Supporting Information

Identification of Lactam-Lactim Tautomers of Aromatic Heterocycles in Aqueous Solution Using 2D IR Spectroscopy

Chunte Sam Peng and Andrei Tokmakoff*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

*Corresponding Author. Telephone: 617-253-4503, Fax: 617-253-7030, tokmakof@mit.edu

Contents

Supporting Information

I. Materials & Experimental methods

Samples

2-pyridone, 6-chloro-2-pyridone, and 4-pyrimidinone were purchased from Sigma-Aldrich and used without further purification. The samples were first dissolved in D_2O and lyophilized in order to exchange the labile hydrogen with deuterium. 2-pyridone was dissolved in 100 mM phosphate buffer in D_2O at 5 mg/ml. 6-chloro-2-pyridone and 4-pyrimidinone were dissolved in 100 mM phosphate buffer in D_2O at 10 mg/ml. All samples were maintained at pH = 7.4.

Spectroscopy

For both FTIR and 2D IR measurements, 25 μL of sample solution was sandwiched between two 1 mm thick CaF_2 windows that are separated by a 50 μm Teflon spacer. Temperature-dependent FTIR spectra were collected using Nicolet 380 FTIR spectrometer at 1.0 cm^{-1} spectral resolution with 16 scans per spectrum. Spectra for both the sample and the solvent were collected with the same procedure and the solvent spectra were subtracted from the sample spectra. Absorptive 2D IR spectra were collected using a 2D IR spectrometer as describe in detail previously.[1] The relative polarizations of the pulses were set to be parallel (ZZZZ). The waiting time (τ_2) between the first two pulses and the third pulse was fixed at 150 fs. The coherence time between the first and the second pulse was scanned in 4 fs steps from -60 fs to 3.0 ps and 2.4 ps for rephasing and non-rephasing spectra, respectively. The coherence time (τ_1) was Fourier-transformed to obtain the first frequency axis ω_1. The heterodyned signal was dispersed in a monochrometer to obtain the ω_3 frequency dimension and collected using a 64 x 2 pixel mercury-cadmium-telluride (MCT) array detector. Linear absorption from the solvent and solute was divided out along both the ω_1 and ω_3 axes to remove spectral distortions.[2]

Ab initio DFT calculation

To help assign the experimental IR spectra, *ab initio* density functional theory (DFT) calculations were performed using QChem.[3] The B3LYP hybrid functional was implemented with the 6-31G (d, p) basis set to optimize the geometry and calculate the vibrational normal modes. A harmonic scaling factor of 0.9614 was applied to help match the calculated frequencies with the experimental frequencies.[4] The calculations were performed in the gas phase or with explicit D_2O molecules in close proximity of a strong hydrogen bond donor/acceptor such as the carbonyl and amino groups. All labile protons were deuterated to match the experimental condition. The same procedure was applied to all possible tautomers of 2-pyridone, 6-chloro-2-pyridone, 4-pyrimdinone.

Supporting Information

II. Concentration-dependent FTIR of 2-pyridone

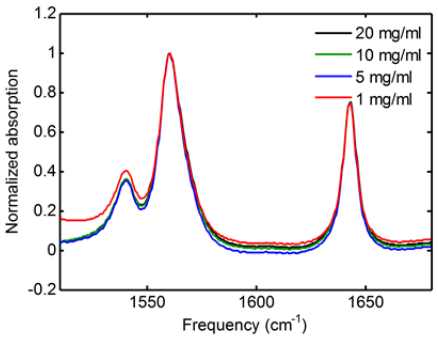


Figure S1: Concentration-dependent FTIR of 2-pyridone in phosphate buffer.

The normalized FTIR spectra of 2-pyridone from 1 mg/ml to 20 mg/ml are almost identical, except for the slight offset seen in the 1 mg/ml sample due to imperfect background subtraction. The concentration dependence indicates that 2-pyridone is in the monomer state within the concentration range in our experiments.

III. UV absorption spectra for 2-pyridone and 6-chloro-2-pyridone

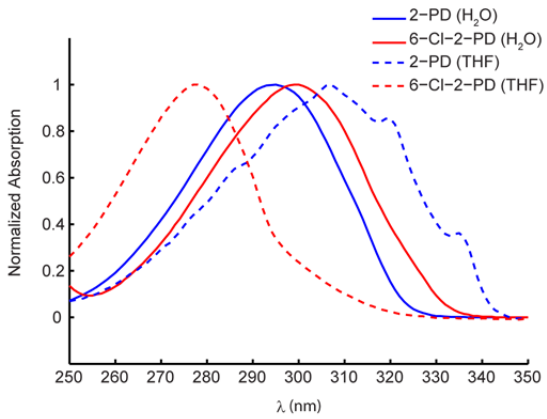


Figure S2: UV absorption spectra of 2-pyridone (blue) and 6-chloro-2-pyridone (red) in H_2O (solid) and THF (dashed). The samples in H_2O were buffered with phosphate buffer at pH = 7.4. The sample concentrations were 50 μg/ml.

Figure S2 shows the first $\pi \rightarrow \pi^*$ absorption band of 2-pyridone and 6-chloro-2-pyridone. Previous calculations[5] have shown that for a given pyridone derivative, the wavelength of the absorption maximum follows a general trend: lactim monomer < lactam monomer. The spectrum of 2-pyridone in H_2O has a maximum at 294 nm, which has been attributed to the lactam tautomer.[6] In general, the lactim tautomers are more populated in non-polar solvents as seen by the spectrum of 6-chloro-2-pyridone in THF, where the absorption maximum is at shorter wavelength 278 nm. However, for 50 μg/ml 2-pyridone in THF, the spectrum displays a maximum at 307 nm (with vibrational bands at 335 nm, 320 nm, 307 nm, and 286 nm) indicating that the lactam tautomer is the dominating species. As the concentration of 2-pyridone in THF increases, 2-pyridone dimerizes and lead to a shift of the band structures.[7] For 6-chloro-2-pyridone in H_2O, the spectrum has a maximum at 300 nm, which would indicate that the lactam form is the predominant species, and a 5 % of lactim was reported by Katritzky and coworkers.[8] Nevertheless, the IR spectra reported in this paper reveal a significant amount of lactim population, demonstrating that IR spectroscopy is more sensitive to the lactam-lactim tautomerism when the two tautomer populations are close to each other, leading to an overlap in the UV absorption.

IV. DFT calculation: effects of tautomerism and hydration on the vibrational modes

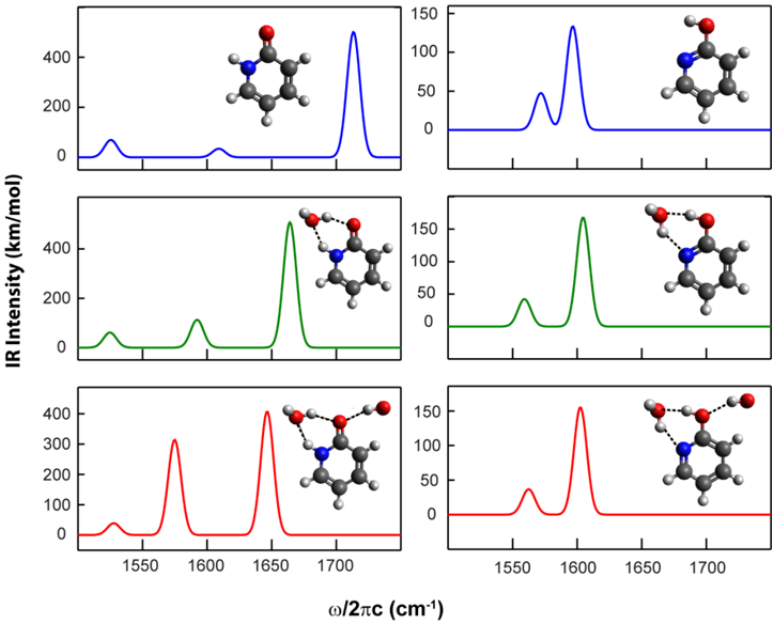


Figure S3: DFT calculated IR spectra of 2-pyridone-d_1 (left) and 2-hydroxypyridine-d_1 (right) with different number of D_2O molecules making HB's to O and N atoms.

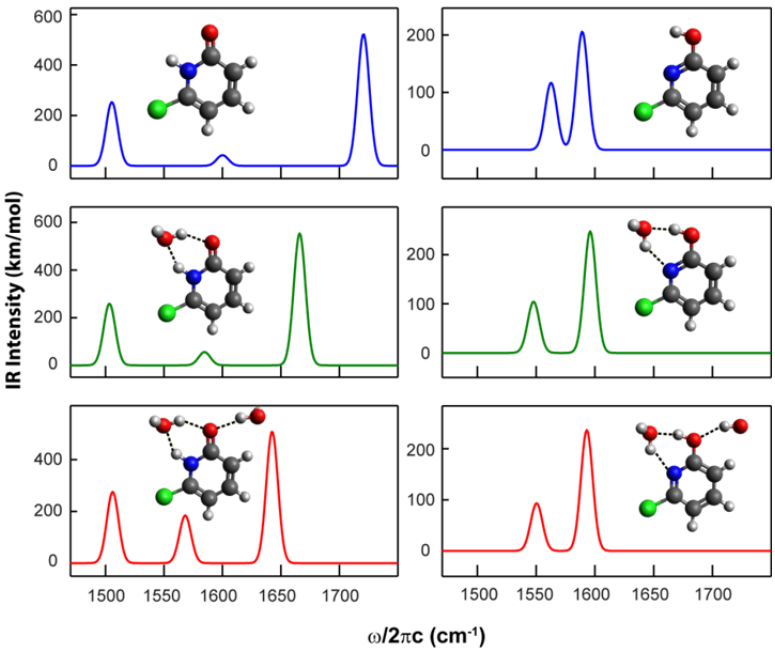


Figure S4: DFT calculated IR spectra of 6-chloro-2-pyridone-d_1 (left) and 6-chloro-2-hydroxypyridine-d_1 (right) with different number of D_2O making HB's to O and N atoms.

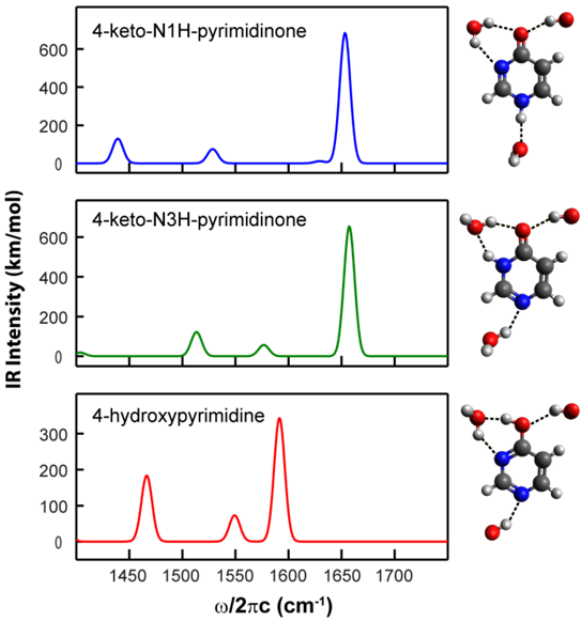


Figure S5: DFT calculated IR spectra of N1H lactam (top), N3H lactam (middle), and lactim (bottom) tautomers of 4-pyrimidinone with 3 D_2O molecules.

Supporting Information

Table S1: Vibrational frequencies of experiment v.s. DFT calculations. The frequencies are in units of cm^{-1}, and the calculated results have been scaled by 0.9614. All calculations were with explicit D_2O molecules.

2-pyridone

Peak	Experiment	DFT calculation	
		Lactam	Lactim
A1	1643	1647	
			1603
A2	1560	1575	1562
A3	1541	1528	

6-chloro-2-pyridone

Peak	Experiment	DFT calculation	
		Lactam	Lactim
B1	1637	1642	
B2	1591		1593
B3	1550	1568	1551
B4	1528	1507	

4-pyrimidinone

Peak	Experiment	DFT calculation		
		N1H lactam	N3H lactam	Lactim
C1	1654		1657	
C2	1646	1654		
C3	1593		1577	1591
C4	1585	---[a]		1549
C5	1538	1529	1513	
C6	1503	1439		1466

[a] The C4 mode is shown to have cross-peaks to C2, C5, and C6 in the 2D IR spectrum, therefore we assign it to be one of the N1H lactam vibration. However, this mode is not observed in the DFT calculations.

V. DFT calculation: relative stabilities of the lactam-lactim tautomers

Numerous work has been done to predict the tautomeric equilibria using computational methods. Here we address some of the issues and challenges in this approach.

First of all, it should be kept in mind that the calculated stabilities of these tautomers are sensitive to not only the solvent environment but also the computational methods used. The relative energies can be differed by as much as 5 kcal/mol between different levels of theory, and sometimes even the sign can be reversed.[9-11] However, a 0.6 kcal/mol energy difference would result in a drastic 36% difference in relative population. It has not been established that a specific computational method works the best when compared to the experimental results. The relative stabilities predicted by these calculations in general agree with the experiments, but the absolute numbers are still under debate.

Secondly, it has become a common practice for experimentalists to run simple DFT calculations in the gas phase to compare to the experimental results. However, tautomerism in biologically relevant processes happen in aqueous solutions, therefore, it is not appropriate to compare the energy difference between tautomers from DFT calculations to experimental results. A proper selection of the solvent model is crucial for even attempting to compare the experimental results with calculations. Take 2-pyridone for example, gas phase experiments have shown that the lactim form is more stable by about -0.58 kcal/mol.[12] On the other hand, the equilibrium is shifted towards the lactam tautomers by about 1 kcal/mol in solution, which has been explained by the larger dipole moment of 2-pyridone and its ability to form hydrogen-bonds with water.[13] Additionally, the activation barrier for the tautomerization of 2-pyridone to 2-hydroxypyridine was calculated to be 38.45 kcal/mol.[11] This value is reduced by about 15 kcal/mol when a single water molecule is included in the calculation and can be further reduced with two hydrating water.[13] The need for a better solvation model is clearly needed to bridge the gap between the experiments and calculations.

Table S2: Relative energies of the tautomers. Zero-point energies have been included. E: electronic energy; H: enthalpy; G: Gibbs free energy. Energies are expressed in units of *kcal/mol*.

2-pyridone

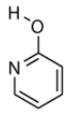
	PD	HP1	HP2	PD D_2O	HP1 D_2O	HP2 D_2O	PD $2D_2O$	HP1 $2D_2O$	HP2 $2D_2O$
E	0	0.036	5.365	0	0.859	12.7	0	3.145	10.87
E+H	0.003	0	5.409	0	0.787	13.43	0	3.195	11.68
E+G	0	0.104	5.348	0	0.939	10.94	0	2.959	8.679

6-chloro-2-pyridone

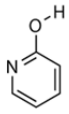
	PD	HP1	HP2	PD D_2O	HP1 D_2O	HP2 D_2O	PD $2D_2O$	HP1 $2D_2O$	HP2 $2D_2O$
E	3.096	0	5.192	2.699	0	12.97	0.4713	0	7.747
E+H	3.156	0	5.257	2.719	0	13.84	0.3453	0	8.521
E+G	3.001	0	5.127	2.696	0	10.72	0.903	0	5.313

4-pyrimidinone

	N1H	N3H	Lactim	N1H $3D_2O$	N3H $3D_2O$	Lactim $3D_2O$
E	9.826	0	1.276	10.9	0	3.656
E+H	9.954	0	1.237	11.81	0	3.697
E+G	9.622	0	1.344	8.052	0	3.409



HP1



HP2

Since the calculations show that HP2 is much higher in energy compared to HP1, we only compared the experimental results to the calculation results for HP1. We can notice that lactim becomes more stable than the lactam tautomers for 6-chloro-2-pyridone compared to 2-pyridone. This observation is consistent with our IR experimental results. However, the experiments show that the lactam population is greater than the lactim population for 6-chloro-2-pyridone (K_{eq} = 2.1 and ΔH = -3.3 kcal/mol), which is contradictory to the calculations (ΔH = +0.35 kcal/mol). This discrepancy demonstrates that current DFT calculations cannot predict accurately the relative stabilities between tautomers, yet predicting a general trend going from one molecule to another is plausible.

VI. Temperature-dependent 2D IR spectra for 6-chloro-2-pyridone

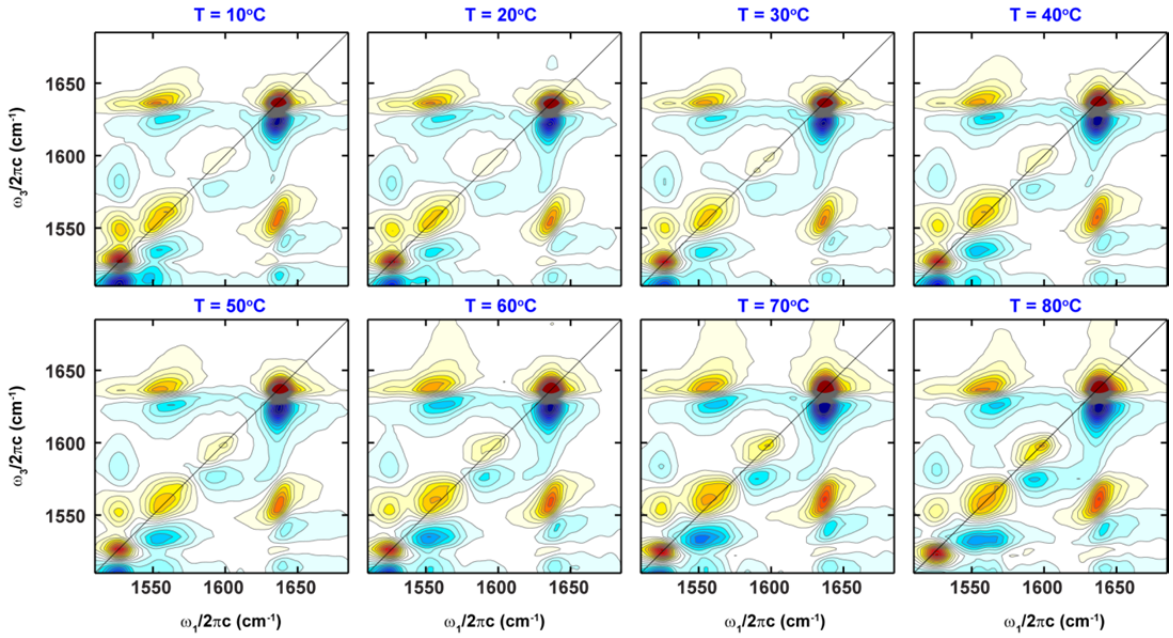


Figure S6: Temperature-dependent 2D IR spectra of 6-chloro-2-pyridone demonstrating the increase of lactim population at the expense of lactam tautomers as the temperature increases. The lactam tautomers also shift to less hydrogen-bonded states.

Since the 2D IR spectra were normalized to the maximum of the absolute value at each temperature, it becomes difficult to see the loss of the lactam tautomer as temperature increases. However, the spectral features were clearly identified in the second SVD component spectrum shown in Fig. 4.

VII. Determination of equilibrium constant for 6-chloro-2-pyridone

For the tautomeric equilibrium of 6-chloro-2-pyridone:

$$1a \rightleftharpoons 1b \tag{S1}$$

Where **1a** and **1b** represent the lactim and lactam tautomers, respectively. The equilibrium constant as a function of temperature therefore is written as the ratio of the populations of two tautomers:

$$K_{eq}(T) = \frac{P_{1b}(T)}{P_{1a}(T)} \tag{S2}$$

We express the tautomer populations using the Boltzmann factors and the relative Gibbs free energy ΔG:

$$P_{1a}(T) = \frac{1}{1+\exp(-\Delta G/kT)} \tag{S3}$$
$$P_{1b}(T) = \frac{\exp(-\Delta G/kT)}{1+\exp(-\Delta G/kT)}$$

From the FTIR spectrum of 6-chloro-2-pyridone, we identify the two separate peaks for the lactim and lactam tautomers at 1591 cm^{-1} and 1637 cm^{-1}, respectively. We can express the integrated intensities of these two peaks using Beer's law:

$$\bar{A}_{1a}(T) = A_{1a}(T)/bC = \varepsilon_{1a}P_{1a}(T) \tag{S4}$$
$$\bar{A}_{1b}(T) = A_{1b}(T)/bC = \varepsilon_{1b}P_{1b}(T)$$

Where b is the path-length, and C is the total concentration of the sample which include both tautomers. If we take the ratio of the two integrated intensities:

$$\frac{\bar{A}_{1b}(T)}{\bar{A}_{1a}(T)} = \frac{P_{1b}(T)}{P_{1a}(T)} \times \frac{\varepsilon_{1b}}{\varepsilon_{1a}} = K_{eq}(T)\frac{\varepsilon_{1b}}{\varepsilon_{1a}} = e^{-\Delta G/kT} \times \frac{\varepsilon_{1b}}{\varepsilon_{1a}} \tag{S5}$$

$$\Rightarrow \ln\left[\frac{\bar{A}_{1b}(T)}{\bar{A}_{1a}(T)}\right] = \frac{-\Delta H}{kT} + \frac{\Delta S}{k} + \ln\left(\frac{\varepsilon_{1b}}{\varepsilon_{1a}}\right) \tag{S6}$$

Here we have made the assumptions that the changes in enthalpy and entropy do not depend on temperature. This should be valid given that the temperature range in the experiment is small (from 10°C to 90°C). Fitting this curve as a function of $1/T$ results in:

$$\frac{-\Delta H}{k} = 1665 \pm 33 \tag{S7}$$
$$\frac{\Delta S}{k} + \ln\left(\frac{\varepsilon_{1b}}{\varepsilon_{1a}}\right) = -4.678 \pm 0.103$$

Once we have obtained ΔH, we can then fit the integrated intensities to get ε_1, ε_2, and ΔS:

$$\bar{A}_{1a}(T) = \frac{1}{1+e^{-\Delta H/kT}e^{-\Delta S/k}}\varepsilon_{1a} \tag{S8}$$
$$\bar{A}_{1b}(T) = \frac{e^{-\Delta H/kT}e^{-\Delta S/k}}{1+e^{-\Delta H/kT}e^{-\Delta S/k}}\varepsilon_{1b}$$

The results are listed:

$$\varepsilon_{1a} = 0.437 \pm 0.016$$
$$\varepsilon_{1b} = 0.563 \pm 0.010 \tag{S9}$$
$$\frac{\Delta S}{k} = -4.931 \pm 0.074$$

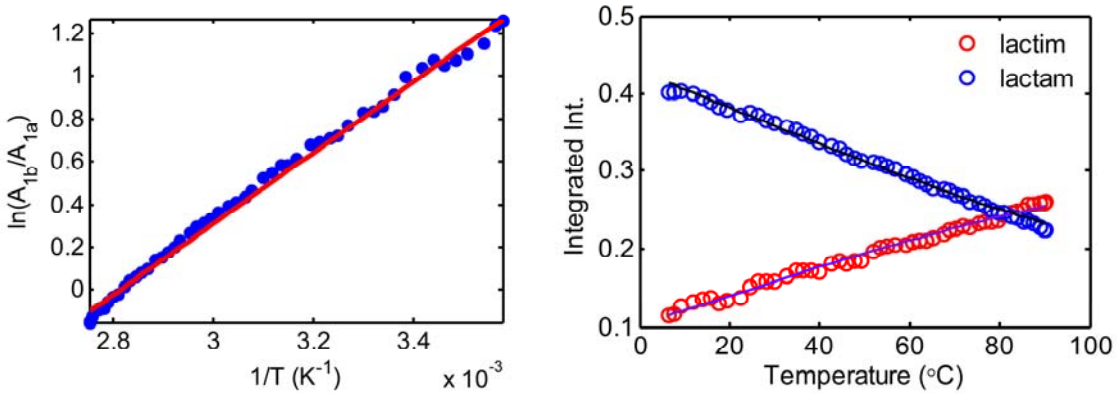


Figure S7: Analysis of the temperature-dependent FTIR spectra of 6-chloro-2-pyridone. (left) Fit to Equation S6. (right) Fit to Equation S8.

VIII. pH-dependent FTIR spectra for 4-pyrimidinone

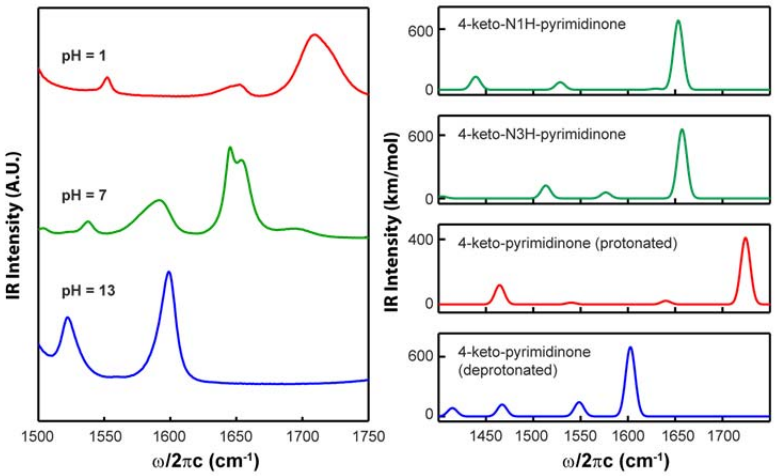


Figure S8: Experimental pH-dependent FTIR of 4-pyrimidinone (left) and DFT calculated FTIR (right) for different protonation states.

References

(1) Chung, H. S.; Khalil, M.; Smith, A. W.; Tokmakoff, A. Transient Two-Dimensional IR Spectrometer for Probing Nanosecond Temperature-Jump Kinetics. *Rev. Sci. Instrum.* **2007**, *78*, 063101-1-10.
(2) Jones, K. C.; Ganim, Z.; Peng, C. S.; Tokmakoff, A. Transient Two-Dimensional Spectroscopy with Linear Absorption Corrections Applied to Temperature-Jump Two-Dimensional Infrared. *J. Opt. Soc. Am. B* **2012**, *29*, 118-129.
(3) Shao, Y., et al. Advances in Methods and Algorithms in a Modern Quantum Chemistry Program Package. *Phys. Chem. Chem. Phys* **2006**, *8*, 3172-3191.
(4) Scott, A. P.; Radom, L. Harmonic Vibrational Frequencies: An Evaluation of Hartree−Fock, Møller−Plesset, Quadratic Configuration Interaction, Density Functional Theory, and Semiempirical Scale Factors. *J. Phys. Chem.* **1996**, *100*, 16502-16513.
(5) Fujimoto, A.; Inuzuka, K. The Lactam - Lactim Tautomerization of Monoamino-Substituted 2-Pyridinols in Tetrahydrofuran. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2292-2299.
(6) Weisstuch, A.; Neidig, P.; Testa, A. C. A Fluorescence Study of Hydroxypyridines. *J. Lumin.* **1975**, *10*, 137-144.
(7) Fujimoto, A.; Inuzuka, K.; Shiba, R. Electronic Properties and Π–Π* Absorption Spectrum of 2-Pyridone. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2802-2806.
(8) Katritzky, A. R.; Rowe, J. D.; Roy, S. K. Potentially Tautomeric Pyridines. Part IX. The Effect of Chlorine Substituents on Pyridone-Hydroxypyridine Tautomerism. *J. Chem. Soc. B* **1967**, 758-761.
(9) Fu, A.; Li, H.; Du, D.; Zhou, Z. Density Functional Study on the Reaction Mechanism of Proton Transfer in 2-Pyridone: Effect of Hydration and Self-Association. *J. Phys. Chem. A* **2005**, *109*, 1468-1477.
(10) Mata, S.; Cortijo, V.; Caminati, W.; Alonso, J. L.; Sanz, M. E.; López, J. C.; Blanco, S. Tautomerism and Microsolvation in 2-Hydroxypyridine/2-Pyridone. *J. Phys. Chem. A* **2010**, *114*, 11393-11398.
(11) Moreno, M.; Miller, W. H. On the Tautomerization Reaction 2-Pyridone ⇌ 2-Hydroxypyridine: An Ab Initio Study. *Chem. Phys. Lett.* **1990**, *171*, 475-479.
(12) Brown, R. S.; Tse, A.; Vederas, J. C. Photoelectron-Determined Core Binding Energies and Predicted Gas-Phase Basicities for the 2-Hydroxypyridine .Dblarw. 2-Pyridone System. *J. Am. Chem. Soc.* **1980**, *102*, 1174-1176.
(13) Barone, V.; Adamo, C. Density Functional Study of Intrinsic and Environmental Effects in the Tautomeric Equilibrium of 2-Pyridone. *J. Phys. Chem.* **1995**, *99*, 15062-15068.