 phenoxy radical intermediate is either formed by the reaction of tyrosine with a) NO2, b) light or c) ozone. A second reaction with NO2 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 NO2 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]\(_{\text{lights}}\) \(\text{NO}_2\)/[HONO]\(_{\text{dark},\text{NO}_2=0}\)) -.

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]\(_{\text{lights}}\)\(\text{NO}_2\)/[HONO]\(_{\text{dark}}\)) . 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]\(_\text{NO}_2\)/[HONO]\(_{\text{NO}_2=20ppb}\)). Red square = BSA coating (16 µg cm\(^{-2}\)) at 161 W m\(^{-2}\) and 50% RH (this study), dark blue triangles pointing up = humic acid coating (8 µg cm\(^{-2}\)) at 162 W m\(^{-2}\) and 20% RH (Stemmler et al., 2006), 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 indicate 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) or at 7 VIS lights (red star). HONO was scaled to HONO concentrations in darkness under dry conditions ([HONO]\(_\text{lights-off} \text{RH}/[\text{HONO}\text{dark RH=0}]\)). 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:
Light-induced protein nitrination and degradation with HONO emission

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Abstract. Proteins can be nitrated by air pollutants (NO\textsubscript{2}), enhancing their allergenic potential. This work provides insight into protein nitration and subsequent decomposition in the presence of solar radiation. We also investigated light-induced formation of nitrous acid (HONO) from protein surfaces that were nitrated either online with instantaneous gas phase exposure to NO\textsubscript{2} or offline by an efficient nitration agent (tetranitromethane, TNM). Bovine serum albumin (BSA) and ovalbumin (OVA) were used as model substances for proteins. Nitration degrees of about 1\% were derived applying NO\textsubscript{2} concentrations of 100 ppb under VIS/UV illuminated condition, while simultaneous decomposition of (nitrated) proteins was also found during long-term (20h) irradiation exposure. Measurements of gas exchange measurements of TNM–nitrated proteins revealed that HONO can be formed and released even without contribution of instantaneous heterogeneous NO\textsubscript{2} conversion. However, fumigation with NO\textsubscript{2} exposure was found to increase HONO emissions substantially. In particular, a strong dependence of HONO emissions on light intensity, relative humidity (RH), NO\textsubscript{2} concentrations and the applied coating thickness were found. The 20 hours long-term studies revealed sustained HONO formation, even if concentrations of the intact (nitrated) proteins were too low to be detected after the gas exchange measurements. A reaction mechanism for the NO\textsubscript{2} conversion based on the Langmuir-Hinshelwood kinetics is proposed.

1 Introduction

Primary biological aerosols (PBA), or bioaerosols, including proteins, from different sources and with distinct properties, are known to influence atmospheric cloud microphysics and public health (Lang-Yona et al., 2016; D’Amato et al., 2007; Pummer et al., 2015). Bioaerosols represent a diverse subset of atmospheric particulate matter that is directly emitted in form of active or dead organisms, or fragments, like bacteria, fungal spores, pollens, viruses, and plant debris. Proteins are found ubiquitously in the atmosphere as part of these airborne, typically coarse-size biological particles (diameter $> 2.5 \mu m$), but also in fine particulate matter (diameter $< 2.5 \mu m$) associated with a host of different constituents such as polymers derived from biomaterials and proteins dissolved in
hydrometeors, mixed with fine dust and other particles (Miguel et al. 1999; Riediker et al., 2000; Zhang and Anastasio, 2003). Proteins contribute up to 5% of particle mass in airborne particles (Franze et al., 2003a; Staton et al., 2015; Menetrez et al., 2007) and are also found at surfaces of soils and plants. Proteins can be nitrated and are then likely to enhance allergic responses (Gruijthuijsen et al., 2006). Nitrogen dioxide (+NO₂) has emerged as an important biological reactant and has been shown to be capable of electron (or H atom) abstraction from the amino acid tyrosine (Tyr) to form TyrO• in aqueous solutions (tyrosine phenoxyl radical, also called tyrosyl radical; Prütz et al. 1984 and 1985; Alfassi 1987; Houée-Lévin et al., 2015), which subsequently can be nitrated by a second NO₂ molecule. Shiraiwa et al. (2012) observed nitration of protein aerosol, but not solely with NO₂ in the gasphase, and demonstrated that simultaneous O₃ exposure of airborne proteins in dark conditions can significantly enhance NO₂ uptake and consequent protein nitration (3-nitrotyrosine formation) by way of direct O₃-mediated formation of the TyrO• intermediate. A connection between increased allergic diseases and elevated environmental pollution, especially traffic-related air pollution has been proposed (Ring et al., 2001). Tyrosine is one of the photosensitive amino acids and it is subject of direct and indirect photo-degradation under solar-simulated conditions (Boreen et al., 2008), especially mediated by both UV-B (λ 280–320 nm) and UV-A (λ 320–400 nm) radiation (Houée-Levin et al., 2015; Bensasson et al., 1993). Direct light absorption or absorption by adjacent endogenous or exogenous chromophores and subsequent energy transfer results in an electronically-excited state of tyrosine (for details see Houée-Lévin et al. 2015 and references therein). If the triplet state of tyrosine is generated, it can undergo electron transfer reactions and deprotonation to yield TyrO• (Fig.1, Bensasson 1993; Davies 1991; Berto et al., 2016). Regardless of how the tyrosyl radical is generated, it can be nitrated by reaction with NO₂, but also hydroxylated or dimerized (Shiraiwa et al., 2012; Reinmuth-Selzle et al., 2014; Kampf et al., 2015).

With respect to atmospheric chemistry, Bejan et al. (2006) have shown that photolysis of ortho-nitrophenols (as is the case for 3-nitrotyrosine) can generate nitrous acid (HONO). HONO is of great interest for atmospheric composition, as its photolysis forms OH radicals, being the key oxidant for degradation of most air pollutants in the troposphere (Levy, 1971). In the lower atmosphere, up to 30% of the primary OH radical production can be attributed to photolysis of HONO, especially during the early morning when other photochemical OH sources are still small (R1, Kleffmann et al., 2005; Alicke et al., 2002; Ren et al., 2006; Su et al., 2008; Meusel et al. 2016).

\[ HONO \rightarrow OH + NO \quad (hν = 300 - 405 \text{ nm}) \]  (R1)

HONO can be directly emitted by combustion of fossil fuels (Kurtenbach et al., 2001) or formed by gas phase reactions of NO and OH (the backwards reaction of R1) and heterogeneous reactions of NO₂ on wet surfaces according to R2. On carbonaceous surfaces (soot, phenolic compounds) HONO is formed via electron or H transfer reactions (R3 and R4-R6; Kalberer et al., 1999; Kleffmann et al., 1999; Gutzwiller et al., 2001; Aubin and Abbatt 2007; Han et al., 2013; Arens et al., 2001, 2002; Ammann et al., 1998, 2005).

\[ 2NO_2 + H_2O \rightarrow HONO + HNO_3 \]  (R2)

\[ NO_2 + \{C-H\}_{red} \rightarrow HONO + \{C\}_{ox} \]  (R3)

\[ ArOH + NO_2 \rightarrow ArO^· \cdot +HONO \]  (R4)

\[ ArOH + H_2O \rightarrow ArO^- + H_3O^+ \]  (R5)

\[ ArO^- + NO_2 \rightarrow NO_2^- + ArO \rightarrow H_3O^+ \rightarrow HONO + H_2O \]  (R6)
Previous atmospheric measurements and modeling studies have shown unexpected high HONO concentrations during daytime, which can also contribute to aerosol formation through enhanced oxidation of precursor gases (Elshorbany et al., 2014). Measured mixing ratios are typically about one order of magnitude higher than simulated ones, and an additional source of 200-800 ppt h\(^{-1}\) would be required to explain observed mixing ratios (Kleffmann et al., 2005; Acker et al., 2006; Sörgel et al., 2011; Li et al., 2012; Su et al., 2008; Elshorbany et al., 2012; Meusel et al., 2016) indicating that estimates of daytime HONO sources are still under debate. It was suggested that HONO arises from the photolysis of nitric acid and nitrate or by heterogeneous photochemistry of NO\(_2\) on organic substrates and soot (Zhou et al., 2001; 2002 and 2003; Villena et al., 2011; Ramazan et al., 2004; George et al., 2005; Sosedova et al., 2011; Monge et al., 2010; Han et al., 2016). Stemmler et al. (2006, 2007) found HONO formation on light-activated humic acid, and field studies showed that HONO formation correlates with aerosol surface area, NO\(_2\) and solar radiation (Su et al., 2008; Reisinger, 2000; Costabile et al., 2010; Wong et al., 2012; Sörgel et al., 2015) and is increased during foggy periods (Notholt et al., 1992). Another proposed source of HONO is the soil, where it has been found to be co-emitted with NO by soil biological activities (Oswald et al., 2013; Su et al., 2011; Weber et al., 2015).

In view of light-induced nitration of proteins and HONO formation by photolysis of nitro-phenols, light-enhanced production of HONO on protein surfaces can be anticipated, which, to the best of our knowledge, has not been studied before.

This work aims at providing insight into protein nitration, the atmospheric stability of the nitrated protein, and respective formation of HONO from protein surfaces that were nitrated either offline in liquid phase prior to the gas exchange measurements, or online with instantaneous gas phase exposure to NO\(_2\), with particular emphasis on environmental parameters like light intensity, relative humidity (RH) und NO\(_2\) concentrations. Bovine serum albumin (BSA), a globular protein with a molecular mass of 66.5 kDa and 21 tyrosine residues per molecule, was chosen as a well-defined model substance for proteins. Nitrated ovalbumin (OVA) was used to study the light-induced degradation of proteins that were nitrated prior to gas exchange measurements. This well-studied protein has a molecular mass of 45 kDa and 10 tyrosine residues per molecule.

2 Materials and methods

2.1 Protein preparation and analysis

BSA (albumin from bovine serum, Cohn V fraction, lyophilized powder, ≥ 96%; Sigma Aldrich, St. Louis, Missouri, USA) or nitrated OVA (ovalbumin) was solved in pure water (18.2MΩ cm) and coated onto the glass tube.

The nitration of ovalbumin (OVA) was described previously (Yang et al., 2010; Zhang et al., 2011). Briefly, OVA (Grade V, A5503-5G, Sigma Aldrich, Germany) was dissolved in phosphate buffered saline PBS (P4417-50TAB, Sigma Aldrich, Germany) to a concentration of 10 mg/ml. 50 µl tetraniitromethane TNM (T25003-5G, Sigma Aldrich, Germany) dissolved in methanol 4% (v/v) were added to a 2.5 ml aliquot of the OVA solution and stirred for 180 min at room temperature. Please note that TNM is toxic if swallowed, can cause skin, eye and respiration irritation, is suspected to cause cancer and causes fire or explosion. Size exclusion chromatography columns (PD-10
nle injection volume was 10 µL. The Na$_2$CO$_3$-impregnated firebrick granules were put into a 0.8 cm inner diameter and 15 cm long glass tube, which was closed by quartz wool plugs on both sides. A constant total flow (1400 mL min$^{-1}$) was provided by means of another N$_2$ mass flow controller (MFC2) that compensated for changes in NO$_2$ addition. Different fractions of total surface areas (50, 70 and 100%) of the reaction tube (50 cm x 0.81 cm i.d.) were coated with 2 mg BSA or nitrated OVA, respectively. Therefore 2 mg protein was dissolved in 600 µL pure water, injected into the tube and then gently dried in a low humidity N$_2$ flow (RH ~ 30-40%) with continuous rotation of the tube. The coated reaction tube was exposed to the generated gas mixture and irradiated with either (i) 1, 3 or 7 VIS lights (400-700 nm; L 15 W/954, lumilux de luxe daylight, Osram, Augsburg, Germany) which is 0, 23, 69 or 161 W m$^{-2}$ respectively or (ii) 4 VIS and 3 UV lights (340-400 nm; UV-A, TL-D 15 W/10, Philips, Hamburg, Germany).

2.2 Coated-wall flow tube system

Figure 2 shows a flowchart of the set-up of the experiment. NO$_2$ was provided in a gas bottle (1 ppm in N$_2$, Carbagas AG, Grümligen, Switzerland). NO$_2$ was further diluted (mass flow controller, MFC3) with humidified pure nitrogen to achieve NO$_2$ mixing ratios between 20 and 100 ppb. Impurities of HONO in the NO$_2$-gas cylinder were removed by means of a HONO scrubber. The Na$_2$CO$_3$ trap was prepared by soaking 4mm firebrick in a saturated Na$_2$CO$_3$ in 50% ethanol / water solution and drying for 24 hours. The impregnated firebrick granules were put into a 0.8 cm inner diameter and 15 cm long glass tube, which was closed by quartz wool plugs on both sides. A constant total flow (1400 mL min$^{-1}$) was provided by means of another N$_2$ mass flow controller (MFC2) that compensated for changes in NO$_2$ addition. Different fractions of total surface areas (50, 70 and 100%) of the reaction tube (50 cm x 0.81 cm i.d.) were coated with 2 mg BSA or nitrated OVA, respectively. Therefore 2 mg protein was dissolved in 600 µL pure water, injected into the tube and then gently dried in a low humidity N$_2$ flow (RH ~ 30-40%) with continuous rotation of the tube. The coated reaction tube was exposed to the generated gas mixture and irradiated with either (i) 1, 3 or 7 VIS lights (400-700 nm; L 15 W/954, lumilux de luxe daylight, Osram, Augsburg, Germany) which is 0, 23, 69 or 161 W m$^{-2}$ respectively or (ii) 4 VIS and 3 UV lights (340-400 nm; UV-A, TL-D 15 W/10, Philips, Hamburg, Germany).

An overview of the experiments performed during this study is shown in table 1. Light induced decomposition of nitrated proteins was studied on OVA. Instantaneous NO$_2$ transformation and its light- and RH- dependence on heterogeneous HONO formation were studied on BSA in short-term experiments. Extended studies on BSA were performed to explore the persistence of the surface reactivity and respective catalytic effects.

A commercial long path absorption photometry instrument (LOPAP, QUMA) was used for HONO analysis. The measurement technique was introduced by Heland et al. (2001). This wet chemical analytical method has an
unmatched low detection limit of 3-5 ppt with high HONO collection efficiency (≥ 99%). HONO is continuously
trapped in a stripping coil flushed with an acidic solution of sulfanilamide. In a second reaction with n-(1-
naphthyl)ethylenediamine-dihydrochloride an azo dye is formed, whose concentration is determined by absorption
photometry in a long Teflon tubing. LOPAP has two stripping coils in series to reduce known interferences. In the
first stripping coil HONO is quantitatively collected. Due to the acidic stripping solution, interfering species are
collected less efficiently but in both channels. The true concentration of HONO is obtained by subtracting the
interferences quantified in the second channel from the total signal obtained in the first channel. The accuracy of the
HONO measurements was 10%, based on the uncertainties of liquid and gas flow, concentration of calibration
standard and regression of calibration.
The reagents were all high-purity-grade chemicals, i.e., hydrochloric acid (37 %, ACS reagent, Sigma Aldrich, St.
Louis, Missouri, USA), sulfanilamide (for analysis, >99 %; Sigma Aldrich) and N-(1-naphthyl)-ethylenediamine
dihydrochloride (>98%; ACS reagent, Fluka by Sigma Aldrich). For calibration Titrisol® 1000 mg NO₂⁻ (NaNO₂ in
H₂O; Merck) was diluted to 0.001 mg/L NO₂⁻. For preparation of all solutions and for cleaning of the absorption
tubes 18MΩ H₂O was used.
NO₂ concentrations were analyzed by means of a commercial chemiluminescence detector from EcoPhysics (CLD
77 AM, Duernten, Switzerland).

3 Results and discussion
3.1 BSA nitration and degradation
Nitratated proteins can lead to a stronger trigger allergic response. The nitration of proteins can be enhanced by O₃
activation (in the dark). In the atmospheric environment, about half a day the time sunlight is present. What happens
with irradiated proteins when exposed to NO₂⁻? Can they be nitrated efficiently? To investigate the degree of protein
nitration under illuminated conditions, BSA coated on the reaction tube (17.5 µg cm⁻²) was exposed to 7 VIS lamps
(40% of a clear sky irradiance for a solar zenith of 48°; Stemmler et al., 2006) and 100 ppb NO₂ at 70% RH. After 20
hours the BSA nitration degree (ND, concentration of nitrated tyrosine residues divided by the total concentration of
tyrosine residues) investigated by means of the HPLC-DAD method was (1.0 ± 0.1)%, significantly higher than the
ND of untreated BSA (0%). Introducing UV radiation (4 VIS plus 3 UV lamps) resulted in a slightly higher ND of
(1.1 ± 0.1)%. Note that no intact protein (nitrated and non-nitrated) could be detected by HPLC-DAD after another
20 hours of irradiation without NO₂⁻, indicating light induced decomposition of proteins. However, the applied
HPLC-DAD technique only detects (nitro-)tyrosine residues in proteins, and does not provide information about
protein fragments or single nitrated or non-nitrated tyrosine residues. Hence, proteins might have been decomposed
while tyrosine remains in its nitrated form, not detectable by our analysis method. Similarly, proteins (here: OVA)
that were nitrated with TNM in aqueous phase prior to coating (21.5 µg cm⁻²) to an extent of 12.5% also decomposed
when illuminated about 6 hours (1-7 VIS lights; with and without 20 ppb NO₂). Thus the nitration of proteins by
light and NO₂ was confirmed, but with simultaneous gradual decomposition of the proteins. Effects of UV irradiation
(240-340 nm) on proteins containing aromatic amino acids were reviewed previously (Neves-Peterson et al., 2012).
It was shown that triplet state tryptophan and tyrosine can transfer electron to a nearby disulfide bridge to form the
tryptophan and tyrosine radical. The disulfide bridge could break leading to conformational changes in the protein but not necessarily resulting in inactivation of the protein. In strong UV light (≈200 nm) the peptide bond could also break (Nikogosyan and Görner, 1999).

Franze et al. (2005) analyzed a variety of natural samples (road dust, window dust and particulate matter PM-2.5) collected in the metropolitan area of Munich, containing 0.08-21 g/kg proteins, and revealed equivalent degrees of nitration (EDN, concentration of nitrated protein divided by concentration of all proteins) between 0.01 and 0.1% only. Such low nitration degree is in line with light induced decomposition of (nitrated) proteins. On the other hand, an EDN up to 10% (average 5%) was found for BSA and birch pollen extract (BPE) exposed to Munich ambient air for two weeks under dark conditions, with daily mean NO₂ (O₃) concentration of 17 to 50 ppb (7 to 43 ppb) in the same study, possibly suggesting the deficiency of decomposition without being irradiated. BSA and OVA loaded on syringe-filters and exposed to 200 ppb NO₂/O₃ for 6 days under dark conditions were nitrated to 6 and 8%, respectively (Yang et al., 2010). Reinmuth-Selzle et al. (2014) found similar ND for major birch pollen allergen Bet v 1 loaded on syringe-filters exposed to 80-470 ppb NO₂ and O₃. When exposed for 3-72 hours to NO₂/O₃ at RH < 92% the ND was 2-4%, while at condensing conditions (RH > 98%) the ND increased to 6% after less than one day (19 hours). The ND of Bet v 1 was considerably increased to 22% for proteins solved in the aqueous phase (0.16 mg mL⁻¹) when bubbling with a 120 ppb NO₂/O₃ gas mixture for a similar period of time (17 hours). Shiraiwa et al. (2012) performed kinetic modelling and found that maximum 30% (conservative upper limit) of N-uptake on BSA could be explained by NO₃ or N₂O₅, which are generated by the reaction of NO₂ and O₃ while overall nitration was governed by an indirect mechanism in which a radical intermediate was formed by the reaction of BSA with ozone, which then reacted with NO₂. On NaCl surface N-uptake was dominated by NO₃ and N₂O₅. Furthermore, NO₃ radicals, which in this study could be formed by photolysis of NO₂ (>410 nm, disproportionation of excited NO₂), are not stable under the light conditions applied (400-700 nm) (Johnston et al., 1996). Therefore, in the present study reactions with NO₃ were neglected. Photolysis of NO₂ forming NO (< 400 nm) can also be neglected (Gardner et al., 1987; Roehl et al., 1994). A photolysis frequency for NO₂ of up to 5 x 10⁻⁴ s⁻¹ under similar experimental light conditions was determined by Stemmler et al., 2007. Other nitration methods, investigated by Reinmuth-Selzle et al. (2014), e.g., nitration of Bet v 1 with peroxynitrite (ONOO⁻, formed by reaction of NO with O₂⁻) or TNM lead to ND between 10 and 72% depending on reaction time, reagent concentration and temperature. Similarly, high NDs of 45-50% were obtained by aqueous phase TNM nitration of BSA and OVA by Yang et al. (2010).

3.2 HONO formation

3.2.1 HONO formation from nitrated proteins

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 NO₂ gas. Strong HONO emissions were found for OVA nitrated in the liquid phase prior to gas-exchange measurements (ND = 12.5%). A strong-high correlation between HONO emission and light intensity was observed (50% RH; Fig. 3). Initially, we did not apply NO₂. Thus the observed HONO formation (up to 950 ppt) originated from decomposing nitrated proteins rather than from heterogeneous conversion of NO₂. However, when exposed to 20 ppb of NO₂ in dark conditions, HONO formation increased 4-fold (50 to 200 ppt), and about 2-fold
with 7 VIS lamps turned on (950 to 1800 ppt). After 7 hours of flow tube experiments (4.5 h irradiation with varying light intensities (0-1-3-7 lights) + 2.5 h irradiation/20 ppb NO$_2$ (7-3-0 lights)), no intact protein was found according to the analysis of HPLC-DAD.

As proteins can efficiently be nitrated by O$_3$ and NO$_2$ in polluted air (Franze et al., 2005, Shiraiwa et al., 2012; Reinmuth-Selzle et al. 2014), the emission of HONO from light-induced decomposing nitrated proteins could play an important role in the HONO budget. As proteins are nitrated at their tyrosine residues (at the ortho position to the OH group on the aromatic ring) the underlying mechanism of this HONO formation should be very similar to the HONO formation by photolysis of ortho-nitrophenols described by Bejan et al. (2006). This starts with a photo-induced hydrogen transfer from the OH group to the vicinal NO$_2$ group (Fig. 1), which leads to an excited intermediate from which HONO is eliminated subsequently.

### 3.2.2 Light dependency

To investigate HONO formation on unmodified BSA coating (31.4 µg cm$^{-2}$) in dependence on light conditions, the radiation intensity (number of VIS lamps) was changed under otherwise constant conditions of exposure at 20 ppb NO$_2$ and 50% RH. Decreasing light intensity revealed a linearly decreasing trend in HONO formation from about 1000 ppt to 140 ppt (red symbols in Fig. 4). After re-illumination to the initial high light intensity the HONO formation was reduced by 32% (blue symbol in Fig. 4). Stemmler et al. (2006) and Sosedova et al. (2011) also observed a similar saturation of HONO formation on humic acid, tannic and gentisic acid at higher light intensities. Stemmler et al. (2006) argued that surface sites activated for NO$_2$ heterogeneous conversion by light (R3) would become de-activated by competition with photo-induced oxidants (X*, R7-8), e.g., primary chromophores or electron donors are oxidized by surface*, which is in line with the observed decomposition of the native protein presented above.

\[
\text{surface} \xrightarrow{\text{hv}} \text{surface}^* \xrightarrow{\text{NO}_2} \text{HONO} + \text{surface}_{\text{ox}} \tag{R7}
\]

\[
X \xrightarrow{\text{hv}} X^* \xrightarrow{\text{surface}^*} \text{surface} - X \tag{R8}
\]

In other studies the NO$_2$ uptake coefficient on soot, mineral dust, humic acid and other solid organic compounds similarly increased at increasing light intensities (George et al., 2005; Stemmler et al., 2007; Ndour et al., 2008; Monge et al., 2010; Han et al., 2016; Brigante et al., 2008). Note that the HONO yield (ratio of HONO formed to NO$_2$ lost) was found to be constant at light intensities in the range of 60-200 W m$^{-2}$ in the work of Han et al. (2016), but have shown a linear dependence on light for nitrated phenols (Bejan et al., 2006).

### 3.2.3 NO$_2$ dependency

At about 50% relative humidity and high illumination intensities (7 VIS lamps, ~161 W m$^{-2}$), heterogeneous formation of HONO strongly correlated with the applied NO$_2$ concentration (Fig. 5). On a BSA surface of about 16.1 µg cm$^{-2}$ (Tab. 1) the produced HONO concentration increased from 56 ppt at 20 ppb NO$_2$ to 160 ppt at 100 ppb NO$_2$. Only at a threshold NO$_2$ level well above those typically observed in natural environments (>>150 ppb) this increasing trend slowed down to some extent, indicative of saturation of active surface sites. A similar pattern of NO$_2$ dependence was also observed for light-induced HONO formation from humic acid (Stemmler et al., 2006) and
phenolic compounds like gentisic and tannic acid (Sosedova et al., 2011) or polycyclic aromatic hydrocarbons (Brigante et al., 2008), and for heterogeneous NO$_2$ conversion on soot under dark conditions (Stadler and Rossi, 2000; Salgado and Rossi, 2002; Arens et al., 2001).

For better comparison of the different studies the HONO concentration measured at different NO$_2$ concentrations was normalized to the HONO concentration at 20 ppb NO$_2$ ([HONO]$_{NO2}$/[HONO]$_{NO2=20ppb}$) in Fig. 5, as variable absolute amounts of HONO were found in different studies and matrices. A cease of the NO$_2$ dependency on heterogeneous HONO formation can be assessed for most of the studies at NO$_2$ concentrations $> 200$ ppb. A very similar correlation (up to 40 ppb NO$_2$) was observed when NO$_2$ was applied additionally during the gas phase photolysis of nitrophenols (fig. 5; Bejan et al., 2006). Even though the matrix (nitrophenols) and conditions (illuminated) of the latter is comparable to the experiment presented here, for BSA no clear indication of saturation was found up to 160 ppb of NO$_2$, pointing to a highly reactive surface of BSA for NO$_2$ under illuminated conditions.

As shown with R7 and R8, the concentration dependence depends on the competing channel R8, therefore, this is strongly matrix dependent, both in terms of chemical and physical properties.

### 3.2.4 Impact of coating thickness

Strong differences in HONO concentrations were found for experiments with different coating thicknesses applying otherwise similar conditions (20 ppb of NO$_2$, 7 VIS lamps and 50% RH). While only 55 ppt of HONO concentration was observed for a shallow homogeneous coating of 16.1 µg cm$^{-2}$ (217.6 nm thickness, see below) applied on the whole length of the tube, up to 2 ppb were found for a thick (more uneven) coating of 31.44 µg cm$^{-2}$ (435.2 nm thickness) covering only 50% of the tube (Fig. 6). Potential explanations are that thicker coating leads to (1) more bulk reactions producing HONO, or (2) different morphologies, e.g., higher effective reaction surfaces. Exposing (20%) different coated surface areas in the flow tube, potentially introduced bias comparing different data sets. Emitted HONO might be re-adsorbed differently by proteins and glass surface. However, as the protein is slightly acidic, a low uptake efficiency of HONO by BSA can be anticipated, which should not differ too much from the uncovered glass tube surface (Syomin and Finlayson-Pitts, 2003). Accordingly, NO$_2$ uptake on glass is assumed to be significantly lower than on proteins.

A strong increase in NO$_2$ uptake coefficients with increasing coating thickness was also observed for humic acid coatings (Han et al., 2016). However, they found an upper threshold value of 2 µg cm$^{-2}$ of cover load (20 nm absolute thickness, assuming a humic acid density of 1 g cm$^{-3}$), above which uptake coefficients were found to be constant. The authors also proposed that NO$_2$ can diffuse deeper into the coating and below 2 µg cm$^{-2}$ the full cover depth would react with NO$_2$, respectively.

For proteins the number of molecules per monolayer depends on their orientation and respective layer thickness can vary accordingly. One (dry, crystalline) BSA molecule has a volume of about 154 nm$^3$ (Bujacz, 2012). In a flat orientation (4.4 nm layer height, and a projecting area of 35 nm$^2$ per molecule) 3.64x10$^{14}$ molecules (40.5 µg; 0.32 µg cm$^{-2}$) of BSA are needed to form one complete monolayer in the flow tube (i.d. of 0.81 cm, 50 cm length, 100% surface coating). Hence, the thinnest BSA coating applied in the experiment (16.1 µg cm$^{-2}$) would consist of 50 monolayers revealing a total coating thickness of 217.6 nm, and the thickest BSA coating (31 µg cm$^{-2}$) would have 99 monolayers and an absolute thickness of 435.1 nm. At the other extreme (non-flat) orientation, more BSA
molecules are needed to sustain one monolayer. With 21.7 nm² of projected area of one molecule and 7.1 nm monolayer height, 5.86x10¹⁴ molecules of BSA are needed to form one complete monolayer in the flow tube. The coatings would consist of between 31 (thinnest) and 61 (thickest) monolayers of BSA. With a flat orientation 1-2% (number or weight) of BSA molecules would build the uppermost surface monolayer, whereas in an upright molecule orientation 1.6-3.3% would be in direct contact with surface ambient air.

In the crystalline form several molecules of water stick tightly to BSA. As BSA is highly hygroscopic, more water molecules are adsorbed at higher relative humidity. At 35% RH BSA is deliquesced (Mikhailov et al., 2004). Therefore the above described number of monolayers and the absolute layer thickness are a lower bound estimate.

In conclusion, the thickness dependence on HONO formation is extremely complex. Activation and photolysis of nitrated Tyr occurs throughout the BSA layer. The heterogeneous reaction of NO₂ may or may be not limited to the surface depending on solubility and diffusivity of NO₂. Also the release of HONO may be limited by diffusion. 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.

3.2.5 RH dependency

The dependence of HONO emission on relative humidity is shown in Fig. 7. Here about 25 ppb of NO₂ was applied to a (not nitrated) BSA coated flow tube (17.5 µg cm⁻²) both in dark and illuminated conditions (7 VIS lights). HONO formation scaled with relative humidity. Kleffmann et al. (1999) proposed that higher humidity inhibits the self-reaction of HONO (2 HONO₁₃,₅ → NO₂ + NO + H₂O), which leads to higher HONO yield from heterogeneous NO₂ conversion.

The RH dependence of HONO formation on proteins is different to other surfaces. For example, no influence of RH has been observed for dark heterogeneous HONO formation on soot particles sampled on filters (Arens et al., 2001). No impact of humidity on NO₂ uptake coefficients on pyrene was detected (Brigante et al., 2008). For HONO formation on tannic acid coatings (both at dark and irradiated conditions) a linear but relatively weak dependence has been reported between 10 and 60% RH, while below 10% and above 60% RH the correlation between HONO formation and RH was much stronger (Sosedova et al., 2011). Similar results were obtained for anthrarobin coatings by Arens et al. (2002). This type of dependence of HONO formation on phenolic surfaces on RH equals the HONO formation on glass, following the BET water uptake isotherm of water on polar surfaces (Finnlayson-Pitts et al., 2003; Summer et al., 2004). For humic acid surfaces the NO₂ uptake coefficients also weakly increased below 20% RH and were found to be constant between 20 and 60% (Stemmler et al., 2007).

While on solid matter chemical reactions are essentially confined to the surface rather than in the bulk, proteins can adopt an amorphous solid or semisolid state, influencing the rate of heterogeneous reactions and multiphase processes. Molecular diffusion in the non-solid phase affects the gas uptake and respective chemical transformation. Shiraiwa et al. (2011) could show that the ozonolysis of amorphous protein is kinetically limited by bulk diffusion. The reactive gas uptake exhibits a pronounced increase with relative humidity, which can be explained by a decrease of viscosity and increase of diffusivity, as the uptake of water transforms the amorphous organic matrix from a glassy to a semisolid state (moisture-induced phase transition). The viscosity and diffusivity of proteins depend strongly on the ambient relative humidity because water can act as a plasticizer and increase the mobility of the
protein matrix (for details see Shiraiwa et al. 2011 and references therein). Shiraiwa et al. (2011) further showed that
the BSA phase changes from solid through semisolid to viscous liquid as RH increases, while trace gas diffusion
coefficients increased about 10 orders of magnitude. This way, characteristic times for heterogeneous reaction rates
can decrease from seconds to days as the rate of diffusion in semisolid phases can decrease by multiple orders of
magnitude in response to both low temperature (not investigated in here) and/or low relative humidity. Accordingly,
we propose that HONO formation rate depends on the condensed phase diffusion coefficients of NO₂ diffusing into
the protein bulk, HONO released from the bulk and mobility of excited intermediates.

3.2.6 Long term exposure with NO₃ under irradiated conditions

To study long-term effects of irradiation on HONO formation from proteins, flow tubes were coated with 2 mg BSA
(17.5 ± 0.4 μg cm⁻²; 90% of total length) and exposed to 100 ppb NO₂, at 80% RH at illuminated conditions for a
time period of up to 20 hours (Fig. 8). Samples illuminated with VIS light only (red and orange colored lines in Fig.
8) showed persistent HONO emissions over the whole measurement period. For reasons unknown, and even though
the observed HONO concentrations were within the expected range with regard to the applied NO₂ concentrations,
RH and cover characteristics, one sample (orange in Fig. 8) showed a sharp short-term increase in the initial phase
followed by respective decrease, not in line with all other samples (compare Fig. 6). However, after 4 hours both VIS
irradiated samples showed virtually constant HONO emissions (-3.8 and +1.6 ppt h⁻¹, respectively). The sample
illuminated with UV/VIS light (3 UV and 4 VIS lamps) showed a sustained sharp increase in the first 4 hours,
followed by persistent and very stable (decay rate as low as -0.5 ppt h⁻¹) HONO emissions at an about 3-fold higher
level compared to samples irradiated with VIS only. HONO formation by photolysis of (adsorbed) HNO₃ is assumed
to be insignificant in this study. With N₂ as carrier gas, gas phase reactions of NO₂ do not produce HNO₃. Even when
small amounts of HNO₃ would be formed by unknown heterogeneous reactions, photolysis of HNO₃ is only
significant at wavelengths < 350 nm, which is close to the lowest limit of the UV wavelength applied in this study.
Likewise, the respective photolysis frequency recently proposed by Laufs and Kleffmann (2016) of about 2.4 x 10⁻⁷
s⁻¹ is very low.
Integrating the 20 hour experiments, 9.23x10¹⁵ (4.6 ppb*h, VISa), 1.53x10¹⁶ (7.7 ppb*h, VISb) and 4.01x10¹⁶ (20
ppb*h, UV/VIS) molecules of HONO were produced. This means between 7.7x10¹⁵ and 3.3x10¹⁶ molecules of
HONO per cm² of BSA geometric surface were formed. With respect to the different experimental conditions
concerning cover thickness, RH, and NO₂ concentrations, this is in a similar order of magnitude as found for humic
acid (2x10¹⁵ molecules cm⁻² in 13 hours) by Stemmler et al. (2006).

If BSA acts like a catalytic converter surface as in a Langmuir-Hinshelwood reaction each BSA molecule can react
several times with NO₂ to heterogeneously form HONO. As described in 3.1, BSA nitration is in competition with
NO₂ surface reactions and only a limited number of NO₂ molecules could react with BSA forming HONO via
nitration of proteins and subsequent decomposition of nitrated proteins. A BSA molecule contains 21 tyrosine
residues, which could react with NO₂. But even a strong nitration agent such as TNM is not capable of nitrating all
 tyrosine residues and a mean nitration degree of 19% was found (Peterson et al., 2001; Yang et al., 2010), i.e., 4
tyrosine residues of one BSA molecule can be nitrated to form HONO. As 2 mg of BSA was applied for each flow
tube coating, a total of 1.8x10¹⁶ protein molecules can be inferred. In 20 hours of irradiating with VIS light 13-22%
of the accessible Tyr residues (4 each BSA molecule) would have been reacted. Irradiating with additional UV lights at least 56% of the tyrosine residues would have been nitrated and decomposed, respectively. But as NO₂ is a much weaker nitrating agent and nitration of only one tyrosine residue is probable (ND of BSA with O₃/NO₂ 6%; Yang et al., 2010) up to 85% BSA molecules would have been reacted when irradiated with VIS lights, and even more HONO molecules as coated BSA molecules would have been generated under UV/VIS light conditions. Other amino-acids of the protein like tryptophan or phenylalanine might also be nitrated but without formation of HONO (Goeschen et al., 2011). Hence, a contribution of heterogeneous conversion of NO₂ can be anticipated.

3.3 Kinetic studies

The experimental results (especially the stability over a long time) indicate that the formation of HONO from NO₂ on protein surfaces likely underlies the Langmuir-Hinshelwood mechanism in which the protein would act as a catalytic converter on a surface (Fig. 9). The first step is the fast, reversible physical adsorption of NO₂ (k₁) and water followed by the slow conversion into HONO (eq.1 and eq.2). In our experiments and in the atmosphere there is always sufficient water and for simplification we assume that the reaction rate only depends on NO₂.

\[
\frac{d([NO_2])_s}{dt} = k_1 \cdot [NO_2]_s \quad \text{(eq.1)}
\]

\[
\frac{d([HONO])_s}{dt} = k_1 \cdot [NO_2]_s \quad \text{(eq.2)}
\]

where index s and g indicate sorbed and gaseous state, respectively.

From the experiments in which higher HONO concentrations were detected with higher light intensities we conclude that the heterogeneous conversion of NO₂ to HONO is light induced or a photochemical reaction. It was observed that the nitration of proteins is a competitive (side) reaction of the direct HONO formation (eq.2) but light induced decomposition of nitrated protein also produces HONO (eq.3).

\[
\frac{d([HONO])_s}{dt} = k_1 \cdot k_2 \cdot [NO_2]_s \quad \text{(eq.3)}
\]

As these two processes cannot be discriminated by the observations presented here, we combine both reactions to formulate an overall formation equation (eq.4) with \( k' = k_1 \cdot k_2 \cdot k_3 \)

\[
\frac{d([HONO])_s}{dt} = [HONO]_s + [HONO]_s = k' \cdot [NO_2]_s \quad \text{(eq.4)}
\]

There are two possible processes for the HONO formation. HONO is formed by heterogeneous NO₂ conversion (k₁) but also via nitration and decomposition of nitrated proteins (k₂, k₃). The final step of the mechanism is the release of the generated HONO into the air. Since proteins are in general slightly acidic, the desorption of HONO (k₅) should be fairly fast (eq.5). Pseudo-first order kinetics are assumed for the reaction of NO₂ to HONO (Stemmler et al. 2007) and the reaction can be described as followed (eq.1).

\[
\frac{d([HONO])_g}{dt} = k'_e \cdot [NO_2]_g \quad \text{(eq.5)}
\]

An effective formation rate of gaseous NO₂ to gaseous HONO \( k''_e \) was calculated according to eq.6,

\[
\frac{d([HONO])_g}{dt} = k_{eff} \cdot [NO_2]_g \quad \text{(eq.16)}
\]

with \( k_{eff} = k_1 \cdot k_2 \cdot k_3 \) the effective pseudo-first order rate constant (for more detailed information check the supplement).
In this study, neither HONO nor NO₂ photolysis is considered, as the overlap of the applied UV/VIS or VIS range (340-700 nm or 400-700 nm) and the HONO and NO₂ photolysis spectrum (<400 nm) is low. Furthermore, the applied light intensity is lower compared to clear sky irradiance and the respective UV light is partly absorbed by the reaction tube although quartz glass was used (transmission ~ 90%) and the photolysis frequency would decrease down to 10⁻⁴ s⁻¹. Hence, the photolysis is assumed to be not significant.

In the first 5-10 min of the long-term experiments HONO increased (Fig. 8 – zoomed in range). This slope was taken as d[HONO]/dt in eq.6. Effective rate constants between 1.48x10⁻⁶ s⁻¹ (VIS a) and 7.40x10⁻⁶ s⁻¹ (VIS b) were calculated. When irradiating with VIS light only, the concentration of HONO was either constant or decreased for 2 h after this first 10 min. When irradiating with additional UV light, the HONO signal showed an enhancement in two steps. In the first 10 min it was strongly increasing (1327 ppt h⁻¹) and then in the next hour it increased less with 170 ppt h⁻¹ prior to stabilization. Therefore two rate constants of 4.10x10⁻⁶ s⁻¹ and 5.2x10⁻⁷ s⁻¹ were obtained, respectively. Reactive uptake coefficients for NO₂ were calculated according to Li et al. (2016). For both irradiation types the uptake coefficient γ was in the range of 7x10⁻⁶ at the very beginning of each experiment. After a few minutes they decreased to a mean of 1x10⁻⁷. The calculated k_eff values and uptake coefficient are in the same range and match the NO₂ uptake coefficients on irradiated humic acid surfaces (coatings) and aerosols obtained by Stemmler et al. (2006 and 2007) which were in between 2x10⁻⁶ and 2x10⁻⁵ (coatings) and 1x10⁻⁶ and 6x10⁻⁶ (aerosols), depending on NO₂ concentrations and light intensities. Similar NO₂ uptake coefficients on humic acid were observed by Han et al. (2016). George et al. (2005) reported about a two-fold increased NO₂ uptake coefficients for irradiated organic substrates (benzophenone, catechol, anthracene) compared to dark conditions, in the order of (0.6-5)x10⁻⁶. NO₂ uptake coefficients on gentisic acid and tannic acid were in between (3.3-4.8)x10⁻⁷ (Sosedova et al., 2011), still being higher than on fresh soot or dust (about 1x10⁻⁷; Monge et al., 2010; Ndour et al., 2008). The NO₂ uptake coefficients on BSA in presence of O₃ (1x10⁻⁵, for 26 ppb NO₂ and 20 ppb O₃) published by Shiraiwa et al. (2012) were somewhat higher than the values calculated here without O₃ but with light.

It was not possible to extract a set of parameters for a Langmuir Hinshelwood mechanism (like Langmuir equilibrium constant, surface accommodation coefficient or second order rate constant) from the presented data. The saturating behavior of photochemical HONO production may be due to either the adsorbed precursor on the surface or due to a photochemical competition process, which also leads to a Lindemann-Hinshelwood type kinetic expression (Minero, 1999).

As proteins can efficiently be nitratated by O₃ and NO₂ in polluted air (Franze et al., 2005, Shiraiwa et al., 2012; Reinmuth-Selzle et al. 2014), the emission of HONO from light-induced decomposing nitratated proteins could play an important role in the HONO budget. As proteins are nitratated at their tyrosine residue (at the ortho position to the NH group on the aromatic ring) the underlying mechanism of this HONO formation should be very similar to the HONO formation by photolysis of ortho-nitrophenols described by Bejan et al. (2006). This starts with a photo induced hydrogen transfer from the OH group to the vicinal NO₂ group (Fig. 1), which leads to an excited intermediate from which HONO is eliminated subsequently.

4. Summary and Conclusion
Photochemical nitration of proteins accompanied by formation of HONO by (i) heterogeneous conversion of NO$_2$ and (ii) by decomposition of nitrated proteins was studied under relevant atmospheric conditions. NO$_2$ concentrations ranged from 20 ppb (typical for urban regions in Europe and USA) up to 100 ppb (representative for highly polluted industrial regions). The applied relative humidity of up to 80% and light intensities of up to 161 W/m$^2$ are common on cloudy days. Under illuminated conditions very low nitration of proteins or even no native protein was observed, indicating a light-induced decomposition of nitrated proteins to shorter peptides. These might still include nitrated residues of which potential health effects are not yet known. An average effective rate constant of the total NO$_2$-HONO conversion of 3.3x10$^{-6}$ s$^{-1}$ (for about 120 cm$^2$ of protein surface, layer thickness 240 nm and a layer volume of 0.003 cm$^3$; surface/volume ratio ~ 40000 cm$^{-1}$) or 8.25x10$^{-8}$ s$^{-1}$ per cm$^2$ BSA layer was obtained. At 20 ppb NO$_2$ 238 ppt h$^{-1}$ HONO would be formed. Projecting this to 1 m$^2$ of pure BSA surface a formation of HONO formation of 19.8 ppb HONO h$^{-1}$ m$^{-2}$ on a pure BSA surface could be estimated. While heterogeneous HONO formation of BSA exposed to NO$_2$ revealed light saturation at intensities higher than 161 W m$^{-2}$, the HONO formation from previously nitrated OVA was linearly increasing over the whole light intensity range investigated. The latter let assume even higher HONO formation under sunny (clear sky) ambient atmospheric conditions. No data about representative protein surface areas on atmospheric aerosol particles are available. However, the number and mass concentration of primary biological aerosol particles such as pollen, fungal spores and bacteria, containing proteins, are in the range of 10-10$^5$ m$^{-3}$ and 10$^{-3}$-1 µg m$^{-3}$, respectively (Despres et al., 2012; Shiraiwa et al., 2012). Typical aerosol surface concentrations in rural regions are about 100 µm$^2$ cm$^{-3}$. Stemmler et al. (2007) estimated a HONO formation of 1.2 ppt h$^{-1}$ on pure humic acid aerosols in environmental conditions. As NO$_2$ uptake coefficients and HONO formation rates on proteins are similar to humic acid, but only about 5% of the aerosol mass can be assumed to consist of proteins, it can be anticipated that HONO formation on aerosols is not a significant HONO source in ambient environmental settings. However, proteins on ground surfaces (soil, plants etc.) might play a more important role. Accordingly, Stemmler et al. (2006 and 2007) suggested that NO$_2$ conversion on soil covered with humic acid would be sufficient to explain missing HONO sources up to 700 ppt h$^{-1}$. Therefore it is difficult to estimate the importance of HONO formation on protein surface and its contribution to the HONO budget. In many studies the calculated unknown source strength of daytime HONO formation is within a range of about 200-800 ppt h$^{-1}$ (Kleffmann et al., 2005; Acker et al., 2006; Li et al., 2012).

Acknowledgment

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Tables and Figures

Tab 1: Details on the different experiments, aims and experimental conditions (coating, applied NO$_2$ concentration, number of lights switched on, relative humidity and time for each exposure step):

<table>
<thead>
<tr>
<th></th>
<th>Coating density (number of monolayers NML$_f$, thickness)</th>
<th>NO$_2$ [ppb]</th>
<th>no. of lamps</th>
<th>RH [%]</th>
<th>time per step [h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>light induced decomposition of nitrated protein and HONO formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>light and NO$_2$ dependency</td>
<td>n-OVA 21.5 ± 0.8 µg cm$^{-2}$ (68 NML$_f$, 298.05 nm)</td>
<td>0-20</td>
<td>0-1-3-7 VIS</td>
<td>50</td>
</tr>
<tr>
<td>B</td>
<td>heterogeneous NO$_2$ transformation on BSA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>NO$_2$ dependency</td>
<td>BSA 16.1±0.4 µg cm$^{-2}$ (50 NML$_f$, 217.6 nm)</td>
<td>0-20-40-60-100</td>
<td>7 VIS</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>light dependency</td>
<td>BSA 31.4±1.4 µg cm$^{-2}$ (99 NML$_f$, 435.2 nm)</td>
<td>20</td>
<td>0-1-3-7 VIS</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>coating thickness</td>
<td>BSA 16.1±0.4 µg cm$^{-2}$ (50 NML$_f$, 217.6 nm), 22.5±0.8 µg cm$^{-2}$ (71 NML$_f$, 310.8 nm), 31.4±1.4 µg cm$^{-2}$ (99 NML$_f$, 435.2 nm)</td>
<td>20</td>
<td>7 VIS</td>
<td>0.5-3</td>
</tr>
<tr>
<td>5</td>
<td>RH dependency</td>
<td>BSA 17.5±0.4 µg cm$^{-2}$ (55 NML$_f$, 241.7 nm)</td>
<td>25</td>
<td>0.7 VIS</td>
<td>0-50-80</td>
</tr>
<tr>
<td>6</td>
<td>time effect</td>
<td>BSA 17.5±0.4 µg cm$^{-2}$</td>
<td>100</td>
<td>7 VIS</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>time effect</td>
<td>BSA 17.5±0.4 µg cm$^{-2}$</td>
<td>100</td>
<td>4 VIS + 3 UV</td>
<td>75</td>
</tr>
</tbody>
</table>

NML$_f$ numbers of monolayers in flat orientation
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$_2$ formation of the tyrosine phenoxyl radical and following NO$_2$ addition 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: MFC = mass flow controller-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 - nitrated in the liquid phase prior to the flow tube experiments (n-OVA: ND 12.5%, coating 21.5 µg cm\(^{-2}\)). With and without 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 in the purging air (20 ppb NO\(_2\)) at 50% RH (HONO is normalized/scaled to the HONO concentration measured without NO\(_2\) and no light ([HONO\(_{\text{lights}}\)]/[HONO\(_{\text{dark}; \text{NO}_2=0}\)]) .
Fig. 4: Light induced HONO formation on BSA. a) Light enhancement of 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 normalized to the HONO concentration in darkness ([HONO\(_{\text{light}}\)]/[HONO\(_{\text{dark}}\)]). 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 NO₂ at different organic surfaces. HONO concentrations are normalized to the HONO concentration at 20 ppb 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 m² m⁻³ at 26% RH and 1x10¹⁷ 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 NO₂ under illuminated conditions (7 VIS lamps). The HONO concentrations were normalized 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, NO₂ 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: the transformation of 25 ppb NO\textsubscript{2} was applied on BSA surface (17.5 µg cm\textsuperscript{-2}) either in darkness (blue triangle) or with 7 VIS lights (red star). HONO was normalized to HONO concentrations in darkness under dry conditions ([HONO]\textsubscript{lights-on-off} / [HONO]\textsubscript{dark; RH=0}). Dotted lines are for guiding the eyes.
Fig. 8: Extended (20 h) 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 regression slopes of which $d$[HONO]/dt were used in the kinetic studies.

Fig. 9: Schematic illustration of the underlying Langmuir-Hinshelwood-mechanism of light induced HONO formation on protein surface. Reaction constants for NO$_2$ uptake, direct NO$_2$ conversion, protein nitration, HONO formation from decomposing nitrated proteins and HONO release are indicated by $k_1$, $k_2$, $k_4$, $k_5$, and $k_3$, respectively.