Supporting information for

Vibrational Coherence Spectroscopy of the Heme Domain in the CO-Sensing Transcriptional Activator CooA

Venugopal Karunakaran[†,$], *Abdelkrim Benabbas*[†], *Hwan Youn*[§], *Paul M. Champion*[†]*

[†]Department of Physics and Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, Massachusetts 02115, § Department of Biology, California State University, Fresno, CA 93740, USA.

p.champion@neu.edu

Vibrational dynamics of CooA

[†]Northeastern University

[§] California State University, Fresno, CA

[$] Present address: Photosciences and Photonics Section, Chemical Sciences and Technology Division, National Institute for Interdisciplinary Science & Technology, CSIR, Trivandrum 695 019, Kerala, India.

Experimental details for vibrational coherence spectroscopy

The degenerate pump-probe coherence experiments are done with the laser system consisting of a tunable (750–960 nm) Ti-Sapphire oscillator (MIRA 900; Coherent, Santa Clara, CA) pumped by a diode laser (Verdi 10; Coherent). The laser pulses of 50–100 fs, 76 MHz with energy of ~10 nJ/pulse are generated by the oscillator. The pulses were frequency-doubled in a 250 μm β-barium borate crystal and then chirp-compensated by a pair of SF10 prisms. Subsequently, the laser light was split with a ratio of 2:1 for the pump and probe beams respectively. An acousto-optic modulator (NEOS Technologies, Melbourne, FL) is used to modulate the pump beam at 1.5 MHz. Before entering the sample, the pump and probe beam polarizations were adjusted to be perpendicular to one another. The average pump and probe laser power at the sample was ~8 and ~4 mW respectively. The time delay between the pump and probe pulse was controlled by a Klinger translation stage (Newport, Irvine, CA). After the sample, the beams were recollimated and the pump light was spatially blocked (using a pinhole) and extinguished by a polarization analyzer that only allowed the probe light to pass.

NSD Analysis.

The differences of the heme geometric distortions induced by the protein environment are quantified by the Normal coordinate structural decomposition (NSD). The planar structure of ferric porphine, [Fe(P)]+ is used as a reference which derived from DFT optimization under D_{4h} symmetry, and the iron as well as the 24 skeletal atoms are included. Each displacement along normal coordinate $\boldsymbol{Q}_{\alpha}$ is determined in the mass-weighted coordinate space using the scalar

product $(\boldsymbol{X} - \boldsymbol{X_0}) \cdot \boldsymbol{Q}_\alpha$, where $\boldsymbol{X}$ and $\boldsymbol{X_0}$ are the mass-weighted atomic coordinates of the input and reference structures, respectively. The quantity $\boldsymbol{Q}_\alpha$ is the unit vector of the mass-weighted normal mode, α, of the reference structure $[Fe(P)]^+$. A Swiss-PdbViewer (ver. 3.7)[1] is used to find the difference between the structures by superimposing with least-squares fitting method. Thus, we can find the displacements along low frequency out-of-plane (OOP) modes of different symmetry (such as propellering, ruffling, saddling, waving*(x)*, waving*(y)*, doming and inverse doming) and use them to describe the observed heme structure. We include both the doming (Fe moves with the porphine nitrogens) and the inverse doming (Fe motion opposite to porphine nitrogens) modes to more accurately specify the iron OOP position, which is approximately the sum of these displacements. A negative displacement is defined only for these two modes, where it indicates the direction of the iron OOP movement (+ is proximal and - is distal).

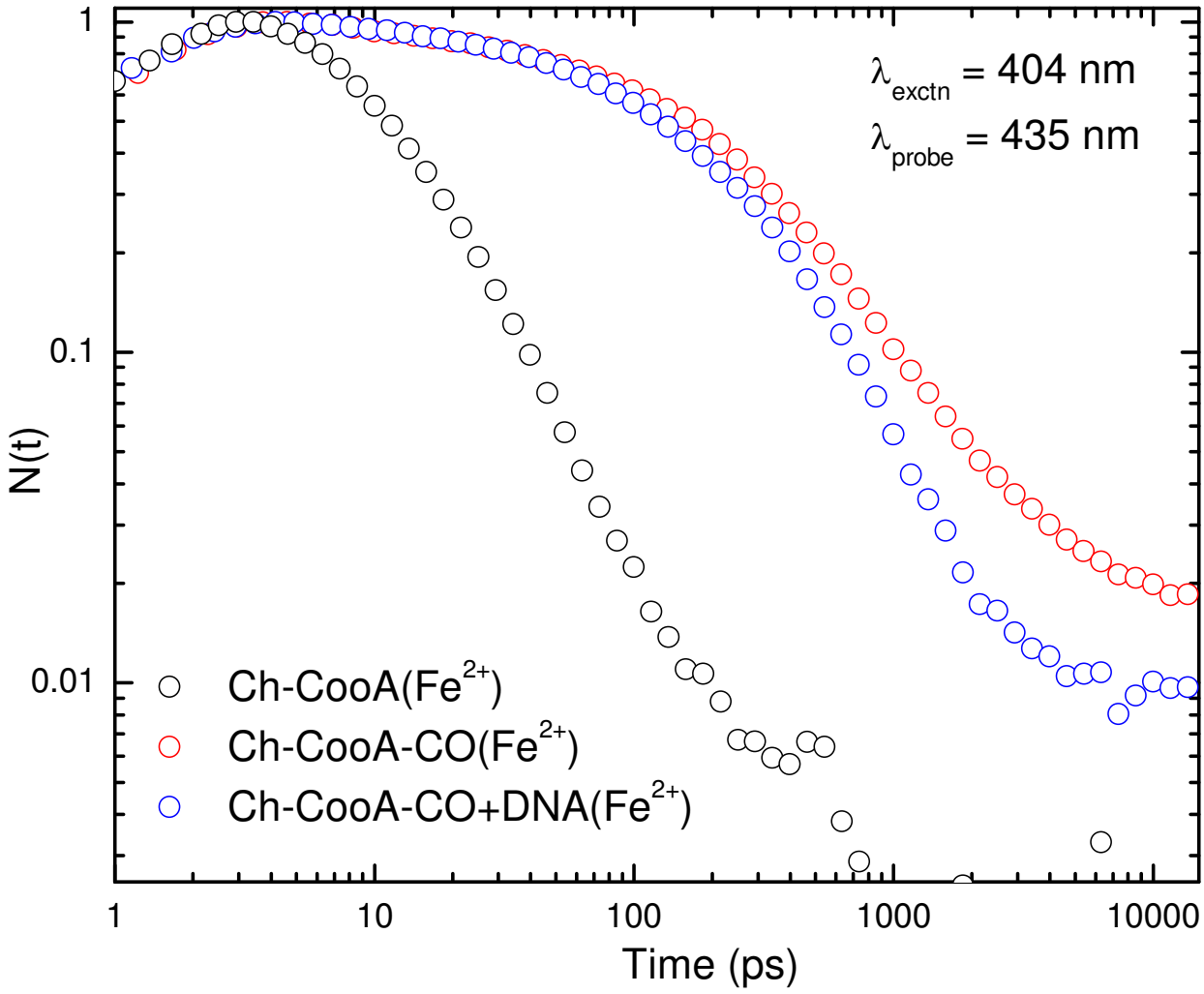

Figure S1 The long time kinetic decays of ferrous (black) and CO bound form of Ch-CooA with (blue) and without (red) specific DNA were measured in a two-color pump-probe setup working at 190 kHz as described previously[2]. The details of kinetic time constants will be published elsewhere (Abdelkrim Benabbas, *et al* under preparation).

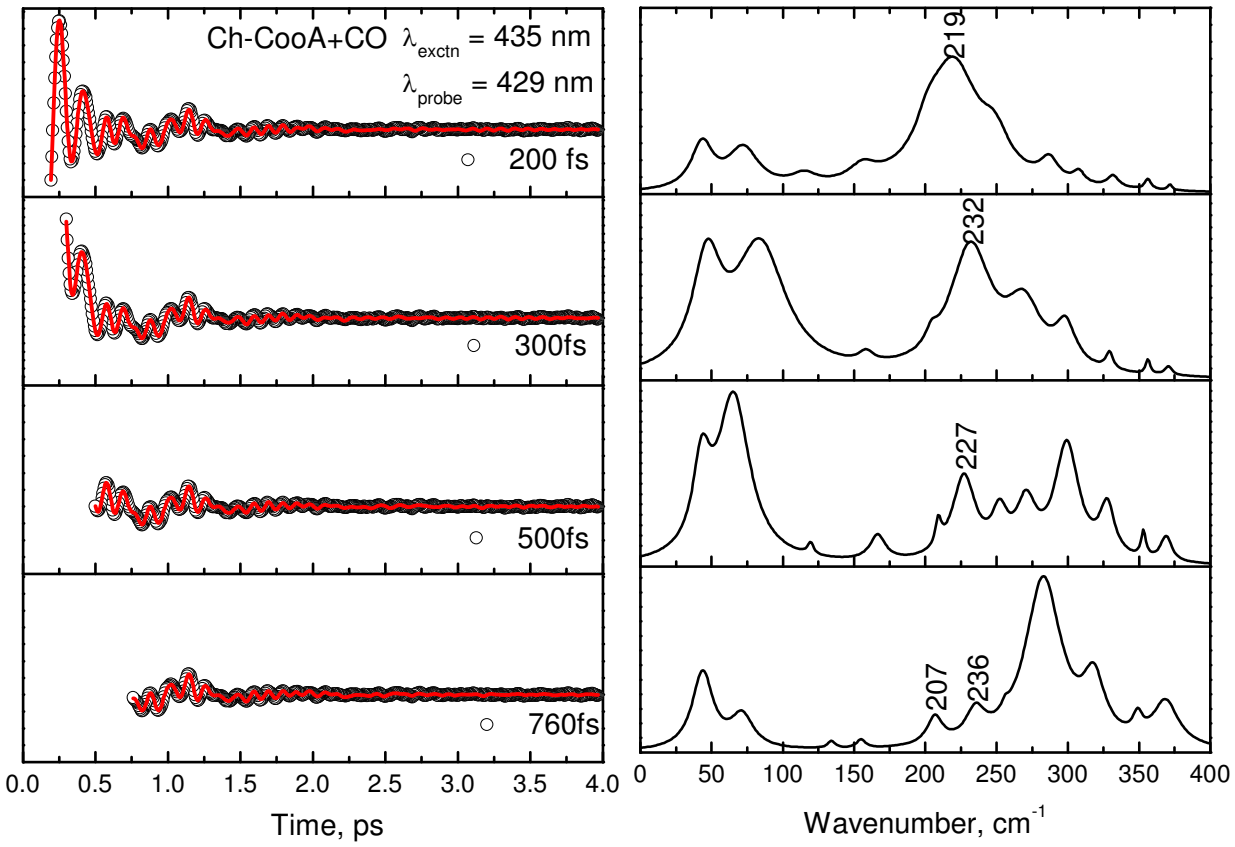

Figure S2 Different time domain (starting with initial time at 200, 300, 500 and 760 fs) analysis with LPSVD for the detune data of CO bound form of Ch-CooA. The left panels present the oscillatory signal and the LPSVD fit. The right panels show the corresponding coherence spectra.

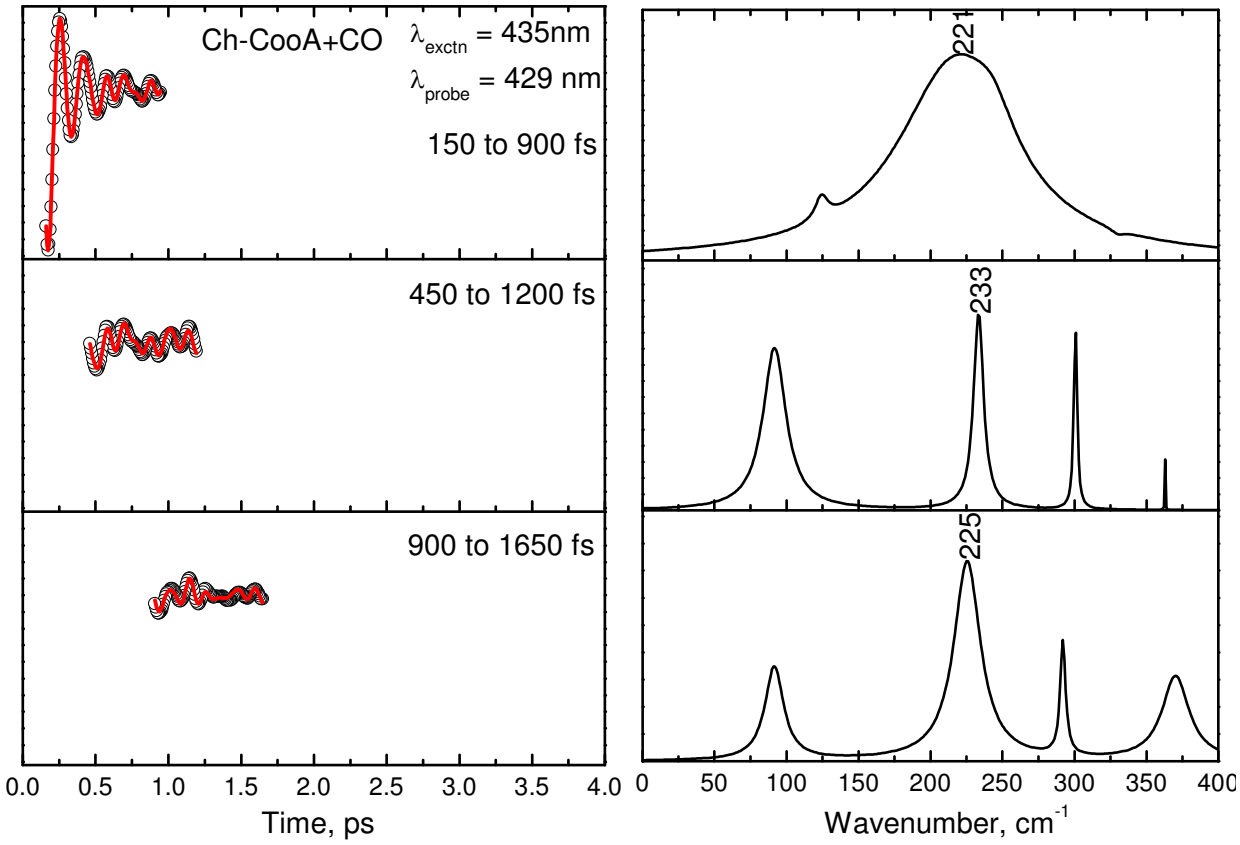

Figure S3 Different specific time domain analysis by LPSVD for the detune data of CO bound form of Ch-CooA. The left panels present the oscillatory signal and the LPSVD fit. The right panels show the corresponding coherence spectra.

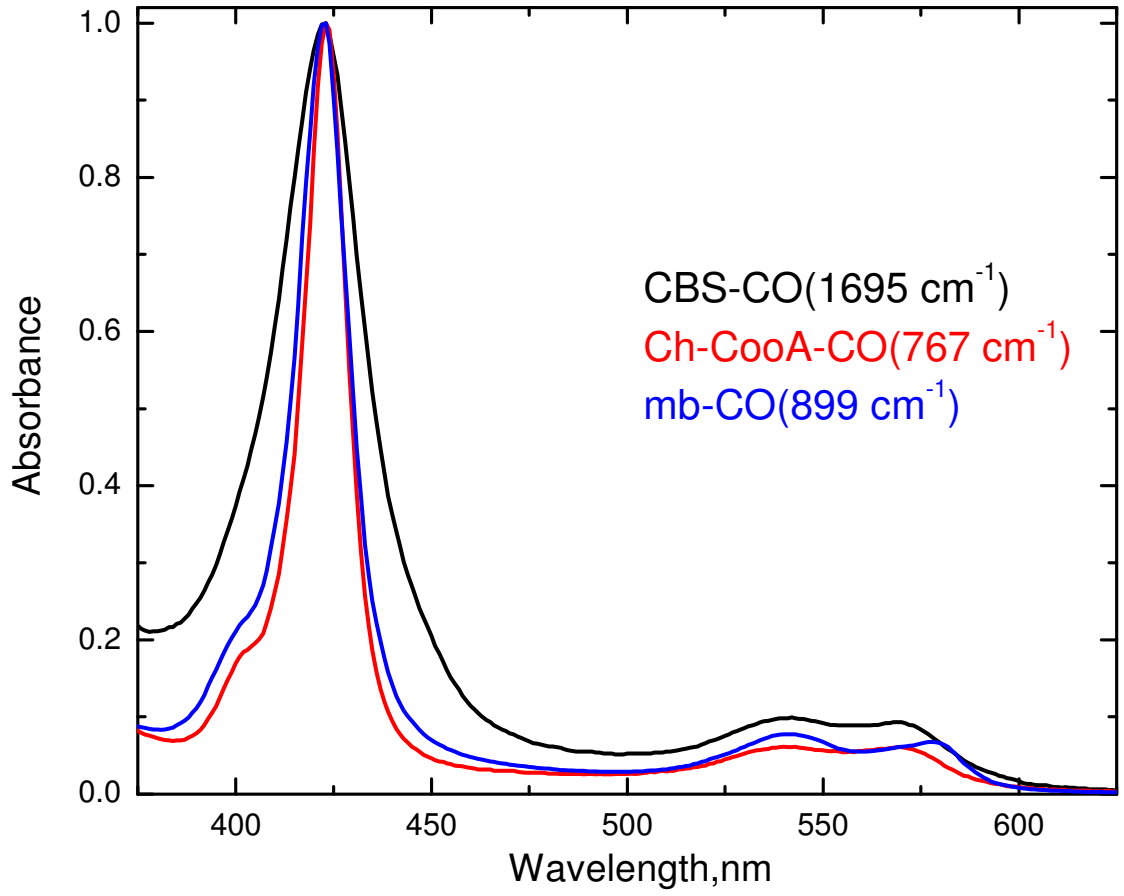

Figure S4 Absorption spectra of the CO complex of various heme proteins: CBS (black), Ch-CooA (red) and myoglobin (blue) and their full width at half maximum (FWHM) values are shown in parenthesis.

Heme Proteins	**ν(Fe–His), cm^{-1}**
soluble guanylate cyclase[3]	204
cytochrome c oxidase[4]	214
cytochrome c [5]	216[a]
myoglobin[6]	220
R-state HbA[7]	221
horseradish peroxidase[8]	244[b]
cytochrome c peroxidase[9]	247
lactoperoxidase[10]	255

Table S1 Fe–His stretching frequencies of various high spin, 5C reduced heme proteins.

[a]Transiently observed after methionine photo-dissociation, [b]Alkaline form.

Reference List

(1) Guex, N.; Peitsch, M. C. *Electrophoresis* 1997, *18*, 2714-2723.

(2) Yu, A. C.; Ye, X.; Ionascu, D.; Cao, W. X.; Champion, P. M. *Rev.Sci.Inst.* 2005, *76*.

(3) Deinum, G.; Stone, J. R.; Babcock, G. T.; Marletta, M. A. *Biochemistry* 1996, *35*, 1540-1547.

(4) Choi, S.; Lee, J. J.; Wei, Y. H.; Spiro, T. G. *J.Am.Chem.Soc.* 1983, *105*, 3692-3707.

(5) Negrerie, M.; Cianetti, S.; Vos, M. H.; Martin, J. L.; Kruglik, S. G. *J.Phys.Chem.B* 2006, *110*, 12766-12781.

(6) Kitagawa, T.; Kyogoku, Y.; Iizuka, T.; Saito, M. I. *J.Am.Chem.Soc.* 1976, *98*, 5169-5173.

(7) Friedman, J. M.; Stepnoski, R. A.; Stavola, M.; Ondrias, M. R.; Cone, R. L. *Biochemistry* 1982, *21*, 2022-2028.

(8) Teraoka, J.; Kitagawa, T. *J.Biol.Chem* 1981, *256*, 3969-3977.

(9) Wang, J.; Boldt, N. J.; Ondrias, M. R. *Biochemistry* 1992, *31*, 867-878.

(10) Manthey, J. A.; Boldt, N. J.; Bocian, D. F.; Chan, S. I. *J.Biol.Chem* 1986, *261*, 6734-6741.