Probing the effects of specific transducin mutations via computational kinetic modeling

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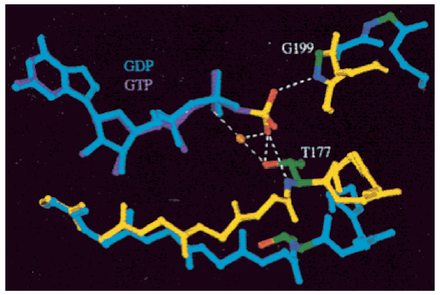
Introduction

Mutations are often used to study the roles of specific protein structures. For proteins with complex activities, however, inferring the mechanism behind an activity change can be difficult. Here we use computational kinetic modeling to aid such inferences.

We study a conserved theronine in the Switch 1 region of Gα subunits. This threonine (T177 in transducin) makes contacts with both the γ phosphate of GTP and an Mg^{2+} ion, as illustrated in **Figure 1** (from [Lambright 1994]).

Figure 1

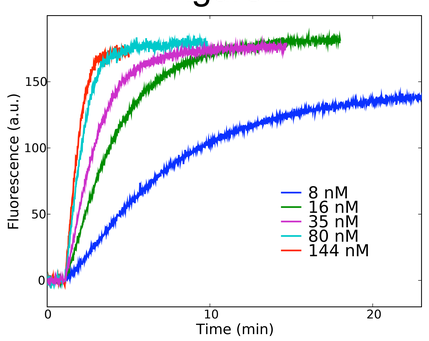


We mutated this threonine to alanine in an α_T/α_{i1} chimera (designated α_T^*) which, unlike wild-type α_T, can be expressed in *E. coli*. Fluorescence measurements provide detailed kinetic information to feed into our computational model.

Experiments

Solubilized bovine rhodopsin, bovine $G_{\beta\gamma}$, and either α_T^* or α_T^*(T177A) were incubated in ambient light at room temperature for 6 minutes. GTPγS, a non-hydrolyzable GTP analog, was added and the accompanying increase in intrinsic tryptophan fluorescence monitored [Phillips 1992]. An example data set is shown in **Figure 2**, where 1 μM GTPγS was added to 4.6 nM of rhodopsin, 279.5 nM of α_T^*, and varying amounts of $G_{\beta\gamma}$.

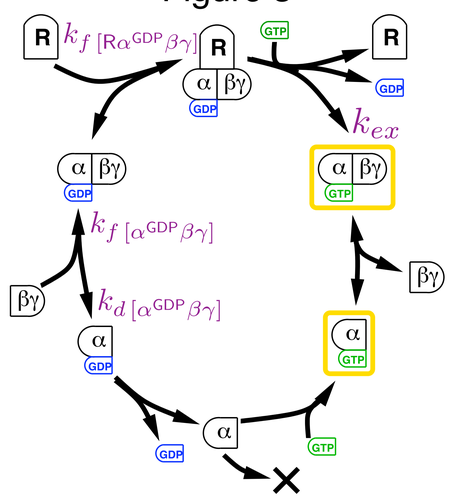
Figure 2



Model

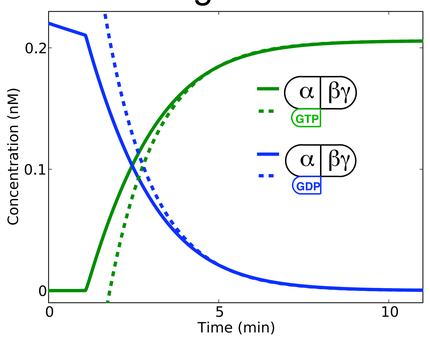
The model is a coupled set of 10 nonlinear ordinary differential equations, parameterized by 10 rate constants. An illustration is shown in **Figure 3**. Fluorescent species are boxed in gold, and selected parameters are indicated in purple. Parameters are fit separately for α_T^* and α_T^*(T177A).

Figure 3



Approach to Equilibrium

Figure 4



Dynamical systems theory offers insight into the rate-determining step of the activation cycle. In particular, as the system approaches equilibrium, the dynamics are dominated by the exponentially decaying slowest mode.

For example, the solid lines in **Figure 4** are trajectories for GDP- and GTP-bound α_T^* $G_{\beta\gamma}$ given the best fit set of parameters and a particular set of experimental conditions. The dashed lines are simple exponentials with a time constant of 1.4 minutes, corresponding to the eigenvalue of the slowest mode. These fit the late-time dynamics quite well, but not early times, suggesting that other modes are significant. Further work will include the effects of these faster modes, and study the parameter dependence of the modes.

Parameter Ensembles

Because there are many sets of parameters that fit the available data similarly, any conclusions must be drawn from the *ensemble* of all sets consistent with the data.

Figure 5 shows the distributions of k_{ex}, the rate constant for GDP→GTP exchange, for α_T^* and α_T^*(T177A). The distributions are well-separated, allowing us to conclude that exchange really is slower in the mutant than the chimera.

Figure 5

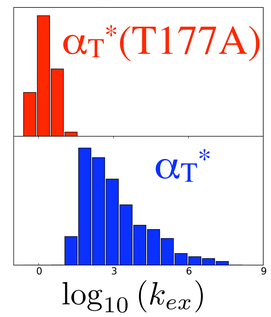
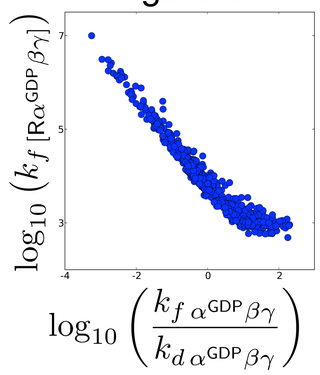


Figure 6 shows the correlation between the rate constant for $\alpha_T^*G_{\beta\gamma}$ dimers binding to rhodopsin and the equilibrium constant for dimer formation. Their ratio determines the flux through the cycle, and is well-constrained by the data, although their absolute values are not.

Figure 6



Acknowledgments

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References

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