Vibrational Spectroscopy

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Probing Vibrational Anisotropy with Nuclear Resonance Vibrational Spectroscopy**

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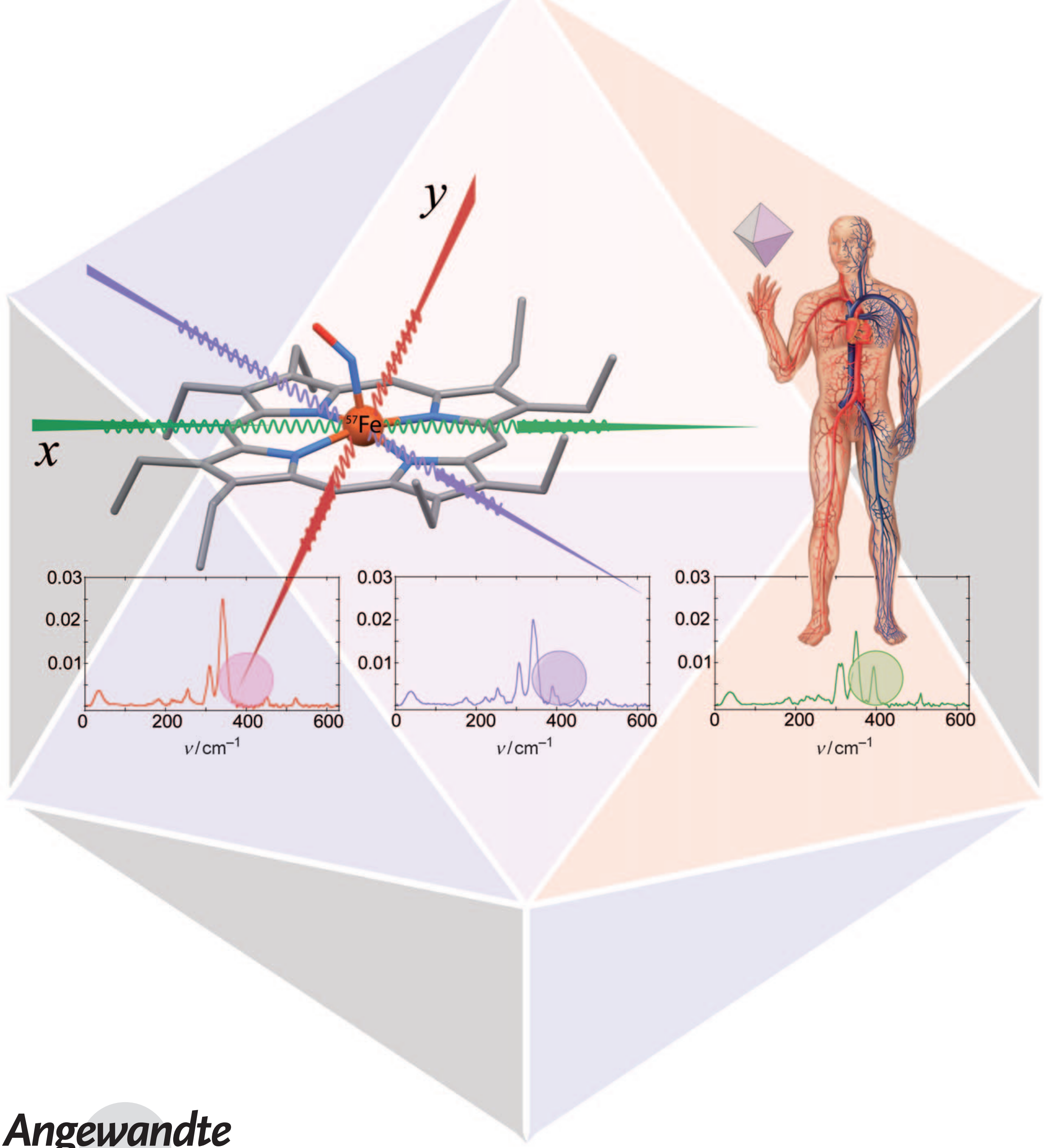
Dedicated to Professor Robert G. Hayes on the occasion of his 74th birthday



One of the important issues of nitrosyl (nitric oxide, NO) iron porphyrinate derivatives (hemes) is to develop a detailed understanding of the molecular basis for selectivity (recognition) between the diatomic ligands NO, CO, and O_2. The sensing of these gaseous molecules is predominantly carried out by heme-based proteins,[1] and heme and heme protein–diatomic interactions continues to be an active area of research.[2] In nature, NO is discriminated from O_2 quite efficiently by a number of systems;[3–5] most prominent are those of soluble guanalyl cyclase and the NO sensing protein of *Clostridium botulinum*. Conformational changes in the protein imparted upon ligand binding is a plausible explanation for such differentiation.[6,7] Very low frequency doming modes, sometimes referred to as reactive modes,[8] may facilitate binding and release of diatomic molecules.[9,10] Infrared and resonance Raman spectroscopy have provided insight into the interplay of structure and function of heme active sites[11] However, these techniques have some inherent limitations, especially in the low frequency regime where mode assignment is hampered by weak signal, spectral congestion and low sensitivity to isotopic substitution.[8] Also, the directionality of iron within particular vibrations, a potential mechanistic indicator, cannot easily be determined. Our recent studies of the nitrosyl (NO) derivatives of iron porphyrinates have emphasized interesting aspects of nitrosyl dynamics of both the five- and six-coordinate NO species,[8,12–16] including nuclear resonance vibrational spectroscopy (NRVS, sometimes termed nuclear inelastic scattering, NIS) studies.[8,15–19]

The direction of metal ion motion in a metal complex will be influenced by the surroundings, with the most significant interactions expected to be between the metal and its donor atoms. Naively, the principal metal motion directions would then be expected to be along the metal–ligand bonds.[20] We report herein the remarkable directional nature of the iron vibrations in [Fe(oep)(NO)],[21] as determined by an oriented single-crystal NRVS experiment; the observed dynamics are only partially in accord with the above expectations. NRVS is a synchrotron-based vibrational technique with unique site selectivity for iron and provides quantitative information on all vibrational frequencies for which there is iron atom motion.[17,22] We have previously used oriented single crystals of heme derivatives[8,15,16,23,24] to assign the in-plane and out-of-plane vibrations, a distinction that is well developed for heme derivatives with the planar equatorial porphyrin ligand. Selectivity between in-plane and out-of-plane modes results because only iron atom motion in the direction of the incident exciting beam will lead to signal intensity in the NRVS experiment. In contrast with conventional approaches to mode assignment based on isotope substitution, single-crystal NRVS measurements simultaneously characterize the directional character of all vibrational motions of the ^{57}Fe probe atom and may distinguish among divergent predictions resulting from quantum chemical calculations. In our previous oriented crystal experiments, we made measurements in which the X-ray beam was either perpendicular to all porphyrin planes in the crystal or parallel to the porphyrin planes; however the in-plane measurement was always made in a single arbitrary general direction in the porphyrin plane.

In a new study, we now explore iron atom motion in specific directions in the porphyrin plane of [Fe(oep)(NO)],[25] which provides an additional dimension of spectral analysis for heme derivatives. Single-crystal measurements probed two orthogonal directions in the porphyrin plane: x, parallel to the intersection of porphyrin and FeNO planes, and y, perpendicular to the FeNO plane. Experimental limitations require that a separate crystal be used for measurements in the z direction, perpendicular to the porphyrin plane. The observed VDOS (vibrational density of states) in the three orthogonal directions are displayed in Figure 1. The uniqueness of the observed vibrational spectra was verified by replicate measurements on separately oriented crystals (see the Supporting Information, Figure S1), by measurement "nn" with the X-ray beam in-plane and parallel to the N_2-Fe-N_4 direction of the porphyrin plane, and by a measurement in a more general direction in the porphyrin plane (Figures S2 and S3). These latter two measurements confirm that the x and y directions can be taken as the fundamental directions. Moreover, the sum of the x, y, and z directional component spectra agree well with the experimental powder (isotropic) spectrum (Figure S4). We also

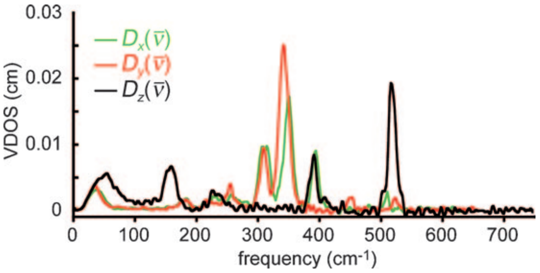


Figure 1. Directional contributions to the VDOS of [Fe(oep)(NO)] for x, y, and z directions. For "x", the beam is parallel to the porphyrin plane and to the Fe-N-O plane, for "y" the beam is parallel to the porphyrin plane and perpendicular to the Fe–N–O plane, and for "z", the beam is perpendicular to the porphyrin plane.

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note a very recent theoretical paper that presented a “First-principles calculation of NRVS” that predicted the possibility of observable anisotropy.[27]

Spectra recorded with the porphyrin core perpendicular to the exciting X-ray beam probe Fe motion along *z* and resolve a total of five distinct features (black line, Figure 1). Two of these represent iron motion with both in-plane and out-of-plane components (394 and ca. 225 cm^{-1}). The remaining three have essentially pure out-of-plane iron motion. The broad absorption at extremely low frequency (< 60 cm^{-1}) is in the region where lattice modes are also expected,[28] but the absorption in the *z* direction has greater intensity and a shift to slightly higher frequency that is consistent with observation of extremely low frequency modes that are intramolecular in character. Differences between the averaged *x* and *y* intensities and the *z* intensity confirms a substantial *z*-direction signal (Figure S5).

DFT calculations[29] predict spectral assignments and kinetic energy distributions that support and enhance the oriented crystal data. The calculations suggest that three modes, predicted at 37, 41, and 47 cm^{-1}, contribute to the broad unresolved observed band below 100 cm^{-1} (Figure S5). All three involve substantial motion of the peripheral ethyl groups as well as motion of both iron and porphyrin core atoms consistent with that of a doming mode (Figure S6).[34] The 158 cm^{-1} mode (predicted 139 cm^{-1}) is also a doming mode. The relatively high frequency of this mode appears related to the peripheral groups of octaethylporphyrin; the analogous signal observed at 140 cm^{-1} in [Fe(oep)(Cl)] is also likely to involve heme doming, although previously misassigned to in-plane vibrations.[20] The very strong signal at 517 cm^{-1} is the Fe–NO stretch; the calculated frequency for this mode is 623 cm^{-1}. The large discrepancy is typical for predicted Fe–NO stretching modes in five-coordinate NO porphyrinate complexes.[8] This band has modest yet interesting shoulders, one on each side of the main peak. These are coincident with the 510 cm^{-1} and 523 cm^{-1} vibrations of Fe observed along the *x* and *y* directions, respectively (Figure 1). The shoulders are also apparent in the powder spectrum shown in Figure S4 which also displays the summation of *x*, *y*, and *z* contributions. The remarkable agreement between these spectra attests to the capability of NRVS for showing subtle features that might have previously been dismissed as adventitious, and we judge the shoulders as possible unforeseen *z* components of the predicted 503 and 522 cm^{-1} modes.

Significant differences between the *x* and *y* NRVS spectra are observed (Figure 1), although in principle the iron should have equivalent interactions with each porphyrin nitrogen atom leading to degeneracy (or nearly so) among in-plane NRVS signals. To facilitate the *x* and *y* comparisons, we show in Figure 2 the observed VDOS in the two directions in a “mirror” plot for the 125–480 cm^{-1} spectral region. Similar diagrams for the (few) remaining peaks at higher or lower frequencies are given in the Supporting Information (Figures S7 and S8). Although the two directional spectra have similarities, there are obvious differences in both peak intensities and frequencies. The most striking difference is the 394 cm^{-1} vibration of Fe along *x* with no corresponding vibration along *y*. This mode was also observed to have substantial iron atom motion along *z*; the 394 cm^{-1} mode is clearly the Fe–N–O bending mode.

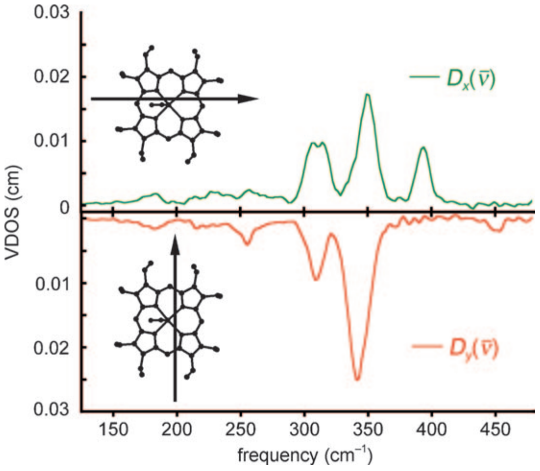

Figure 2. Observed contributions to the Fe VDOS in the 125–480 cm^{-1} region. The inset porphyrin diagrams illustrate the direction of the incident X-ray beam. The two portions of the mirror diagram are on the same scale.

Measurements as a function of direction within the porphyrin plane allow for a more detailed examination of the mode assignments than previous (general) in-plane studies had permitted. The strongly developed directionality of the observed NRVS spectra provide strong constraints on the spectral predictions by DFT calculations in the issue of the appropriate choice of functional. One feature of major importance is the description of the magnetic properties of the $S = 1/2$ system; the treatment of the unpaired spin systems is a well-known difficulty of DFT calculations, which seems especially true for nitrosyl systems.[33] Although these issues will be investigated in more detail in future calculations, the results from the present calculation using the BP86 functional appears to have captured the essentials of the vibrational directionality.

A comparison of the observed and predicted x and y spectra is given in Figure 3; the analogous diagram for the *z* component is given in Figure S9. As can be seen in these figures, the peak at 394 cm^{-1} clearly has only *x*- and *z*-observed components, a directional dependency identical to that of the predicted 417 cm^{-1} mode. The components of motion[34] are illustrated in Figure 4 and as noted above are those expected for an FeNO bend, although only 60 % of the energy is localized on the FeNO unit. This vibration was not cleanly identified in a previous study on [Fe(tpp)(NO)] (tpp = tetraphenylporphyrin). Interestingly, experimentally the *x* and *z* components are equal in intensity (Figure 1) whereas the predicted intensities are not quite equal.

The predicted modes in the 200–380 cm^{-1} region are consistent with the observed peaks in both their directional character and intensities (Figure 3). The minimally resolved 307 and 314 cm^{-1} Fe vibrations along *x* correspond to the predicted modes at 297 and 318 cm^{-1} as they are “*x*-only” in predicted character and have appropriate separation. The predicted mode at 348 cm^{-1} agrees quite well with the observed 350 cm^{-1} Fe vibration along *x* and even has predicted modes consistent with the observed band asymme-

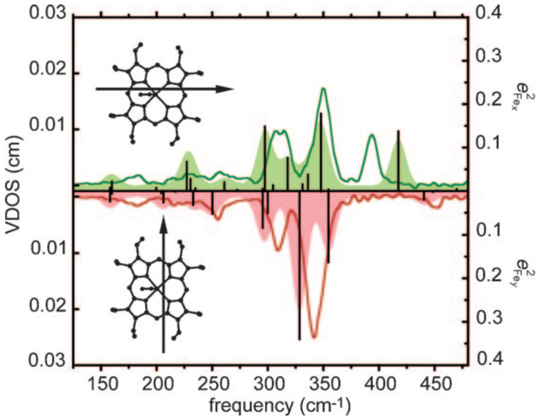

Figure 3. Measured (lines) and predicted (shaded) contributions to the VDOS from Fe atom motion along *x* (green) and *y* (red) directions, together with calculated $e^2_{Fe_x}$, $e^2_{Fe_y}$ values (bars) over the 125–480 cm^{-1} region. Porphyrin diagrams to the left present the in-plane directions of Fe motion, parallel (top panel) and perpendicular to the FeNO plane (bottom panel).

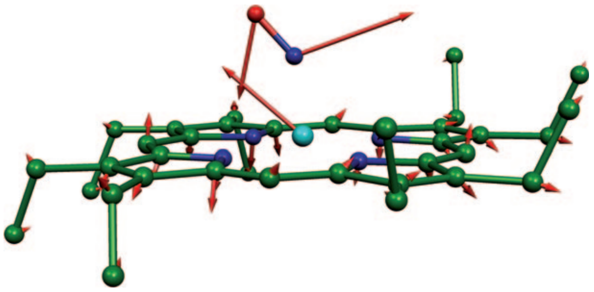

Figure 4. Depiction of the predicted 417 cm^{-1} mode. Cyan, Fe; blue, N; green, C. Hydrogen atoms have been omitted for clarity. In this figure, and in Figures S10—S15 each arrow is 100 $(m_j/m_{Fe})^{1/2}$ times longer than the zero-point vibrational amplitude of atom *j* (with mass m_j)), and the bonds to the Fe atom are omitted to enhance the visibility of the Fe motion.

try at 331 cm^{-1} and 336 cm^{-1}. The observed 308 cm^{-1} Fe vibration along *y* correlates well with predicted modes at 295 and 300 cm^{-1}, all with "*y*-only" character. The largest amplitude Fe vibration along *y* at 342 cm^{-1} is bracketed by predicted modes at 328 cm^{-1} and 355 cm^{-1}, which evidently have a smaller separation than predicted.

The experimental vibrational data for [Fe(oep)(NO)] over the entire range of iron atom motion are thus seen to be strongly anisotropic. The polarized in-plane modes are best referred to a coordinate system where the Fe−N−O group defines the *xz* plane. The contributions of Fe motion along the *x*, *y*, and *z* directions for all modes for which Fe motion constitutes more than 1 % of the total kinetic energy are tabulated in Table S1 of the Supporting Information. We have examined the character of the predicted vibrations and find that there are a number of approximately degenerate pairs. These are predicted at frequencies 295 and 297 cm^{-1}, 300 and 318 cm^{-1}, 328 and 348 cm^{-1}, 336 and 355 cm^{-1}, 503 and 522 cm^{-1}, and 158 and 160 cm^{-1}. These are illustrated in the Supporting Information (Figures S10 to S15). Only the last pair has iron atom motion that has both substantial *x* and *y* character; the remaining five pairs have motion that is either along *x* (underlined values) or along *y*. Only two modes have predicted directional character that is not seen in the experimental spectra; the peaks at 297 and 348 cm^{-1} are predicted to have some *z* character that however is not apparent in the experimental directional data. Still, the *z* component is a relatively small predicted component of these two modes.

Figure 5 depicts the predicted *x*, *y*, and *z* components of all modes with significant iron contributions and which are in strong agreement with the experimental data. This stacked bar graph clearly shows the predominant *x* or *y* character of all major in-plane modes. In all cases but one, the in-plane iron motion, and that with relatively low intensity, is directed between adjacent pairs of equatorial Fe−N_p bonds, that is either in the direction parallel or perpendicular to the Fe−N−O plane. What features of the [Fe(oep)(NO)] molecule lead to such strongly polarized vibrational spectra? We believe that the vibrational spectra reflect the strong asymmetry in the interaction between the Fe d_π orbitals and the NO π^* orbitals parallel and perpendicular to the FeNO plane, owing to the nonlinear Fe−N−O group. The in-plane iron to porphyrin nitrogen bonds reveal the same asymmetry.[26,35] The strong asymmetry in the in-plane as well as the out-of-plane spectra reveal the wealth of information available in oriented-crystal NRVS data. These data can now be used for the construction of more definitive tests of theoretical calculations and ultimately to better define ligand association and dissociation pathways.[36] Further experiments will determine how widespread the axial ligand-based anisotropy is and the bonding character required for its observation.

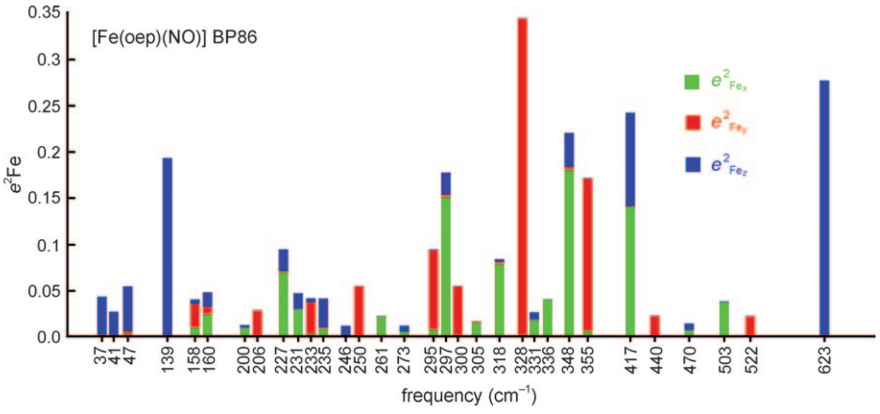

Figure 5. Bar graph showing DFT-predicted directional characteristics of all 32 modes with e^2_{Fe} >0.01. $NRVS_x$ green; $NRVS_y$ red; $NRVS_z$ blue. The values of the predicted frequencies are given at each tick mark, but the horizontal scale is only approximately linear in frequency to avoid overlaps.

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[25] Single crystals of the triclinic form of [Fe(oep)(NO)][26] have a pair of inversion symmetry related molecules in the unit cell. These parallel molecules are equivalent to a single molecule in these oriented NRVS experiments. ^{57}[Fe(oep)(NO)] was prepared by reductive nitrosylation of [Fe(oep)(Cl)] in chloroform. X-ray quality crystals were obtained by liquid diffusion using methanol as the non-solvent. The complex crystallizes in the triclinic space group $P\bar{1}$, $a = 10.4204(1)$, $b = 10.5562(7)$, $c = 14.0425(7)$ Å, $\alpha = 79.830(5)$, $\beta = 89.585(4)$, $\gamma = 80.264(4)°$, $V = 1498.10(13)$ Å^3, $Z = 2$.

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