Supporting Information

Insight into the rescue of oxidized soluble guanylate cyclase by the activator cinaciguat

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Figure S1: Loss of heme from ferrous sGC. UV–visible spectra of ferrous sGC (1.9 μM) in the presence of the apoMb H64Y/V68F trap (50 μM) over the 15 hr timecourse. The activity of ferrous sGC after a 15 hr incubation with apoMb H64Y/V68F trap (inset).

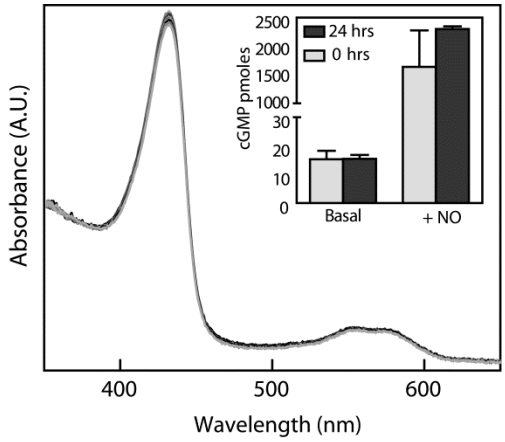


Figure S2: The rates of heme dissociation from oxidized sGC heme domains (β1(1-194), β1(1-385) and β2(1-217)) were determined in a similar manner to full length sGC (A). The maximum spectral changes were fitted to a one phase exponential growth to obtain rate constants. The half-life ($t_{1/2}$) calculated from these rate constants are shown in comparison to oxidized full length sGC (B).

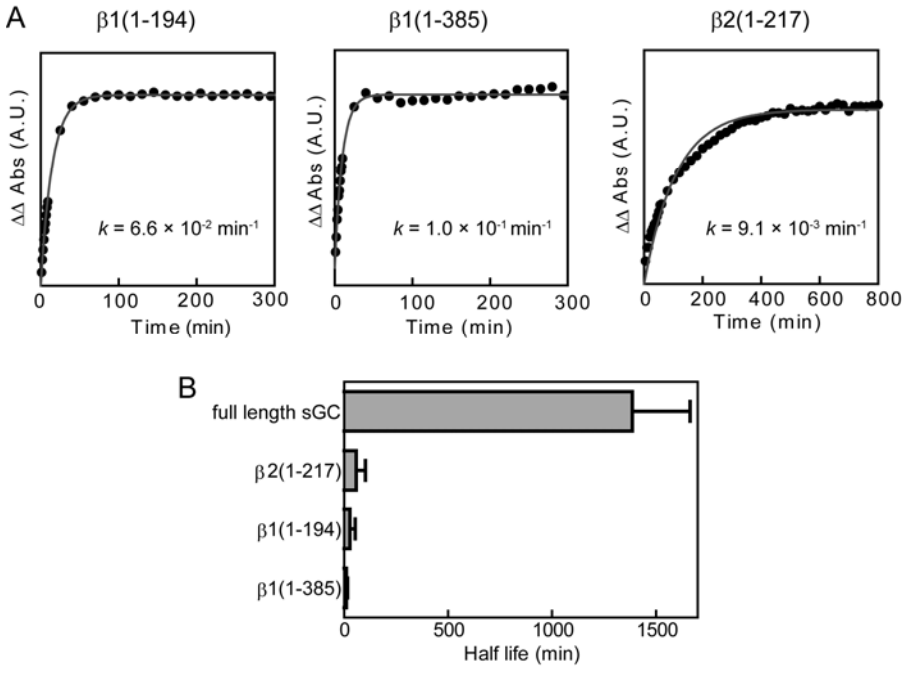


Figure S3: No heme loss from ferrous sGC (0.8 μM) was observed in the presence of cinaciguat (8 μM) with the apoMb H64Y/V68F trap (50 μM) during 15 hr of incubation at 22°C. The changes in UV-visible spectra are due to light scattering rather than heme loss since there was no shift in the Soret absorbance maximum (430 nm).

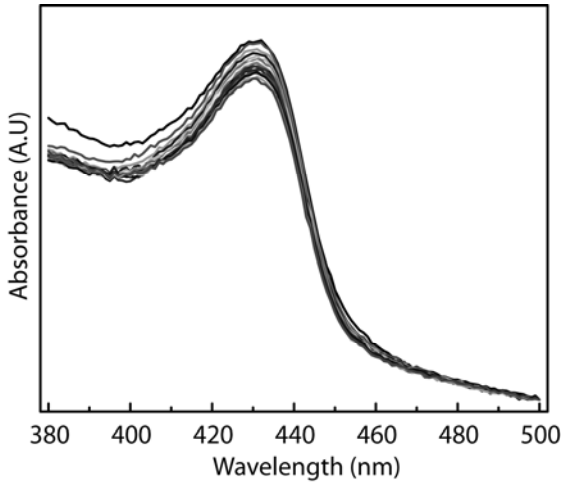


Figure S4: Change in activity of oxidized sGC during heme replacement by cinaciguat. Correlation of oxidized sGC (1.8 μM) heme loss with activity in the presence of cinaciguat (20 μM) is shown.

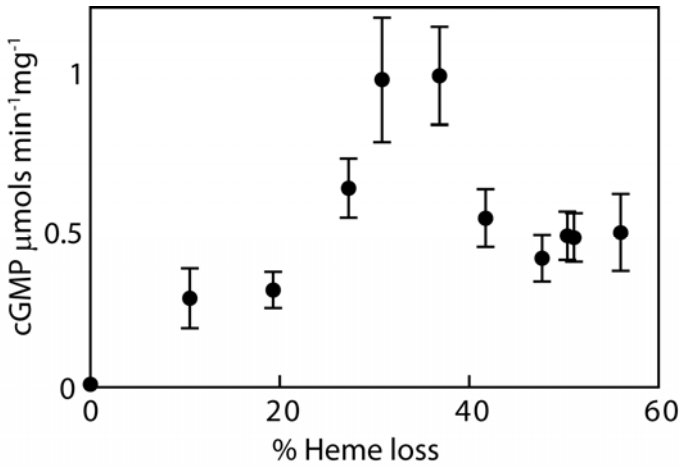
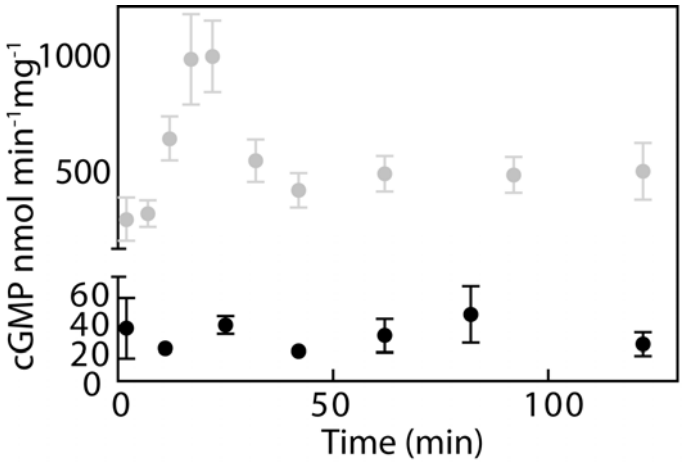


Figure S5: Comparison of oxidized sGC (1.8 μM) activity alone (black) with time and in the presence of cinaciguat (20 μM) (gray).



SUPPORTING EXPERIMENTAL PROCEDURES

Purification of Mb(H64Y/V68F): RP523 *E. coli* cells containing the pCW vector with Mb(H64Y/V68F) were grown in TB media containing ampicillin (100 µg/mL) and hemin (30 µg/mL) at 37°C. Expression of Mb(H64Y/V68F) was induced by addition of IPTG (1 mM) and was allowed to occur for 16-18 hrs at 22°C. Cells were harvested by centrifugation and frozen at -80 °C. Cell pellets of were thawed on ice and resuspended in buffer A (100 mL, 50 mM sodium phosphate at pH 8.0, 200 mM NaCl, 1 mM benzamidine hydrochloride). Pefabloc (1 mM) and DNase I were added during lysis. The resuspended cells underwent homogenization (EmulsiFlex-C5 homogenizer) and centrifugation (Optima XL-100K) at 40,000 rpm for 1-2 hrs. The supernatant was applied at 0.5 mL/min to a Ni_2NTA Superflow column (Qiagen) that had been equilibrated with buffer A. The column was washed at 1 mL/min with buffer A containing 25 mM imidazole until the absorbance at 280 nm was constant. The protein was then eluted at 1 mL/min with buffer A containing 250 mM imidazole. The eluate was concentrated to 3 mL using a Vivaspin 20 5,000 MWCO PES concentrator and loaded onto the size exclusion column (HiLoad 26/60 Superdex 75 column,GE Healthcare). The protein was then separated with an isocratic flow of buffer B (50 mM HEPES at pH 7.5, 50 mM NaCl) at 0.4 mL/min and 4 mL fractions were collected.

Purification of β1(1-194): Expression and purification of β1(1-194) was identical to Mb(H64Y/V68F) except the following changes. Cell lysis was accomplished in buffer C (50 mM sodium phosphate at pH 8.0, 300 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol, and1 mM benzamidine hydrochloride). The Ni_2NTA Superflow column was also equilibrated with buffer C. After loading the supernatant the column was washed with buffer C containing 25 mM imidazole. The protein was eluted in buffer C containing 150 mM imidazole. During the size exclusion step the protein was separated with an isocratic flow of buffer D (50 mM TEA at pH 7.5, 150 mM NaCl, 5 % glycerol and 5 mM dithiothreitol).

Heme-dissociation from sGC heme-domains: As in with full length sGC, the rates of heme dissociation were determined from the decrease in Soret absorbance of sGC heme domains, and the concurrent increase in myoglobin H64Y/V68F Soret absorbance. The experiments were conducted identical to the full length experiments with the following changes. The proteins (18-23 µM) were oxidized with potassium ferricyanide (10 mM) in buffer E (50 mM Hepes at pH 7.4, 50 mM NaCl). Excess ferricyanide was removed by buffer exchange to buffer E using PD MiniTrap™ G-25 (GE Healthcare). The proteins were then diluted to a final concentration of 1 µM and apoMb(H64Y/V68F) was added to a final concentration of 20 µM in buffer E.

Determination of heme-dissociation rate constants: The maximal absorbance difference between two wavelengths (391 nm and 417 nm for ferric sGC) was chosen to fit equation (1) below to obtain rate constants where *k* is the rate constant, *t* is time, and $\Delta\Delta Abs = Abs_{391} - Abs_{417}$.

$$\Delta\Delta Abs = y0 + (Plateau - y0) \times (1 - e^{-kt}) \qquad (1)$$