A Proposed Mechanism for *in vivo* Programming Transmembrane Receptors

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Abstract. Transmembrane G-protein coupled receptors (GPCRs) are ideal drug targets because they resemble, in function, molecular microprocessors for which outcomes (e.g. disease pathways) can be controlled by inputs (extracellular ligands). The inputs here are ligands in the extracellular fluid and possibly chemical signals from other sources in the cellular environment that modify the states of molecular switches, such as phosphorylation sites, on the intracellular domains of the receptor. Like in an engineered microprocessor, these inputs control the configuration of output switch states that control the generation of downstream responses to the inputs.

Many diseases with heterogeneous prognoses including, for example, cancer and diabetic kidney disease, require precise individualized treatment. The success of precision medicine to treat and cure disease is through its ability to alter the microprocessor outputs in a manner to improve disease outcomes. We previously established *ab initio* a model based on maximal information transmission and rate of entropy production that agrees with experimental data on GPCR performance and provides insight into the GPCR process. We use this model to suggest new and possibly more precise ways to target GPCRs with potential new drugs.

We find, within the context of the model, that responses downstream of the GPCRs can be controlled, in part, by drug ligand concentration, not just whether the ligand is bound to the receptor. Specifically, the GPCRs encode the maximum ligand concentration the GPCR experiences in the number of active phosphorylation or other switch sites on the intracellular domains of the GPCR. This process generates a memory in the GPCR of the maximum ligand concentration seen by the GPCR. Each configuration of switch sites can generate a distinct downstream

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response bias. This implies that cellular response to a ligand may be programmable by controlling drug concentration. The model addresses the observation paradox that the amount of information appearing in the intracellular region is greater than amount of information stored in whether the ligand binds to the receptor. This study suggests that at least some of the missing information can be generated by the ligand concentration. We show the model is consistent with assay and information-flow experiments.

In contrast to the current view of switch behavior in GPCR signaling, we find that switches exist in three distinct states: inactive (neither off nor on), actively on, or actively off. Unlike the inactive state, the active state supports a chemical flux of receptor configurations through the switch, even when the switch state is actively off. Switches are activated one at a time as ligand concentration reaches threshold values and does not reset because the ligand concentration drops below the thresholds. These results have clinical relevance. Treatment with drugs that target GPCR-mediated pathways can have increased precision for outputs by controlling switch configurations. The model suggests that, to see the full response spectrum, fully native receptors should be used in assay experiments rather than chimera receptors.

Inactive states allow the possibility for novel adaptations. This expands the search space for natural selection beyond the space determined by pre-specified active switches.

1 Introduction

Protein receptors that span the cell membrane are molecular microprocessors [20]. They gather information from outside the cell and process and transmit the information to the intracellular space, where it is directed to chemical pathways that lead to cellular response to extracellular conditions. The molecular microprocessors are programmed by natural selection and chemical conditions within the organism. For disease control, the programming is achieved mostly through the directed application of drugs. It requires higher-resolution drug targets and increased understanding of the effects of high-resolution drug targeting [2].

The most important class of these transmembrane receptors, both scientifically and medically, are the G-protein coupled receptors (GPCRs). More than 30% of all prescription drugs target GPCRs [18]. The advent of precision medicine has increased the importance of understanding how information can be controlled at higher levels of resolution. As an example, the hormone angiotensin II increases blood pressure and prolonged hypertension drives diabetic kidney disease (DKD). Renin angiotensin system inhibitors (RASi), such as angiotensin converting enzyme inhibitors (ACEis) and angiotensin II receptor blockers (ARBs) block the formation and action of angiotensin II and lower systemic blood pressure. Interestingly, when compared with other antihypertensive agents, ACEIs and ARBs stabilize kidney function at the same level of achieved blood pressure better than conventional antihypertensive therapy [22].

This suggests that angiotensin II also operates in other processes [2,13]. Indeed, the angiotensin receptor and other G-protein coupled receptors can trigger distinct multiple downstream responses that depend on the cellular environment [6,11,17] and thereby may lead to heterogeneous disease progression and effect of therapy.

As the angiotensin example illustrates, the era of precision medicine requires much higher resolution drug targeting to reduce the heterogeneity of disease progression. This requires a better understanding of the details of how the information is processed by GPCRs. Recent attempts [6] at making progress on this ambitious goal have relied on teleonomy, the imparting of goals to natural selection. In this case, a model was developed that imparted goals of maximal rate of energy production and information transmission. In this study, we use the model developed in [6] to improve the resolution of GPCR information processing and increase our understanding of how this resolution can be used to program the molecular microprocessors to individualize patient treatments for improved prognosis.

Information is a type of entropy [5,7,10,21]. Systems in equilibrium are those in which entropy is maximized and entropy flow is zero. Systems in equilibrium are not alive. Living systems are those in which entropy, and hence information, flows. Entropy flow can occur, for instance, when a chemical concentration is far from its equilibrium value as is the case with adenosine triphosphate (ATP) in biological systems. Entropy and information flow can also occur in the presence of spatial gradients such as those found at cell membranes. Both types of entropy flow are important in this study. Figure 1A illustrates a case in which information flows across a cell membrane. Figure 1C illustrates an active phosphorylation switch in which a chemical flux is driven by excess of ATP.

Information contained in the extracellular space is transmitted to the intracellular space by receptors (Fig. 1A). Ligands, such as hormones, auxins, nutrients, neurotransmitters, and many other molecules in the extracellular fluid, announce their presence to the cell by binding to extracellular domains of the receptor and allosterically altering the intracellular properties of the receptor. The intracellular changes in the receptor effect the cell's response to the ligand stimulus. In many cases, the extracellular changes take the form of phosphorylated and unphosphorylated sites on the C tail of the receptor protein (Figs. 1B and C) [11]. The sites form a barcode that is read by intracellular processes that respond to the information in the code [3,4,11,23–26]. Other receptor conformations are possible but the barcode process described here is a good representative exemplar.

A number of questions emerge. How is the barcode programmed? In other words, which sites are phosphorylated and which are unphosphorylated? How many active sites, or switches, are there? How is the phosphorylation state of the barcode changed? What determines the number of active phosphorylation sites and how is that number changed? The issue is that any set of molecules that store information, as does the barcode, must be stable to thermal and other fluctuations [19]. Yet, rearrangement of the barcode must be possible. Since many phosphorylation sites may be present, this requires a significant amount of energy to effect a global change. Typically sites are phosphorylated and dephosphorylated by catalyst kinases and phosphatases, respectively. How can both kinases and

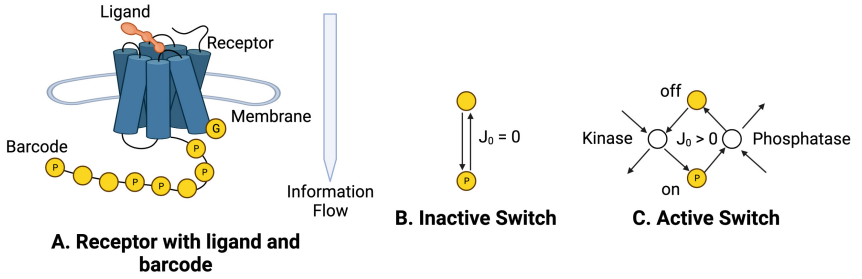

Fig. 1. A. A ligand binds to the extracellular domains of a receptor that spans the cell membrane. A number of phosphorylation sites occupy the C tail of the receptor. Some of the sites are inactive (not shown); some are active and phosphorylated (designated by P); and some are active and unphosphorylated. Phosphorylated sites are designated on, while unphosphorylated states are designated off. The site labeled G is a GTPase switch found in G-protein coupled receptors (GPCRs). **B.** An inactive PdPC switch. Every physical path from unphosphorylated (off) to phosphorylated (on) has a microscopically reversed path. The chemical flux in the forward path is equal to the flux in the microscopically reversed path. The flux is in detailed balance and the reaction is in equilibrium. **C.** An active PdPC switch. The reactions in a forward circular direction have significant chemical flux J_0 and are catalyzed by kinases and phosphatases. The microscopically reversed pathways in the opposite direction have negligible flux and are not displayed. The switch is driven far from equilibrium by the flux J_0.

phosphatases act in global concert to change the information content across an entire barcode of several phosphorylation switches?

This study attempts to address some of these questions. Our approach is grounded in recent theoretical work [6] fitting biochemical data [17], site-directed spectroscopy observations [11], molecular dynamic simulations [11] and information-flow measurements [9]. Our approach differs from the mass-action-driven modeling approaches on signaling bias, e.g. [1], thus it may be unfamiliar to some due to its new approach and its heavy reliance on non-equilibrium thermodynamic principles. However, the fundamentals of the original model are well described by [6]. Therefore, we only introduce here some of the relevant features of the approach in Figs. 1 and 2. The new findings are illustrated and supported by data in Table 1 and Figs. 3 and 4. Briefly, we show that in addition to information for output (e.g. effector coupling, MAPK cascading, gene expression) induced by ligand binding to the GPCR, the dynamics of ligand concentration presented to this receptor, both concentration and time, provide information that is transmitted to downstream responses. Consequently, new drug presentation strategies may provide an additional tool in targeting GPCRs for disease control.

2 Approach and Results

2.1 Relation Between Ligand Concentration and Number of Active Phosphorylation Sites

We build on the study in [6]. That work used maximum entropy production along with maximum information storage and transfer to generate the bag-of-independent-switches (BOIS) model picture, which imposes a number of constraints on the barcode. The model predicts that three switch configurations are possible, on, off, and inactive and that the phosphorylation sites (switches) are effectively uniform, except for a small number of differences that divide the switches into the three configurations. Active switches are distinguished from inactive switches in that active switches support a finite chemical flux through the switches [16], while inactive switches have zero flux Fig. 1B and C). Active switches are divided into phosphorylated (on) and unphosphorylated (off) sites. The chemical flux in active switches serves as a local energy/heat source that drives the process. The basic exemplar switch is a phosphorylation-dephosphorylation cycle (PdPC) [16] displayed in Fig. 1B for the inactive switch and Figure C for an active switch. The number of switches N, in this case, is equal to the number of phosphorylation sites on the C tail. The receptor concentration is designated R_T.

For a receptor that has never been exposed to a particular ligand, all switches in the barcode are inactive with no chemical flux. As ligand concentration increases, the switches activate one at a time (Fig. 2). The first switch activates when the receptor concentration reaches an experimentally accessible [17] reference concentration of $R_{ref} = R_T/N$ that is independent of the type of ligand or the ligand concentration [6]. As ligand concentration increases, the switches activate one-by-one until all N switches are activated. If the ligand concentration is then reduced, the switches that have been activated do not deactivate. This is conceptually similar to a ratchet and pawl that ratchets to a higher number of activated states (Supplement A.1).

According to the BOIS model, the maximum concentration L^* that the ligand reaches, the total number N of switches, and the number M of activated switches obey the relationship (Supplement A.2)

$$\frac{R_L}{R_{ref}} = \frac{L^*}{K_D + L^*}\,(N - M)\; < 1 \tag{1}$$

where K_D is the ligand/receptor dissociation constant. Equation 1 is a constraint on the number of activated switches M. If the ligand concentration is very large, $L_{max} \gg K_D$, then all the phosphorylation sites are activated, $M = N$. If the receptor has never encountered the ligand, $L^* = 0$, then the arguments of Supplement A.2 indicate that no switches are activated and $M = 0$. The predicted value for M is the minimum value of M for which the inequality Eq. 1 is true. This can be written

$$M = \text{floor}\,\left[(N+1)\frac{L^*}{K_D + L^*}\right] \tag{2}$$

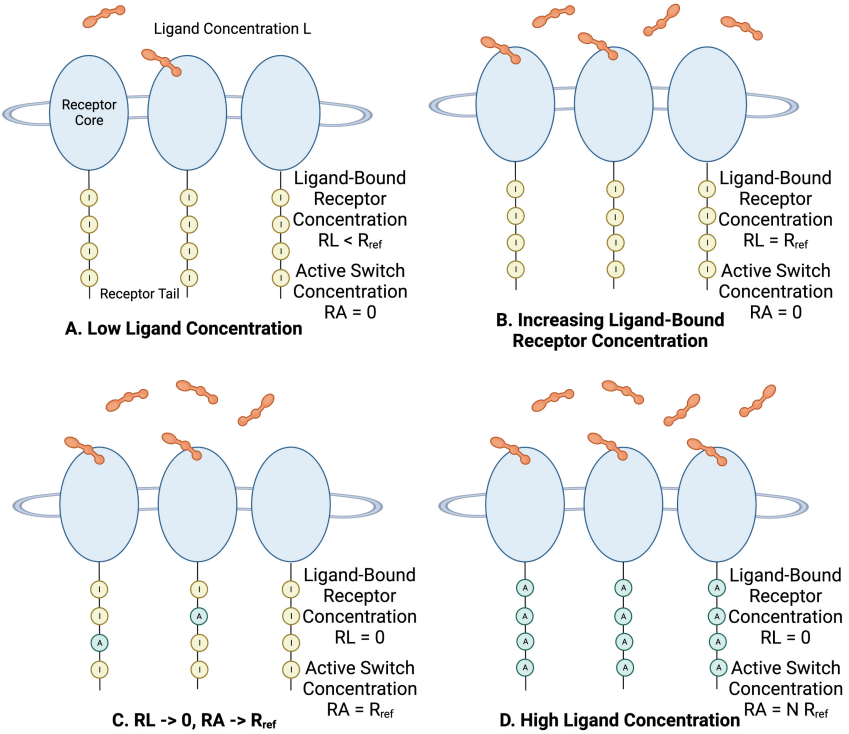

Fig. 2. ratchet. **A.** Early time, low ligand concentration. The ligand-bound core concentration $R_L = 0$. The concentration of active switches $R_A = 0$ All switches are inactive (I). **B.** As ligand concentration increases, the ligand-bound core concentration R_L approaches the reference concentration R_{ref}. **C.** When the ligand-bound core concentration R_L equals R_{ref}, the core concentration R_L goes to zero and the concentration R_A of active switches goes to R_{ref}. **D.** The process repeats until the number M of active switches is equal to the number pf phosphorylation sites N. At any given time the concentration of active switches is $R_A = M\ R_{ref}$. The number of active switches M does not decrease when the ligand concentration decreases.

where floor chooses the largest integer less than the argument. When the maximum ligand concentration is zero, $M = 0$. When the argument of floor becomes slightly greater than one, then $M = 1$. When the maximum ligand concentration becomes very large, then $M = N$. The ligand concentrations $L(M)$ at which switch M turns on is determined by

$$M = N \frac{L^*(M)}{K_D + L^*(M)} \tag{3}$$

The quantity $L^*(M)$ can be approximated by the half-maximal effective concentration $\mathrm{EC}_{50}(M)$ for switch M. This prediction is consistent with observations

that significant downstream response can be observed even when a significant fraction of receptors are not engaged causing EC_{50} values to be potentially much smaller than the value of the dissociation constant K_D [27].

We see from Eq. 2 that an approximation of the value of the largest ligand concentration the receptor has encountered is coded into the number of active switches, according to the BOIS model. The amount of information per bit I_M stored in this manner is (Supplement A.2)

$$I_M = \log_2 \frac{N!}{(N-M)!} \tag{4}$$

where M is given by Eq. 2. The BOIS model thus provides a mechanism for encoding and transmitting information related to extracellular ligand concentration. The maximum information I_B stored in the binding of ligand with the receptor is

$$I_B = \log_2 2 = 1 \text{ bit} \tag{5}$$

Therefore, the amount of information we have identified in the extracellular fluid that can be transmitted to the intracellular fluid is the sum of Eqs. 4 and 5. This extra information about the maximum ligand concentration may contribute to the observed excess transmitted information [9].

2.2 Application to G-Protein Coupled Receptors

An important GPCR feature, in the context of this study, is that, in addition to the barcode on the C tail, there is a GTPase switch [16] that activates particular downstream responses to ligand stimuli [14].

The BOIS model predictions of the previous section can be compared with GPCR assay observations [17]. The results are summarized in Table 1 and Fig. 3. The assays examined the response in two receptors, adrenergic and angiotensin, when tested with several ligands. The responses in two downstream pathways were measured, the response to the G_α GTPase switch turning on and the response of the recruitment of β arrestin (βarr) to the C tail barcode of the receptor GPCR [12]. The βarr is thought to be a scaffold for other responses mediated by the barcode [12]. To reduce noise, the C tails of the light receptors in the assay were replaced with C tails of the vasopressin. Receptors with this alternate tail are known as chimeric. While the βarr recruitment site was preserved, the remaining phosphorylation sites on the C tail may not have been preserved on the chimeric-receptor tail.

The ligand concentration was slowly increased from 0 molar to a concentration at which the response was saturated. It was found that the maximum response was approximately equal to a common reference concentration R_{ref} for each receptor in agreement with the BOIS predictions [6]. This can be seen in Fig. 3C and D where the maximum values of the response concentrations normalized to R_{ref} are found at the corners, (00), (10), (01), and (11), of a hypercube. Moreover, the switches turned on one at a time also as indicated by the BOIS

Table 1. Summary of Assay Data. Two receptors were tested with several ligands. The detailed results and the ligands are given in the Supplement and reference [17]. The purple row is for those outcomes in which both the G_α and the βarr recruitment switches are turned on by the ligands. The red cells indicate assays in which the G_α switch is turned on by the ligands, but the βarr recruitment switch is off. The blue cells indicate assays in which the G_α switch is turned off by the ligands, but the βarr recruitment switch is on. The yellow cell indicates a ligand that did not turn on either the G_α or the βarr switch. The G_α column indicates the mean logarithm of the molar ligand concentration at which the G_α switch turns on. The βarr column indicates the concentration at which the βarr switch turns on. An X indicates that the switch did not turn on. The Order columns indicate the order in which the switch turns on as ligand concentration increases. We see, for balanced ligands, that the second switch turns on at a ligand concentration approximately one order of magnitude higher than the concentration at which the first switch turns on. For the biased ligands, the order of the switch turning on is determined by comparison with the concentrations of the balanced ligands. For example, the biased G_α ligands are determined to be the second switch turning on by noting that the concentration -7.76 for the biased turn on is approximately equal to -7.95, the concentration of the second switch to turn on for the balanced ligands.

Adrenergic					**Angiotensin II**				
	Order	G_α	Order	βarr		Order	G_α	Order	βarr
Bal	1^{st}	**-9.44**	2^{nd}	**-7.95**	**Bal**	1^{st}	**-8.06**	2^{nd}	**-7.14**
Bias	2^{nd}	**-7.76**		X	**Bias**		X	2^{nd}	**-6.86**
None		X		X					

model (Table 1, Fig. 3A and B). In the case we have here in which the ligand concentration of the second switch is much greater than the ligand concentration of the first switch, then, from Eq. 2, the dissociation constant K_D is approximately the $L^*(1) = EC_{50}(1)$ of the first switch, which is given in Table 1 as $10^{-9.44}$ M for the adrenergic receptor and $10^{-8.06}$ M for the angiotensin II receptor. This implies, from Eq. 3, that the total number N of switches is

$$N = 2 \tag{6}$$

This means that the experimenters [17] observed all the states in the two receptors that were affected by the increasing ligand concentration. Moreover, it implies that the replacement of the adrenergic and angiotensin II C tails with vasopressin tails did not preserve the ability of the barcode to trigger responses other than βarr recruitment.

From Eq. 4, we see that two bits ($2 \log_2 2$) of information is transmitted across the cell membrane to the intracellular space.

The BOIS model predicts that these receptors are able to detect if a ligand is attached and whether the ligand concentration is greater than or less than the half maximal effective concentration $\mathrm{EC}_{50}(1)$ for the first switch.

Four distinct responses were observed, a balanced response to the bound ligand in which both the G_α switch and the βarr recruitment switch turn on

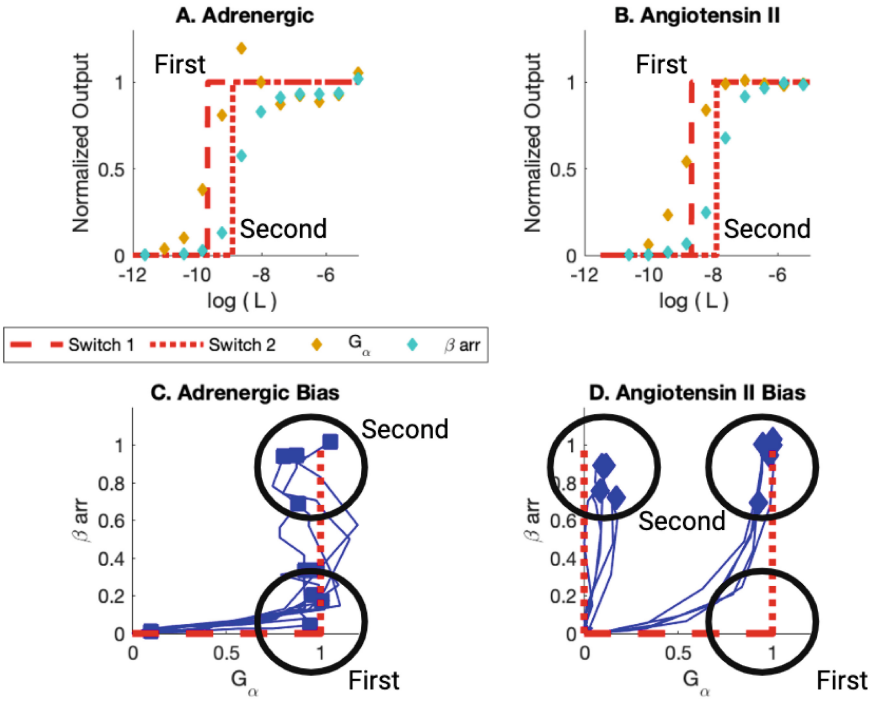

Fig. 3. A. Adrenergic Receptor with Formoterol as Ligand. Here, L is the ligand concentration. The theory is displayed in red. The dashed line is the simulation of the activation of the first switch. The dotted curve is the activation of the second switch. The yellow markers are the observed assay dose response for the G_α pathway. The cyan markers are the observed assay dose response for β arr. **B.** Angiotensin II Receptor with Angiotensin II as Ligand. **C.** Bias Plot for All Ligands for Adrenergic Receptor (see Table 1). The simulation results are displayed in red. Note that some ligands are G_α biased; their endpoints lie close to the G_α axis. Other ligands are balanced; their endpoints lie at (1,1). No β arr bias is seen in this set of ligands. **D.** Bias Plot for All Ligands for angiotensin II Receptor. This plot illustrates balanced bias and β arr bias. For balanced bias the first switch is the GTPC and the second switch is the PdPC that activates arrestin recruitment. For β arr bias, the GTPC is not activated. The BOIS model predicts that the first ACTIVATED is a PdPC that is not observed. Here, G_α bias is only seen for ligand concentrations that are associated with sub-maximal response.

(purple row in Table 1), a situation in which just the G_α switch turned on (red cells), a situation in which just the βarr switch turned on (blue), and a situation in which no switches turned on (yellow). For the balanced ligands, both switches are seen to activate, first the G_α switch and then the βarr recruitment switch (Fig. 4). The first switches to activate in the case of biased ligands is now

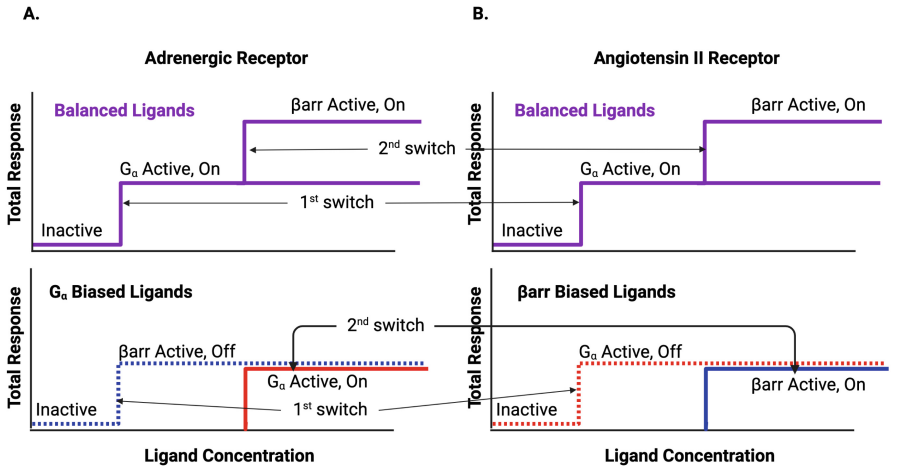

Fig. 4. BOIS interpretation of bias data. Ligands can be classified by whether the response is balanced or biased. The upper curve is the response for balanced ligands. The lower curve is the response for biased ligands. The system supports two switches that activate sequentially. For low initial values of ligand concentration, all switches are inactive. As ligand concentration increases, the first switch is activated. For balanced ligands, the first switch observed to be activated is G_α, while for biased ligands the first switch can be either G_α **A.** or βarr **B.**. For biased ligands, the first switch is activated in the off state. For the adrenergic receptor **A.**, The G_α switch does not need to be activated in order to activate the βarr switch into the off state for biased ligands. After the first switch activates, but before the second switch activates, half the switches are active and half are inactive. As ligand concentration increases further, the second switch activates activating the βarr recruitment pathway for balanced ligands and the remaining inactive pathways for the biased ligands. Note that the BOIS model predicts that if the ligand concentration is lowered from its maximum that the activated switches do not deactivate. Therefore, the number of active switches is a measure of the maximum ligand concentration.

observed, however. The adrenergic receptor responds with G_α response when the second switch activates, while the angiotensin II receptor responds with the βarr recruitment response when the second switch activates.

The BOIS model predicts that, for biased ligands in these observations, the first switch is activated, but set to the off state. In other words, the switch sites are absorbing energy and dissipating heat but they are in the off position. This means, for example, that if the switch is a phosphorylation site, it is active and generating chemical flux through the site but the site spends most of its time unphosphorylated and not activating downstream response.

The BOIS model predicts that, for the one ligand that displayed no response, the switches may have been activated but they were in the off state.

3 Discussion

This study starts from an abstract model, the BOIS model [6] that was built on the assumptions of maximum rate of entropy production and maximum storage and flow of information. To validate the model with experiment, the BOIS model was specialized in [6] to address information flow in GPCRs. The process of this specialization was continued in this study and further hypotheses were generated that were compared with assay observations of [17] and information-flow experiments [28].

Specifically, switches can exist as phosphorylation sites on the C tail or on the GPCR or they can exist as GTPase switches on the core of the receptor. The switches can exist in three distinct states [6], inactive, active/on, and active/off. Active states are distinguished from inactive states in that active states support a chemical flux of receptor states through the switches while the inactive states have zero flux. For switches in the on state the receptor spends most of the time in the phosphorylated state in the case of phosphorylation switches. In the off state, the receptor spends most of the time in the unphosphorylated state.

Each active switch associates with a quantum of the total receptor concentration equal to $R_{ref} = R_T/N$ where R_T is the receptor concentration and N is the number of switches. This is true for both inactive and active switches [6]. This is a collective process. Each switch has a local property R_{ref} affected by a global quantity N, which is non-local. It is not clear how an individual switch gains access to how many switches, active and inactive, there are.

The switches are activated one at a time in units of R_{ref} as ligand concentration increases from an initial zero value and assuming all switches in the receptor are inactive. Therefore N is the total number of switches than can be activated. Whether a switch is in the on state or to the off state seems to be determined at activation. If a switch becomes activated, then it remains active if the ligand concentration drops. Therefore, the number of active switches is a measure of the maximum concentration the receptor has experienced. The expression for this is given in Eqs. 3 and 4.

These predictions are consistent with experimental observations that significant response can occur when only a small number of total receptors are engaged with active switches [27]. They also suggests that observations that do not use chimeric-receptor C tails may be of importance. Chimeric-receptor tails were used to reduce noise, but the model predicts that the only switches available in assay experiments [17] were the βarr recruitment switch and the G_α switch when chimeric-receptor tails were employed. One expects many more phosphorylation switches [11]. This hints that the native C tails had relevant phosphorylation sites that did not appear on the chimeric-receptor tails. This suggests that the some of the noise that was eliminated by the chimeric-receptor tails was actually signal. If one redoes the assay experiments with native tails and measures more than two downstream responses, then the model predicts a series of signal switches that turn on sequentially and that the EC_{50}s of those switches would be closer together.

The amount of information transmitted through the membrane, Eq. 4, from the ligand concentration can account for some of the information found missing in information flow measurements [28]. In the observations, two bits or more of information was observed transmitting through the membrane, but if the only source of the information was whether the ligand was bound or not to the receptor, then only one bit, at most, was available to be transmitted. This suggests that there might be information other than simple binding that is associated with the ligand. At least some of the missing information seems to originate with the value of the ligand concentration.

Comparison of the BOIS model with assay experiments [17] indicates that the first switch that is activated may be either a G_α switch or a βarr switch for biased ligands (Fig. 4). For the two receptors observed, the first switch was G_α for balanced ligands and the second switch was for βarr recruitment. Bias may be determined at the time of switch activation. Our small observational sample [17] suggests that the first state activated is activated to the off state and determines the bias, at least in the case of a small number of switches ($N = 2$).

This picture differs from the canonical model of GPCR activation [15]. In that model, the G_α switches activates and turns on first. Subsequently, the βarr switch activates and turns on. Somewhat surprisingly, for the adrenergic receptor in this study coupled with the assay study [17], the G_α switch does not need to be activated in order to activate the βarr switch into the off state for biased ligands. Within the context of the BOIS model and the assay experiments [17], the βarr switch is first to activate, but it is turned off. The G_α switch may be turned on after the βarr recruitment switch, but the mechanism for this is currently unclear. These observations may also be affected by the information lost in the chimeric-receptor tail.

These results suggest some options for programming the receptor microprocessors with drugs for application in precision medicine. The new information in this study relates to the importance of ligand concentration in determining the downstream response.

In addition to ligand type, downstream response seems to be determined by the ligand concentration. Ligand concentration determines the number of active switches and the downstream response. Presumably there is a different downstream set of responses for each value of the number of active switches. Bias is determined by first switch activated. Ligand selection indicates that ligands determine the G_α pathway and the βarr pathway.

This study indicates that bias can be determined at the time that switches are activated. It is still not clear, however, if or how switch states can be altered between on and off after the switch is activated. Altering switch states at this level is a competition between the longevity and stability requirements for useful information and the need to be able to change the states for adaptability [19].

The observation that a biological switch can have three possible states rather than the two in a Boolean world leads to implications for adaptability. The switches in inactive states are switches that can be either on or off when activated. They represent the possibility of future information encoding for unknown

future cell responses. The possible information in the barcode is no longer constrained by the number of active switches that are definitely in on and off states. The number of potential barcode configurations has increased significantly over the number encoded by on and off. This concept of increasing the size of the available search space to respond to future unknown events has been dubbed adjacent possibility [8] and has been suggested to be a necessary requirement for natural selection.

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