Photoisomerization Action Spectrum of Retinal Protonated Schiff Base in the Gas Phase

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(Dated: 16 September 2014)

The photophysical behaviour of the isolated retinal protonated n-butylamine Schiff base (RPSB) is investigated in the gas phase using a combination of ion mobility spectrometry and laser spectroscopy. The RPSB cations are introduced by electrospray ionisation into an ion mobility mass spectrometer where they are exposed to tuneable laser radiation in the region of the $S_1 \leftarrow S_0$ transition (420-680 nm range). Four peaks are observed in the arrival time distribution of the RPSB ions. On the basis of predicted collision cross sections with nitrogen gas, the dominant peak is assigned to the all-*trans* isomer, whereas the subsidiary peaks are assigned to various single, double and triple *cis* geometric isomers. RPSB ions that absorb laser radiation undergo photoisomerization, leading to a detectable change in their drift speed. By monitoring the photoisomer signal as a function of laser wavelength an action spectrum, extending from 480 to 660 nm with a clear peak at 615 ± 5 nm, is obtained. The photoisomerization action spectrum is related to the absorption spectrum of isolated retinal RPSB molecules and should help benchmark future electronic structure calculations.

Keywords: retinal, electrospray ionization, ion mobility spectrometry, photoisomerization, laser spectroscopy

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I. INTRODUCTION

Retinal and its analogues play key functions in vision and photosynthesis, transforming photon energy into the structural reorganisation of proteins with prodigious efficiency. For example, the photoisomerization of the protonated retinal Schiff base (RPSB) plays a role in rhodopsin, the primary unit for vision, with the photoisomerization from 11-*cis* to the *trans* configuration being the primary event in the visual transduction process. In bacteriorhodopsin and halorhodopsin, which function as light activated proton and chloride pumps, respectively, the *trans* form of RPSB is photoisomerised to the 13-*cis* form. Remarkably, the absorption maximum of retinal can be tuned over a broad wavelength range by changing the RPSB configuration, protein environment and proximity of the counter ion, and occurs at 489 nm in rhodopsin, 568 nm in bacteriorhodopsin, 578 nm in halorhodopsin and 446 nm in ethanol solution.^{1,2} For retinal embedded in a protein, photoisomerization occurs on a 450-500 fs timescale,³ has a high quantum yield (0.64), and proceeds with extraordinary specificity for a particular product photo isomer.⁴ Understanding how the wavelength of the retinal absorption is governed by conformation and interactions with the surrounding protein and counter ion has long stood as a challenge for experimental and theoretical biophysicists.⁵ The current degree of control is highlighted by the recent encapsulation of all-*trans* RPSB in selectively modified versions of the human cellular retinol binding protein II (hCRBP II),⁶ in which electrostatic interactions controlling the electronic properties of the RPSB were modulated by introducing point mutations in the protein, thereby allowing tuning of the absorption maximum from 425 to 644 nm. In other recent studies, control over the excited state lifetime, the photoisomerization pathway and photoisomerization yield for RPSB in solution has been achieved chemically through the introduction of polarisable backbone substituents.^{7,8}

Although intermolecular interactions between the protein and the retinal molecule are capable of shifting the absorption maximum by several hundred nm, there is less certainty about the role of the inherent retinal conformation on the absorption wavelength. Indeed, within a protein environment it is difficult to disentangle intermolecular and intramolecular effects. In principle, the role of conformation can be elucidated more clearly by removing the protein scaffold and investigating the isolated retinal molecule. A common target for theoretical^{5,9} and experimental studies¹⁰⁻¹⁵ is the retinal protonated

n-butylamine Schiff base (Figure 1), which includes the n-butylamine group that usually tethers the retinal moiety to the surrounding native protein.

FIG. 1. The all-*trans* retinal protonated Schiff base. DFT calculations by Valsson *et al.* at the M06-2X/cc-pVDZ level predict that the isomers are 6*s*-*cis*(-) with $\phi = -38.8^\circ$ $\Delta E = 0$ kJ/mol, 6*s*-*trans* with $\phi = -170.1^\circ$ $\Delta E = +0.5$ kJ/mol, 6*s*-*cis*(+) with $\phi = +41.8^\circ$ $\Delta E = +1.4$ kJ/mol.⁹

Generating experimental spectroscopic data for isolated RPSB ions in the gas phase against which different computational approaches can be calibrated requires deploying a sufficiently sensitive spectroscopic technique for ultra-low concentrations, and preferably ensuring that the probed molecules have a single configuration so that an unambiguous connection can be established between the configuration and absorption spectrum. In pioneering studies, Andersen and coworkers probed the electronic transitions of n-butylamine RPSB ions in an ion storage ring by photodissociating them with a pulsed, tunable laser beam while monitoring neutral photofragments.^{10,11,13,14} The most recent photodissociation (PD) spectrum,¹³ which differs somewhat from previously reported versions,^{10,11} exhibits a broad peak with a flat-topped maximum extending from 610 nm down to 530 nm.¹³ The PD spectrum was interpreted as reflecting overlapping contributions from 6*s*-*trans* and 6*s*-*cis* configurations of RPSB (see Fig. 1) with peak absorptions at 610 and 530 nm, respectively.¹³

Here we investigate the electronic transitions and photoisomerization of RPSB using an alternative form of action spectroscopy, PhotoIsomerization Action (PISA) spectroscopy.^{16,17} The approach is a modification of ion mobility spectrometry (IMS), whereby ions drifting under the influence of an electric field through a buffer gas (usually helium or nitrogen) are separated spatially and temporally according to their collision cross section, which in turn depends on the molecular conformation. In the current work, we monitor photoisomerization from all-*trans* RPSB to different *cis* versions of RPSB as the ions drift under the influence of an electric field through a cell filled with N₂ buffer gas. Generally, the *cis* forms of RPSB are more compact than the *trans* form and travel through the drift cell

more rapidly. We have previously deployed the approach to investigate the electronic absorptions and photoisomerization of the carbocyanine dye cations HITC⁺ and DTC⁺ in the gas phase.^{16,17}

Our approach has several potential advantages for investigating the photophysical behaviour of RPSB. First, even without laser excitation, IMS provides information on the different RPSB isomers generated by the electrospray ionisation source. Second, by monitoring the intensity of the photo isomer peaks or depletion of the parent peak as a function of laser wavelength, one can generate an action spectrum that is related to the absorption spectrum convoluted with isomerization probability (which may depend on wavelength). For RPSB, the action spectrum obtained in this way complements the PD action spectra obtained by monitoring photo fragments.^{10,11,13,14} *Trans-cis* isomerization barriers for RPSB are typically around 100 kJ/mol, much less than bond dissociation energies (300-400 kJ/mol), ensuring that the entire thermal population potentially contributes to the PISA spectrum.

II. METHODS

The experimental approach involves irradiating protonated RPSB in a custom built ion mobility apparatus.^{16,17} The experimental layout is shown in Figure 2. RPSB cations were electrosprayed from a 10⁻⁵ M solution of *trans* RPSB in 1:1 methanol:H₂O with a few drops of acetic acid (electrospray voltage 3 kV, flow rate ~5 μ L/min). The ions passed through a heated desolvation capillary ($T=500$ K) and were accumulated in an ion funnel before being launched in a 150 μ s pulse into a 0.9 m drift tube filled with N₂ buffer gas ($P=14$ torr). The electric field in the drift tube (44 V/cm) was sustained by a series of 90 ring electrodes. At the end of the drift tube, the ions were gathered radially by a second ion funnel before passing through a 0.3 mm orifice into an octopole ion guide from which they exited through a 3 mm orifice into a quadrupole mass filter (QMF) set to $m/z=340$ amu. Ions exiting the QMF were sensed by a channeltron detector connected to a discriminator and a multichannel scaler (MCS). The apparatus was run at 20 Hz and the ions' arrival time distribution (ATD) built up as a histogram of ion counts versus time. The mobility resolution for the RPSB ions was typically $t_d/\Delta t_d=60$, which can be compared to a diffusion-limited resolution under the prevailing conditions of 120.¹⁶ Under typical

FIG. 2. Schematic illustration of the gas-phase RPSB photoisomerization experiment. Electro-sprayed RPSB ions are collected by an ion funnel and injected into a 0.9 m drift tube filled with N_2 buffer gas where they are exposed to tuneable visible radiation. The ions then drift under the influence of an electric field through the buffer gas before being collected by a second ion funnel, and passed through an octopole and a quadrupole mass filter. Photoisomerization changes the ions' collision cross section and speed, such that they arrive at the ion detector earlier or later than the parent ions.

operating conditions with a drift field of 44 V/cm, N_2 buffer gas pressure and temperature of 14 torr and 295 K, respectively, the effective temperature of the ions is predicted to be $T_{eff}=303$ K.¹⁸

For the photoisomerization studies, alternate ion packets were irradiated immediately after exiting the first ion funnel with the 10 Hz output of a pulsed, tuneable optical parametric oscillator (OPO, $\lambda=410-710$ nm, 10 ns pulse width, 5 cm^{-1} bandwidth, 5-10 mJ/pulse, ~ 5 mm beam diameter). The laser-induced change was recorded as the difference between laser-on and laser-off ATD histograms. Typically, the energy was 5-10 mJ/pulse over the RPSB absorption band and had a cross section of $\sim 5\text{ cm}^2$, corresponding to energy fluxes

of 1-2 mJ/cm²/pulse. The photoisomerization signal was normalised by photon number. All RPSB isomers present in the ion packet are simultaneously exposed to light at the beginning of the drift region and are therefore potentially depleted or augmented by the light pulse.

At the beam intensities used in this study photoisomerization was the dominant process with the number of RPSB ions undergoing photoisomerization at least 100 times greater than the number photodissociating (see Section III A). However, if we increased the laser beam intensity by an order of magnitude (to 20-30 mJ/cm² at 532 nm), we clearly observed $m/z=248$ amu photofragments, as found in previous fast ion beam studies.¹⁵ These photofragments have the same drift mobility as background $m/z=248$ amu ions generated directly by the ion source. We believe that in our experiment the $m/z=248$ amu photofragments at these higher intensities (which are well above those used for the photoisomerization investigations described in this paper) arise from multi-photon excitation of the RPSB.

The all-*trans* RPSB was synthesized under exclusion of light in an Ar atmosphere according to previous methods.¹⁹ Briefly, all-*trans* retinal (Sigma Aldrich) was added to an equimolar amount of n-butylamine (Sigma Aldrich) in dry CHCl₃. The mixture was heated to reflux conditions until ¹H NMR showed complete disappearance of the carbonyl signal at approximately 10 ppm (*ca* 1 hr) following which the product was collected by evaporation of the solvent under reduced pressure. The isomeric purity of the product was established by HPLC.

III. RESULTS AND DISCUSSION

A. Arrival time distribution of electrosprayed RPSB cations

The arrival time distribution (ATD) for RPSB ions ($m/z=340$ amu) without laser radiation is shown in Figure 3a. Although the electrosprayed sample was prepared as *trans* RPSB, the ion mobility data provide clear evidence that at least 4 different isomers with $m/z=340$ amu are present in the drifting ion population (Peaks A-D). Peak arrival times, collision cross sections, and tentative assignments are given in Table I. We discuss the peak assignments later in Section IV, remarking here only that the dominant, slow

FIG. 3. (a) Arrival time distribution for electrosprayed RPSB. (b) Difference between laser-off and laser-on ATDs. Peaks have each been fitted with Gaussian functions (fwhm=0.7 ms) as indicated by the solid lines. The dotted line is proportional to the integral of the laser on/off difference signal with respect to arrival time. (c) Simulated ATD assuming a Boltzmann distribution of isomers at $T=600$ K (solid line), and difference between ATDs at $T=800$ K and 600 K (dotted line). Ranges for predicted arrival times for single, double and triple *cis* isomers are indicated by horizontal lines.

peak (peak A) is almost certainly due to the all-*trans* RPSB isomer and that the earlier peaks arise from either geometric RPSB isomers, RPSB protomers (structures in which the proton is localised on a C atom), or possibly cyclic forms of RPSB. These other isomers are presumably formed either in the electrospray process or during passage through the desolvation capillary where the temperature is ~ 500 K.

The effect of laser excitation at 590 nm on the drifting RPSB population is illustrated in Figure 3b where the difference between the laser-on and laser-off ATDs is plotted. There is

TABLE I. Arrival times (t_a), collision cross sections (σ_{meas}), and assignments for experimental ATD peaks shown in Figure 3.

Peak	t_a ms	σ_{meas} $\text{\AA}^2$	assignment	formation
A	44.15	227.9	<i>trans</i> , 8c, 12c, 13c	thermal/photo
B	43.25	222.6	9c	thermal/photo
C	41.30	212.5	triple- <i>cis</i> , cyclic	thermal/photo
D	40.10	206.3	triple- <i>cis</i> , cyclic	thermal
E	42.60	219.2	double- <i>cis</i> /triple- <i>cis</i>	photo

an obvious depletion of the lowest mobility species (peak A) accompanied by formation of the isomer(s) associated with peaks B and C. Peak D is enhanced only very slightly by laser irradiation. Significantly, a new photo-induced isomer peak appears at 42.60 ms (peak E), which is in the region expected for double-*cis* or triple-*cis* isomers (see Section IV). The isomer(s) responsible for peak E is (are) either formed by a direct photochemical transformation or requires more energy than is available thermally. Crucially, the depletion of peak A is completely balanced by enhancements of peaks B, C and E, as demonstrated by the fact that the integral of the difference ATD signal **with respect to arrival time** (dotted curve in Figure 3b) returns to zero (to within 1%) by $t_a=45.5$ ms. Given that the ATD was recorded with the quadrupole mass filter set to $m/z=340$ amu, this is compelling evidence that photoisomerization is the only significant photo-process and that photodissociation and other loss mechanisms are negligible **under the operative experimental conditions**.

B. Photoisomerization Action Spectrum

We turn now to a discussion of the wavelength dependence for the RPSB photoisomerization. The difference between the laser-on and laser-off ATDs as a function of OPO wavelength is presented as a two-dimensional plot in Figure 4 where it can be seen that the wavelength dependence for the depletion of the slowest ions (peak A), which is assigned to the all-*trans* isomer of RPSB, is very similar to the wavelength dependence for the

FIG. 4. Difference between laser-on and laser-off ATDs as a function of wavelength (x-axis) and ion arrival time (y-axis) with the mass spectrometer set to $m/z=342$ amu. The blue region corresponds to a laser-induced depletion of *trans* RPSB ions (Peak A in Fig. 3), and the red/yellow regions to augmentation of faster, more compact isomeric structures (peaks B, C, E in Fig. 3).

growth of the photo isomers (peaks B, C & E). The wavelength dependence of the photo activity is more apparent in Figure 5 where the depletion of the all-*trans* RPSB signal (Peak A) is plotted as a function of wavelength. The observed band, which corresponds to the $S_1 \leftarrow S_0$ transition, ranges from 440 to 660 nm with a distinct maximum at 615 ± 5 nm. The photo-enhancements of peaks B, C and E are also plotted as function of wavelength in Figure 5 and display a similar wavelength dependence, although, the maximum of the curve for peak C is perhaps slightly blue-shifted by 10 nm to 605 nm. The gas-phase PISA absorption band is red-shifted by 170 nm from the peak of the $S_1 \leftarrow S_0$ band for RPSB in acetonitrile ($\lambda_{max}=445$ nm). There is also evidence for photoisomerization below 450 nm in the spectral region associated with the onset of the $S_2 \leftarrow S_0$ transition.¹¹

Notably, the shape of the PISA $S_1 \leftarrow S_0$ band differs from the most recently reported photodissociation (PD) spectrum of RPSB, which exhibits a broader peak with a flat top extending from 530 to 610 nm.¹³ The PD spectrum was interpreted as reflecting overlapping contributions from the 6*s-trans* and 6*s-cis* configurations of RPSB with peak absorptions at 610 and 530 nm, respectively.¹³ This explanation was supported by complete active space self-consistent field method (CASSCF) calculations that predict absorptions at 620 and 547 nm for the 6*s-trans* and 6*s-cis* forms, respectively, and also on the basis of separately obtained spectra of RPSB derivatives in which the β -ionone ring was chemically modified.

FIG. 5. PISA spectrum of RPSB in the gas phase normalised by photon flux. The raw spectrum and the laser power curve are available in the supplemental material (SM).²⁰

The CASSCF methodology was subsequently criticised as lacking appropriate dynamical correlation by Valsson and Filippi,⁹ who, using an alternative multiconfigurational perturbative (NEVPT2) approach, predicted that the RPSB absorption wavelengths were 561 nm for the 6s-*trans*, 537 nm for 6s-*cis*(-), and 532 nm for 6s-*cis*(+) configurations, a range of 29 nm. The PISA spectrum, which does not exhibit a plateau but rather a well defined peak at 615 nm, is consistent with the 6s-*trans*, 6s-*cis*(-) and 6s-*cis*(+) isomers having similar absorption wavelengths, near the experimental peak maximum (615 nm). This assumes, as predicted by our calculations and those of others,^{9,14} that the three 6s isomers have similar energies and comparable populations at the effective temperature of the ions in the drift tube (303 K). It is worth noting that the experimental band maximum is very close to the wavelength (619 nm) predicted recently for the 6s-*cis*(-) isomer of RPSB (with an H atom rather than a butyl group bonded to the N atom) using the SF-BHHLYP/DH(d,p) and XMC-QDPT2/cc-pVDZ methods for the S_0 and S_1 states, respectively.²¹ Corresponding calculations for the 6s-*cis*(+) and 6s-*trans* isomers were not reported.

It is conceivable that some of the signal to shorter wavelength in the PISA spectrum (between 490 and 580 nm) is due to different isomeric forms. However, it is likely that at least some of this signal comes from unresolved vibrational progressions that are excited due to changes in C-C bond lengths accompanying the $S_1 \leftarrow S_0$ transition.^{9,21}

In considering the differences between PD and PISA spectra it is first useful to examine why we observe photoisomerization of RPSB and not photodissociation to produce

$m/z=248$ amu photofragments as detected previously.¹⁵ As explained in Section III A, photodissociation is negligible at the light intensities used in the present experiment ($<1\%$ of the photoisomerization signal), although at much higher light intensities ($\times 10$) we do observe $m/z=248$ amu photofragments. There are several possible explanations for the absence of PD in the drift cell at low light levels depending on whether the photon energy (E_{ph}) is more or less than the energy of the dissociation barrier (E_d).

1. A single visible photon provides sufficient energy to fragment the RPSB cation ($E_{ph} > E_d$), however, collisional quenching in the PISA apparatus rapidly deprives the RPSB ions of energy so that they are unable to overcome the dissociation barrier yet are still able to surmount the much lower isomerization barriers. This would imply that the dissociation rate following single photon absorption is much lower than the collision rate ($5 \times 10^8 \text{ s}^{-1}$). In this case, the PISA and PD spectra should be similar (assuming identical laser beam intensities, ion internal energies, and isomer distributions).
2. Absorption of a single visible photon does not provide most RSPB ions at 303 K with sufficient energy to overcome the dissociation barrier ($E_{ph} < E_d$) but still gives them enough energy to photoisomerize. If this was true, previous PD studies of RPSB may have involved either hot ions and or multiphoton absorption, both of which can lead to spectral distortions. Regarding the possibility for multiple photon absorption, it is worth emphasising that measuring a linear dependence for the photodissociation signal against laser power over a limited intensity range does not necessarily exclude multiphoton absorption as the $S_1 \leftarrow S_0$ transition of RPSB is strong and easily saturated at low light intensity. Beam intensities were not provided for previous PD spectroscopic studies of RPSB,^{10,11,13} making it impossible to compare them with those in the current PISA investigation ($1\text{-}2 \text{ mJ/cm}^2/\text{pulse}$).

Obviously, the magnitude of the dissociation barrier is a key issue in determining whether the PD spectrum will resemble the absorption spectrum. Assuming that single photon fragmentation occurs (Option 1 above - which at this stage we believe is not certain for cold RPSB ions), there are several possible explanations for the discrepancy between the PD and PISA spectra. For example, it is conceivable that the differences

between the PD and PISA spectra are due to different isomeric compositions for the ion populations in the two experiments. Certainly, the presence of several isomer peaks in the ATD for electrosprayed RPSB cations (Figure 3) would be consistent with the PISA spectrum containing contributions from several low energy RPSB conformers, presumably including those in which the β -ionone ring is rotated around the 6s bond. The fact that the peak in the PD spectrum is broader than the PISA spectrum might imply that an even larger range of isomers was present in the PD experiment.

It is also possible that the RPSB ions in the PD experiment are vibrationally hotter than those in the PISA experiment, leading to a broadening of the visible band in the PD spectrum. In the PISA experiment, the ions have undergone roughly 10^6 collisions with N_2 buffer gas molecules after exiting the first ion funnel and before being intercepted by the laser beam and therefore should be thermalised vibrationally at $T_{eff}=303$ K. The RPSB cations in the PD experiment, which were also created using an electrospray source, were presumably also thermalised in the source. However, conceivably they were heated through gas collisions while being stored or accelerated to 22 keV to circulate in the ion storage ring. In principle, the effects of the creation, storage and acceleration process on the internal energy of the ions could be assessed by obtaining PD spectra of molecular ions that have well-resolved vibronic structure and which have previously been spectroscopically characterised under controlled conditions, including the para-dichlorobenzene cation,²² protonated tryptophan or tyrosine cations.²³

The differences might also stem from a wavelength dependence for the photoisomerization yield. For example, it is conceivable that the pronounced 615 nm peak in the PISA spectrum of RPSB arises because the photoisomerization yield peaks near 615 nm and decreases for shorter wavelengths. The photoisomerization barriers (40-130 kJ/mol; see Section IV) are much lower than the photon energies, so any variation of the photoisomerization yield with wavelength is unlikely to arise because of energy constraints. The evidence from condensed phase studies for a wavelength dependence of the photoisomerization yield is equivocal; for all-*trans* RPSB embedded in proteins and in acetonitrile solution, the photoisomerization quantum yield has been found to be independent of wavelength, whereas for all-*trans* RPSB in methanol the photoisomerization yield decreases by 25% going from 540 to 440 nm (although the variation is within the error range for the yield measurements).²⁴ Although we cannot entirely discount the possibility for

FIG. 6. Isomeric forms of RPSB determined from M06-2X/cc-pVDZ DFT calculations. In each case, the energy in kJ/mol is given with respect to the all-*trans* isomer of RPSB. Energetic and structural details of these and other isomers are given in the SM.²⁰

a strong wavelength dependence for the photoisomerization yield in the gas phase, we consider it unlikely.

IV. ATD PEAK ASSIGNMENTS

We now consider the RPSB isomers that are responsible for the observed ATD peaks. Although the conformational landscape of RPSB in the gas phase is extensive and complicated, the relative simplicity of the ATDs shown in Figure 3 which contain just 5 peaks suggests that only a restricted range of RPSB isomers are present in the IMS apparatus or that several isomers have very similar collision cross sections. As noted earlier, the most intense peak at 44.15 ms (peak A) most likely corresponds to all-*trans* RPSB, which has a predicted collision cross section of $220 \text{ \AA}^2$, 3.5% less than the measured value ($227.9 \text{ \AA}^2$). Assignment of the other peaks is less secure but they presumably belong to one of the three classes of molecules illustrated in Figure 6. 1. RPSB geometric isomers in which there are one or more *cis* bonds in the retinal chain (Figure 6a). 2. RPSB protomers, structures in which the excess proton is localised on a C atom rather than on the N atom (Figure 6b). 3. Cyclized versions of RPSB, akin to those previously observed in solution by Okamura et al. (Figure 6c).²⁵ Below, we consider these explanations in turn.

To help assign the ATD peaks we employed M06-2X/cc-pVDZ DFT calculations to predict the equilibrium geometries, vibrational frequencies, energies, free energies of single, double and some triple *cis* geometrical isomers of RPSB, along with protomers, and cyclic forms. The M06-2X functional with its intermediate amount of exact exchange has been found to give an accurate description of RPSB's geometrical and energetic features.⁹ The geometrical structures were used to predict collision cross sections and mobilities using the classical trajectory method (TM) as instituted in the Mobcal program.^{26,27} Atom-atom potential energy parameters for the interaction between N_2 and the RPSB molecule were taken from ref. 28. Partial atomic charges were calculated using the Merz-Singh-Kollman scheme,²⁹ constrained to reproduce the molecular electrostatic potential. The DFT calculations were carried out using the Gaussian09 suite.³⁰

1. Geometric Isomers

Perhaps the simplest and most likely explanation for the 5 observed ATD peaks is that they correspond to various geometric isomers of N-protonated RPSB of the type shown in Figure 6a. This explanation is consistent with the laser induced depletion of the

all-*trans* isomer (Peak A) and enhancement of peaks B-E because in solution *trans* RPSB is known to photoisomerize to other *cis* isomers including 11-*cis*, 13-*cis* and 9-*cis* RPSB.^{31,32} These single *cis* RPSB isomers have similar absorption band profiles to the all-*trans* isomer but have 20-50% lower absorption cross sections.^{31,32} Therefore, simultaneous irradiation of an ion packet consisting of various geometric isomers of RSPB can be expected to result in net conversion of all-*trans* RPSB isomer to other *cis* isomers.

Considering only the conjugated retinal chain and ignoring rotations about the 6s bond and about the bonds in the butyl chain, there are 256 different *trans-cis* isomers. Here we have undertaken preliminary DFT explorations of the geometries and energies of ~ 100 different isomers, including single *cis*, double *cis*, and selected triple *cis* isomers of the retinal chain. We also calculated barriers for isomerization between the all-*trans* isomer to the single *cis* isomers and from single *cis* isomers to selected double *cis* isomers. Computed properties of the single *cis* isomers (averaged for internal rotation around the 6s bond, *vide infra*) are compiled in Table II. More comprehensive data for single *cis*, double *cis* and some triple *cis* isomers are provided in the SM.²⁰

The single *cis* isomers are predicted to lie 6-24 kJ/mol above the all-*trans* isomer and are possibly present in significant abundance in the drifting ion population. Barriers for *trans-cis* isomerization about the retinal bonds are relatively high and exhibit an alternation such that barriers for rotation about the formal double bonds are higher than for rotation about the single bonds, although the difference diminishes moving away from the β -ionone ring. Isomerization of the all-*trans* isomer to form the 7-*cis*, 9-*cis*, 11-*cis* and 13-*cis* isomers is calculated to require surmounting 100-130 kJ/mol barriers on the S_0 surface whereas barriers for isomerization around the 8, 10, 12 and 14 single bonds range from 40 to 80 kJ/mol (see Table II). One can expect that the 8-*cis*, 9-*cis*, 12-*cis* and 13-*cis* isomers, which all have energies ≤ 15 kJ/mol above the ground state, will be formed from the *trans* isomer in the heated desolvation capillary where the temperature is ~ 500 K, and will probably survive the passage through the drift region.

It is important to recognise that isomers that have similar energies and are separated by low barriers will interconvert as they drift through the apparatus and so will generate a single ATD peak. For example, as previously noted,⁹ in the gas phase RPSB has 3 low-lying conformers corresponding to rotation around the 6s bond, that have similar energies and relatively low interconversion barriers. DFT calculations by Valsson *et al.* at the M06-2X/cc-

TABLE II. Data for low energy conformers of RPSB determined from M06-2X/cc-pVDZ DFT calculations. In each case the 6s bond is in the (-) configuration (see Fig. 1). Included are zero-point corrected energies (ΔE) and free energies (ΔG at $T=298$ K) relative to that of the lowest energy isomer, barrier height for isomerization from the *trans* isomer, and average collision cross section (σ) in N_2 calculated using Eqn. 1. More complete calculated data for RPSB isomers are reported in the SM.²⁰

	ΔE	ΔG	barrier	σ_{calc}
	kJ/mol	kJ/mol	kJ/mol	$\text{\AA}^2$
				(calc)
<i>trans</i>	0	0		219.9
7- <i>cis</i>	16.2	18.0	117	216.0
8- <i>cis</i>	11.5	10.8	42	218.4
9- <i>cis</i>	6.0	8.3	131	216.4
10- <i>cis</i>	23.6	27.1	60	217.9
11- <i>cis</i>	20.2	15.8	113	217.6
12- <i>cis</i>	10.7	14.2	68	220.3
13- <i>cis</i>	5.5	3.8	100	219.2
14- <i>cis</i>	17.9	19.8	81	219.0

pVDZ level predict that the isomers are 6s-*cis*(-) with $\phi=-38.8^\circ$ $\Delta E=0$ kJ/mol, 6s-*trans* with $\phi=-170.1^\circ$ $\Delta E=+0.5$ kJ/mol, 6s-*cis*(+) with $\phi=+41.8^\circ$ $\Delta E=+1.4$ kJ/mol.⁹ Energy barriers between the 6s-*cis*(-) and 6s-*cis*(+) forms and between 6s-*cis*(-) and *trans* forms are 6 and 14 kJ/mol, respectively.⁹ Energies and geometries from our M06-2X/cc-pVDZ calculations are very similar (see SM²⁰). Under the conditions prevailing in the drift region ($T_{eff}\sim 303$ K, collision rate $\sim 5\times 10^8$ s⁻¹), these 6s isomers are expected to interconvert on timescales short compared to the drift time (~ 45 ms) and so should appear as a single peak in the ATD. Comparable DFT calculations show that the barriers for internal rotation around the 6s bond are also low for the 7-*cis*, 8-*cis*, 9-*cis*, 10-*cis*, 11-*cis*, 12-*cis* and 13-*cis* isomers, and that in each case the three 6s conformers should interconvert as they traverse the drift region. To take this conformational averaging into account, we estimated the average

collision cross-section ($\bar{\sigma}$) for interconverting population of 6s isomers as,

$$\bar{\sigma} = \sum_{i=1}^3 \sigma_i e^{\frac{-\Delta G_i}{RT}} / \sum_{i=1}^3 e^{\frac{-\Delta G_i}{RT}} \quad (1)$$

where the sum is over the 6s-*cis*(-), 6s-*cis*(+) and 6s-*trans* isomers, and in each case ΔG_i is the relative free energy at $T=298$ K (close to the effective temperature in the drift region). Averaged cross-sections for selected single *cis* isomers along with isomerization barriers are listed in Table II.

The n-butyl chain of RPSB is also expected to undergo conformational rearrangements as the RPSB ions drift through the buffer gas because typical barrier heights for rotation around the N-C and C-C bonds in the alkyl chain are expected to be ≤ 20 kJ/mol. At this stage, we have not taken these butyl chain rearrangements into account, restricting our attention to isomers in which the butyl chain is fully extended. Conformational rearrangements of the butyl chain should lead on average to somewhat more compact forms with slightly higher mobilities than the extended forms. Here we assume that the conformational averaging of the butyl chain has a similar effect on the drift mobilities for the different RPSB isomers. Eventually, estimating the effect of the conformational rearrangements of the butyl chain on the drift mobility is probably best accomplished using a molecular mechanics approach.

To explore whether the measured ATD is consistent with a thermal population of single, double and triple *cis* RPSB isomers, we have simulated the ATD using calculated energies and mobilities for ~ 100 different energetically low-lying geometric isomers with averaging over the 6s rotation for the collision cross section. This was achieved by associating each isomer with a Gaussian peak (fwhm=0.6 ms) centred at the predicted arrival time (determined from its calculated mobility), and an intensity that assumes a Boltzmann distribution of isomers at $T=600$ K (100 K higher than the temperature of the electrospray desolvation capillary). The collision cross sections for the different isomers were scaled such that the predicted arrival time for the all-*trans* isomer corresponded to the measured arrival time for peak A. The simulation assumes that the ions rearrange relatively slowly over the barriers connecting the various geometric isomeric forms, which typically have heights of ~ 100 kJ/mol for rotation around the odd C-C bonds of the retinal chain and ~ 50 kJ/mol for rotation around the even bonds (Table II).

Notwithstanding the crudity of the underlying assumptions, the simulated ATD shown

in Figure 3c reproduces two fastest peaks in the ATD (peaks A and B) reasonably well. Several single *cis* isomers (8-*cis*, 12-*cis*, 13-*cis*) are predicted to have a similar collision cross-sections to the all-*trans* isomer and lie sufficiently low in energy (≤ 15 kJ/mol with respect to the all-*trans* isomer) to also contribute to the dominant peak A. The slightly earlier peak B, centered at 43.25 ms corresponds to ions with 2% smaller collision cross section, and can be assigned primarily to the 9-*cis* form with contributions from the 7-*cis* and 11-*cis* forms (predicted to lie 6.6, 16.5 & 21.3 kJ/mol above the all-*trans* form, respectively). A simulated laser on/off ATD generated by assuming that the laser pulse merely heats the molecules from 600 to 800 K is shown in Figure 3c. The resemblance to the measured laser on/off ATD is also reasonable in the region associated with the single and double *cis* isomers (peaks A, B and E).

Generally, the simulated ATD is less satisfactory in the region near the earlier peaks C and D (arrival times 41.30 and 40.10 ms), which correspond to ions with collision cross sections that are respectively 7 and 9% smaller than the all-*trans* isomer, and must be due to more compact isomers. Calculated collision cross sections based on DFT structures suggest that these earlier peaks are possibly associated with geometric isomers possessing three adjacent *cis* bonds (e.g. 9-*cis* 10-*cis* 11-*cis*, 11-*cis* 12-*cis* 13-*cis* or 12-*cis* 13-*cis* 14-*cis* isomers). These triple-*cis* isomers lie ≥ 30 kJ/mol above the ground state and are predicted to have very low populations at $T=600$ K but become significantly populated at higher temperatures and may become important following photoexcitation.

2. Protomers

As noted above, an alternative explanation for the faster isomers (peaks C and D and perhaps peak E) is that they correspond to RPSB protomers, structures in which the excess proton is localised on a C atom rather than on the N atom (Figure 6b). DFT calculations demonstrate that these protomer structures lie 79-191 kJ/mol above the all-*trans* form of RPSB and therefore they are less likely to be formed in the electrospray ion source than the *trans-cis* geometric isomers (see SM²⁰ for protomer structures and energies). Preliminary DFT and mobility calculations suggest that the protomers in their extended configurations have mobilities that are comparable to the geometric isomers of RPSB and have predicted arrival times ranging from 42.7 to 44.5 ms. The lowest energy protomer is predicted to

have the excess proton attached to the 14 C, lie 79 kJ/mol above the N protonated form, and have an arrival time of 43.7 ms. Intriguingly, if the peaks C and E indeed correspond to protomers, then one would be obliged to conclude that visible photo-excitation can induce intramolecular proton transfer in RPSB. Whether this process is feasible depends of course on the magnitude of the energy barrier separating them from the N protonated version.

3. Cyclic versions of RPSB

A third explanation for the faster, more compact isomers (peaks C and D) is that they are due to cyclic versions of RPSB similar to those previously observed by Okamura et al.²⁵ (Figure 6c). Generally, these proposed cyclic versions, two examples of which are illustrated in Figure 6c, have collision cross sections that are 10-15% less than all-*trans* RPSB and are predicted to arrive in the 35-41 ms range. For example, the lowest energy cyclic form we have managed to locate computationally is shown on the right of Figure 6c and lies 12.9 kJ/mol below the all-*trans* form of RPSB. Its predicted collision cross section is ~10% less than the all-*trans* RPSB isomer and its predicted arrival time is 39.6 ms, slightly less than peak D. Again, assignment of peaks C and D to cyclic structures would imply that RPSB can be photochemically transformed into cyclic versions of RPSB in the gas phase.

We also considered the tricyclic structure shown in Figure 6d, which has been proposed as an intermediate in the photodissociation of RPSB under collision free conditions.¹⁵ It has been postulated that the tricyclic isomer forms rapidly from RPSB following excitation of the $S_1 \leftarrow S_0$ transition and then decomposes slowly (on the ms timescale) to give a charged bicyclic fragment with $m/z=248$ amu and a toluene molecule (or isomer thereof). The most likely stereoisomer of the tricyclic structure is calculated to have an energy 30 kJ/mol above all-*trans* RPSB and a collision cross section with N_2 of $188 \text{ \AA}^2$, around 15% lower than that of the all-*trans* isomer. Its predicted arrival time is 38 ms, well before any of the observed ATD peaks. Therefore, this tricyclic intermediate appears unlikely to be formed, either through collisions or photo excitation, under the higher pressure conditions prevailing in our apparatus.

In principle, the absorption spectra of the isomers can be used as a means for their

identification, deciding whether they are indeed due to geometric RPSB isomers (Figure 6a) or to various protomers and cyclic versions (Figures 6b & c). These latter species possess shorter conjugated sequences than N-protonated RPSB and will therefore have distinctly different absorption bands, most likely occurring to shorter wavelength. As shown in Section III B below, there is no indication that the different isomers (peaks A-E) have significantly different absorption profiles, although admittedly we have not yet conducted systematic scans at wavelengths shorter than 410 nm.

V. PHOTOISOMERIZATION MECHANISM

As far as we are aware this is the first experimental work to address photoisomerization of RPSB in the gas phase. In solution and when embedded in a protein, *trans* RPSB is hypothesised to photoisomerize to *cis* isomers (e.g. 11-*cis*, 13-*cis*) through a conical intersection (CI) linking the S_1 and S_0 surfaces.³³ Data on the photoisomerization pathways of RPSB in methanol solution are summarised in Table III. The photoisomerization for all-*trans* RPSB in methanol has been found to proceed with high specificity and has a measured quantum yield of 0.17 ± 0.03 .³² HPLC analysis of photoproducts shows that *trans* RPSB in methanol photoisomerizes primarily to 11-*cis* RPSB with formation of 13-*cis*, 9-*cis*, and 7-*cis* isomers being less efficient by an order of magnitude.³² On the other hand, both the 11-*cis* and 13-*cis* forms thermally rearrange primarily to give all-*trans* RPSB.³²

Is there a similar specificity in the gas phase? Does photoisomerization proceed through a CI, driving isomerization around, for example, the 11,12 bond to form the 11-*cis* isomer (direct photochemical mechanism), or is it essentially a statistical process, that follows internal conversion (IC) with ~ 200 kJ/mol of electronic energy transformed into vibrational energy and subsequent statistical exploration of the isomeric landscape? Recent theoretical studies predict that the photo-excited RPSB molecule in the gas phase is rather floppy and that there are multiple relaxation paths as the molecule moves away from the FC region (with rotations around formal double and single bonds); some of these paths move towards CIs whereas others do not.³⁴ Ultimately, the fate of molecules passing through a CI linking the S_1 and S_0 surfaces may be the same as for molecules that undergo IC. This is because collisions are much less frequent in the gas phase than in solution and there is no effective means for dissipating vibrational energy liberated during passage from the S_1 FC

TABLE III. Photophysical data for all-*trans*, 13-*cis*, 11-*cis* and 9-*cis* RPSB dissolved in methanol taken from ref. 32. Included are wavelengths of absorption maxima (λ_{max}), extinction coefficients (ϵ), photoisomerization quantum yields (ϕ_{iso}), and main photoisomerization products.

	λ_{max} nm	ϵ $M^{-1}cm^{-1}$	ϕ_{iso}	products
<i>trans</i>	445	51800	0.17 ± 0.03	0.02(9c), 0.14(11c), 0.01(13c)
9- <i>cis</i>	432	39400	0.13 ± 0.03	0.07(t), 0.06(11c)
11- <i>cis</i>	442	23300	0.22 ± 0.05	0.22 (t)
13- <i>cis</i>	434	23900	0.13 ± 0.02	0.11(t), 0.01(11c)

region, down through the CI, and then on the S_0 surface. Under these circumstances, even if the system reaches a particular isomeric form on the S_0 surface it possesses sufficient vibrational energy to rearrange to other forms. Eventually, collisions with buffer gas ($k_c \sim 5 \times 10^8 s^{-1}$) cool the system below the isomerization barriers ($V_b \sim 100$ kJ/mol). This statistical picture of photoisomerization in the gas phase is supported by the reasonable agreement between observed and predicted laser on/off difference ATDs if we presume that the laser pulse merely heats the ion population from 600 to 800 K (Figure 3c).

Previously, we used a statistical approach to explain the photoisomerization behaviour of the polymethine dye DTC^+ , which consists of two aromatic groups connected by 4 conjugated C-C bonds, and which we have previously studied using PISA spectroscopy.¹⁷ For DTC^+ , a time-dependent master equation simulation successfully predicted the preferential formation of the all-*trans* and 8,9-*cis* isomers following laser excitation and the absence of the 2,8-*cis* form. Simulations of the relaxation on the ground state electronic surface demonstrated that following IC, the DTC^+ molecules explore the conformational landscape as they slowly cool through buffer gas collisions. In line with experimental observations, the simulations showed that the 8,9-*cis* isomer is preferred over the 2,8-*cis* isomer because it lies lower in energy (by ~ 5 kJ/mol) and because it is separated from the *trans* isomer by a substantially higher barrier (89.7 versus 43.6 kJ/mol). Because of rapid

isomeric interconversion, the preference for the more stable 8,9-*cis* isomer was predicted to persist even when it was assumed that the 2,8-*cis* isomer was initially populated by passage through a CI. RPSB is a significantly more complicated system than DTC⁺ and we have not yet modelled its isomerization behaviour using the master equation approach. However, as for DTC⁺, IC with subsequent slow collisional relaxation should favour energetically low-lying isomers that are separated from other forms by large isomerization barriers.

VI. CONCLUSIONS

Protonated retinal Schiff base ions have been formed using electrospray ionisation and introduced into a ion mobility spectrometer where they are irradiated with tuneable visible radiation. The RPSB ion population is dominated by the all-*trans* geometric isomer but also contains several more compact isomeric forms that can also be formed through photo excitation of the all-*trans* isomer. The photoisomerization action spectrum of RPSB exhibits a single, well-defined $S_1 \leftarrow S_0$ peak at 615 ± 5 nm. The band differs in shape from the recently reported photodissociation spectrum which displays a broad band with a flat top that extends from 530 to 610 nm.¹³ The differences between the PISA and PD spectra are suggested to arise either from differences in the wavelength dependence for the photodissociation yield or photoisomerization yield, differences in the internal energy of the ion populations, or presence of different isomers in the probed populations. **Notably, the PISA data suggests that the $S_1 \leftarrow S_0$ absorption wavelengths of the 6s-*cis*(-), 6s-*cis*(+) and 6s-*trans* forms of RPSB are similar.**

There are several obvious extensions to the current work. First, it would be useful to prepare and characterise other RPSB isomers (9-*cis* , 11-*cis* , 13-*cis* isomers), measuring their collision cross sections and PISA spectra so that the data can be compared with the all-*trans* RPSB results presented here. This would hopefully help assign the ion mobility peaks observed in this study. Second, it would be useful to subject the RPSB ions to an IMS step before laser irradiation so that a particular isomer population can be irradiated cleanly without interference from other isomers. This would also help in assigning the ATD peaks and would allow one to determine whether other RPSB protomers or cyclized

versions are present in the drifting ion population as they should have distinct absorption spectra. From a theoretical perspective, reliable calculations for the dissociation barrier would enable us to understand whether photodissociation of cold RPSB is a single or multi-photon process and perhaps help identify the source of differences between the PISA and PD spectra.

ACKNOWLEDGMENTS

This research was supported under the Australian Research Council's Discovery Project funding scheme (Project Numbers DP110100312 and DP120100100). We acknowledge Catrin Goeschen and Luke Gamon for helping with the RPSB synthesis and HPLC characterisation. We thank Professor Matthew Bush for providing a modified version of the Mobcal code with N₂ collision parameters described in ref. 28.

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