ELECTROSTATIC AND LIPID-ANCHOR CONTRIBUTIONS TO THE INTERACTION OF TRANSDUCIN WITH MEMBRANES: MECHANISTIC IMPLICATIONS FOR ACTIVATION AND TRANSLOCATION

SUPPLEMENTARY MATERIAL

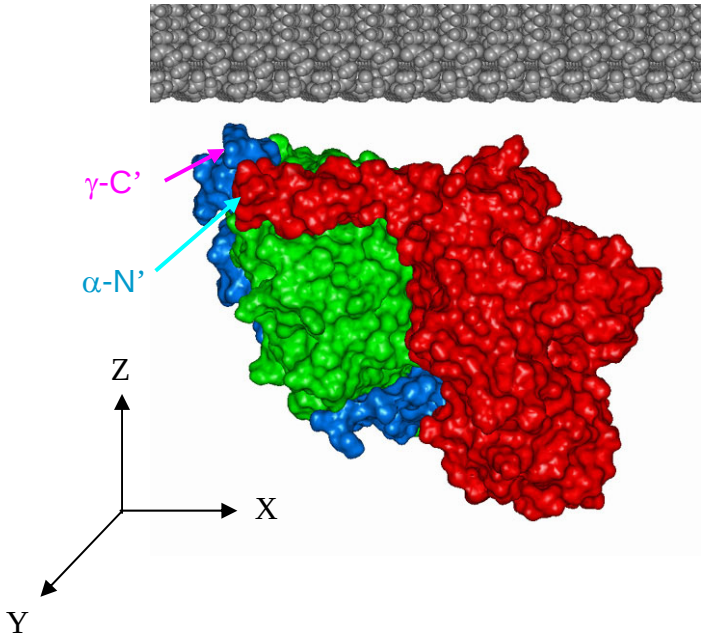

Figure S1. Initial orientation of the G_t heterotrimer relative to the membrane. G_t subunits are shown in Connolly surface representation and are colored red (α), green (β) and blue (γ). The lipid bilayer is depicted in gray CPK representation. For the initial orientations of dissociated $G_t\alpha$ and $G\beta_1\gamma_1$, each of these structures was superimposed on their relevant counterparts in the heterotrimeric structure.

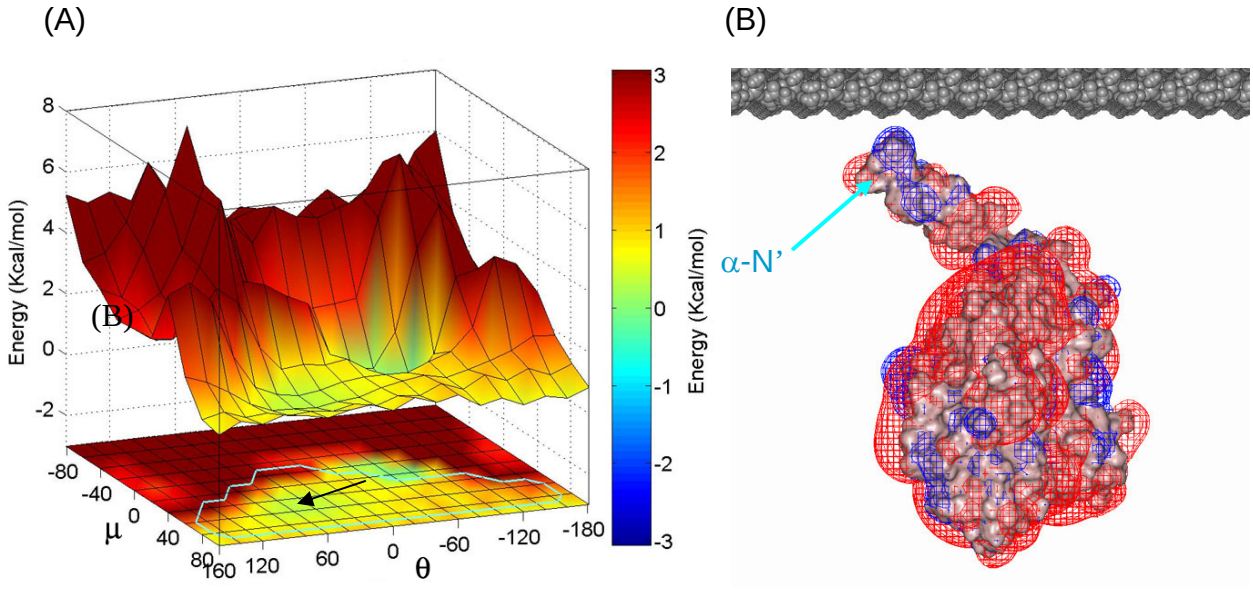

Figure S2. Interactions of a "heterotrimeric-like" conformation of $G_t\alpha$ with the membrane. A. Global orientation sampling of $G_t\alpha$ in a hypothetical "heterotrimeric" conformation. The cyan line encloses "lipid-allowed" orientations, which enable the $G_t\alpha$ N-terminal lipid to reach the membrane. The lipid-allowed orientation with minimal free energy (which is repulsive, ΔG_{el}=0.4 kcal/mole) is marked with a black arrow. $\langle \Delta G_{el} \rangle$ = 0.8 kcal/mol. B. Visualization of the minimal free energy orientation of the heterotrimeric conformation of $G_t\alpha$. Note that the extended N-terminal helix enables the negatively charged bulk of $G_t\alpha$ to remain far away from the membrane and results in lower repulsion.

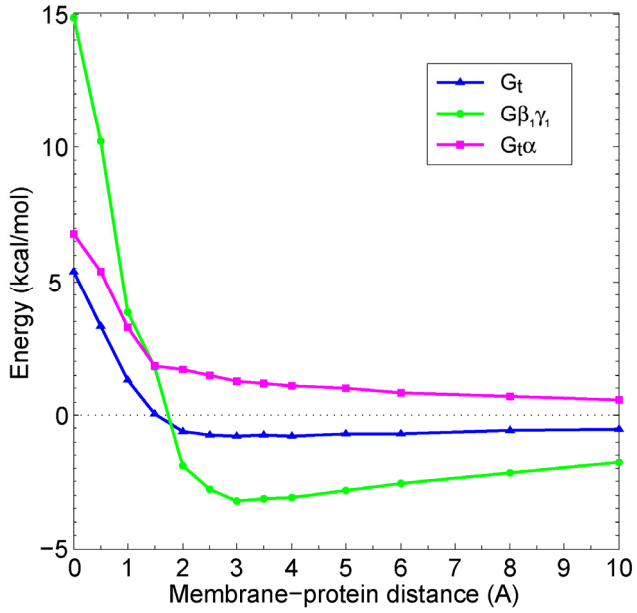

Figure S3. Distance-dependence of the electrostatic interaction of different transducin states with the membrane. Each G_t state is in the orientation of minimum free energy (Figures 1, 3, 5), as in previous studies (1-7). The *x*-axis depicts the distance between the molecular surfaces of the proteins and the lipid bilayer.

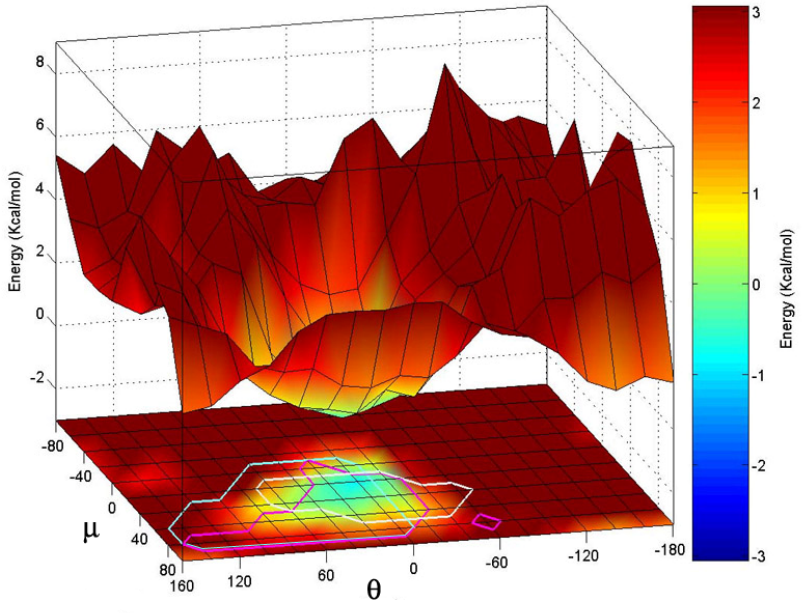

Figure S4. Overlap of G_t's "allowed" orientations and orientations enabling direct interaction with rhodopsin. The graph shows the same data as Figure 1A, but in addition a white line encloses the orientations that are predisposed to bind rhodopsin, i.e. where the residues of G_t shown to bind rhodopsin (the C-terminals of both $G\beta_1$ and $G\gamma_1$, and $G_t\alpha$'s N-terminus, α4-β6 loop, and most importantly $G_t\alpha$'s C-terminus (8-12)) face the membrane without any obstruction and the $G_t\alpha$ C-terminus is at least 15Å away from the membrane surface. The latter distance corresponds to the estimated protrusion of rhodopsin into the cytosol, based on available crystal structures. Increasing this distance to 20Å has a negligible effect on these results.

SUPPLEMENTARY MATERIAL REFERENCES:

1. Ben-Tal, N., Honig, B., Peitzsch, R. M., Denisov, G., and McLaughlin, S. (1996) *Biophys J* **71**(2), 561-575
2. Murray, D., McLaughlin, S., and Honig, B. (2001) *J Biol Chem* **276**(48), 45153-45159.
3. Murray, D., and Honig, B. (2002) *Mol Cell* **9**(1), 145-154.
4. Murray, P. S., Li, Z., Wang, J., Tang, C. L., Honig, B., and Murray, D. (2005) *Structure* **13**(10), 1521-1531
5. Murray, D., Arbuzova, A., Hangyas-Mihalyne, G., Gambhir, A., Ben-Tal, N., Honig, B., and McLaughlin, S. (1999) *Biophys J* **77**(6), 3176-3188
6. Murray, D., Hermida-Matsumoto, L., Buser, C. A., Tsang, J., Sigal, C. T., Ben-Tal, N., Honig, B., Resh, M. D., and McLaughlin, S. (1998) *Biochemistry* **37**(8), 2145-2159
7. Murray, D., Ben-Tal, N., Honig, B., and McLaughlin, S. (1997) *Structure* **5**(8), 985-989
8. Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) *Nature* **379**(6563), 311-319
9. Onrust, R., Herzmark, P., Chi, P., Garcia, P. D., Lichtarge, O., Kingsley, C., and Bourne, H. R. (1997) *Science* **275**(5298), 381-384
10. Hamm, H. E. (1998) *J Biol Chem* **273**(2), 669-672
11. Preininger, A. M., and Hamm, H. E. (2004) *Sci STKE* **2004**(218), re3
12. Chabre, M., and le Maire, M. (2005) *Biochemistry* **44**(27), 9395-9403