Helix dipole movement and conformational variability contribute to allosteric GDP release in $G\alpha_i$ subunits

Supplementary Information

Anita M. Preininger[1#], Michael A. Funk[1#], Scott M. Meier[1], William M. Oldham[1], Christopher A. Johnston[2,†], Suraj Adhikary[3], Adam J. Kimple[2], David P. Siderovski[2], Heidi E. Hamm[1,], and Tina M. Iverson[1,4,*]*

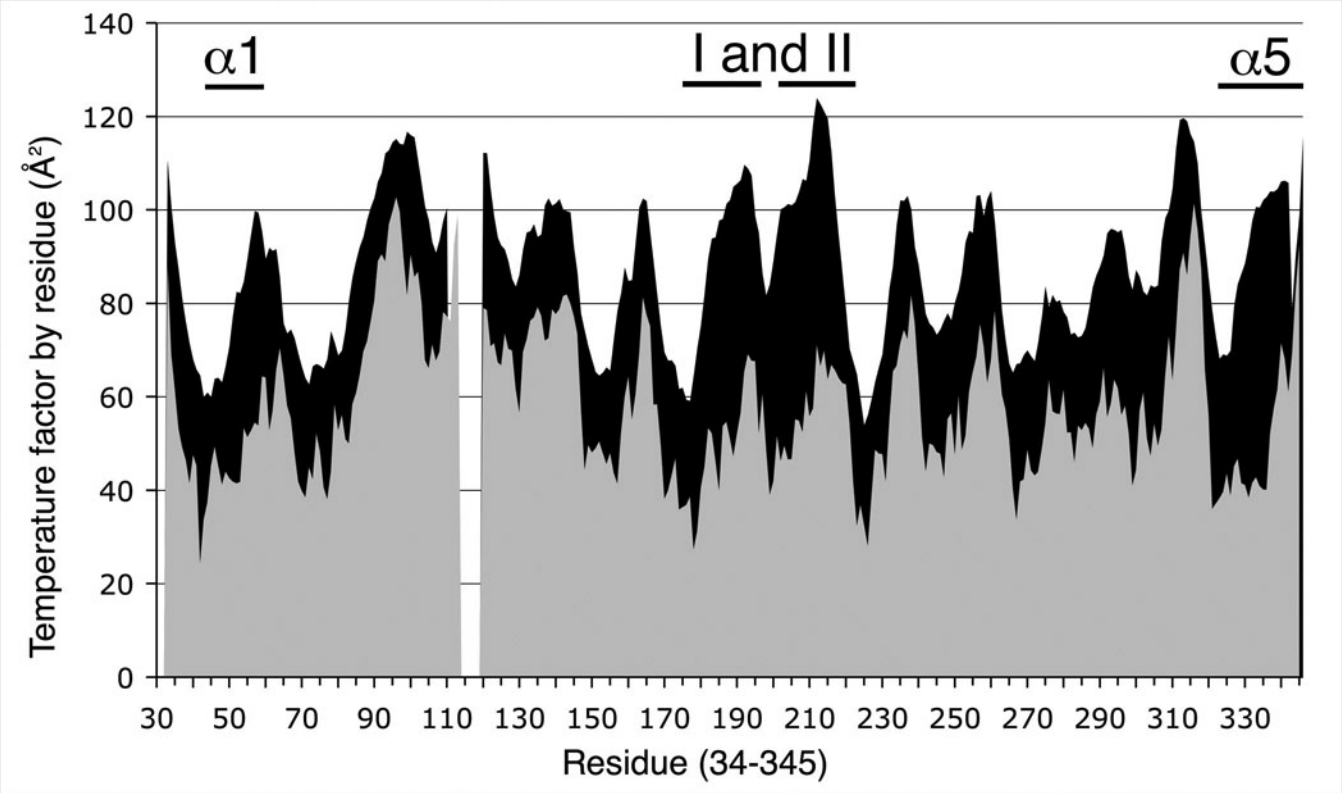
[1]Departments of Pharmacology, [3]Biological Sciences and [4]Biochemistry, Vanderbilt University Medical Center, Nashville, TN 37232-6600 and [2]Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7365

#These authors contributed equally to this work.

† Present address: Institute of Molecular Biology, University of Oregon, Eugene, OR, 97403

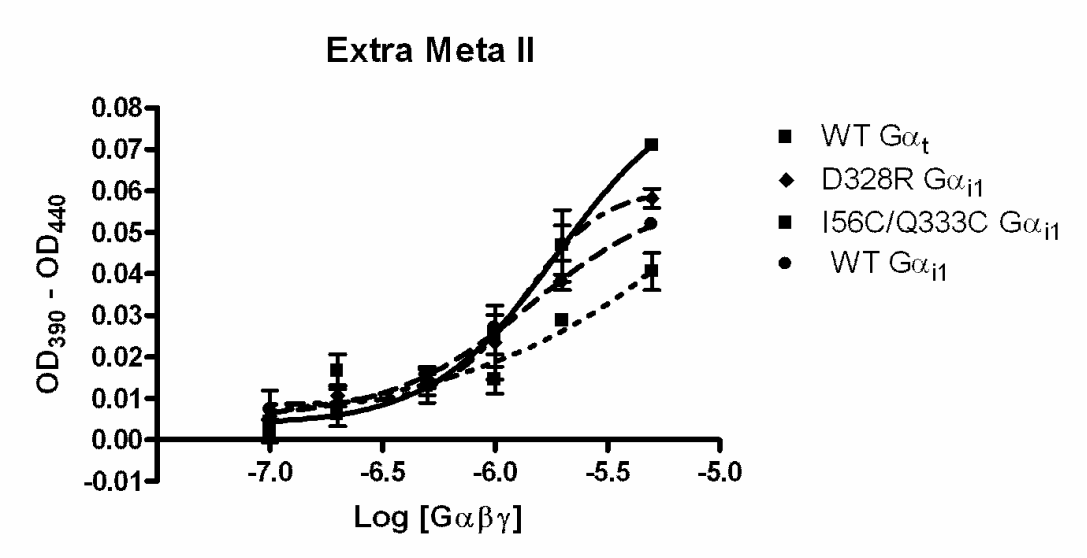
*Correspondence should be addressed to HEH: fax, (615) 343-1084; phone, (615) 343-3533; email, heidi.hamm@vanderbilt.edu or TMI: fax, (615) 343-6532; phone, (615) 322-7817; email, tina.iverson@vanderbilt.edu

SUPPLEMENTARY FIGURE 1



Supplementary Fig. 1 Temperature factor analysis by residue. Crystallographic temperature-factor values for the I56C/Q333C $G\alpha_{i1}$ are shown in *black* (average 87.8 $Å^2$), while temperature-factor values for the wild type crystallized in the same space group are shown in *light grey* (average 57.3 $Å^2$). Sequence positions for the α1 helix (α1), switch I and switch II (I and II), and the α5 helix (α5) are marked. Residues 111-119 are not observed in the crystallographic electron density. As a result, the temperature factors cannot be compared in this region.

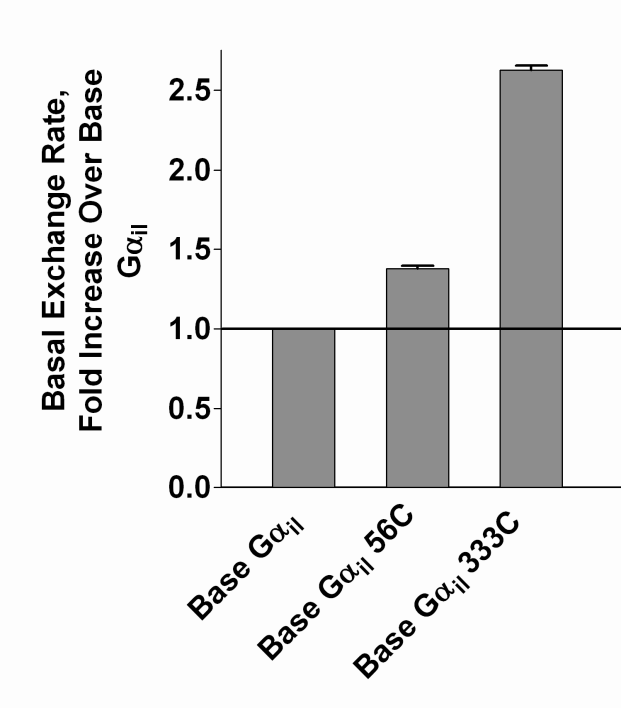
SUPPLEMENTARY FIGURE 2



Supplementary Fig. 2

Urea-washed dark-adapted ROS membranes (5 μM) were incubated on ice for 5 minutes with indicated concentrations of Gα proteins (in complex with $G\beta_1\gamma_1$). Absorbance of each ROS-Gαβγ combination was scanned from 350 to 650 nm using a DW2000 Spectrophotometer (Olis) in 50mM HEPES, 0.1M NaCl, 1mM $MgCl_2$, 1mM DTT, pH 8.0 both before and after light activation, and extra meta II was calculated as the difference between Abs_{390} (light - dark) less Abs_{440} (light - dark).

SUPPLEMENTARY FIGURE 3



Supplementary figure 3: Basal intrinsic nucleotide release was determined as described in materials and methods for $G\alpha_{il}$ subunits (*1*) with individual cysteine mutations at the indicated residues. The I56C/Q333C $G\alpha_{i1}$ has a 6.2-fold enhancement of basal nucleotide exchange over wild-type.

During the review process, all Supplementary movies can be downloaded from:

http://structbio.vanderbilt.edu/~tina/Galpha

Or http://structbio.vanderbilt.edu/~tina/movies.tar.gz

Supplementary Movie 1. Roto-translation of the α5 helix is associated with global changes in the I56C/Q33C $G\alpha_{i1}$ subunit. The α5 helix (*yellow*) roto-translates from the position that it adopts in the GDP-AlF_4^--bound wild-type $G\alpha_{i1}$ subunit structure (*2*) (PDBID 1GFI) into the position that is observed in the I56C/Q333C $G\alpha_{i1}$ subunit structure and the movie pauses briefly. Following the pause, the other secondary structural elements are moved into the position observed in the I56C/Q333C $G\alpha_{i1}$ subunit structure. Coloring is similar to that in Figure 1 with the α1 helix is shown in *orange*, and the GTPase-helical domain linker in *light blue*, and the GDP-AlF_4^- molecule is shown as a *red* space filling model. The exact order of structural changes is not known.

Supplementary Movie 2. Modeling of a continuous movement of the main chain elements at the back door egress pathway from the nucleotide pocket from the positions observed in a resting state to positions observed in rapidly-exchanging $G\alpha_{i1}$ subunits. Switch II is shown in *bright green*, the β2 and β3 strands are in *dark green*, the α1 helix is in *orange*, switch III is in *purple*, and the GDP-AlF_4^- molecule is shown as a *red* space filling model. Some of the residues of the switch II region were not observed in the density of the KB-752 peptide-bound structure. The movie begins with these structural elements in the positions that they adopt in the GDP-AlF_4^--

bound wild-type $G\alpha_{i1}$ subunit structure (*2*) (PDB-ID 1GFI). These elements then morph into the positions observed in the I56C/Q333C $G\alpha_{i1}$ structure, and the movie pauses briefly. Following the pause, each of these structural elements morphs once again into the positions observed in the GDP -SO_4^{2-}-bound $G\alpha_{i1}$ subunit in complex with peptides KB-752 peptide and D2N (*3*) (PDB-ID 2HLB).

Supplementary Movie 3. Close-up view of the structural rearrangements of the back door egress pathway shows potentially altered bonds to the γ-phosphate of the guanine nucleotide. The protein is shown in *blue*, the GDP-AlF_4^- molecule is shown with the nucleotide base and the two phosphates as a *red* space filling model and the AlF_4^- shown as a *blue* space filling model. The P-loop and γ-phosphate binding side chains from the positions observed in the GDP-AlF_4^--bound wild-type $G\alpha_{i1}$ subunit structure (*2*) (PDB-ID 1GFI) into the positions observed in the I56C/Q333C $G\alpha_{i1}$ subunit structure showing a slight opening of the back door.

1. Oldham, W.M., N. Van Eps, A.M. Preininger, W.L. Hubbell, and H.E. Hamm (2006). Mechanism of the receptor-catalyzed activation of heterotrimeric G proteins*, Nature Structural and Molecular Biology. 13*, 772-777.
2. Coleman, D.E., A.M. Berghuis, E. Lee, M.E. Linder, A.G. Gilman, and S.R. Sprang (1994). Structures of Active Conformations of $G_{i\alpha 1}$ and the Mechanism of GTP Hydrolysis*, Science. 265*, 1405-1412.
3. Johnston, C.A. and D.P. Siderovski (2007). Structural basis for nucleotide exchange on G alpha i subunits and receptor coupling specificity*, Proc Natl Acad Sci U S A. 104*, 2001-2006.