SUPPORTING INFORMATION

REVERSIBLE HEME-DEPENDENT REGULATION OF HUMAN CYSTATHIONINE β-SYNTHASE BY REDUCTIVE CARBONYLATION[†]

Omer Kabil[1], Colin L.Weeks[2], Sebastián Carballal[3,4], Carmen Gherasim[1], Beatriz Alvarez[3], Thomas G. Spiro[2] and Ruma Banerjee[1]

EXPERIMENTAL PROCEDURES

Purification of CBS and MSR—Recombinant human CBS (*1*) and MSR (*2*) were purified as described previously. The activity of ferric, ferrous and ferrous-CO CBS were measured under anaerobic conditions using the radiolabeled assay as described (*1*).

Reductive Carbonylation of CBS with MSR and CO—The reaction mixture for reductive carbonylation of CBS contained 0.5 μM MSR, 0.5 mM NADPH and 5 μM CBS in 100 mM potassium phosphate buffer, pH 7.4, prepared under anaerobic conditions in a quartz cuvette. The spectrum of reduced MSR was recorded and served as the baseline before addition of CBS and changes in the heme spectrum were monitored by UV-visible absorption spectroscopy. The cuvette was sealed and the headspace was purged with CO for15 min. Alternatively, the CO-saturated buffer was employed. Ferrous-CO CBS was oxidized by exposing the sample to air for 30 to 45 min.

CBS-CO Photolysis—The CO adduct of CBS was prepared by adding an excess of sodium dithionite to a solution of ferric CBS (23-50 μM) in 100 mM Tris buffer, pH 8.6 saturated with CO. The ferrous-CO CBS solution was left for 2 h after initiation of CO binding to allow time for conversion of PLP to the enolimine tautomer. The sample (1-1.5 mL) was then placed in a 5 mL pear-shaped flask in an ice-bath, and evacuated/Ar-purged three times prior to the start of photolysis. Photolysis was carried out using a lamp equipped with a halogen bulb (300 W, 120 V, Osram ELH) with a gentle stream of Ar blowing over the solution while it was slowly stirred for 40-60 min. A 3 L glass beaker filled with water was employed as a simple lens to focus the light onto the ferrous-O CBS solution. The sample was evacuated twice during the irradiation period, then Ar purging was resumed.

Spectroscopic Characterization—Fluorescence emission spectra were recorded on a Perkin-Elmer LS-50B fluorimeter with 410 nm excitation as previously described (*3*). Raman spectra were recorded using 390 nm excitation from a Ti:sapphire 1 kHz pulsed laser as previously described (*3*). UV-visible absorption spectra of CBS solutions in a 1.0 cm quartz cuvette or the 0.4 cm quartz fluorescence cuvette

were recorded using an Agilent 8453 diode array spectrophotometer. The concentrations of the CBS solutions were determined using ε_{428} = 81 mM^{-1} cm^{-1} for ferric CBS (*4*).

SI References

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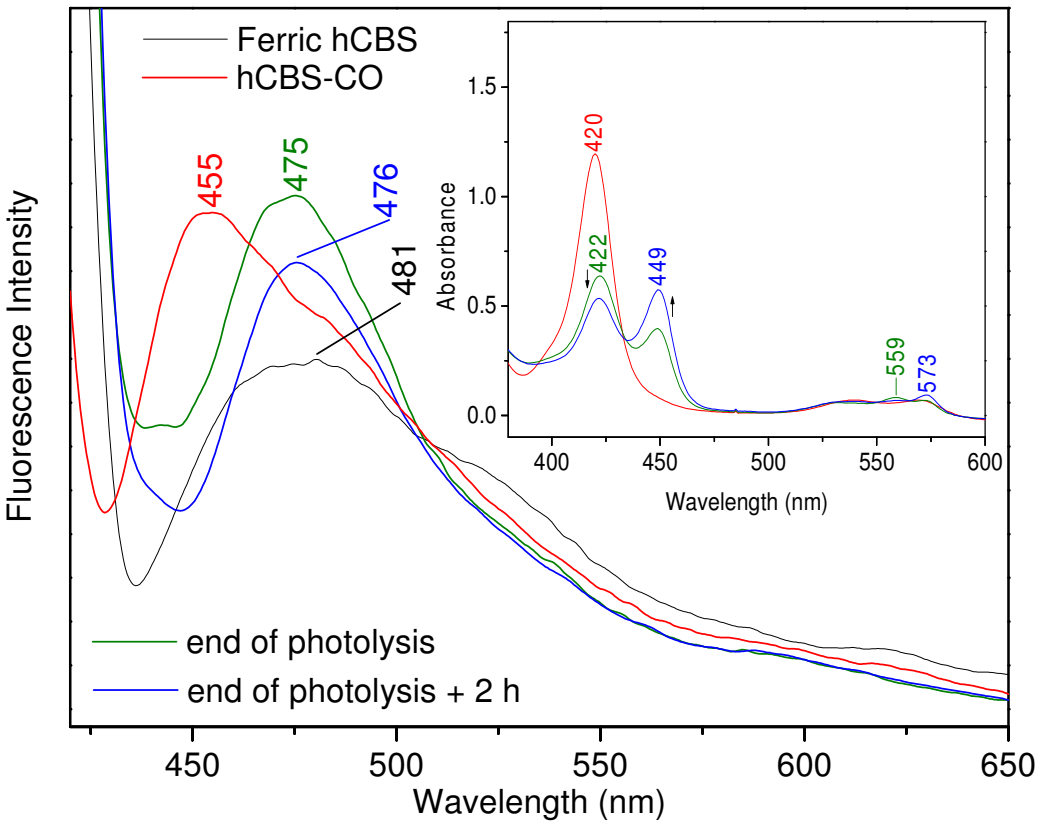

Figure S1 Fluorescence emission spectra of the indicated forms of 23 μM CBS in Tris buffer pH 8.6 (100 mM) recorded using excitation at 410 nm. Spectra were obtained from a sample that was converted from ferric CBS to ferrous-CO CBS and then photolyzed. *Inset*: UV-visible absorption spectra.

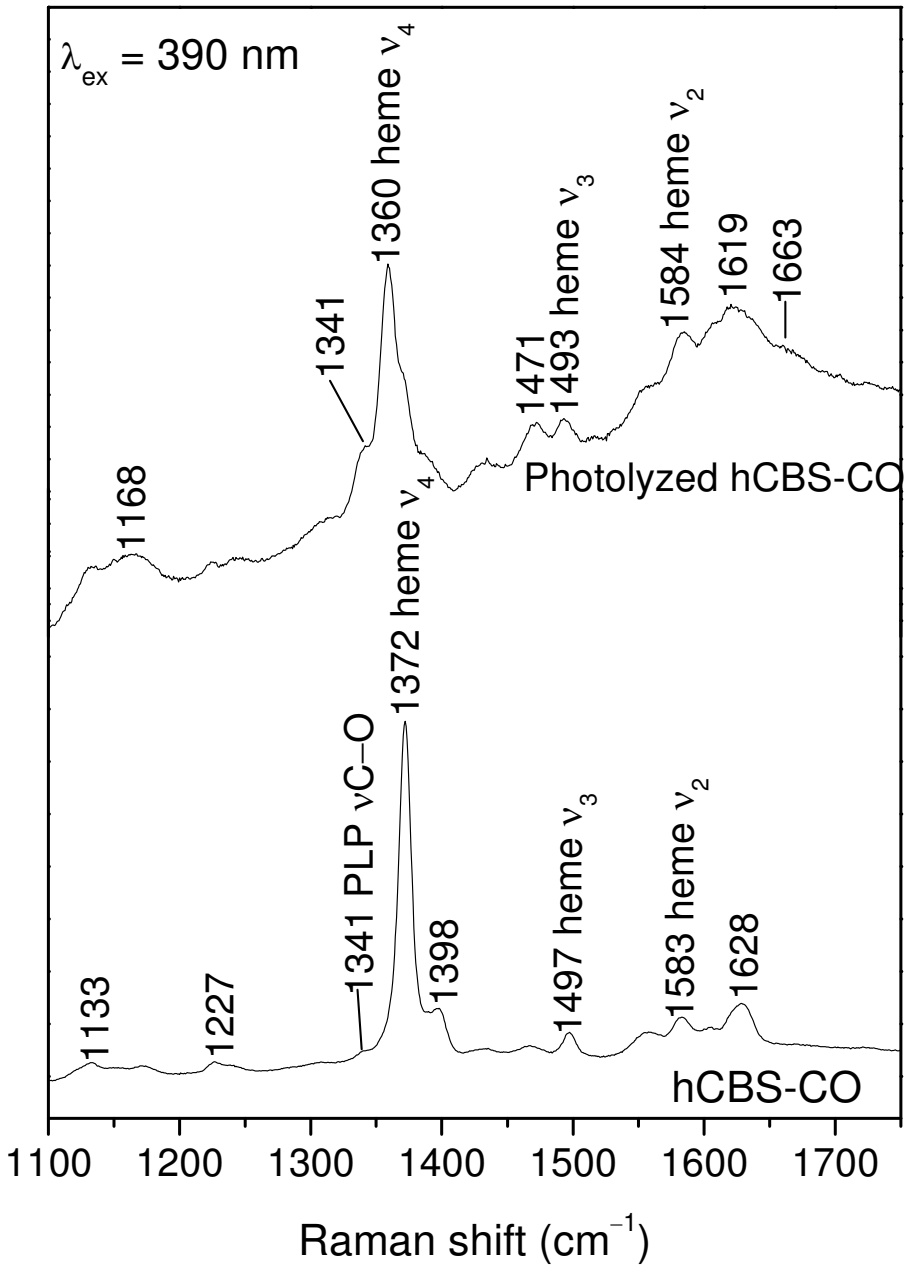

Figure S2. Raman spectra of photolyzed CBS-CO and ferrous-CO CBS obtained using 390 nm excitation. These spectra were used for deconvolution as shown in Fig. 2.