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CONSTRUCTION OF 40 NANOSECOND TIME RESOLUSION STEP-SCAN FTIR DIFFERENCE SPECTROMETER

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A step-scan time-resolved Fourier transform infrared (FTIR) spectrometer was constructed. Using 1064 nm radiation from a 9 ns pulsed Nd:YAG laser as the IR source, we have obtained both the time-resolved spectra in the frequency domain and the change of the transient signals in the time domain. The latter allows us to measure the time resolution of the instrument to be about 40 nanoseconds.

Keywords: Time resolution; nanoseconds; step-scan Fourier transform infrared spectroscopy

Time-resolved vibrational spectroscopy is a very powerful technique to elucidate protein dynamics. In the past few years our lab has developed time-resolved ultraviolet resonance Raman (UVRR) spectroscopy, which monitors vibrational signatures of aromatic residues, to study the allosteric reaction coordinate in hemoglobin (Hb) after photolysis of HbCO[1]. UVRR spectroscopy is limited, however, by the availability of UV-absorbing chromophores. To continue our investigation of Hb dynamics *via* vibrational spectroscopy we are now developing time resolved step-scan FTIR difference spectroscopy, which can provide information about structural elements not accessible to UVRR spectroscopy. In collaboration with Heinz Frei at Lawrence Berkeley National Laboratory, we have

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established [2] that time-resolved FTIR spectroscopy is capable of monitoring protein structure changes as well as ligand recombination in Hb on time scales down to tens of nanoseconds. Both geminate and second-order ligand recombinations were determined to be in excellent agreement with previous visible absorption measurement [3].

Recently several other applications of nanoseconds step-scan FTIR spectroscopy have appeared in the literature [4–6]. However, the time resolution of the step-scan FTIR spectrometer has not been measured experimentally but was only estimated by the response time of the detector [4, 6]. In this report we used 1064 nm radiation from a 9 ns pulsed Nd:YAG laser as the IR source, and obtained both the time-resolved spectra in the frequency domain and the change of the transient signals in the time domain at the same time (Fig. 1). As shown in Figure 2, a nearly symmetric time response with a full width at half-maximum of 40 ns was observed, showing that the time-resolution of the system is better than 40 ns, which includes both rise time and decay time.

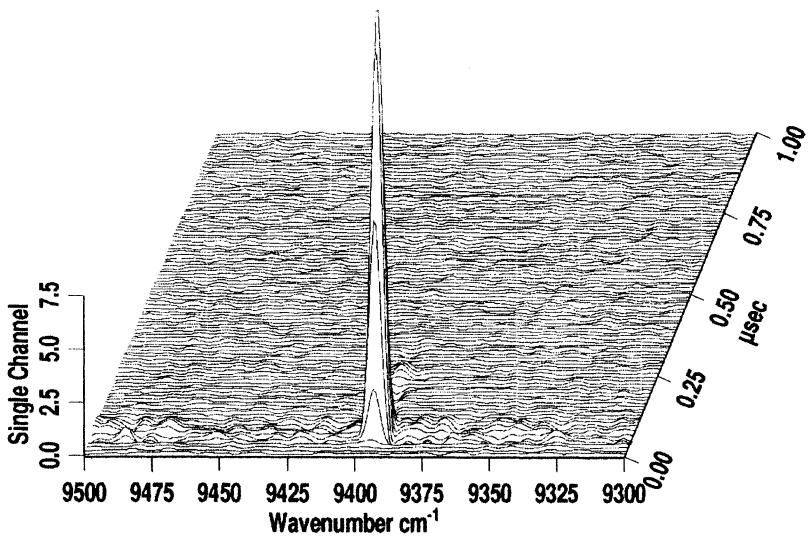

FIGURE 1 Step-scan time-resolved FTIR spectra of a 1064 nm (939 cm^{-1}) radiation of the 9 ns laser pulse. A Bruker IFS 66/v FTIR spectrometer with step-scan option was used. The data were recorded at 10 ns intervals using a 200 MHz digitizer and spectral resolution was 4 cm^{-1}.

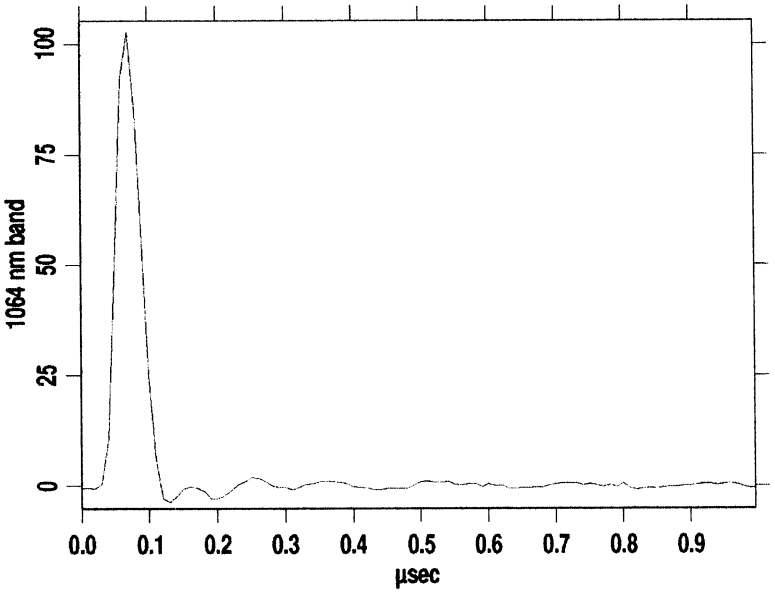

FIGURE 2 The observed time response of the band 1064 nm (939 cm^{-1}).

References

[1] Jayaraman, V., Rodgers, K. R., Mukerji, I. and Spiro, T. G. (1995). *Science*, **269**, 1843.
[2] Hu, X., Frei, H. and Spiro, T. G. (1996). *Biochemistry*, **35**, 13001.
[3] Hofrichter, J., Sommer, J. H., Henry, E. R. and Eaton, W. A. (1983). *Proc. Natl. Acad. Sci. USA*, **80**, 2235.
[4] Hage, W., Kim, M., Frei, H. and Mathies, R. A. (1996). *J. Phys. Chem.*, **100**, 16026.
[5] Siebert, F. (1993). *Biomolecular Spectroscopy*, Part A; Clark, R. J. H. and Hester, R. E. Eds., John Wiley & Sons Ltd.: New York, pp. 1–49.
[6] Schoonover, J. R., Strouse, G. F., Omberg, K. M. and Dyer, R. B. (1996). *Comments Inorg. Chem.*, **18**, 165.