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Kinetic Isotope Effect Provides Insight into the Vibrational Relaxation Mechanism of Aromatic Molecules: Application to Cyano-phenylalanine

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Abstract

Varying the reduced mass of an oscillator via isotopic substitution provides a convenient means to alter its vibrational frequency and hence has found wide applications. Herein, we show that this method can also help delineate the vibrational relaxation mechanism, using four isotopomers of the unnatural amino acid *p*-cyano-phenylalanine (Phe-CN) as models. In water, the nitrile stretching frequencies of these isotopomers, Phe-¹²C¹⁴N (**1**), Phe-¹²C¹⁵N (**2**), Phe-¹³C¹⁴N (**3**), and Phe-¹³C¹⁵N (**4**), are found to be equally separated by $\sim 27\text{ cm}^{-1}$, whereas their vibrational lifetimes are determined to be 4.0 ± 0.2 (**1**), 2.2 ± 0.1 (**2**), 3.4 ± 0.2 (**3**), and 7.9 ± 0.5 ps (**4**), respectively. We find that an empirical relationship that considers the effective reduced mass of CN can accurately account for the observed frequency gaps, while the vibrational lifetime distribution, which suggests an intramolecular relaxation mechanism, can be rationalized by the order-specific density of states near the CN stretching frequency.

Graphical abstract

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Supporting Information

Experimental methods, details of the Gaussian calculation, additional results on density of states, and temperature dependence of the CN stretching vibration of Phe-CN.

Isotopic substitution is a widely used technique in vibrational spectroscopy to 1) identify the nature of a vibrational mode in question, 2) shift the vibrational frequency of interest to a different position, 3) enhance/break vibrational couplings, and 4) provide a site-specific vibrational probe. For example, the strategy of selectively labeling individual amide carbonyl groups, using either $^{13}\text{C}=^{16}\text{O}$ or $^{13}\text{C}=^{18}\text{O}$, has found many novel applications in elucidating the structure, dynamics and functions of proteins.^{1–15} More recently, isotopic substitution has been shown to affect the nature of chemical bonds,¹⁶ indicating that this small perturbation may have more profound effects than previously thought. An important, while much less pursued, application of isotopic labeling is to facilitate the study and understanding of the mechanism of vibrational energy relaxation in the condensed phase.¹⁷ Vibrational energy transfer or redistribution is ubiquitous in chemistry, and thus has been extensively studied. While we now know a great deal about this process, especially in small and isolated molecules in the gas phase¹⁸ and a handful of cases in the condensed phase, such as ion clusters,¹⁹ a comprehensive, predictive, and quantitative understanding of how it occurs is still lacking. Herein, we show, using *p*-cyano-phenylalanine (hereafter referred to as Phe-CN, Figure 1) as an example, that insight into the vibrational relaxation mechanism of the molecule of interest can be obtained by investigating the vibrational relaxation dynamics of a series of isotopomers.

We chose Phe-CN as our model system because 1) its CN stretching vibration is a useful site-specific infrared (IR) probe of proteins,²⁰ 2) Fayer and co-workers have shown that the CN vibrational lifetime of benzonitrile is shorter than that of its naturally abundant ^{13}CN isotopomer,²¹ and 3) it is relatively straightforward to synthesize the four CN isotopomers of Phe-CN,²² wherein the mass of the C (N) atom is either 12 (14) or 13 (15). An additional goal of this study is to identify which one of these CN variants has the longest vibrational lifetime, as in certain applications the vibrational lifetime determines the detection time window of the measurement. For simplicity, the four CN isotopomers are hereafter referred to as **1** (Phe- $^{12}\text{C}^{14}\text{N}$), **2** (Phe- $^{12}\text{C}^{15}\text{N}$), **3** (Phe- $^{13}\text{C}^{14}\text{N}$), and **4** (Phe- $^{13}\text{C}^{15}\text{N}$).

As shown (Figure 1 and Table 1), the CN stretching frequencies of these Phe-CN isotopomers in water were found to be centered at 2236.7 ± 0.5 (**1**), 2210.1 ± 0.5 (**2**), 2183.5 ± 0.5 (**3**), and $2156.1 \pm 0.5 \text{ cm}^{-1}$ (**4**). This presents an interesting trend as it cannot be readily explained by the change of the apparent reduced mass of the CN oscillator (Table 1). For example, the apparent CN reduced mass of **2** is almost identical to that of **3**, yet their vibrational frequencies differ by approximately 27 cm^{-1} . In fact, when the mass of the C or N atom is changed each time, the CN stretching frequency is varied by almost a constant value (i.e., 27 cm^{-1}).

To better understand this trend, we performed normal mode frequency calculations using Gaussian²³ on **1-4**. As shown (Table 1), the calculated CN stretching frequencies accurately capture the frequency variation arising from each isotopic substitution. Upon further inspection, it became clear that the CN stretching vibration is not a pure local mode consisting of displacements of only the C and N atoms, but also involves small displacements of the phenyl carbon atoms, especially the one bound to the nitrile group. To account for this effect, Gaussian employs a weighted reduced mass approach, which includes contributions from all atoms displaced during the CN vibration in the frequency calculations.

Inspired by the approach used by Gaussian, we sought to develop a simple empirical relationship that, in the absence of quantum mechanical calculations, can be used to predict the vibrational frequency change arising from an isotopic substitution. The working hypothesis was that when a diatomic oscillator, A-B, is covalently linked to a third atom (D) to form a linear D-A-B structure, the effective masses of A and B ($m_{e,A}$ and $m_{e,B}$) will be different from their respective atomic masses (i.e., m_A and m_B) and can be calculated using the following equations:

$$m_{e,A} = m_A - \frac{m_A}{m_A + m_D} \cdot \left(\frac{r_1}{r_1} \right)^2 = m_A - \frac{m_A}{m_A + m_D} \quad (1)$$

$$m_{e,B} = m_B + \frac{m_A + m_B}{m_A + m_B + m_D} \cdot \left(\frac{r_1 + r_2}{r_1} \right)^2, \quad (2)$$

where r_1 and r_2 are the equilibrium bond lengths of D-A and A-B bonds. These equations include weighting effects on atoms A and B resulting from their covalent attachment to one another and to atom D. The effective mass of atom A is reduced due to a dampening effect from atom D, and the effective mass of B is increased with a weighting including contributions from both atoms A and B. In addition, we assumed that the frequency of a specific isotopically labeled A-B variant (ν_i) can be predicted from that of the nonlabeled molecule (ν_0) using the harmonic frequency relationship, namely,

$$\nu_i = \nu_0 \sqrt{\frac{\mu_{e,0}}{\mu_{e,i}}}, \quad (3)$$

where $\mu_{e,0}$ and $\mu_{e,i}$ are the corresponding effective reduced masses calculated based on the effective masses of A and B. As indicated (Table 1), this empirical method does an excellent job in predicting the CN stretching frequencies of the other three Phe-CN isotopomers using the experimentally determined frequency of Phe- $^{12}\text{C}^{14}\text{N}$. To further substantiate the validity of the empirical model, we use it to predict the change in the nitrile stretching frequency of SCN^- and CH_3CN upon isotopic substitution. For SCN^- , the nitrile stretching frequency²⁴ and the bond lengths²⁵ are: 2064 cm^{-1} , 1.689 Å (S-C), and 1.149 Å (C-N), respectively. Using these parameters and the aforementioned method, the nitrile stretching frequencies of $\text{S}^{12}\text{C}^{15}\text{N}^-$, $\text{S}^{13}\text{C}^{14}\text{N}^-$, and $\text{S}^{13}\text{C}^{15}\text{N}^-$ are calculated to be 2035, 2017, and 1988 cm^{-1} , which are in excellent agreement with their corresponding experimental values^{24,26} (i.e., 2038, 2015, and 1991 cm^{-1}). As reported by Binev and co-workers,²⁷ the nitrile stretching frequencies of $\text{CH}_3\text{-}^{12}\text{C}^{14}\text{N}$ and $\text{CH}_3\text{-}^{12}\text{C}^{15}\text{N}$ are 2049.0 and 2222.4 cm^{-1} , respectively, in water. In addition, the C-C and C-N bond lengths are 1.458 and 1.157 Å.²⁸ Using the traditional reduced mass equation, the nitrile stretching frequency of $\text{CH}_3\text{-}^{12}\text{C}^{15}\text{N}$ is determined to be 2214 cm^{-1} . However, when using the effective reduced mass method, the frequency is predicted to be 2221 cm^{-1} , resulting in a much closer agreement with the experimental value. In addition, we performed a more stringent test of this method by applying it to calculate the nitrile stretching frequency of SeCN^- , based on that of SCN^- . We chose these molecules because the electronegativity (2.58) of Se is similar to that (2.55) of S and hence, the difference in their nitrile stretching frequencies is expected, based on the current empirical model, to arise largely from the difference in the masses of Se and S. Indeed, the predicted nitrile stretching frequency of SeCN^- , 2075 cm^{-1} , exactly matches the experimental value determined by Zheng and co-workers.²⁹ Thus, taken together, these examples demonstrate the applicability and usefulness of the proposed empirical method.

To explore the effect of the CN reduced mass (μ_{CN}) on the vibrational relaxation dynamics of Phe-CN, we performed IR pump-probe measurements on **1-4** under the magic angle polarization condition with the polarizer correctly placed.³⁰ As shown (Figure 2), the time-resolved spectra clearly reveal the existence of at least four distinguishable spectral features, with one pair (i.e., a negative-going band plus a positive-going band) decaying away in less than 20 ps and the other persisting for more than the longest delay time used (i.e., 60 ps). Based on the signal strength and position, we believe that the short-lived features report on the relaxation dynamics of the vibrationally excited state of the CN oscillator. On the other hand, the long-lived spectral features could arise from either a thermal effect³¹ or a dark state.³² In the case of our samples, the solvent (water) has a high absorbance at the IR pump wavelength. Thus, we tentatively assign this long-lived spectral pair to a thermal effect, i.e., the temperature-induced shift of the CN stretching vibrational band. As shown (Figure S1), this assignment is consistent with the temperature dependence of the CN vibrational mode. To accurately capture the vibrational relaxation dynamics, a representative frequency within the excited state absorption spectrum was chosen, and the corresponding kinetic trace from 0.5 to 20 ps was fit to a single-exponential function with an offset (corresponding to the residual signal of the long-lived thermal component). As shown (Figure 3), the fits in all cases are satisfactory and the resultant time constants indicate that the CN vibrational relaxation dynamics of **1-4** vary with μ_{CN} in a nonmonotonic manner. In particular, the longer vibrational lifetime (7.9 ± 0.5 ps) of **4** (i.e., Phe- $^{13}\text{C}^{15}\text{N}$) makes it more useful in

applications, such as two-dimensional IR measurements, whereby the CN vibration is used to probe the fluctuation dynamics of its environment.

The unusual dependence of the CN vibrational lifetime of Phe-CN on μ_{CN} warrants further exploration. Water can form hydrogen bonds with the CN group, which, in principle, could assist in the direct vibrational energy relaxation to solvent. In the current case, however, the different nitrile vibrational lifetimes cannot be attributed to this (or any other intermolecular vibrational energy transfer) mechanism as the isotopic substitutions are not expected to change the electronic potential of the CN group or the molecule. In other words, these differences most likely result from a change in the intramolecular vibrational relaxation (IVR) rate in response to an isotopic substitution. An indirect, but substantial, piece of evidence that corroborates this notion is that the vibrational lifetime of the nitrile stretching mode of R-SeCN is much longer than that of R-SCN,³³ although their vibrational frequencies only differ by a few wavenumbers.

The mechanism of IVR has been extensively studied in the past, both experimentally and theoretically.^{34–71} A convenient framework to describe vibrational energy flow in a large molecule is to organize the available vibrational states into tiers, wherein the initially prepared vibrational state will transfer its energy, through anharmonic couplings, to states in the first tier, and the subsequent, sequential tier-to-tier energy transfers eventually lead to energy equilibration in the system. For example, using an ultrafast technique combining Raman probe and IR pump, Dlott and co-workers⁷² were able to show that the vibrational relaxation dynamics of liquid benzene following excitation of the C-H (or C-D for benzene-*d*₆) stretching mode occurs in a three-tiered fashion, and that isotopic substitution can have a significant effect on the tier structure. Similarly, the study of Crim and co-workers⁷³ on the vibrational relaxation mechanism of methylene iodide (CH₂I₂) indicated that the IVR process of the fundamental C-H stretching vibration likely includes contributions from two paths: a primary state-specific relaxation pathway involving a few strongly coupled states with low coupling orders, and a minor pathway involving many weakly-coupled states with higher coupling orders. However, when the C-H stretching overtone transitions were excited (3000–9000 cm⁻¹), they showed that states with lower order couplings (less than 8) were primarily responsible for the IVR process.⁷⁴ For a high-frequency oscillator that is covalently attached to an aromatic parent molecule, the mechanism of vibrational energy transfer is potentially even more complicated. In a more recent study involving nitrobenzene and *o*-methylnitrobenzene, Dlott and co-workers⁷⁵ found that the flow of vibrational energy between the NO stretching and phenyl ring modes is unidirectional and dependent on the structure of the ring, proceeding only from NO₂-to-ring in nitrobenzene and only from ring-to-NO₂ in *o*-methylnitrobenzene. A computational study by Leitner and coworkers⁷⁶ indicated that the experimentally determined IVR rates of these systems can be qualitatively explained by considering only low-order anharmonic interactions. Furthermore, in cases where the intramolecular coupling is weak, a change in the apparent reduced mass of a high-frequency oscillator via isotopic substitution is expected to have much less of an effect on its vibrational relaxation rate, as observed for the CO stretching mode of several metalloporphyrin-CO complexes.^{77,78} Thus, in the context of these previous studies and findings, our results are not only interesting, but also present a unique opportunity to yield new insight into the mechanism of vibrational relaxation in complex molecules.

To provide a qualitative but plausible interpretation of the nonmonotonic dependence of the nitrile vibrational lifetime of Phe-CN on μ_{CN} , we performed harmonic frequency calculations using Gaussian on the four isotopomers of the side chain of Phe-CN, using *p*-tolunitrile (hereafter referred to as Tol-CN) as a model. As expected (Table S1), the harmonic frequencies of the 42 normal modes of Tol-CN are distributed in a wide spectral region. Moreover, besides the CN stretching vibration, 10 low-frequency modes also exhibit a significant dependence on μ_{CN} (Figure S2). Those vibrations, the frequencies of which are red-shifted by at least 0.25% from the corresponding Tol- $^{12}\text{C}^{14}\text{N}$ frequencies, are considered to be mechanically coupled to the CN stretching mode and, hence, could play a specific role in assisting its vibrational relaxation. To test this possibility, we calculated, using those specific states for each Tol-CN isotopomer, the number of combination and overtone states that have a frequency located within a 50 cm^{-1} region around the corresponding nitrile stretching frequency. Specifically, we used a recursive computational algorithm to identify and count the combination and overtone states that meet this requirement, wherein the number of vibrational quanta used for the combination is referred to as the coupling order.⁷⁴ For example, when considering a total of 5 states (i.e., $|n_1 n_2 n_3 n_4 n_5\rangle$) and a coupling order of 3, the following combinations will be evaluated: $\{|30000\rangle, |03000\rangle, |00300\rangle, \dots, |10200\rangle, \dots, |00111\rangle\}$. As indicated (Table S2), we found that the numbers thus determined by including only the aforementioned 10 mechanically coupled states for the four Tol-CN isotopomers do not yield a trend, at least for coupling orders of 2–12, that matches that observed for the CN vibrational lifetimes of the Phe-CN isotopomers, suggesting that those mechanically coupled vibrations are not primarily responsible for the relaxation of the CN mode. Results obtained from an additional calculation that includes more mechanically coupled states (i.e., 16) also support this notion (Table S3). Furthermore, we repeated this calculation using those modes that are significantly coupled to the CN stretching vibration, identified via anharmonicity calculation with Gaussian. As shown (Table S4), the results obtained with 9 normal modes of Tol- $^{12}\text{C}^{14}\text{N}$, which all have an anharmonicity coupling constant with the CN stretching mode of greater than 1 cm^{-1} (Table S5), also suggest that the IVR process in the current case is not controlled by a few specific vibrational modes.

Further analysis of the Tol-CN isotopomer combination and overtone states revealed that the order-specific density of states around the CN frequency may be the underlying cause for the observed difference among the vibrational relaxation dynamics of the four Phe-CN isotopomers. Using the 34 normal modes that have a lower energy than that of the CN stretching vibration, we first calculated the total number of states (combination and overtone) with frequencies lying in a $\pm 25\text{ cm}^{-1}$ window around the calculated CN stretching frequency for each isotopomer, and then used this number to determine the density of states in this 50 cm^{-1} region. As indicated (Figure 4 and Table S6), lower order combinations (i.e., those involving 3–5 quanta) exhibit a relatively constant density over the four isotopomers, but increasing the number of quanta used to produce the combination states to 6 and above begins to reveal a distinct trend: the highest density of states for Tol- $^{12}\text{C}^{15}\text{N}$, moderately increased density for Tol- $^{13}\text{C}^{14}\text{N}$, and a decreased density of states for Tol- $^{13}\text{C}^{15}\text{N}$, in comparison to that of Tol- $^{12}\text{C}^{14}\text{N}$. This trend coincides with the nonmonotonic reduced-mass dependence of the CN vibrational lifetime of Phe-CN, suggesting that the vibrational energy initially relaxes through many nearby, weakly coupled combination states rather than

through a few strongly coupled modes. It is worth noting that the low-frequency methyl torsion vibration (ν_1 , 29 cm^{-1}) of Tol-CN, which contributes to the calculated density of states, does not exist in Phe-CN. Thus, to further validate the conclusions reached above, we carried out similar calculations on the four Phe-CN isotopomers. In this case, there are 55 modes that have a lower frequency than that of the CN stretching vibration and are used in the calculation of all possible combination and overtone states for a specific coupling order. As shown (Table S7), the same nonmonotonic trend begins to emerge at a coupling order of 6, thus supporting the above assessment. Despite this agreement, however, we caution that this calculation alone does not definitively rule out the possibility of a tiered vibrational relaxation process. It remains plausible that low-order anharmonic interactions are at least partly implicated in the CN vibrational relaxation process,⁷⁹ and the low accuracy of the low frequency modes generated by Gaussian could lead to significant error in the higher order combination states. For example, a 10% increase in the vibrational frequency of the methyl torsion mode of the Tol-CN isotopomers would result in a trend (Table S8) that is different from that shown in Figure 4. In spite of these potential pitfalls, we believe that the isotopic approach presented here provides a useful strategy to facilitate the study and understanding of one of the most important phenomenon in chemistry, vibrational energy relaxation.

In summary, we assessed the nitrile stretching frequencies and vibrational lifetimes of four CN isotopomers of an unnatural amino acid, Phe-CN, in aqueous solution. The primary goal was to understand how a change in the reduced mass, via isotopic substitution, of a high-frequency oscillator that is covalently attached to an aromatic molecule affects its vibrational frequency and energy relaxation dynamics. Since isotopic substitutions do not alter the electronic potential of the molecule of interest and hence its interactions with the solvent, the resultant changes in the vibrational properties of the oscillator in question can be understood from its interactions with other vibrational degrees of freedom of the system. Interestingly, the nitrile stretching frequencies of the four Phe-CN isotopomers were found to be approximately evenly spaced, with each isotopic substitution decreasing the vibrational frequency by $\sim 27\text{ cm}^{-1}$ in the order of Phe- $^{12}\text{C}^{14}\text{N}$, Phe- $^{12}\text{C}^{15}\text{N}$, Phe- $^{13}\text{C}^{14}\text{N}$, and Phe- $^{13}\text{C}^{15}\text{N}$. This result indicated that due to the covalent connection to a third atom, the reduced mass of the nitrile group, calculated based only on the apparent masses of the constituent atoms, becomes a less accurate predictor of its vibrational frequency. To solve this problem, we proposed an empirical equation to calculate the effective masses of these atoms and hence the effective reduced mass of the oscillator. For both Phe-CN and SCN^- , we found that the effective reduced masses thus calculated can accurately predict the vibrational frequency of CN isotopomers based on the frequency of the respective $^{12}\text{C}^{14}\text{N}$ variant. Furthermore, our results showed that the excited-state decay kinetics of the four Phe-CN isotopomers follow a nonmonotonic trend, with Phe- $^{13}\text{C}^{15}\text{N}$ having the longest vibrational lifetime (i.e., 7.9 ps), which suggests an intramolecular vibrational relaxation mechanism wherein the underlying dynamics are controlled by the density of low-frequency states.

Supplementary Material

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Figure 1. Normalized CN stretching vibrational bands of **1-4**, as indicated. The corresponding peak frequencies and bandwidths are given in Table 1. Shown in the top panel is the structure of Phe-CN.

Figure 2.
Time-resolved spectra in the nitrile stretching band region of **1-4**, as indicated.

Figure 3.

Transient absorption kinetics of **1-4** at representative probing frequencies for the excited state population decay, as indicated. The smooth line in each case corresponds to a fit of the kinetic trace to a single-exponential function with an offset, and the resultant vibrational lifetimes are 4.0 ± 0.2 (**1**), 2.2 ± 0.2 (**2**), 3.4 ± 0.2 (**3**), and 7.9 ± 0.5 ps (**4**), respectively.

Figure 4. Density of states in the nitrile stretching frequency region of the four Tol-CN isotopomers obtained with different coupling orders, as indicated. Also shown are the CN vibrational relaxation rates (open circles) of the corresponding Phe-CN isotopomers (right axis).

Table 1

Molecular and Spectroscopic Properties of the Nitrile Oscillator, Including the Reduced Mass (μ), Effective Reduced Mass (μ_e), Experimentally Determined Peak Frequency (ν) and Bandwidth (FWHM), and Frequencies Obtained from the Empirical Model (ν_M) and Gaussian Calculation (ν_G)

isotopomer	μ (amu)	μ_e (amu)	ν (cm ⁻¹)	fwhm (cm ⁻¹)	ν_M (cm ⁻¹)	ν_G (cm ⁻¹)
Phe- ¹² C ¹⁴ N (1)	6.46	6.73	2236.7	10.8	-	2232.9
Phe- ¹² C ¹⁵ N (2)	6.67	6.90	2210.1	11.6	2209.0	2204.8
Phe- ¹³ C ¹⁴ N (3)	6.74	7.06	2183.5	11.3	2183.9	2179.8
Phe- ¹³ C ¹⁵ N (4)	6.96	7.25	2156.1	9.7	2155.6	2150.8