pathways and subnetworks that are critical for cell physiology and diseases. One such pathway is the PI3K/Akt/mTOR pathway, which is critical for regulating cell growth, proliferation, apoptosis, and glucose metabolism, and is frequently dysregulated in cancer. On the other hand, we also take a systems biology approach to analyzing signaling networks. For instance, in collaboration with Heng Zhu and Jiang Qian's laboratories at JHU, we developed a strategy based on functional protein microarrays and bioinformatics to experimentally identify substrates for 289 unique human kinases. We further constructed a high-resolution map of phosphorylation networks that connects 230 kinases to 2591 *in vivo* phosphorylation sites in 652 substrates, providing global insights into kinase-mediated signaling pathways. In short, I could also say we work on whichever projects excite us most.

Tell us something about your work that is exciting for you right now

I am very excited about testing our new 'activity architecture' hypothesis. The assembly/disassembly and enzymatic activities of protein nanomachines underlie all cellular functions, and dysregulated nanomachines are the ultimate culprits in cancer. Knowing when and where these nanomachines are active is, therefore, critical to understanding the molecular drivers for normal cellular functions as well as for tumorigenesis, yet current efforts to characterize the molecular constituents of the cellular machinery overlook this critical dimension. We seek to establish a new conceptual framework to specifically understand the cellular organization of molecular activities. We hypothesize that cellular biochemical activities are spatially organized into an 'activity architecture' via the specific organization of active molecules and their regulatory partners. This activity architecture, together with the structural and mechanical architecture of the cell, encodes all the information needed to drive cellular function. We further hypothesize that perturbations to this activity architecture, even by a few dysregulated driver molecules, could lead to detrimental effects on cellular functions, such as loss of control over cell growth, division, and death. We are developing a new generation of biosensor and imaging technologies to characterize the activity architecture of the cell and examine the dysregulated activity architecture in cancer cells.

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Letter

Cooperativity Has Empirical and Ultimate Levels of Explanation

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Controversy over the meaning of pharmacological parameters often arises because of a lack of appreciation of different hierarchical levels of analysis. In a recent letter in *Trends in Pharmacological Sciences*, Zhang and Kavana [1] concluded that my two-state model for allosterism lacks cooperativity, even though Figures 5 and 6 in my review [2] illustrate examples of how the two-state model yields specific cooperativity values. Here, I explain how the two-state model (receptor-state analysis) gives rise to the cooperativity parameter (α) of the allosteric ternary complex model (receptor-population analysis).

Figure 1A shows the allosteric ternary complex model [3]. No states are illustrated, only receptor complexes (*R*, *DR*, *RA*, and *DRA*). The parameters K_1 and K_2 represent the observed affinity constants (reciprocal of the concentration of ligand required for half-maximal occupancy) of the orthosteric (*D*) and allosteric (*A*) ligands, and α, the cooperativity constant. Thus, αK_1 represents the observed affinity constant of *D* when the receptor population is saturated with the allosteric ligand, and αK_2, the observed affinity constant of *A* when the receptor population is saturated with the orthosteric ligand *D*.

In this population analysis, receptor activation is denoted by efficacy terms, (ε_D, ε_A, and ε_0), which represent the fractions of the populations of the *DR*, *RA*, and unoccupied receptor (*R*) complexes in the active state, respectively. The parameter, β_1, represents the scalar by which allosteric ligand *A* alters the efficacy of orthosteric ligand *D* ($\beta_1\varepsilon_D$, fraction of the population of DRB complexes in the active state). The product of the allosteric effects on affinity (α) and efficacy (β_1) is denoted by the parameter, γ_1 ($\gamma_1 = \alpha\beta_1$). This parameter is also equivalent to the ratio $\varepsilon_A/\varepsilon_0$ and, hence, is determined by the allosteric ligand and constitutive activity.

In Figure 1B, the allosteric ternary complex model is illustrated by four populations of receptors representing the unoccupied (*R*) and the three types of occupied receptor complex (*DR*, *RA*, and *DRA*). In this example, each population contains 1000 receptors, and the active and inactive receptor states are denoted by yellow and blue colors, respectively. Given that each receptor complex (e.g., *DR*) represents a mixture of structures, there is no real receptor species that has an observed affinity of K_1 or an activity of ε_D. Rather, these parameters represent the weighted average values of the receptor population.

If we turn up the zoom lens (Figure 1C), the ligand-binding sites on each state can be seen to isomerize concertedly as the receptor transitions between states. The affinities of ligands for these states of the receptor are designated in the two-state

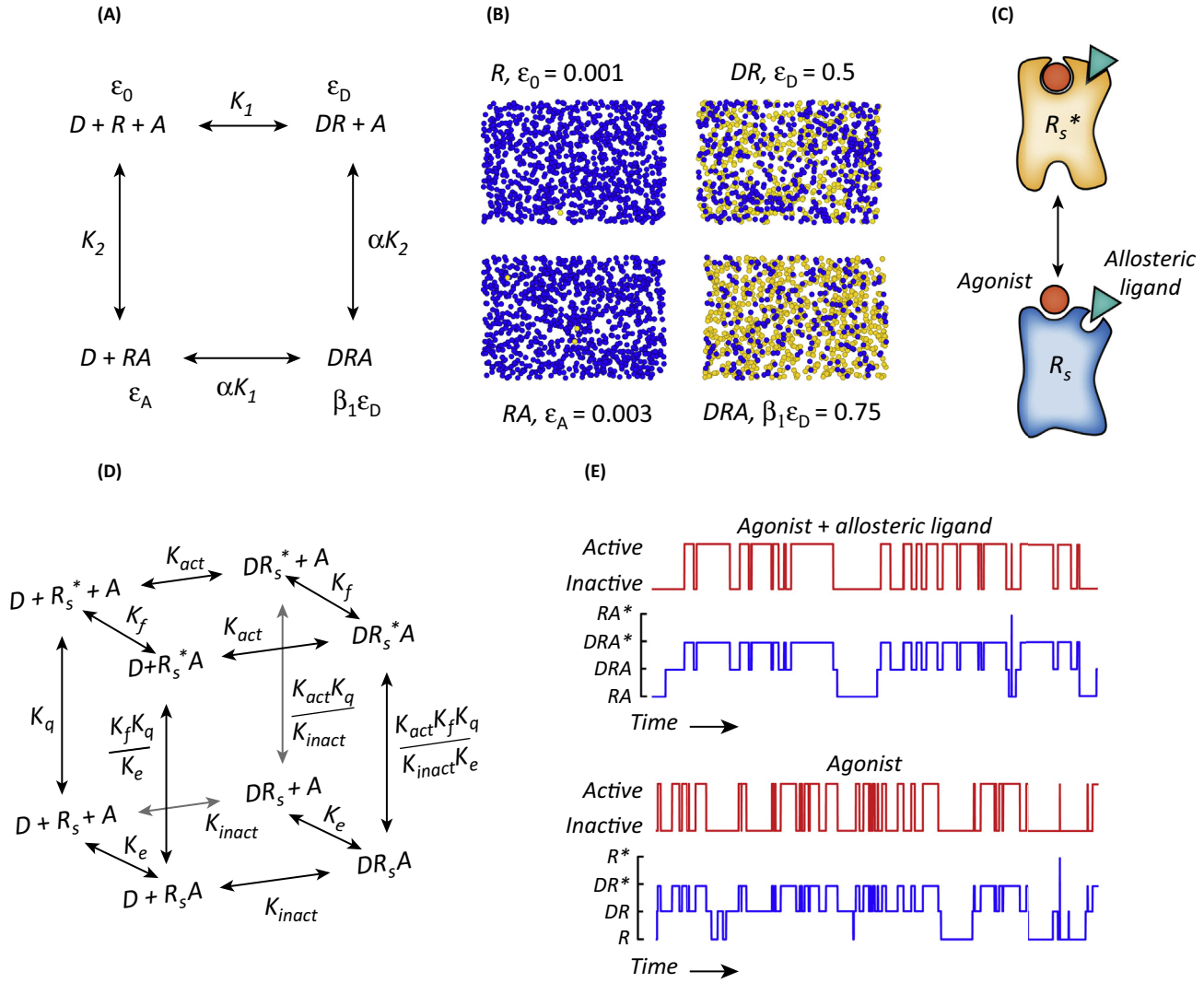

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Figure 1. Two Different but Consistent Ways of Quantifying Cooperativity Based on the Aggregate Receptor Population (Population Analysis) or Individual Receptors (State Analysis) as the Unit of Analysis. (A) This allosteric ternary complex model addresses the observed affinity of orthosteric (*D*) and allosteric (*A*) ligands for the receptor population (K_1 and K_2, respectively). These constants have inverse molar units and are defined accordingly (i.e., M^{-1}, K_1 = [*DR*]/[*D*][*R*]). The cooperativity constant, α, represents the scalar change in the observed affinity of each ligand caused by the binding of the other ligand to the receptor complex. This constant (α = 3.0 in this example) represents the measure of cooperativity at the population level of analysis. The fractional amount of the population of each type of receptor complex in the active state is denoted by an efficacy term (i.e., ε_0, ε_D, ε_A, and $\beta_1\varepsilon_D$ for *R*, *DR*, *RA*, and *DRA*, respectively). (B) This illustration is intended to represent the receptor population (1000 receptors) at an instant in time under four conditions: in the absence of ligand (*R*); in the presence of receptor-saturating concentrations of *D* (*DR*); *A* (*RA*); and both *D* and *A* (*DRA*). Active and inactive receptors are denoted by yellow and blue symbols, respectively. During this snapshot, the unoccupied receptor population (*R*) contains a single constitutively active receptor near the middle of its lower border and the allosteric ligand-occupied receptor population (*RA*) contains three, two near the center and one in the upper left quadrant. The active receptors in the *DR* and *DRA* populations are obvious. (C) A view of a single receptor isomerizing between active (R_s*) and inactive states (R_s). The structures of the two states are sufficiently distinctive such that the active state has the capacity to catalyze a signal, whereas the inactive does not. Both ligands (*D* and *A*) have characteristic affinity constants (microscopic constants) for the active and inactive receptor states. (D) The two-state model for allosteric interactions. Unlike the allosteric ternary complex model, the affinities of ligands are determined by the state or structure of the binding pocket regardless of whether one or two ligands are bound to the receptor complex. Cooperativity is determined by the selectivity of the ligands for the active state (i.e., K_{act}/K_{inact} and K_f/K_e for *D* and *A*, respectively). For this example of positive cooperativity (K_{act}/K_{inact} = 1000 and K_f/K_e = 3.0), both ligands have the effect of increasing the isomerization constant of the receptor (K_q). Thus, the cooperative effect of the allosteric ligand on the binding of the orthosteric ligand is equivalent to K_f/K_e (3.0). (E) The two-state model can be used to simulate single receptor activity as a continuous Markov process in the absence and presence of allosteric modulator. The transitions between the various types of receptor complex are indicated in blue, whereas the activity of the receptor is indicated in red. Here the positive cooperative effect of

(Figure legend continued on the bottom of the next page.)

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