CHAPTER 5

PROTEIN LOGIC

In recent years both experimentalists and theorists have begun to appreciate that cellular decision making can be performed at the level of a single molecule. Receptor proteins that bind two types of ligand can act as logic gates, initiating different cellular responses depending on which combination of ligands is present. Here we investigate the versatility of receptor function by applying a well known statistical mechanical model to receptors which bind either one or two ligands, and their associated dimers. Notably, we find that a single heterodimer can realize any of the 16 possible logic gates, including the XOR gate, by variation of biochemical parameters. We then investigate versatility by recombination, asking whether a set of only three receptors with fixed parameters can encode the four functionally unique logic gates (OR, AND, ANDNOT, and XOR) simply by forming the possible dimer combinations. An exhaustive search reveals that the simplest set (two single-ligand receptors and one double-ligand receptor) can realize several different groups of three gates, a result for which the previous analysis of single receptors and dimers provides a clear interpretation. Both results underscore the surprising functional freedom readily available to cells at the single-molecule level.

Based on manuscript W.H. de Ronde, P.R. ten Wolde and A.M. Mugler. Protein Logic In preparation.
5.1 Introduction

Cells depend on cues from their environment to initiate behaviors, including growth, division, differentiation, and death. For this purpose, cells have evolved surface receptors that detect environmental signals and relay them to their interior. Intracellular proteins respond to the states of these receptors, triggering a network of biochemical interactions that ultimately leads to a specific action. Although a particular environmental signal often elicits a particular cellular response, it is well established that signals can also act in combination [51, 129, 130, 131]. In this case the response triggered when two signals are present can be distinct from the responses triggered by each signal alone. The cell thereby acts as a logic gate, integrating two inputs to produce a single output. The performance of logical computations by cells has been studied in depth both experimentally, e.g. for engineered circuits [132, 133] and molecules [134, 135, 136], and theoretically, e.g. for biochemical networks [137] and gene regulation [138, 139, 140].

Remarkably, it is becoming clear that such logical computations can be encoded entirely within a single receptor molecule [84]. That is, while some receptors respond quite specifically to a single type of signaling molecule (or ligand), many respond to multiple ligands [141, 142, 143, 144]. Thus, the local concentrations of two or more ligands can act as the logical input, while the activity state of the receptor acts as the logical output. Moreover, many receptors exist in the form of dimers or larger oligomers. For example, G Protein-Coupled Receptors (GPCR) and ErbB receptors can each form dimers consisting of receptors of the same type (homodimers) or a receptor of each type (heterodimers) [141, 145, 146]. One might intuit (and indeed we will see) that a heterodimer, in particular, is well poised to act as a logical integrator of two ligand signals. Both of these mechanisms — multi-ligand specificity and dimerization — allow for the possibility that a cell encodes decisions directly at the detection level, even before further intracellular processing leads to the ultimate phenotypic response.

The focus of this study is to elucidate the functional capabilities available to a cell directly at the level of its receptors. We use a statistical mechanical model to investigate the versatility of logical functions attainable by single receptor monomers or dimers. Versatility is explored in two contexts. First, for a given monomer or dimer, we identify the set of logical functions that can be explored by variation of its biochemical parameters. Notably, we find that all 16 possible logic gates can be performed by a single dimer, a result that we support analytically. Second, motivated by the fact that receptors can diffuse, bind, and unbind on the cell surface [147], we ask whether a minimal set of receptor monomers with fixed parameters can perform a maximally distinct set of logical functions simply by forming the possible dimer combinations. We find several parameter settings at which dimers realize three out of four unique functions, a result for which the first analysis provides a clear interpretation. These results make clear that single receptor molecules can encode diverse functions that cells can exploit both on long evolutionary timescales, via parameter exploration, or on the short timescales of cell signaling, via diffusive recombination.
5.2 Methods

We study receptor function by appealing to an equilibrium statistical mechanical model. Statistical mechanical models have been used quite fruitfully in the study of many molecular biology problems, including receptor activity and gene regulation [148]. In the case of receptors, several models are well known. All assume that a receptor can exist in either an active (A) or an inactive (I) state, and that binding of a ligand changes the receptor bias for each state. In the Koshland-Nemethy-Filmer (KNF) model, ligand binding directly activates the receptor [149]. That is, the bias is complete: a ligand-bound receptor is active, and an unbound receptor is inactive. This condition is relaxed in the Monod-Wyman-Changeux (MWC) model, in which ligand-bound receptors can be in either state, but coupled receptors switch between states in synchrony [150]. Finally, in the conformational spread (CS) model [151], both conditions are relaxed: a ligand-bound receptor can be in either state, and coupled receptors can be in different states. Because we are interested in the minimal model that can capture the ability to perform logic gates, we adopt the MWC model; the KNF model prohibits certain logic gates by construction, while the CS model allows excess parametric freedom (clearly, what can be achieved by the MWC model can be achieved by the CS model). Furthermore, the MWC model has been shown to agree with experiments on receptors [87, 152].

The input in our model is the pair of concentrations $[S_1]$ and $[S_2]$ of two different ligands. The output is the probability for a receptor monomer or dimer to be in its active state. We consider three monomer types and the associated dimers (Fig. 5.1): a monomer that binds ligand 1 (U), one that binds ligand 2 (V), and one that binds both ligands (W). In the last case, ligand binding is competitive: there is only one binding pocket, so only one ligand type can
bind at a time. Noncompetitive binding, in which both ligand types can bind simultaneously, is captured by the $Q_{UV}$ dimer (Fig. 5.1).

The probability $p^A$ for a receptor to be in the active state is computed from the partition functions, which enumerate all possible ways a receptor can be in either the active ($Z^A$) or inactive ($Z^I$) state:

$$p^A = \frac{Z^A}{Z^I + Z^A}. \quad (5.1)$$

The explicit forms of the partition functions under the MWC model are presented as each monomer and dimer is discussed in the Results section. For intuition, we provide an example here: the partition functions for monomer $W$ are

$$Z^A = \omega_0 \left(1 + q_1^A + q_2^A\right), \quad (5.2)$$

$$Z^I = 1 + q_1^I + q_2^I, \quad (5.3)$$

where $\omega_0 = e^{-E_0/kBT}$ is the Boltzmann probability corresponding to the energy difference $E_0$ between the active and inactive state, and $q_j^i = [S_i]/K_j^i$ is the ratio of the concentration of ligand $i \in \{1, 2\}$ to the dissociation constant in activity state $j \in \{A, I\}$. In Eq. 5.2, the three terms correspond to the receptor being active when no ligand is bound, when ligand 1 is bound, and when ligand 2 is bound, respectively. The same holds for Eq. 5.3 with the receptor being inactive.

The dependence of $p_A$ on $[S_1]$ and $[S_2]$ defines the receptor’s function (Fig. 5.1, bottom row). Functions are categorized based on the idealized behavior prescribed by the 16 possible two-input binary logic gates (Fig. 5.1). Mathematically, the function approaches binary logic when the output is either minimal ($p^A \rightarrow 0$) or maximal ($p^A \rightarrow 1$) in each of the four states defined by each input being absent ($[S_i] = 0$) or present ($[S_i] > 0$). Numerically, when varying parameters to assess whether a receptor can realize a particular logic gate, we use an evolutionary algorithm based on the Wright-Fisher model [80, 81]. Briefly, the difference between $p^A$ and the binary logic gate is evaluated over a grid of points and used to define a “fitness”. Parameters for which $p^A$ better approximates the logic gate yield a higher fitness, permitting optimization. A detailed description of the optimization procedure is provided in App. 5.A.1.

### 5.3 Results

First, we identify the logic gates that each receptor monomer and dimer can perform by parameter variation. Here, several derived analytic constraints support the numerical results. Then, we investigate the extent to which a set of monomers can access distinct logic gates by dimerizing. Several combinations of distinct gates are possible, a finding for which the first results provide a clear interpretation.
### 5.3 Results

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<th>NAND</th>
<th>NOR</th>
<th>XOR</th>
<th>ORN₁</th>
<th>NONE</th>
<th>NOTₚ₁</th>
<th>ORN₂</th>
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**Figure 5.2:** Functional versatility by parameter variation. For all monomers and dimers, we show the possible functions attainable by varying parameters. Attainability is assessed by numerical optimization and interpreted based on analytic constraints derived in the text.

#### 5.3.1 Functions accessible by parameter variation

Figure 5.2 shows the set of logic gates that each monomer and dimer can perform, as determined by numerical optimization of model parameters. The most striking feature is that one of the dimers can perform all 16 possible gates. This and the other numerical results in Fig. 5.2 can be understood intuitively by appealing to analytic results derived from the underlying model, which we will describe in turn for each monomer and dimer.

**Monomers**

The first two monomers, receptors U and V, respond to only one input each. Therefore, they are trivially constrained to gates which depend on neither input (ALL, NONE) or on only one input (YESₚ₁, NOTₚ₁). Receptor W, on the other hand, allows competitive binding of both inputs, and can therefore realize several nontrivial gates.

At this point it is useful to observe that the gates exist in antagonistic pairs (a gate and its inverse), shown consecutively in Fig. 5.2: (AND, NAND), (OR, NOR), etc. Any receptor that can perform one member of a pair can perform the other, simply by inverting certain parameter values. Furthermore, several gates are equivalent under reversal of the two ligands (those with subscripts in Fig. 5.2): (AND₁ₛ₁, AND₂ₛ₂), (ORN₁ₛ₁, ORN₂ₛ₂), etc. Again, any receptor that can perform one of these can perform the other, simply by switching certain parameter values (corresponding to exchanging the effect of S₁ and S₂). Eliminating these redundancies, we arrive at four unique gates that respond nontrivially to both inputs:

\[
\text{AND, OR, ANDN}_S₁, \text{ XOR.} \tag{5.4}
\]

We will consider only these four unique gates from this point on.

The third monomer, receptor W, whose partition functions are given in Eqs. 5.2-5.3, can realize two of the four unique gates: OR and ANDNₚ₁. The OR-gate follows straightforwardly from the situation where both ligands activate the receptor individually; their combination will then activate it as well. The ANDNₚ₁-gate can be formed if ligand 1 binds more strongly.
than ligand 2 \((q_1^A \gg q_2^A)\), but ligand 1 only weakly biases the receptor toward the active state \((q_1^A \sim q_1^I)\), while ligand 2 strongly biases it \((q_2^A \gg q_2^I)\). In this scenario, a receptor that is inactive in the absence of both ligands \((\omega_0 \ll 1)\) will only be active in the presence of ligand 2 and not 1.

Receptor \(W\) cannot realize the other two unique gates, \(A\mathcal{N}D\) and \(X\mathcal{O}R\). Both gates require a cooperative effect when both ligands are present: in the \(A\mathcal{N}D\)-gate, neither ligand activates the receptor individually, but both activate it together; in the \(X\mathcal{O}R\)-gate, each ligand activates the receptor individually, but both suppress activation together. Such cooperative effects are not possible with competitive binding. As we will see next, dimerization is required to perform these gates.

Dimers

The three monomers admit six possible dimer combinations — three homodimers and three heterodimers. The homodimers \(Q_{UU}\) and \(Q_{VV}\) respond to only one input each and are therefore trivially constrained like monomers \(U\) and \(V\). Moreover, heterodimers \(Q_{UV}\) and \(Q_{VU}\) are equivalent upon ligand exchange and can therefore realize equivalent sets of logic gates upon parameter variation. This leaves three dimers that can realize unique sets of logic gates upon parameter variation: \(Q_{UV}, Q_{UV},\) and \(Q_{VU}\).

The first dimer, receptor \(Q_{UV}\), is the simplest heterodimer: it is formed by combining monomer \(U\), which responds only to ligand 1, and monomer \(V\), which responds only to ligand 2. Unlike receptor \(W\), which is limited to competitive binding, the dimeric receptor \(Q_{UV}\) has two binding pockets and therefore allows noncompetitive (i.e. cooperative) binding. Accordingly, its partition functions extend those of receptor \(W\) (Eqs. 5.2-5.3) to include a cooperative term:

\[
Z^A = \omega_0(1 + q_1^A + q_2^A + \omega_{12} q_1^A q_2^A),
\]

\[
Z^I = 1 + q_1^I + q_2^I + \omega_{12} q_1^I q_2^I,
\]

The cooperative term contains an additional Boltzmann factor \(\omega_{12} = e^{-E_{12}/kBT}\) corresponding to the cooperative binding energy \(E_{12}\), which could originate from, e.g., a conformational change of the receptor upon binding of one ligand that opens the binding pocket for the other ligand. For example, in the actin regulatory protein N-WASP, the binding affinity of each of its inputs, Cdc42 and PIP2, is increased by a factor of \(~400\) when the other input is bound \[130\].

Receptor \(Q_{UV}\) can realize three of the four unique gates: \(0R, A\mathcal{N}D_{1},\) and \(A\mathcal{N}D\). The \(0R\) and \(A\mathcal{N}D_{1}\) gates follow straightforwardly from the fact that \(Q_{UV}\) reduces to \(W\) for no cooperativity \((\omega_{12} = 0)\), and receptor \(W\) can realize these gates as previously discussed. The \(A\mathcal{N}D\)-gate is formed when the receptor is inactive in the presence of each ligand alone but, due to the cooperative interaction, is active in the presence of both ligands together. Receptor \(Q_{UV}\) cannot realize the \(X\mathcal{O}R\)-gate: if the receptor is activated by either one of the two ligands, it must also be activated by both ligands together. The cooperative interaction enhances the effect that each ligand individually has on the activation of the receptor, but it
cannot reverse it.

The intuition behind why receptor \(Q_{UV}\) can realize the AND-gate can be quantified by considering the constraints that an AND-gate places on the partition functions:

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<tr>
<th>(S_1)</th>
<th>(S_2)</th>
<th>(p^A)</th>
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<tbody>
<tr>
<td>0</td>
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(5.7)

Here, we have recognized that a low output requires \(Z^A \ll Z^I\) (see Eq. 5.1); therefore, the first three lines reflect that in an AND-gate the output is low in the first three input conditions. Similarly, a high output requires \(Z^A \gg Z^I\), which is reflected in the last line. Receptor \(Q_{UV}\) can realize the AND-gate precisely because the constraints in Eq. 5.7 can be met simultaneously. For example, taking for illustration the simplifying case of intermediate cooperativity (\(\omega_{12} \gtrsim 1\)) and symmetric, saturating ligand concentrations (\(q_1^I = q_2^I \gg 1\)), Eq. 5.7 reduces to

\[
1 \ll 1/\sqrt{\omega_0} \ll q_1^A/q_1^I \ll 1/\omega_0.
\]

(5.8)

Indeed, we see that the AND-gate requires a bias upon ligand binding that is too weak to activate the receptor individually (\(q_1^A/q_1^I \ll 1/\omega_0\)), but strong enough to activate the receptor cooperatively (\(q_1^A/q_1^I \gg 1/\sqrt{\omega_0}\)). The strength of the cooperativity influences the quantitative properties of the AND-gate: an increase in \(\omega_{12}\) shifts the transition region of the gate to smaller ligand concentrations, as indeed observed in studies of the AND-like N-WASP protein [130].

The second dimer, receptor \(Q_{UV}\), is also a heterodimer: it is formed by combining monomer \(U\), which responds only to ligand 1, and monomer \(W\), which responds competitively to both ligands. The partition functions for this receptor are

\[
Z^A = \omega_0 \left(1 + q_{1,U}^A + q_{1,W}^A + q_2^A + \omega_{11} q_{1,U}^A q_{1,W}^A + \omega_{12} q_{1,U}^A q_{2,W}^A\right),
\]

(5.9)

\[
Z^I = 1 + q_{1,U}^I + q_{1,W}^I + q_2^I + \omega_{11} q_{1,U}^I q_{1,W}^I + \omega_{12} q_{1,U}^I q_{2,W}^I.
\]

(5.10)

Here, since ligand 1 can bind to either monomer \(U\) or \(W\), we distinguish these cases with the second subscript on \(q_1^A\). There are now two cooperative terms, corresponding to the cases where monomers \(U\) and \(W\) bind, respectively, ligands 1 and 1 (\(\omega_{11}\), or ligands 1 and 2 (\(\omega_{12}\)). Eqs. 5.9-5.10 make clear that receptor \(Q_{UV}\) reduces to receptor \(W\) (Eqs. 5.2-5.3) in the limit \(q_{1,U}^I \to 0\), and to receptor \(Q_{UV}\) (Eqs. 5.5-5.6) in the limit \(q_{1,W}^I \to 0\).
Receptor \( Q_{W} \) can realize all four unique gates (and therefore all 16 possible gates; Fig. 5.2). The OR, ANDN\(_1\), and AND gates follow straightforwardly from the fact that receptor \( Q_{U} \), which can realize these gates, is a limiting case. The XOR-gate is less trivial. Below we offer an intuitive argument for why receptor \( Q_{W} \) can realize an XOR-gate, and in App. 5.A.2 we prove analytically that the output can be a nonmonotonic function of the two inputs for this receptor, which is required for an XOR-gate.

The XOR-gate is formed when each ligand individually activates the receptor by binding to monomer \( W \), but when both ligands are present, ligand 1 is outcompeted and thus binds to monomer \( U \), in turn suppressing activation. It is instructive here to describe this process in more detail. Suppose that ligand 1 promotes activation when bound to \( W \) but suppresses activation when bound to \( U \). Further, suppose that ligand 1 binds more strongly to \( W \) than to \( U \), such that in the presence of ligand 1 alone, the receptor is active. Now suppose that ligand 2 promotes activation when bound to \( W \). Since ligand 2 can only bind to \( W \), in the presence of ligand 2 alone, the receptor is also active. Finally, suppose that ligand 2 "interferes" with ligand 1, i.e. binds more strongly to \( W \) than ligand 1 does. Then, in the presence of both ligands, ligand 2 binds to \( W \), leaving ligand 1 to bind to \( U \). If \( U \) suppresses activation more strongly than \( W \) promotes activation, then in the presence of both ligands, the receptor is inactive. The resulting logic is the XOR-gate.

The third dimer, receptor \( Q_{W} \), is a homodimer: it is formed by combining two \( W \) monomers, each of which responds competitively to both ligands. The partition functions for this receptor are

\[
Z^A = \omega_0 \left( 1 + 2 q_1^A + 2 q_2^A + \omega_{11} q_1^A q_1^A + 2 \omega_{12} q_1^A q_2^A + \omega_{22} q_2^A q_2^A \right), \quad (5.11)
\]

\[
Z^A = 1 + 2 q_1^I + 2 q_2^I + \omega_{11} q_1^I q_1^I + 2 \omega_{12} q_1^I q_2^I + \omega_{22} q_2^I q_2^I. \quad (5.12)
\]

Here, the factors of two account for the fact that each ligand can be bound to either of two symmetric monomers. There are now three cooperative terms, corresponding to the cases where both monomers bind ligand 1 (\( \omega_{11} \)), both bind ligand 2 (\( \omega_{22} \)), or one binds ligand 1 and the other binds ligand 2 (\( \omega_{12} \)).

Receptor \( Q_{W} \) can realize three of the four unique gates: OR, ANDN\(_1\), and AND. The OR and ANDN\(_1\) gates follow straightforwardly from the fact that each monomer alone can realize these gates as previously discussed. The AND-gate relies on strong suppression of cooperation between monomers if they are bound to the same ligand type (i.e. \( \omega_{11} \rightarrow 0, \omega_{22} \rightarrow 0 \); this suppression prevents activation when only one ligand is present. In fact, this limit reduces Eqs. 5.11-5.12 to Eqs. 5.5-5.6 (up to factors of 2), meaning the AND-gate constraint, Eq. 5.8, also holds here under the same conditions for which it was derived. Receptor \( Q_{W} \) cannot realize the XOR-gate: because the individual monomers are identical, no negative interference is possible, as it is for receptor \( Q_{U} \).

5.3.2 Functions accessible by recombination

In the previous section we identified the logic gates accessible by individual receptors via variation of intrinsic biochemical parameters. In this section we ask a second question. Recognizing that receptors bind and unbind to the cell membrane, diffuse within the membrane,
and bind to each other (Fig. 5.3a), we here seek the logic gates that a set of monomers can realize — at fixed parameters — simply by forming the possible dimer combinations. This question is key to functional control at the single-molecule level: if diverse logic gates can be realized by a small set of monomers, cellular function could be strongly tuned by simply expressing a particular pair of monomers in abundance over the others. Alternatively, a signaling mechanism such as covalent modification could determine when a particular dimer was active, such that even if all dimer combinations were represented simultaneously on the membrane, only one would dominate the functional response of the cell. This question is also critically related to the challenge that all cells face: to encode reliable responses using limited resources (here, a limited set of monomers) and on short timescales (here, set by expression and diffusion).

The three monomers we study form four functional dimers: $Q_{UV}$, $Q_{UW}$, $Q_{WV}$, and $Q_{WW}$ (the dimers $Q_{UU}$ and $Q_{VV}$ respond to only one input each and are neglected; additionally we neglect the monomer $W$ itself although it is functional because its behavior is captured by $Q_{WW}$). This fact leads to the enticing question of whether there exist parameters at which the four dimers perform the four unique logic gates (Eq. 5.4). Such a finding would be highly nontrivial: all monomers are present in at least two dimers, and therefore the performance of a particular logic gate by one dimer places heavy constraints on the parameters of the other dimers.

An exhaustive search, in which we numerically optimize for each of the $4! = 24$ dimer-to-logic gate mappings in turn (App. 5.A.1), suggests that no parameter set exists at which all four unique logic gates are performed. Moreover, replacing any subset of gates with the corresponding inverse gates and repeating gives the same result in each of the $2^4 = 16$ cases. Interestingly, the result seems to be due to the fact that the parameters which support the XOR-gate in receptor $Q_{UV}$ (or its counterpart $Q_{WW}$) prohibit the AND-gate in any of the other receptors. Next we support this numerical observation with an intuitive argument.

Suppose that receptor $Q_{UV}$ performs an XOR-gate. As described in the previous section, the XOR-gate requires that when ligand 1 is present alone, it activates the receptor by binding to monomer $W$. Since the AND-gate requires the opposite behavior, namely that the receptor is inactive when ligand 1 is present alone, then the AND-gate cannot be formed by any receptor in which ligand 1 only binds to $W$. This group includes receptors $Q_{UW}$ and $Q_{WV}$, leaving only receptor $Q_{UV}$. Then, as also described in the previous section, the XOR-gate requires that ligand 1 suppresses activation when bound to monomer $U$. Since the AND-gate requires that the receptor is active when both ligands are present, in receptor $Q_{UV}$ this suppression would have to be overpowered by activation via ligand 2 binding to $V$. However, if this were the case, the receptor would surely be active in the presence of ligand 2 alone, which is inconsistent with the behavior of an AND-gate. These arguments make clear that if receptor $Q_{UV}$ performs an XOR-gate, no other receptor can form an AND-gate. The same arguments, but with the ligands exchanged, hold if receptor $Q_{WV}$ performs the XOR-gate instead of receptor $Q_{UW}$. Since receptors $Q_{UW}$ and $Q_{WV}$ are the only receptors that can perform the XOR-gate, we conclude that the XOR and AND gates are not mutually accessible by recombination of monomers $U$, $V$, and $W$ at fixed parameters.

Even though all four unique logic gates cannot be performed at fixed parameters, we
do find six parameter sets at which unique groups of three logic gates are performed by	hree of the dimers. We denote these parameter sets as \( \phi_k \), for \( k \in \{1, 2, \ldots, 6\} \), and show the logic gates and the dimers that perform them in Fig. 5.3b. We stress that this result is still nontrivial: two of the groups are performed by receptors \( Q_{WW} \), \( Q_{UW} \), and \( Q_{WV} \), which all contain monomer \( W \); additionally, two groups are performed by receptors \( Q_{UV} \), \( Q_{VV} \), and \( Q_{UV} \), in which each monomer is represented in two of the three dimers. Due to the high degree of monomer overlap in both cases, one might have expected the three dimers to be constrained to similar functionality at fixed parameters; instead, we find that three unique logic gates can be formed. Further, Fig. 5.3b reveals that all four logic gates are represented among the six groups (but, as expected, never XOR and AND in the same group).

Finally, the optimal solutions shown in Fig. 5.3b are robust to parametric perturbation: as shown in App. 5.A.3, for all \( \phi_k \), most random perturbations in which each parameter changes by an average of \( \sim 20\% \) change the fitness of none of the three logic gates by more than 10%. All of these features underscore the functional versatility available to cells by dimeric recombination.

In the remainder of this section, we provide for parameter sets \( \phi_1 \) and \( \phi_2 \) the intuition behind how the three logic gates in Fig. 5.3b are performed by the corresponding receptors.

In App. 5.A.4, we provide similar intuition for parameter sets \( \phi_3 \), \( \phi_4 \), \( \phi_5 \), and \( \phi_6 \). Furthermore, in App. 5.A.4, we argue why the groups observed in Fig. 5.3b (and their counterparts obtained upon ligand exchange) are the only groups of three unique logic gates that one expects to observe under this model.

Parameter set \( \phi_1 \) (Fig. 5.3b, first row) corresponds to a case where each ligand only weakly promotes activation in the presence of monomer \( W \). This feature allows receptor \( Q_{VW} \) to remain inactive when each ligand is present individually but become activated when both ligands are present together, forming the AND-gate. Furthermore, ligand-bound \( U \) both promotes activation and strongly enhances the binding of ligand 2 to \( W \). This feature allows receptor \( Q_{UW} \) to perform the OR-gate: when ligand 1 is present alone, it promotes activation by binding to \( U \); when ligand 2 is present in abundance and ligand 1 is present only in a small amount (and thus still in the "off" state), the small amount of ligand 1 is nonetheless sufficient to promote activation via enhanced binding of ligand 2 to \( W \); and when both ligands are present in abundance, the two effects combine, resulting in activation. Finally, ligand-bound \( V \) both suppresses activation and strongly enhances the binding of ligand 1 to \( W \). This feature allows receptor \( Q_{VW} \) to perform the AND\textsubscript{2} gate: when ligand 2 is present it suppresses activation via \( V \), independent of ligand 1; but when ligand 1 and not (very much of) ligand 2 is present, the small amount of ligand 2 strongly enhances binding of ligand 1 to \( W \), thus promoting activation.

Parameter set \( \phi_2 \) (Fig. 5.3b, second row) corresponds to a case where ligand-bound \( W \) promotes activation. This feature is sufficient for receptor \( Q_{WW} \) to perform the OR-gate. Furthermore, ligand 2 binds more strongly to \( V \) than to \( W \), and ligand-bound \( V \) suppresses activation more strongly than ligand-bound \( W \) promotes activation. These features allow receptor \( Q_{VV} \) to perform the AND\textsubscript{2} gate, since only in the presence of ligand 1 and not 2 will activation be promoted via \( W \) and not suppressed via \( V \). Finally, (i) ligand 1 binds more strongly to \( W \) than to \( U \), (ii) ligand 2 binds more strongly to \( W \) than ligand 1 does, and
(iii) ligand-bound U suppresses activation more strongly than ligand-bound W promotes activation. These are the precise features that allow receptor $Q_{UIW}$ to perform the XOR-gate, as outlined in detail in the previous section.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5_3.png}
\caption{Functional versatility by recombination. a) Given the three monomer types, four functional units can be formed by receptors diffusing and dimerizing on the membrane (arrows). b) Four parameter sets $\phi_i$ are shown at which three of the four dimers perform functionally unique logic gates.}
\end{figure}

5.4 Discussion

We have used a statistical mechanical model to investigate the versatility of receptor function in two contexts: (i) the ability of a single receptor to access logical functions by parameter variation, and (ii) the ability — at fixed parameters — for a set of receptor monomers to access logical functions by dimerizing. The first context is arguably important on evolutionary timescales, on which mutations and environmental pressures act to change a cell’s intrinsic biochemical parameters. The second context is more critical at short timescales, e.g. for cell signaling, during which gene expression and diffusion can potentially change cellular function at the molecular level by favoring the dimerization of particular receptors over others.

In the first context, we find that a single heterodimer, receptor $Q_{UIW}$, can realize all 16 possible logic gates by parameter variation. Our analysis reveals that such complete func-
tional freedom, while perhaps surprising, is in fact quite intuitive for this receptor. In particular, receptor $Q_{UV}$ performs the most challenging function, the XOR-gate, by exploiting an interference between the two ligands (i.e., when both ligands are present, one outcompetes the other for the activating binding pocket, ultimately causing suppression). Such a non-monotonic response requires competitive binding and asymmetric activation biases, both of which are possible by heterodimerization.

In the second context, we find that the simplest combination of monomers that yields four functional dimers cannot in fact perform the four unique logic gates at fixed parameters, an observation we explain by arguing that the AND-gate and the XOR-gate are not mutually accessible. Nonetheless, an exhaustive numerical search reveals that several distinct groups of three unique gates are performable, a result that is nontrivial given the high degree of overlap among dimers’ parameter spaces. We offer intuitive explanations for the emergence of these groups, and further, we argue that these groups are exhaustive. The ability to perform diverse functions with a limited set of simple components is of critical importance to the question of how cells encode reliable responses with limited resources.

We have adopted a minimal model (the MWC model) to describe a minimal set of components, and we have explored the functional capabilities available under these conditions. We are further encouraged by the fact that the MWC model has been shown to agree with experiments on receptors [87, 152]. Nonetheless, several extensions to the model or the study itself are natural choices for further exploration. First, the conformational spread (CS) model [151] generalizes the MWC model, and thus it would allow for more functional freedom in logic gate construction. However, it is always a concern that generalizing one’s model can reduce the fraction of functional parameter space simply by increasing the total volume of parameter space.

Second, it would be straightforward to introduce one or more additional monomers when considering recombination. For example, introducing an additional monomer that binds a single ligand might in fact admit parameter sets at which all four unique logic gates are performed, at the expense of increasing the number of individual components that the cell must produce. The impact of such a finding, however, would be reduced by the fact that more than four functional dimer combinations would be possible. Third, it would also be straightforward to consider more complex dimers (or higher oligomers), such as $Q_{W_1W_2}$, in which each pocket binds both ligands competitively, but with asymmetric parameters. Of course, such increasing complexity would only be justified in the context of correspondingly detailed biological examples.

It is well established that receptors are responsive to multiple ligands. Interestingly, recent experiments have even exploited this fact to synthetically construct proteins that perform certain logic gates [84]. At the same time, receptor dynamics on the membrane — diffusion, binding, unbinding, dimerization — suggest that receptors can as functional signaling units by recombination. Indeed, experiments have shown that for many receptors, such as ErbB and GPCR, monomers combine to form different dimers that have different functionality [141, 145, 146]. It is our hope that this study will contribute to a predictive framework in which experiments like these can be interpreted and extended. The findings we report — that a single receptor can function as any logic gate and that a limited set
of monomers can access diverse logic gates by dimerizing — speak to the large degree of functional control available to cells at the level of individual receptor molecules.

5.5 Acknowledgements

I would like to thank Jose Alvarado for a critical reading of this chapter.
5. A Supplementary Information

5. A. 1 Optimization details

In this section we describe in detail the numerical optimization algorithm. In Table. 5.1 we list the optimization parameters.

Single receptor

We use an evolutionary algorithm based upon the Wright-Fisher algorithm to obtain the parameters that correspond to a specific logic gate for every receptor type (Fig. 5.1). The Wright-Fisher algorithm models the evolution of a population. Evolution occurs in discrete, synchronous steps, and the population size remains constant. At each step, each member of the population produces offspring in proportion to its fitness. Then, mutations occur, and the mutated offspring comprise the population for the next step.

In our case, for a given receptor, we have a “population” of $R$ initial parameter points $\phi_r$. Each point has fitness $f_r$, and the total fitness for the receptor is $F = \sum f_r$. At each step, $R$ new points (“offspring”) are drawn from the distribution $p_r = f_r/F$, which weights each point by its fitness. Each new point is then “mutated” by multiplying a randomly selected parameter by the factor $(1 + \delta)$, where $\delta$ is drawn uniform randomly from the range $[-\Delta : \Delta]$; we take $\Delta = 0.3$.

We define fitness as the agreement between the real-valued output of the statistical mechanical model, $p^A$, and the binary output of a specific ideal logic gate. The ideal logic gate is prescribed by the goal function $G([S_1], [S_2])$, which takes the value 0 or 1 depending on whether each input is “off” ($[S_1] < [S^*]$) or “on” ($[S_1] > [S^*]$), where $[S^*]$ sets the scale of ligand concentrations to which the receptor responds. We compare $p^A$ and $G$ over an $N \times N$ grid of input values, spaced logarithmically over the ranges of $[S_1]$ and $[S_2]$, which we take to be $[S_i]/[S^*] \in [10^{-2}, 10^2]$. The fitness is thus

$$f_r = -\sum_{n,n'=1}^{N} p^A \left([S^*_n],[S^*_n']\right) \cdot G \left([S^*_1],[S^*_2]\right). \quad (A5.1)$$

The results in this article are obtained for $N = 4$. Taking $N = 2$ leads to suboptimal results, while taking different values of $N > 2$ yields similar results to $N = 4$. Similar results are also obtained for a fitness function with $N = 2$ and an additional central point at $[S_1] = [S_2] = [S^*]$, at which $G$ is the average of the truth table for the gate.

The optimization parameters, as well as the bounds within which the model parameters are initialized and constrained during optimization, are given in Table. 5.1.

Multiple receptors

In the case where $M$ different receptors (e.g. $Q_{UV}, Q_{AV}, Q_{UV}$) are combined to act in different combinations as unique logic gates, the optimization algorithm follows a specific order in the optimization. A straightforward extension of the model for a single receptor is the optimization of three (or four) gates simultaneously, and taking as fitness $F$ the summed
<table>
<thead>
<tr>
<th>Model input</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[S_i]/[S^*]$</td>
<td>$10^{-2} - 10^2$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Bounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{i,k}^j/[S^*]$</td>
<td>$10^{-4} - 10^3$</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>$10^{-3} - 10^3$</td>
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<tr>
<td>$\omega_1l$</td>
<td>$10^{-2} - 10^2$</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Optimization parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>$\Delta$</td>
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</tr>
<tr>
<td>$N$</td>
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<tr>
<td>$R$</td>
<td>50000</td>
</tr>
<tr>
<td>Steps</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Table 5.1:** Overview of parameters. During optimization, model parameters are initialized and constrained within the indicated bounds.
fitness of every gate $F_m$:

$$\mathcal{F} = \sum_{m=1}^{M} F_m.$$  \hfill (A5.2)

However, this optimization is not capable of optimizing all the gates independently. Instead, the algorithm optimizes either one (or two) gates, but then cannot optimize the third gate. To optimize the third gate, the already optimized gates decrease (temporarily) in fitness. This decrease is larger than the increase in fitness for the third gate and the algorithm finds suboptimal peaks in this rugged fitness landscape.

Instead of optimizing all gates simultaneously, we optimize gates in order. For the homodimer construction ($Q_{WW}, Q_{IY}, Q_{VY}$), we first optimize $Q_{WW}$, then $Q_{IY}$, where we only change the parameters of $U$, and then $Q_{VY}$, only changing $V$. The achieved results greatly outperform the results where we optimize all three gates simultaneously.

For the heterodimer construction ($Q_{UW}, Q_{WV}, Q_{UV}$), we again start by optimizing gate $Q_{UW}$, then the two gates $Q_{UW}$ and $Q_{WV}$ simultaneously, and finally $Q_{UW}, Q_{WV},$ and $Q_{UV}$. Again this procedure gives much better results than simultaneous optimization of all three gates.

For the construction with $Q_{WW}, Q_{UW}, Q_{UV}$, we start by optimizing gate $Q_{WW}$, then the two gates $Q_{WW}$ and $Q_{UW}$ simultaneously, and finally $Q_{WW}, Q_{UW},$ and $Q_{UV}$.

5.A.2 Formal proof for a XØR-gate for receptor $Q_{UW}$

The probability to be active $\rho^A ([S_1], [S_2])$ in an XØR-gate is a nonmonotonic function of $[S_1]$ and $[S_2]$ simultaneously. More specifically, for constant $[S_2] = [S_2]$, $\rho^A ([S_1], [S_2])$ is either monotonically increasing or decreasing, depending on the value $[S_2]$; for small $[S_2]$, $\rho^A$ is monotonically increasing, while for large $[S_2]$, $\rho^A$ is monotonically decreasing.

A positive derivative $\partial \rho^A / \partial [S_2]$ reflects a monotonically increasing function, while a negative derivative reflects a monotonically decreasing function. Therefore, in an XØR-gate, the derivative of the probability with respect to $[S_2]$ at constant $[S_1]$ should change sign as function of $[S_1]$. Again due to symmetry, the derivative of the probability with respect to $[S_1]$ at constant $[S_2]$ should change sign as function of $[S_2]$.

We will prove that the XØR-gate is possible for the $Q_{UW}$ receptor even with $\omega_{11} = \omega_{21} = 1$. Recalling Eq. 1, the derivative can be written $\partial \rho^A / \partial [S_2] = f / (Z^A + Z^I)^2$, where the numerator

$$f ([S_1], [S_2]) = \frac{\partial Z^A}{\partial [S_2]} Z^I - Z^A \frac{\partial Z^I}{\partial [S_2]}$$  \hfill (A5.3)

alone determines the sign. We therefore must show that $f$ changes sign as function of $[S_1]$. 


Specifically, the XOR-gate requires
\[
\begin{align*}
f > 0 & \text{ for } [S_1^c] < [S_1^c]^*, \quad (A5.4) \\
f < 0 & \text{ for } [S_1^c] > [S_1^c]^*, \quad (A5.5)
\end{align*}
\]
for some $[S_1^c]^*$ and all $[S_2]$.

The partition functions $Z^A$ and $Z^I$ for the Q_{UW} receptor are given by Eqs. 9-10 with $q_i = [S_1] / K_i^I$. Performing the derivatives in Eq. A5.3 reveals that $f$ is a third order polynomial in $[S_1]$ in which all dependence on $[S_2]$ drops out. Only one root is potentially positive:

\[
[S_1]^* = \frac{K_{1,W}^I K_{1,W}^A (K_2^I - K_2^A)}{K_{1,W}^I K_2^A - K_{1,W}^A K_2^I}.
\]

To satisfy Eqs. A5.4-A5.5, we require that the zeroth order term (the intercept) is positive and that the leading order term is negative; enforcing these conditions yields
\[
\begin{align*}
K_2^I - K_2^A > 0, \quad (A5.7) \\
K_{1,W}^I K_2^A - K_{1,W}^A K_2^I > 0, \quad (A5.8)
\end{align*}
\]
which are in fact the precise conditions that maintain positivity of the root (Eq. A5.6). Parameters that satisfy these conditions enable the sign of $\partial p^A / \partial [S_2]$ to depend on constant $[S_1]^*$, which is one of the two conditions necessary to perform the XOR-gate. Notably, Eq. A5.7 directly shows that the binding of ligand 2 to the W monomer in Q_{UW} in the active state is less likely than binding in the inactive state.

The second requirement is that the sign of $\partial p^A / \partial [S_1]$ depends on constant $[S_2]^*$. Specifically, in Eqs. A5.4-A5.5, the XOR-gate requires that the numerator $g([S_1], [S_2]^*)$ of the derivative satisfies
\[
\begin{align*}
g > 0 & \text{ for } [S_2^c] < [S_2^c]^*, \quad (A5.9) \\
g < 0 & \text{ for } [S_2^c] > [S_2^c]^*. \quad (A5.10)
\end{align*}
\]
for some $[S_2^c]^*$ and all $[S_2]$. Performing the derivative reveals that $g$ is a second order polynomial whose coefficients depend on $[S_1]$. To satisfy Eqs. A5.9-A5.10, we again require that the intercept is positive and that the leading order term is negative; enforcing these conditions yields
\[
\begin{align*}
h \left( [S_1], K_{1,U}^I, K_{1,U}^A, K_{1,W}^I, K_{1,W}^A \right) > 0, \quad (A5.11) \\
k_{1,U}^I - K_{1,U}^A < 0, \quad (A5.12)
\end{align*}
\]
where the function $h$ results straightforwardly from the derivative but is unwieldy, such that we do not reproduce it here. The roots of the polynomial $[S_2^c]^*$ are similarly unwieldy, but noting positivity requirements ($[S_2^c]^* > 0, K_{1,W}^I > 0, K_{1,U}^I > 0, K_2^I > 0$), parameter
regimes can be derived that satisfy both Eqs. A5.7-A5.8 and Eqs. A5.11-A5.12 simultaneously. As an example we present one possible regime here:

\[
\frac{K_{1,W}^A}{K_{1,W}^I} \frac{K_{2}^A}{K_{2}^I} < 1, \quad (A5.13)
\]

\[
0 < \frac{K_{1,W}^A}{K_{1,W}^I} \leq \frac{K_{1,U}^I}{K_{1,U}^I + K_{1,W}^I}, \quad (A5.14)
\]

\[
K_{1,W}^A + K_{1,U}^A > K_{1,U}^I + K_{1,W}^I. \quad (A5.15)
\]

Eq. A5.13 states that the W monomer is activated by [S\textsubscript{1}] and [S\textsubscript{2}], and that activation by [S\textsubscript{1}] is stronger than activation by [S\textsubscript{2}]. Note that for small concentrations of either [S\textsubscript{1}] or [S\textsubscript{2}], W is inactive. The two more interesting constraints are in Eq. A5.14 and Eq. A5.15. Eq. A5.15 states that [S\textsubscript{1}] bound to monomer U deactivates the receptor (\(K_{1,U}^A > K_{1,U}^I\)), since, from Eq. A5.13, we have seen that \(K_{1,W}^A < K_{1,W}^I\). More importantly the deactivation of U by binding [S\textsubscript{1}] is stronger than the activation of W by [S\textsubscript{1}], and following Eq. A5.13, it is thus also stronger than activation of W by [S\textsubscript{2}]. This is precisely the interference interaction as described in the main text. The last constraint, Eq. A5.14, provides the required binding strength of [S\textsubscript{1}] to U and W to satisfy all constraints. In the main text we argue that W should preferably bind [S\textsubscript{2}], such that in the presence of both ligand [S\textsubscript{2}] binds to W and [S\textsubscript{1}] binds to U with as result that Q\textsubscript{UW} is inactive.

Here we have shown that the Q\textsubscript{UW} receptor is capable of the nonmonotonic derivatives required by the XOR-gate. This capability is necessary but not sufficient to perform the gate, as an ideal logic gate requires that the output be maximally high and low at the appropriate input values. Our numerical results, however, indeed confirm that the Q\textsubscript{UW} receptor can perform the XOR-gate.

5A.3 Parameter sensitivity

In this section we discuss the sensitivity to parameter variation of the results at the six parameter sets \(\varphi_k\) shown in Fig. 5.3. Robustness against parameter fluctuations generally is considered an important quality of biochemical systems, due to stochastic nature of intra- and extracellular processes. If the observed logic gates only function within a very narrow parameter regime, this could lead to unreliable functioning.

Parameters are varied according to

\[
\varphi_{\text{new}} = \varphi_{\text{old}} (1 + n^z)
\]

where \(n^z\) is the \(z\)th component of a uniformly distributed random vector \(n\) with norm \(|n| = \eta\). Under this implementation, \(\eta\) sets the average (root mean square) factor by which each parameter changes via \(\langle \frac{\delta \varphi^z}{\varphi^z} \rangle = \eta / \sqrt{Z}\), where \(Z\) is the number of parameters. We sample \(10^6\) different vectors \(n\).

Sensitivity is measured by computing the fraction of new parameter sets for which, for
each individual gate $m$, the relative change in fitness is less than a factor $\lambda$:

$$\left| \frac{F_{m}^{\text{new}} - F_{m}^{\text{old}}}{F_{m}^{\text{old}}} \right| < \lambda \quad \forall m. \quad (A5.17)$$

Figure 5.4 reveals that for all $\varphi_k$, most random perturbations in which each parameter changes by an average of $\langle \delta \varphi^2 / \varphi^2 \rangle \sim 20\%$ change the fitness of none of the three logic gates by more than $\lambda = 10\%$.

![Graphs showing robustness to parameter variation](image)

**Figure 5.4:** Robustness to parameter variation for the six parameter sets at which dimers can form three unique logic gates (Fig. 5.3): a) $\varphi_1$, b) $\varphi_2$, c) $\varphi_3$, d) $\varphi_4$, e) $\varphi_5$, and f) $\varphi_6$. An increase in $\langle \delta \varphi^2 / \varphi^2 \rangle$ reflects a larger range of parameter fluctuations and an increase in $\lambda$ reflects a loosening of the robustness constraint. The dashed black lines indicate that a significant fraction of random perturbations in which each parameter changes by an average of $\langle \delta \varphi^2 / \varphi^2 \rangle \sim 20\%$ change the fitness of none of the three logic gates by more than $\lambda = 10\%$.

5.A.4 Figure 5.3b is exhaustive

Here, we argue why the groups observed in Fig. 5.3b (and their counterparts obtained upon ligand exchange) are the only groups of three unique logic gates that one expects to observe
under this model. The overall logic is presented first, with the arguments subsequently given in subsections.

There are 4 ways to choose a group of three from the four functional dimers \(Q_{VW}, Q_{UV}, Q_{WV}, \) and \(Q_{UV}\) to perform the three unique logic gates, namely: \(\{Q_{VW}, Q_{UV}, Q_{WV}\}\), \(\{Q_{UV}, Q_{WV}, Q_{UV}\}\), \(\{Q_{WV}, Q_{UV}, Q_{VW}\}\), and \(\{Q_{VW}, Q_{UV}, Q_{UV}\}\). The last two groups are symmetric upon ligand exchange; we therefore consider only the first three groups.

The first group is \(\{Q_{VW}, Q_{UV}, Q_{WV}\}\). As shown in the main text, receptor \(Q_{VW}\) is capable of performing an AND-gate, an OR-gate, or an ANDNI-gate, but not an XOR-gate (Fig. 5.2). If receptor \(Q_{VW}\) performs an AND-gate, receptor \(Q_{UV}\) can perform an ANDNI-gate or an OR-gate, but not an XOR-gate (Argument 1). Receptor \(Q_{WV}\) then performs the OR-gate or the ANDNI-gate, respectively (it also cannot perform an XOR-gate by the same argument). These two possibilities are represented by parameter set \(\varphi_1\) (Fig. 5.3b) and its counterpart upon ligand exchange. If receptor \(Q_{VW}\) performs an OR-gate, receptor \(Q_{UV}\) can perform an ANDNI-gate or an XOR-gate, but not an AND-gate (Argument 2). Receptor \(Q_{WV}\) then performs the XOR-gate or the ANDNI-gate, respectively (it also cannot perform an AND-gate by the same argument). These two possibilities are represented by parameter set \(\varphi_2\) (Fig. 5.3b) and its counterpart upon ligand exchange. If receptor \(Q_{VW}\) performs an ANDNI-gate, three unique gates cannot be performed (Argument 3). Therefore, this group is exhaustively represented by \(\varphi_1\) and \(\varphi_2\).

The second group is \(\{Q_{UV}, Q_{WV}, Q_{UV}\}\). As shown in the main text, receptor \(Q_{UV}\) is capable of performing an ANDNI-gate, an OR-gate, or an AND-gate, but not an XOR-gate (Fig. 5.2). If receptor \(Q_{UV}\) performs an ANDNI-gate, receptor \(Q_{UV}\) can perform an XOR-gate or an OR-gate, but not an AND-gate (Argument 4). Receptor \(Q_{WV}\) then performs the OR-gate or the XOR-gate, respectively (it also cannot perform an AND-gate by the same argument). These two possibilities are represented by parameter set \(\varphi_3\) (Fig. 5.3b) and its counterpart upon ligand exchange. If receptor \(Q_{UV}\) performs an OR-gate, receptor \(Q_{UV}\) can perform an ANDNI-gate or an ANDNI-gate, but not an XOR-gate (Argument 5). Receptor \(Q_{WV}\) then performs the ANDNI-gate or the AND-gate, respectively (it also cannot perform an XOR-gate by the same argument). These two possibilities are represented by parameter set \(\varphi_4\) (Fig. 5.3b) and its counterpart upon ligand exchange. If receptor \(Q_{UV}\) performs an AND-gate, three unique gates cannot be performed (Argument 6). Therefore, this group is exhaustively represented by \(\varphi_3\) and \(\varphi_4\).

The third group is \(\{Q_{VW}, Q_{UV}, Q_{UV}\}\). We note that this group is different from the first two groups, since it does not contain the two receptors \(Q_{UV}\) and \(Q_{UV}\) which are symmetric upon ligand exchange. \(Q_{UV}\) and \(Q_{WV}\) is not present in the receptors \(Q_{UV}\), \(Q_{UV}\), receptor \(Q_{UV}\). To obtain a set which includes the XOR-gate, this gate has to be accessed by receptor \(Q_{UV}\). As shown in the main text, receptor \(Q_{VW}\) is capable of performing an AND-gate, an OR-gate, or an ANDNI-gate, but not an XOR-gate (Fig. 5.2). If receptor \(Q_{VW}\) performs an AND-gate, receptor \(Q_{UV}\) can perform an ANDNI-gate or an OR-gate, but not an XOR-gate (Argument 1). If receptor \(Q_{UV}\) performs an ANDNI-gate, receptor \(Q_{UV}\) cannot perform an OR-gate (Argument 7); since receptor \(Q_{UV}\) also cannot perform an XOR-gate (Fig. 5.2), three unique gates cannot be performed. Therefore, receptor \(Q_{UV}\) must perform an OR-gate, leaving receptor \(Q_{UV}\) to perform an ANDNI-gate. This possibility is represented by parameter set \(\varphi_6\) (Fig. 5.3b).
5.A Supplementary Information

If receptor $Q_{UVW}$ performs an OR-gate, receptor $Q_{UVW}$ can perform an ANDN-gate or an XOR-gate, but not an ANDN-gate (Argument 2). If receptor $Q_{UVW}$ performs as an ANDN-gate, receptor $Q_{UV}$ cannot perform an AND-gate (Argument 8); since receptor $Q_{UV}$ also cannot perform an XOR-gate, three unique gates cannot be performed. Therefore, receptor $Q_{UVW}$ must perform an XOR-gate, leaving receptor $Q_{UV}$ to perform an ANDN-gate. This possibility is represented by parameter set $\varphi_6$ (Fig. 5.3b). If receptor $Q_{UVW}$ performs as an ANDN-gate, three unique gates can not be performed (Argument 9). Therefore, this group is exhaustively represented by $\varphi_5$ and $\varphi_6$.

This completes the logic arguing that the groups observed in Fig. 5.3b are exhaustive.

Argument 1

If receptor $Q_{UVW}$ performs an AND-gate, ligand 2 alone does not promote activation when binding to monomer $W$. Therefore, because ligand 2 does not bind to monomer $U$, the receptor $Q_{UVW}$ is always inactive if ligand 2 is present alone. This behavior is inconsistent with the logic of the XOR-gate.

Argument 2

If receptor $Q_{UVW}$ performs an OR-gate, ligand 2 alone promotes activation when binding to monomer $W$. Therefore, because ligand 2 does not bind to monomer $U$, the receptor $Q_{UVW}$ is always active if ligand 2 is present alone. This behavior is inconsistent with the logic of the AND-gate.

Argument 3

If receptor $Q_{UVW}$ performs an ANDN-gate, receptors $Q_{UV}$ and $Q_{UV}$ can each perform neither an XOR-gate nor an AND-gate, thereby preventing the group $\{Q_{UVW}, Q_{UV}, Q_{UVV}\}$ from performing three unique gates. The reason is straightforward: if receptor $Q_{UVW}$ performs an ANDN-gate, one ligand must suppress activation via $W$ while the other ligand promotes activation via $W$. This feature immediately excludes the XOR-gate since, as described in the main text, an XOR-gate requires both ligands to promote activation via $W$. This feature also excludes an ANDN-gate since, as also described in the main text, an ANDN-gate requires either that activation via $W$ is promoted only weakly or that both ligands suppress activation via $W$. In the first case, activation of receptor $Q_{UVW}$ (or $Q_{UVV}$) is only achieved cooperatively when both ligands are present. In the second case, activation is achieved with both ligands present via $U$ (or $V$) due to an interference effect similar to that underlying the XOR-gate (see discussion of parameter set $\varphi_4$ above).

Argument 4

If receptor $Q_{UV}$ performs an ANDN-$S_1$ gate, $Q_{UV}$ cannot function as an AND-gate. To function as an AND-gate (see Eq. 9), this requires that $\omega_0 q_1^A q_2^A \gg q_1^L q_2^L$, while $\omega_0 q_1^A \ll q_1^L$ and $\omega_0 q_2^A \ll q_2^L$. This conditions cannot be satisfied simultaneously.

Argument 5

If receptor $Q_{UV}$ performs an OR-gate, ligand 1 activates the receptor via $U$. However, for receptor $Q_{UVW}$ to perform the XOR-gate, ligand 1 must suppress activation via $U$, as described
in the main text.

Argument 6
If $Q_{UV}$ functions as an AND-gate $U$ is activated by $S_1$ and $V$ is activated by $S_2$, but both activation biases alone are insufficient to activate the receptor. This excludes the formation of a XOR-gate for either the $Q_{UV}$ or $Q_{WV}$. As we have discussed in the previous section, the XOR-gate is obtained by the deactivation of $U$ ($V$) by ligand $S_1$ ($S_2$). However, it is possible that $Q_{UV}$ is a OR-gate, while $Q_{WV}$ is an ANDN-gate. The $Q_{UV}$-OR-gate requires that $W$ is activated by $S_2$ and $S_1$, since monomer $U$ is not active in the presence of $S_1$. The $Q_{WV}$-ANDN-gate requires that $W$ is strongly deactivated by $S_1$. These two conditions on $W$ are mutually exclusive.

Argument 7
If receptors $Q_{WV}$ and $Q_{UV}$ perform an AND-gate and an ANDN-gate, respectively, the ANDN-gate must be ANDN$_{S_1}$, not ANDN$_{S_2}$. The reason is that the AND-gate requires ligand 2 to promote activation via $W$, while the ANDN$_{S_2}$ gate requires ligand 2 to suppress activation via $W$. Then, if $Q_{UV}$ indeed performs the ANDN$_{S_1}$ gate, receptor $Q_{UV}$ cannot perform an OR-gate. The reason is that the AND and ANDN$_{S_1}$ gates require ligand 1 to suppress activation via $U$ and not via $W$, while the OR-gate requires ligand 1 to promote activation via $U$ de-activated by $S_1$. Therefore, receptor $Q_{UV}$ cannot function as an OR-gate.

Argument 8
If receptors $Q_{WV}$ and $Q_{UV}$ perform an OR-gate and an ANDN-gate, respectively, the ANDN-gate must be ANDN$_{S_1}$, not ANDN$_{S_2}$. The reason is the OR-gate requires ligand 2 to promote activation via $W$, while the ANDN$_{S_2}$ gate requires ligand 2 to suppress activation via $W$. Then, if $Q_{UV}$ indeed performs the ANDN$_{S_1}$ gate, receptor $Q_{UV}$ cannot perform an AND-gate. The reason is that the OR and ANDN$_{S_1}$ gates require ligand 1 to suppress activation via $U$ and not via $W$, while the AND-gate requires ligand 1 to promote activation via $U$ de-activates $U$.

Argument 9
If receptor $Q_{WV}$ performs an ANDN-gate, receptor $Q_{UV}$ cannot perform a XOR-gate, since this requires that both ligands activate $W$. If receptor $Q_{WV}$ performs an ANDN$_{S_1}$ gate, receptor $Q_{UV}$ cannot perform an AND-gate, since $Q_{UV}$ is active if only ligand 2 is present. If receptor $Q_{WV}$ performs an ANDN$_{S_1}$ gate, receptor $Q_{UV}$ can perform an OR-gate if ligand 1 activates $U$ more strongly than it deactivates $W$. However, receptor $Q_{UV}$ is then always active if ligand 1 is present, and this is inconsistent with the logic of the AND-gate. If receptor $Q_{WV}$ performs an ANDN$_{S_2}$ gate, receptor $Q_{UV}$ cannot perform an AND-gate, since $Q_{UV}$ is active if only ligand 1 is present (ligand 1 activates $U$) or $Q_{WV}$ is never active (ligand 1 deactivates $U$ more strongly than it activates $W$). If receptor $Q_{WV}$ performs an ANDN$_{S_2}$ gate, receptor $Q_{UV}$ can perform an OR-gate, if (i) ligand 1 activates $U$ and (ii) in the presence of small ligand 1 and an abundance of ligand 2 the receptor $Q_{UV}$ is active. However, receptor $Q_{UV}$ is then always active if ligand 1 is present and this is inconsistent with the logic of the AND-gate.