Protein Dynamics in Cytochrome P450 Molecular Recognition and Substrate Specificity using 2D IR Vibrational Echo Spectroscopy

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Supporting Information
Fifth Order Signals

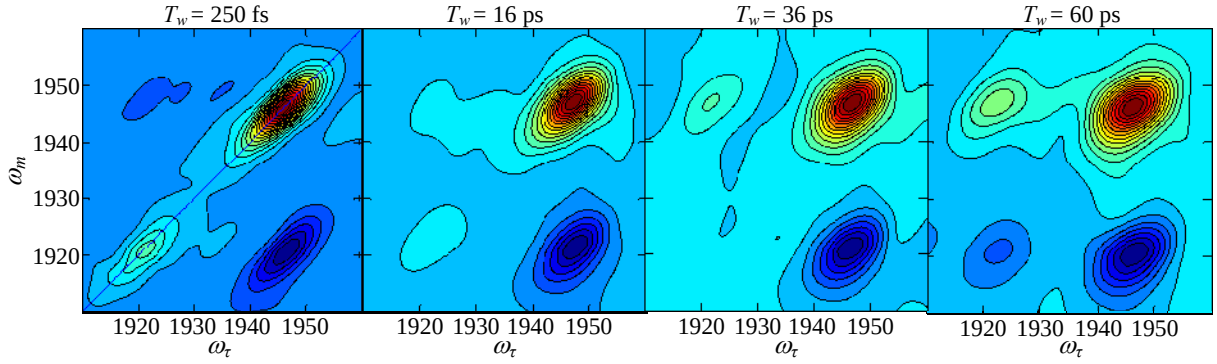

Figure SI-1. 2D IR spectra of the cyt $P450_{cam}$-CO-norcamphor complex at varying waiting times.

Figure SI-1 displays 2D IR spectra of the cyt $P450_{cam}$-CO-norcamphor complex with an expanded frequency axis. The large positive going peak (red) on the diagonal (upper right corner) of the T_w = 250 fs spectrum is the 3rd order 0-1 transition, and the negative going peak (blue) directly below it is from the 3rd order 1-2 transition emission. The other positive going peak on the diagonal (lower left corner) is a 5th order peak involving the 1-2 transition. The small negative going peak directly above the diagonal 5th order peak (upper left corner) is another 5th order peak involving the 0-1 transition.

These bands result from 5th order signals, not additional conformational states or chemical exchange. The bands disappear when the experiment is performed with lower pulse energies, and their intensities display an unusual T_w dependence that can be described by consideration of 5th order field-matter interactions and the differences in the lifetimes of the

second vibrational excited state and the first vibrational excited state. As time progresses in the figures, the 5th order peaks vanish and then reappear with opposite sign. By 60 ps, the diagonal 5th order peak has gone from positive to negative, and the off-diagonal 5th order peak has gone from negative to positive. This behavior can be explained in quantitative detail and will be the subject of a subsequent publication.

The identification and discussion of the 5th order signals in 3rd order vibrational echo experiments have previously been reported,[1,2] but the features shown above have not been observed previously or given a theoretical explanation. A full treatment of these effects is not relevant to the studies of enzyme dynamics presented in the main paper. Most importantly, those

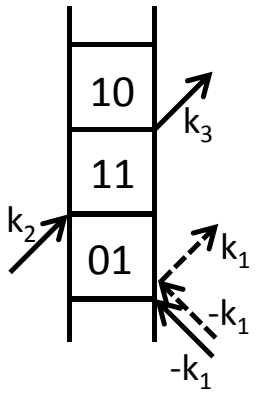

Figure SI-2. An example of a fifth order diagram that contributes to the 0-1 diagonal band. The field-matter interactions are indicated by arrows. Additional interactions (represented by dashed arrows) with the bra in the first pulse lead to a fifth order signal. Further diagrams reflecting the possible combinations where additional interactions occur with second or third pulse, with both the bra and ket, are not shown. See ref. 1 for a complete description.

Feynman diagrams involving five field-matter interactions that could lead to signals in the phase-matched direction and result in a contribution to the 0-1 diagonal band that is used in the data analysis of the enzyme dynamics can only arise when the two additional interactions occur within one a single pulse, both acting on either the bra or ket (Figure SI-2). The 5th order diagrams contributing to the 0-1 diagonal band differ only from the corresponding third order diagrams by a period of population evolution that is at most the duration of the IR pulse (~100 fs). This aspect of the 5th order signals has been discussed in detail previously.[1] Because this time period is short compared to the duration of T_w, the 5th order

signals do not influence the shape of the diagonal 0-1 bands, and thus the analysis of the bands to determine the FFCF.

References

(1) Finkelstein, I. J.; McClain, B. L.; Fayer, M. D. *J. Chem. Phys.* **2004**, *121*, 877.

(2) Hamm, P.; Lim, M.; Asplund, M.; Hochstrasser, R. M. *Chem. Phys. Lett.* **1999**, *301*, 167.