

Observation of Terahertz Vibrations in the Nitrogenase FeMo Cofactor by Femtosecond Pump–Probe Spectroscopy**

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Human reliance on synthetic nitrogen fertilizers produced in the Haber–Bosch ammonia synthesis process has a host of undesirable consequences,^[1] including dead zones from eutrophication, contributions to acid rain and greenhouse gases, and the consumption of large quantities of natural gas and coal.^[2] Increased understanding of the alternative route, biological nitrogen fixation, could lead to better options for the delivery of fixed nitrogen to crop plants.^[3,4] In any case, it would be instrumental in solving one of the longstanding questions in catalysis: How does nature reduce dinitrogen to ammonia at low temperatures and at atmospheric pressure?

One of the best-studied biological nitrogen-fixation systems is the Mo-dependent nitrogenase (N₂ase) from *Azotobacter vinelandii*.^[3–5] The active site of this enzyme employs a MoFe₇S₉X-homocitrate “FeMo-cofactor”, where “X” is an unidentified interstitial light atom, and this cluster is extractable into organic solvents as the small molecule “FeMoco”.^[6–9] Dramatic progress has been made recently using electron nuclear double resonance (ENDOR) of nitrogenase mutants under special conditions to observe nitrogenous intermediates at various states of reduction.^[10–14] However, techniques are still needed to characterize nitrogenase from other points of view, preferably on short time scales and in solution. In this work, as a prelude to measure-

ments on complex samples, we have investigated *N*-methylformamide (NMF) solutions of isolated FeMoco using impulsive coherent vibrational spectroscopy (ICVS). The results are compared to those from nuclear resonance vibrational spectroscopy (NRVS),^[15,16] and the combination of data from both of these techniques allows a more comprehensive description of FeMoco vibrational activity.

In the ICVS experiment, an ultrashort pump laser pulse, resonant with the sample absorption, promotes a small fraction of the molecules to an electronic excited state. A probe pulse delayed by time τ measures the time-dependent differential transmission ($\Delta T/T$) signal. If the pump pulse duration is significantly shorter than the periods of the vibrations of interest, then a coherent vibrational wave packet can be formed in the excited and/or in the ground electronic states. Periodic motion of this packet along displaced bond coordinates will modulate, by means of Franck–Condon factors, the molecular absorption. Fourier transformation of the oscillatory component of the time-dependent $\Delta T/T$ signal yields vibrational frequencies coupled to the electronic transition for the chromophore under study. To date, ICVS has been applied to the study of several biochemical systems, including heme proteins,^[17–19] green fluorescent protein,^[20] blue copper proteins such as azurin,^[21] plastocyanin,^[22–25] and umecyanin,^[26] and the Fe(Cys)₄ site in the electron-transfer protein rubredoxin.^[27]

In our ICVS experiments, a benzenethiolate-treated FeMoco solution was pumped by 15 fs pulses centered at 450 nm, and probed by sub-10 fs pulses with a broadband spectrum spanning the 500–700 nm range.^[28,29] Figure 1a shows the steady-state absorption spectrum for this FeMoco solution, together with the spectra of the pump and probe pulses. The relatively featureless FeMoco absorption spectrum is one indicator of sample integrity, because air-oxidized FeMoco exhibits a variety of distinct features in its visible spectrum.^[30] No changes in the absorption spectrum were observed during the ICVS experimental sessions. Over a period of several weeks of storage, the sample bleached and a distinct absorption band grew in at 470 nm (see the Supporting Information), indicating the presence of air-oxidized FeMoco.^[30] These observations indicate that the integrity of the sample was maintained during the period when the ICVS measurements were conducted.

Figure 1b shows the 2D differential transmission map $\Delta T/T(\lambda, \tau)$ following excitation at 450 nm. $\Delta T/T$ spectra at two different time delays are shown in Figure 1c, whereas a typical dynamics together with an exponential fit is reported in Figure 1d. The response is strongest around 540 nm, but absorption changes are clear out to 700 nm. The raw pump–

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