Quaternary Structure Controls Ligand Dynamics in Soluble Guanylate Cyclase

Byung-Kuk Yoo, Isabelle Lamarre, Jean-Louis Martin, and Michel Negrerie[1]

From the Laboratoire d'Optique et Biosciences, INSERM U696, CNRS UMR 7645 Ecole Polytechnique, 91128 Palaiseau, France.

michel.negrerie@polytechnique.fr

Table S1. Time constants and relative amplitudes of each exponential components for NO rebinding to β_1(190) from fitted kinetics at single wavelengths in Figure 1F. The excited state decay (τ_{ex} = 4.3 ps) was included for fitting, but not for calculating the relative amplitudes which represent only the heme and diatomic dynamics. To calculate the amplitudes listed in Table 1, we used the values at 426 nm where both 4c-coordinate heme and 6c-NO heme have their absorption maximum, assuming similar absorption coefficients.

Wavelength	τ_1 (*ps*)	A_1	τ_2 (*ps*)	A_2	τ_3 (*ns*)	A_3	*Cst*
396 nm	7.1	*-0.89*	–	–	–	–	*-0.11*
415 nm	7.0	*0.58*	240	*-0.10*	1.98	*-0.07*	*-0.25*
428 nm	6.8	*0.33*	235	*-0.17*	2.0	*-0.12*	*-0.38*
435 nm	6.9	*0.58*	222	*-0.09*	2.0	*-0.11*	*-0.22*
450 nm	7.2	*0.63*	220	*0.10*	1.95	*0.07*	*0.20*

Table S2. Fitted parameters for excited states kinetics in Figure 2.

Kinetics	τ_1 (*ps*)	A_1	τ_2 (*ps*)	A_2	τ_3 (*ns*)	A_3	*Cst*
at 443 nm	4.3	*0.74*	22	*0.23*	0.3	*0.03*	*0*
at 427 nm	4.3	*-0.43*	22	*-0.12*	0.4	*-0.13*	*-0.32*
SVD1	4.3	*-0.44*	22	*-0.12*	0.4	*-0.12*	*-0.32*
SVD2	4.3	*0.55*	11	*0.33*	–	–	*0.12*

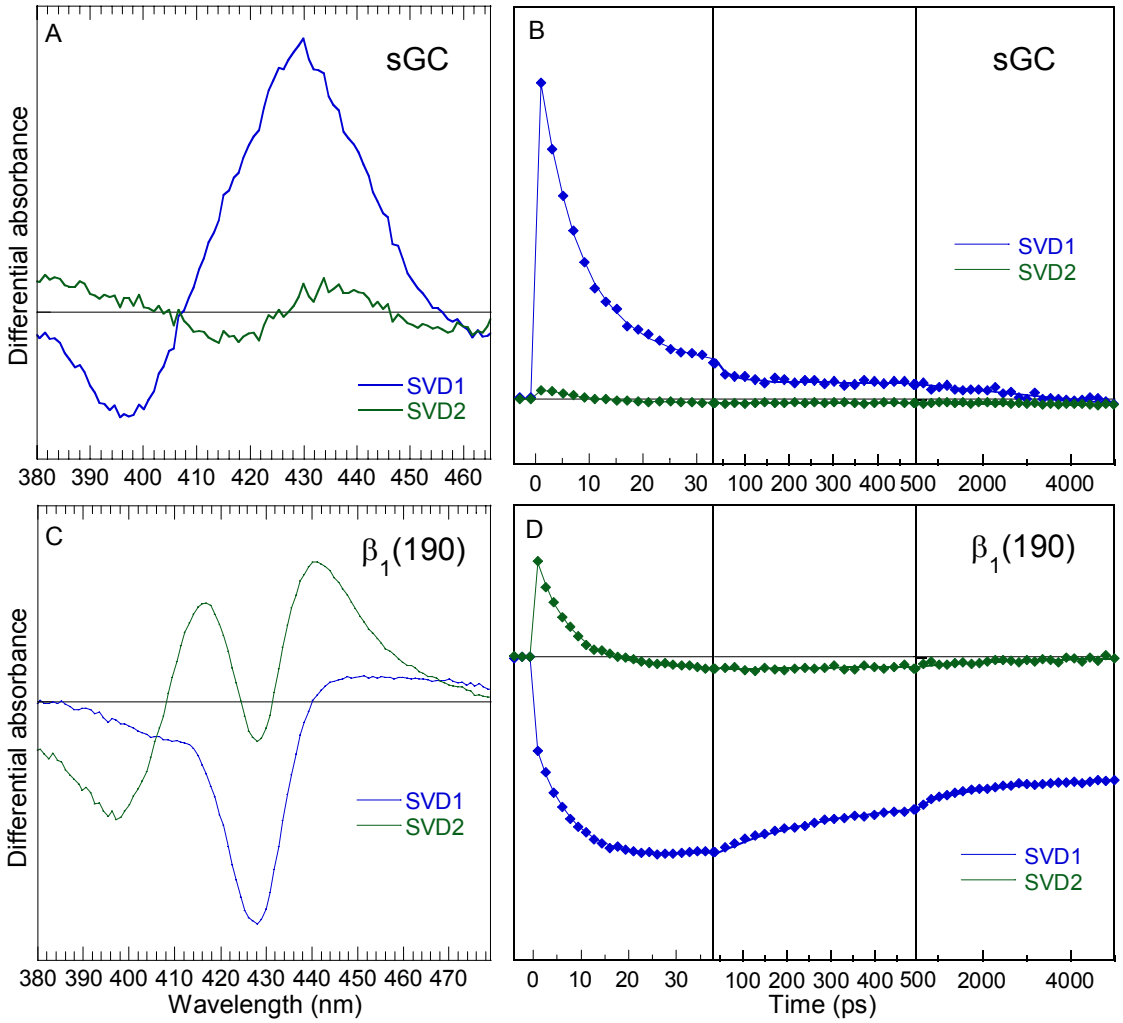

Figure S1. Global analysis by Singular Value Decomposition of the time-wavelength data matrices. Panels **A** and **B** represent respectively the spectral and kinetic orthogonal components for the entire sGC. **C** and **D** are the same for the heme domain. While in the first case NO geminate rebinding could be extracted, this was not possible in the second case because the photodissociated 6c-NO heme and the photoproduct 4c-heme have Soret absorption maximum at the same position. We therefore also fitted the kinetics at single wavelengths (Figure 1F).

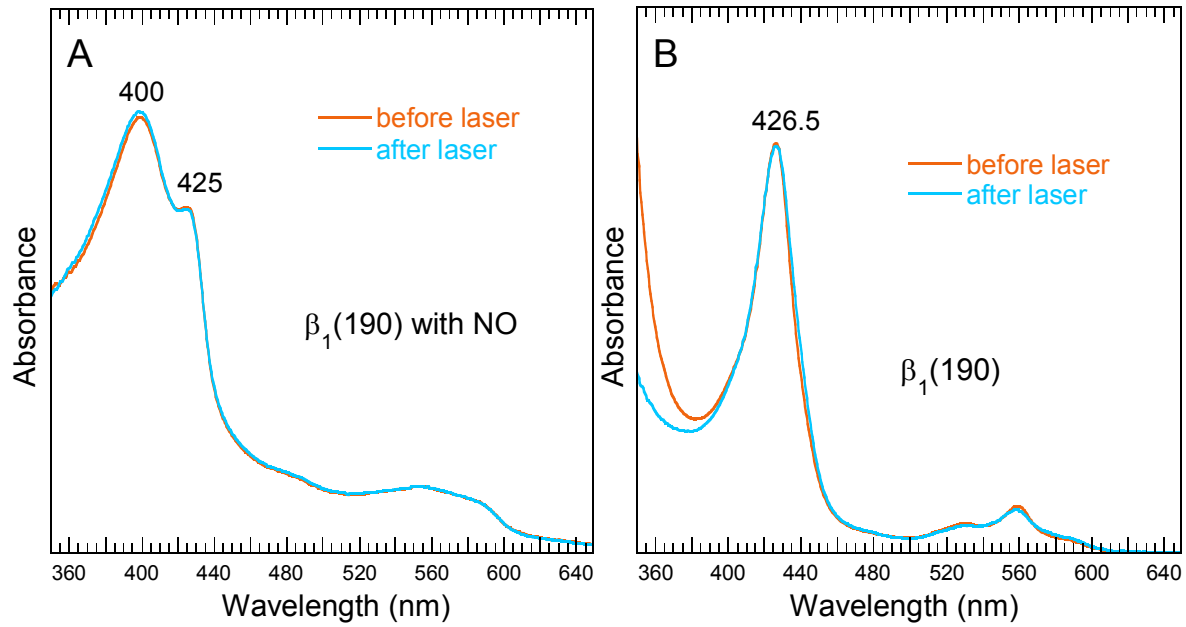

Figure S2. Steady-state absorption spectra comparison. **A**: Reduced NO-liganded β_1(190) heme domain immediately before and after laser experiment. **B**: Reduced unliganded β_1(190) heme domain immediately before and after laser experiment. In this case, the decrease of absorption below 370 nm is due to slow oxidation of dithionite.

The shoulder at 425 nm in panel A is due to a minor ferrous 6-coordinate species which could not be removed by chromatography. We thus discarded the possibilty of a denatured protein. Two possibilities can account for its formation: 1- the heme is bis-ligated, with an internal side-chain bound to the iron in the distal side; 2- the formation of a 6-coordinate His-Fe-NO (opposed to the 5c-NO in sGC). This shoulder did not disappear when using 100% NO. Because we do not observed any induced absorption at 430-435 nm at 5 ns (Fig. 1E), we discarded the possibility of His-Fe-NO, which can be photo-dissociated. We thus believe that this 6-coordinate species is a bis-ligated heme with an internal side-chain also bound to the distal side. Consequently, one should hypothesize that either a small proportion is present together with β_1(190)-CO, with its minor contribution hiden by the strong β_1(190)-CO absorption, or CO can displace it. We believe that this species is formed because of the more flexible character of the isolated heme domain β_1(190) with respect to the full-length protein.

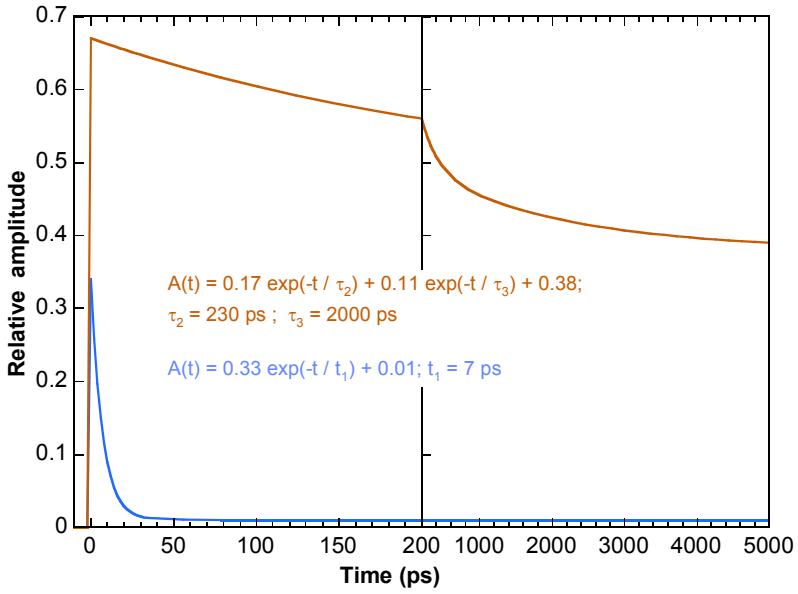

Figure S3. Calculated kinetics of the respective contributions of NO geminate rebinding to the 4c-heme (blue) and of the back-reduction after photo-oxidation (orange). The relative amplitudes are based upon the differential absorbances measured at 428 nm, assuming similar absorption coefficients for the 4c-heme and the 5c-His reduced heme. This similarity is assumed from the ratio between induced absorption and bleaching absorbance in transient spectrum of sGC at +2 ps, which is similar to the ratio between absorption coefficients of 5c-NO and 5c-His (Ref. S1, S2). The constant term is assigned to oxidized species, with no hypothesis about its back-reduction which takes place after 5 ns.

S1. Karow, D. S., Pan, D. H., Davis, J. H., Behrends, S., Mathies, R. A., and Marletta, M. A. (2005) *Biochemistry* **44**, 16266-16274

S2. Martin, E., Berka, V., Tsai, A. L., and Murad, F. (2005) *Met. Enzymol.* **396**, 478-492

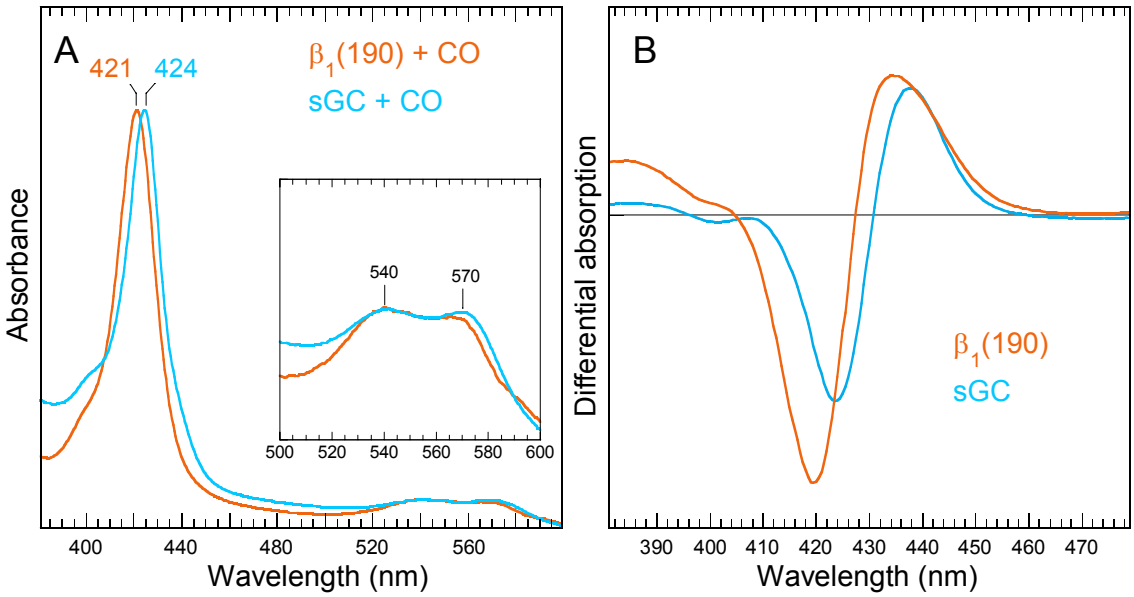

Figure S4. A: Steady-state absorption spectra comparison of CO-liganded β_1(190) and CO-liganded sGC. **B**: Difference of steady-state spectra of ferrous unliganded minus ferrous CO-liganded for sGC and β_1(190).

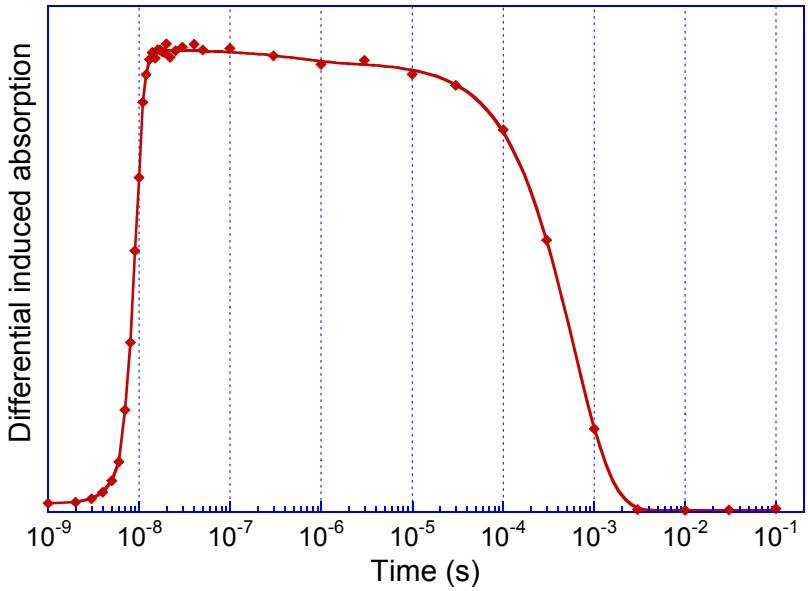

Figure S5: Kinetics of CO rebinding to myoglobin on extended time scale after CO photodissociation with a 5-ns pulse in the presence of [CO] = 1.33 mM. Data were fitted to a sum of two exponentials, yielding a bimolecular time constant τ_{bi} = 0.6 ± 0.02 ms (A_{bi} = 98%). The association rate constant is k_{on} = 1.25 ± 0.1 × 10^6 $M^{-1}{\cdot}s^{-1}$.