Ultrafast light-induced dynamics in solvated biomolecules:
The indole chromophore with water

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Interactions between proteins and their solvent environment can be studied in a bottom-up approach using hydrogen-bonded chromophore-solvent clusters. The ultrafast dynamics following UV-light-induced electronic excitation of the chromophores, potential radiation-damage, and their dependence on solvation are important open questions. The microsolvation effect is challenging to study due to the inherent mix of the produced gas-phase aggregates. We used the deflector to spatially separate different molecular species in combination with pump-probe velocity-map-imaging experiments. We demonstrated that this powerful experimental approach reveals intimate details of the UV-induced dynamics in the near-UV-absorbing prototypical biomolecular indole-water system. We determined the time-dependent appearance of the different reaction products and disentangled the occurring ultrafast processes. This novel approach ensures that the reactants are well-known and that detailed characteristics of the specific reaction products are accessible – paving the way for the complete chemical-reactivity experiment.

It is a long-held dream of chemistry to follow chemical reactions in real time [1–3]. Here, the observation of the transition state [1] and the recording of electronic and nuclear motion [4–8] during the making and breaking of bonds are of special interest. These processes at the heart of chemistry occur on ultrafast attosecond (as) to picosecond (ps) timescales. One of the fundamental challenges in their investigation is to initiate the reactions effectively instantaneously on the timescale of the dynamics [2–6]. In the ultimate chemical-reaction-dynamics experiment the reactants are well-defined and well-known, the characteristics of the products, e.g., their yields, momenta, and structures, are precisely observed, and the intermediate electronic and nuclear structures are precisely recorded with high spatial and temporal resolution.

In the ongoing quest for this ultimate experiment, scientists designed increasingly advanced machines to prepare well-defined reactants [7, 8] and to probe the characteristics of the products in high detail [9]. These ingredients already enabled extremely detailed studies of elementary chemical reactions involving atoms and small molecules [10–11] including full details on the quantum-state-correlations of reactants and products [12, 13], albeit without temporally resolving the chemical dynamics.

Alternatively, investigating photochemically-triggered reactions in pump-probe experiments with ultrashort laser pulses enabled the direct study of chemical dynamics in real time [2–4]. Using intense ultrashort laser pulses to excite strong electronic transitions with subsequent photochemical rearrangements and possibly breaking of the molecules has two crucial advantages: it directly defines the starting time of the chemical reaction and it also yields significantly higher densities of molecules undergoing the chemical reaction. These dynamical molecular systems can then be probed using ion- [15–16] or electron-imaging techniques [17, 18], high-harmonic-generation spectroscopy [19, 20] and laser-induced electron diffraction [1, 21, 22], or x-ray [23, 24] and electron diffraction [3, 25].

Severe limitations still exist for such time-resolved studies of bimolecular reaction systems. The ultrafast dynamics of these complex reaction systems are studied through so-called half-collisions [26], in which a chemical bond between two molecules is broken due to the laser excitation. Bimolecular aggregates, so-called molecular clusters, are produced in a supersonic expansion. However, this yields a broad distribution of cluster sizes and whereas the mean and width of the distribution can be tuned [11, 27], it does not allow the production of samples of individual aggregates. In combination with the necessarily broad bandwidth of ultrashort laser pulses, one always simultaneously initiates and probes the dynamics of multiple molecular systems, with different structures and sizes. This leads to overlapping signals from different chemical reactions [14].

Small hydrogen-bonded aggregates of aromatic molecules or chromophores with polar solvent molecules like ammonia (NH₃) and water (H₂O) are important model systems for the interactions between proteins and their solvent environment [28, 29]. For instance, these interactions affect the folding and thereby directly the function of proteins [30]. Microsolvated biomolecular chromophores and their ultrafast dynamics were extensively studied, but current knowledge of their dynamics is strongly limited due to the problem of overlapping signals...
described above [14, 31].

Here, we show the first ultrafast pump-probe experiment that utilised the electrostatic deflector to produce a high-purity bimolecular solute-solvent aggregate sample, in combination with velocity-map imaging (VMI) mass spectrometry, to disentangle the reaction products. We utilised these novel capabilities to study the ultraviolet-light (UV) induced dynamics of the prototypical indole-water (indole(H$_2$O), C$_8$H$_7$N–H$_2$O) complex including eventual dissociation on the picosecond timescale. This is of high relevance, as indole is the chromophore of the most strongly near-UV absorbing common amino acid tryptophan [32] and thus proteins. It was shown that the environment strongly affects the photochemical properties of tryptophan [33]. These characteristics were frequently used to investigate the structure and dynamics of proteins [34].

In addition, the UV-light-induced dynamics of bare and microsolvated indole (C$_8$H$_7$N) was studied extensively both theoretically and experimentally [29, 31, 35–39]. When hydrogen-bonded aggregates of chromophores and polar solvent molecules are irradiated by UV light, they are typically electronically excited to one or several πσ$^*$ states [40]. These states often possess a conical intersection with an optically dark and dissociative πσ$^*$ state that plays an important role in the photochemistry of these species [29, 41]. For indole in this πσ$^*$ state it is expected that it ejects an electron into the aqueous environment leading to the formation of a charge-separated state, a solvated electron [32]. For the indole(H$_2$O) aggregate it was predicted that this electron-transfer process is followed by the transfer of a proton, leading to a net hydrogen-transfer reaction from indole to H$_2$O [29]. However, so far this hydrogen transfer has not been observed experimentally, although it was observed for the similar indole-ammonia (indole(NH$_3$)$_n$) system [42], which was ascribed to ammonia being a better hydrogen acceptor than water [29].

Previous studies on UV-excited indole(H$_2$O) clusters found dynamics occurring on multiple timescales of 20–100 fs, 150–500 fs, and 14 ps [31, 39]. These were tentatively ascribed to an internal conversion between electronically excited states, relaxation dynamics along the πσ$^*$ state, and a coupling of the πσ$^*$ state with the electronic ground state, respectively [31, 39]. However, these studies suffered from the overlapping signals of different cluster sizes described above. Therefore, product channels could not be investigated and the long-time relaxation dynamics were dominated by contributions from fragmentation of larger clusters.

We demonstrate that our high-purity sample allows us to investigate product channels and to follow the dy-
dynamics of indole(H$_2$O) on long timescales. This provides significant additional insight into these prototypical reaction dynamics, i.e., it provides evidence for an incomplete hydrogen-transfer process in indole(H$_2$O). Overall, our novel experimental approach, with the bimolecular reactants well-defined and the product channels clearly identifiable in real time, propels us a major step forward to unravel the complex pathways in bimolecular reaction systems.

RESULTS

The ultrafast chemical dynamics of indole(H$_2$O) was investigated in a molecular-beam apparatus containing an electrostatic deflector and a velocity-map-imaging ion detector, see Figure 1 a. Using the deflector, we spatially separated indole(H$_2$O) from the other species in the molecular beam, e.g., indole, H$_2$O, and helium seed gas [43]. This resulted in a high-purity indole(H$_2$O) sample, as shown in Supplementary section “Species separation”. UV pump pulses with a central wavelength of 269 nm electronically excited indole(H$_2$O). The reaction products were detected through ionisation with a delayed NIR probe pulse with a central wavelength of 1320 nm and velocity-map imaging of the generated ions. The high-purity sample provided by the deflector allowed us to investigate all product channels in a background-free manner, since the product molecules, i.e., indole and water, are not present in the molecular beam in the interaction region – whereas in traditional experiments they were present in the direct molecular beam in large amounts that obscured these product signals. Thus the purified reactants allowed us to identify the appearance of indole$^+$ and H$_2$O$^+$ product ions that originate from indole(H$_2$O).

Figure 1 a, b show the measured indole(H$_2$O)$^+$ (blue dots), indole$^-$ (red diamonds), and H$_2$O$^+$ (green squares) ion signals as function of the pump-probe delay. The H$_2$O$^+$ and indole(H$_2$O)$^+$ signals are scaled up for improved visibility by factors 10 and 5, respectively. Figure 1 a shows the long-time relaxation dynamics, whereas Figure 1 b zooms in on the short delays for indole(H$_2$O)$^+$ and indole$^+$. Dynamic effects are clearly visible in all three signals: All channels exhibit a constant ionisation signal for negative delays that increases when the laser pulses temporally overlap. The indole(H$_2$O)$^+$ signal shows a fast increase followed by a decay with a fast and a slow component, whereas the indole$^+$ product signal contains a fast increase followed by a slow increase. We could clearly observe a delay of the fast increase in the indole$^+$ signal compared to the fast increase in the indole(H$_2$O)$^+$ signal. The H$_2$O$^+$ product signal shows a slow increase as function of the pump-probe delay, see Figure 1 c. On top of these general dynamics, we observed oscillations with a period of 1.67 ps for indole(H$_2$O)$^+$ and indole$^+$, which are in phase between the indole(H$_2$O)$^+$ and indole$^+$ signals. High-temporal-resolution measurements for indole$^+$ at ~120 ps showed that the oscillations are not damped on that timescale, see Figure S5. Our measurements did not reveal any oscillations in the H$_2$O$^+$ signal.

To disentangle the underlying dynamics of these time-dependent ion yields we employed a reaction model, see Methods. The observations could be described well by a five-level model:

\[
\begin{align*}
\text{indole}(H_2O) S_0 & (1) \xrightarrow{UV} \text{indole}(H_2O) \pi\pi^* (2) \\
\tau_1 \rightarrow \text{indole}(H_2O) \pi\sigma^+ (3) \\
\tau_2 \rightarrow \text{indole}(H_2O) S_0 (4) \\
\tau_3 \rightarrow \text{indole} + H_2O (5)
\end{align*}
\]

with the time constants $\tau_1$ coupling states $i$ and $i + 1$. The dynamics in indole(NH$_3$)$_n$ [42] and indole(H$_2$O)$_n$ [31] clusters were previously described with similar models, albeit with six and four states. Here, we needed five levels to accurately describe the dynamics we observed in indole(H$_2$O). We started with all population in the electronic ground state $S_0$ (1) of indole(H$_2$O) and used Maxwell-Bloch equations to find the time-dependent populations of all states. The ion yields are calculated as linear combinations of these populations, which for indole$^+$ and indole(H$_2$O)$^+$ were multiplied by a cosine function representing the oscillations. We fitted the coefficients for the linear combinations as well as the time constants to the experimental data and obtained $\tau_2 = 445 \pm 71$ fs, $\tau_3 = 13 \pm 2$ ps, and $\tau_4 = 96 \pm 10$ ps. These results were used to calculate the yields of indole(H$_2$O)$^+$, indole$^+$, and H$_2$O$^+$ shown by the blue, red, and green solid lines in Figure 1 a, b, respectively. Figure 1 a shows the signals computed from the Maxwell-Bloch equations directly, whereas Figure 1 b includes the cosine functions describing the oscillations. This model matches the experimental data very well.

Additional information about the underlying dynamics was obtained from the kinetic-energies of H$_2$O$^+$, i.e., the delay dependence of the total projected kinetic energy release (TKER), see Supplementary section “Evolution of the total kinetic energy release”. To separate the delay-dependent signal from the constant H$_2$O$^+$ rest-gas background signal we first determined the mean TKER distribution for negative pump-probe delays, when the H$_2$O$^+$ ion yield is constant. This distribution, which is shown in Figure 2 a, was subtracted from all TKER resulting in the TKER changes shown in Figure 2 b. For short delays, the TKER distribution is similar to the static background. However, a dynamical signal develops when the delay increases: The mean TKER decreases and the distribution gets narrower.

DISCUSSION

Our reaction model yields the dynamics depicted in Figure 3. In short: The UV laser excites indole(H$_2$O) in the electronic ground state $S_0$ (1) to the electronically excited
πσ* (2) state. The system then interconverts to the optically dark πσ* (3) state and, subsequently, to the S0 (4) state. The resulting vibrationally-hot electronic-ground-state indole(H2O) dissociates into separate vibrationally-excited indole and H2O molecules (5) via so-called statistical unimolecular decay. Moreover, vibronic-wavepacket dynamics occur in this system, which are taken into account in the reaction model. In the following, the different steps will be discussed in more detail.

When the UV laser (purple arrow in Figure 3) excites indole(H2O) to the ππ* state, the system can be ionised by the subsequent absorption of three NIR photons (left red arrows) through the electronic ground state of the cation, which has a similar geometry as the ππ* state [44] and an ionisation energy of $E_i = 7.37$ eV [45]. This results in the fast increase in the indole(H2O)+ signal. Due to the excess energy above the ionisation potential, fragmentation of indole(H2O)+ can occur [43], leading to a small contribution to the fast increase in the indole+ signal, see Supplementary section “Contributions of Bloch states to the ion yields”.

The subsequent interconversion from the ππ* state to the πσ* state with $\tau_2 = 445 \pm 71$ fs is related to nuclear rearrangement and electron transfer, see the inset of Figure 2. In the equilibrium geometry of the πσ* state, depicted in the lower part of the inset of Figure 3, the H2O molecule tilts along the in-plane-bending coordinate of the cluster and the N-O distance decreases, whilst the N-H distance increases with respect to the geometry in the S0 state, depicted in the upper part of the inset. Moreover, an electron is transferred from a π-orbital on the indole moiety to a σ*-orbital localised around the H2O molecule, often referred to as a solvated electron [32]. This is depicted by the calculated isosurfaces of the orbital amplitudes plotted in the structures in the inset of Figure 3.

In order to efficiently ionise the system from this πσ* state, i.e., in a vertical transition, a higher-energy cationic state needs to be accessed, depicted by the dashed horizontal line in Figure 3, likely requiring four NIR photons. We suspect that this cationic state is above the binding energy of indole(H2O)+ of 0.6 eV [46], resulting in more fragmentation and correspondingly more indole+ signal [46]. Overall, this leads to a decrease in the indole(H2O)+ signal and a corresponding increase in the indole+ signal. This explains why the fast increase in the indole+ signal is delayed with respect to the fast increase in the indole(H2O)+ signal.

We attribute the 1.67 ps oscillations overlayed on these electronic and nuclear dynamics to wavepacket dynamics in C–H stretch vibrations modifying the transition strengths in the system, see Supplementary section “Oscillations due to wavepacket dynamics”. These dynamics occur due to the coherent excitation of vibrational states within the bandwidth of the pump laser, depicted by the shaded purple area in Figure 3 and manifest themselves as a modification of the ionisation probability, leading to the observation of the oscillations.

Subsequently, the indole(H2O) complexes in the πσ* state interconvert within $\tau_3 = 13 \pm 2$ ps to the S0 state through nonadiabatic coupling as described before [47]. This interconversion leads to vibrationally-excited indole(H2O) clusters in the electronic ground state, which are ionised less likely due to the increased $E_i$ and which result in vibrationally-hot ions that fragment leading to indole+ signal. Therefore, this results in a slow decrease in the indole(H2O)+ signal and a slow increase in the indole+ signal.

The vibrationally-hot indole(H2O) dissociates on a timescale of $\tau_3 = 96 \pm 10$ ps into separate neutral indole and H2O molecules containing a lot of vibrational energy. Both products can be ionised, leading to increased indole+ and H2O+ signals. At least three NIR photons are needed to reach the ionisation energy for the vibrationally-hot indole, like for indole(H2O), whereas a minimum of 14 NIR photons is needed for the ionisation of ground-state H2O. An alternative pathway for the formation of H2O molecules would be direct dissociation in the πσ* state. However, our measured TKER distributions, Figure 2 match Maxwell-Boltzmann distributions for delays >65 ps, which strongly indicates that the H2O molecules are formed in a statistical unimolecular-decay process in the S0 state instead of a direct dissociation in the πσ* state, see Supplementary section “Evolution of the total kinetic energy release”.

Figure 2. Time evolution of the total kinetic-energy release (TKER) distribution of H2O+. (a) TKER distribution from intact indole(H2O), which is given by the mean TKER distribution for negative pump-probe delays. (b) Delay-dependent TKER-distribution differences after subtraction of the delay-independent signal. The blue dashed line represents the mean TKER obtained from an ion-dipole interaction model, see Supplementary section “Evolution of the total kinetic energy release”.

Figure 3.
χ₂ ≈ 445 fs
χ₃ ≈ 13 ps
χ₄ ≈ 96 ps

Figure 3. Schematic of the UV-induced photochemistry in indole(H₂O). The purple arrow indicates the UV pump-photon energy yielding the overall energy in the system after absorption of an UV photon, indicated by the purple line, which is conserved throughout the dynamics. The shaded purple area represents the corresponding bandwidth. Blue arrows depict the different steps in the observed photochemistry with corresponding timescales; see text for details. The red arrows indicate the absorption of NIR probe photons leading to ionisation. The black dashed line represents the higher-energy cationic state that needs to be accessed to efficiently ionise from the πσ* state; see text for details. The indole + H₂O product channel contains a lot of vibrational energy. The inset shows computed isosurface representations of the relevant molecular orbitals of indole(H₂O), the upper plot shows the highest occupied molecular orbital (HOMO), a π-orbital, for the equilibrium geometry in the S₀ state, whereas the lower plot shows the σ* orbital in the equilibrium geometry of the πσ* state.

The mean of the TKER distributions changes as a function of the delay, see Figure 2. This time evolution can be explained by considering the ion-dipole interaction: When the H₂O molecule is ionised and the separate indole and H₂O⁺ molecules are still relatively close, they will repel each other due to the ion-dipole interaction, leading to an increase in the kinetic energy. For longer delays, the increased distance between the two moieties results in a correspondingly lower kinetic energy. The width of the TKER distribution is governed by the wavepacket evolution along the ion-dipole interaction potential and decreases for longer delays. We used a classical ion-dipole interaction model to describe the evolution of the TKER; details are provided in Supplementary section “Evolution of the total kinetic energy release”. This yielded the dashed blue line in Figure 2 which is in very good agreement with our experimental results, indicating that the two moieties slowly move apart with a speed v ≈ 12 m/s. This is another confirmation for the statistical unimolecular-decay process in the S₀ state.

In order to shed light on the possible net hydrogen-
transfer process taking place in the πσ* state of indole(H$_2$O), we investigated the H$_3$O$^+$ signal. However, we did not find any delay-dependent effect in the H$_3$O$^+$ signal coming from neutral dissociation of indole(H$_2$O). This indicates that, although the N–H bond is stretched in the πσ* state, the electron transfer is not followed by proton transfer to H$_2$O for dissociation. We conclude that after UV excitation at 4.61 eV the indole(H$_2$O) aggregate either survives or dissociates into the individual indole and H$_2$O molecules, which themselves stay intact and slowly move away from each other.

Our pump-probe experiments on pure indole(H$_2$O) samples in combination with our reaction model based on a five-level system provide new insight into the ultrafast processes occurring in this prototypical solvated-biomolecule system, especially regarding the combined electronic and nuclear dissociation dynamics and its product channels. The time constant $\tau_2 = 445 \pm 71$ fs that we assigned to the interconversion from the πσ* to the πσ* state is within the broad range $\tau_2 = 150 \ldots 500$ fs previously tentatively attributed to dynamics on the πσ* surface [39]. We note that the previous study did not describe the interconversion to the πσ* state and that $\tau_2$ in the present study is on the same order as the instrument response function (IRF). We could not resolve the fastest time constant of $20 - 100$ fs observed before [39], since it is shorter than the IRF. Regarding the time constant $\tau_3 \approx 13$ ps, our interpretation is consistent with previous tentative assignments of a 14 ps decay constant to interconversion from the πσ* state to the S$_0$ state [31]. While previous work was not able to distinguish products from different cluster sizes, our well-defined-reactant study fully supports this finding. Furthermore, the $\tau_1 = 96 \pm 10$ ps time constant we obtained for the bond breaking and formation of H$_2$O is comparable to the ones found for the production of (NH$_3$)$_{n-1}$NH$_4^+$ resulting from dissociation of indole(NH$_3$)$_n$ clusters with $n \leq 2$ [42].

In conclusion, we demonstrated that our novel experimental approach to perform pump-probe studies of ultrafast chemical dynamics on species-selected samples provides unprecedented details on intermediates and reaction products and thus the chemical reaction dynamics. Creating a high-purity sample of the prototypical solvated biomolecule indole(H$_2$O) enabled us to investigate its UV-induced dissociation dynamics in intimate detail, well beyond previous experimental studies. We observed an initial delay in the indole$^+$ ion, which we ascribed to the ionisation of indole(H$_2$O) from the ππ* and πσ* states resulting in different cationic states of indole(H$_2$O) with distinct fragmentation probabilities. Moreover, we could follow the long-time relaxation dynamics in the reaction products, which revealed clear evidence for an incomplete hydrogen-transfer process and thus indicates that the indole chromophore is protected by the attached water against UV-induced radiation damage. This is opposite to earlier theoretical predictions [29], but fully in line with previous experiments [31].

While in previous experiments the H$_2$O$^+$ product signal was completely obscured by the unavoidable large amount of H$_2$O in the molecular beam, our purified samples allowed us, for the first time, to experimentally determine the time constant of the hydrogen-bond-breaking process to 96 ps. Moreover, based on the kinetic-energy distributions of the H$_2$O products we conclude that this biochemically important process [45] occurs via statistical unimolecular decay in the electronic ground state. As ultrafast excited-electronic-state deactivation after the absorption of UV photons could be essential for the photostability of proteins [19], our results demonstrate how such mitigation of UV-induced radiation damage through solvent interactions works.

Overall, these results demonstrate that our novel experimental approach combining the deflector, velocity-map imaging, and pump-probe ultrafast time-resolved spectroscopy enables the observation of complete chemical-reactivity pathways in chemical reactions of complex molecular systems. The investigation of a bimolecular half-collision reaction allowed for the precise triggering of the dynamics and presents a promising approach for more complex chemical systems.

Our approach can directly be combined with shorter, i.e., few-femtosecond or attosecond, laser pulses and with tunable wavelength to follow the energy-dependent ultrafast electronic and chemical processes in complex reaction systems. This could be further aided by coincidence measurements [16,50]. Moreover, diffractive imaging of the nuclear dynamics [1,23,25] would provide complementary detailed information on the actual atomic structural dynamics. For instance, photoelectron-momentum-imaging and laser-induced-electron-diffraction experiments would be a direct extension of the current experiments and initial experiments are ongoing [51,52]. These approaches should reveal the complete reaction pathway of the nuclear and electronic dynamics that occur in these reactions. Such detailed insights will ultimately yield a deep understanding of the formation and breaking of bonds and allow to develop a truly dynamical basis of chemistry.

**METHODS**

**Experimental setup**

The experimental setup, schematically shown in Figure 1c was described previously [53,54]. In brief, 95 bar of helium was bubbled through room-temperature water before passing through the sample reservoir of an Even-Lavie valve containing indole (Sigma-Aldrich, ≥ 99 %). The valve was operated at 110°C and a repetition rate of 250 Hz. After passing through two skimmers, the beam travelled through a 15.4 cm long electrostatic deflector [8]. Applying a voltage of 20 kV between the electrodes of the deflector created inhomogeneous electric fields that spatially dispersed and separated indole and indole(H$_2$O) based on their Stark effect [8,33], see Supplementary section “Species separation”. Passing through another
The generated ions were accelerated towards a multichannel-plate and phosphor-screen detector using the VMI spectrometer. Images were recorded with a CMOS camera at 500 Hz, alternating between molecular-beam signal and background frames. The MCP was temporally gated in order to record ion images for individual mass-to-charge ratios, which we refer to as mass-gated images. Indole$^+$ and indole(H$_2$O)$^+$ signals were measured in a detuned-VMI mode to avoid saturation and damage of the central part of the detector. H$_2$O$^+$ ions were measured in VMI mode at an increased NIR intensity of $\sim$1 $\cdot$ 10$^{14}$ W/cm$^2$, accounting for the relatively high ionisation energy $E_i \approx 12.6$ eV of H$_2$O.

The delay between pump and probe laser pulses was scanned back and forth multiple times using a motorised translation stage. Indole(H$_2$O)$^+$ and indole$^+$ signals were recorded in the same measurements, by scanning the pump-probe delay and the mass-gating simultaneously. We used a step size of 417 fs for t = -1.535 $\ldots$ 6.805 ps, made one step of 834 fs, and used a step size of 1.668 ps for t > 7.639 ps. We used $\sim$8000 laser shots per data point. The H$_2$O$^+$ signal was recorded for t = -5.705 $\ldots$ 140.245 ps with a step size of 4.17 ps and $\sim$41 000 laser shots per data point. Data at 119.395, 123.565, and 140.245 ps was discarded due to instabilities in the spatial overlap of the UV and NIR beams for the longest delays. The delays t > 114 ps do not significantly alter the fit, but show that the H$_2$O$^+$ signal starts to level off. To improve the statistics for Figure 2, this data was combined with a second measurement for which the H$_2$O$^+$ signal was recorded for t = -19.391 $\ldots$ 122.390 ps with a step size of 1.668 ps and $\sim$340 000 laser shots per data point. The two data sets were merged and averaged over 4 ps, and the data points at 118, 122, and 142 ps were discarded due to the non-perfect spatial overlap of the laser beams.

**Reaction model**

We described the time-dependent ion signals using a reaction model for a five-level system, see [1]. The Maxwell-Bloch equations that describe the evolution of the populations $\rho_{ij}$ of state $i$ corresponding to this model are given by [14, 47]

$$
\rho_{11} = \frac{i}{2} \Omega_0 g(t) (\rho_{12} - \rho_{21})
$$

$$
\rho_{22} = -\frac{i}{2} \Omega_0 g(t) (\rho_{12} - \rho_{21}) - \Gamma_{22} \rho_{22}
$$

$$
\rho_{21} = -\frac{i}{2} \Omega_0 g(t) (\rho_{11} - \rho_{22}) - (\Gamma_{21} - i\Delta \omega) \rho_{21}
$$

$$
\rho_{12} = \frac{i}{2} \Omega_0 g(t)^* (\rho_{11} - \rho_{22}) - (\Gamma_{21} + i\Delta \omega) \rho_{12}
$$

$$
\rho_{33} = \Gamma_{22} \rho_{22} - \Gamma_{33} \rho_{33}
$$

$$
\rho_{44} = \Gamma_{33} \rho_{33} - \Gamma_{44} \rho_{44}
$$

$$
\rho_{55} = \Gamma_{44} \rho_{44}
$$

where $\Gamma_{i} = 1/\tau_i$ and $g(t) = \exp(-\frac{1}{2}(t/\tau_{IRF})^2)$, which represents the instrument-response function (IRF) with $\tau_{IRF} = 381$ fs, see Supplementary section “Instrument response function”. We assumed $\Delta \omega = 0$ and $\Gamma_{21} = \Gamma_{22}/2$ [14]. The Rabi frequency was estimated to $\Omega_0 \approx 3.4$ ps$^{-1}$ based on the peak intensity and duration of the UV pulses and an estimated transition dipole moment $\mu_{12} \approx 15 \, e\, \text{pm}$ [17].

Initially, all population was in state 1, i.e., $\rho_{11} = 1$. Integrating (2) yields the delay-dependent populations of the different states. The simulated ion-signal intensities for indole(H$_2$O)$^+$, indole$^+$, and H$_2$O$^+$ are given by linear combinations of the different populations $\rho_{ii}$:

$$
I_{\text{indole(H}_2\text{O)}^+} (t) = \rho_{\text{indole(H}_2\text{O)}^+} (t) \sum_{i=1}^{4} A_i \rho_{ii}(t)
$$

$$
I_{\text{indole}^+} (t) = \rho_{\text{indole}^+} (t) \sum_{i=2}^{5} B_i \rho_{ii}(t)
$$

$$
I_{\text{H}_2\text{O}^+} = C \rho_{55}.
$$

with the population $\rho_{ii}(t) = f(t) \otimes \rho_{ij}(t)$ of state $i$ after convolution with a Gaussian function $f(t)$ with a FWHM of 70 fs, which represents the intensity envelope of the NIR pulse [14]. The decay constants $\tau_2$, $\tau_3$, and $\tau_4$ as well as the coefficients $A_i$, $B_i$, and $C$ in (3) were fit using a Levenberg-Marquardt algorithm and a reduced-$\chi^2$ objective function of simulated against background-corrected experimental ion signals, Figure 1 a.

The oscilations in the indole$^+$ and indole(H$_2$O)$^+$ signals were modelled as $\rho_{jj}^\text{osc}(t) = a_j + b_j \cos(\omega t + \phi)$, with $j = \text{indole(H}_2\text{O)}^+$ or indole$^+$. These parameters were optimised once using a high-resolution measurement of the oscillations and then fixed in the fitting procedure to $a_{\text{indole(H}_2\text{O)}^+} = 1.04$, $b_{\text{indole(H}_2\text{O)}^+} = 0.14$, $a_{\text{indole}^+} = 0.92$, $b_{\text{indole}^+} = 0.11$, $\omega = 2 \pi \cdot 0.60$ THz = 3.77 THz and $\phi = 1.77$. 

The delay between pump and probe laser pulses was scanned back and forth multiple times using a motorised translation stage. Indole(H$_2$O)$^+$ and indole$^+$ signals were recorded in the same measurements, by scanning the pump-probe delay and the mass-gating simultaneously. We used a step size of 417 fs for $t = -1.535 \ldots 6.805$ ps, made one step of 834 fs, and used a step size of 1.668 ps for $t > 7.639$ ps. We used $\sim$8000 laser shots per data point. The H$_2$O$^+$ signal was recorded for $t = -5.705 \ldots 140.245$ ps with a step size of 4.17 ps and $\sim$41 000 laser shots per data point. Data at 119.395, 123.565, and 140.245 ps was discarded due to instabilities in the spatial overlap of the UV and NIR beams for the longest delays. The delays $t > 114$ ps do not significantly alter the fit, but show that the H$_2$O$^+$ signal starts to level off. To improve the statistics for Figure 2, this data was combined with a second measurement for which the H$_2$O$^+$ signal was recorded for $t = -19.391 \ldots 122.390$ ps with a step size of 1.668 ps and $\sim$340 000 laser shots per data point. The two data sets were merged and averaged over 4 ps, and the data points at 118, 122, and 142 ps were discarded due to the non-perfect spatial overlap of the laser beams.

skimmer the molecules were intersected by the focused pump and probe laser beams in the centre of a VMI spectrometer.

Indole(H$_2$O) was electronically excited by ultraviolet-light (UV) pulses with a central wavelength of 269 nm, a pulse duration of $\sim$560 fs (full width at half maximum, FWHM), and a peak intensity of $\sim 2 \cdot 10^9$ W/cm$^2$. Near-infrared (NIR) pulses centred around 1320 nm with a duration of $\sim$70 fs (FWHM) and a peak intensity of $\sim 1 \cdot 10^{13}$ W/cm$^2$ were used to ionise the complex and its fragments. Both laser beams were linearly polarised along $Y$, i.e., parallel to the detector plane, and focused into the centre of the molecular beam with $4\sigma \approx 90$ µm and $4\sigma \approx 60$ µm for the intensities of the UV and NIR beams, respectively. Unless mentioned otherwise, we adjusted the laser powers such that the ionisation signal of indole(H$_2$O) with either of the two beams alone was negligible.
The best fit yielded $\tau_2 = 445 \pm 71$ fs, $\tau_3 = 13 \pm 2$ ps, and $\tau_4 = 96 \pm 10$ ps with a reduced $\chi^2$ of 1.42 and a coefficient of determination $R^2 = 0.999$. The resulting time-dependent contributions of the individual states to the ion signals are shown in Figure 4 in the Supplementary section “Contributions of Bloch states to the ion yields”.

**Ab initio calculations**

We performed *ab initio* calculations on indole(H$_2$O) using Psi4 [55]. The ground state geometry was optimised using density-fitted second-order Møller-Plesset perturbation theory (DF-MP2) calculations using an aug-cc-pVTZ basis set. We used equation-of-motion coupled-cluster singles and doubles (EOM-CCSD) in combination with an aug-cc-pVDZ basis set to optimise the geometry in the $\pi\sigma^*$ state.

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COMPETING INTERESTS

The authors declare no competing interests.