Supporting Materials

Structural and functional insights into heme-binding domain of human soluble guanylate cyclase α2 subunit and heterodimeric α2β1

Hongyan Wang · Fangfang Zhong · Jie Pan · Wei Li · Jihu Su · Zhong-Xian Huang · Xiangshi Tan

Primers for cloning of hsGC α2_H and hsGC β1_H-α2_H

P1: 5’-ATGGATCCGGCGGCGGAGCGGTTCGCTCAGACGATAC-3’
P2: 5’-TCTAAGCTTTCAGGAGGTCCCTCTGGAAG-3’
P3: 5’-ATGGATCCGGCGGCGGAGCGGTATGTACGGATTTGTGAATC-3’
P4: 5’-CTGTATCGTCTGAGGCGAGCTGCCACCGCTGCCGCTACCGCTGCCGCAGCTCGCCTCAGACGATACAG-3’
P5: 5’-GATTTTTATGAAGATCTTTGGGCGAGCGGTAGCGGCGAGCGGTGGCCAGCTCGCCTACGACGATACAG-3’

Fig.S1 Heme loss of hsGC monitored via UV/Vis indicating heme transfer from hsGC in oxidized state to apo-myoglobin (black line) forming holo-myoglobin (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2_H; (B), hsGC β1_H-α2_H; (C) hsGC β1_H; (D) hsGC α1_H.

Fig.S2 Heme loss of hsGC monitored via UV/Vis indicating no heme transfer from
reduced hsGC or hsGC-CO complex to apo-myoglobin (black line) to form holo-myoglobin (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2H ; (B), hsGC β1H-α2H ; (C) hsGC β1H; (D), hsGC α2H-CO; (E), hsGC β1H-α2H-CO; (F) hsGC β1H-CO.

**Fig. S3** Heme loss of hsGC in NO-bound form monitored *via* UV/Vis indicating heme transfer from hsGC-NO to apo-myoglobin (black line) forming metmyoglobin-NO complex (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2H; (B), hsGC β1H-α2H; (C) hsGC β1H; (D) hsGC α1H; (E) hsGC β1H H105G

**Fig. S4** Signal prediction analysis of the N-terminal 100 residues of hsGC α2 subunit.

**Fig. S5** Time courses for heme transfer from hsGC H-NOX domain constructs to apo-myoglobin. Heme dissociation rate constants (koff) were measured by UV-vis spectrometer with kinetic mode from the heme transfer experiments using the apo-myoglobin assay. The heme transfer process was monitored at 409 nm for ferric hsGC α2H (A), hsGC β1H-α2H (B), hsGC β1H (C), hsGC α1H (D) and 421 nm for NO-complex hsGC β1H (E), hsGC β1H-α2H (F), hsGC α1H (G).
**Fig. S1** Heme loss of hsGC monitored *via* UV/Vis indicating heme transfer from hsGC in oxidized state to apo-myoglobin (black line) forming holo-myoglobin (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2_H; (B), hsGC β1_H–α2_H; (C) hsGC β1_H; (D) hsGC α1_H.

**Fig. S2** Heme loss of hsGC monitored *via* UV/Vis indicating no heme transfer from reduced hsGC or hsGC-CO complex to apo-myoglobin (black line) to form holo-myoglobin (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2_H; (B), hsGC β1_H–α2_H; (C) hsGC β1_H; (D), hsGC α2_H–CO; (E), hsGC β1_H–α2_H–CO; (F) hsGC β1_H–CO.
**Fig. S3** Heme loss of hsGC in NO-bound form monitored via UV/Vis indicating heme transfer from hsGC-NO to apo-myoglobin (black line) forming metmyoglobin-NO complex (red line). Protein concentration: hsGC H-NOX domain (2 μM); apo-myoglobin (20 μM). (A), hsGC α2H; (B), hsGC β1H-α2H; (C) hsGC β1H; (D) hsGC α1H; (E) hsGC β1H H105G.

**Fig. S4** Signal prediction analysis of the N-terminal 100 residues of hsGC α2 subunit
**Fig. S5** Time courses for heme transfer from hsGC H-NOX domain constructs to apo-myoglobin. Heme dissociation rate constants ($k_{off}$) were measured by UV-vis spectrometer with kinetic mode from the heme transfer experiments using the apo-myoglobin assay. The heme transfer process was monitored at 409 nm for ferric hsGC α2H (A), hsGC β1H-α2H (B), hsGC β1H (C), hsGC α1H (D) and 421 nm for NO-complex hsGC β1H (E), hsGC β1H-α2H (F), hsGC α1H (G).