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# Photo-induced processes within noncovalent complexes involved in molecular recognition

Marwa Abdelmouleh,<sup>[a]</sup> Mathieu Lalande,<sup>[a]</sup> Violaine Vizcaino,<sup>[a]</sup> Thomas Schlathölter,<sup>[b]</sup> and Jean-Christophe Pouilly\*<sup>[a]</sup>

**Abstract:** Investigating the intrinsic properties of molecular complexes is crucial for understanding the influence of noncovalent interactions on fundamental chemical reactions. Moreover, specific molecular recognition between a ligand and its receptor is a highly important biological process, but little is known about the effects of ionizing radiation on ligand/receptor complexes. We have probed the processes triggered by VUV photoabsorption on isolated noncovalent complexes between the glycopeptide antibiotic vancomycin and a mimic of its receptor, by means of mass spectrometry and synchrotron radiation. In the case of protonated species, the glycosidic bond of vancomycin is cleaved with low activation energy, regardless of the molecular environment. In sharp contrast, for deprotonated species, electron photodetachment from carboxylate groups only triggers CO<sub>2</sub> loss whereas the glycosidic bond remains intact. Importantly, we also find that the noncovalent complex survives VUV photoabsorption only when the native structure is conserved in the gas phase.

## Introduction

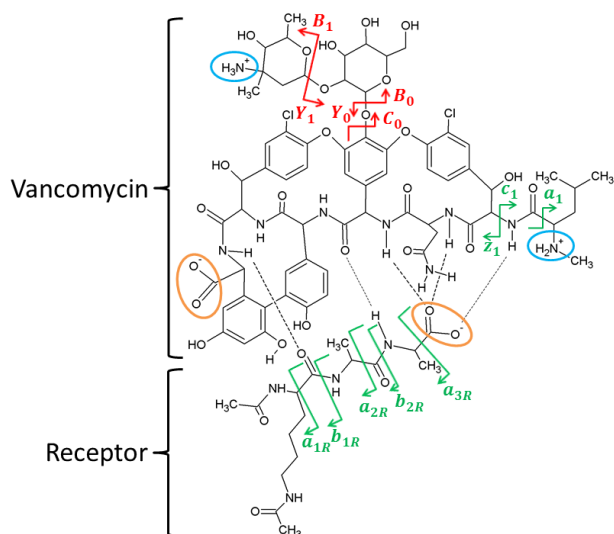
Noncovalent interactions are essential in molecular physics and chemistry, notably regarding molecular assemblies. Describing and understanding these interactions therefore allows for instance controlling the properties of new materials, catalyzing chemical reactions or creating sensors allowing for the detection of gases, explosives or drugs. Molecular recognition of a receptor by a drug is needed for medical efficiency and specificity, the latter being crucial for avoiding side-effects. Drug-receptor binding is governed by noncovalent links such as H-bonds, charge-dipole, cation- $\pi$  or  $\pi$ - $\pi$  interactions, which can be revealed by structural techniques like NMR, X-ray crystallography or IR spectroscopy. These methods have also allowed visualizing the complementary structures of ligands and receptors, a striking example of the structure/function relationship in biology. Particular classes of systems, such as intrinsically disordered proteins, at first seemed to challenge this relationship when they were first discovered. Over the last decades, their study led to extension of the structure/function paradigm to the relationship between *dynamical* structure and biological function. For instance, specific binding of particular disordered proteins to their targets (other proteins, DNA, oligosaccharides...) can trigger a conformational change (the so-called "induced fit") that leads to a well-defined structure and

activates the system. Proteins also allow repairing harmful damage due to oxidants, *via* for instance specific recognition of damaged regions of DNA by enzymes. Oxidants are ubiquitous in tissues of living organisms, and include endogenous agents such as ionizing radiation. Despite their relevance, little is known about radiation effects on noncovalent complexes involved in molecular recognition. Stisova *et al.*<sup>[1]</sup> have shown that gamma rays damage a subtype of the estrogen receptor protein and destabilize its complex with a DNA sequence. Bond cleavages within a protein-DNA complex have been observed by means of X-ray crystallography.<sup>[2]</sup> However, such condensed-phase investigations often suffer from a lack of knowledge about the stoichiometry of noncovalent complexes. Gas-phase mass spectrometry techniques, on the other hand, allow for complete control over the system under study. The combination of mass spectrometry with synchrotron radiation has for instance recently led to the identification of the binding site of a ligand to the intrinsically disordered protein IB5.<sup>[3]</sup>

In the present study, we combine synchrotron radiation and mass spectrometry in order to investigate photoabsorption in isolated molecular noncovalent complexes. As a model system for specific molecular recognition, we choose the vancomycin-Ac<sub>2</sub>-L<sup>D</sup>A<sup>D</sup>A noncovalent complex, denoted V+R in the following. Vancomycin is a last-resort antibiotic against infections caused by Gram-positive bacteria: when vancomycin specifically binds to a precursor of the main constituent of the bacterial cell wall, it prevents its renewal and leads to death of the bacterium. Previous studies have established that binding is enantiospecific: the C-terminal <sup>D</sup>A<sup>D</sup>A sequence is mandatory for high affinity between vancomycin and the receptor.<sup>[4]</sup> The native molecular interactions responsible for this behavior have been unraveled by structural investigation of the V+R complex in a crystal by X-ray diffraction.<sup>[5]</sup> The resulting structure is depicted in Fig. 1, emphasizing the H-bonds involving the CO<sub>2</sub><sup>-</sup> group of the receptor. By means of IR spectroscopy, this biologically-active structure has been found to be conserved for deprotonated complexes in the gas phase,<sup>[6]</sup> but not for protonated species,<sup>[7]</sup> in consistence with the results from early low-energy collision-induced dissociation experiments of Roepstorff and co-workers.<sup>[8]</sup> Indeed, in the protonated complex, the neutral carboxyl group of Ac<sub>2</sub>-L<sup>D</sup>A<sup>D</sup>A strongly disturbs the intermolecular H-bonding network, causing it to leave the binding pocket of vancomycin and being linked to the disaccharide moiety.<sup>[7]</sup> In the following, we report experimental results from tandem mass spectrometry and synchrotron radiation, and describe the intrinsic processes triggered by photoabsorption in noncovalent molecular complexes involving vancomycin.

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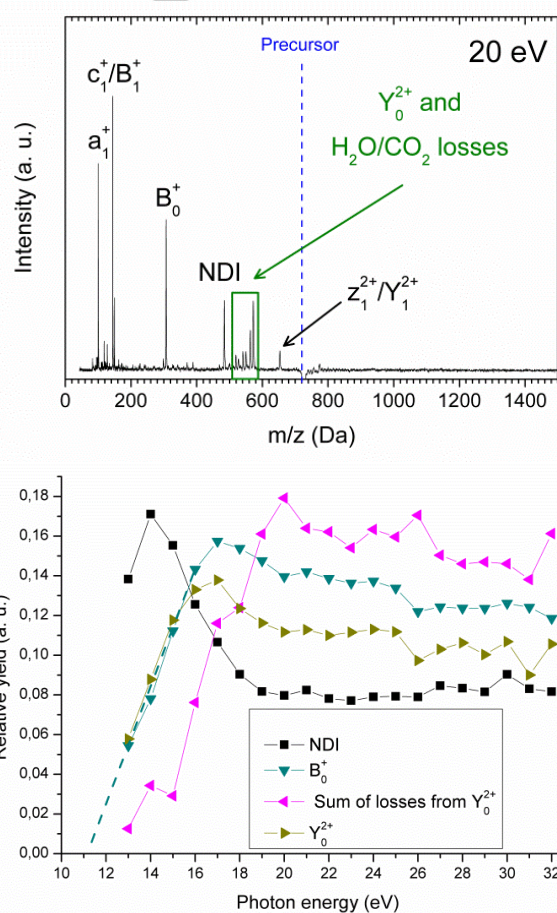
**Figure 1.** Chemical structure of the V+R complex in the condensed phase, showing native intermolecular H-bonds as dashed lines. The most basic and acidic sites are protonated and deprotonated, and encircled in blue and orange, respectively. The usual nomenclature for peptide backbone as well as oligosaccharide fragmentation is used for the main fragments observed after photoabsorption. Green arrows indicate on which side of the cleaved bond the charge is located.

## Results and Discussion

### Protonated species

First, let us consider the mass spectrum as well as the evolution of the yields of cationic products of isolated doubly-protonated vancomycin  $[V+2H]^{2+}$  after single VUV photon absorption in the energy range from 13 to 32 eV (cf. Fig. 2). At all photon energies, we observe a peak at  $m/z$  483.7 that can be assigned to non-dissociative ionization (NDI) of the precursor ion, leading to  $[V+2H]^{3+}$ . This is consistent with measured ionization energies of peptide dications, which have been found to be in the 10–12 eV range.<sup>[9]</sup> All the other peaks in the mass spectrum are due to fragment ions. At  $m/z$  306 and 572.5, we find the complementary  $B_0^+$  and  $Y_0^{2+}$  fragments formed after cleavage of the glycosidic bond separating the disaccharide moiety from the central phenyl ring (see Fig. 1). Cleavage of the bond linking the two saccharide groups (leading to  $B_1^+$  and  $Y_1^{2+}$  product ions at  $m/z$  144 and 653.5, respectively) is also observed. However, these peaks could also be due to peptide backbone fragmentation into  $c_1^+$  and  $z_1^{2+}$  ions. The peak at  $m/z$  100 can be assigned to the  $a_1^+$  peptide backbone fragment ion. All these findings are consistent with the data reported previously<sup>[10]</sup> from collision-induced dissociation (CID) experiments on gas-phase argon and helium at a relatively high kinetic energy of 4 keV that is sufficient for ionizing collisions. The yields of  $Y_0^{2+}$  and  $B_0^+$  are almost identical from 13 to 16 eV, and show the same evolution all over the energy range (see Fig. 2), indicating that both are formed by photoionization-induced cleavage of the glycosidic bond. Besides, their yields have an extrapolated appearance

energy of about 11 eV, which can be accounted for by a low activation energy for this cleavage. Interestingly, additional peaks corresponding to  $H_2O$  and  $CO_2$  sequential losses from  $Y_0^{2+}$  are also observed. These peaks are probably due to further fragmentation of  $Y_0^{2+}$  after ionization and internal conversion, followed by intramolecular vibrational energy redistribution. Such neutral molecule losses after glycosidic bond cleavage have already been reported to occur in glycopeptides after CID and UV photoabsorption, and have been attributed to radical-mediated processes.<sup>[11,12]</sup> The latter have also been put forward to explain the loss of neutral molecules from side chains of peptides after photoionization, with low appearance energies of a few eV.<sup>[13]</sup> In excellent agreement, we observe a systematic 2 eV shift of the yields of  $H_2O$  and  $CO_2$  sequential losses from  $Y_0^{2+}$  with respect to the  $Y_0^{2+}$  yield itself (see Fig. 2).



**Figure 2.** Top: mass spectrum of  $[V+2H]^{2+}$  after absorption of one 20 eV photon (the usual nomenclature for fragmentation of peptides and oligosaccharides has been used and NDI stands for non-dissociative ionization); bottom: relative yield of photo-induced channels of  $[V+2H]^{2+}$  as a function of photon energy, normalized by detector efficiency and the sum of all peak areas (see the experimental section in SI). The green dashed line is here to guide the eye.

To investigate the effects of complexation on photofragmentation, we studied the triply-protonated vancomycin dimer. Most of the peaks in the mass spectrum appearing in Fig. 3 can be assigned to the same species as for isolated vancomycin, with a noticeable exception: the peak at  $m/z$  865, which corresponds to the triply-charged complex between vancomycin and the  $Y_0^+$  fragment. This peak is due to loss of the  $B_0^+$  fragment ion from vancomycin after ionization of the dimer, without cleavage of all noncovalent bonds linking the two molecules. This process has been reported for the triply-protonated eremomycin (an antibiotic similar to vancomycin) dimer after electron-capture dissociation, where radical reactions dominate.<sup>[14]</sup> It probably implies an activation energy lower than the binding energy between the two vancomycin molecules.

When looking at the crystal structure of the vancomycin dimer in Fig. 3, we can see that it is stabilized by four intermolecular H-bonds. Furthermore, the sugar group of each vancomycin is bound to the rest of the molecule only *via* the glycosidic bond. If this structure is conserved in the gas phase, photo-induced cleavage of the glycosidic bond can lead to the formation of the sugar radical cation ( $B_0^+$ ) and the complementary  $[V+Y_0]^{3+}$  complex ion. Additional information can be obtained from the photon energy dependence of the relative partial ion yields of these species (*cf.* Fig. 3). First, as for isolated vancomycin, the almost identical yields of the  $[V+Y_0]^{3+}$  and  $B_0^+$  ions support our assignment. Their linear rise from 14 to 19 eV shows that these fragments result from ionization of  $[V_2+3H]^{3+}$ , whose appearance energy has been extrapolated to about 12 eV, a value close to the ionization energy of triply-charged peptides,<sup>[9]</sup> but also of sugars (about 9 eV for neutral deoxyribose,<sup>[15]</sup> plus 3 eV to account for the 3+ charge state of the system,<sup>[9]</sup> which gives 12 eV). This indicates that ionization can take place either in the sugar or peptidic moieties, followed by glycosidic bond cleavage with low activation energy. The appearance energy of 12 eV is also very similar to the one obtained for isolated vancomycin (see Fig. 2), which indicates that dimerization hardly affects this process.

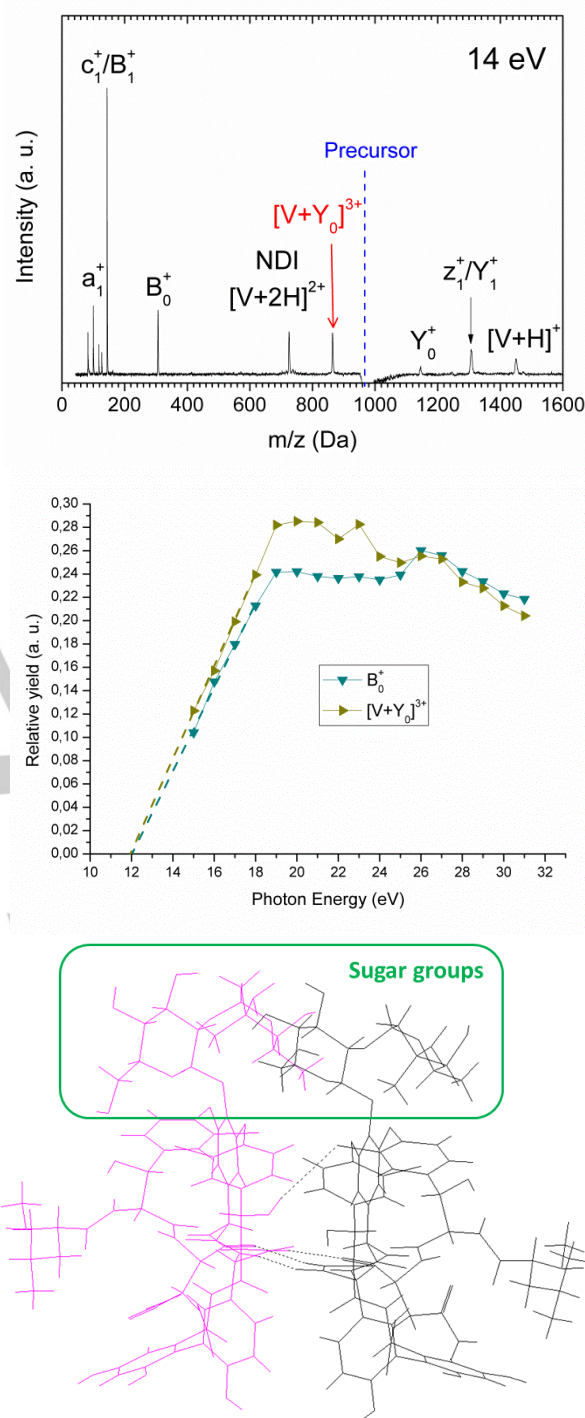


Figure 3. Top: mass spectrum of  $[V_2+3H]^{3+}$  ( $m/z$  967) after absorption of one 14 eV photon. The same nomenclature as in Fig. 2 has been used for fragments. The bond cleavages accounting for their formation are indicated in Fig. 1. Middle: evolution of the yields of  $[V+Y_0]^{3+}$  and  $B_0^+$  after single photoabsorption of  $[V_2+3H]^{3+}$  as a function of photon energy, normalized by the detector efficiency and the sum of all peak areas (see the experimental section in SI). The dashed lines are here to guide the eye. Bottom: Geometrical structure of the vancomycin dimer in a crystal (PDB 1AA5), with intermolecular H-bonds depicted in dashes and each molecule in a different color. The sugar groups are encircled in green.

In order to identify the species formed by ionization and fragmentation of the  $\text{Ac}_2\text{-K}^{\text{DA}}\text{A}$  receptor mimic (denoted R) in the mass spectra obtained by irradiation of V+R complexes, let us take a look at the results for the protonated R dimer after absorption of a single 18 eV photon (cf. Fig. 4). We mostly observe singly-charged ions due to fragmentation of the peptide backbone ( $a_1^+$ ,  $a_2^+$ ,  $b_1^+$  and  $b_2^+$ ), but also an internal fragment at  $m/z$  126 ( $\text{K}_{\text{sc}}^+$ ), due to cleavage of two bonds close to the lysine residue. The  $b_1^+$  and  $b_2^+$  fragments have previously been observed by Z. Yang *et al.*<sup>[17]</sup> after surface-induced dissociation of  $[\text{V}+\text{R}+\text{H}]^+$ , where ground-state processes after vibrational energy deposition dominate, as they do in low-energy CID. Furthermore, it has been demonstrated that the same mechanism is responsible for the presence of backbone fragments in the mass spectra of other protonated peptides ionized by one VUV photon.<sup>[18]</sup> Vibrational energy redistribution also induces intermolecular dissociation of the dimer, which accounts for the presence of the intact protonated monomer  $[\text{R}+\text{H}]^+$  at  $m/z$  373. Interestingly, a peak at  $m/z$  417 is also present: we attribute it to a complex between  $[\text{R}+\text{H}]^+$  and  $\text{CO}_2$ . The latter would thus come from the other R peptide, possibly after ionization, H transfer from the  $\text{CO}_2\text{H}$  group and dissociation. This scenario is supported by the presence of a peak at  $m/z$  328, which corresponds to the ionized peptide lacking one  $\text{CO}_2$  molecule. More insight can be gained by investigating the structure of the protonated R dimer. We have performed a conformational search by replica-exchange molecular dynamics and the structure of the lowest-energy conformer is shown in Fig. 4. The  $\text{CO}_2\text{H}$  group of neutral R is linked to  $[\text{R}+\text{H}]^+$  by two H-bonds not involving the H atom. Moreover, the latter is H-bound to a  $\text{C}=\text{O}$  group of the same peptide, allowing for intramolecular transfer. These structural features are consistent with our mechanism. Therefore, the observation of  $\text{CO}_2$  attached to  $[\text{R}+\text{H}]^+$  would mean that these non-covalent intermolecular bonds remain intact after covalent bond cleavage. This can be due to a very low appearance energy, possibly pointing to a radical-mediated process, akin to the case of electron detachment-induced  $\text{CO}_2$  loss from deprotonated peptides containing carboxylate moieties.<sup>[19]</sup> This is a second example of the influence of the molecular 3D structure on the processes induced by photoionization, after the vancomycin dimer.

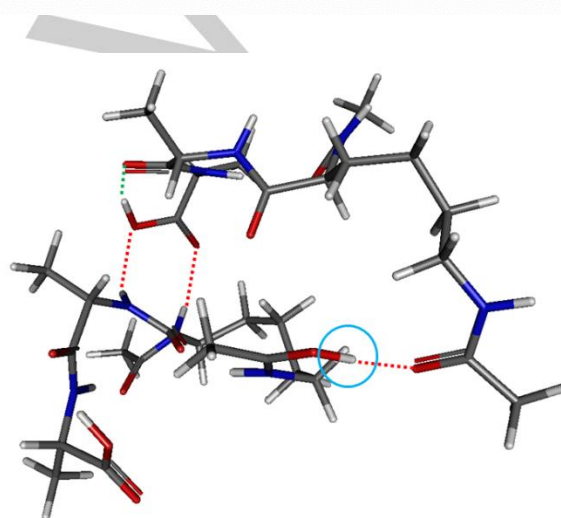
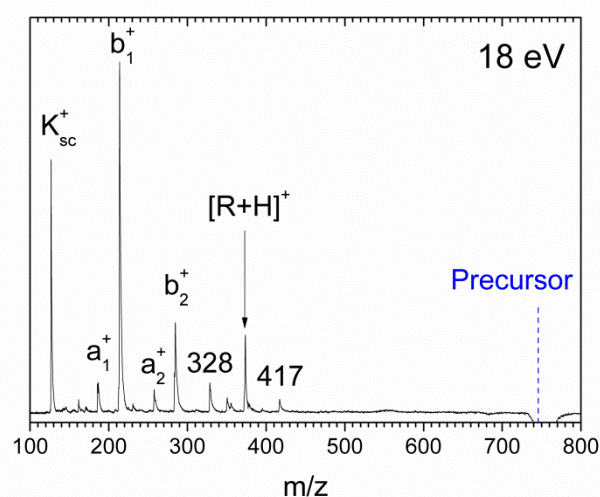


Figure 4. Top: mass spectrum of  $[\text{R}_2+\text{H}]^+$  ( $m/z$  745) after absorption of one 18 eV photon. The same nomenclature as in Fig. 2 has been used for fragments. The bond cleavages accounting for their formation are indicated in Fig. 1. Bottom: structure of the lowest-energy conformer after REMD, optimized at the B3LYP/6-31g(d) level (see the "Experimental set-up and simulations" section for details). Intermolecular H-bonds are depicted with red dashes. The intramolecular H-bond involving the transferred H (see text) is highlighted in green dashes. C, N, O and H atoms are colored in grey, blue, red and white, respectively. The proton is encircled in blue.

The mass spectrum of the doubly-protonated noncovalent complex between vancomycin and R after single photon absorption at 14 eV is shown in Fig. 5. The intact ionized species is not observed despite an ionization energy that is probably lower than 14 eV. The complex thus always fragments after photoabsorption, likely due to a low binding energy. We also detect doubly-charged vancomycin as well as singly-charged R, which are complementary fragments after ionization. Interestingly, R is observed in protonated form and not as a radical cation. Since in the complex, vancomycin and R have been reported to be doubly-protonated and neutral, respectively,<sup>[7]</sup> the presence of  $[\text{R}+\text{H}]^+$  might be explained by

intermolecular proton transfer from ionized vancomycin to neutral R and/or H transfer from vancomycin to ionized R. It has been previously shown that for amino acid clusters, proton transfer can be triggered by ionization.<sup>[20]</sup> Moreover, this process is supported by the evolution of the main channels as a function of photon energy (*vide infra*). Fragments originating from both R and vancomycin can be clearly identified and all of them also appear in the spectra of isolated vancomycin (*cf.* Fig. 2) or the R dimer (*cf.* Fig. 4). This can be explained by dissociation of the complex followed by fragmentation of vancomycin and/or R, due to internal energy transfer. Besides, H<sub>2</sub>O and CO<sub>2</sub> sequential losses from Y<sub>0</sub><sup>2+</sup> are much less intense than for isolated vancomycin (see Fig. 2), which supports their formation being due to vibrational energy transfer, this energy being now redistributed over more degrees of freedom. Interestingly, we observe a new peak at *m/z* 678 that is absent from all previous spectra. We attribute this peak to a complex between the B<sub>0</sub><sup>+</sup> fragment of vancomycin and neutral R (denoted [B<sub>0</sub>+R]<sup>+</sup> in the following). The complementary fragment would thus be Y<sub>0</sub>, which we detect in two charge states: 1+ and 2+. Observing [B<sub>0</sub>+R]<sup>+</sup> is consistent with the structure of the gas-phase complex that has been reported previously from infra-red multi-photon dissociation (IRMPD) spectroscopy.<sup>[7]</sup> Indeed, R is H-bound to the sugar moiety, implying that glycosidic bond cleavage occurs without dissociation of all noncovalent bonds to form [B<sub>0</sub>+R]<sup>+</sup>. To figure out if this process is triggered by ionization, as for the vancomycin dimer, let us look at the relative yields of [B<sub>0</sub>+R]<sup>+</sup>, Y<sub>0</sub><sup>2+</sup> and Y<sub>0</sub><sup>+</sup> as a function of photon energy (*cf.* Fig. 5). The yields of [B<sub>0</sub>+R]<sup>+</sup> and Y<sub>0</sub><sup>2+</sup> both increase smoothly between 14 and 18 eV, and their extrapolated appearance energy is 12 eV. The same behavior has been observed for the vancomycin dimer (see Fig. 3), suggesting the same underlying mechanism, namely ionization of vancomycin within the complex, followed by glycosidic bond cleavage with low activation energy. The yield of Y<sub>0</sub><sup>+</sup> does not correlate with that of [B<sub>0</sub>+R]<sup>+</sup>, which shows that they are probably formed by different channels. This further confirms the influence of geometrical structure on fragmentation of noncovalent complexes induced by photoionization. Even more information can be obtained by plotting the sum of yields of all fragments of R as well as vancomycin, along with those of intact R and vancomycin (*cf.* Fig. 5). The most striking is the opposite trends for the sum of vancomycin and R fragments between 14 and 20 eV and their crossing around 15 eV. Fragmentation of R and vancomycin dominate at low and high energy, respectively. The yield of intact protonated receptor follows a trend opposite to that of the receptor fragments, but the sum of their yields is not constant, which means that all fragments do not come from the protonated R. We attribute the complementary amount to ionization of neutral R within the complex, followed by fragmentation. This process would thus be quenched by ionization-induced proton transfer between vancomycin and R, followed by dissociation of the complex and release of [R+H]<sup>+</sup>, whose yield is rising between 14 and 20 eV, as seen above for ionization-induced species from vancomycin monomer and dimer. Further increasing photon energy leads to a slight decrease of the yield of intact [R+H]<sup>+</sup>, attributed to its

fragmentation, and consistently compensated by an increase of the sum of all yields of R fragments.

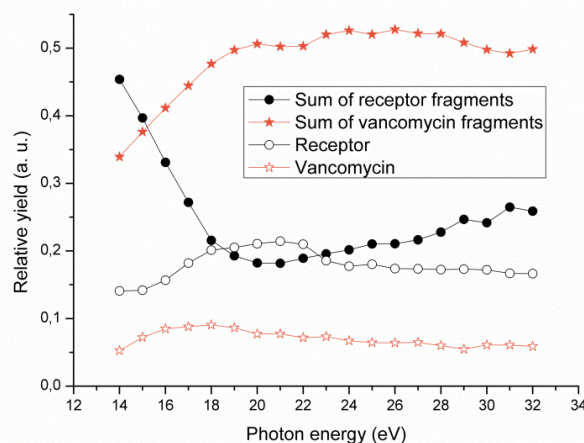
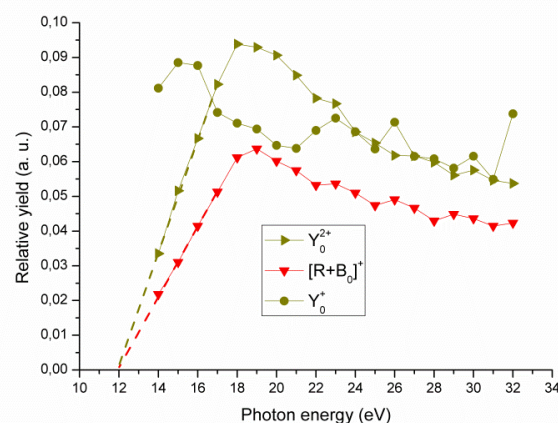
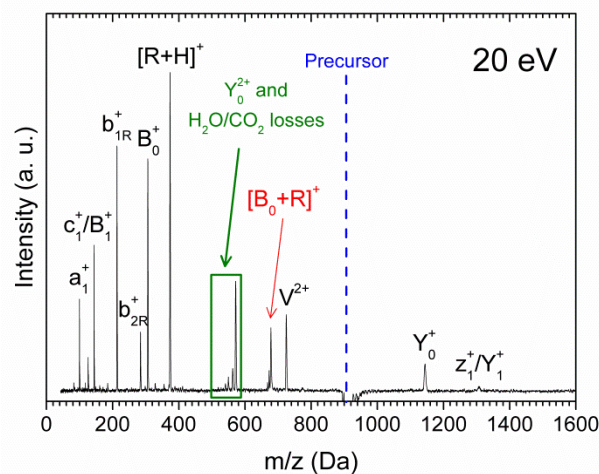


Figure 5. Top: mass spectrum of [V+R+2H]<sup>2+</sup> after absorption of one 14 eV photon. The same nomenclature as in Fig. 2 has been used for fragments. The bond cleavages accounting for their formation are indicated in Fig. 1. Middle and bottom: relative yield of the main species formed after single photoabsorption of [V+R+2H]<sup>2+</sup> as a function of photon energy, normalized by the detector efficiency and the sum of all peak areas (see the experimental section in SI). The dashed lines are here to guide the eye. The yields of the intact protonated receptor and intact vancomycin are indicated by "Receptor" and "Vancomycin", respectively.

### Deprotonated species

In order to probe the effects of charge state and structure on the photoabsorption of V+R noncovalent complexes, doubly-deprotonated species have been studied, the results being shown in Fig. 6. The most intense peaks in the mass spectrum of doubly-deprotonated vancomycin after absorption of one 16 eV photon are attributed to loss of CO<sub>2</sub> after photodetachment, forming the [V-CO<sub>2</sub>]<sup>-</sup> radical anion, and to the C<sub>0</sub><sup>-</sup> fragment due to cleavage of the bond linking the central phenyl ring to the oxygen atom bound to the disaccharide group. Minor ions Y<sub>1</sub><sup>-</sup> and Y<sub>0</sub><sup>-</sup>-CO<sub>2</sub> are formed by glycosidic bond cleavage. The mass spectra of [V+R-2H]<sup>2-</sup> also contain the same intense peak corresponding to [V-CO<sub>2</sub>]<sup>-</sup>, and the other intense peak is assigned to [V+R-2H-CO<sub>2</sub>]<sup>-</sup>. Interestingly, the latter fragment means that CO<sub>2</sub> loss is observed in the complex without breakage of all noncovalent bonds between vancomycin and R (see Fig. 1). It is thus clear that the most abundant channel triggered by single VUV photoabsorption in deprotonated species is loss of CO<sub>2</sub>, which is fundamentally different from the case of protonated systems. CO<sub>2</sub> loss induced by excitation or detachment of one electron from valence orbitals has been previously reported for deprotonated peptides.<sup>[21–23]</sup> This has been rationalized by electron transfer or detachment from CO<sub>2</sub><sup>-</sup>, followed by a radical reaction and CO<sub>2</sub> loss,<sup>[24]</sup> and is perfectly plausible in deprotonated vancomycin as well as [V+R-2H]<sup>2-</sup> since they contain one and two CO<sub>2</sub><sup>-</sup> groups, respectively, according to previous IR spectroscopy studies.<sup>[6]</sup> Therefore, electron detachment from CO<sub>2</sub><sup>-</sup> can explain the observed CO<sub>2</sub> loss. In [V+R-2H]<sup>2-</sup>, there is one CO<sub>2</sub><sup>-</sup> in R and one in vancomycin (see Fig. 1), electron detachment can thus occur from R or vancomycin. The other major peak in the mass spectrum of the complex (see Fig. 6) can be assigned to vancomycin *without* CO<sub>2</sub>, which can be interpreted as a consequence of electron detachment from R followed by vibrational energy transfer and dissociation of the complex, together with CO<sub>2</sub> loss from deprotonated vancomycin, due to excess vibrational energy. This is supported by experimental results from low-energy collision-induced dissociation<sup>[8]</sup> and IRMPD of deprotonated V+R complexes,<sup>[6]</sup> where processes are triggered by vibrational energy deposition. In the mass spectra of the complex after photoabsorption, two peaks corresponding to deprotonated R and CO<sub>2</sub> loss are also observed (see Fig. 6): BOTH are most probably due to dissociation of the complex after electron detachment from vancomycin followed by vibrational energy transfer. Their weak intensity points to a higher binding energy of the complex after electron detachment from vancomycin than from R. This is consistent with the structure of the precursor complex in the gas phase, which has been shown to be the native structure with strong H-bonds linking the CO<sub>2</sub> group of R to the binding pocket of vancomycin (see Fig. 1).<sup>[6]</sup> After electron detachment from R, these charge-dipole interactions do not exist anymore, thus decreasing the complex binding energy, whereas little structural effect is expected after electron detachment from the CO<sub>2</sub><sup>-</sup> group of vancomycin, because it is not bound to R, and thus can leave the complex without breaking it, leading to [V+R-2H-CO<sub>2</sub>]<sup>-</sup>. The

influence of the native structure is also supported by the much lower intensity of the peak attributed to further loss of CO<sub>2</sub>, leading to [V+R-2H-2CO<sub>2</sub>]<sup>-</sup>: when both CO<sub>2</sub> groups are lost, it mostly leads to dissociation of the complex. When photon energy rises from 16 to 24 eV (*cf.* Fig. 6), we observe an increase of [V-H-CO<sub>2</sub>]<sup>-</sup> and [R-H-CO<sub>2</sub>]<sup>-</sup>. In this energy range, we have previously demonstrated that the amount of transferred vibrational energy increases,<sup>[13,25]</sup> it is therefore in line with our hypothesis of [V-H-CO<sub>2</sub>]<sup>-</sup> and [R-H-CO<sub>2</sub>]<sup>-</sup> being due to internal conversion and intramolecular vibrational energy redistribution after electron detachment.

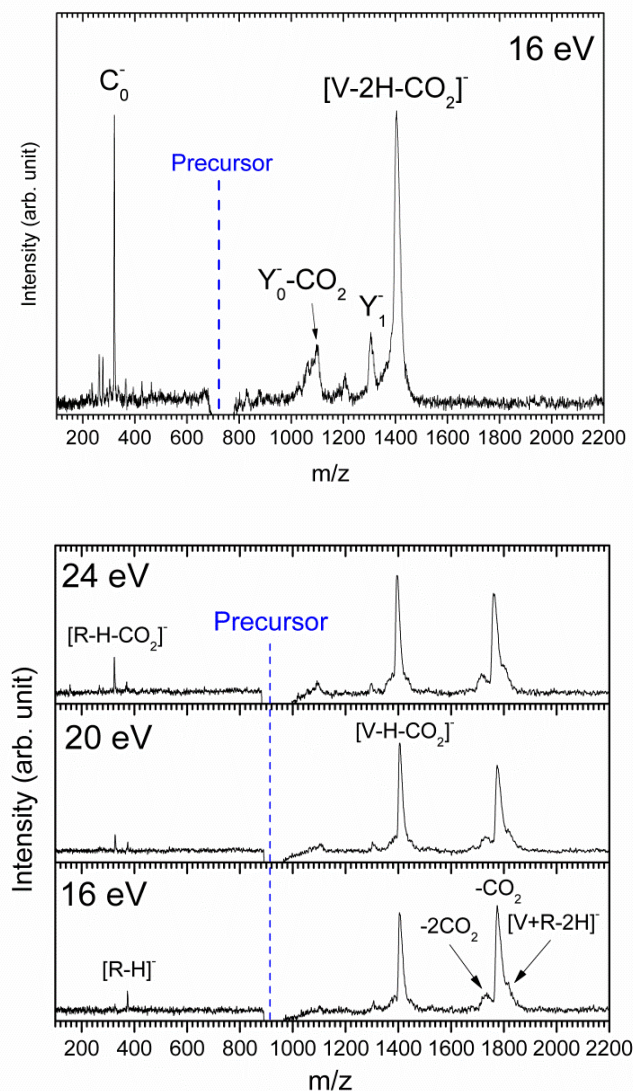


Figure 6. Mass spectra of [V-2H]<sup>2-</sup> (top) and [V+R-2H]<sup>2-</sup> (bottom) after absorption of one VUV photon. Loss of one and two CO<sub>2</sub> molecules from the latter after electron detachment is denoted -CO<sub>2</sub> (*m/z* 1775) and -2CO<sub>2</sub> (*m/z* 1731), respectively. The same nomenclature as in Fig. 2 has been used for fragments. The bond cleavages accounting for their formation are indicated in Fig. 1.

It is insightful to compare our results on complexes of vancomycin and  $\text{Ac}_2\text{-K}^{\text{D}}\text{A}^{\text{D}}\text{A}$  receptor mimic to those obtained by Roepstorff and co-workers:<sup>[8]</sup> they have shown that CID at 70 eV kinetic energy in the laboratory frame leads to the same fragmentation yield for doubly-deprotonated as for doubly-protonated complexes. The fact that our results indicate that VUV photoabsorption induces less fragmentation for the doubly-deprotonated complex might come from the radical species created after electron removal: radical-mediated processes have been shown to play an important role, both for protonated and deprotonated peptides for instance.<sup>[13,19,26]</sup> In low-energy CID, these processes are negligible for closed-shell species and instead, charge-remote or charge-directed mechanisms are dominant.<sup>[27]</sup> Therefore, one can expect different kinetics and energetics between radical and closed-shell species.

## Conclusions

In this article, we have shown that photoabsorption of vancomycin within isolated noncovalent complexes leads to very different products depending on the initial charge state: for protonated species, the glycosidic bond is cleaved with low activation energy, in contrast to the deprotonated species containing carboxylate moieties that undergo  $\text{CO}_2$  loss and intermolecular dissociation. A competition between dissociative ionization and ionization-induced intermolecular proton transfer is observed for the doubly-protonated vancomycin- $\text{Ac}_2\text{-K}^{\text{D}}\text{A}^{\text{D}}\text{A}$  complex. An influence of geometrical structure is also observed in all noncovalent complexes investigated. Furthermore, we show that loss of vancomycin- $\text{Ac}_2\text{-K}^{\text{D}}\text{A}^{\text{D}}\text{A}$  noncovalent interactions after single electron removal occurs to a much lower extent when the native structure of the complex is retained. Further work is needed to know if the receptor moves away from the binding pocket of vancomycin after photoabsorption: this structural change could be investigated by tandem ion mobility spectrometry. Such studies have already been successfully performed on deprotonated eosin Y.<sup>[28]</sup>

## Experimental and Theoretical Section

Vancomycin hydrochloride and  $\text{Ac}_2\text{-K}^{\text{D}}\text{A}^{\text{D}}\text{A}$  were purchased from Sigma-Aldrich as powders of over 80 % purity, and were used without further purification. Solution were prepared in 50:50 (volume ratio) water/methanol at 50  $\mu\text{M}$  concentration with 1 % of formic acid for protonated species and potassium hydroxide for deprotonated species.

A home built tandem mass spectrometer, described in detail elsewhere,<sup>[29]</sup> has been used to record mass spectra of the ionic photo-products from the interaction between molecular systems and synchrotron radiation. Briefly, protonated or deprotonated molecular systems are produced with an electrospray ion source and transported into the vacuum chamber through a heated capillary. The molecular ion beam is then focused into an ion funnel and guided into an octopole before being mass-over-charge selected with a quadrupole mass-filter and subsequently accumulated in a 3D radiofrequency ion trap. Trapping is facilitated by collisions with a helium buffer gas injected into the ion trap during the filling process. Molecular ions have been irradiated by

VUV photons at the U125-2\_NIM<sup>[30]</sup> beamline of the BESSY II synchrotron (Helmholtz-Zentrum Berlin). Photon beam exposure of the trap content, typically during 300 ms to 1000 ms, is controlled with a mechanical shutter in order to guarantee that more than 90 % of the product cations result from the absorption of a single photon. To do so, the irradiation time is tuned to induce a depletion of the precursor peak below 10 %. Since the absorption of multiple photons is a sequential process at these fluxes ( $10^{12-13}\text{s}^{-1}$ ), the absorption events are independent, thus a probability  $p$  for absorbing one photon gives the probability  $p^2$  for two photons. Neglecting the absorption of more than two photons, we obtain  $p^2 + p < 0.1$  and thus  $p < 0.09$ . Precursor ions and cationic fragments are then extracted from the trap and analysed by time-of-flight mass spectrometry. Mass spectra of the non-irradiated trap content and irradiated residual gas are recorded as well in order to spot background peaks due to photoionization of residual gas molecules. Then, the beam-on mass spectrum is subtracted from the beam-off one, and the resulting spectrum shows the precursor depletion with a negative intensity. Assuming that absorption of one photon leads to ionisation and/or fragmentation of the precursor ion, this depletion is proportional to the total photo-absorption yield. All mass spectra are normalised by the precursor depletion, as well as by the sum of the abundances of all ions formed by photoabsorption.

Replica-exchange molecular dynamics have been performed for the protonated  $\text{Ac}_2\text{-K}^{\text{D}}\text{A}^{\text{D}}\text{A}$  dimer to get insight into its geometrical structure. This method has already been used in order to find the structure of the lowest-energy conformations of  $[\text{V}+\text{R}+2\text{H}]^{2+}$ , because it ensures an efficient sampling of the potential energy surface of a molecular system.<sup>[7]</sup> The chosen protonation site of  $[\text{R}+\text{H}]^+$  within the dimer is the oxygen atom of the lysine side-chain acetyl group, which substitutes the  $\text{NH}_2$  group that would have had the highest proton affinity in the molecule. We used the AMBER99 force field and 30 replicas were sampled from 20 to 600 K with a geometric progression. Each replica was propagated for 20 ps for thermalisation to occur, and then for an additional 2 ns with a 1 fs time step. For thermal equilibrium, we used a Berendsen thermostat with a 0.1  $\text{ps}^{-1}$  coupling constant. Exchange between neighboring replicas was attempted every 100 fs. For each replica, a snapshot was taken every 40 ps and the geometry of the conformers obtained was further optimized with the AMBER99 force field. The structure of the lowest-energy conformer has further been optimized at the B3LYP/6-31g(d) level thanks to the Gaussian09 software.<sup>[31]</sup>

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