Electronic Supplementary Information

Experiment and theory confirm that UV laser photodissociation

spectroscopy can distinguish protomers formed via electrospray

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Electronic Supporting Information

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S1. Laser Power Dependence of the Photodepletion of Protonated para-Aminobenzoic acid and Evidence of Secondary Fragmentation

i. Laser Power Studies

The effect of laser power on the logarithmic depletion in ion signal ($\ln[I_0/I]$) of PABA·H⁺, electrosprayed from an aqueous solution, has been studied at four excitation energies: 3.63, 3.90, 4.90 and 5.06 eV (342, 318, 253 and 245 nm). These excitation energies span the regions of high absorption in the water-ESI absorption spectrum, given in Figure 2a. Figure S1 shows that under the experimental conditions, power ~ 1.0 mJ, photodepletion increases near-linearly at 3.90, 4.90 and 5.06 eV, indicating that the experiment is operating under one-photon conditions. With an excitation photon energy of 3.63 eV, the photodepletion flattens above 0.4 mJ. Under these conditions, it can be assumed that absorption is saturated due to complete fragmentation of all ions that overlap with the laser beam.



Fig. S1Gaseous dependency of the laser power on the photodepletion of PABA·H+ions with excitation wavelengths of: 342, 318, 253 and 245 nm. PABA·H+was produced by electrospraying an aqueous solution of PABA.

An average of the data points in figure S1 was fitted to a function of the form:

$$f(x) = a \times b^x$$

Where a = 54.2 and b = 0.462. The experimentally recorded average laser power (P^{-0.462}) is used to correct the logarithmic depletion in ion intensity.

ii. Secondary Fragmentation of Photofragments

Figure S2a and S2b shows the normalised absorption spectra of PABA·H⁺ electrosprayed from solutions of water and acidified MeCN respectively. The absorption spectra are represented by photodepletion (given as blue dots) and the sum of all observed photofragments (given as red dots), these spectra are normalised to the absorption value at 4.90 eV (253 nm). Also included is the difference between the photodepletion and photofragment absorption spectra (given as black dots). Figure S2a shows that there is a large difference between depletion and fragment production between 3.6 - 3.8 eV, this is attributed to the secondary fragmentation of primary photofragments to masses outside the range of our mass spectrometer (m/z must be greater than 50) which is caused by photofragments absorbing UV photons. Figure S2b shows that for the MeCN-ESI absorption spectra, secondary fragmentation is significant above 5.4 eV and is a minor process around 3.6 eV.



Fig. S2 Absorption spectra of PABA·H⁺, electrosprayed from solutions of a) water and b) acidified-MeCN, which are normalised to the absorption value at 4.90 eV (253 nm). Blue dots are photodepletion, red dots are the sum of the observed photofragments and the black dots are the difference between the blue and red dots.

S2. Computational Results: Protonation, Stability and Excited States.

i. Structures and Energies of protonated PABA

Scheme S1 Calculated structures and relative electronic energies of the potential structures of protonated PABA. Relative energies are given in kJ mol⁻¹.

	Isomer 1	Isomer 2	Isomer 3	Isomer 4
Oxygen Protonated	0.0	11.8	20.4	48.8
Nitrogen Protonated	33.7	75.9		
Carbon Protonated	51.1	135.7	53.6	

ii. Description of the MRCI and SORCI Excited State Calculations

The excited state calculations were performed using the MRCI and SORCI modules of the ORCA quantum chemistry package. The B3LYP optimised structures of the lowest energy tautomers of **PABA-OH**⁺ and **PABA-NH**₃⁺ were re-optimised with the MP2/6-311+G(2d,2p) level of theory using Gaussian 09. Frequency calculations were performed on the structures which showed that they were energy minima.

When using ORCA, the aug-cc-pVDZ basis set and its auxiliary basis set (aug-cc-pVDZ/C) were used for all calculations. Natural orbitals were generated using MP2 theory, specifying a relaxed density. The resolution of the identity (RI) approximation was specified when using MP2 theory (RI-MP2) and throughout the CASSCF, SORCI and MRCI calculations. The MP2 natural orbitals were extracted from the generated GBW file using the orca_plot utility and were visually assessed. Orbitals which possessed significant electron density above and below the plane of the molecule were classified as π -orbitals, the orbitals were re-ordered such that the π -orbitals would be in the active space of CASSCF calculations. CASSCF(10,9) and CASSCF(8,8) calculations were run for the PABA-OH⁺ and PABA-NH₃⁺ structures respectively. A restricted basis set containing six electrons in six orbitals of the CASSCF reoptimised orbitals were used as the active space in the excited state MRCI and SORCI calculations, the active space was reduced to speed up calculations. For **PABA-OH**⁺, an initial MRCI calculation using the B3LYP optimised structure and MP2 natural orbitals was found to most accurately reproduce the experimentally observed absorption transitions. All active space orbitals of PABA-OH⁺ and PABA-NH₃⁺ are given in figures S3 – S6. The electronic transitions responsible for producing the first four absorption bands in the MRCI and SORCI excitation spectra of PABA-OH⁺ and PABA-NH₃⁺ are summarised in tables S1 and S2 respectively.

Table S1Orbital transitions responsible for the first four absorption bands of PABA-OH+in the MRCI and SORCI calculations. Percentage contributions were calculatedfrom the weightings predicted for each transition. Orbital transitions whichcomprise less than 10% of the total absorption are omitted. * predicts a twoelectron excitation from orbital 35 – 36.

	MRCI		SORCI			
PABA-OH ⁺	Initial Orbital	Final Orbital	Percentage Contribution	Initial Orbital	Final Orbital	Percentage Contribution
$S_1 \leftarrow S_0$	35	36	95.0	35	36	90.2
$S_2 \leftarrow S_0$	35	37	41.4	35	37	23.1
	34	36	57.7	34	36	71.8
$S_3 \leftarrow S_0$	35	37	57.9	35	37	70.5
	34	36	40.6	34	36	21.7
$S_4 \leftarrow S_0$	33	36	93.7	35	38	11.3
				35	36*	41.2
				33	36	39.2

Table S2 Orbital transitions responsible for the first four absorption bands of PABA NH₃⁺ in the MRCI and SORCI calculations. Percentage contributions were calculated from the weightings predicted for each transition. Orbital transitions which comprise less than 10% of the total absorption are omitted.

	MRCI			SORCI		
PABA-NH ₃ ⁺	Initial	Final	Percentage	Initial Orbital	Final Orbital	Percentage
	Official	Orbital	Contribution	Official	Official	Contribution
$S_1 \leftarrow S_0$	35	37	16.7	35	36	26.1
	34	36	81.5	35	37	21.2
				34	36	30.7
				34	37	17.9
$S_2 \leftarrow S_0$	35	36	92.0	35	36	40.4
				35	37	19.7
				34	36	25.7
				34	37	14.2
$S_3 \leftarrow S_0$	35	37	66.7	35	36	30.9
	34	36	13.1	34	37	65.1
	34	37	16.5			
$S_4 \leftarrow S_0$	34	37	85.1	35	37	55.6
				34	36	42.4



Fig. S3 Reference space orbitals used within a MRCI calculation to predict the gaseous absorption spectrum of PABA-OH⁺. Details of the calculation are given in the text.



Fig. S4Reference space orbitals used within a SORCI calculation to predict the gaseous absorptionspectrum of PABA-OH+. Details of the calculation are given in the text.



Fig. S5Reference space orbitals used within a MRCI calculation to predict the gaseous absorption spectrum

of **PABA-NH**³⁺. Details of the calculation are given in the text.



Fig. S6Reference space orbitals used within a SORCI calculation to predict the gaseous absorption spectrum

of **PABA-NH**³⁺. Details of the calculation are given in the text.

Time dependent density functional theory (TD-DFT) calculations were performed on the B3LYP optimised structures of **PABA-OH**⁺ and **PABA-NH**³⁺ using Gaussian 09. The B3LYP functional and 6-311+G(2d,2p) basis set were used to calculate the first 30 excited states of **PABA-OH**⁺ and **PABA-NH**³⁺. The resulting TD-DFT excitation spectra are presented in figure S7, with the transitions and oscillator strengths of the individual excitations given in tables S3 and S4.



Fig. S7 Calculated TD-DFT excitation spectra of PABA protonated at the carboxylic acid (PABA-OH⁺) and amine (PABA-NH₃⁺), calculated using the B3LYP functional. The oscillator strengths of individual transitions are given by the vertical bars. The full line spectrum represents a convolution of the calculated electronic transitions with Gaussian functions (0.25 eV HWHM).

Table S3Calculated transition energies and oscillator strengths of the PABA-OH+
structure from TD-DFT calculations with the B3LYP functional. Only
transitions under 7 eV with oscillator strength > 0.005 are listed.

Transition Energy (eV)	Oscillator Strength	
$\pi \rightarrow \pi^*$ Transitions		
4.10	0.5594	
4.42	0.0159	
5.08	0.0557	
6.28	0.0225	
6.64	0.2079	
6.98	0.3551	

Table S4Calculated transition energies and oscillator strengths of the PABA-NH3+structure from TD-DFT calculations with the B3LYP functional. Only
transitions under 7 eV with oscillator strength > 0.005 are listed.

Transition Energy (eV)	Oscillator Strength			
$\pi \rightarrow \pi^*$ Transitions				
5.11	0.0168			
5.62	0.1302			
5.93	0.0070			
6.29	0.0909			
6.64	0.5490			
6.73	0.2534			
$\pi \rightarrow \sigma^*$ Transitions				
5.62	0.0052			
n (Oxygen Lone Pair) $\rightarrow \sigma^*$ Transitions				
6.68	0.0300			
6.99	0.0089			

S3. Solution Phase UV Spectra of PABA Under Neutral and Acidic Conditions

The solution phase absorption spectra of PABA in water and MeCN under neutral and mildly acidic conditions are presented in figure S8. The spectra show that in the pure solvent, neutral PABA absorbs broadly between 4.0 - 5.0 eV, peaking at 4.45 and 4.33 for water and MeCN respectively. PABA also absorbs above 5.3 eV. Upon the addition of HCl, the absorption cross section of the band between 4.0 - 5.0 eV significantly decreases and the higher energy band shifts to lower energies. The band between 4.0 - 5.0 eV develops fine structure (most clearly observed in the MeCN spectrum) which peaks at 4.45 and 4.59 eV and 4.48 and 4.60 eV for water and MeCN respectively. The higher energy band is now resolvable for both solvents, peaking at 5.50 and 5.54 eV for water and MeCN respectively.



Fig. S8 Solution phase absorption spectra of PABA (~ 2 x 10⁻⁴ mol L⁻¹) across the range 3.2-5.65 eV in solutions of: a) water and b) MeCN. Absorption spectra are recorded in the pure solvent (black line) and with 1 drop of 3M HCl in 3 mL of the PABA solution (blue line).

The change in absorption spectra is caused by a change in protonation state of PABA, from neutral to monoprotonated. It is notable that even under acidic conditions, no significant absorption is recorded below 4 eV.

S4. Additional Photofragment Mass Spectrum

Figure S9 shows the photofragment mass spectrum of isolated PABA·H⁺ irradiated with an excitation photon energy of 5.56 eV (223 nm), PABA·H⁺ was electrosprayed from a solution of acidified MeCN. This photon energy is within band **IV** of the experimental absorption spectra. Figure S9 is significantly from figure 3b of the main text, which is the MeCN-ESI photofragment mass spectrum of PABA irradiated at 4.56 eV (272 nm). Figure S9 shows that as the photon energies increase, **PABA-NH3**⁺ fragments preferentially into m/z 137 and 65, which associates a reduction in the production of m/z 139 and 121.



Fig. S9Photofragment mass spectrum of $PABA \cdot H^+$ electrosprayed from acidifiedMeCN and irradiated with an excitation photon energy at 5.56 eV (223 nm).

S5. Gaussian Fitting of the Photofragment Production Spectra

Fig. S10 shows the Gaussian fitted photoproduction spectra of fragments associated with the **PABA-OH**⁺ (Fig. S10a) and **PABA-NH3**⁺ (Fig. S10b) structures of protonated PABA, the data plotted in Fig. S10 is the same as the data in Fig. 4 of the main text. Fig. S10a can be described well using eight Gaussian functions, which show that bands **I** and **II** can be broken down into two sets of absorption features that peak at 3.51, 3.59 and 3.77 eV and 4.89, 5.06 and 5.26 eV respectively. The photofragment production spectrum also shows that two weak bands, centred at 4.47 and 5.72 eV, are resolvable. The Gaussian fitting of Fig. S10b shows that band **III** can be deconvoluted into three separate absorption features which peak at 4.56, 4.67 and 4.84 eV. Band **IV** can be fitted with two Gaussians which peak at 5.56 and 5.84 eV.

The maximum of the lowest energy Gaussian curve within bands I - IV is tentatively assigned as the vertical transition energy of that electronic transition. The higher energy Gaussian curve(s) are likely to represent averages of vibronic transitions. However, absolute assignments of the vibronic transitions are not possible in a low-resolution photodissociation experiment.



Fig. S10 Photofragment production spectra of the photofragments with a) m/z 93, from PABA electrosprayed in water; b) the sum of fragments m/z 121, 137 and 139, from PABA electrosprayed in MeCN. Fragments are produced following photoexcitation of mass-selected PABA·H⁺ ions, across the range 3.2 – 5.8 eV.