N–H Stretching Modes of Adenosine Monomer in Solution Studied by Ultrafast Nonlinear Infrared Spectroscopy and Ab Initio Calculations

Christian Greve,† Nicholas K. Preketes,‡ Rene Costard,† Benjamin Koeppen,† Henk Fidder,† Erik T. J. Nibbering,*† Friedrich Temps,§ Shaul Mukamel,‡ and Thomas Elsaesser†

†Max-Born-Institut für Nichtlineare Optik und Kurzzeitspektroskopie, Max-Born-Strasse 2 A, D-12489 Berlin, Germany
‡Department of Chemistry, University of California, Irvine, California 92697-2025, United States
§Institut für Physikalische Chemie, Christian-Albrechts-Universität zu Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany

ABSTRACT: The N–H stretching vibrations of adenine, one of the building blocks of DNA, are studied by combining infrared absorption and nonlinear two-dimensional infrared spectroscopy with ab initio calculations. We determine diagonal and off-diagonal anharmonicities of N–H stretching vibrations in chemically modified adenosine monomer dissolved in chloroform. For the single-quantum excitation manifold, the normal mode picture with symmetric and asymmetric NH2 stretching vibrations is fully appropriate. For the two-quantum excitation manifold, however, the interplay between intermode coupling and frequency shifts due to a large diagonal anharmonicity leads to a situation where strong mixing does not occur. We compare our findings with previously reported values obtained on overtone spectroscopy of coupled hydrogen stretching oscillators.

1. INTRODUCTION

Nucleic acid bases, building blocks of DNA and RNA, have been investigated with numerous experimental and theoretical methods. In light of the hydrogen-bonding interactions between complementary nucleic acid bases, linear vibrational spectroscopy has been one of the favored techniques as N–H stretching modes provide a direct local probe of these interactions. Typically, one follows either a top-down approach, where a DNA (or RNA) double helix formed by a specific base pair sequence is studied,1–3 or a bottom-up approach, where only a limited number of building blocks, that is, nucleic acid bases, are included in the study. In recent years, individual bases and base pairs have been studied in the gas phase4–7 and in solution.8–12 Despite the fact that both in the gas phase and in solution such a bottom-up approach involves the existence of several different hydrogen-bonded complexes, a characterization of infrared (IR) spectra has been possible by applying a comparison to theoretical spectra resulting from quantum chemical calculations.

Until now, most of these studies have relied on linear IR spectra that give insight into the frequencies and cross sections of the fundamental ν = 0–1 transitions. Apart from leaving much ambiguity in the assignment of N–H stretching bands, the diagonal anharmonicities and the couplings between different modes, that is, the off-diagonal anharmonicities, have remained unspecified. Ultrafast nonlinear pump–probe and two-dimensional (2D) infrared spectroscopy give direct insight into such behavior, as has been demonstrated for N–H and C=O stretching excitations of DNA sequences and oligomers.2,3,13–19 To a lesser extent, nucleobase pairs and monomers in solution12,20–22 have been studied by such techniques. As a result, the diagonal and off-diagonal anharmonicities of uncomplexed nucleic bases have so far remained elusive.

In this article, the N–H stretching modes of adenosine monomer in chloroform solution (Figure 1) are studied by a combination of ultrafast nonlinear infrared spectroscopy and ab initio calculations of vibrational modes and couplings. The adenosine monomer contains an NH2 group with two local N–H stretching oscillators. We deduce from linear and 2D-IR spectra the diagonal and off-diagonal anharmonicities of the N–H stretching modes. We analyze the observed frequency shifts in the linear and 2D-IR spectra using both normal and local mode representations, the latter allowing for a detailed comparison with theory. We substantiate these results by ab initio calculations, which provide local diagonal anharmonicities and intermode couplings.

Received: April 21, 2012
Revised: June 11, 2012
Published: June 23, 2012

© 2012 American Chemical Society
The Journal of Physical Chemistry A

2. EXPERIMENTAL AND THEORETICAL METHODS

Linear and Nonlinear IR Spectroscopy. Chemically modified 2'-deoxyadenosine nucleoside was synthesized in a similar fashion as reported before.11 To increase the solubilities in weakly polar solvents, the hydroxyl groups of the ribose units were substituted with tert-butylidimethylsilyl (TBDMs) groups. We denote the chemically modified nucleic base 3',5'-TBDMs protected 2'-deoxyadenosine as species A (Figure 1a). CHCl₃ (Uvasol, ≥99.0% purity) was obtained from Aldrich and CDCl₃ from Deutero. The solvent was dried over molecular sieves with pore diameters of 0.3 nm. Deuteration of the amino group of adenosine was obtained by dissolving A in a 100-fold excess of methanol-d₅. After a few minutes, the solvent was removed in vacuum. This procedure was repeated once before the substance was taken up in chloroform and the solution was transferred to a cuvette, leading to about a 70% degree of deuteration.

Linear IR spectra were recorded at room temperature with a Varian 640 FT-IR spectrometer (resolution = 1 cm⁻¹). The solutions were held between 1 mm CaF₂ windows with absorption path lengths of 0.2 mm using Teflon spacers. Femtosecond IR pump–probe and 2D-IR photon echo experiments were performed as described before.12 In short, the mid-IR pulses, generated by parametric down-conversion of the output of an amplified Ti:sapphire laser system, had a center frequency of 3350 cm⁻¹ and a pulse duration of 50 fs. In the pump–probe experiments, the probe pulses were spectrally dispersed after interaction with the sample (resolution = 4 cm⁻¹). The 2D-IR spectra have a resolution of 10 cm⁻¹ along the detection frequency νₚ. For the ultrafast experiments, we used a 0.04 M solution of A in CHCl₃ which was held between 1 mm CaF₂ windows with an absorption path length of 0.2 mm.

Quantum Chemical Calculations. Ab initio calculations were performed on 9-methyladenine (9-mA) and 9-methyl-adenine-d₂ (9-mA-d₂), where the amino hydrogens have been exchanged with deuterium. All calculations were performed in the gas phase, at the HF/6-311++G** level of theory using Gaussian 09.23 Linear absorption and absorptive 2D-IR spectra (κᵣ + κ₀) were calculated using the sum-over-states expressions as implemented in SPECTRON.24 All calculations were performed in the impulsive limit using a homogeneous dephasing of 4 cm⁻¹. For the 2D-IR calculations, the sum-over-states expressions were used (for a complete description, see ref 25) with the population time (tₚ) fixed to 0 fs. To account for inhomogeneous broadening, a stochastic Gaussian frequency fluctuation was added to the symmetric (⟨(ω₀)² − (ωₙ)²⟩)²/2 = 21 cm⁻¹) and asymmetric (⟨(ω₀)² − (ωₙ)²⟩)²/2 = 30 cm⁻¹) fundamental (eigenstate) frequencies over 500 snapshots.24 Anharmonic shifts were fixed to the gas-phase values.

3. EXPERIMENTAL RESULTS AND DISCUSSION

Figure 1 shows the linear FT-IR spectra of A in chloroform, before and after partial H/D exchange of the hydrogen atoms in the adenosine NH₂ group. In a normal mode picture, the infrared band of nondeuterated A at 3413 cm⁻¹ is assigned to the symmetric NH₂ stretching mode υ(NH₂)ₛ while the asymmetric stretching band υ(NH₂)ₐ occurs at 3525 cm⁻¹. From the frequency difference between these two transitions, that is, 2J_H = υ(NH₂)ₛ − υ(NH₂)ₐ = −112 cm⁻¹, a “mechanical” coupling of Jₚₔ = −560 cm⁻¹ between the two local N–H stretching modes can be deduced. Slightly larger values for Jₚₔ of −57.5 to −59.0 cm⁻¹ are found for gas-phase 9-H-adenine and 9-mA.26–28 9H-adenine in helium nanodroplets29 and in Ar matrices,29 and 9-ethyladenine in CDCl₃ solution.8

In the partially H/D-exchanged sample, the symmetric ND₂ stretching mode υ(ND₂)ₛ is observed at 2489 cm⁻¹, and the asymmetric ND₂ stretching mode υ(ND₂)ₐ is at 2639 cm⁻¹, leading to a value for the splitting of 2J_D = υ(ND₂)ₛ − υ(ND₂)ₐ = −154 cm⁻¹, that is, Jₚₔ = −77.0 cm⁻¹. The H/D exchange yield is estimated to be ~70%, with the result that not only A monomers with fully exchanged ND₂ groups contribute to the spectrum but also partially exchanged A monomers, for which both the local N–H and the N–D stretching modes of an NHD group are observed. This partial exchange allows determination of the transition frequencies of the virtually decoupled local N–H and N–D stretching modes of the NHD group, υ(NH) = 3469 cm⁻¹ and υ(ND) = 2565 cm⁻¹. These values lie perfectly halfway between the symmetric and asymmetric normal mode stretching frequencies, showing that the exchange of a single hydrogen atom with a deuterium effectively decouples the two local N–H stretching modes. Moreover, it explicitly confirms the validity of regarding the normal modes as linear combinations of the local N–H(D) modes, with a splitting caused by the extracted couplings. From the decoupled local mode frequencies, it follows that the H/D isotope effect on the local NH stretching mode frequency is equal to 1.352, whereas the isotope effect on the coupling between the two local modes is Jₚₔ/Jₚₐ = (1.375)⁻¹.

To determine the vibrational population dynamics, we performed time- and frequency-resolved pump–probe mea-
measurements. In Figure 2a, transient pump–probe spectra are plotted for delay times between 0.2 and 40 ps. On the fundamental transitions at $\nu(NH_2)_A \approx 3415 \text{ cm}^{-1}$ and $\nu(NH_2)_S \approx 3525 \text{ cm}^{-1}$, one observes a prominent decrease of absorption that is due to the depletion of the respective $\nu = 0$ ground state and stimulated emission from the respective $\nu = 1$ state. The bleaching components below 3380 and 3500 cm$^{-1}$ are caused by transitions of complexed A molecules (cf. asterisks in Figure 1a). The complexed molecules that will not be considered in the following, show a somewhat higher transition dipole $\mu$, which we assign to a vibrationally heated ground state formed after the decay of the $\nu = 1$ states.

The oscillatory component on the pump–probe transients of Figure 2b, obtained after correction for the slow picosecond exponential population decay, is shown in Figure 2c,d, and the corresponding Fourier transforms are given in Figure 2e,f. From this analysis, we derive an oscillation frequency of 112 cm$^{-1}$ (corresponding to a period of 298 fs), with a dephasing time of $\sim 1$ ps. The pump spectrum covers both the $\nu = 0$–1 transitions of the $\nu(NH_2)_S$ and $\nu(NH_2)_A$ modes. An excitation scheme where spectrally distinct components of the pump field induce the two transitions simultaneously generates a coherent superposition of quantum states. As a result, the coherent superpositions induced by the pump pulse result in quantum beats between the $\nu(NH_2)_S$ and $\nu(NH_2)_A$ normal modes with a frequency of $2|J|_d = \nu(NH_2)_A - \nu(NH_2)_S = 112 \text{ cm}^{-1}$ in the subsequent time period. The relatively long dephasing time of this quantum beat is fully in line with the observed difference in line widths of the inhomogeneously broadened transitions of the $\nu(NH_2)_S$ and $\nu(NH_2)_A$ modes (FWHM = 21 and 30 cm$^{-1}$, respectively).

Figure 3 shows 2D-IR spectra of A dissolved in CHCl$_3$, obtained with the $(k_1, k_2, k_1, k_{LO}) = (ZZYY)$ polarization scheme where spectrally distinct components of the pump field result in quantum superposition of quantum states. As a result, the coherent superpositions induced by the pump pulse result in quantum beats between the $\nu(NH_2)_S$ and $\nu(NH_2)_A$ normal modes with a frequency of $2|J|_d = \nu(NH_2)_A - \nu(NH_2)_S = 112 \text{ cm}^{-1}$ in the subsequent time period. The relatively long dephasing time of this quantum beat is fully in line with the observed difference in line widths of the inhomogeneously broadened transitions of the $\nu(NH_2)_S$ and $\nu(NH_2)_A$ modes (FWHM = 21 and 30 cm$^{-1}$, respectively).
geometry. We plot absorptive 2D-IR spectra, that is, the sum of the rephasing and nonrephasing signals. The 2D spectra were recorded for (population) waiting times of (c) $T = 0.2$, (d) 0.3, (e) 0.5, and (f) 1.0 ps. Each 2D spectrum is normalized to the maximum positive signal at $(\nu_1[\text{cm}^{-1}], \nu_3[\text{cm}^{-1}]) = (3413, 3413)$. The 2D spectra display two prominent positive diagonal peaks at (3413,3413) and (3525,3525), which originate from the $\nu = 0 \rightarrow 1$ transitions of the $\nu(NH_2)^S$ and $\nu(NH_2)^A$ normal modes (cf., linear infrared spectra in Figure 3a,b). In addition, there are positive cross peaks at (3413,3525) and (3525,3413) and negative off-diagonal peaks at (3413,3320), (3413,3390), (3525,3280), and (3525,3465). As a function of population time $T$, the spectral positions, line shapes, and relative intensities of the different peaks display minor changes. This observation suggests a minor role of spectral diffusion on this time scale and a negligible energy transfer from the $\nu(NH_2)^A$ mode at 3525 cm$^{-1}$ to the $\nu(NH_2)^S$ mode at 3413 cm$^{-1}$, as is to be expected given the 6 and 10 ps lifetimes.

In Figure 3g, we show the spectrum of Figure 3c on a magnified intensity scale. Cuts along the detection frequency axis for $\nu_1 = 3525$ and 3413 cm$^{-1}$ (dashed lines in Figure 3g) are plotted in Figure 3h, together with the (intensity) spectrum of the femtosecond infrared pulses in Figure 3h. In addition to the strong peaks originating from the monomeric
species A, there are a number of weaker peaks caused by complexed molecules.

We now interpret the 2D spectra by assigning the different peaks to different vibrational transitions in the normal mode picture. The corresponding level scheme is shown in Figure 4, together with a schematic 2D spectrum including the assignments. The fundamentalν = 0→1 transitions of ν(NH3) and ν(NH2) give rise to the diagonal peaks at (νS01,νA01) = (3413,3413) and νA01 = (3525,3525). In addition, there are the ν = 1→2 transitions of each of the two normal modes and the transition to the combination state in which both modes are excited on their fundamental transition. The frequency spacing of the positive peak at (νS01,νA01) = (3525,3413) and the negative peak at (νA01,νS01) = (3525,3280) has a value of ΔSA ≈ 133 cm⁻¹. The detection frequency spacing between the positive peak at (νS01,νA01) = (3413,3525) and the negative peak at (νS01,νA01) = (3413,3392) has the same value, within the experimental accuracy. Thus, we assign the two peak patterns to the two-quantum excitation of the coupled normal modes where both modes are excited on the fundamental transition and the combined excitation is anharmonically down-shifted by ΔSA ≈ 133 cm⁻¹ compared to the sum of the two fundamental frequencies of 3413 + 3525 = 6938 cm⁻¹. The pairs of positive and negative off-diagonal peaks at the two excitation frequencies νS01 = 3413 cm⁻¹ and νA01 = 3525 cm⁻¹ are characteristic for this double excitation.33 In addition to such pairs of off-diagonal peaks, there are negative peaks at (νS01,νA12) = (3413,3320) and (νA01,νS01) = (3525,3465) that represent the ν = 1→2 contributions of ν(NH3) and ν(NH2). From their detection frequency values, we derive diagonal anharmonicities ΔS = 3413−3320 = 93 cm⁻¹ and ΔA = 3525−3465 = 60 cm⁻¹.

4. THEORETICAL ANALYSIS AND DISCUSSION

The measured 2D spectra are in qualitative agreement with the normal mode description, discussed in section 3. An alternative choice of vibrational coordinates are the local N−H stretching modes. In principle, the two types of mode descriptions lead to equivalent predictions of the observed transitions as they are linked by a mere basis set transformation. For a more detailed analysis with the help of theoretical calculations, we now introduce the local mode picture, which has been used extensively in overtone spectroscopy.34−37 Using local modes has been shown to be advantageous in the explanation of the interplay of couplings and anharmonic shifts of overtone states of, in particular, hydrogen stretching modes. Comparison of the results obtained on the local N−H stretching modes of A with previously published data on O−H and N−H stretching modes of a variety of molecules is therefore straightforward.

In Figure 5, we show an energy level scheme appropriate for a discussion of the local mode picture. On the left-hand side, the ground state, the (degenerate) one-quantum and two-quantum states, as well as the combination tone are shown for the uncoupled local oscillators. The right-hand side of the scheme represents the coupled oscillators with arrows indicating the transitions between the single- and double-excitation manifolds and n indicating the number of excitation quanta in the system. We analyzed this level scheme with the n = 1 and 2 manifolds in the local mode representation33,35−41 by adopting the symmetrized local mode basis with the elements

\[
\text{li, } f^2 = \frac{1}{\sqrt{2}} (\text{li, j} \pm \text{lj}, i) \quad \text{for } i < j
\]

(1a)

\[
\text{li, } i^2 = \text{li, i}
\]

(1b)

The fundamental n = 0→1 transitions occur at 3413 and 3525 cm⁻¹. From each of these n = 1 states, two excited-state n = 1 → 2 absorption transitions are strongly allowed. With these excited-state absorption transitions, the energy location of the three levels in the n = 2 manifold can be accurately determined, 6735, 6805, and 6990 cm⁻¹ (accuracy = ±5 cm⁻¹). Furthermore, realizing that the uncoupled fundamental local N−H stretching frequency is equal to 3469 cm⁻¹, we can deduce from this level scheme that the total diagonal anharmonicity in the double-excitation manifold is (6 × 3469 − 6735 − 6805 − 6990) = 284 ± 13 cm⁻¹.

Using the intermode coupling J12 = −56.0 cm⁻¹ as input, we obtain a local mode diagonal anharmonicity of Δmin ≈ 135 cm⁻¹ which leaves for the diagonal anharmonic downshift of the combination mode 1,1 about Δ ≈ 14 cm⁻¹. The local mode anharmonicity Δloc ≈ 135 cm⁻¹ is comparable to those of free (non-hydrogen-bonded) N−H stretching vibrations in heterocyclic nucelic bases or base model systems.12,30,31 We note that, in contrast to Cho,40,41 Child and Halonen35−37 and Hamm and Zanni30 do not introduce an anharmonic shift Δ, for the combination level 1,1. The 14 cm⁻¹ value obtained here suggests that this anharmonic shift is small for the N−H stretching local modes of adenosine. In fact, the value barely exceeds the error margin on the total anharmonicity for the n = 2 manifold.

To obtain insight into the microscopic origin of the anharmonicities and couplings of A, we now discuss results from ab initio quantum chemical calculations. The HF/6-311++G** potential energy surface was expanded to sixth order in the two local N−H stretching coordinates.42,45

\[
H = \sum_i \frac{p_i^2}{2m_i} + \sum_{ij} \frac{1}{2} G_{ij} p_i p_j + V_0 + \sum_i f_i \frac{p_i}{r_i} + \frac{1}{2!} \sum_{ijkl} f_{ijkl} r_i r_j
\]

\[
+ \frac{1}{3!} \sum_{ijkl} f_{ijkl} r_i r_j r_k + \frac{1}{4!} \sum_{ijkl} f_{ijkl} r_i r_j r_k r_l + \frac{1}{5!} \sum_{ijklmn} f_{ijklmn} r_i r_j r_k r_l r_m r_n
\]

(2)

where Gij are elements of the Wilson G matrix44,45 and f(nk) are the nth order force constants

\[
f(nk)_{kk...kn} = \left( \frac{\partial^n V}{\partial r_k \partial r_k ... \partial r_n} \right)_0
\]

(3)

The nuclear dipole moment was truncated at linear order

\[
\mu = \mu_0 + \sum_i \left( \frac{\partial \mu}{\partial r_i} \right)_0 r_i
\]

(4)

The equilibrium force constants and transition dipole moments were determined by least-squares fits to the computed potential energy surface and electric dipole, respectively. The optimized geometry agrees well with previous results.46 The NH3 group is roughly 10° out of plane with respect to the ring. The potential energy surface and the electric dipole moment were calculated on a grid whose points are displacements from equilibrium ranging from −0.2 to 0.2 Å in steps of 0.05 Å.

7640 dx.doi.org/10.1021/jp303864m J. Phys. Chem. A 2012, 116, 7636−7644
The Hamiltonian can be written in an equivalent form by transforming the internal coordinates using

$$
\begin{align*}
 r_i &= \sqrt{\frac{\hbar}{2m_i \omega_i}} (B_i + B_i^\dagger) \\
 p_i &= i \sqrt{\frac{\hbar m_i \omega_i}{2}} (B_i - B_i^\dagger)
\end{align*}
$$

where $B_i$ and $B_i^\dagger$ are the bosonic annihilation and creation operators, which satisfy the commutation relation $[B_i, B_j^\dagger] = \delta_{ij}$. Using these relations, the Hamiltonian can now be written as

$$
H = -\frac{1}{2} \sum_{i \neq j} \gamma_{ij} (B_i^\dagger - B_i)(B_j^\dagger - B_j) + V_0
+ \sum_i \gamma_i (B_i^\dagger + B_i) + \frac{1}{2} \sum_{i \neq j} \omega_{ij} (B_i^\dagger + B_i)(B_j^\dagger + B_j)
+ \frac{1}{4!} \sum_{i < j < k} \gamma_{ijk} (B_i^\dagger + B_j^\dagger)(B_j^\dagger + B_k^\dagger)(B_i^\dagger + B_k^\dagger)
+ \frac{1}{5!} \sum_{ijklm} \gamma_{ijklm} (B_i^\dagger + B_j^\dagger)(B_j^\dagger + B_k^\dagger)(B_k^\dagger + B_l^\dagger)
+ \frac{1}{6!} \sum_{ijklm} \gamma_{ijklm} (B_i^\dagger + B_j^\dagger)(B_j^\dagger + B_k^\dagger)(B_k^\dagger + B_l^\dagger)
\begin{align*}
\gamma_{ij} &= \gamma_{ij}^\prime - \varphi_{ij} \\
\gamma_{ij}^\prime &= \gamma_{ij}^\prime - \varphi_{ij} = -\frac{\sqrt{\omega_i \omega_j \omega_{ij} G_{ij}}}{2\sqrt{G_{ii} G_{jj}}} - \frac{\sqrt{\omega_i \omega_j \omega_{ij} f_{ij}}}{2\sqrt{f_{ii} f_{jj}}}
\end{align*}
\tag{6}
$$

where the dimensionless force constants are related to those in Eq. 2 by $\gamma_{ijklm} = [\hbar/2m_i \omega_i 2m_j \omega_j 2m_n \omega_n]^{1/2} f_{ijklm} \omega_{klm}$ and $\omega_{klm}$ are the reduced mass and harmonic local mode frequency of the internal coordinate $r_{klm}$. The bilinear ("mechanical") coupling, which causes the splitting between the symmetric and asymmetric fundamentals, includes both a kinetic ($\gamma_{ij}^\prime$) and potential energy ($\varphi_{ij}$) contribution.

The calculated anharmonic energy levels and the expansion coefficients of the Hamiltonian for 9-mA and 9-mA-$d_2$ are listed in Tables 1 and 2. All frequencies and anharmonicities are scaled by a factor of 0.9183. This factor, which is greater than the standard harmonic scaling factor 47 and less than the recently determined anharmonic scaling factor, based on second-order vibrational perturbation theory calculations, was chosen to match with experiment. The calculated anharmonic energy levels match qualitatively with the anharmonicity parameters derived from the 2D-IR experiments (Figure 4). Due to the negative bilinear coupling, the symmetric stretch is lower in energy than the asymmetric stretch, as previously assigned. Upon isotopic substitution, $\gamma_{ij}^\prime$ increases by a factor of $\sqrt{2}$, which explains the greater splitting in 9-mA-$d_2$. A similar effect has recently been observed for water.

The intensity ratio $R$ of the symmetric and asymmetric peaks in the linear absorption spectrum (Figure 1) is estimated at $\sim 1.45 \pm 0.2$. Regarding these modes as positive and negative linear combinations of $\mathrm{N-H}$ local modes, the angle $\theta$ between the local mode transition dipoles is predicted from $\cos \theta = (R - 1)/(R + 1)$ to be $\sim 79 \pm 4^\circ$. Our ab initio calculations produce an angle between the local mode transition dipoles of $95^\circ$, which confirms that the $\mathrm{N-H}$ stretching local mode dipoles are not parallel to the bond vectors (Figure 5). This is similar to the amide I mode, where the transition dipole moment makes a $20^\circ$ angle with the C=O bond vector. The angle between the symmetric and asymmetric transition dipoles is $84^\circ$.

Figure 5 compares the measured and calculated 2D-IR spectra for $k_{zz} k_{zz} k_{zz} k_{zz} + k_{zz} k_{zz} k_{zz} k_{zz}$ (ZZZZ) and (ZZZY) polarization geometries. The nearly perpendicular orientation of the two transition dipole moments is reflected in the relative intensities of the cross peaks compared to those of the diagonal peaks in the 2D-IR spectra for these polarization geometries. The local mode diagonal anharmonicity, $\Delta_{loc}$, was calculated by expanding Eq. 2 in one dimension and calculating the resulting anharmonic shift. A comparison of the ab initio results to the

| $\nu_a$ | 3525 | 3530 (2861) |
| $\nu_s$ | 3413 | 3406 (2704) |
| $\Delta_{\nu}$ | 60 | 46 (31) |
| $\Delta_{\tau}$ | 93 | 69 (35) |
| $\Delta_{\tau}$ | 133 | 106 (68) |
| $\Delta_{\tau}$ | 284 | 219 (134) |
| $\Delta_{\tau}$ | 135 | 107 (65) |
| $\Delta_{\tau}$ | 14 | 6 (5) |

*Values for 9-MA-$d_2$ are given in parentheses.

### Table 2. Anharmonic Expansion Coefficients$^a$ of 9-mA in cm$^{-1}b$

| $g_1$ | $-0.2$ | $-0.2$ |
| $g_2$ | $-0.1$ | $-0.1$ |
| $g_{1111}$ | 149 (6.8) |
| $g_{1112}$ | 22.6 (10.3) |
| $g_{1122}$ | 22.9 (10.4) |
| $g_{1212}$ | 151 (6.9) |
| $g_{1222}$ | $-6.7$ | $-6.7$ |
| $g_{1111}$ | 149 (6.8) |
| $g_{1112}$ | 22.6 (10.3) |
| $g_{1122}$ | 22.9 (10.4) |
| $g_{1212}$ | 151 (6.9) |
| $g_{1222}$ | $-6.7$ | $-6.7$ |
| $g_{1111}$ | 149 (6.8) |
| $g_{1112}$ | 22.6 (10.3) |
| $g_{1122}$ | 22.9 (10.4) |
| $g_{1212}$ | 151 (6.9) |
| $g_{1222}$ | $-6.7$ | $-6.7$ |

*Values for 9-MA-$d_2$ are given in parentheses.

The force constants are listed as $g_{ijkl} = [\hbar/2m_i \omega_i 2m_j \omega_j 2m_n \omega_n]^{1/2} f_{ijkl} \omega_{klm}$.
experimental parameter values derived using the coupled local mode model is shown in Table 1. Our calculations confirm that the total diagonal anharmonicity is dominated by $\Delta_{\text{loc}}$ while $\Delta$ is small. Whereas the main features observed in the experiments are reproduced in the calculated 2D-IR spectra, the absolute value of the total diagonal anharmonicity, a measure of the three anharmonic shifts, is underestimated by a factor of 1.3, resulting in smaller spectral shifts of peaks in the 2D-IR spectra related to excited-state $n = 1 \rightarrow 2$ absorption transitions. The relative magnitudes of the three anharmonic shifts are roughly equal to the experimentally measured values, suggesting that a further refinement of the calculations should point at a better estimation of the N–H stretching anharmonicities.

We now discuss our findings on the diagonal anharmonicities and couplings of the N–H stretching modes of A, relating to previously reported work on hydrogen stretching oscillators, as well as indicating the repercussions that the obtained parameter values have on the fundamental $n = 0 \rightarrow 1$ and excited-state $n = 1 \rightarrow 2$ absorption transitions. It has been shown that for hydrogen stretching overtone states with $n = 2$–5 molecules, such as H$_2$O and C$_2$H$_2$, quenching of the intermode $J_{\text{HH}}$ coupling by the anharmonicity of the individual bond potentials $\Delta_{\text{loc}}$ causes a localization of vibrational excitation, that is, a local mode representation provides a better description for the experimentally determined frequency shifts of these overtone transitions. For C$_2$D$_2$ and SO$_2$, the normal mode picture is more beneficial to describe the observed features. Child and Halonen have provided the formalism to connect the local mode and normal mode representations. Depending on the relative magnitudes of coupling $J_{\text{HH}} = -56$ cm$^{-1}$ and diagonal anharmonicity $\Delta_{\text{loc}}$ it follows which mode description is closer to the real situation for the overtone states. In particular, when the value of the parameter $\tilde{\xi}$, defined as:

$$\tilde{\xi} = \frac{2}{\pi} \arctan\left(\frac{2J}{\Delta_{\text{loc}}}\right)$$

(8)

is close to zero, the local mode limit applies (i.e., $|J| \ll \Delta_{\text{loc}}$). In contrast, when $\tilde{\xi}$ becomes equal to $\pm 1$, that is when $|J| \gg \Delta_{\text{loc}}$, the normal mode limit is reached. For the A monomer, with $J_{\text{HH}} = -56$ cm$^{-1}$ and $\Delta_{\text{loc}} = 135$ cm$^{-1}$, we find $\tilde{\xi} = -0.44$, indicating that the character of the N–H stretching modes of A in overtone states is intermediate between local and normal limits. The characteristics of the N–H stretching modes of the A monomer can at best be compared with the O–H stretching modes of gas-phase H$_2$O, with $\tilde{\xi} = -0.34$, where similar diagonal anharmonicities and couplings have been derived using the local mode picture. The local mode basis has also been applied in the interpretation of overtone spectra of amines and anilines. From such studies, it has been derived that for aliphatic amines such as methylene and cyclopropylamine $|J_{\text{HH}}| = 32$ cm$^{-1}$ and $\Delta_{\text{loc}} = 158$ cm$^{-1}$. For aromatic amines such as aniline, where the resonance between the nitrogen lone pair and the $\pi$-molecular orbitals leads to a planarization of the NH$_2$ group, the local mode coupling increases to $|J_{\text{HH}}| = 43.65$ cm$^{-1}$ and $\Delta_{\text{loc}} = 163.4$ cm$^{-1}$. Comparing these literature values with those that we have found for the A monomer suggests that the magnitude of the local mode coupling increases upon increased interaction of the nitrogen lone pair with an extended aromatic $\pi$-molecular orbital system, whereas the magnitude of the local diagonal anharmonicity remains similar. To validate this, N–H stretching parameters for other molecular systems having the NH$_2$ functionality should be investigated.

The coupling $J_{\text{HH}} = -56.0$ cm$^{-1}$ leads to strong mixing of the resonant $\nu = 1$ states of the two local modes (i.e., $|J_{\text{HH}}| \gg \omega_{\nu} - \omega_{\nu - 1}$, and therefore, the normal modes are the proper eigenstates in the $n = 1$ manifold and, thus, the strong coupling regime applies to the single-excitation manifold. In the double-excitation manifold, on the other hand, the $|1,1\rangle$ local mode state is coupled to the local mode states $|0,2\rangle, |2,0\rangle$ that are both lowered by the large diagonal anharmonicity $\Delta_{\text{loc}}$. Transforming the basis set to $|1,1\rangle, |1,2\rangle, |2,0\rangle$ eliminates the coupling between $|0,2\rangle$ and the other two states and produces a modified coupling of $2J_{\text{HH}}$ between $|1,1\rangle$ and $|1,2\rangle$, with uncoupled energies of $2\omega_{\nu} - \Delta_1$ and $2\omega_{\nu} - \Delta_{\text{loc}}$, respectively. Thus, in the double-excitation manifold, the symmetric stretch overtone is only a proper eigenmode if $(\Delta_{\text{loc}} - \Delta_1)/(2J_{\text{HH}}) > 1$. In the present case, this ratio is $(135 - 14)/(2 \times 56) \approx 1.1$; therefore, clearly, the intermediate coupling regime applies to the double-excitation manifold, and neither the local nor the normal mode representations provide an accurate description of these vibrational overtone eigenstates. As a result, the $|1,1\rangle$ and $|1,2\rangle$ states of A are strongly mixed (see Figure 5), with the mixing angle $\phi$ defined as:

$$\phi = \arctan\left(\frac{4J}{\Delta_{\text{loc}} - \Delta_1}\right)$$

(9)

Finding $\phi = 118^\circ$ for A, such strong mixing results also in the weak allowance of the harmonically forbidden three-quantum transitions. Even though in our 2D-IR experimental data sets we find indications of these forbidden transitions becoming weakly allowed, which would lead to negative cross peaks located at $(3525,3210)$ and $(3413,3575)$, the minute signal strengths are just above detection threshold and of similar magnitude as peaks due to A complexes, preventing us from making definitive statements on this. The calculated 2D-IR spectra indicate that the magnitudes of these additional cross peaks are within a few percent of the main peaks. Such behavior of "forbidden" transitions having small cross sections has been observed before for the excited-state $n = 1 \rightarrow 2$ transitions in 2D-IR spectra obtained on the C$\equiv$O stretching modes of Rh(CO)$_5$(C$_2$H$_5$O)$_3$ (1) complexes. For such complexes with carbonyl stretching modes, however, $\Delta_{\text{loc}} \approx |J|$, and mixing between the symmetric stretching overtone and the combination state is less pronounced with concomitant weaker cross sections for these "forbidden" transitions.

Therefore, we feel that such rich effects of vibrational spectroscopy can be grasped best on hydrogen stretching oscillators, that is, C–H, N–H, and O–H stretching modes, with additional features that may take place upon formation of hydrogen bonds. Here, ultrafast 2D-IR spectroscopy provides a clear alternative to overtone spectroscopy to obtain direct insight into these vibrational coupling effects for molecules in the condensed phase. With ultrafast 2D-IR spectroscopy, one not only discerns the information on vibrational frequency shifts and band intensities into two spectral dimensions (as opposed to overtone spectroscopy where the transitions are recorded in one dimension), but additional orientational degrees of freedom can be explored using the polarization properties of the femtosecond IR laser pulses. A difference between the N–H and O–H stretching modes is the typically much larger spectral broadening for O–H stretching modes upon formation of hydrogen bonds, a consequence of a larger
susceptibility of O–H stretching modes to fluctuations imposed by the surrounding solvent shells.\textsuperscript{60,61} Dynamical decoupling effects may then be expected to hinder a full determination of the interplay between coupling and diagonal anharmonicities. As a result, full characterization of the rich spectral structure of the nonlinear 2D-IR spectra is likely to happen for N–H stretching manifolds of hydrogen-bonded nucleobase pairs rather than for O–H stretching bands of hydrogen-bonded topographies consisting of multiple OH groups.

5. CONCLUSIONS

We have investigated the linear FT-IR and nonlinear femtosecond 2D-IR spectra of the N–H stretching modes of the adenosine (A) monomer in chloroform solution. On the basis of the frequency positions of the symmetric and asymmetric NH\textsubscript{2} stretching, of symmetric and asymmetric ND\textsubscript{2} stretching, and of the N–H and N–D stretching modes of NH\textsubscript{D} groups, couplings \(J_{\text{A}} = -56 \text{ cm}^{-1}\) and \(J_{\text{D}} = -77 \text{ cm}^{-1}\) were found. We have applied the local mode representation to fully explore the first \(n = 1\) and second \(n = 2\) excitation manifolds using the formalism developed to describe results from overtone spectroscopy of stretching oscillators. Whereas the local N–H stretching modes of A are degenerate in frequency, that is, \(|J_{\text{D}}| > |\omega_{\text{A}} - \omega_{\text{D}}| = 0\), making them strongly coupled in the \(n = 1\) single excited states, and the symmetric and asymmetric normal modes are proper eigenmodes of the molecular system, the large local diagonal anharmonicity \(\Delta \omega_{\text{A}} \approx 135 \text{ cm}^{-1}\) leads to a distinctly different behavior for the \(n = 2\) double-excitation manifold, where intermediate coupling leads to the situation that neither local nor normal modes are the appropriate eigenmodes for A. We anticipate that the analysis presented here can be further refined when comparing 2D-IR spectra of N–H stretching modes in the A monomer with N–D stretching modes of deuterated A monomer, as well as extending to linear and 2D-IR spectroscopy on hydrogen-bonded A.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: nibberin@mbi-berlin.de.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Research Council under the European Union’s Seventh Framework Programme (FP7/2007-2013/ERC Grant Agreement No. 247051; T.E.), the German Science Foundation (Deutsche Forschungsgemeinschaft; F.T.), the National Institutes of Health (Grants GM059230 and GM091364; S.M.), as well as the National Science Foundation (Grant CHE-1058791; S.M.). N.K.P. is supported by a National Science Foundation Graduate Research Fellowship. We cordially acknowledge assistance in the pump–probe experiments by Dr. Ismael A. Heisler.

■ REFERENCES

(6) Dong, F.; Miller, R. E. Science 2002, 298, 1227.