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DOI:
10.1016/j.cplett.2016.05.061

Document Version
Accepted author manuscript

Citation for published version (APA):
Taherkhani, M., Armentano, A., Cern, J., & Muller-Dethlefs, K. (2016). Threshold ionization spectroscopic investigation of supersonic jet-cooled, laser-desorbed Tryptophan. *Chemical Physics Letters*, *657*, 142-147. https://doi.org/10.1016/j.cplett.2016.05.061

Published in:
Chemical Physics Letters

Threshold ionization spectroscopic investigation of supersonic jet-cooled, laser-desorbed Tryptophan

Mehran Taherkhani [a, b], Antonio Armentano [a, b, d], Jiří Černý [c],
and Klaus Müller-Dethlefs [a, b] *

[a] The Photon Science Institute, School of Chemistry, The University of Manchester, Alan Turing Building, Oxford Road, Manchester M13 9PL, United Kingdom

[b] School of Chemistry, The University of Manchester, Oxford Road, Manchester, M13 9PL, United Kingdom.

[c] Institute of Biotechnology, The Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20, Prague, Czech Republic.

[d] Now at: Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Chemistry Departement, Via Manfredonia 20, 71121, Foggia-Italy.

* corresponding author, k.muller-dethlefs@manchester.ac.uk, Tel: +44 161 2751006

Abstract

Tryptophan (Trp) was studied by two-colour Photoionization Efficiency (PIE) and Mass Analysed Threshold Ionization (MATI) spectroscopy using a laser desorption apparatus. Conformer A of Trp was excited into the S_1 state (34878cm^{-1}) and the second laser was scanned around the D_0 cation ground and the D_1 excited state. No ionisation signal into the D_0 state could be found, but a clear threshold was observed for the D_1 state with an ionisation energy of 66704 ± 3 cm^{-1} (8.27 eV). This observation is explained in terms of the electronic configurations of the S_1 and cationic states.

Introduction

Electron transport (ET) plays a key role in many biochemical processes.[1] Efficient long-range transport can take place via hopping mechanisms involving intermediates. Examples include ET through, effectively "one-dimensional", DNA and peptide chains. Therefore, for better understanding of these processes, an appropriate investigation regarding the structures and the stability of the cationic state of biological molecules such amino-acids and nucleic acid bases is a fundamental starting point.

While a large numbers of spectroscopic studies have been carried out on the neutral and first excited states of many biological molecules[2-7], only few experimental studies are available in literature on the ground or electronically excited ionic states (D_0, D_1).[8, 9] Photoelectron spectroscopy of the biological molecules mentioned above has provided only approximate values for the Ionization Energies (IE) of these systems. In contrast to traditional photoelectron spectroscopy, it is possible to resolve vibrational spectra in the cationic state and obtain very accurate values for ionization energies with high-resolution Zero Electron Kinetic Energy (ZEKE)[10] spectroscopy and its variant, Mass Analyzed Threshold Ionization (MATI).[11] The first spectroscopic investigation of a neutral isolated biomolecule using MATI spectroscopy provided the adiabatic ionization energy and cationic vibrational structure of the jet-cooled DNA-base thymine.[12]

Electrospray Ionization–Mass Spectrometry (ESI-MS)[13-16] has become one of the most versatile tool for analysing biomolecules of ionic systems. Also, laser assisted ejection of biological molecules from a matrix (specifically matrix-assisted laser desorption/ionization, MALDI)[17] represents a broad, active research area.[18, 19]

In contrast to these methods, producing a sufficient vapour pressure in a supersonic rare gas expansion of isolated neutral bio-molecules such as Tryptophan (Trp) for spectroscopic investigation still remains a significant challenge.[20] Since Trp is characterized by an extremely low vapour pressure and decomposes easily upon heating, carefully designed laser desorption sources are required to convey this molecule into a supersonic jet expansion cleanly and with sufficient number density to allow its spectroscopic study under molecular beam conditions.

For this purpose, we recently developed a laser desorption system with improved signal stability and extraordinary long sample lifetime.[20] This long sample lifetime of above one month at the laser desorption repetition rate of 20 Hz has been achieved by optimizing cylindrical sample rods (1:1 weight ratio graphite/Tryptophan, 3 mm diameter and 6 mm length) from which the substance is laser-desorbed. Furthermore, the signal stability and signal-to-noise ratio has been very significantly improved by averaging the signal over the entire sample rod surface.[20] This was achieved by synchronizing the data acquisition with the rotation of the sample rod. This system has been already successfully tested on Trp recording the Resonance-Enhanced Two-Photon Ionization, R2PI (one-colour and two-colour) spectra[20] with a resolution quality comparable to those obtained previously by Piuzzi et al.[21]

In the present work, this laser desorption system[20] was used to extend the spectroscopic investigation of Trp towards cationic states by employing PIE and MATI spectroscopy. These methods allowed the determination of an accurate value of the Trp ionisation energy (IE) albeit, surprisingly, for the excited cation D_1 state and not the D_0 ground state (see results section).

Theoretical methods

Density Functional Theory (DFT) employing the B3LYP functional[22, 23] with the 6-31G* basis set[24] was used for the ground state and cationic state geometry optimizations, while time dependent DFT (TDDFT) with the same functional and basis set was used for the excited state geometry optimizations. These calculations were performed using the TurboMole 5.10 package of programs.[25]

Numerical vibrational analysis was performed using the NumForce module of TurboMole and all the structures represented minima. The restricted active space DFT (RASDFT) as implemented in the MOLCAS package[26] was used for the further analysis of the optimized structures. The calculations were performed employing the B3LYP functional and ANO-S basis set. The active orbitals included the top 10 occupied orbitals and 14 virtual orbitals defining the active space of 20 electrons in 24 orbitals. The excitations were restricted within the active space to up to two holes in the occupied orbitals as well as up to two electrons in the virtual orbitals. The ANO-S basis set was chosen (after qualitative agreement with smaller active space ANO-L calculation) as a less demanding still well performing basis set allowing us to use such a large active space.

Experiment

A detailed description of the experimental set-up can be found elsewhere.[20, 27] Briefly, the experiment consists of a home-built vacuum system[28] and a Nd:YAG (Continuum Minilite II) laser system for the desorption and two frequency doubled dye lasers (Radiant Narrowscan) synchronously pumped by a Nd:YAG laser (Continuum 8020 Powerlite) for the excitation and ionization of the molecules. The vacuum system is divided into two parts separated by a skimmer. The first part (source chamber) contains the desorption source which is attached to a pulsed supersonic jet valve (General Valve). The pellet is pressed from a mixture of graphite and tryptophan (1:1 mass ratio). The pellet, is placed in the desorption source at a distance of a few millimeters from the nozzle and it is rotated by a motorized actuator. Following expansion of the desorbed sample together with the carrier gas (argon), the supersonic jet is skimmed and the internally cooled sample molecules enter the second part of the vacuum system (ionization chamber). Here the molecular beam is crossed in a perpendicular fashion by two counter-propagating laser beams produced by the frequency doubled dye lasers. Perpendicular to the axis of the molecular beam the ions produced by the R2PI are extracted by a delayed electric field into a Wiley MacLaren type Time of Flight Mass Spectrometer (TOF) formed by a pair of parallel electrodes (Ion repeller). The extracted ions travel through the drift tube, where their jet velocity component is compensated by a small dc field imposed by a pair of parallel plates, after which they are focused by an einzel lens. After passing the ion lens, the ions are reflected in the reflectron and detected by a dual stage microchannel plate (MCP). The signal from the MCP is amplified and displayed on a digital oscilloscope. The data acquisition is carried out by a PC via lab-developed software that also controls the lasers.

The spectra were recorded at a fixed desorption wavelength of 1064 nm with a desorption energy of 3.5 mJ (0.04 J /cm^2) and 2.5 bars of argon backing pressure. The Trp molecules were excited and ionized using the two dye lasers with a resolution of 0.04 cm^{-1} (in the UV) using Rhodamine 6G for the excitation into the S_1 state and DCM for the threshold excitation/ionization. The fundamental output of the dye lasers is frequency doubled in BBO crystals. The wavelengths of the dye lasers were calibrated (± 0.02 cm^{-1}) with reference to simultaneously recorded iodine absorption spectra, corrected from air to vacuum.[29] The pulse energies were set to 0.3 mJ for the excitation laser and to 4 mJ for the ionization (scanned) laser. For the optimization of the UV power, the frequency doubling crystals were tracked (either by auto-tracking or using a lookup table) to the optimum position and a set of mass spectra for each wavelength was collected and averaged. Typically 30-40 mass spectra are averaged for a given laser wavenumber at a repetition rate of 20 Hz.

To record PIE spectra, the first laser was set resonant to the S_1 origin and the photon energy of the second laser was scanned while mass-selected ion currents ("sliced" out of the corresponding mass-spectrum) were recorded. For the MATI spectra, only ions resulting from the pulsed field ionization of high-*n* Rydberg states must be detected. These long-lived ZEKE Rydberg states are only present in a small energy range of a few wavenumbers below each energy level of the cation. MATI spectroscopy is experimentally more challenging compared to ZEKE electron detection since the molecules in long-lived ZEKE Rydberg states must first be separated from omnipresent spontaneous ions, before they are pulsed-field ionized. This separation is carried out by applying a small pulsed separation field (*ca.* 1 to 2 V/cm) which moves the spontaneous ions away from the (neutral) ZEKE Rydberg molecules. Compared to ZEKE pulsed field electron detection and discrimination against energetic electrons this means that the separation of the Rydberg molecules from the spontaneous ions needs a much longer delay time of ten or more μs between photoionization and pulsed-field ionization. Therefore the ionization area (defined by the intersection of ionization laser beam and the molecular beam) needed to be moved away from the centre of the analyser to a point upstream (by *ca.* 1 to 1.5 cm) in the molecular beam. About 0.5 μs after laser excitation, an offset field of typically ~1 V/cm is applied to the ion accelerator. After a delay time of 12-20 μs, separation of ZEKE Rydberg molecules from spontaneous ions has been completed. Also, the ZEKE Rydberg molecules have drifted with the molecular beam velocity to the centre line of the analyser where they are pulsed field ionized by a high voltage pulse of +1.5kV applied to the ion repeller.

Results

For the following experiments, conformer A of Trp was chosen because previous resonant two-photon ionization (R2PI) measurements[20] have shown that the most intense signal of Trp in the gas phase corresponds to this conformer A, suggesting that conformer A has the highest population of all neutral (S_0) Trp conformers under jet expansion conditions. All measurements were carried out with the excitation laser fixed at the $S_1 \leftarrow S_0$ origin band of the conformer A of Trp (34878 cm^{-1}). Our first measurements focussed on searching for a threshold ionization signal for the D_0 cation ground state known from single photon He(I) VUV photoelectron spectroscopy (PES) as reported by Seki *et al.*[30] The ionization energy for the D_0 threshold was reported to lie at 58072 cm^{-1} (7.3± 0.1 eV).[30] In several attempts, using several laser dyes, we scanned the ionization laser in the range of 22300-24300 cm^{-1}. This range is more than sufficiently wide to contain the D_0 threshold, considering the limited accuracy of the IE measurement by PES.[30] However, no measurable signal could be detected within this scanned range in spite of increasing the laser pulse energy of the scanned ionisation laser (for the frequency doubled UV) to 4 mJ. Finally, we extended our search into the region above 32000 cm^{-1} and then we were able to discover a PIE signal. Fig. 1a (top) displays the PIE spectrum of laser desorbed jet cooled Trp via the $S_1 \leftarrow S_0$ intermediate state of conformer A (34878 cm^{-1}, Rhodamin 6G laser dye). The PIE spectrum in Fig. 1a (top) was obtained by applying a voltage pulse of 660 V and 1μs width to the ion repeller, delayed by 18 μs relative to the excitation event. The ionization threshold was measured by scanning the ionization laser over the range 31446-32252 cm^{-1} (using DCM as laser dye) and 4 mJ pulse energy for the frequency doubled UV of the ionization laser. The PIE curve (Fig. 1a, top) shows a well-defined baseline with a small step in the threshold region followed by a sharp rise. The first jump in the spectrum corresponds to the adiabatic IE (AIE), which can be estimated to be at 66694 ± 20 cm^{-1} (8.269 ± 0.002 eV). Since the spectrum in Fig. 1a was obtained under delayed pulsed field extraction the molecules are excited under field free conditions.

MATI of Tryptophan

A more accurate value for the IE of Trp into the excited cation D_1 state can be obtained from the analysis of the MATI spectra of Tryptophan. The MATI spectra were difficult to obtain due to the challenge of separating the prompt background ions from the high-*n* Rydberg molecules of interest.

The MATI spectrum of Trp displayed in Fig. 1b was again recorded via the $S_1 \leftarrow S_0$ origin band of the conformer A of Tryptophan at 34878 cm^{-1}. The prompt ions were separated from the Rydberg molecules by a small pulsed field of 0.5V/cm (1.5V applied to the electron repeller/3cm). Following our usual MATI procedure[38] the TRP Rydberg molecules were then finally ionized by a high-voltage pulse of 660V applied to the ion repeller. The ionization threshold was measured by scanning the ionization laser in the range of 31574 - 32258 cm^{-1}. The MATI spectrum presents a broad structure with a width of 440 cm^{-1} that includes a vibrational progression of an almost equal spacing of 110 cm^{-1}. The first signal at 66704 cm^{-1}, which is in accordance with the ionizing laser photon energy of 31826 cm^{-1}, corresponds to the IE of Trp in the D_1 state, not the D_0 state. The ionization energy measured from the MATI spectrum was field corrected by $4\sqrt{F}$ (F = applied field in V/cm)[31] in order to account for the small negative difference between the energy of the pulsed field ionized Rydberg states and the true ionization energy defined as an ion with an electron of zero energy at infinity.

In this Letter, we report an explanation for only observing the D_1 but not the D_0 cation state by analysing the electronic structure of the (cation) D_0 and D_1 states as well as the (neutral) S_0 and S_1 states using the RASDFT method. As a comparison, structural changes upon electronic excitation and ionization were also studied at the TDDFT-B3LYP level. A full vibrational analysis of the excited D_1 state was attempted but found to presently be untractable.[34]

Theoretical results

Originally we were looking for an explanation of the broad MATI spectrum in terms of a significant change of geometry of the Trp moiety upon ionization. This required to re-visit the analysis of the different conformations of Trp by exploring the usefulness of a variety of theoretical methods. In order to account for suspected geometrical changes upon excitation and ionization we (re-)optimized the six most stable conformers of Trp[32] in their neutral S_0 ground, S_1 excited, and D_0 and D_1 cationic states. The resulting ground state geometries show no significant structural differences compared to the parent conformers obtained at a high level of theory (RI-MP2 and DFT-D).[32] Figure 2 summarizes the relative energy of different conformers in their ground state at the B3LYP/6-31G* level of theory as well as the geometrical changes between the neutral ground state, the first singlet excited state, and the cationic ground state structures. Even at the dispersion uncorrected B3LYP level the relative energies of conformers differ but the global minimum still corresponds to the experimentally relevant[20] conformer A geometry. The differences include in most cases only a minor rotation of the indole moiety and reorientation of the COOH and NH_2 groups. In the S_1 state of the most stable A conformer the excitation induces a slight out-of-plane displacement of the aromatic hydrogen atoms (the out of plane angle in the S_1 state is about 13 degrees, the change in relative indole/backbone orientation is about 7 degrees). All the optimized geometries represent energy minima, all with real vibrational frequencies.

The TDDFT approach was first tried out because it seems to have been successful for the study of biomolecular building blocks.[33] However, when attempting to optimize also the first excited state of the cation (D_1) using both restricted open shell and unrestricted TDDFT we did not succeed in obtaining viable results. This is probably due to instability of the reference ground state or too low excitation energy, respectively. These observations suggest possible multi-reference features of the cationic states.

After the failure to optimize the D_1 geometry at the TDDFT level we were looking for a functional capable of reliable CAS/RAS DFT calculations which is implemented in available programs. This led (in order to find a consistent description of the ground, excited, and ionized states) to the final selection of B3LYP.[34] We performed single point multi-configuration RASDFT calculations for the ground state and three lowest excited states of the Trp conformers in their neutral and cationic forms at the above mentioned S_0

and S_1 optimized geometries. The results for the most stable Trp conformer A are summarized in Fig. 3. The scheme explains the observed experimental results in terms of the electronic configuration of Trp. The experimental procedure involves electronic excitation of the conformer A of Trp originally present in its electronic ground state geometry. This S_1 state is allowed to relax its geometry and further ionized, ending unexpectedly in the excited state of the cation (D_1 state) as opposed to the expected cationic ground state D_0. We can decompose the process into three steps, excitation, relaxation and ionization.

This experimental behaviour is captured in the scheme. The Trp in the geometry of the S_0 is excited to the S_1 state (this calculation models the excitation sub-process; left group of columns, electron excited from HOMO-1 and HOMO-2 to LUMO+5 and LUMO+10). Still at the S_0 geometry the electronic configuration of the cationic ground state (D_0, second group of columns) and excited state (D_1, third group of columns) are also shown for further comparison. The geometric relaxation of the S_1 state is modelled by optimization of the S_1 geometry. The electronic configurations obtained at this geometry are summarized in the fourth to sixth group of columns. It clearly connects the first and sixth group of columns. The original S_1 state, after geometry relaxation (keeping the same electronic configuration within occupied orbitals while losing the electrons originally present in the virtual orbitals) and ionization, corresponds to the D_1 state at the S_1 relaxed geometry. The key step for facilitating the $D_1 \leftarrow S_1$ transition is that there is enough time for the geometry relaxation of the S_1 state. In contrast, for VUV photoelectron spectroscopy the ionization starts from the S_0 state and is determined by a vertical process related only to the S_0 geometry and electronic configuration.

Discussion

The feature at 66704 cm^{-1} (8.27 eV) is assigned to the IE for Trp since no further features can be observed at low energy in the MATI spectrum. This value is in full agreement with the value found from the PIE spectrum in Fig. 1a, and reflects a transition to the D_1 state. Based on the theoretical calculation and the fact that the value in the range of 7.3 ± 0.1 eV determined by PES for D_0 of Trp could not be detected by the R2PI technique, allows us to assign the obtained value of 66704 cm^{-1} (8.27 eV) to the D_1 state of the cationic Trp. It has to be stressed that this peak is not the most intense in the spectrum due to the fact that the $\Delta\nu = 0$ propensity rule does not hold and the lowest vibrational state is not seen as the most intense. as the adiabatic ionization is not the strongest transition. The first overtone ($\nu = 2$) in the progression at 66926cm^{-1} is the one of highest intensity. The broad structural shape of the spectrum is probably due to the presence of vibrational progressions for each of the conformers, which indicates a geometrical rearrangement of the Trp in the cationic state following the ionization. Increasing intensity of the vibrational modes reflects a change of the stability of different Trp conformers upon ionization. The observation of a geometrical change of Trp following ionization is in good agreement with threshold photoelectron spectra (TPES) measurements obtained by Gaie-Levrel et al.[35]

The IE values determined for Trp by TPES[35] and VUV photoelectron spectroscopy (PES) [30,36] are about 1eV above the value of the IE of Trp obtained from R2PI presented here. As outlined above, this can be explained by conformational relaxation in the S_1 state during the R2PI process. A quite similar result is noted for phenylalanine (Phe) when comparing the direct VUV photoionization experiments of Gaie-Levrel et al.[35] with R2PI spectra by Lee et al.[37] The latter authors selected each of the six known conformers by their specific S_1 band origins and then scanned the second ionizing laser photon energy for each selected S_1 origin. The IEs for Phe determined via R2PI lie in the range of 8.80 to 9.15 eV and are considerably higher than the experimental value of 8.63 ± 0.02 eV determined via VUV photoelectron spectroscopy.[36]

The wavelength selectivity is achieved in R2PI when the excitation frequency is resonant with an electronic state, whereas in VUV direct photoionization no such selectivity is available since this process does not proceed through an intermediate state. This leads to the consequence that an observed difference in the IE for a R2PI process can be due to the different electronic transitions which are not the same as in a single photon ionization (SPI) process. In the SPI technique used in VUV-PES the ionization of the Trp molecule occurs by removing an electron directly from the ground state aromatic ring. In contrast, in resonant two-photon ionization used for MATI/PIE the preferred ionization route of the molecule takes place by removing an electron from the first exited state (S_1) to produce the exited state of the cation (D_1) of the aromatic indole ring of Trp and not the cation ground state (D_0).

Conclusions

The A conformer of the amino acid Trp has been investigated using PIE/MATI spectroscopy employing a laser desorption source. An accurate value for the ionization energy of Trp, which corresponds to the necessary energy to remove one electron from the first exited state S_1 into the first excited cationic state D_1, has been obtained from the MATI spectrum. The significant difference of 1eV between the IE's determined by the R2PI and the SPI process outlines the importance of the techniques used to determine the IE. The structural shape of the vibrational modes in the MATI spectrum of Trp provides key experimental evidence of the geometry change that occurs upon ionization. Further knowledge on the cationic state of Trp conformers could be obtained by recording MATI spectra *via* different frequencies of the vibrational modes.

Acknowledgements

This study was supported by BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF and RVO: 86652036. MT and AM acknowledge PhD studentship support from the University of Manchester.

Figures

Fig . 1a/1b

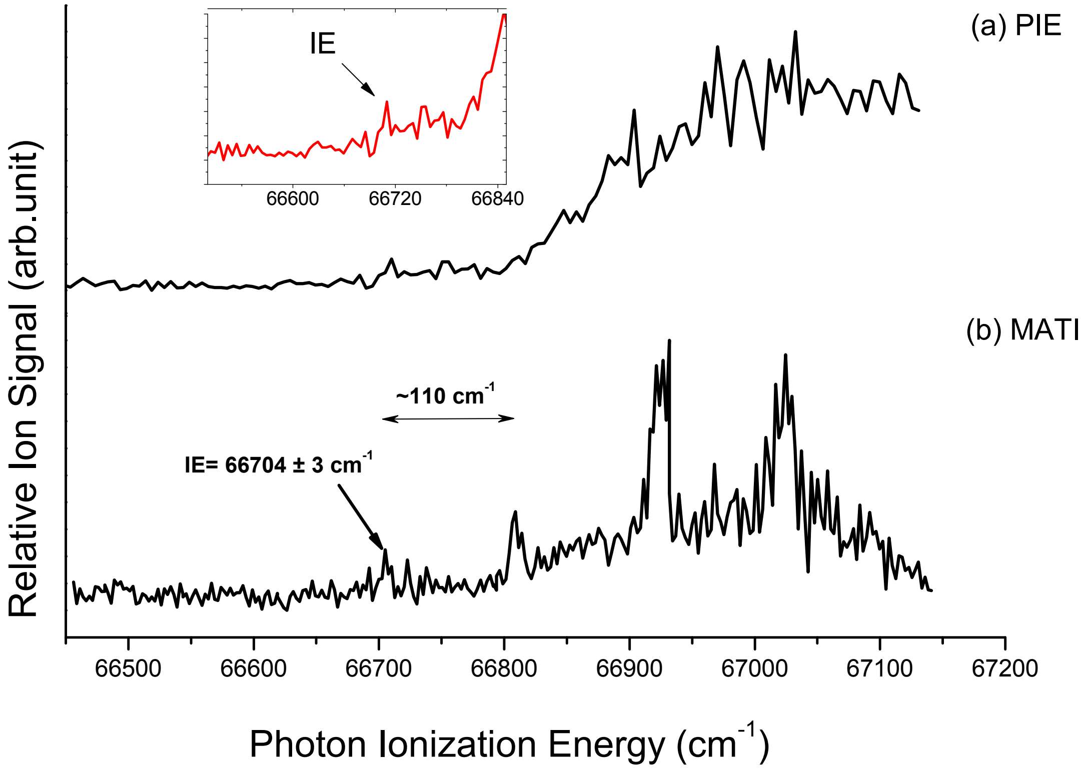


Fig. 2

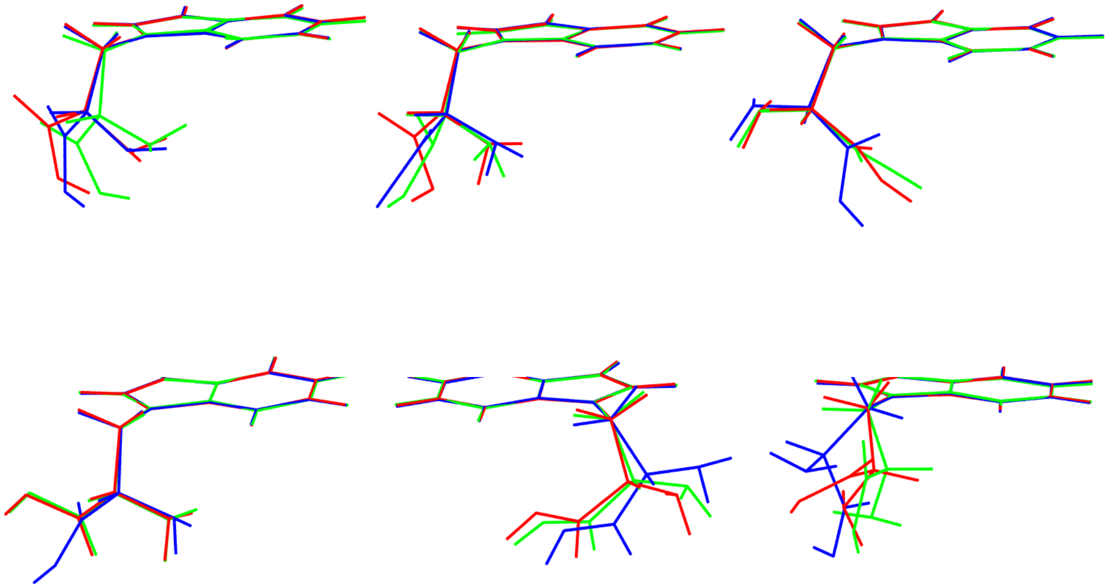
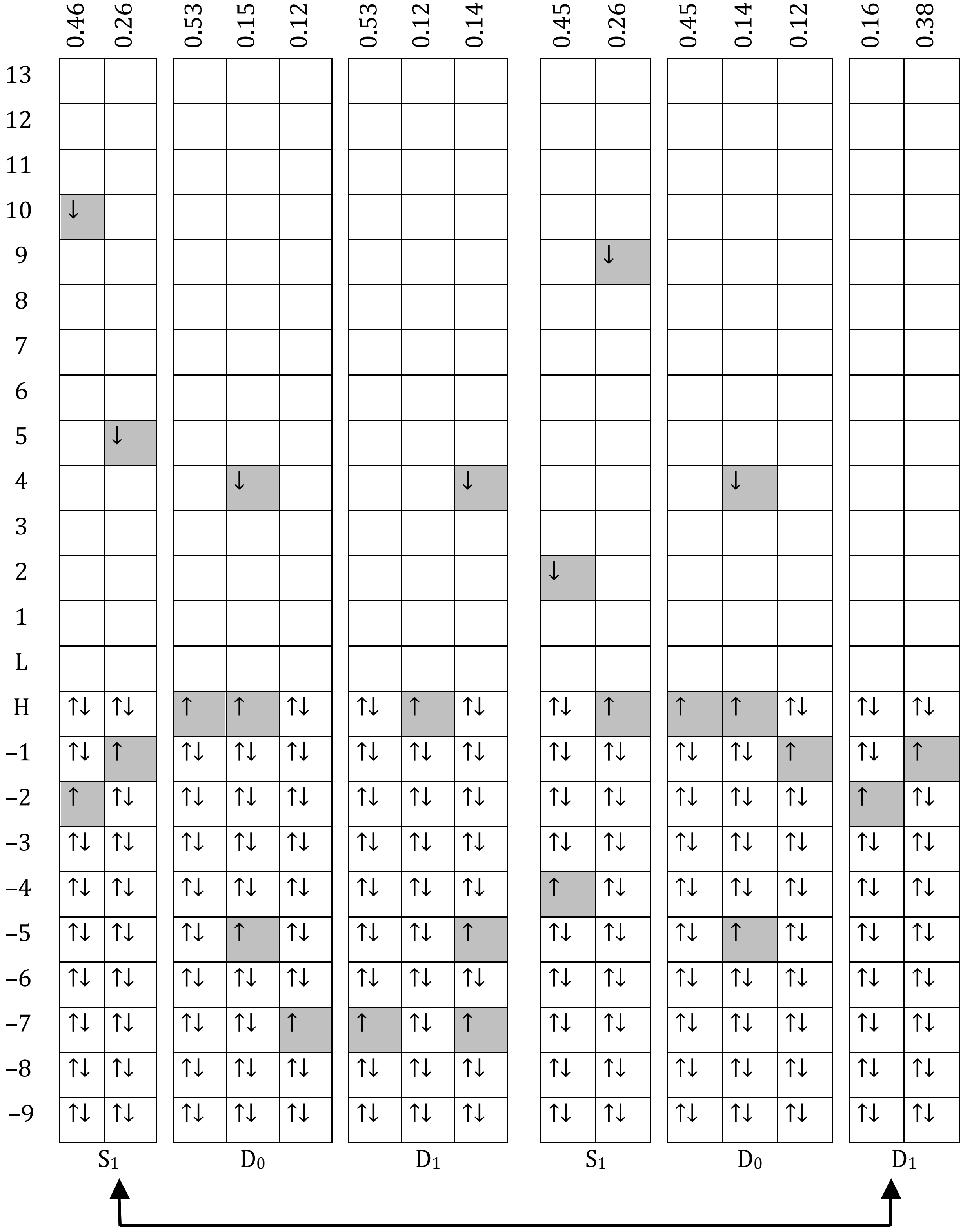


Fig. 3



	0.46	0.26	0.53	0.15	0.12	0.53	0.12	0.14	0.45	0.26	0.45	0.14	0.12	0.16	0.38
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12															
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H	↑↓	↑↓	↑	↑	↑↓	↑↓	↑	↑↓	↑↓	↑	↑	↑	↑↓	↑↓	↑↓
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	S_1		D_0			D_1			S_1		D_0			D_1	

electronic configuration at the S_0 geometry

electronic configuration at the S_1 geometry

Figure captions

Fig. 1: (a) PIE (with enlarged section at threshold in red) and (b) MATI spectra of the cationic excited state of laser desorbed jet cooled Trp obtained by (1+1′) R2PI recorded via the $S_1 \leftarrow S_0$ intermediate state of conformer A.

Fig. 2: Summary of geometrical changes of the six Trp conformers[14] in the neutral ground state (S_0, red), upon excitation (S_1, green) and in the cationic ground state (D_0, blue). The relative S_0 energies (in kcal/mol) at the optimized B3LYP/6-31G* geometry (left value) and previously obtained CCSD(T) values (right) are shown, respectively.

Fig. 3: The electronic configuration of the Trp conformer A in the neutral ground state geometry (S_0) and the optimized geometry of the first excited state (S_1). At these geometries the RASDFT/ANO-S electronic configurations and their weights within the S_1, D_0 and D_1 states are shown (top row above the table). The symbols H and L stand for HOMO and LUMO orbitals, respectively. The scheme explains the observed experimental results in terms of the electronic structure of Trp. The experimental procedure involves electronic excitation of the conformer A of Trp originally present in its electronic ground state S_0 geometry into the S_1 state. This S_1 state is allowed to relax its geometry and is further ionized, ending unexpectedly in the excited state of the cation (D_1 state) instead of the expected cationic ground state D_0. We can decompose the process into three steps, excitation, relaxation and ionization. This experimental procedure is captured in the scheme. The Trp in the geometry of the S_0 is excited to the S_1 state (this calculation models the excitation sub-process; left group of two columns, electron excited from HOMO-1 and HOMO-2 to LUMO+5 and LUMO+10). Still at the S_0 geometry the electronic configuration of the cationic ground state (D_0, second group of three columns) and the excited state (D_1, third group of three columns) are also shown for further comparison. The geometry relaxation of the S_1 state is modelled by the optimization of the S_1 geometry. The electronic

configurations obtained at this geometry are summarized in the fourth to sixth group of columns. The clear connection between the first and sixth group of columns (S_1 to D_1 arrow) explains why the S_1 state prefers to ionize into the D_1 and not the D_0 state. The original S_1 state, after geometry relaxation (keeping the same electronic configuration within occupied orbitals while losing the electrons originally present in the virtual orbitals) and ionization, corresponds to the D_1 state at the S_1 relaxed geometry. In contrast, no such connection can be seen between the original S_1 state (first group of two columns) and the D_0 state (fifth group of three columns) electronic configuration at the S_1 relaxed geometry.

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