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editorial

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Can we use Nature's Structure-Activity-Relationships to Make Better Drugs?

The article by Bofill *et al.* [1] in this issue presents some intriguing ideas on the ever-present problem of how to make more effective drugs for therapy. In data-based systems of discovery and development, the more information available (the more input), presumably the better (quality) will be the output. Thus, the datasets compiled by Bofill *et al.* [1] offer a unique opportunity to learn from what is the culmination of an enormous amount of scientific effort, *i.e.* the production of a therapeutically-useful drug entity coupled with millions of years of evolution. This approach is applied with the obvious advantage of using the human body as a discovery laboratory (what better probe of pharmacological systems than molecules that do not cause harm in humans?), an idea that is being exploited to discover new leads to drug targets from approved drugs ('drug repurposing') [2].

The analyses in this paper offer some empirical relationships between drug activities that can be thought of in terms of protein dynamics, specifically the idea that proteins exist as 'ensembles' of different conformations of related free energy, but otherwise different in terms of ligand recognition [3]. In this model, receptor 'activation', in the sense of a ligand modifying the behavior of the receptor towards its host cell, is depicted as being due to conformational selection [4] whereby the selective affinity of a ligand for various states stabilizes those states at the expense of others to create a new ligand-bound ensemble. Nature's metabolome for receptor targets consists of agonists and there is evidence to suggest that the ensembles for agonists are larger than they would be for antagonists. This is consistent with the known thermodynamic flexibility of GPCRs (nature's prototypic allosteric protein), a notion supported by the experimental procedures needed to constrain receptor protein movement to obtain crystals of GPCRs for determination of structure [5]. Within this context, the inactive form of the receptor could be considered to be the 'special' one with regard to cellular activation and that when the receptor is free from dynamic restraint through mutation, a number of the resulting conformations can still function as active states and produce response. For example, substitution of 19 amino acids in position 293 of the α_{1B}-adrenoceptor receptor leads to the creation of 19 constitutively-active receptors with only one amino acid in the natural conformation locking the receptor into an inactive state [6]. Thus, promiscuous binding in an ensemble to form different conformations may more likely produce a receptor active state than an inactive state.

There may be teleological reasons for a broad ensemble activity for agonists. Multiple receptor active states may be programmed to respond heterogeneously to signaling pathways to selectively activate some signaling pathways at the expense of others (a phenomenon referred to as 'signaling bias' [7]). This is seen in the known differences in signaling bias of seemingly redundant

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natural agonists for receptor types (*i.e.*chemokines). For instance, the chemokine receptor CCR7 interacts with two endogenous natural chemokine agonists, CCL19 and CCL21. However, while both of these agonists produce full cellular responses through this receptor, the responses are not of equal quality. Specifically, both produce G protein activation but only CCL19 (not CCL21) causes receptor agonist-dependent phosphorylation and recruitment of β -arrestin to terminate the G protein stimulus [8]. The cause of this biased signaling is proposed to be the selective stabilization of different agonist receptor active states [9], thus nature makes use of the multiple mix and match of receptor states with signaling proteins in the cell to modify cellular signaling. Signaling bias is a product of natural probe dependent allosterism and it is probable that it is used to fine tune signaling [8].

In terms of protein dynamics, it might be supposed that the array available for selection for an agonist in the receptor ensemble conformational cafeteria might be larger than the corresponding selection for antagonists (which are pharmacologically-selected to have affinity for the less prevalent inactive receptor states). In fact, antagonists may be selected (by the pharmacological discovery process) for a special and less prevalent set of receptor conformations. Protein dynamics suggests that affinity and efficacy are thermodynamically- linked and that it is imperative that the binding of a ligand to a receptor ensemble will change conformation and thus change the ensemble, *i.e.* binding is not a passive process [10]. This being the case, pharmacological selection in the drug discovery candidate selection process is aimed at producing antagonist bound receptor states that do not produce agonism, *i.e.* will be different from the agonist natural metabolome. This difference in target conformational states could account for the closer relative activity of agonists with members of the metabolome (see Bofill *et al.*, Fig 2A [1]) compared to that of antagonists (see Bofill *et al.*, Fig 2B [1]).

Irrespective of how these datasets can lead to speculation about molecular mechanisms of receptor function, as discussed by Bofill *et al.* [1], they can furnish practical and empirical guidelines that may be used to evaluate new drugs in terms of activity and safety. Pharmacology is a science notoriously dependent on the observation of drug activity in systems that pharmacologists do not fully understand (*i.e.*'black box' activity). Historically, drug discovery was, and in many cases is still, based on null methods rather than detailed analyses of known molecular mechanisms, thus there is a tradition of applying ideas the origin of which are not yet clearly understood. In this sense, the use of metabolome data to guide discovery is in keeping with this tradition. Within this context, the authors make a strong and appropriate case for exploration and exploitation of the metabolome to further evaluate and validate these relationships. In addition, the findings of this paper suggest how similar approaches may be used to exploit other pharmacological databases. For instance, it would be interesting to examine a corresponding 'synthetic metabolome' of the natural metabolites made in humans of approved synthetic drugs. If the metabolites of drugs are considered close analogs of the active drug (*i.e.*metabolic transformation products are similar in chemical structure to the parent [11]) then metabolism provides a comparison of active drugs and closely similar structures interacting with the ensemble, in essence a structure activity system provided by nature. In addition, such a database might shed light on the intrinsic properties of orthosteric (binding to the natural endogenous agonist binding site on the target) and allosteric (binding to a unique binding site on the target) ligands. Structure activity relationships for allosteric scaffolds are known to demonstrate 'activity switching' whereby very small changes in ligand structure lead to very large differences in ligand activity [12]. Given this, it might be predicted that there would be a greater difference in affinity between the parent and metabolite for allosteric molecules (as opposed to orthosteric ones).

The paper by Bofill *et al.* [1] brings conclusive evidence that the affinity of endogenous metabolites for their native receptors can serve as baseline for the primary pharmacology of drugs, and that nearly 96% of drugs have bioactivity values within 2 orders of magnitude of the corresponding metabolite bioactivities for the same protein. Based on this analysis, the authors further propose a relatively straightforward method to evaluate drug safety. Importantly, this method redefines drug safety margins based on *in vitro* affinities of the endogenous ligands, and not on the relative differences between target and off-target bioactivities. This may have practical consequences in terms of anticipating side effects linked to secondary pharmacology. In particular, one ought to consider that the biological machinery of related animal species is composed of similar yet mutated proteins, the function of which is nonetheless regulated by the same endogenous ligand. It seems as the chemical composition of biological systems seems to be more robust than their protein composition. Accordingly, the *in vitro* binding affinities of an endogenous ligand and drugs for a given protein might differ across species, and so may also their relative differences. Since some animal species are used as surrogate human models (*e.g.*, mouse, rat, guinea pig) both drug efficacy and safety findings in those species may not always be translatable to humans. In this respect, the paper by Bofill *et al.* [1] highlights the relevance of collecting data on the endogenous metabolome, in humans but also in human-model species, as a means to deepening our understanding of the enormous complexity of drug discovery.

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