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Measurements of ligand bias and functional affinity

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Regarding our article (Signalling bias in new drug discovery: detection, quantification and therapeutic impact; *Nature Rev. Drug Discov.* **12**, 205–216 (2013))[1], we would like to thank Sudarshan Rajagopal for his comments.
In his correspondence piece (Quantifying biased agonism: understanding the links between affinity and efficacy; *Nature Rev. Drug Discov.* 17 May 2013 (doi:10.1038/nrd3954-c1))[2], he considers that two models — namely allosteric and pharmacological models — can be used to determine ligand bias at seven transmembrane receptors (also known as G protein-coupled receptors) and that we have incorrectly used a pharmacological model combined with a conditional affinity value. In our view, the choice of model used to describe receptor function is not an issue, as both types of model are just (imperfect) views of the molecular events that lead to agonism. The pertinent question is whether the affinity of the agonist can change depending upon the receptor state. For example, if the receptor is coupled to a specific signalling protein, isomerized into its active state[3], uncoupled from any signalling proteins in the inactive state, and so on.

We stand by the opinion presented in our Perspective article that a ligand can have differing affinities at these different receptor states and these differing affinities must be taken into account when measuring biased agonism. For example, the structure of the β-adrenergic receptor differs when it is complexed with a nanobody to simulate a G protein and when it is not coupled to a nanobody[4,5]. These differences manifest as changes in ligand affinity. For example, structural changes in transmembrane domains six and seven of the κ-opioid receptor upon binding of specific G protein subunits produce an 18-fold change in the affinity of this receptor to salvanorin[6].

In our Perspective, we defined the term functional affinity (also referred to as conditional affinity) as the K_A term described in the Black–Leff model of agonism[7]. Indeed, each of the methods discussed in our Perspective that incorporate the Black–Leff model — whether it be our transduction coefficients, the methods favoured by Rajagopal (that is, determining σ_{lig} values) or, indirectly with relative activity values — implicitly use a measure of functional affinity. Therefore, when using the Black–Leff model, we must abide with what the model defines as affinity, namely the "equilibrium-dissociation constant of the agonist–receptor complex"[7].

In our opinion, arbitrarily assigning a binding affinity for a full agonist to calculate a τ value (that is, a transducer ratio) is a sterile exercise if that binding affinity is incorrect with respect to the Black–Leff model; that is, the use of an incorrect K_A value will yield an incorrect τ value (and an incorrect σ_{lig} value). Also, for full agonists, an infinite combination of τ values and K_A values will fit the concentration–response curve, and this is precisely why we use τ/K_A ratios, as it is only the ratio that becomes a unique identifier of the concentration–response curve produced by a specific full agonist.

A more practical problem has emerged from the literature whereby τ values (and therefore σ_{lig} values) for two pathways for weak partial agonists cannot be calculated using a single affinity value of K_A. Specifically, these systems show that the EC_{50} values (the concentration of agonist that produces a 50% maximal response) of low-efficacy partial agonists for two pathways are significantly different from each other. As the EC_{50} for weak partial agonists approximates the K_A (see equation 9 of REF. 8), this precludes fitting concentration–response curves for the two pathways using the same K_A (examples of which we point out in Supplementary information S2 that accompanies our article[1]). For example, when clenbuterol acts as a partial agonist at β_1-adrenergic receptors, there is a 16-fold difference in the EC_{50} value generated from measurement of G protein interactions compared to measurement of β-arrestin interactions[9].

When τ values for two pathways for weak partial agonists cannot be calculated using a single value of K_A the choices are to either discard the operational model as it cannot fit pathway-selective agonism using a value of single affinity, or consider that functional affinities may differ in the cell and use these different functional affinities. If one discards the operational model, then it negates transduction coefficients, relative activity values as well as σ_{lig} values. We agree with Rajagopal that relative activity values are of merit for measuring bias, but take the view that transduction coefficients are also useful. Indeed, relative activity values are identical to transduction coefficients for agonist concentration–response curves that have slopes of 1 (REF. 10). We also feel that σ_{lig} values are useful if it is shown that the binding affinity approximates what the Black–Leff model used to estimate τ (namely the K_A for the operational model equation). Without this confirmation we feel it is a hazardous step to make this assumption and go on ascribing what could be inaccurate values for bias.

Both transducer ratios and relative activity ratios use functional affinity and yield estimates of signalling bias in all systems. In contrast, a single estimate of affinity cannot be used to accommodate concentration–response data for two signalling pathways for a growing number of agonists. Direct measurements of partial agonist affinity through pharmacological means need to be carried out to examine why a single estimate of affinity will not furnish consistent τ values for some agonists that activate two signalling pathways from the same receptor. The use of any affinity value will furnish a value for τ, but whether this value is consistent for agonists that activate two signalling pathways from the same receptor depends on the use of the correct value of K_A being used.

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Competing interests statement
The authors declare no competing financial interests.