

Supplementary Materials for
**Guanylyl Cyclases A and B Are Asymmetric Dimers That Are
Allosterically Activated by ATP Binding to the Catalytic Domain**

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- Fig. S1. Detectable amounts of GC-A or GC-B protein are only found in HEK 293T cells specifically transfected with plasmids encoding each receptor.
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- Fig. S3. Kinetic values for substrate-velocity experiments presented in the indicated figures.

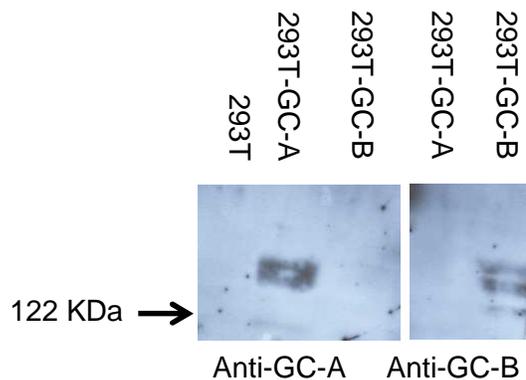


Fig. S1. Detectable amounts of GC-A or GC-B protein are only found in HEK 293T cells specifically transfected with plasmids encoding each receptor. Membrane protein (12 μ g) from untransfected HEK 293T cells or HEK 293T cells stably expressing rat GC-A (293T-GC-A) or rat GC-B (293T-GC-B) was resolved by SDS-PAGE and transferred to an Immobilon membrane as previously described (45). The membrane was cut in half and analyzed by Western blotting with rabbit polyclonal anti-serum against the C-terminus of GC-A (Anti-GC-A) or antiserum against the C-terminus of GC-B (Anti-GC-B) as shown. GC-A and GC-B are doublets in stably expressing HEK 293T cells, similar to the pattern of endogenous proteins in cell lines (59).

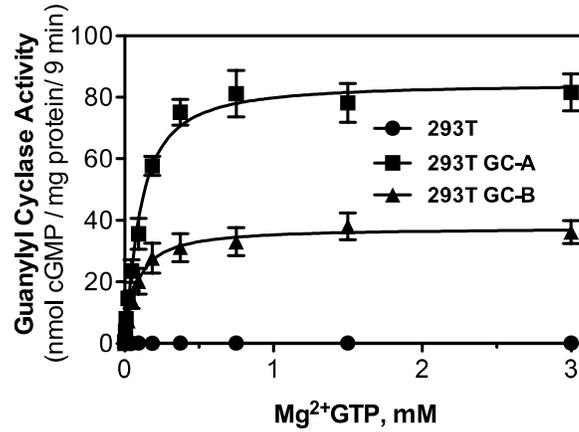


Fig. S2. HEK 293T cells do not have detectable membrane GC activity. GC activity was measured in membranes prepared from HEK 293T, 293T-GC-A, and 293T-GC-B cells in the presence of 1 mM ATP and 1 μ M ANP for HEK 293T and 293T-GC-A cells or 1 μ M CNP for 293T-GC-B cells, with increasing concentrations of Mg²⁺GTP. The Michaelis constants for GC-A and GC-B were 0.1 mM and 0.08 mM, respectively, which are similar to values measured for these receptors in cells with endogenous GCs (32). The Michaelis constant of untransfected cells was too low to be determined. Data are from two independent experiments.

Figure 1A (GC-A)		
	- ATP	+ ATP
Vmax	2.60	3.92
Km (mM)	1.8	1.6
nH	1.5	1.1*

Figure 1B (GC-B)		
	- ATP	+ ATP
Vmax	3.79	4.22
Km (mM)	1.0	1.0
nH	1.4	1.0*

Figure 1D (GC-A)		
	- ANP	+ ANP
Vmax	4.42	103.6*
Km (mM)	1.1	0.5
nH	1.3	1.2

Figure 1E (GC-B)		
	- CNP	+ CNP
Vmax	3.94	48.8*
Km (mM)	2.2	1.0
nH	1.3	1.3

Figure 1G (GC-A)		
	- ATP	+ ATP
Vmax	69.4	84.4
Km (mM)	0.7	0.1*
nH	1.7	1.2*

Figure 1H (GC-B)		
	- ATP	+ ATP
Vmax	48.6	41.3
Km (mM)	0.6	0.1*
nH	1.5	1.1*

Figure 2A (GC-A)			
	ATP, μ M		
	0	10	100
Vmax	64.5	68.5	78.7
Km (mM)	0.5	0.2*	0.08*
nH	1.4	1.2	0.9*

Figure 2B (GC-B)			
	ATP, μ M		
	0	10	750
Vmax	27.9	50.4	30.2
Km (mM)	0.9	0.4*	0.2*
nH	1.4	0.9*	0.9*

Figure 3B (GC-A)		
	GTP	2'dGTP
Vmax	50.4	63.2
Km (mM)	2.5	1.5
nH	1.5	1.0*

Figure 3E (GC-A)				
	Control	2'dATP	ADP	ATP
Vmax	110.6	96.0	92.7	103.9
Km (mM)	0.8	0.4*	0.2*	0.1*
nH	1.4	1.2	1.1	1.0*

Fig. S3. Kinetic values for substrate-velocity experiments presented in the indicated figures. Vmax refers to nmol cGMP generated per mg protein per unit of time (in min), Km is given in mM, and nH is the Hill coefficient. * $P < 0.05$ based on an extra sum of squares F test.