

Supporting Information

Towards a detailed description of pyridoxamine tautomeric species

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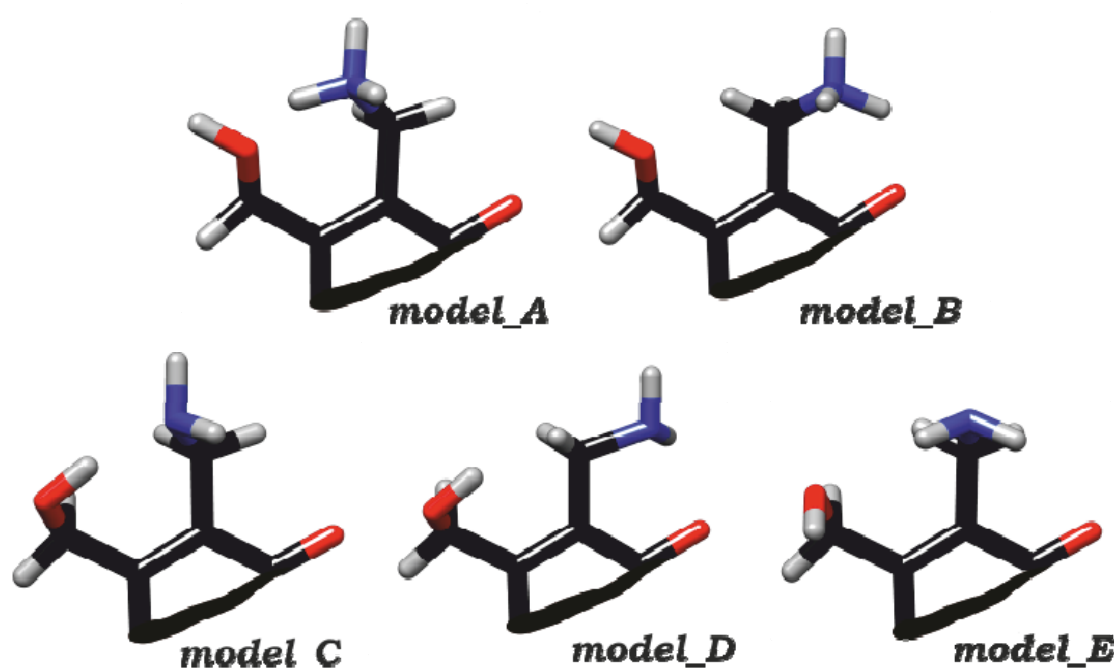


FIGURE S1. Initial input models used to optimize the geometry of each PM form at PCM-MP2/6-311++G(d,p) level of theory. Models A, B were used to compute the structure of those PM forms that possess an amminium ion. Models C, D, E were used to optimize the geometry of PM forms with a non-protonated methylamino group. Displayed models are built to represent all PM forms. For clarity O3' is shown as unprotonated whereas the aromatic ring is truncated.

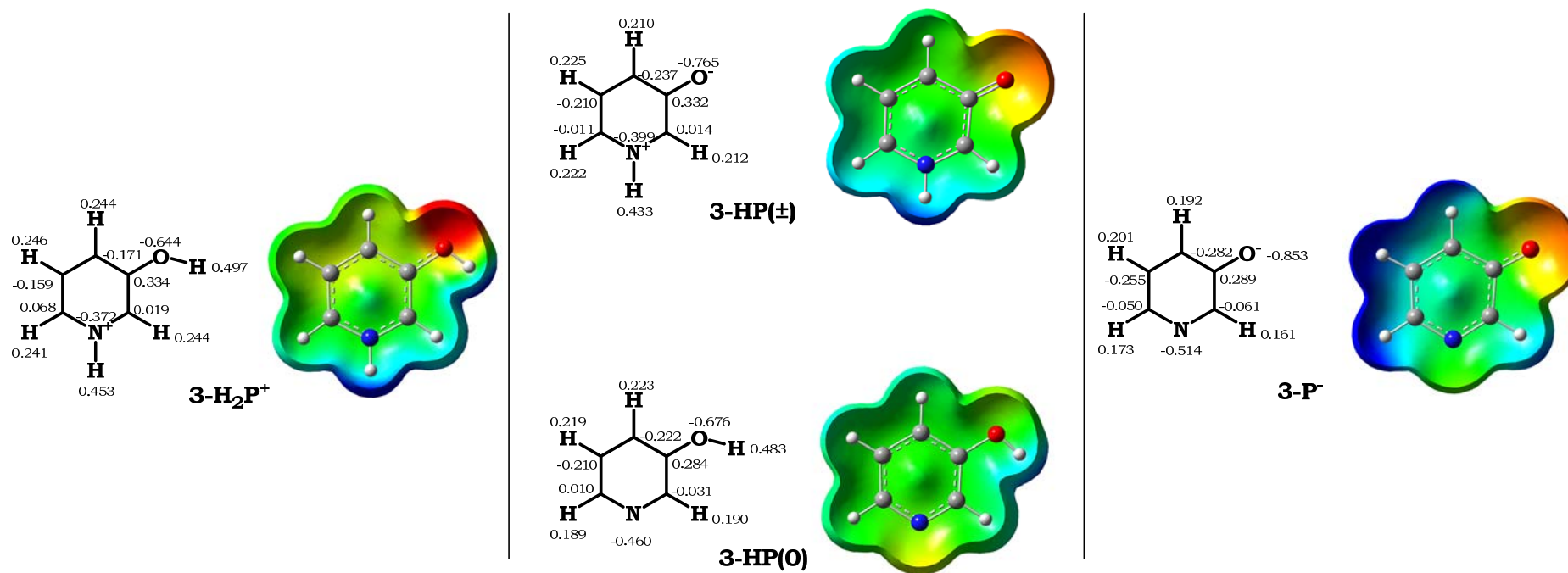


Figure S2. NBO atomic charges and molecular electrostatic potential (MEP) maps of 3-H₂P⁺, 3-HP(0), 3-HP(±) and 3-P⁻ computed at PCM-MP2/6-311++G(d,p) level of theory. The isosurface value in MEPs is 0.002 with a range of 0.1 to 0.3 a.u. for 3-H₂P⁺; -0.2 to 0.2 a.u. for 3-HP(0) and 3-HP(±); and -0.3 to -0.1 a.u. for 3-P⁻.

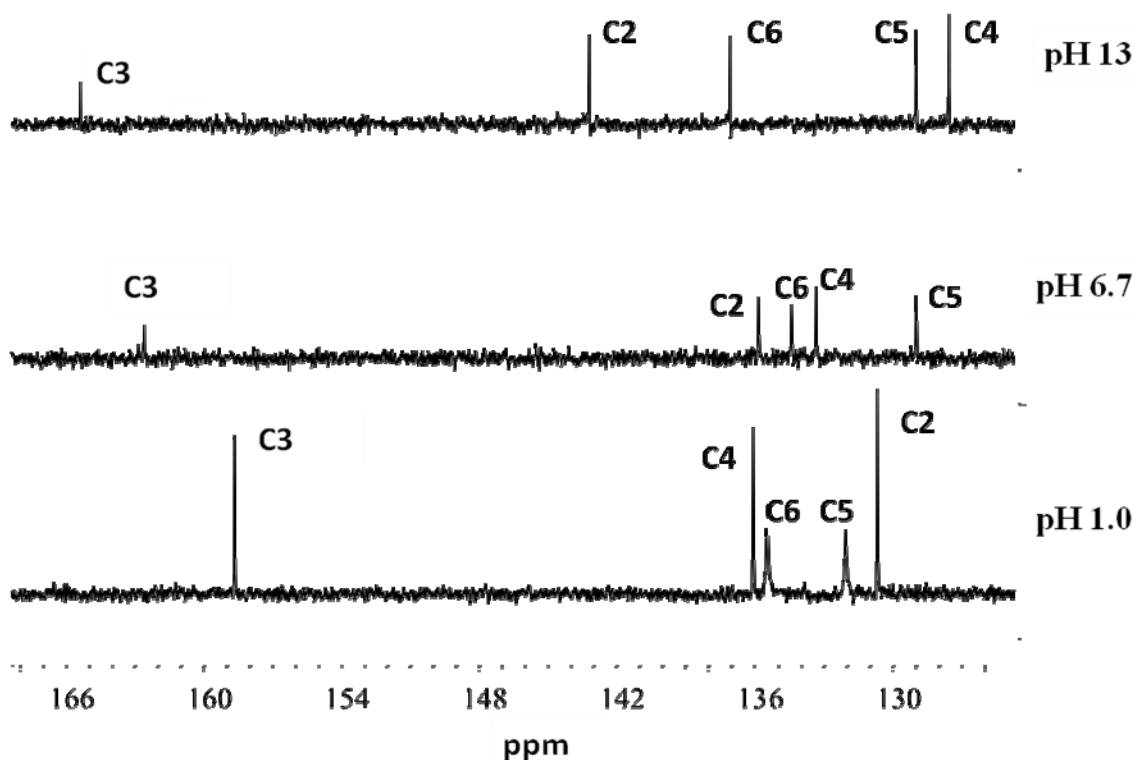


Figure S3. ^{13}C NMR spectra for solutions containing 50 mM 3-HPY in 0.1 M HCl, 0.5 M phosphate buffer at pH 6.7 and 0.1 M NaOH. All solutions were prepared in 90:10 $\text{H}_2\text{O}/\text{D}_2\text{O}$ mixtures and the spectra were collected at 25 °C.

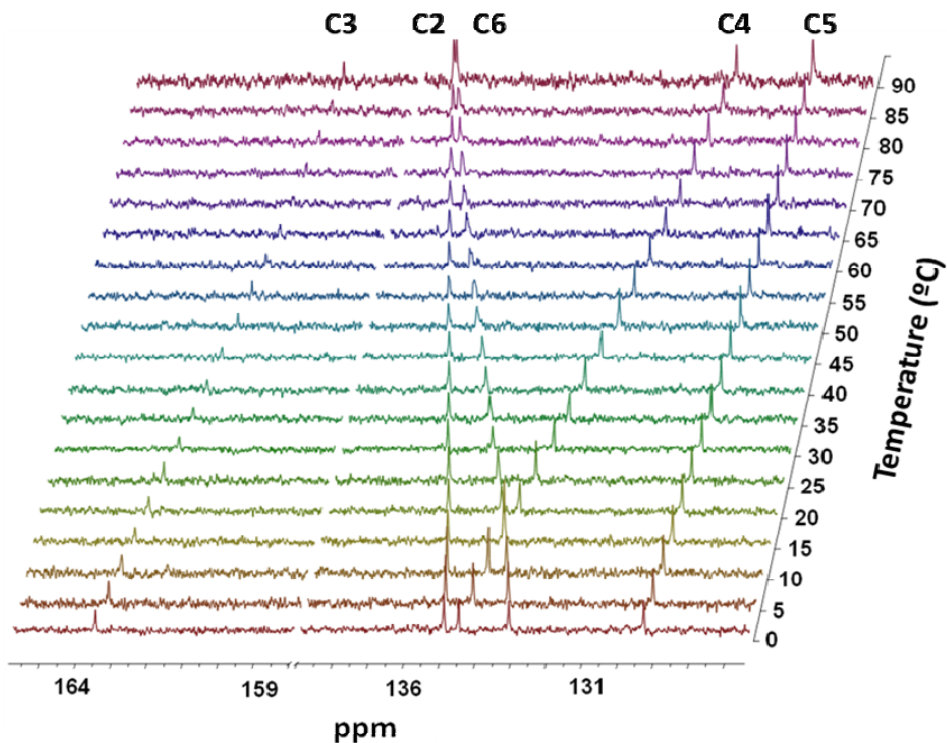


Figure S4. Temperature variation of ^{13}C NMR spectra of 50 mM 3-HPY solution buffered at pH 6.7 with sodium phosphate. The solution was prepared in 90:10 $\text{H}_2\text{O}/\text{D}_2\text{O}$.

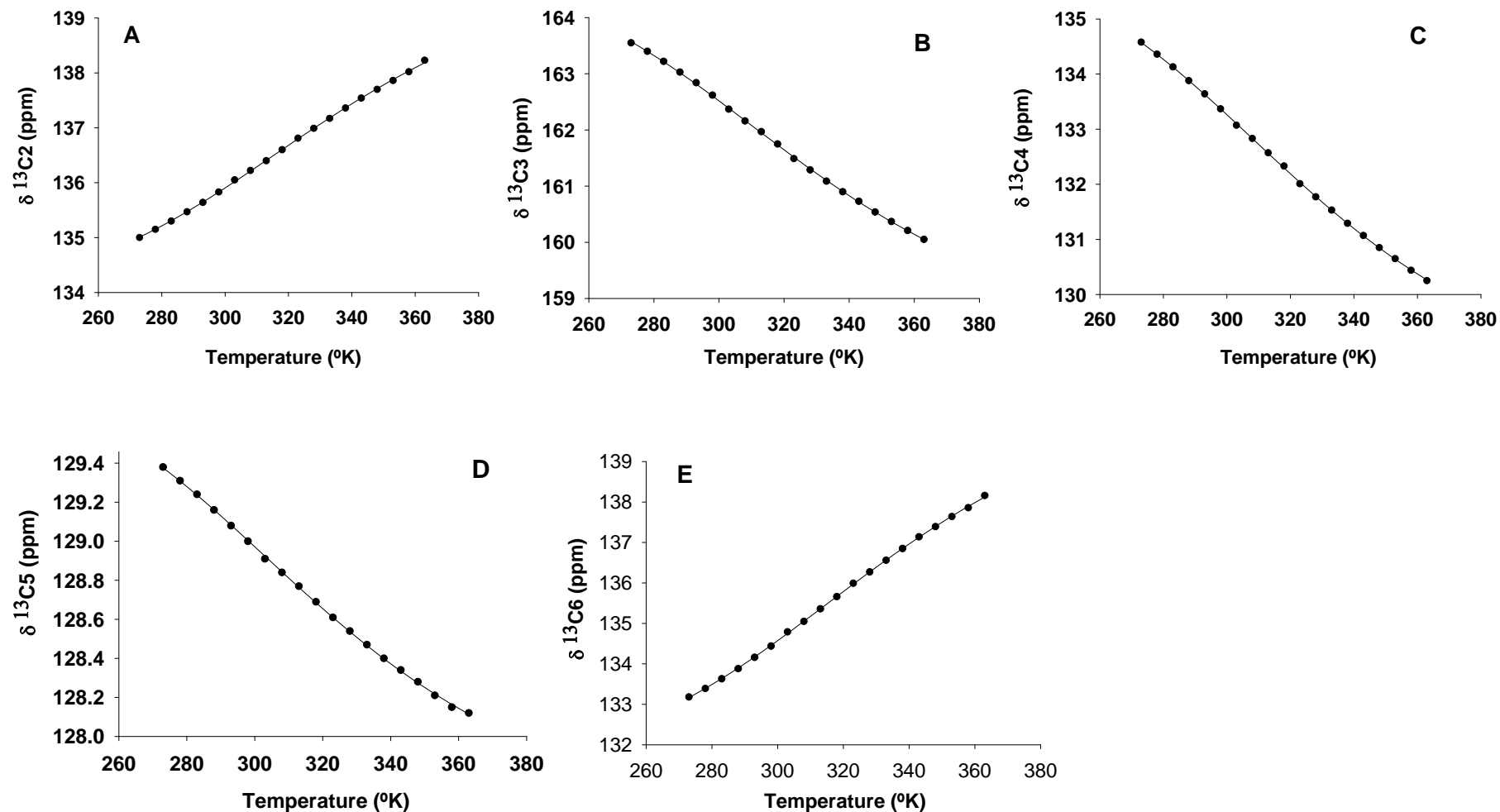


Figure S5. Temperature dependence of ^{13}C NMR average chemical shifts for the equilibrium between 3-HP(0) and 3-HP(\pm) in water. The continuous line is the theoretical curve, and dots are experimental chemical shifts at each given temperature. A) refers to C2; B) to C3; C) to C4; D) to C5; and E) to C6. The numbering is according to Scheme 1.

Table S1. Comparison between experimental ^{13}C NMR chemical shifts values (ppm) of H_3PM^{2+} , $\text{H}_2\text{PM}(\pm)^+$, $\text{HPM}(-)$ and PM^- (determined from a PM solution at pH 1.0, 6.0, 9.5 and 13 respectively) with their theoretical equivalents^a computed for the high stabilization energy conformations of H_3PM^{2+} , $\text{H}_2\text{PM}(\pm)^+$, $\text{HPM}(-)$ and PM^- .

Structure	^b C2	^b C2'	^b C3	^b C4	^b C4'	^b C5	^b C5'	^b C6
H₃PM²⁺_B	148.6	12.2	158.5	130.8	42.0	147.2	63.7	139.6
^cPM (pH 1.0)	145.6	17.8	152.5	138.9	37.3	140.8	61.3	133.9
H₂PM(±)⁺_A	142.0	18.7	175.3	124.0	47.4	149.4	66.8	133.6
^cPM (pH 6.0)	147.4	17.6	165.7	135.4	39.1	138.6	61.7	125.6
HPM(-)⁻_A	155.7	18.8	168.3	134.6	46.4	140.7	66.2	142.6
^cPM (pH 9.5)	151.6	20.4	--	128.3	40.5	139.9	61.5	133.4
PM⁻_D	158.5	23.7	171.8	138.1	39.6	145.8	67.0	131.6
PM⁻_E	158.8	22.9	173.3	140.8	45.0	137.3	66.7	130.7
^cPM (pH 13)	153.5	21.6	162.7	135.3	38.8	140.3	62.7	135.1

^a ^{13}C NMR chemical shifts were calculated by using GIAO method at PCM-B3LYP/6-311++G(d,p) level. Chemical shifts values are relative to TMS.
^bAtom numbers are illustrated in Scheme 2.
^cValues determined experimentally.

3-HPY absorption spectra and band deconvolution analysis.

3-HPY absorption spectra were recorded at 25 °C on a Shimadzu UV-2401 PC double-beam spectrophotometer. Quartz cells of 1cm path length were used to obtain electronic spectra. Spectroscopic data were acquired over the energy range from 3.10 to 5.64 eV. The buffer solution background spectrum was used as spectral reference. UV-vis spectra were obtained in various 0.02 M buffers having an ionic strength of 0.1 M adjusted by addition of KCl as required. The reagents used to prepare the buffer solutions were HCl (pH 1), sodium chloroacetate (pH 2 and 3), sodium acetate (pH 4 and 5), succinic acid (pH 6), potassium dihydrogen phosphate (pH 7, 7.5 and 8), boric acid (pH 8.5 and 9), potassium bicarbonate (pH 10 and 11) and NaOH (pH 12 and 13). Each buffer was used to prepare a solution containing 0.15 mM of 3-HPY. All the reagents were purchased to Sigma-Aldrich and used as received. Milli-Q water was used throughout.

The individual spectra obtained at each pH were used as inputs for the factor analysis software SPECFIT/32 (1). This software performs a global analysis of equilibrium systems with singular value decomposition and non-linear regression. Based on an equilibrium scheme between the different ionic forms, the software analyses mixed pH-dependent spectra and splits them into an individual spectrum for each ionic species in equilibrium.

Electronic transitions reflect in the presence of individual bands in the UV-vis spectrum of 3-HPY. Such bands are described in terms of four parameters, namely: position (excitation energy, E_{exc}), intensity (molar absorption coefficient, ϵ), oscillator strength (f), width (W) and symmetry (ρ). These bands were obtained from UV-vis spectra deconvolution by fitting the obtained spectra to a sum of 2-4 log-normal functions to resolve the contribution of each individual band to the overall absorption. Our curves were fitted by using the commercial software PeakFit v.4.0 (2). The input data consisted of an initial set of parameters estimated for each peak which the software iteratively optimized by reducing the difference between the experimental values and the combined curve obtained from the initial log-normal curve estimates.

The number of variables to be optimized was minimized by using $W=3.5 \cdot 10^3 \text{ cm}^{-1}$ for the bands with $E_{exc} > 5.1 \text{ eV}$, the corresponding value for the electronic transition with $E_{ex} < 5.1 \text{ eV}$ was $2.8 \cdot 10^3 \text{ cm}^{-1} \leq W \leq 3.4 \cdot 10^3 \text{ cm}^{-1}$. The bands with $E_{exc} > 5.1 \text{ eV}$ were assumed to have $\rho=1.4 \text{ eV}$. The UV-vis spectra for the 3-HPY ionic forms were deconvoluted into log-normal curves. The obtained results are listed in Table S2, which include excitation energy, band width, asymmetry, area and intensity values, all of them obtained from the fitting.

The 3- H_2P^+ spectrum was deconvoluted into two log-normal bands (Fig. S7A), band I centered at 4.38 eV and band II centered at 5.58 eV. Fig. S7B shows the UV-vis spectrum for the species 3-HP, which occurs as two tautomers in equilibrium. Band I and IV correspond to the first and second transition of the 3-HP(0) tautomer, whereas band II and III correspond to the 3-HP(\pm) tautomer. Fig. S7C shows the UV-vis spectrum for the 3- P^- ionic form. This spectrum was deconvoluted in three different bands: band I centered at 4.16 eV; band II centered at 4.91 eV; and band III centered at 5.26 eV.

Table S2. Excitation energies values, molar absorption coefficients, band width, asymmetry and molar area for the log-normal bands used in deconvolution processes done on each 3-HPY ionic form.

	Parameter	3-H₂P⁺	3-HP	3-P⁻
Band I	E_{exc} (eV)	4.38 ± 0.01	3.95 ± 0.01	4.16 ± 0.01
	ϵ (M ⁻¹ ·cm ⁻¹)	5643 ± 2	2781 ± 1	4262 ± 4
	Band width ·10 ⁻³ (cm ⁻¹)	3.27 ± 0.01	3.48 ± 0.01	3.45 ± 0.01
	Asymmetry (ρ)	1.41 ± 0.01	1.34 ± 0.01	1.40 ± 0.01
	Area (km/mol)	201.3 ± 10.1	104.9 ± 5.2	160.0 ± 8.0
Band II	E_{exc} (eV)	5.58 ± 0.2	4.50 ± 0.01	4.91 ± 0.04
	ϵ (M ⁻¹ ·cm ⁻¹)	3129 ± 3	1858 ± 2	809 ± 161
	Band width ·10 ⁻³ (cm ⁻¹)	4.48 ± 0.04	3.54 ± 0.01	2.58 ± 0.28
	Asymmetry (ρ)	1.43 ± 0.01	1.54 ± 0.01	1.0 ^a
	Area (km/mol)	153.0 ± 7.7	72.6 ± 3.6	22.21 ± 1.11
Band III	E_{exc} (eV)	--	5.04 ± 0.01	5.26 ± 0.01
	ϵ (M ⁻¹ ·cm ⁻¹)	--	4068 ± 4	9547 ± 49
	Band width ·10 ⁻³ (cm ⁻¹)	--	3.46 ± 0.01	3.88 ± 0.05
	Asymmetry (ρ)	--	1.41 ± 0.01	1.35 ± 0.02
	Area (km/mol)	--	153.4 ± 7.7	401.7 ± 20.1
Band IV	E_{exc} (eV)	--	6.2 ± 0.05 ^b	--
	ϵ (M ⁻¹ ·cm ⁻¹)	--	26670 ± 5087	--
	Band width ·10 ⁻³ (cm ⁻¹)	--	5.36 ± 0.3	--
	Asymmetry (ρ)	--	1.05 ^a	--
	Area (km/mol)	--	1295 ± 65	--

^aThe values without error value were fixed in the fitting process.

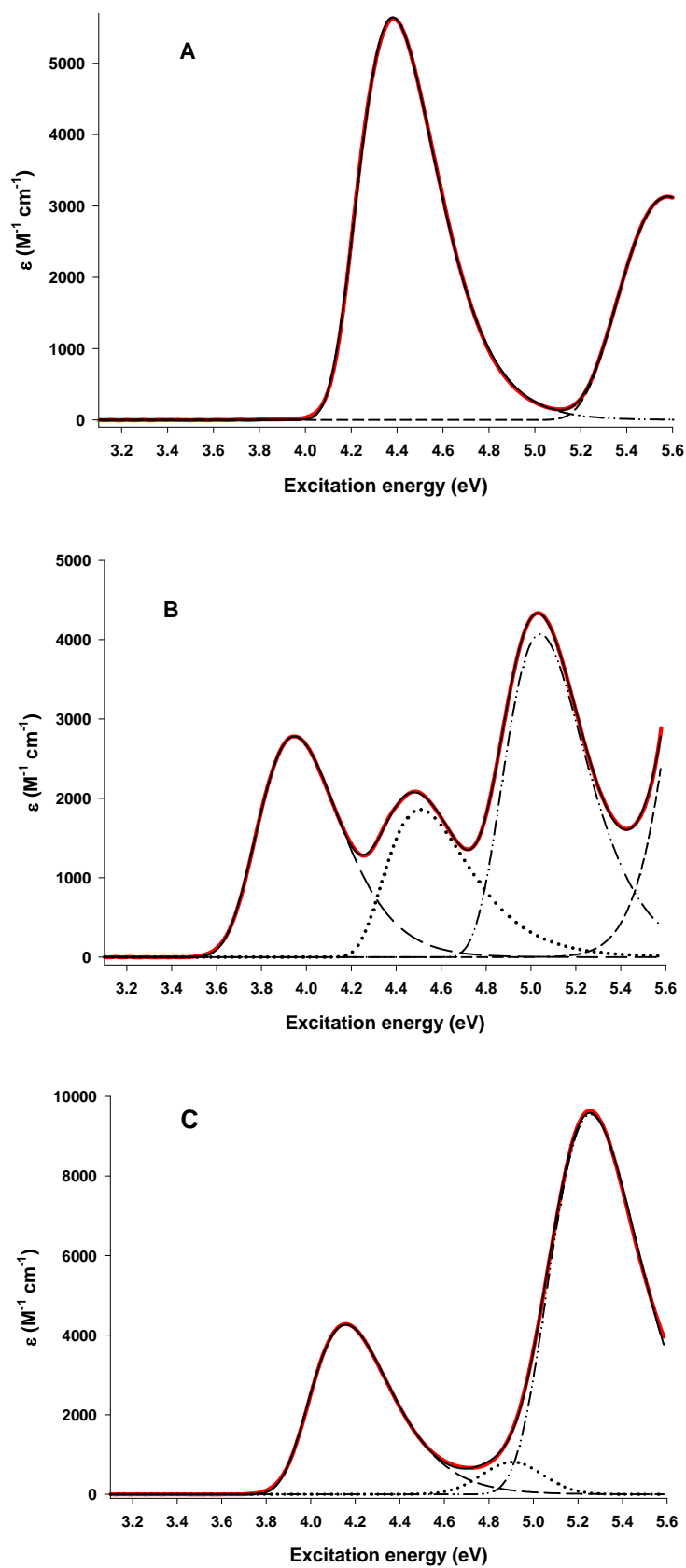


Figure S6. Experimental UV-vis spectra for 3- H_2P^+ (A), 3-HP (B), 3- P^- (C) (red lines). Fitted curves (—) obtained as combinations of a variable number of log-normal curves are shown as dashed lines.

Table S3. Calculated excitation energy values (eV) for different PM models.

	H₃PM²⁺_B	H₃PM²⁺	H₂PM(±)_A	H₂PM(±)⁺	^bH₂PM(+)_C	H₂PM(+)⁺_E	H₂PM(+)⁺	H₂PM(O)⁺_B	H₂PM(O)⁺		
<i>E_{exc}</i> (eV)	4.330 ^a	4.216 ^b	3.524 ^c	3.819 ^b	3.531 ^a	3.071 ^a	4.216 ^b	4.627 ^a	4.474 ^b		
	HPM(-)_A	HPM(-)	^bHPM(±)_D	HPM(±)_E	HPM(±)	^bHPM(O)_E	HPM(O)_C	HPM(O)	^bPM_D	PM_E	PM
<i>E_{exc}</i> (eV)	4.321 ^c	4.017 ^b	3.432 ^c	3.523 ^c	3.844 ^b	4.722 ^a	4.627 ^a	4.377 ^b	5.027 ^c	4.997 ^c	4.030 ^b
^a <i>E_{exc}</i> values were calculated by using the TD-DFT method at PCM-HCTH/6-311++G(d,p) level. Models for each structure are shown in Fig. 1 and S1. ^b Experimental <i>E_{exc}</i> values. ^c <i>E_{exc}</i> values were calculated by using the TD-DFT method at PCM-B3LYP/6-311++G(d,p) level. Models for each structure are shown in Fig. 1 and S1.											

References

1. R. A. Binstead, A. D. Zuberbühler and B. Jung. SPECFIT/32, V. 3.0.23, Spectrum Software Associates (1993-2001).
2. PeakFit v.4.0, AISN software, Ekrath, Bermany (1995)