Review

CellPress

Unraveling structural mechanisms of allosteric drug action

Ruth Nussinov[1,2] and Chung-Jung Tsai[1]

[1] Cancer and Inflammation Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA
[2] Sackler Institute of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Orthosteric drugs block the active site to obstruct function; allosteric drugs modify the population of the active state, to modulate function. Available data lead us to propose that allosteric drugs can constitute anchors and drivers. The anchor docks into an allosteric pocket. The conformation with which it interacts is unchanged during the transition between the inactive and active states. The anchor provides the foundation that allows the driver to exert a 'pull' and/or 'push' action that shifts the receptor population from the inactive to the active state. The presence or absence of driver atom in an allosteric drug can exert opposite agonism. We map a strategy for driver identification and expect the allosteric trigger concept to transform agonist/antagonist drug discovery.

The concept and merit of driver and anchor atoms in allosteric drug design

A specific function of a protein is determined by the extent that a macromolecule populates its active conformation [1–10]. Nature co-optimized allosteric regulation of protein hosts and ligands for signal transduction [11], enzyme activation [12], metabolism [13], cell death [14], and transcription control [15]. Similarly, a drug can exploit optimized or byproduct pockets at allosteric sites to exert analogous or opposite regulation [16]. The recent unified view of allostery [17] (Figure 1) indicated that the pre-existing propagation pathway [18,19] specifies a tight structural coupling between the active and allosteric sites and that the extent of the relative increase of the active state population is determined by specific ligand–host interactions that stabilize the active conformation and/or destabilize the inactive conformation. Guided by these principles, we here address questions such as how very similar ligands can bind at the same allosteric site, with one acting as an agonist and the other as antagonist.

The modes of action of allosteric drugs differ from those of orthosteric drugs. Orthosteric drugs bind in the active site and block it; allosteric drugs bind elsewhere on the protein and alter the population of the conformations at the active site [20]. Orthosteric drugs shut off native protein function; allosteric drugs modulate it. Some modulators act as agonists, to enhance function, whereas others act as antagonists, to reduce it. The chemical difference between allosteric agonists and antagonists – which bind at the same site – can be surprisingly small: even a change of a single atom [21] or a small group [16] may result in opposite effects or in large differences in efficacy. Further, even the same allosteric ligand bound at the same site can lead to opposing effects in different environments [22]. Questions include the following. (i) How can minor or subtle chemical changes, or identical compounds in different tissues, lead to such large, sometimes opposite effects? (ii) Can we predict *a priori* whether an allosteric modulator would be an agonist or antagonist? (iii) Can we establish some principles that can help guide allosteric drug discovery toward one or the other? Unlike orthosteric drugs, for which the key determinant of drug outcome is high affinity [20], in the case of allosteric modulators the extent of the stabilization of the active (or inactive) conformation (allosteric efficacy) can be pivotal in specifying the drug action. The past few years witnessed landmark publications, including agonist- and antagonist-bound structures, that provided hitherto unavailable clues [23–25]. Observations made by several laboratories, including those of Biondi [26,27], Kuriyan [11,28], Anand [21], Taylor [21,29], Wells [16,30], Melacini [31,32], Kalodimos [33,34], and Schwartz [35–37], emphasize that the affinity and concentration of modulators determine their residence time on the receptor and thus the duration of drug action; however, the detailed interactions may determine whether the drug is an agonist or antagonist. Ligand atoms can be divided into anchor and driver. The anchor docks into the allosteric pocket, forming favorable interactions with an already highly populated conformation in the inactive and active states; by contrast, an attractive 'pulling' or repulsive 'pushing' by driver atoms can stabilize the active conformation and/or destabilize the inactive conformation. Figure 2 illustrates how pulling or pushing by even a single driver atom can favor a specific state in contrast to an anchor atom having the same interactions in both states. Thus, it is unsurprising that even subtly varied ligand interactions may result in different – agonist or antagonist – outcomes. Further, it is not necessarily the case that the same atoms in a given drug or ligand always play the driver role in different proteins or that distinct atoms fulfill the roles of anchor and driver. Below we discuss the characteristic properties of

Corresponding author: Nussinov, R. (NussinoR@helix.nih.gov, ruthnu@helix.nih.gov).
Keywords: allosteric drug discovery; protocol; agonist; antagonist; driver atom; anchor atom; allostery.

0165-6147/
 http://dx.doi.org/10.1016/j.tips.2014.03.006

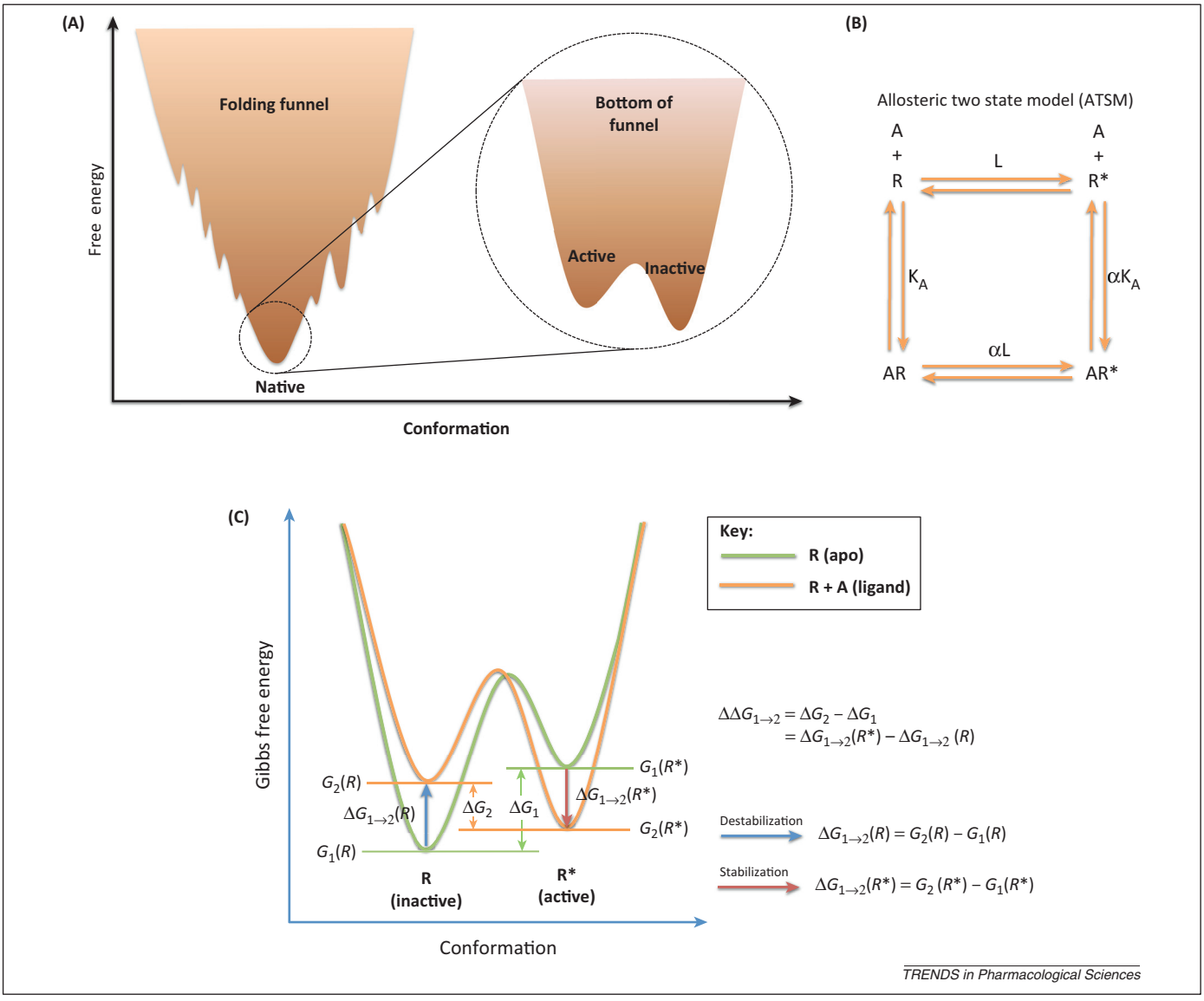

Figure 1. A brief graphic summary of 'how allostery works'. This unified view of allostery [17] considers allostery from the thermodynamic standpoint [61,62], in terms of the energy landscape of population shift [63,64], and from a simplified structural view of allostery with exactly the same allosteric descriptors. **(A)** At the bottom of the folding funnel are protein conformations optimized by evolution. They occupy two distinct free-energy minima with the population of the conformations dominated by either the active form or the inactive form. The relative population of the two states depends on the stabilization energy, which reflects two distinct sets of specific interactions. **(B)** The allosteric two-state model (ATSM) presents an equilibrium between the inactive R state and the active R* state binding to an allosteric ligand, A. The relative population between R and R* is defined by the equilibrium constant, $L = [R^*]/[R]$. Given the equilibrium constant for ligand A bound to the inactive state, $K_A = [AR]/([A][R])$, and to the active state, $\alpha K_A = [AR^*]/([A][R^*])$, the equilibrium constant between AR and AR* is deduced to be $\alpha L = [AR^*]/[AR]$ due to the complete circle of equilibrium. The forward reaction AR → AR* with allosteric efficacy $\alpha > 1$ implies a population shift due to the allosteric binding event. **(C)** The free-energy landscape presentation of the ATSM defines $\Delta G_1 = G_1(R^*) - G_1(R)$ before binding depicted as a light-green curve, the relative free energy between the inactive (R) and active (R*) states, and $\Delta G_2 = G_2(R^*) - G_2(R)$ after binding (orange curve). The extent of population shift as measured by the free-energy change due to binding, $\Delta\Delta G_{1\to2}$, can also be expressed by the difference between the stabilization energy with respect to the active conformation, $\Delta G_{1\to2}(R^*) = G_2(R^*) - G_1(R^*)$ (red arrow), and the destabilization energy with respect to the inactive conformation, $\Delta G_{1\to2}(R) = G_2(R) - G_1(R)$ (blue arrow). Adapted from [17].

driver, anchor, and structural pathway and propose guidelines for agonist versus antagonist drug discovery and for improving the efficacy of same-site allosteric modulators.

Although the merit of allosteric drugs is well recognized and considerable efforts have been invested in their development, how to improve the drugs, as well as how to a priori differentiate between allosteric agonist and antagonist, is currently (largely) conducted via costly trial-and-error protocols. We believe this is because allosteric drug discovery has mainly followed in the footsteps of orthosteric drug design protocols, despite their fundamentally different mechanistic foundation.

An overview of small molecule allosteric versus orthosteric drugs: pluses and minuses

A growing number of allosteric modulators are either already on the market or in the pipeline. They target diseases ranging from chronic kidney failure to cognitive deficits of Alzheimer's disease or schizophrenia, gastroesophageal reflux disease, HIV, pain, Parkinson's disease and more [38–41]. Their increasing popularity is understandable: they are generally safer because they are more specific; they do not compete with the natural ligand; and sometimes they do not affect a receptor unless the natural ligand is already present. Unlike the on/off effects of