

Supplemental Data

“A New Approach to Producing Functional $G\alpha$ Subunits Yields the Activated and Deactivated Structures of $G\alpha_{12/13}$ Proteins”

Barry Kreutz, Douglas M. Yau, Mark Nance, Shihori Tanabe, John J. G. Tesmer, and Tohru Kozasa

Figure S1. Alignment of the amino-terminal regions of $G\alpha_{i1}$, $G\alpha_{12}$, and $G\alpha_{13}$. The amino terminal 28 amino acids of rat $G\alpha_{i1}$ (GenBank Accession ID: M17527), mouse $G\alpha_{13}$ (M63660), and $G\alpha_{12}$ (NM_010302) were aligned by CLUSTALW (1) with minor modifications. Residues contributed by respective proteins used in generating $G\alpha_{i13}$ or $G\alpha_{i12}$ are depicted by boxes, and the point of chimeric protein fusion is marked by an arrow. Conserved residues are boxed in black.

Figure S2. $G\alpha_{i13}$ is activated by AlF_4^- . (A) $G\alpha_{i13}$ is protected from tryptic proteolysis in an activation-dependent manner. The 100,000 x g supernatant of Sf9 cell lysate containing His₆- $G\alpha_{i13}$ was diluted two-fold with either 2X GDP reaction buffer (20 mM HEPES pH 8.0, 19 mM MgCl₂, 190 μM GDP) or 2X GDP· AlF_4^- reaction buffer (2X GDP reaction buffer supplemented with 60 μM AlCl₃ and 20 mM NaF). After incubation for 15 min on ice, 8 μg of bovine pancreatic trypsin (Calbiochem, San Diego, CA) was added to the indicated samples and incubated at 30°C for 10 min. Sample mixtures (~9 μg each) were boiled, resolved by SDS-PAGE, and immunoblotted (IB) with anti- $G\alpha_{13}$ C-terminus antibody. (B) AlF_4^- -dependent inhibition of GTPγS binding to Ni-NTA column eluates containing $G\alpha_{i13}$. Ni-NTA column eluates containing $G\alpha_{i13}$ or purified $G\alpha_{i1}$ (from *E. coli*) were incubated for 90 min at 30°C in the absence or presence of AlF_4^- ,

as indicated. GTP γ S binding to G α subunits was monitored as described in Experimental Procedures. Data are the mean of duplicate determinations.

Figure S3. Pseudo-effector crystal contact between the effector-binding region of G $\alpha_{i/12}$ (colors as in Fig. 4a) and a symmetry-related α -helical domain (yellow). Corresponding residues in G $\alpha_{i/13}$, if different, are given in parentheses. The pseudo-effector complex buries 1800 \AA^2 of accessible surface area, but cannot occur in G $\alpha_{i/13}$ due to lack of sequence conservation in its α -helical domain.

Movie 1. Conformational changes proposed to occur upon deactivation of G $\alpha_{i/12/13}$ subunits. Colors are assigned as in Figure 4A. The movie starts with the structure of G $\alpha_{i/12}$ ·GDP·AlF $_4^-$, and then “morphs” into its deactivated, GDP-bound conformation (modeled using the G $\alpha_{i/13}$ ·GDP structure). Elements that become disordered upon deactivation fade from view as the movie progresses. The side chain of Glu175, which packs against the bound guanine nucleotide only in the deactivated state, is shown as a stick model. The α D- α E linker, wherein Glu175 is found, thus acts like a spring that “pushes” the α -helical domain away from the Ras-like domain upon deactivation. The intermediate steps between the activated and deactivated states of G $\alpha_{i/12}$ were generated via adiabatic mapping (2, 3).

Supplemental References

1. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice, *Nucleic Acids Res.* 22, 4673-4680.
2. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination, *Acta Crystallogr D Biol Crystallogr* 54, 905-21.
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Figure S1

$G\alpha_{i1}$ 1 -----MGCTLSAEDKAAVERSKMIDRNLREDGEKAAREVKKILLGAGESGKS 47
 $G\alpha_{13}$ 1 -----MADFLPSRSVLSVCFPGCVLTNGEAEQQRKSKEDKCLSREKTYVKRLVKILLGAGESGKS 62
 $G\alpha_{12}$ 1 MSGVVRTLRSRCLLPAEAGARERRAGAARDAER-EARRRSRDIDALLAREERRAVRRLVKILLGAGESGKS 69

Figure S2

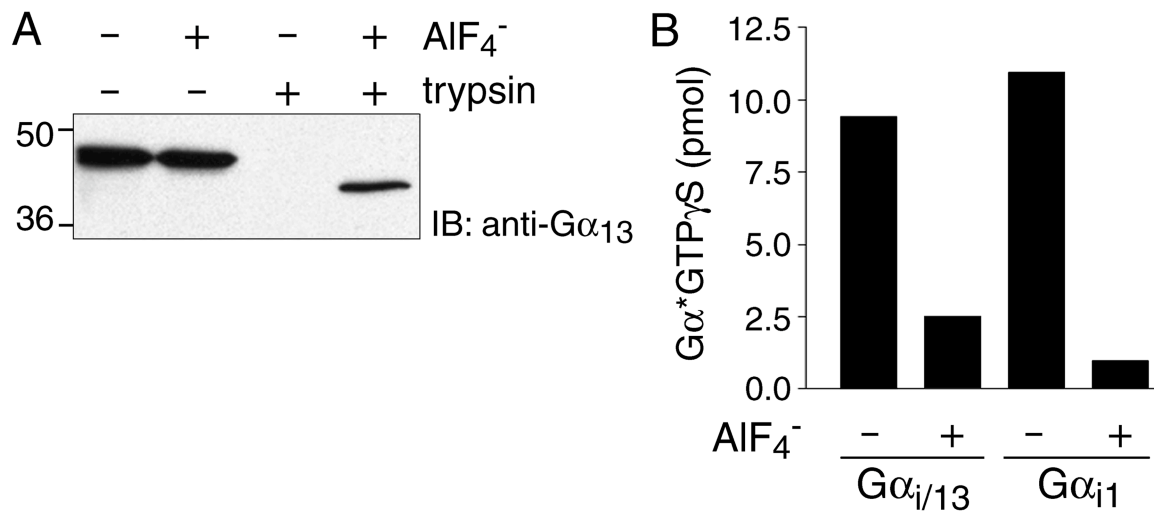


Figure S3

