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Far-IR Spectroscopy

Gas-Phase Peptide Structures Unraveled by Far-IR Spectroscopy: Combining IR-UV Ion-Dip Experiments with Born–Oppenheimer Molecular Dynamics Simulations**

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Abstract: *Vibrational spectroscopy provides an important probe of the three-dimensional structures of peptides. With increasing size, these IR spectra become very complex and to extract structural information, comparison with theoretical spectra is essential. Harmonic DFT calculations have become a common workhorse for predicting vibrational frequencies of small neutral and ionized gaseous peptides.[1] Although the far-IR region (< 500 cm^{-1}) may contain a wealth of structural information, as recognized in condensed phase studies,[2] DFT often performs poorly in predicting the far-IR spectra of peptides. Here, Born–Oppenheimer molecular dynamics (BOMD) is applied to predict the far-IR signatures of two γ-turn peptides. Combining experiments and simulations, far-IR spectra can provide structural information on gas-phase peptides superior to that extracted from mid-IR and amide A features.*

Thus far, most peptide spectroscopy studies have considered the structurally diagnostic amide NH stretching, C=O stretching, and NH bending modes. Including the far-IR region in a structural analysis has several advantages. Where mid-IR vibrations are localized modes containing information on local electrostatic and H-bonded environments, soft vibrations in the far-IR range are typically delocalized over the peptide and are hence expected to be highly sensitive to secondary structural motifs.[2a] Moreover, even in a supersonic molecular beam expansion, IR spectra of larger peptides suffer from increasing spectral congestion to an extent where only families of structures can be identified rather than a single conformation.[1c,3] The far-IR spectrum instead shows a wealth of well-resolved absorption bands[4] allowing for improved structural assignments, provided theoretical predictions are reliable.

Especially for larger peptides, DFT spectra are usually computed within the harmonic approximation, ignoring the intrinsic anharmonic character of the vibrations. In the mid-IR region, an empirical uniform scaling factor is usually applied to correct for this deficiency[5] (nonuniform scaling is used in some studies[6]). However, this approach does not work well in the far-IR region because the anharmonic mode-couplings are difficult to approximate by scaling factor(s) only. BOMD simulations provide anharmonic vibrational spectra directly, as reviewed earlier,[7] and are employed here as theoretical support for our far-IR experiments.

The performance of BOMD in calculating anharmonic far-IR spectra is demonstrated here for two γ-turn-capped dipeptides, Ac-Phe-Gly-NH_2 (FG) and Ac-Phe-Ala-NH_2 (FA), see Figure 1. In BOMD, the nuclei are treated classically, while the electrons are treated quantum-mechanically using DFT. The forces on the nuclei are derived from the Kohn–Sham energy and propagation in time is achieved by solving Newton's equations of motion.[8] The vibrational spectrum is computed as the Fourier transform of the dipole time correlation function over trajectories of 30 ps (averaged over 3 runs).[7,9] This approach has been successful in predicting the room-temperature IR spectra of gas-phase peptides[10] and sugars.[11] The same methodology can also be applied to liquids and interfaces.[7,12] A detailed description of the BOMD method is given in the Supporting Information and in a review.[7] Briefly, dynamical simulations are performed with CP2K,[8] employing the dispersion-corrected BLYP-D3 representation,[13] the aug-TZV2P Gaussian basis set, and a 400 Rydberg plane-wave basis set. CP2K is a freely

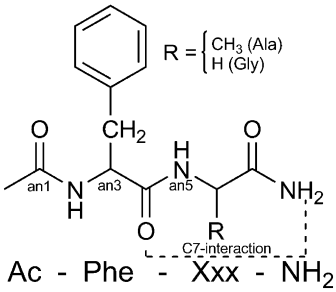

Figure 1. Chemical structure of Ac-Phe-Xxx-NH_2 (Xxx = Ala (FA) or Gly (FG)).

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available program to perform simulations on condensed-phase and biological systems. No ad hoc corrections are applied to the calculated spectra. Although the temperature in the supersonic beam is around 10–15 K, simulations are run at 50 K, as the dynamics are expected to be too slow at low temperatures.

Exploiting the free electron laser FELIX, IR spectra were recorded from 1850 to 100 cm^{-1} using IR-UV ion-dip spectroscopy (IR-IDS) in a seeded molecular beam expansion.[14] The IR spectra of the two peptides show resolved absorption bands down to 135 cm^{-1} (Figure 2), with a full-width-at-half-maximum (FWHM) bandwidth of about 3 cm^{-1}, limited by the bandwidth of the laser. IR spectra in the amide A region were previously recorded for the same peptides by Mons and co-workers.[15]

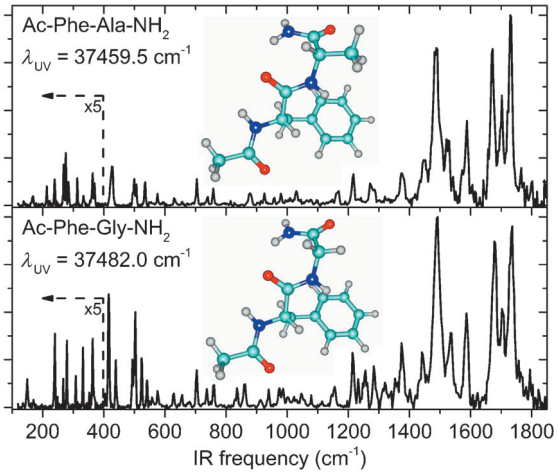

Figure 2. Experimental IR-UV spectra of Ac-Phe-Ala-NH_2 (top) and Ac-Phe-Gly-NH_2 (bottom) recorded over the IR range from 100 to 1850 cm^{-1}.

Details of the conformational search for FG and FA are given in the Supporting Information. Comparisons of scaled harmonic spectra for the low-energy conformers (FA1 for FA, FG3/FG4 for FG) using different DFT functionals are shown in Figure SI-7-9. Using harmonic B3LYP results in the mid-IR, identification of FA as structure FA1 is straightforward, confirming the previous assignment based on the amide A region alone.[15] Following the same strategy for the FG peptide is less trivial. Two similar, almost iso-energetic structures FG3 (C7eq) and FG4 (C7ax) (Figure SI-4) have indistinguishable harmonic spectra. Mons and co-workers were unable to attribute the experimental spectrum to either one of these conformers.[15]

Figure 3 presents experimental IR-IDS and BOMD calculated spectra for FA1 and FG3/FG4 in the 100–800 cm^{-1} region. The far-IR vibrations are reliably reproduced by the BOMD dynamical spectra. Especially the bands below 500 cm^{-1} are in excellent agreement, with deviations smaller than 10 cm^{-1}. Between 500 and 800 cm^{-1}, slightly larger deviations are observed (<20 cm^{-1}). Importantly, the dynamical far-IR spectra of FG3 and FG4 (Figure 3 f,g) show that only the FG3 bands match the experiment. The overall

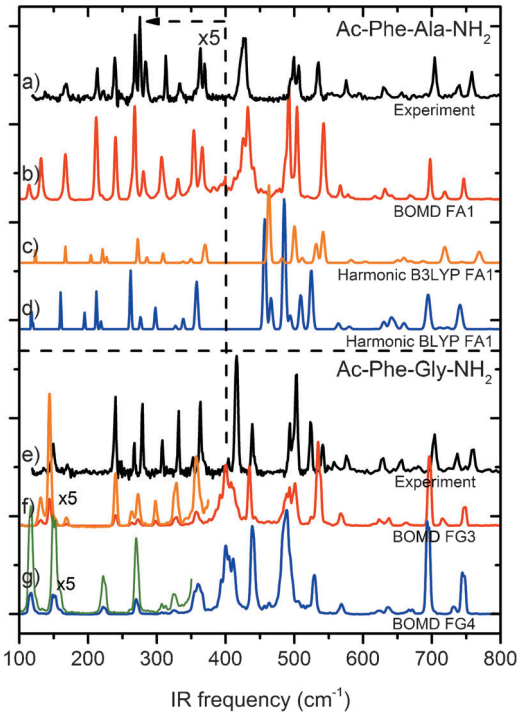

Figure 3. a) Experimental IR spectrum of Ac-Phe-Ala-NH_2 (FA). Computed IR spectrum of FA1 using b) anharmonic dynamical BOMD, c) scaled harmonic DFT (B3LYP/6-311+G(d,p)) and d) DFT (BLYP/6-311+G(d,p)). e) Experimental IR spectrum of Ac-Phe-Gly-NH_2 (FG). Theoretical anharmonic dynamical BOMD IR spectrum of f) FG3 and g) FG4. The orange and green traces of (f) and (g) are five-fold zooms.

pattern of bands in the 100–375 cm^{-1} region is well-reproduced by the dynamical spectrum of FG3. Moreover, the double peak at 500 cm^{-1} is clearly because of FG3 and not because of FG4.

The dynamical anharmonic spectra are undoubtedly more useful for a definite assignment of the FA1 and FG3 structures than the scaled/unscaled harmonic spectra employing common DFT functionals (B3LYP, B97D, and BLYP), as illustrated in Figure 3 and Figure SI-7-9. The harmonic vibrations are unable to accurately reproduce the far-IR signatures of the peptides (see Refs. [4b,c]), independent of the functional. Positions and number of active bands from the harmonic calculations do not agree with experiment, especially in the 400–600 cm^{-1} interval, although B97-D appears to perform better for FA1 (Figure SI-7–9). Moving to higher frequencies, however, the dynamical frequencies start to deviate from experiment and resemble the unscaled harmonic frequencies calculated at the same BLYP level for both FA and FG. The dynamical NH amide II modes are hence red-shifted by 30–40 cm^{-1} from experiment, while the C=O amide I modes are red-shifted by about 70 cm^{-1}. The peak spacing between the experimental C=O and N–H modes is however reproduced reasonably well in the dynamical spectra. The strong redshifts from experiment for the C=O stretches are due to the BLYP functional; the same redshift is found for N-methyl-acetamide (NMA) calculated at the same BLYP level of theory (Figure SI-6). Local modes such as C=O

and N–H stretches can be very sensitive to local deviations in the potential energy surface (PES), which are possibly quite large for the BLYP functional, as suggested by the unusual scaling factor larger than 1.0 in the mid-IR. In contrast, delocalized far-IR modes involve more global motions over the PES and are therefore less sensitive to small local errors in the computed PES, explaining the good agreement found between dynamical spectra and experiments in the far-IR region.

To identify the vibrational character of far-IR bands, Fourier transforms of intramolecular coordinate (IC) time correlation functions (TCF) are calculated over the trajectories. For instance, the 542 cm^{-1} band in FA1 is assigned to an NH out-of-plane bending vibration by monitoring the O_{Phe}-C_{Phe}-N_{Ala}-H_{Ala} dihedral angle TCF (Figure 4b).

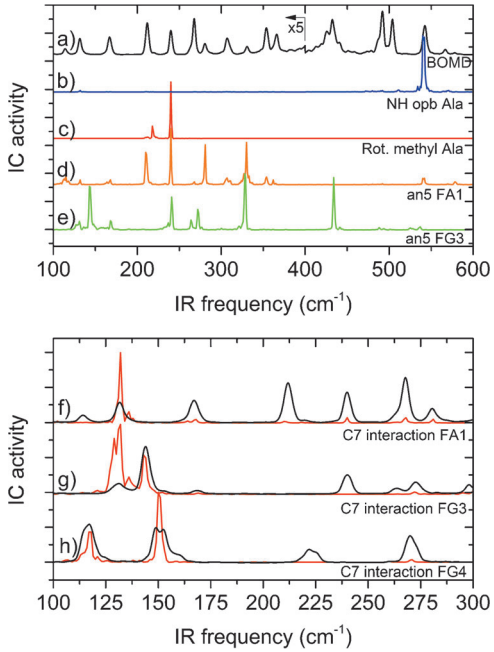

Figure 4. a) BOMD anharmonic IR spectrum of FA1. b–e) Fourier transforms of IC-TCF. IC for b) O_{Phe}-C_{Phe}-N_{Ala}-H_{Ala} (opb = out-of-plane bending), c) C_{Ala}-$C\alpha_{Ala}$-$C\beta_{Ala}$-Hβ dihedral, d) angle an5 in FA1, and e) angle an5 in FG3. Bottom panel: Black is the anharmonic BOMD IR spectrum, red are the Fourier transforms IC-TCF of O_{Phe}-N_{NH2} (C7 interaction) for f) FA1, g) FG3, and h) FG4.

One noticeable difference between the far-IR spectra of FA and FG is the doublet band around 210 cm^{-1} in the spectrum of FA, which does not appear for FG, suggesting that it may be due to the methyl group in FA. However, the C_{Ala}-$C\alpha_{Ala}$-$C\beta_{Ala}$-Hβ dihedral TCF of FA1 reveals that methyl rotation contributes to both normal modes at 210 and 240 cm^{-1}, though more dominant at 240 cm^{-1} (Figure 4c). The only ICs found to have a significant contribution to the band at 210 cm^{-1} are an1–an5 (Figure 1), exemplifying the delocalized character of normal modes in this frequency range. an5 also contributes to the bands at 240, 281, and 330 cm^{-1} (trace 4d). The activity of angle an5 in FG3 (trace 4e) is noticeably different from that of FA1, demonstrating the complex behavior of far-IR vibrations. Even minor structural differences result in significantly different far-IR responses.

Another crucial spectroscopic signature involves the C7 interactions present in both the FG and FA peptides, which can be monitored using the O_{Phe}-N_{NH2} distance TCF (Figure 4f–h). For the identified structures FA1 and FG3 (C7eq), this interaction gives rise to a common band at 131 cm^{-1}. For FG4 (C7ax), this band is shifted to 150 cm^{-1}. Because of the delocalized nature of these vibrations, the C7eq interaction in FG3 also produces a band at 144 cm^{-1}, while the C7ax interaction in FG4 produces an extra feature at 117 cm^{-1}. BOMD is able to distinguish between subtle differences in intramolecular interactions.

In conclusion, theoretical far-IR anharmonic spectra for the gas-phase FA and FG peptides obtained with BOMD show excellent agreement with experimental spectra down to 100 cm^{-1}. Dynamical anharmonic spectra combined with experimental far-IR spectra are thus a promising tool for the detailed structural characterization of peptides in the gas phase. Particularly for larger peptides, where the diagnostic amide vibrations in the mid-IR spectrum may suffer from spectral congestion, delocalized vibrations in the far-IR spectrum may form an interesting alternative; BOMD dynamical spectra are able to reproduce and assign vibrational features in terms of atomic motions. Special signatures such as the C7eq/C7ax interactions can be predicted and used for the analysis of far-IR and THz spectra of peptides of increasing size and complexity. Of specific interest to the peptides studied here, BOMD has been able to discriminate between two similar secondary structures, which could not be differentiated in the mid-IR spectrum.

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