Supplemental Information

Structure and monomer/dimer equilibrium for the guanylyl cyclase domain of the optogenetics protein RhoGC

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Running title

Structure and monomer/dimer equilibrium of guanylyl cyclase

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	457
RhoGC	TEAKEYESVTVFFSDITNFTVISSRTSTKDMMATLNKLWLEYDAIAKR
Human-GUCY1A3	SMQGQVVQAKKFSNVTMLFSDIVGFTAICSQCSPLQVITMLNALYTRFDQQCGE
Human-GUCY1B3	-MHKRPVPAKRYDNVTILFSGIVGFNAFCSKHASGEGAMKIVNLLNDLYTRFDTLTDSRK
Chlamydomonas	SAPAQEHPEATVLFSDIVGFTEIASRSSPLEVCSLLDELYQRFDAAIEE
Synechocystis	GIDPFTKMGGDRRPITILTSDLRGFTSTSEGLNPEEVVKVLNIYFGKMADVITH
	497 501 *** *** ** ** ** 545
RhoGC	W-GVYKVETIGDAYLGVTGAPDVVPDHAERACNFAVDIIEMIKSFKTITGESINIR
Human-GUCY1A3	L-DVYKVETIGDAYCVAGGLHKESDTHAVQIALMALKMMELSDEVMSPHGEPIKMR
Human-GUCY1B3	NPFVYKVETVCDKYMTVSGLPEPCIHHARSICHLALDMMEIAGQV-QVDGESVQIT
Chlamydomonas	YPQLYKVETIGDAYMVVCNVTVPCDDHADVLLEFALRMHEEASRVASSLGEPVRIR
Synechocystis	-HGGTIDEFMGDGILVLFGAPTSQQDDALRAVACGVEMQLALREVNQQVTGLGLQPLEMG
	* * * 566 * 573 577 * * * * * * * * * *
RhoGC	IGLNSGPVTAGVLGD-LNPHWCLVGDTVNTASRMESTSKAGHIHISESTYHFIKSK
Human-GUCY1A3	IGLHSGSVFAGVVGV-KMPRYCLFGNNVTLANKFESCSVPRKINVSPTTYRLLKDC
Human-GUCY1B3	IGIHTGEVVTGVIGQ-RMPRYCLFGNTVNLTSRTETTGEKGKINVSEYTYRCLMSPENSD
Chlamydomonas	VGMHSGPVVAGVVGR-KMPRFCLFGDTVNTASRMESHGEAGQIHISEACYCCLRSK
Synechocystis	IGINTGEVVVGNIGSEKRTKYGVVGAQVNLTYRIESYTTGGQIFISSTTLEAAGDRV
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RhoGC	FVTQPLDVMEVKGKGKMQTYWVLGRKM
Human-GUCY1A3	PGFVFTPRSREELPPNFPSEIPGICHFLDAYQQGTNSKPCFQKKDVEDGNANFLGKASGID
Human-GUCY1B3	PQFHLEHRGPVSMKGKKEPMQVWFLSRKNTGTEETKQDDDAENLYFQ
Chlamydomonas	ERFEIRERGNITVKGKGTMRTYLLSPLERT
Synechocystis	HVNGNRTVQPKGVKDPVVIWDVAGVGEPYNLSLAVEEQ

Figure S1. Sequence alignment of guanylyl cyclase catalytic domains from proteins with annotated crystal structures. Sequence comparison of guanylyl cyclase domains from *B. emersonii*, human, *C. reinhardtii*, and *Synechocystis* PCC6803. The multiple sequence alignment was generated using Clustal Omega(1). Conserved amino acid residues involved in catalysis are boxed and numbered according to RhoGC, the guanylyl cyclase domain from *B. emersonii* as reference.



Figure S2. Enzyme activity as a function of substrate concentration. The concentration of GTP was varied while enzyme ($[GC_{Rho}] = 10 \ \mu\text{M}$) and metal ion ($[Mn^{2+}] = 10 \ \text{mM}$) concentrations were fixed. The data (solid circles) were fit to the equation $Rate = \frac{V_{max}(S)^n}{(S_{0.5})^n + S^n}$ (solid line) where V_{max} is the maximum velocity, *S* is the concentration of GTP, $S_{0.5}$ is the substrate concentration where the $Rate = V_{max}/2$, and *n* is the Hill coefficient: $S_{0.5} = 0.9 \pm 0.1 \ \text{mM}$, $V_{max} = 16 \pm 1 \ \mu\text{M/s}$, and $n = 1.2 \pm 0.3$. While it is not possible to confidently distinguish cooperative from non-cooperative rate behavior with these data, a Hill-type equation was used here for consistency with other guanylyl cyclases.(2)



Figure S3. Dependence of guanylyl cyclase activity on enzyme concentration. *A*, Data for GCwCC_{Rho}-Mn from Figure 8*A*, replotted on an expanded scale to more clearly illustrate non-linear dependence of activity on enzyme concentration. *B*, Control experiment showing that non-linear dependence of activity on enzyme concentration is not a consequence of non-specific protein interactions. The conditions of this experiment were identical to those in Figure 8*A* except that the total protein concentration was maintained at 50 μ M using bovine serum albumin (BSA) to compensate for decreasing concentrations of GC_{Rho}. The same non-linear dependence of activity on enzyme concentration was observed in this experiment as was found in the absence of BSA, clearly showing that the non-linear behavior does not result from non-specific protein interactions. The dotted curve is a fit of the data to equation 1 with $K_D = 49 \pm 30 \ \mu$ M and $k_{cat} = 13 \pm 3 \ s^{-1}$.



Figure S4. Structural superposition of the *B. emersonii* GC_{Rho} monomer with the crystal structure of the mammalian AC (PDB entry 1CJU) complex with substrate analog ddATP showing the location of conserved catalytic residues. GC_{Rho} molecule A was superposed on the mammalian AC molecule A. GC_{Rho} molecule B was duplicated from A and superposed on mammalian AC molecule B. Cartoon representation of GC_{Rho} and mammalian AC molecules are colored in cyan and salmon, respectively. Active site residues are shown as sticks. Active site residues for GC_{Rho} and AC are identified before and after the slash, respectively. Only one of the two symmetry-related active sites in the structure is shown for clarity.

REFERENCES

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