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Adding Substituent Nonadditivity in Protein Allostery by NMR

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Allostery is a common regulatory mechanism in proteins. Many proteins exist in dynamic equilibrium between active and inactive states (1). Transitions between these states are facilitated by a network of allosteric interactions that cannot be easily identified by comparative analysis of structure and dynamics of residues found in the end points. Current structure-based virtual screening and lead optimization protocols rely on static structures of these various states to predict the impact of substituent modifications on binding free energies (2). Coupling between substituents, nonadditivity, severely limits the accuracy of linear scoring functions (3–5). Although allostery is a frequent cause of nonadditivity, determining the effects of allostery on the energetic landscape of substrate binding presents a major challenge. Boulton et al. have started to address this in this issue of *Biophysical Journal* (6). By analyzing the thermodynamic cycle of the cAMP-binding domain (CNBD) of the HCN4 channel, they present a new NMR-based approach to characterize the allosteric mechanisms underlying nonadditivity resulting from simultaneous base substitution (adenine to guanine in cGMP) and phosphate substitution (equatorial exocyclic oxygen to sulfur in Rp-AMPS) of cAMP.

The CNBD apo state of HCN4 is a stereotypical allosteric switch, dynamically sampling both an autoinhibitory (P_1) and an active (P_2) state at a rate that is fast on the NMR timescale. cAMP binding selects and stabilizes the active state, whereas the three analogs of cAMP (Rp-AMPS, cGMP, and Rp-cGMPS) modulate the $P_1 \Leftrightarrow P_2$ equilibrium (Fig. 1; for details, please refer to manuscript). Therefore, nonadditivity could result purely from the $P_1 \Leftrightarrow P_2$ exchange (γ_{12}) and/or from state-specific nonadditivity (γ_1, γ_2). This requires precise knowledge of the population of P_1 ($\chi_{1,j}$) and P_2 ($\chi_{2,j}$) when saturated with each ligand (S_j), which NMR is ideally suited to provide. In this case, because the exchange rate is fast, the observed chemical shift is a population-weighted average of P_1 and P_2 states, allowing for determination of the populations from two-dimensional spectra. They found that the conformational exchange (γ_{12}) contributed to the dominant source of nonadditivity was due to unfavorable interactions in the active state conformation (γ_2).

Molecular dynamics simulations showed that Rp-AMPS induces "steric frustration" between F689 and L663 in the active state. Chemical shift covariance analyses (CHESCA), a method developed by the Melacini lab in 2011 (7,8), revealed the extensive allosteric network, including F689, regulating the $P_1 \Leftrightarrow P_2$ transition. A frustration-silencing mutation, F689A, was found to elevate both the γ_{12} and γ_2 sources of nonadditivity. Subsequent CHESCA analysis of the F689A mutants revealed a dramatic reduction in the allosteric network. Although Boulton et al. do not discuss the implications of altering the allosteric network on the thermodynamic cycle, the effect is clear.

These results demonstrate a powerful first step in the development of a robust method for interrogating the thermodynamics of ligand binding to allosteric receptors in addition to breaking down the contributions of conformational exchange (γ_{12}) and state-specific effects (γ_1, γ_2). Boulton et al. clearly demonstrate how changes in the allosteric network can directly affect substituent nonadditivity. It is clear that incorporation of the allosteric networks identified through CHESCA analysis into structure-based virtual screening could be highly beneficial. It will be interesting to see the application of this method to slow to intermediate exchange systems using chemical exchange saturation transfer (CEST) or relaxation dispersion (RD) experiments to determine populations (9,10).

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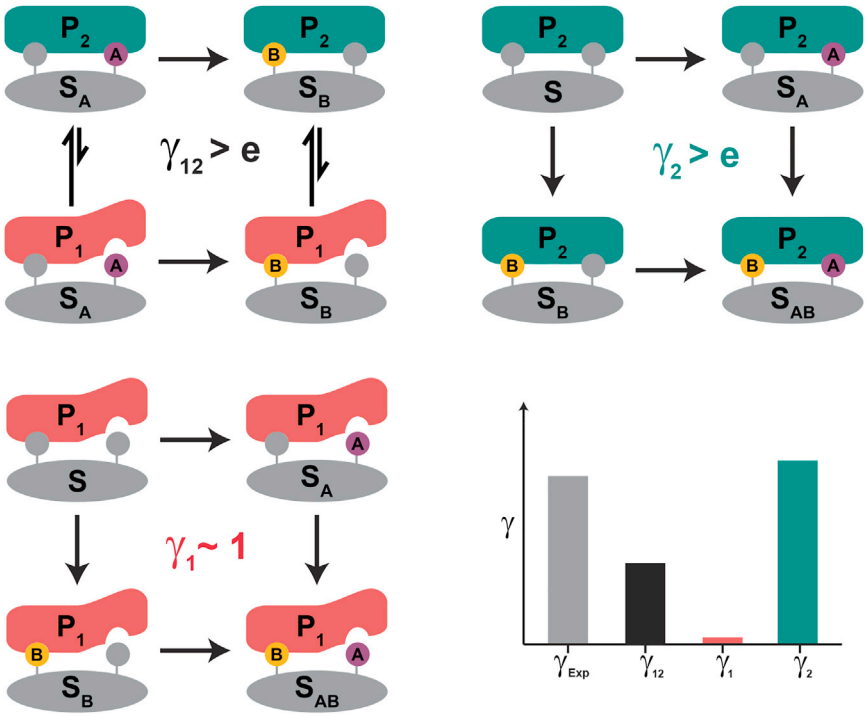

FIGURE 1 Schematic of the Observed Equilibria. To see this figure in color, go online.

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