

Two-Dimensional UV–vis/NMR Correlation Spectroscopy: A Heterospectral Signal Assignment of Hydrogen-Bonded Complexes

Benjamin Koeppe,[†] Peter M. Tolstoy,^{*,†,‡,§} Jing Guo,[†] Erik T. J. Nibbering,[‡] and Thomas Elsaesser[‡]

[†]Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, D-14195 Berlin, Germany

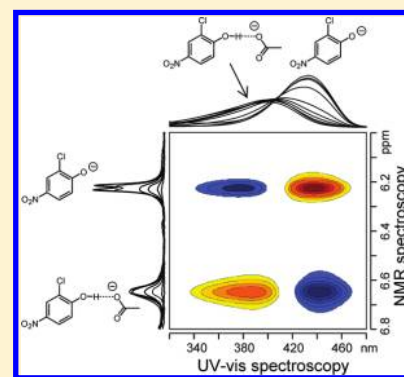
[‡]Max Born Institut für Nichtlineare Optik und Kurzzeitspektroskopie, Max-Born-Straße 2A, D-12489 Berlin, Germany

[§]V.A. Fock Institute of Physics, St. Petersburg State University, Uljanovskaja 1, 198504, St. Petersburg, Russia

S Supporting Information

ABSTRACT: We explore in a combined UV–vis/NMR approach the hydrogen-bonded complexes of 2-chloro-4-nitrophenol (CNP) with acetate anion as a model for amino acid side-chain interactions. For the first time, we present two-dimensional UV–vis/NMR correlation spectra, measured simultaneously for the same sample inside of the magnet of a NMR spectrometer. Synchronous and asynchronous 2D plots allow us to monitor the formation of hydrogen-bonded complexes, assign the signals to specific species, and finally estimate the geometry of a hydrogen-bonded 1:1 heteroconjugated anion coexisting with four other anionic species formed in CD₂Cl₂ solution at 180 K. Combined analysis of NMR and UV–vis spectra with the help of previously published hydrogen-bond correlations shows that the hydrogen bond in the heteroconjugate is of the CNP–O[−]···HOOCCH₃ type with $r(\text{O}\cdots\text{O}) \approx 2.48$ Å and the average bridging proton displacement from the hydrogen bond center of about 0.18 Å.

SECTION: Kinetics, Spectroscopy



The position of bridging protons in hydrogen bonds formed by amino acid side chains within the tertiary structure of a protein is often crucial for its function. However, locating bridging protons in solution and, more generally, understanding function are challenging experimental tasks that often require application of various spectroscopic techniques. Optical spectroscopy gives insight into photoinduced functional processes which can be mapped even on the time scale of vibrational motions. Such methods give, however, no direct insight into structure, and thus, an assignment of different optical spectra to different molecular species is challenging. On the other hand, NMR probes local geometries and interactions under conditions close to equilibrium and in a highly specific way. Optical techniques combined with NMR can provide complementary insight, allowing for a broader understanding of processes determining function.

In this Letter, we focus on anionic model complexes with short OHO bonds, which mimic Asp/Glu side-chain interactions with a Tyr side chain or a cofactor in a nonaqueous environment. Such short anionic phenol/carboxylate H-bonds are formed, for example, in active sites of photoactive yellow protein^{1,2} or a ketosteroid isomerase complex with equilenin inhibitor.³ While modeling phenol/carboxylate interactions in (polar) aprotic solutions, one has to keep in mind that the stoichiometry and geometry of intermolecular H-bonded complexes are quite sensitive to the experimental conditions (concentration, temperature, solvent composition, etc.). This makes it difficult to

investigate such systems in independent spectroscopic experiments because usually a new sample is prepared for each type of the measurements, leading to a possible mismatch of the experimental conditions. It is advantageous to build a spectrometer capable of measuring both types of spectra from the same sample. Recently, this approach was used by some of us for measuring liquid-state UV–vis and NMR spectra of a sample placed inside of the magnet of an NMR spectrometer (UVNMR).⁴ Other examples of various combinations of spectroscopic methods have been proposed in refs 5–8. Having recorded a set of combined spectra, a task remains to cross-assign them, that is, to find out which signals in different types of spectra stem from the same species.

These considerations have determined the scope of our work. We have selected to investigate the formation and geometry of the H-bond in the 1:1 heteroconjugated anion formed by 2-chloro-4-nitrophenol (CNP) with acetate in an aprotic environment (see the structure in Figure 1f; in this Letter, it is called also a heteroconjugate for brevity) by considering UVNMR spectra of three-component mixtures of CNP, acetic acid, and tetraethylammonium (TEA) acetate (Figure 1a) dissolved in CD₂Cl₂ at 180 K.⁴ A number of species can be formed in such a solution, as illustrated in Figure 1b–f, and the choice of the sample composition

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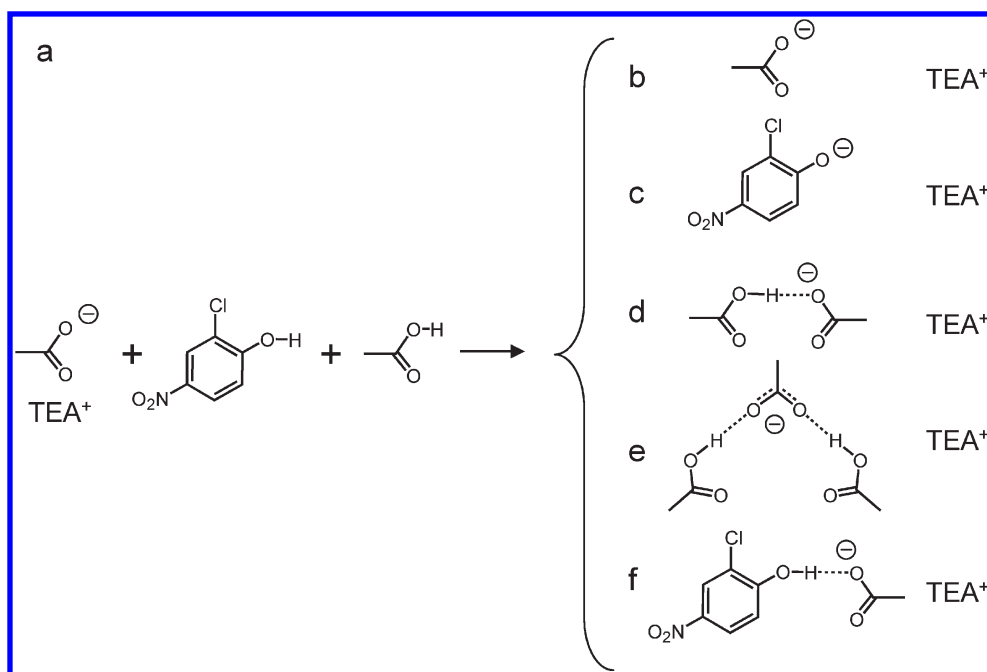


Figure 1. (a) TEA acetate, 2-chloro-4-nitrophenol, and acetic acid used to prepare three-component mixtures; (b–f) anionic species, which are formed in a three-component mixture dissolved in CD₂Cl₂.

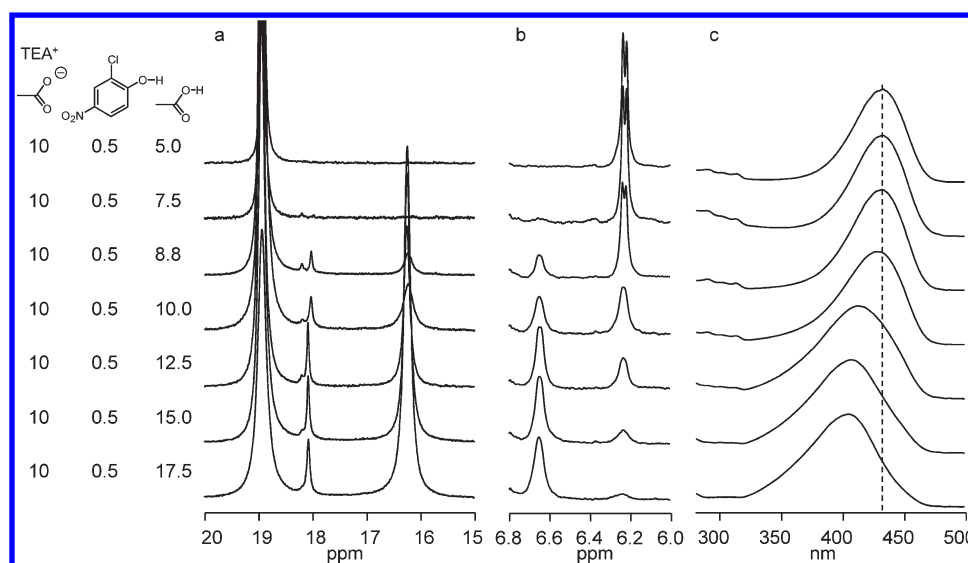


Figure 2. (a,b) ¹H NMR spectra and (c) simultaneously measured UV–vis absorption spectra of mixtures of CNP, acetic acid, and TEA acetate dissolved in CD₂Cl₂ at ~180 K. The numbers on the left-hand side represent the concentration of the added chemicals in mM.

will be justified below. The goal of the work is two-fold. First, we want to identify spectroscopic features of the heteroconjugate by combining information content of optical (UV–vis) and NMR spectra. Second, we want to use the spectroscopic parameters to locate the bridging proton in the OHO bond with the help of previously published correlations.^{9–11}

To find a correspondence between UV–vis and NMR spectra, we use two-dimensional correlation spectroscopy of Noda's type (see Supporting Information for the exact mathematics).^{12,13} This method belongs to the class of spectral processing techniques; it quantifies and displays in a graphical fashion the similarity between the spectral intensity dependencies on an external variable

at two spectroscopic observables. Used mostly for the analysis of sets of vibrational spectra,^{14,15} 2D correlation spectroscopy could also be applied to correlate spectral measurements of different types.^{16–20} This is especially an attractive option in our case because identical experimental conditions of UV–vis and NMR measurements minimize potential artifacts in the 2D plots. Here, for the first time, we demonstrate that 2D UVNMR correlation spectroscopy can be applied successfully.

In Figure 2, series of concentration-dependent UVNMR spectra of mixtures of CNP, acetic acid, and TEA acetate dissolved in CD₂Cl₂ at ~180 K is presented. The numbers on the left-hand side represent concentrations of the added chemicals

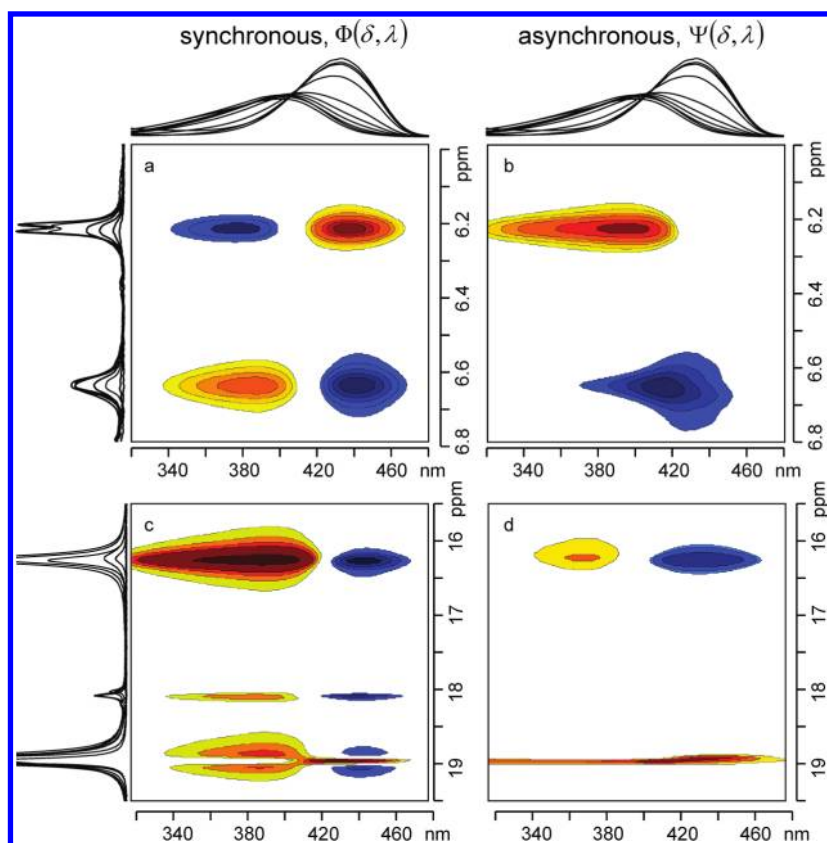


Figure 3. Synchronous (left) and asynchronous (right) two-dimensional UVNMR correlation spectra of three-component mixtures, containing CNP, acetic acid, and TEA acetate dissolved in CD_2Cl_2 at ~ 180 K. The NMR spectral regions of ortho proton signals of the CNP moiety (a,b) and H-bonding proton signals (c,d) are correlated with UV–vis spectra. Positive intensity is shown in yellow and red, and the negative one is in blue; z-axis values are in arbitrary units; intensity change between contour lines is ca. 10%.

in mM. Upon addition of acetic acid to the solution already containing all three components, three NMR signals of hydrogen-bonded protons at 16.2, 18.1, and 18.9 ppm (Figure 2a) grow at unequal rates, while two NMR signals of ortho protons of the CNP moiety at 6.25 and 6.65 ppm exchange intensities (Figure 2b). In the simultaneously measured UV–vis spectra (Figure 2c), the band at ~ 430 nm diminishes, and a new band appears at ~ 400 nm upon addition of acetic acid to the solution.

The UV band at 430 nm is characteristic for nitrophenolate anions and thus assigned to the CNP anion (Figure 1c).²⁶ The NMR signal of phenolic ortho protons at 6.25 ppm (the only signal visible in the top spectrum of Figure 2b) should thus also be assigned to this species. The NMR signal at 18.9 ppm is characteristic for the homoconjugated anion of acetic acid (Figure 1d).²¹ This means that when the sample mixture contains a large excess of TEA acetate and small amounts of acetic acid and CNP, only TEA hydrogen diacetate is formed. In other words, CNP gives the OH proton away to TEA acetate, leading to the formation of TEA hydrogen diacetate and leaving a free (not H-bonded) CNP anion. In the following stepwise addition of acetic acid (which is considered a “titration” of the sample), the incoming acid could form complexes with any of the existing species in solution; with TEA acetate, it would form TEA hydrogen diacetate (Figure 1d), with TEA hydrogen diacetate, it would form TEA dihydrogen triacetate (Figure 1e),²¹ and, finally, with CNP anion, it would form the desired heteroconjugated anion (Figure 1f).

Now let us consider Figure 3, where synchronous (Figure 3a) and asynchronous (Figure 3b) plots are given. Positive intensity

is shown in yellow and red, and negative intensity in blue. In the synchronous plot for ortho proton NMR signals and UV–vis spectra, we clearly see two positive cross-peaks; $\Phi(6.25 \text{ ppm}, 440 \text{ nm})$ corresponds to the signals of the CNP anion, and $\Phi(6.65 \text{ ppm}, 390 \text{ nm})$ must arise from the heteroconjugate as it is the only other species that involves the CNP moiety. Figure 3b shows that these two pairs of signals are in perfect synchronicity.

2D UVNMR correlations for the low-field region of NMR spectra are shown in Figure 3c,d. This region is characteristic for complexes with short and strong hydrogen bonds ($\text{O}\cdots\text{O}$ distance of about 2.5–2.4 Å). All three visible NMR signals have positive synchronous cross correlations with the UV band of the heteroconjugate. However, only the NMR signal at 18.1 ppm is in a perfect synchronicity with all of the changes of UV–vis spectra and thus can be assigned to the heteroconjugate. This conclusion is confirmed by 2D NMR/NMR correlation plots, given in the Supporting Information, which show that signals at 18.1 and 6.65 ppm are perfectly synchronous.

Let us now interpret the spectral features in terms of H-bond geometry. Previously, homoconjugated anions of acetic acid have been studied by low-temperature ^1H and ^{13}C NMR²¹ and vicinal H/D isotope effects²² on ^1H NMR chemical shifts; therefore, here, we will focus only on the heteroconjugated anion. From previously published hydrogen-bond correlations,¹⁰ it follows that ^1H NMR chemical shift of 18.1 ppm corresponds to the average $\text{O}\cdots\text{O}$ distance of about 2.48 Å and the average displacement of the bridging proton from the H-bond center

of about 0.18 Å. Unfortunately, the ^1H NMR chemical shift alone does not tell us on which side of the H-bond center the bridging proton is located. To determine this, we consider the position of the UV–vis band; the absorption at around 400 nm is closer to the band of the CNP anion (440 nm) than to the band of the neutral CNP (320 nm).⁴ This means that the geometry of the complex is closer to $\text{CNP}-\text{O}^-\cdots\text{HOOCH}_3$ than to $\text{CNP}-\text{OH}\cdots^-\text{OOCCH}_3$. Note, however, that the UV–vis band of the heteroconjugate is rather broad. This broadening may be governed by a distribution of rapidly fluctuating solvent shell configurations,²³ leading to different H-bond geometries and UV–vis band positions, possibly even to some degree of proton tautomerism.^{24,25}

In summary, we note that 2D heterospectral correlations were applied for the first time to a combination of UV–vis and NMR spectra, obtained using a novel UVNMR technique, guaranteeing identical experimental conditions for both types of measurements. The heterospectral signal assignment allowed us to identify signals of 1:1 heteroconjugated H-bonded complexes of CNP and the acetate anion present in CD_2Cl_2 solution at 180 K in a mixture with four other species. We find that the H-bond geometry in the heteroconjugate is close to $\text{CNP}-\text{O}^-\cdots\text{HOOCH}_3$ with an $\text{O}\cdots\text{O}$ distance of ~ 2.48 Å and proton displacement from the hydrogen bond center of ~ 0.18 Å. In general, establishing UVNMR cross-correlations allows one to identify and even predict spectroscopic properties of H-bonded complexes from incomplete or ambiguous spectral information. This feature could be especially useful for biomolecules in aqueous solution, where the observation of bridging proton signals is often hindered by the proton exchange with solvent molecules, while UV–vis spectra are often readily available.^{26,27}

EXPERIMENTAL SECTION

UVNMR Setup. The experimental setup for the UVNMR measurements is based on a 500 MHz Bruker NMR spectrometer and a custom NMR probe equipped with a fiber optic reflection probe (Avantes, Eerbeek, Netherlands). An **Avantes-2048 fiber-coupled UV spectrometer** was used to record optical spectra. Samples were measured in quartz 5 mm flat-bottomed “NMR cuvettes” custom-made by Hellma (Müllheim, Germany). Inside of the NMR cuvettes, PTFE inserts with 0.2 mm spacers were placed, providing a 0.4 mm optical path length for absorption measurements in reflection. The setup is described in more detail in ref 4.

Sample Preparation. CD_2Cl_2 (Eurisotope, 99.7%) was dried over molecular sieves of 4 Å. CNP and acetic acid (Aldrich) were used without further purification. Tetraethylammonium (TEA) acetate was prepared by mixing acetic acid with 2 equiv of TEA hydroxide in methanol solution (Aldrich), removing the solvents by azeotropic distillation with CH_2Cl_2 . Mixing of the components for the sample preparation was based on 10 and 100 mM stock solutions of CNP, acetic acid, and TEA acetate in dry CD_2Cl_2 . The overall sample volumes were about 400 μL . The composition was checked using the relative integrated intensities of NMR signals of nonexchangeable protons.

UVNMR Measurements. UV–vis spectra were acquired in ~ 1 min with internal averaging. Blank UV–vis measurements were performed prior to the actual sample measurements using CD_2Cl_2 solvent at 200 K. ^1H NMR spectra were processed with 15 Hz line broadening (to minimize the artifacts in 2D correlations²⁸) and calibrated to CHDCl_2 (5.32 ppm).

ASSOCIATED CONTENT

S Supporting Information. Equations used to construct 2D correlation spectra, another example of 2D UVNMR correlation (recovery of photoisomerized 4-aminoazobenzene), and 2D NMR/NMR correlation plots for the three-component mixture. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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