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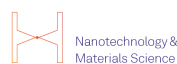
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ABSTRACT

The assignment of the hydrogen bonded O–H stretch vibration in the proline matrix IR spectrum has sparked controversy. Employing constrained nuclear electronic orbital methods, we provide a clear assignment that the vibrational frequency drops to near 3000 cm^{-1} as a result of the interplay between electronic effects, nuclear quantum effects, and matrix effects.

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Hydrogen bonding interactions play a crucial role in a variety of biological systems, impacting critical biological processes such as protein folding and enzyme catalysis.^{1–5} Recently, nuclear quantum effects (NQEs) have been shown to be an important factor that modulates the properties of hydrogen bonds, especially within enzyme active sites.^{6–10} Given that amino acids and short peptides are the fundamental building blocks of proteins, it is essential to develop a comprehensive understanding of the hydrogen bonding interactions in these systems and of the influence of NQEs on these hydrogen bonds. However, the impact of NQEs on the hydrogen bonding of amino acids and short peptides remains largely unexplored, representing an important direction for research.

Among the proteinogenic amino acids, proline asserts a singular influence over protein structure and function due to its unique ring structure.^{11,12} Unlike other amino acids, proline's side chain is covalently bonded to the nitrogen, thereby forming a characteristic pyrrolidine loop.^{11,13} Consequently, proline can disrupt and terminate both alpha helices and beta sheets^{11,12,14–17} as well as form a distinct type of protein secondary structure known as a polyproline helix.^{12,18,19} Furthermore, beyond its biological functions, proline can act as a catalyst in important organic reactions such as the aldol and Mannich reactions.^{20–22}

As with other amino acids, proline can exist in multiple conformers, which can be categorized by their intramolecular hydrogen bonding patterns as shown in Fig. 1.^{13,23–30} Type I features an

N–H···O=C interaction, type II features an H–N···H–O interaction, and type III features an N–H···O–H interaction.^{13,23–30} Types I and III are characterized by hydrogen bonded N–H stretches and free O–H stretches, while type II is characterized by a free N–H stretch and a hydrogen bonded O–H stretch. Typically, type I and type III conformers are highly similar in their vibrational spectra, and we here collectively refer to them as type I/III. The ring structure of proline introduces an additional source of conformational isomerism: the pyrrolidine ring can pucker either up or down with the lower-case letters *a* and *b* referring to the lower and higher energy ring puckers, respectively.¹³ The energy ordering of these conformers can vary depending on the amino acid. For proline, past experimental and theoretical investigations indicate that the type II conformer is the lowest in energy and that the lower-energy ring-pucker conformers dominate under experimental matrix conditions.^{13,31–40}

Amino acid conformers can be investigated with vibrational spectroscopy. Experimentally, gas-phase FT-IR results are available, but these experiments are conducted at high temperatures, which tend to break hydrogen bonds and thus eliminate the distinction between conformers.⁴¹ In contrast, matrix isolation spectroscopy is conducted at cryogenic temperatures within a frozen inert gas matrix.^{42–44} These experimental conditions stabilize different types of conformers, allowing them to be identified through the obtained IR spectra.^{42–44} It has been observed that, in general, the O–H

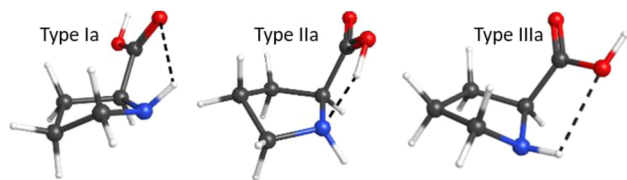


FIG. 1. Three major types of proline conformers with different hydrogen bonding interactions, which are depicted by dashed lines.

stretching frequencies for type I, type II, and type III amino acids are highly consistent when measured under the same matrix conditions.¹³ For instance, in argon matrices at ~ 12 K, the free O–H stretches for type I and type III amino acids appear near 3560 cm^{-1} , and the hydrogen-bonded O–H stretch for type II appears near 3200 cm^{-1} .^{13,25–30,45–59}

However, experimental studies on proline did not yield any identifiable signal near 3200 cm^{-1} ,^{13,60} even though computationally, type IIa proline is shown to be the lowest-energy conformer, and experimentally, two peaks occur in the C=O stretch region, which are believed to correspond to the type Ia/IIIa and type IIa conformers.^{13,60} This raises a perplexing question: where is the proline type IIa hydrogen-bonded O–H vibrational signal in the matrix IR spectrum?

Adamowicz and co-workers originally proposed that this signal appears in the C–H stretching region and is thus obscured by the C–H stretch peaks.¹³ This assignment was based on the experimental difference spectrum between proline (proline- D_0) and doubly deuterated proline (proline- D_2 , with N–H and O–H deuterated), which shows a broad peak centered at 3025 cm^{-1} .¹³ Computationally, density functional theory (DFT)-based harmonic analysis with an empirical scale factor of 0.92 yielded a frequency of 3031 cm^{-1} , which further supported this assignment.¹³ However, pure DFT-based harmonic analysis is unable to capture anharmonicity and NQEs and is of questionable reliability when using empirical scale factors to account for these effects.

Recently, using the adiabatically switched semiclassical initial value representation (AS-SCIVR),^{39,61–63} a new assignment has been proposed, suggesting that the type IIa hydrogen bonded O–H stretch is at 3329 cm^{-1} ,³⁹ near the previously assigned N–H stretching region and significantly outside the C–H stretching region.^{13,60} However, this new peak assignment is also not fully convincing, as the experimental spectrum shows only a weak and broad N–H

stretch signal in the $3300\text{--}3400\text{ cm}^{-1}$ region,^{13,60} and it would be unusual if this weak signal could correspond to both O–H and N–H stretches.

In this work, we will identify this missing and disputed type IIa hydrogen bonded O–H stretch signal in the matrix IR spectrum of proline through constrained nuclear–electronic orbital (CNEO)^{64–66} calculations. The CNEO framework, which has recently been developed in our group, can incorporate NQEs, especially quantum nuclear delocalization effects, in quantum chemistry calculations and molecular dynamics simulations through a quantum-corrected effective potential energy surface.^{67,68} This framework has been shown to be remarkably successful for hydrogen-related vibrations.^{69–73} Herein, our study will provide strong evidence supporting the original assignment of the type IIa hydrogen bonded O–H stretch as appearing within the C–H stretching region as a result of the interplay between electronic effects, NQEs, and matrix effects.

The hydrogen bonded O–H vibrational frequencies for the type IIa proline conformer are provided in Table I. The disputed experimental result is 3025 cm^{-1} , which is the maximum position of a wide and relatively faint peak in the difference spectrum of proline- D_0 and proline- D_2 . Without NQEs, unscaled DFT/B3LYP harmonic analysis predicts the frequency to be 3359 cm^{-1} , which is about 300 cm^{-1} higher than the experimental assignment. When the empirical scale factor of 0.92 as used by Adamowicz and co-workers is applied,¹³ the frequency drops sharply to 3090 cm^{-1} . However, the reliability of empirical scale factors can be questionable, as they often need to vary with vibrational modes as well as electronic structure methods. With AS-SCIVR, this hydrogen bonded O–H vibrational frequency was reported to be 3329 cm^{-1} ,³⁹ which is similar to the unscaled DFT harmonic result and does not support the experimental assignments.

Using the same B3LYP functional^{74–76} as selected in previous studies,^{13,34,39,62} we performed both VPT2^{77,78} calculations and CNEO-DFT harmonic analysis. The predicted frequencies of the type IIa hydrogen-bonded O–H stretch are 3069 and 3021 cm^{-1} for VPT2 and CNEO-DFT, respectively. These predictions align with the original experimental difference spectrum assignment and scaled DFT harmonic analysis by Adamowicz and co-workers.¹³ We note that our current study and the original study by Adamowicz did not include dispersion corrections, whereas the AS-SCIVR study used D3 corrections. One might expect that dispersion corrections are needed as hydrogen bonds are not real chemical bonds. However, the necessity of dispersion corrections for hydrogen-bonded systems is less clear-cut compared to other weak interaction systems,

TABLE I. O–H stretching frequencies (cm^{-1}) in type IIa proline.

Experiment ^a	CNEO ^b			AS-SCIVR ^c	Conventional DFT ^b		
	DFT	MD	VPT2 ^b		Unscaled	Scaled ^d	AIMD
3025 (disputed)	3021	3036	3069	3329	3359	3090	3382

^aExperimental value from difference spectrum in Ref. 13.

^bValues obtained in this work with B3LYP/aug-cc-pVDZ.

^cAS-SCIVR value from Ref. 39, obtained with B3LYP-D3/aug-cc-pVDZ.

^dValue from this work with a scale factor of 0.92 from Ref. 13 applied.

since the nature of hydrogen bonding is more electrostatic than dispersion.⁷⁹ To further support this treatment, we provide the DFT harmonic results and VPT2 results with the D3(0)⁸⁰ and D3(BJ)⁸¹ corrections, along with the corresponding DFT and VPT2 values without the corrections, in Table S3. This analysis shows that essentially the dispersion correction can only change the frequencies by at most ~ 30 cm^{-1} , which can be neglected compared to the peak assignment dispute of around 200 cm^{-1} .

According to previous studies by our group on hydrogen-related vibrations, CNEO-DFT harmonic analysis can reduce errors by 90% compared to conventional unscaled DFT harmonic analysis.^{66,69–71} Furthermore, although VPT2 may face challenges with strongly hydrogen-bonded systems or shared-proton systems,^{70,82} the hydrogen bond in type IIa proline is comparatively weaker, with its expected vibrational frequency being above 3000 cm^{-1} . Therefore, VPT2 is reliable for this system. The agreement between the CNEO-DFT harmonic frequencies and the corresponding VPT2 values indicates that this assignment is robust. However, to achieve even more accurate results, we additionally performed CNEO-MD simulations. Compared to CNEO harmonic analysis, CNEO-MD can better capture mode coupling effects through thermal motions at finite temperatures and therefore

yield more accurate peak positions and qualitatively correct peak broadenings,^{69–71} however, we acknowledge that the descriptions of overtones, combination bands, and Fermi resonances are still challenging and that high-level quantum methods, such as MCTDH and VSCF/VCI, are still needed for accurate descriptions of these subtle features.^{70,71} The improved performance of CNEO-MD over CNEO-DFT harmonic analysis is usually more significant for hydrogen-bonded O–H stretch modes, which often couple strongly with some low-frequency vibrations, than it is for free O–H stretches, which have relatively weak couplings to other modes.^{70,71}

The CNEO-MD simulated IR spectra for both un-deuterated proline- D_0 and doubly deuterated proline- D_2 are shown in Figs. 2(a) and 2(b), respectively. For each species, we performed MD simulations for the lowest energy type IIa conformer as well as for the type Ia and type IIIa conformers. The Fourier transform is applied to the dipole autocorrelation functions of the MD trajectories to obtain their respective IR spectra. To compare with the experimental spectra, which are believed to be a mixture of mainly type Ia and type IIa conformers, we also created mixed spectra for both proline- D_0 and proline- D_2 with a 50%–50% mixing ratio. In principle, if equilibrium is reached, the mixing ratio should follow a Boltzmann distribution. However, since the experiment involved first sublimating the

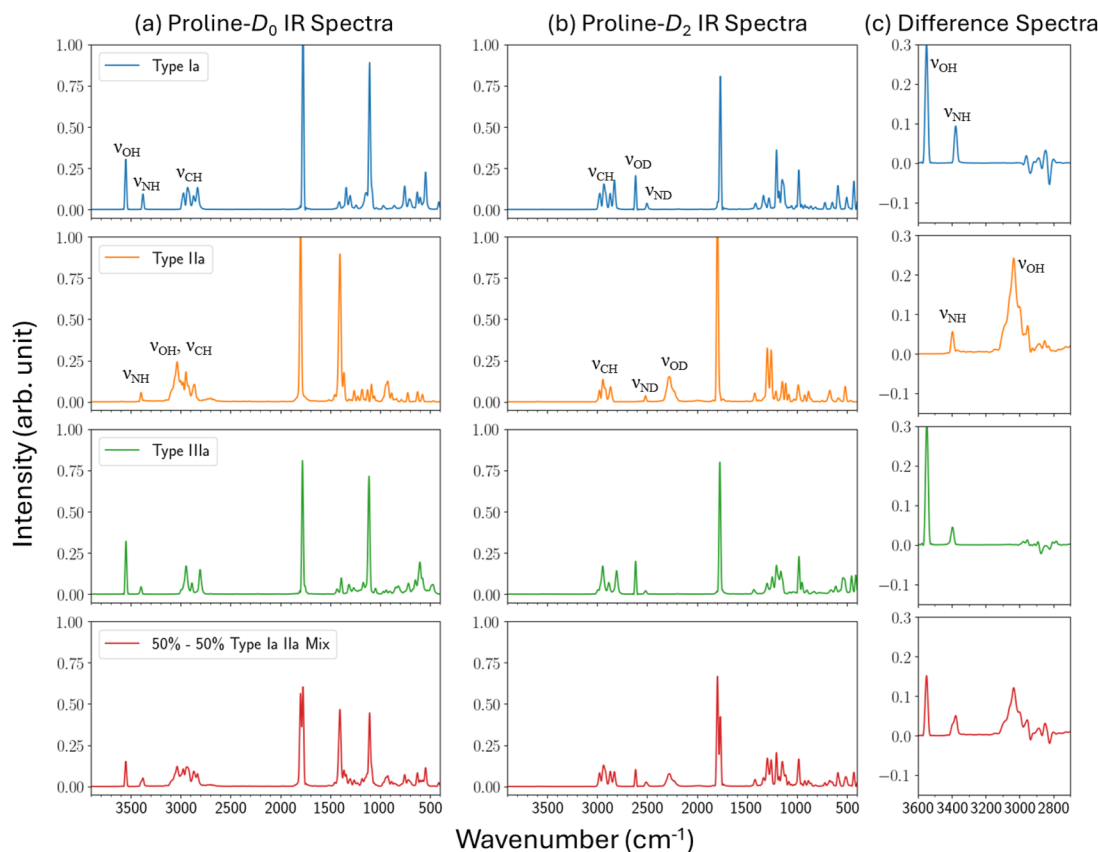


FIG. 2. Simulated IR spectra by CNEO-MD-B3LYP for different types of conformers of (a) un-deuterated proline- D_0 and (b) doubly deuterated proline- D_2 . (c) Difference between (a) and (b) in the high frequency 2600 – 3600 cm^{-1} region.

amino acid—potentially breaking hydrogen bonds and allowing bond rotation—and then cooling it down to a matrix environment, the relative ratio of different conformers formed and stabilized during the cooling process is uncertain.⁸³ For qualitative investigation, we simply chose a 50% type Ia and 50% type IIa mixing ratio. Our peak assignments for the type Ia and type IIa conformers are shown in the simulated spectra presented in Fig. 2.

For both the type Ia and type IIIa conformers, the un-deuterated proline-*D*₀ and doubly deuterated proline-*D*₂ CNEO-MD IR spectra show great resemblance above the fingerprint region, except for the strong O–H stretch and weak N–H stretch peaks, which shift to lower frequencies upon deuteration. In contrast, for the type IIa conformer, while the weak N–H(D) stretch remains clear in both the un-deuterated and deuterated species, the O–H stretch does not display a distinct single peak in the un-deuterated species. Fortunately, the O–D stretch in proline-*D*₂ is distinct with a peak lower in frequency than the N–D stretch. This peak serves as evidence that in the un-deuterated species, the type IIa hydrogen bonded O–H stretch frequency is also very likely lower than that for the N–H stretch and thus likely appears close to the C–H stretch region around 3000 cm⁻¹.

Upon further examination of the C–H stretch region in the CNEO-MD spectra, we can clearly observe differences between proline-*D*₀ and proline-*D*₂ for the type IIa conformer. The progression of bands around 3000 cm⁻¹ is distinctly broader and stronger in the proline-*D*₀ spectrum than that in the proline-*D*₂ spectrum. In contrast, for the type Ia and type IIIa conformers, the two species have essentially the same line shape and intensities for both isotopes in the C–H stretch region. These observations suggest that the type IIa hydrogen-bonded O–H stretch overlaps with the C–H stretch region.

This assignment is further supported by the simulated CNEO-MD difference spectra shown in Fig. 2(c). For each conformer, the difference spectrum was obtained by subtracting that conformer's CNEO-MD calculated proline-*D*₀ spectrum from the corresponding proline-*D*₂ spectrum. Because the OH, NH, and CH stretch modes are of the main interest, we show the difference spectra focused in on the 2700–3600 cm⁻¹ range. For the type IIa difference spectrum, a clear and broadened peak is observed with a maximum intensity at 3036 cm⁻¹, whereas the difference in this same region is essentially negligible for the type Ia and type IIIa conformers.

Quantitatively, the simulated difference spectra for both the type IIa conformer and the 1:1 type Ia–IIa conformer mixture show a narrower broadening compared to the experimental result, with a peak broadening of about 200 cm⁻¹ in our simulations vs about 400 cm⁻¹ in the experiment. Several factors could contribute to this difference. One is that the choice of the electronic functional can affect the peak position as well as the mode couplings. Another factor is that these simulations are performed in the pure gas phase, which neglects the matrix environment that might also couple with the molecular modes and further broaden the spectrum. Both factors will be discussed in more detail in the following paragraphs. Nonetheless, the qualitative similarity in peak broadening and the consistent peak centering near the experimental value of 3025 cm⁻¹ strongly support the conclusion that the type IIa hydrogen-bonded O–H stretch overlaps with the C–H stretch region.

Furthermore, we performed conventional DFT-based AIMD simulations as a comparison. The results are presented in Fig. S1. AIMD gives a peak value of 3382 cm⁻¹ for the type IIa hydrogen bonded O–H stretch, which is more than 300 cm⁻¹ higher than the CNEO-MD result and is significantly separated from the C–H stretch region. This large difference between the CNEO-MD and AIMD results demonstrates again the crucial role of quantum nuclear delocalization during MD simulations of hydrogen bonding systems.

In addition to nuclear quantum effects and mode coupling effects, matrix effects in experiments and the electronic structure method used in computations influence the alignment between experimental spectra and theoretical predictions. In experiments, the molecule is frozen within an inert gas matrix, which typically weakens the bonds and causes a redshift in the corresponding vibrational frequencies.^{84,85} This effect is particularly significant for hydrogen-bonded O–H stretches.⁸⁴ Previous studies of glycine, the simplest amino acid, have shown that different inert gas matrices can produce varying redshifts due to the varying coupling strength between the matrix gas and the amino acid.⁴⁷ In particular, it was found that the argon (Ar) matrix can cause a redshift of nearly 100 cm⁻¹ for the hydrogen bonded O–H stretch in the type II glycine conformer.⁴⁷ Given the stronger basicity of the secondary amine in proline compared to the primary amine in glycine, we may anticipate a similar, if not greater, redshift for proline. Therefore, if a low temperature gas phase spectrum of proline were to be obtained via advanced spectroscopic techniques such as isotopomer-selective photofragmentation vibrational spectroscopy,^{86,87} we expect that the hydrogen-bonded O–H stretch frequency would be around 100 cm⁻¹ higher than the current matrix spectrum due to the absence of matrix effects. This would result in a clearer peak separation of the hydrogen bonded O–H stretch from the C–H stretch region.

For the electronic structure method, although the B3LYP functional is used in this study as well as in previous studies, it is in fact not the most accurate electronic functional. To assess the error introduced by the electronic structure method, we performed harmonic analysis using coupled cluster singles and doubles (CCSD),⁸⁸ with the results shown in Table S1. Although harmonic analysis with CCSD does not account for NQEs, it can serve as a relatively accurate reference to assess errors in the electronic functional. Compared to the CCSD harmonic results, pure DFT-B3LYP harmonic analysis underestimates the hydrogen-bonded O–H stretch in type IIa by 156 cm⁻¹, indicating that the B3LYP functional significantly underestimates the vibrational frequency. Therefore, although CNEO-CCSD is not yet developed, we anticipate that its predicted hydrogen bonded O–H vibration for type IIa proline would be around 150 cm⁻¹ higher than the result predicted by CNEO-B3LYP, resulting in a separation from the C–H stretch region. In addition to B3LYP, we performed harmonic analysis and MD simulations with the PBE0^{89–91} and M06-2X⁹² functionals (Table S2 and Figs. S2–S5). For PBE0, the overall performance is highly similar to B3LYP, with underestimated vibrational frequencies relative to CCSD. A major difference is that CNEO-MD with PBE0 gives an even broader peak than that with B3LYP, which aligns better with the matrix experimental result. In contrast, M06-2X suffers less from functional error and predicts vibrational frequencies similar to CCSD. As a result,

CNEO-MD simulated spectra with M06-2X see a distinct peak separation between the hydrogen-bonded O–H stretch and the C–H stretches.

Interestingly, the 100 cm^{-1} or more redshift caused by experimental matrix effects and the 150 cm^{-1} functional underestimation relative to CCSD due to the choice of the B3LYP functional play nearly the same role of shifting the hydrogen bonded O–H stretch into the C–H stretch region, leading to a fortuitous match between the matrix IR experiment spectrum and the simulated CNEO-B3LYP-MD spectrum. If pure gas phase IR spectra of proline are recorded, without matrix effects, we estimate that the hydrogen bonded O–H stretch will better separate from the C–H stretch region as predicted by CNEO-MD using the more accurate M06-2X functional.

Given that the proline type IIa hydrogen bonded O–H stretch has been unequivocally assigned, now the remaining question is: why is this frequency significantly redshifted to around 3000 cm^{-1} compared to the 3200 cm^{-1} results observed for other amino acids? A reasonable explanation was provided by Adamowicz *et al.* in their original paper: because secondary amines are more nucleophilic than primary amines, the corresponding $\text{N}\cdots\text{H}\cdots\text{O}$ hydrogen bond in proline will be stronger than those in other amino acids. This stronger hydrogen bond weakens the O–H bond more and thus leads to a lower O–H vibrational frequency.

We further support this explanation by comparing the optimized geometries of type IIa proline and type II glycine. With CNEO-DFT, the O–H and $\text{N}\cdots\text{H}$ distances are 1.031 and 1.711 Å for proline and 0.986 and 1.924 Å for glycine, respectively. The longer O–H distance and shorter $\text{N}\cdots\text{H}$ distance in proline indicate a stronger hydrogen bond compared to glycine. Similar results are also observed with conventional DFT-optimized geometries (0.991 Å proline O–H, 1.868 Å proline $\text{N}\cdots\text{H}$, 0.986 Å glycine O–H, and 1.924 Å glycine $\text{N}\cdots\text{H}$), leading to consistent conclusions.

In summary, by employing CNEO methods to account for NQEs in proline, we have unequivocally assigned the type IIa proline hydrogen-bonded O–H stretch to a broad peak overlapping with the C–H stretch region near 3000 cm^{-1} . CNEO-DFT harmonic analysis qualitatively supports this assignment, and the CNEO-MD difference spectrum result further confirms the broad O–H stretching peak observed experimentally with the predicted central peak position in good alignment with the experimental difference spectrum. We find that experimental matrix effects and electronic errors in the employment of the B3LYP functional in computations both lead to a redshift of 100 cm^{-1} or more for the type IIa hydrogen bonded O–H stretch peak position, contributing to the overlap with the C–H stretch region and thereby aligning computational predictions with experimental results. The unique secondary amine group in proline, with its high nucleophilicity, accounts for the stronger hydrogen bond and weakened O–H bond in its type IIa conformer, which is the primary reason for the significantly redshifted peak position compared to other amino acids.

The [supplementary material](#) includes computational details and example input files, functional benchmark tests, complete O–H stretch vibrational frequencies and vibrational spectra by different methods, and effects of dispersion corrections.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

James Langford: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (supporting); Visualization (supporting); Writing – original draft (equal). **Yuzhe Zhang:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (equal); Visualization (supporting); Writing – review & editing (supporting). **Zehua Chen:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (equal); Supervision (supporting); Writing – review & editing (supporting). **Yang Yang:** Conceptualization (lead); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Supervision (lead); Visualization (lead); Writing – original draft (equal); Writing – review & editing (lead).

DATA AVAILABILITY

The data that support the findings of this study are available within the article and its [supplementary material](#). More detailed data are available from the corresponding author upon reasonable request.

REFERENCES

- G. J. Narlikar and D. Herschlag, “Mechanistic aspects of enzymatic catalysis: Lessons from comparison of RNA and protein enzymes,” *Annu. Rev. Biochem.* **66**(1), 19–59 (1997).
- T. C. Bruice and S. J. Benkovic, “Chemical basis for enzyme catalysis,” *Biochemistry* **39**(21), 6267–6274 (2000).
- S. J. Benkovic and S. Hammes-Schiffer, “A perspective on enzyme catalysis,” *Science* **301**(5637), 1196–1202 (2003).
- R. E. Hubbard and M. Kamran Haider, “Hydrogen bonds in proteins: Role and strength,” in *eLS* (John Wiley & Sons, Ltd., 2010).
- D. Herschlag and M. M. Pinney, “Hydrogen bonds: Simple after all?,” *Biochemistry* **57**(24), 3338–3352 (2018).
- J.-K. Hwang and A. Warshel, “How important are quantum mechanical nuclear motions in enzyme catalysis?,” *J. Am. Chem. Soc.* **118**(47), 11745–11751 (1996).
- J. Pu, J. Gao, and D. G. Truhlar, “Multidimensional tunneling, recrossing, and the transmission coefficient for enzymatic reactions,” *Chem. Rev.* **106**(8), 3140–3169 (2006).
- L. Wang, S. D. Fried, S. G. Boxer, and T. E. Markland, “Quantum delocalization of protons in the hydrogen-bond network of an enzyme active site,” *Proc. Natl. Acad. Sci. U. S. A.* **111**(52), 18454–18459 (2014).

- ⁹A. Vardi-Kilshain, N. Nitoker, and D. T. Major, "Nuclear quantum effects and kinetic isotope effects in enzyme reactions," *Arch. Biochem. Biophys.* **582**, 18–27 (2015).
- ¹⁰T. E. Markland and M. Ceriotti, "Nuclear quantum effects enter the mainstream," *Nat. Rev. Chem.* **2**(3), 0109 (2018).
- ¹¹V. Madison, "Flexibility of the pyrrolidine ring in proline peptides," *Biopolymers* **16**(12), 2671–2692 (1977).
- ¹²A. A. Morgan and E. Rubenstein, "Proline: The distribution, frequency, positioning, and common functional roles of proline and polyproline sequences in the human proteome," *PLoS One* **8**(1), e53785 (2013).
- ¹³S. G. Stepanian, I. D. Reva, E. D. Radchenko, and L. Adamowicz, "Conformers of nonionized proline. Matrix-isolation infrared and post-Hartree–Fock *ab initio* study," *J. Phys. Chem. A* **105**(47), 10664–10672 (2001).
- ¹⁴L. Piela, G. Némethy, and H. A. Scheraga, "Proline-induced constraints in α -helices," *Biopolymers* **26**(9), 1587–1600 (1987).
- ¹⁵R. Sankaramakrishnan and S. Vishveshwara, "Conformational studies on peptides with proline in the right-handed α -helical region," *Biopolymers* **30**(3–4), 287–298 (1990).
- ¹⁶S. C. Li, N. K. Goto, K. A. Williams, and C. M. Deber, "Alpha-helical, but not beta-sheet, propensity of proline is determined by peptide environment," *Proc. Natl. Acad. Sci. U. S. A.* **93**(13), 6676–6681 (1996).
- ¹⁷C. M. Deber and A. G. Therien, "Putting the β -breaks on membrane protein misfolding," *Nat. Struct. Biol.* **9**(5), 318–319 (2002).
- ¹⁸A. A. Adzhubei and M. J. E. Sternberg, "Left-handed polyproline II helices commonly occur in globular proteins," *J. Mol. Biol.* **229**(2), 472–493 (1993).
- ¹⁹A. A. Adzhubei, M. J. E. Sternberg, and A. A. Makarov, "Polyproline-II helix in proteins: Structure and function," *J. Mol. Biol.* **425**(12), 2100–2132 (2013).
- ²⁰S. Bahmanyar and K. N. Houk, "Origins of opposite absolute stereoselectivities in proline-catalyzed direct mannich and aldol reactions," *Org. Lett.* **5**(8), 1249–1251 (2003).
- ²¹S. K. Panday, "Advances in the chemistry of proline and its derivatives: An excellent amino acid with versatile applications in asymmetric synthesis," *Tetrahedron: Asymmetry* **22**(20–22), 1817–1847 (2011).
- ²²R. B. Sunoj, "Proline-derived organocatalysis and synergism between theory and experiments," *WIREs Comput. Mol. Sci.* **1**(6), 920–931 (2011).
- ²³R. D. Suenram and F. J. Lovas, "Millimeter wave spectrum of glycine. A new conformer," *J. Am. Chem. Soc.* **102**(24), 7180–7184 (1980).
- ²⁴C. H. Hu, M. Shen, and H. F. I. Schaefer, "Glycine conformational analysis," *J. Am. Chem. Soc.* **115**(7), 2923–2929 (1993).
- ²⁵A. Y. Ivanov, G. Sheina, and Y. P. Blagoi, "FTIR spectroscopic study of the UV-induced rotamerization of glycine in the low temperature matrices (Kr, Ar, Ne)," *Spectrochim. Acta, Part A* **55**(1), 219–228 (1998).
- ²⁶S. G. Stepanian, I. D. Reva, E. D. Radchenko, M. T. S. Rosado, M. L. T. S. Duarte, R. Fausto, and L. Adamowicz, "Matrix-isolation infrared and theoretical studies of the glycine conformers," *J. Phys. Chem. A* **102**(6), 1041–1054 (1998).
- ²⁷S. G. Stepanian, I. D. Reva, E. D. Radchenko, and L. Adamowicz, "Conformational behavior of α -alanine. Matrix-isolation infrared and theoretical DFT and *ab initio* study," *J. Phys. Chem. A* **102**(24), 4623–4629 (1998).
- ²⁸S. G. Stepanian, I. D. Reva, E. D. Radchenko, and L. Adamowicz, "Combined matrix-isolation infrared and theoretical DFT and *ab initio* study of the nonionized valine conformers," *J. Phys. Chem. A* **103**(22), 4404–4412 (1999).
- ²⁹G. Bazsó, E. E. Najbauer, G. Magyarfalvi, and G. Tarczay, "Near-infrared laser induced conformational change of alanine in low-temperature matrices and the tunneling lifetime of its conformer VI," *J. Phys. Chem. A* **117**(9), 1952–1962 (2013).
- ³⁰S. G. Stepanian, A. Y. Ivanov, and L. Adamowicz, "Conformational composition of neutral leucine. Matrix isolation infrared and *ab initio* study," *Chem. Phys.* **423**, 20–29 (2013).
- ³¹M. Ramek, A.-M. Kelterer, and S. Nikolić, "*Ab initio* and molecular mechanics conformational analysis of neutral L-proline," *Int. J. Quantum Chem.* **65**(6), 1033–1045 (1997).
- ³²A. G. Császár and A. Perczel, "*Ab initio* characterization of building units in peptides and proteins," *Prog. Biophys. Mol. Biol.* **71**(2), 243–309 (1999).
- ³³A. Lesarri, S. Mata, E. J. Cocinero, S. Blanco, J. C. López, and J. L. Alonso, "The Structure of neutral proline," *Angew. Chem., Int. Ed.* **41**(24), 4673–4676 (2002).
- ³⁴E. Czinki and A. G. Császár, "Conformers of gaseous proline," *Chem. - Eur. J.* **9**(4), 1008–1019 (2003).
- ³⁵W. D. Allen, E. Czinki, and A. G. Császár, "Molecular structure of proline," *Chem. - Eur. J.* **10**(18), 4512–4517 (2004).
- ³⁶S. Mata, V. Vaquero, C. Cabezas, I. Peña, C. Pérez, J. C. López, and J. L. Alonso, "Observation of two new conformers of neutral proline," *Phys. Chem. Chem. Phys.* **11**(21), 4141–4144 (2009).
- ³⁷F. Fathi and H. Farrokhpour, "Valence ionization of L-proline amino acid: Experimental and theoretical study," *Chem. Phys. Lett.* **565**, 102–107 (2013).
- ³⁸R. Hadidi, L. Nahon, D. K. Božanić, H. Ganjtabar, G. A. Garcia, and I. Powis, "Conformer-dependent vacuum ultraviolet photodynamics and chiral asymmetries in pure enantiomers of gas phase proline," *Commun. Chem.* **4**(1), 72 (2021).
- ³⁹G. Botti, C. Aieta, and R. Conte, "The complex vibrational spectrum of proline explained through the adiabatically switched semiclassical initial value representation," *J. Chem. Phys.* **156**(16), 164303 (2022).
- ⁴⁰Y. Yang and Z. Lin, "Extensive exploration of the conformational landscapes of neutral and terminally blocked prolines in the gas phase: A density functional theory study," *J. Chem. Res.* **46**(4), 174751982211104 (2022).
- ⁴¹R. Linder, M. Nispel, T. Häber, and K. Kleinermanns, "Gas-phase FT-IR-spectra of natural amino acids," *Chem. Phys. Lett.* **409**(4–6), 260–264 (2005).
- ⁴²T. Bally, "Matrix isolation," in *Reactive Intermediate Chemistry*, edited by R. A. Moss, M. S. Platz, and M. Jones, Jr. (John Wiley & Sons, Ltd., 2003), pp. 795–845.
- ⁴³P. Dubey, J. Saini, K. Verma, G. Karir, A. Mukhopadhyay, and K. S. Viswanathan, "Chapter 14 - Matrix isolation spectroscopy—A window to molecular processes," in *Molecular and Laser Spectroscopy*, edited by V. P. Gupta (Elsevier, 2018), pp. 317–340.
- ⁴⁴D. F. Dinu, M. Podewitz, H. Grothe, T. Loerting, and K. R. Liedl, "On the synergy of matrix-isolation infrared spectroscopy and vibrational configuration interaction computations," *Theor. Chem. Acc.* **139**(12), 174 (2020).
- ⁴⁵Y. Grenie, J. Lassegues, and C. Garrigou-Lagrange, "Infrared spectrum of matrix-isolated glycine," *J. Chem. Phys.* **53**(7), 2980–2982 (1970).
- ⁴⁶Y. Grenie and C. Garrigou-Lagrange, "Infrared spectra of glycine isotopic species isolated in an argon or nitrogen matrix," *J. Mol. Spectrosc.* **41**(2), 240–248 (1972).
- ⁴⁷F. Huisken, O. Werhahn, A. Yu. Ivanov, and S. A. Krasnokutski, "The O–H stretching vibrations of glycine trapped in rare gas matrices and helium clusters," *J. Chem. Phys.* **111**(7), 2978–2984 (1999).
- ⁴⁸B. Lambie, R. Ramaekers, and G. Maes, "On the contribution of intramolecular H-bonding entropy to the conformational stability of alanine conformations," *Spectrochim. Acta, Part A* **59**(6), 1387–1397 (2003).
- ⁴⁹B. Lambie, R. Ramaekers, and G. Maes, "Conformational behavior of serine: An experimental matrix-isolation FT-IR and theoretical DFT(B3LYP)/6-31++G** study," *J. Phys. Chem. A* **108**(47), 10426–10433 (2004).
- ⁵⁰R. Ramaekers, J. Pajak, B. Lambie, and G. Maes, "Neutral and zwitterionic glycine.H₂O complexes: A theoretical and matrix-isolation Fourier transform infrared study," *J. Chem. Phys.* **120**(9), 4182–4193 (2004).
- ⁵¹R. Ramaekers, J. Pajak, M. Rospenk, and G. Maes, "Matrix-isolation FT-IR spectroscopic study and theoretical DFT(B3LYP)/6-31++G** calculations of the vibrational and conformational properties of tyrosine," *Spectrochim. Acta, Part A* **61**(7), 1347–1356 (2005).
- ⁵²S. Jarmelo, L. Lapinski, M. J. Nowak, P. R. Carey, and R. Fausto, "Preferred conformers and photochemical ($\lambda > 200$ nm) reactivity of serine and 3,3-dideutero-serine in the neutral form," *J. Phys. Chem. A* **109**(25), 5689–5707 (2005).
- ⁵³A. Kaczor, I. D. Reva, L. M. Proniewicz, and R. Fausto, "Importance of entropy in the conformational equilibrium of phenylalanine: A matrix-isolation infrared spectroscopy and density functional theory study," *J. Phys. Chem. A* **110**(7), 2360–2370 (2006).
- ⁵⁴J. C. Dobrowolski, M. H. Jamróz, R. Kołos, J. E. Rode, and J. Sadlej, "Theoretical prediction and the first IR matrix observation of several L-cysteine molecule conformers," *ChemPhysChem* **8**(7), 1085–1094 (2007).
- ⁵⁵C. Espinoza, J. Szczepanski, M. Vala, and N. C. Polfer, "Glycine and its hydrated complexes: A matrix isolation infrared study," *J. Phys. Chem. A* **114**(18), 5919–5927 (2010).

- ⁵⁶G. Bazsó, G. Magyarfalvi, and G. Tarczay, "Tunneling lifetime of the *ttc*/VIp conformer of glycine in low-temperature matrices," *J. Phys. Chem. A* **116**(43), 10539–10547 (2012).
- ⁵⁷B. Boeckx, W. Nelissen, and G. Maes, "Potential energy surface and matrix isolation FT-IR study of isoleucine," *J. Phys. Chem. A* **116**(12), 3247–3258 (2012).
- ⁵⁸B. Boeckx and G. Maes, "The conformational behavior and H-bond structure of asparagine: A theoretical and experimental matrix-isolation FT-IR study," *Biophys. Chem.* **165–166**, 62–73 (2012).
- ⁵⁹E. E. Najbauer, G. Bazsó, R. Apóstolo, R. Fausto, M. Biczysko, V. Barone, and G. Tarczay, "Identification of serine conformers by matrix-isolation IR spectroscopy aided by near-infrared laser-induced conformational change, 2D correlation analysis, and quantum mechanical anharmonic computations," *J. Phys. Chem. B* **119**(33), 10496–10510 (2015).
- ⁶⁰I. D. Reva, S. G. Stepanian, A. M. Plokhotnichenko, E. D. Radchenko, G. G. Sheina, and Y. P. Blagoi, "Infrared matrix isolation studies of amino acids. Molecular structure of proline," *J. Mol. Struct.* **318**, 1–13 (1994).
- ⁶¹R. Conte, L. Parma, C. Aieta, A. Rognoni, and M. Ceotto, "Improved semiclassical dynamics through adiabatic switching trajectory sampling," *J. Chem. Phys.* **151**(21), 214107 (2019).
- ⁶²G. Botti, M. Ceotto, and R. Conte, "On-the-fly adiabatically switched semiclassical initial value representation molecular dynamics for vibrational spectroscopy of biomolecules," *J. Chem. Phys.* **155**(23), 234102 (2021).
- ⁶³R. Conte and M. Ceotto, "Semiclassical molecular dynamics for spectroscopic calculations," in *Quantum Chemistry and Dynamics of Excited States* (John Wiley & Sons, Ltd., 2020), pp. 595–628.
- ⁶⁴X. Xu and Y. Yang, "Full-quantum descriptions of molecular systems from constrained nuclear–electronic orbital density functional theory," *J. Chem. Phys.* **153**(7), 074106 (2020).
- ⁶⁵X. Xu and Y. Yang, "Constrained nuclear–electronic orbital density functional theory: Energy surfaces with nuclear quantum effects," *J. Chem. Phys.* **152**(8), 084107 (2020).
- ⁶⁶X. Xu and Y. Yang, "Molecular vibrational frequencies from analytic hessian of constrained nuclear–electronic orbital density functional theory," *J. Chem. Phys.* **154**(24), 244110 (2021).
- ⁶⁷Z. Chen and Y. Yang, "Incorporating nuclear quantum effects in molecular dynamics with a constrained minimized energy surface," *J. Phys. Chem. Lett.* **14**(1), 279–286 (2023).
- ⁶⁸Y. Wang, Z. Chen, and Y. Yang, "Calculating vibrational excited state absorptions with excited state constrained minimized energy surfaces," *J. Phys. Chem. A* **127**(25), 5491–5501 (2023).
- ⁶⁹X. Xu, Z. Chen, and Y. Yang, "Molecular dynamics with constrained nuclear electronic orbital density functional theory: Accurate vibrational spectra from efficient incorporation of nuclear quantum effects," *J. Am. Chem. Soc.* **144**(9), 4039–4046 (2022).
- ⁷⁰Y. Zhang, X. Xu, N. Yang, Z. Chen, and Y. Yang, "Describing proton transfer modes in shared proton systems with constrained nuclear–electronic orbital methods," *J. Chem. Phys.* **158**(23), 231101 (2023).
- ⁷¹Y. Zhang, Y. Wang, X. Xu, Z. Chen, and Y. Yang, "Vibrational spectra of highly anharmonic water clusters: Molecular dynamics and harmonic analysis revisited with constrained nuclear–electronic orbital methods," *J. Chem. Theory Comput.* **19**(24), 9358–9368 (2023).
- ⁷²X. Zhao, Z. Chen, and Y. Yang, "Constrained nuclear–electronic orbital QM/MM approach for simulating complex systems with quantum nuclear delocalization effects incorporated," *Chem. Phys. Rev.* (to be published).
- ⁷³X. Xu, "Constrained nuclear–electronic orbital density functional theory with a dielectric continuum solvent model," *J. Phys. Chem. A* **127**(30), 6329–6334 (2023).
- ⁷⁴A. D. Becke, "Density-functional exchange-energy approximation with correct asymptotic behavior," *Phys. Rev. A* **38**(6), 3098–3100 (1988).
- ⁷⁵A. D. Becke, "Density-functional thermochemistry. III. The role of exact exchange," *J. Chem. Phys.* **98**(7), 5648–5652 (1993).
- ⁷⁶C. Lee, W. Yang, and R. G. Parr, "Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density," *Phys. Rev. B* **37**(2), 785–789 (1988).
- ⁷⁷V. Barone, "Anharmonic vibrational properties by a fully automated second-order perturbative approach," *J. Chem. Phys.* **122**(1), 014108 (2005).
- ⁷⁸P. R. Franke, J. F. Stanton, and G. E. Doublerly, "How to VPT2: Accurate and intuitive simulations of CH stretching infrared spectra using VPT2+K with large effective Hamiltonian resonance treatments," *J. Phys. Chem. A* **125**(6), 1301–1324 (2021).
- ⁷⁹A. D. Boese, "Density functional theory and hydrogen bonds: Are we there yet?," *ChemPhysChem* **16**(5), 978–985 (2015).
- ⁸⁰S. Grimme, J. Antony, S. Ehrlich, and H. Krieg, "A consistent and accurate *ab initio* parametrization of density functional dispersion correction (DFT-D) for the 94 elements H–Pu," *J. Chem. Phys.* **132**(15), 154104 (2010).
- ⁸¹S. Grimme, S. Ehrlich, and L. Goerigk, "Effect of the damping function in dispersion corrected density functional theory," *J. Comput. Chem.* **32**(7), 1456–1465 (2011).
- ⁸²L. C. Dzigan, R. J. DiRisio, L. R. Madison, and A. B. McCoy, "Spectral signatures of proton delocalization in H⁺(H₂O)_{n=1–4} ions," *Faraday Discuss.* **212**, 443–466 (2018).
- ⁸³T. F. Miller III, D. C. Clary, and A. J. H. M. Meijer, "Collision-induced conformational changes in glycine," *J. Chem. Phys.* **122**(24), 244323 (2005).
- ⁸⁴F. Huisken, M. Kaloudis, and A. A. Vigin, "Vibrational frequency shifts caused by weak intermolecular interactions," *Chem. Phys. Lett.* **269**, 235–243 (1997).
- ⁸⁵O. Bludský, J. Chocholoušová, J. Vacek, F. Huisken, and P. Hobza, "Anharmonic treatment of the lowest-energy conformers of glycine: A theoretical study," *J. Chem. Phys.* **113**(11), 4629–4635 (2000).
- ⁸⁶N. Heine, M. R. Fagiani, M. Rossi, T. Wende, G. Berden, V. Blum, and K. R. Asmis, "Isomer-selective detection of hydrogen-bond vibrations in the protonated water hexamer," *J. Am. Chem. Soc.* **135**(22), 8266–8273 (2013).
- ⁸⁷N. Yang, C. H. Duong, P. J. Kelleher, M. A. Johnson, and A. B. McCoy, "Isolation of site-specific anharmonicities of individual water molecules in the I⁻·(H₂O)₂ complex using tag-free, isotopomer selective IR-IR double resonance," *Chem. Phys. Lett.* **690**, 159–171 (2017).
- ⁸⁸I. Shavitt and R. J. Bartlett, *Many-Body Methods in Chemistry and Physics: MBPT and Coupled-Cluster Theory* (Cambridge University Press, 2009).
- ⁸⁹J. P. Perdew, K. Burke, and M. Ernzerhof, "Generalized gradient approximation made simple," *Phys. Rev. Lett.* **77**(18), 3865–3868 (1996).
- ⁹⁰J. P. Perdew, M. Ernzerhof, and K. Burke, "Rationale for mixing exact exchange with density functional approximations," *J. Chem. Phys.* **105**(22), 9982–9985 (1996).
- ⁹¹C. Adamo and V. Barone, "Toward reliable density functional methods without adjustable parameters: The PBE0 model," *J. Chem. Phys.* **110**(13), 6158–6170 (1999).
- ⁹²Y. Zhao and D. G. Truhlar, "The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: Two new functionals and systematic testing of four M06-class functionals and 12 other functionals," *Theor. Chem. Acc.* **120**(1–3), 215–241 (2008).
- ⁹³Center for High Throughput Computing, "Center for high throughput computing," 2006; <https://doi.org/10.21231/GNT1-HW21>.