Using Nature’s “Tricks” To Rationally Tune the Binding Properties of Biomolecular Receptors

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INTRODUCTION

Nature employs proteins and nucleic acids for high affinity, high specificity recognition of an enormous range of molecular targets. The immune system, for example, can generate antibodies against effectively any protein and many small molecules. Hybridization is, likewise, generalizable to the high-specificity, high-affinity detection of any nucleic acid sequence. These observations have motivated decades of research aimed at harnessing the power of biological recognition in such technologies as sensors (reviewed in ref 1), “smart” responsive adhesives (reviewed in ref 2) and materials (reviewed in ref 3), synthetic biology (reviewed in ref 4), and molecular computing (reviewed in ref 5).

THE TYRANNY OF THE LANGMUIR ISOTHERM

Despite the many positive attributes of—and successful development of technologies based on—biological recognition, the physics of single-site binding nevertheless limits the utility of bioreceptors in many applications. Perhaps the easiest way to visualize these limitations is to consider the “useful dynamic range”, which is the range of target concentrations over which a receptor is sensitive (i.e., can differentiate between small changes in target concentration) and specific (i.e., can differentiate between small change in target chemistry). Both the location and width of this useful dynamic range are, for
most single site receptors, fixed by the hyperbolic curve (first described by Hill but now often called the "Langmuir isotherm"; for a readable history, see ref 8) relating receptor occupancy to ligand concentration for saturable, noninteracting binding sites.

\[
\text{Occupancy} = \frac{[\text{target}]}{K_D + [\text{target}]}
\]  

(1)

The useful dynamic range (here defined as the concentrations over which occupancy shifts from 10% to 90%) of such receptors spans an 81-fold change in concentration centered on the dissociation constant, \(K_D\) (Figure 1). Misalignment between the placement of this range and the expected range of target concentrations reduces both sensitivity (Figure 1, bottom left) and specificity (Figure 1, bottom right).9,10 The fixed width of the useful dynamic range is likewise often limiting. Clinically relevant viral loads, for example, vary over ranges that dwarf an 81-fold range (e.g., from \(\sim 50\) to \(>10^8\) HIV copies/mL).11 In the other direction, the physiologically relevant ranges of many drugs and metabolites are as narrow as 4-fold,12 an 81-fold dynamic range reduces the extent to which receptor occupancy varies over such a narrow range of target concentrations, reducing in turn the precision with which a sensor’s output can define the concentration of its target.

In response to the limitations of single-site binding, evolution has invented a number of mechanisms by which the useful dynamic range of receptors can be tuned, extended, narrowed, or otherwise optimized.13 The ubiquity with which nature employs these mechanisms suggests that they may be of value in the development of improved biotechnologies. Thus motivated, recent years have seen significant efforts to rationally adapt allostery, cooperativity, and other such mechanisms to the receptors employed in artificial biotechnologies, efforts that we believe will only grow in importance as the subtlety and nuance with which we incorporate biomolecules into artificial technologies improves. Here we review these efforts, placing emphasis on the route taken by our research group in the hopes that our experience will serve to illustrate the potential scope of this approach.

**THE POPULATION-SHIFT MECHANISM**

Our trip down this road began with our work on using binding-induced "structure switching" as means of transducing binding events into a robust electrochemical output.14,15 As with the optical analogues that preceded them,16 this broad class of biosensors takes its inspiration from nature: naturally occurring chemoreceptors almost invariably respond to their targets by undergoing binding-induced changes in conformation or oligomerization (Figure 2). This "switch", in turn, triggers a specific output, such as the opening of an ion channel or the activation of an enzyme.

Conformation switching provides a useful means of transducing binding events into specific outputs.21 The mechanism is, for example, quite selective, allowing both naturally occurring "sensors" and artificial biosensors to perform even in complex, multicomponent samples, such as blood serum.22 The mechanism is also reagentless, single-step, and rapidly reversible, allowing it to respond continuously as a target concentration rises and falls.\(^{23,24}\) Finally, the mechanism is versatile, since it can be engineered into a wide range of receptors and coupled to a wide range of optical, electrochemical, or catalytic outputs (reviewed in ref 17).

The physics of structure-switching receptors are well described by the population-shift model,26 which, in turn, provides a route by which, as described below, we can tune the useful dynamic range of such receptors more-or-less at will. In this, a receptor switches between two states: a more stable conformation that does not bind and a less stable but binding-competent conformation. Binding stabilizes the latter, shifting the pre-existing equilibrium and thus coupling recognition with the sort of large-scale conformational switch needed to generate robust outputs (Figure 2A). An important consideration here is that, in the absence of the target, the conformational change is unfavorable, and thus the overall observed affinity of the receptor, \(K_{D_\text{off}}\), depends on its switching equilibrium constant, \(K_S\), and the intrinsic affinity of its binding-competent conformation, \(K_{D_\text{on}}\), by

\[
K_D = K_{D_\text{off}} \left(\frac{1 + K_S}{K_S}\right)
\]  

(2)

An important consequence of this coupling between affinity and the switching equilibrium constant is that it supports a number of mechanisms by which the binding properties of such receptors can be precisely controlled.

**TUNING THE PLACEMENT OF THE DYNAMIC RANGE USING DISTAL SITE MUTATIONS**

Optimal dynamic range tuning is generally achieved when the midpoint of the binding curve matches the midpoint of the expected target range. When this condition is met, the receptor...
achieves maximum sensitivity (a large change in occupancy with a small change in concentration) and specificity (a large change in occupancy with a small change in chemistry). Such tuning can be achieved by altering the target-receptor interface via, for example, mutation, but such changes also alter specificity. The population-shift mechanism provides a means of altering affinity without altering specificity via modifications that alter the switching equilibrium constant, $K_S$. Shown are examples of naturally occurring and designed receptors that exploit this mechanism: (B) the erythropoietin receptor,18 (C) the purine-sensing riboswitch,19 and (D) an SH3 domain artificially re-engineered to undergo binding-induced folding in response to its target ligand.20

Figure 2. Nature commonly employs the population-shift mechanism to couple binding events to specific outputs.12 (A) In this, a receptor populates two conformations: a more stable but nonbinding state and a less stable binding-competent state. Ligand binding shifts the conformational equilibrium to the latter state, thus coupling recognition with an unfavorable conformational change and rendering the overall affinity of the receptor sensitive to the switching equilibrium constant, $K_S$. Shown are examples of naturally occurring and designed receptors that exploit this mechanism: (B) the erythropoietin receptor,18 (C) the purine-sensing riboswitch,19 and (D) an SH3 domain artificially re-engineered to undergo binding-induced folding in response to its target ligand.20

As our first demonstration of the effectiveness of using the population-shift mechanism to tune the dynamic range of artificial receptors, we employed molecular beacons,16 a fluorescent biosensor for the detection of specific nucleic acid sequences. (top left) The coupling between a beacon’s switching equilibrium constant and its affinity renders it possible to tune its dynamic range upward by orders of magnitude via the introduction of GC base pairs, which stabilize the stem. (bottom right) Because these modifications leave the binding interface unchanged, the specificity profiles of the variant sensors remain constant. Adapted with permission from ref 26. Copyright 2009 National Academy of Sciences.

Figure 3. (top) The population-shift mechanism can be used to tune the dynamic range of receptors without altering their specificity. (middle) As a demonstration we have employed molecular beacons,16 a widely used biosensor for the optical (fluorescent) detection of specific nucleic acid sequences. (bottom left) The coupling between a beacon’s switching equilibrium constant and its affinity renders it possible to tune its dynamic range upward by orders of magnitude via the introduction of GC base pairs, which stabilize the stem. (bottom right) Because these modifications leave the binding interface unchanged, the specificity profiles of the variant sensors remain constant. Adapted with permission from ref 26. Copyright 2009 National Academy of Sciences.

TUNING THE PLACEMENT OF THE DYNAMIC RANGE USING ALLOSTERY

Although mutational control over the switching equilibrium constant provides an important route toward optimizing useful dynamic ranges, this approach must be performed during design and fabrication and does not allow for adjustments to be made after the receptor is deployed. To achieve this nature...
typically employs allostery, a mechanism in which the binding of one ligand modulates the affinity with which a second ligand binds to a distal site on the same receptor. To date a number of authors have re-engineered catalytic systems (enzymes, ribozymes, DNAzymes) to render them subject to allosteric control.28−33 We have likewise explored this mechanism, focusing on its use in tuning the dynamic range of structure-switching biosensors.

Allosteric activation and inhibition occur when an allosteric effector binds to and thus stabilizes either the binding-competent or nonbinding states (respectively) of the receptor, raising or lowering the population of the binding-competent state and thus improving (Figure 5, top) or reducing (Figure 6, top) affinity. To explore these mechanisms we again employed molecular beacons as our initial model, rendering them allosterically controllable via the introduction of single-stranded tails on each of the beacon’s two termini.34 A single-stranded DNA that binds one tail acts as an activator by partially invading (and thus destabilizing) the beacon’s stem, pushing the useful dynamic range to lower concentrations (Figure 5). To achieve allosteric inhibition, we designed a single-stranded DNA that binds both tails simultaneously, thus hindering stem opening and pushing the useful dynamic range to higher concentrations (Figure 6).

Given our success in engineering allostery into molecular beacons, we next expanded this to a structurally more complex receptor. Specifically, we engineered allosteric inhibition into the cocaine-binding aptamer described above.27 To do this we designed short DNA sequences complementary to portions of the aptamer sequence that stabilize a nonbinding, partially double-stranded conformation. Using this approach, we achieved dynamic range tuning over ca. 3 orders of magnitude, with the extent of inhibition depending on both the length and concentration of the inhibitor. Thus, allosteric control provides a more predictable route to tuning the dynamic range of this more complex receptor than was provided by the mutational approaches described above. Following this, we used thymine−thymine and cytosine−cytosine mismatches, respectively, to fabricate mercury(II) and silver(I) ion sensors that are allosterically tuned by DNA effectors.35

Figure 4. Use of distal site mutations to alter the switching equilibrium constant provides a means of tuning the dynamic range of structurally complex receptors. (top) Here, for example, we have used this approach to tune the dynamic range of an aptamer-based cocaine sensor. Specifically, we designed variants of a cocaine-binding aptamer (bottom left) in which the switching equilibrium constant, and thus the aptamer’s overall affinity for its target, was reduced via truncations or circular permutations that destabilize the binding-competent conformation (bottom right). Adapted with permission from ref 27. Copyright 2012 American Chemical Society.

Figure 5. Allostery provides a means of tuning the dynamic range “on-the-fly”, after a receptor was designed and fabricated. (top) Allosteric activation, for example, can be used to push the useful dynamic range to lower concentrations. (middle) To demonstrate this we used a “tailed” molecular beacon and designed activators that bind to the tail and partially invade the stem, destabilizing it. (bottom) This, in effect, increases the switching equilibrium constant, shifting the beacon’s dynamic range to lower target concentrations. Adapted with permission from ref 34. Copyright 2012 American Chemical Society. See also ref 35.

Figure 6. (top) Allostery can also be used to push the useful dynamic range to higher concentrations. (middle) To achieve this with molecular beacons, we added two tails that work in concert to form a single effector-binding site. An inhibitor binding the two stabilizes the beacon’s nonbinding conformation, reducing the switching equilibrium constant (bottom) and pushing the useful dynamic range to higher concentrations. For examples of this using other receptors, see refs 27 and 35. Adapted with permission from ref 34. Copyright 2012 American Chemical Society.
BROADENING AND SHAPING THE DYNAMIC RANGE USING MATCHED RECEPTOR SETS

Shifting midpoint of the dynamic range, of course, is not the only way with which nature optimizes her receptors: evolution has likewise invented mechanisms that overcome the intrinsic limitation of the fixed width and shape of single site binding curves. Perhaps the simplest of these to conceive is the use of sets of receptors differing in affinity to broaden the dynamic range (Figure 7A), an approach that nature employs in many naturally occurring systems and that several groups have previously applied to artificial biosensors. These prior examples, however, generated their receptor variants via binding-site mutations that cause the specificity of the sensor to change as a function of target concentration, a problem that the population-shift mechanism circumvents.

Our first efforts to broaden dynamic ranges employed the variant molecular beacons described above (Figure 7). Using optimized mixtures of up to four different beacons, we have expanded the dynamic range of this system to up to 900 000-fold (Figure 7C) without altering their specificity profiles. We then extended this to other, more complex receptors. For example, using a mixture of four cocaine-binding aptamer variants, we produced a receptor set with a 330 000-fold dynamic range. In parallel, we have shown that mixtures of variants, we produced a receptor set with a 330 000-fold dynamic range.27 In parallel, we have shown that mixtures of variants, we produced a receptor set with a 330 000-fold dynamic range.27 In parallel, we have shown that mixtures of variants, we produced a receptor set with a 330 000-fold dynamic range.27 In parallel, we have shown that mixtures of variants, we produced a receptor set with a 330 000-fold dynamic range.27 Using this same strategy, we have also expanded the dynamic range of our silver(I) and mercury(II) sensors, broadening their dynamic ranges by orders of magnitude.35

While achieving extended dynamic range improves the usefulness of biosensors for many applications, others could benefit from yet more complex input–output behavior. It may, for example, prove beneficial, in some circumstances to “trade-off” sensitivity to small changes in concentration within a window of useful concentrations (e.g., a drug’s clinically relevant concentration range) to achieve enhanced sensitivity above or below this range. That is, some applications could benefit from “three-state” behavior that balances enhanced responsiveness at the extremes of the dynamic range against a “dead-zone” in the middle of the dynamic range over which sensitivity, and thus measurement precision, will be poorer. We have realized such behavior by combining receptors differing dramatically in affinity. For example, by combining molecular beacons differing 500-fold in dissociation constant, we created a system that responds robustly to fluctuating molecular concentrations above or below an intermediate 100-fold concentration span at the cost of exhibiting much poorer responsiveness over this intermediate range (Figure 7D).

NARROWING THE DYNAMIC RANGE USING SEQUESTRATION

The 81-fold dynamic range of single-site binding limits the ability of many biomolecular-based systems to respond sensitively to small changes in target concentration. In response, however, nature has invented a number of mechanisms by which biomolecular receptors can be made to respond much more sensitively and that can likewise be adapted to artificial bioreceptors.

The first mechanism that we exploited to this end is “sequestration”, an effect that underlies the extraordinary responsiveness of many genetic networks. In sequestration, low concentrations of the target are sequestered via binding to a high affinity but nonresponsive receptor (the “depletant”) that serves as a sink to prevent the accumulation of free target (Figure 8, top, left). When the total target concentration surpasses the concentration of the depletant, this sink saturates, causing a large rise in the relative concentration of free target. This, in turn, activates a second, lower affinity receptor (the “probe”) that, unlike the depletant, generates an output (Figure 8, top, right). The resultant threshold effect generates a far steeper input–output curve than that seen for simple single site binding. That said, there is no “free lunch”; this increase in sensitivity comes at the cost of poorer detection limits because, of course, the detection limit is defined by the (poorer) affinity of the receptor and not the higher affinity of the depletant. Still, for some applications (i.e., applications that require high measurement precision and for which sufficiently high affinity receptors are available), this represents a worthwhile trade off.

Prior to our work, other groups had recapitulated sequestration to create bistable transcriptional networks with sharp, adjustable thresholds. We, in contrast, have used sequestration to improve the responsiveness of a number of structure-switching biosensors, including optical and electrochemical molecular beacons, aptamer-based sensors, and transcription-factor beacons. Doing so, we have succeeded in pushing the 81-fold dynamic range of these sensors down to as low as 1.5-fold, significantly increasing their ability to detect small changes in relative target concentration (Figure 8, bottom).
NARROWING THE DYNAMIC RANGE USING COOPERATIVITY

Sequestration provides a ready route toward achieving improved sensitivity to small changes in target concentration, but it does so at the cost of requiring an extra reagent (the depletant) added at a precisely controlled concentration. In contrast, allosteric cooperativity, a second method that nature uses to produce either steeper or broader input-output behavior, does not suffer from this limitation. First described empirically in 1910 (ref 47) before being explained theoretically some four decades later, 48, 49 cooperativity is the dominant mechanism that allows, for example, hemoglobin to saturate in the lungs and yet deliver a large fraction of its cargo in the peripheral tissues over only a 3-fold drop in oxygen.

Cooperativity is achieved when the binding of one copy of a target molecule modulates the affinity with which subsequent molecules bind to other distal sites on the same receptor, thus rendering the cooperativity subset of allostery in which both the activator and the target are the same ligand. If earlier binding events render subsequent binding less favorable, the resultant negative cooperativity broadens the dynamic range. If, instead, earlier binding events render subsequent binding more favorable, the outcome is positive cooperativity and a steeper, more responsive output curve, given by:

\[
\text{Occupancy} = \frac{[\text{target}]^{n_H}}{K_{1/2}' + [\text{target}]^{n_H}}
\]

(3) in which \(n_H\) the “Hill coefficient,” reflects the system’s now steeper, higher-order dependence on target concentration. Ideal behavior (i.e., the steepest, most cooperative binding curve) is achieved when binding becomes “all or none” and \(n_H\) equals the number of interacting binding sites on the receptor. The dynamic range, in turn, goes as the \(n_H\)-th root; that is, for a perfectly cooperative, two-site receptor \(n_H = 2\) and the useful dynamic range falls to \(81^{1/2}\) or a 9-fold change in target concentration. This improved sensitivity once again, however, comes at a cost; the midpoint of a positively cooperative binding curve, \(K_{1/2}'\), is necessarily at higher ligand concentrations than the dissociation constant of the first (higher-affinity) binding event.

While a handful of rationally designed cooperative receptors had been described prior to our entry into the field,50, 51 these early approaches are not readily transferable to any receptors other than those explored in the original works. Thus motivated, we set out to explore more generalizable approaches to the rational design of allosterically cooperative receptors. Our first efforts focused, as always, on molecular beacons (Figure 9). 52 Specifically, we fabricated a “tailed” molecular beacon analogous to the modified beacon that we employed in the allosteric activation studies described above save that the tail and loop share a common sequence (Figure 9, middle left). We thus converted the system from heterotropic allostery, in which the activator and target differ, to homotropic allostery, in which the two are identical. Under these circumstances hybridization of the first copy of the target weakens the stem and improves the affinity with which the second copy binds, leading to cooperative behavior and improved responsiveness: the tailed beacon binds its target molecule with a Hill coefficient of 1.54 ± 0.10, corresponding to a (17 ± 3)-fold dynamic range (Figure 9, middle right).

To achieve greater cooperativity requires a larger difference in affinity between the first and second binding events. This can be generated in molecular beacons by altering the sequence, and thus stability, of their double-stranded stems (Figure 3). Unfortunately, however, because the stem of the tailed beacon also serves as one of its target-binding sites, we cannot arbitrarily change the stability of the stem without also changing the beacon’s specificity. To circumvent this, we explored an allosteric design that places both binding sites within the single-stranded loop (Figure 9, bottom left). 53 The strain associated with the binding of a single copy of the target to this two-site loop is sufficient to destabilize the stem and shift the population, improving the affinity of the second binding event. Using a relatively high stability stem, this system achieves a Hill coefficient of 1.94 ± 0.17 and a dynamic range of only (9.6 ± 1.6)-fold (Figure 9, bottom right), thus achieving a degree of cooperativity within experimental error of an ideal two-site receptor.

Although they serve as illustrations of the principles involved, the approaches that we have taken to the design of cooperative molecular beacons are likewise not transferable to structurally more complex receptors. More recently, however, we have developed a population-shift-based architecture general enough that it can even be implemented in the absence of detailed knowledge of the structure of the receptor. 54 This approach employs a tandem repeat of two copies of one-half of the receptor linked to a tandem repeat of its other half via a long, unstructured loop (Figure 10). The first binding event, which causes the association of the two sets of paired half-receptors to form two complete receptors, must overcome the unfavorable entropy of closing the loop. The second binding event need not “pay” this loop closure cost, improving its affinity and generating a cooperative, high sensitivity response. We have
used this mechanism to produce cooperative two-site mercury-
(II), cocaine, and doxorubicin binding receptors achieving Hill
coefficients of up to $1.98 \pm 0.04$ and dynamic ranges of as little
as 9.2-fold (Figure 10). 53

CONCLUSIONS
To our reading, the dominant focus of the biosensor
community to date has been on achieving the lowest possible
detection limits, with specificity and sensitivity largely being
relegated to secondary considerations that are solved by the
inclusion of washing and dilution steps or via the careful control
of stringency (i.e., optimizing the temperature, pH, or ionic
strength to tune dynamic range placement). Nature, in contrast,
cannot afford the luxury of washing and dilution steps, nor can
she arbitrarily alter temperature, pH, or ionic strength to
increase stringency, forcing evolution to expend at least as
much effort on achieving optimal sensitivity and specificity as
on achieving low detection limits. As the use of biomolecular
receptors in artificial technologies matures, and these
technologies begin to mimic the single-step convenience of
naturally occurring chemoperception systems, we suspect that
the biosensor community’s focus will likewise begin to shift
toward these important, if perhaps less obvious, issues. Given
the ease with which we have been able to rationally introduce
Nature’s solutions to these problems into a wide range of DNA-
based receptors and given the growing success of research
groups working on biomolecular design, we are optimistic that,
moving forward, these and similar approaches will offer viable
solutions to a wide range of bioanalytical problems.

Figure 9. Allosteric cooperativity provides another route by which the useful dynamic range of single-site receptors can be narrowed, improving the sensitivity with which they respond to small changes in target concentration. (top) Such cooperativity arises when the binding of one copy of a target molecule enhances the affinity with which subsequent molecules bind to other distal sites on the same receptor. (middle) Initially, we designed a cooperative molecular beacon by adding a target-binding tail appended to (and partially overlapping with) the beacon’s double-stranded stem. The binding of the first target molecule thus destabilizes the stem, improving the affinity with which the second target molecule binds and producing a steeper, more responsive binding curve. (bottom) We have also designed a “symmetric”, allosterically cooperative beacon that places both binding sites within the single-stranded loop. The strain associated with the binding of a single copy of the target to this two-site loop is sufficient to destabilize the stem and shift the population, improving the affinity of the second binding event. Using a relatively high stability stem, this system achieves a dynamic range of just $(9.6 \pm 1.6)$-fold, which is within error of the 9-fold dynamic range expected for a perfectly cooperative two-site receptor. Figure adapted with permission from ref 52. Copyright 2014 Wiley.

Figure 10. (top) We have developed a loop-closure mechanism for engineering allosteric cooperativity into structurally more complex receptors. In this, a tandem repeat of one-half of a receptor is linked to a tandem repeat of the second half of the same receptor via a long, unstructured loop. The binding-induced association of the first pair of receptor “halves” must pay the entropic cost of loop closure, reducing the affinity of the first binding event relative to that of the second, thus producing a cooperative response. We demonstrate this approach using an otherwise noncooperative (bottom, left) cocaine-binding aptamer and achieved a cooperative response covering just 13-fold (bottom right). Figure adapted with permission from ref 53. Copyright 2014 National Academy of Sciences.
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The authors declare no competing financial interest.

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REFERENCES
(3) Studart, A. Biologically inspired dynamic material systems. Angew. Chem., Int. Ed. 2015, 54, 3400–16.


(47) Hill, A. V. The possible effects of the aggregation of the molecules of haemoglobin on its oxygen dissociation curve. J. Physiol. London 1910, 40, IV–VII.

