**Supporting Text**

**An Allosteric Model for $G_{s\alpha}$ Function**

Consider a two-state equilibrium between a basal state of G protein (stabilized by GDP G (G) (which binds $G_{\beta\gamma}$) and an active state of G protein (stabilized by GTP) (G*) [which binds adenylyl cyclase (AC)] that is coupled to nucleotide exchange (see Fig. 2), where $L_{GDP}$ is the equilibrium constant for the reaction in the GDP-bound state, and $L_{GTP}$ is that in the GTP-bound state. The apparent equilibrium binding constant (dissociation constant) for interaction of AC or $G_{\beta\gamma}$ with the GTP- or GDP-bound states of $G_{s\alpha}$ will depend on the allosteric constants in the following manner:

$$K_{app,GTP}^{AC} = K_{AC}\left[1+\frac{1}{L_{GTP}}\right]$$

$$K_{app,GDP}^{AC} = K_{AC}\left[1+\frac{1}{L_{GDP}}\right]$$

$$K_{app,GTP}^{\beta\gamma} = K_{\beta\gamma}\left[1+L_{GTP}\right]$$

$$K_{app,GDP}^{\beta\gamma} = K_{\beta\gamma}\left[1+L_{GDP}\right],$$

where $K_{AC}$ is the intrinsic binding constant of adenylyl cyclase to the G* state, and $K_{\beta\gamma}$ is the intrinsic binding constant of $G_{\beta\gamma}$ to the G state. To understand the effect of mutations that alter the allosteric binding constants, we plot the apparent binding constants for AC and $G_{\beta\gamma}$ for all values of $L$ in Fig. 7.

Consider the effect of mutants that cause equal left or right shifts of the allosteric constants $L_{GDP}$ and $L_{GTP}$ on the binding affinities of adenylyl cyclase or $G_{\beta\gamma}$. Shifts to the right will cause the ratio of affinities of adenylyl cyclase for the GTPγS and GDP states (the nucleotide sensitivity) to decrease relative to WT but will cause the same ratio for $G_{\beta\gamma}$ to increase relative to WT. Shifts of $L_{GTP}$ and $L_{GDP}$ to the left will cause exactly the opposite effect. In contrast, mutations that perturb the absolute affinities for cyclase or

$G_{\beta\gamma}$ need not (and generally will not) display a reciprocal effect on the nucleotide sensitivities.