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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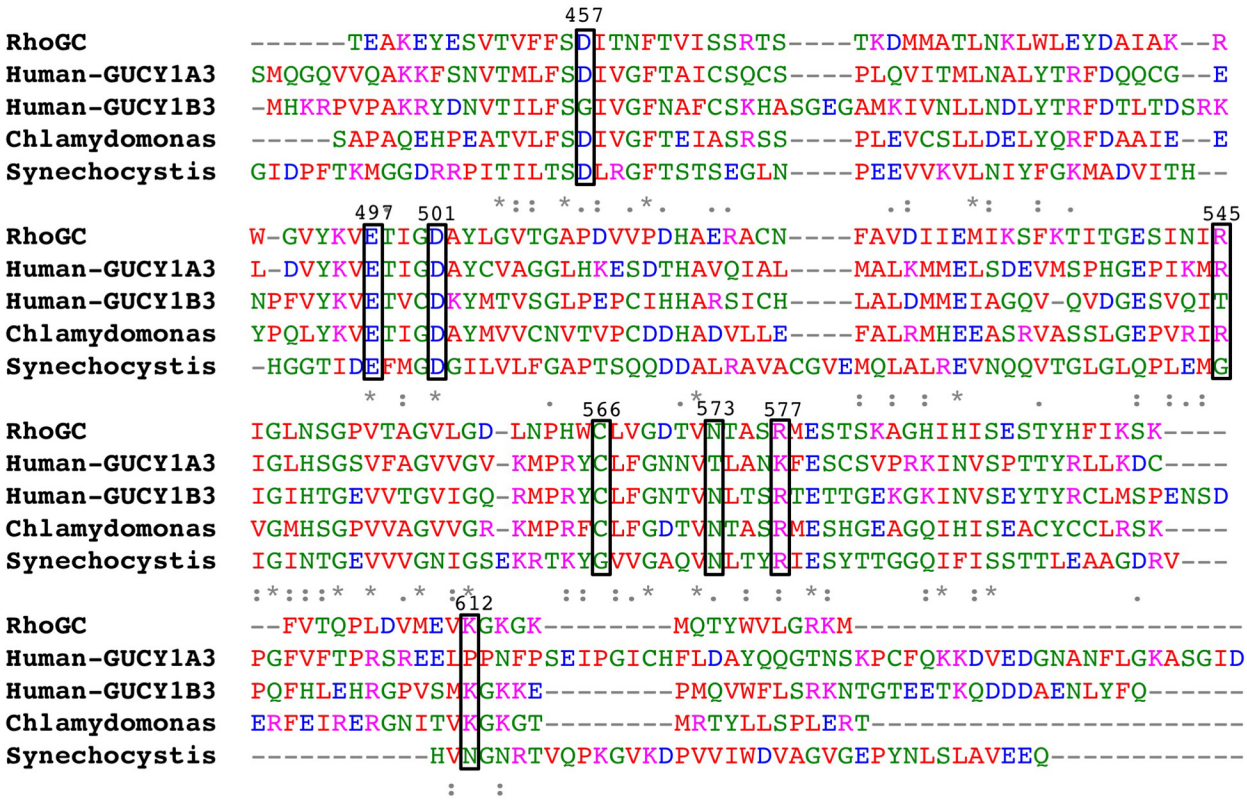

Figure S1. Sequence alignment of guanylyl cyclase catalytic domains from proteins with annotated crystal structures. Sequence comparison of guanylyl cyclase domains from *B. emersonii*, human, *C. reinhardtii*, and *Synechocystis* PCC6803. The multiple sequence alignment was generated using Clustal Omega(1).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

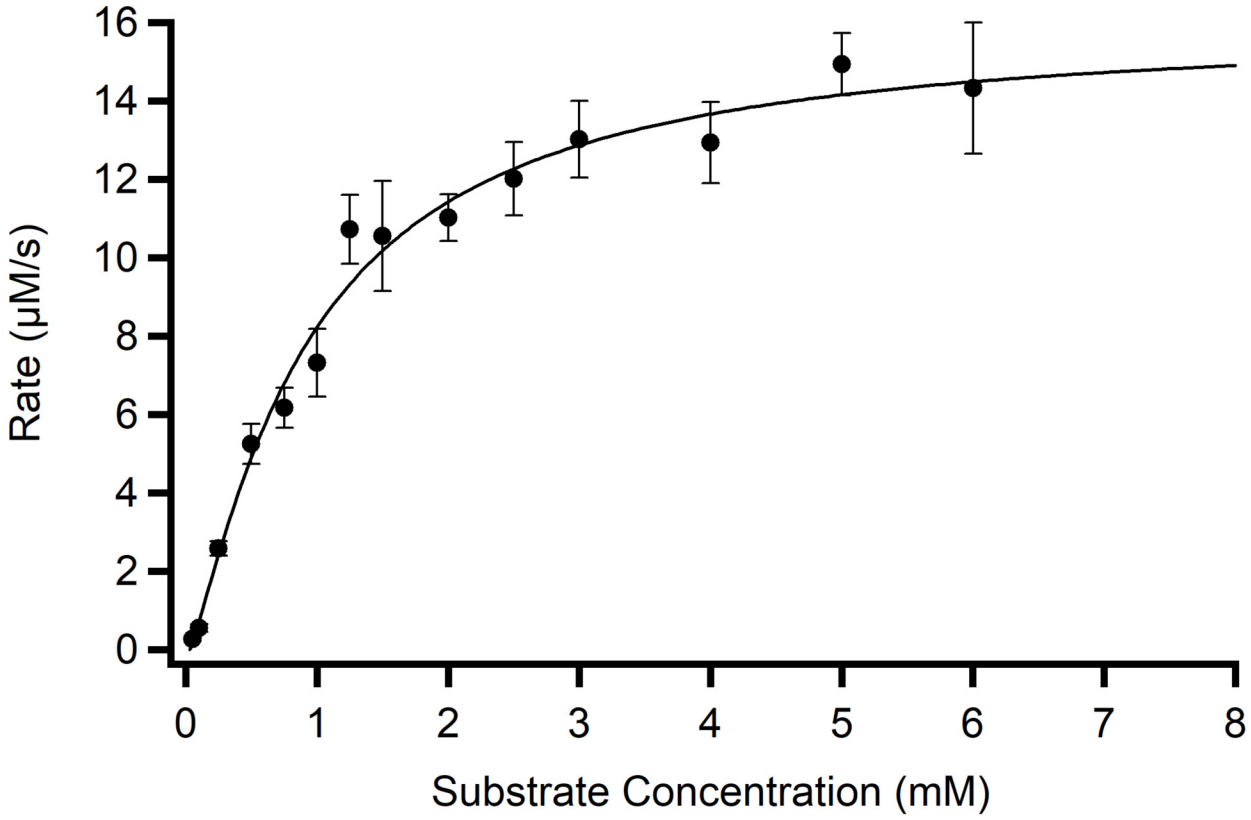

Figure S2. Enzyme activity as a function of substrate concentration. The concentration of GTP was varied while enzyme ([GC_{Rho}] = 10 μM) and metal ion ([Mn^{2+}] = 10 mM) concentrations were fixed. The data (solid circles) were fit to the equation $Rate = \frac{V_{max}(S)^n}{(S_{0.5})^n + S^n}$ (solid 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 ± 0.1 mM, V_{max} = 16 ± 1 μM/s, and n = 1.2 ± 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)

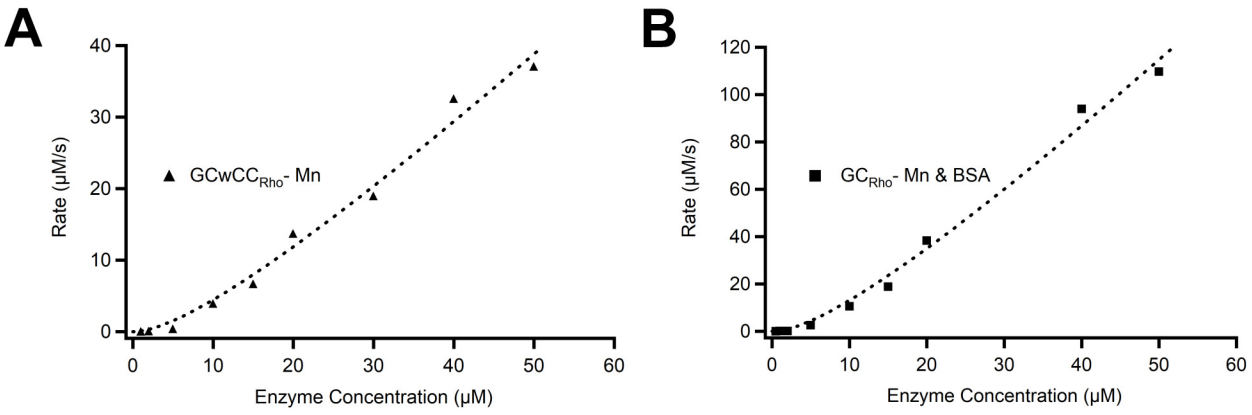

Figure S3. Dependence of guanylyl cyclase activity on enzyme concentration. *A*, Data for GCwCC$_{Rho}$-Mn from Figure 8*A*, replotted on an expanded scale to more clearly illustrate non-linear dependence of activity on enzyme concentration. *B*, Control experiment showing that non-linear dependence 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 μM 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 $K_D = 49 \pm 30$ μM and $k_{cat} = 13 \pm 3$ s^{-1}.

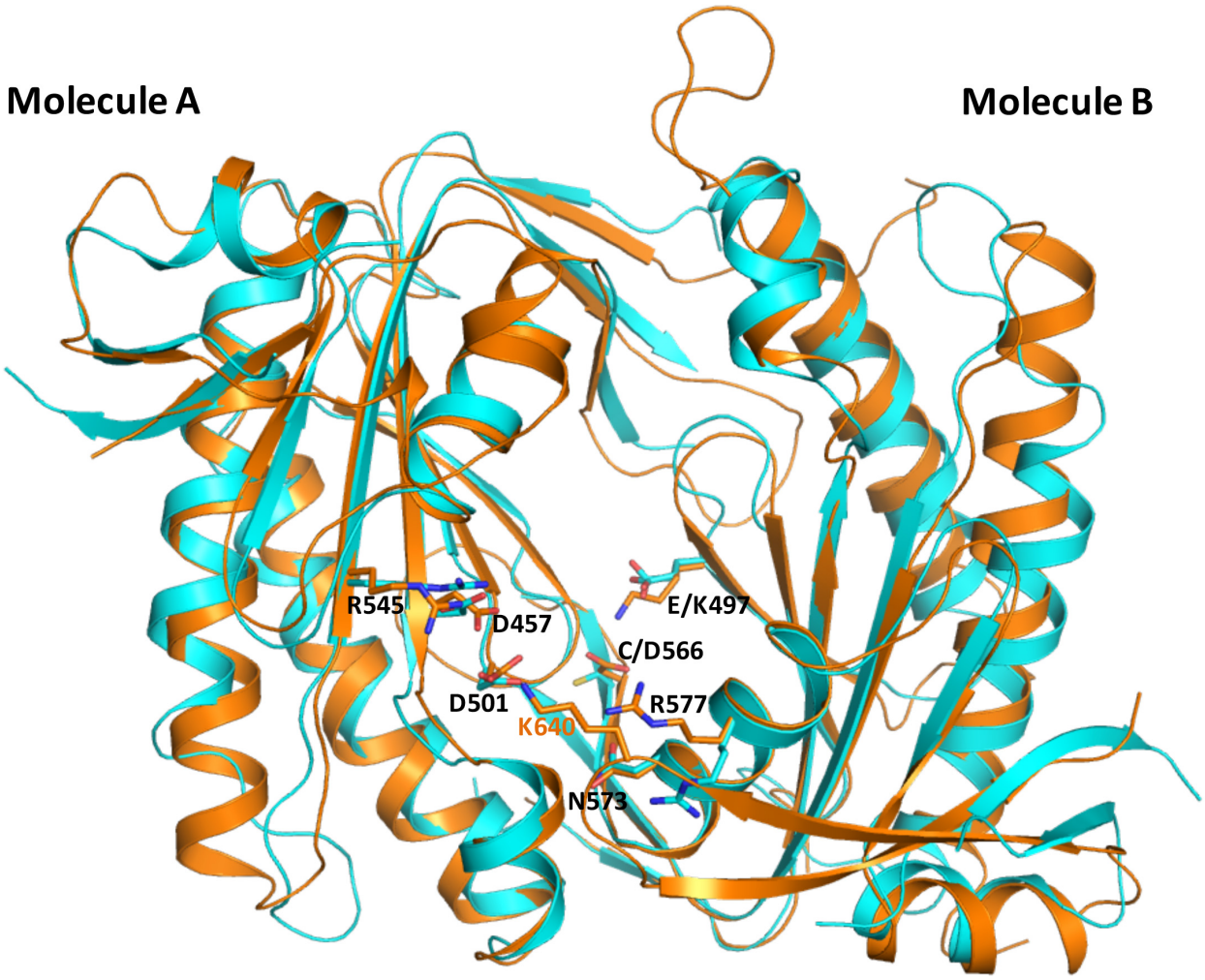

Figure S4. Structural superposition of the *B. emersonii* GC_{Rho} monomer with the crystal structure of the mammalian AC (PDB entry 1CJU) complex with substrate analog ddATP showing the location of conserved catalytic residues. GC_{Rho} molecule A was superposed on the mammalian AC molecule A. GC_{Rho} 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

1. McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., Cowley, A. P., and Lopez, R. (2013) Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Res.* **41**, W597-600.
2. Winger, J. A., Derbyshire, E. R., Lamers, M. H., Marletta, M. A., and Kuriyan, J. (2008) The crystal structure of the catalytic domain of a eukaryotic guanylate cyclase. *BMC Struct. Biol.* **8**, 42