Multiplexing and its upper bound in biological sensory receptors

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Biological sensory receptors provide perfect examples of microscopic scale information transduction in the presence of non-negligible thermal fluctuations. For example, studies of ligand-receptor reveal that accurate concentration sensing is achieved by integrating out noise in the sensor’s stochastic trajectories. However, we argue that the stochastic trajectory is not always an adversary—it could allow a single sensor to perform multiplexing (or muxing) by simultaneously transducing multiple environmental variables (e.g., concentration, temperature, and flow speed) to the downstream sensory network. This work develops a general theory of stochastic sensory muxing and a theoretical upper bound of muxing. The theory is demonstrated and verified by an exactly solvable Markov dynamics model, where an arbitrary sensor can achieve the upper bound of muxing without optimizing parameters. The theory is further demonstrated by a realistic Langevin dynamics simulation of a ligand receptor within a bath of ligands. Simulations verify that even a binary state ligand receptor with short-term memory can simultaneously sense two out of three independent environmental variables—ligand concentration, temperature, and media’s flow speed. Both models demonstrate that the upper bound for muxing is tight. This theory provides insights on designing novel microscopic sensors that are capable of muxing in realistic and complex environments.

I. INTRODUCTION

Sensory receptors play an important role in the functions of cells and these molecular complexes are able to accurately perceive their environments and transmit information inside the cell through various signalling mechanisms, despite the thermal fluctuations and other sources of noises [1–3]. A classical example of cellular sensing is the ligand-receptor binding mechanism for concentration sensing. The average fraction of time when the receptor binds with the ligand provides an intuitive physical characterization of the ligand concentration. The accuracy of the concentration estimation, as a result, is determined by the ability to average out thermal fluctuations and obtain an average binding fraction. The seminal work of Berg and Purcell [4], which studied the diffusion of ligands around a sensor, first showed that there exists a limitation to a receptor’s concentration sensory accuracy due to the presence of noise. Later, Bialek and Setayeshgar sharpened the Berg and Purcell’s accuracy bound by including the receptor’s kinetic back actions to the surrounding ligand solution [5]. Kaizu et al further utilized the theory of diffusion influenced reactions and improved the result of Bialek-Setayeshgar [6]. In 2019, Mora and Nemenman [7] developed a theory of concentration sensing when concentration itself is a function of time.

To date, the studies of ligand-receptor chemical sensing have been extended to more complex models. An incomplete set of examples include cooperative sensing by multiple ligand-receptor sensors [8, 9], multiple ligand sensing by single sensor [10, 11], ligand-receptor networks [12, 13], etc. Additionally, Markovian signal detection [14], effect of receptor diffusion [15], etc have also been considered in the study of ligand-receptor accuracy. The majority of studies on the accuracy of biological sensors treat each sensor as a single-variable information transducer, which measures only one of the multiple independent traits of a complex environment (e.g., mechanical force, temperature, or concentration) [17–19]. As an example, most studies of ligand-receptor sensors have shared an assumption that the receptor is mainly capable of measuring the ligand concentration [20]. In this regime, the sensor indeed estimates the concentration by performing statistical averaging of the fraction of the binding time, which is approximately proportional to the ligand concentration. Through the statistical averaging, either in the form of a time average or the form of a space (ensemble) average, fluctuations and noise can be eliminated, yielding a highly accurate estimation of the concentration. Here we would like to take a step back from the presumption of what a sensor can sense, and recover the information that is lost in performing direct statistical averages. We find that even though averaging over time removes the unwanted thermal noise, it also averages out useful environmental information other than ligand concentration hidden in the sensor’s time-correlated stochastic trajectories.

In this work, by removing the presumption of the sensor’s functionality, we argue that biological sensors (as simple as a single-molecule ligand-receptor) could perform multiplexing sensing or muxing—sensing multiple independent environmental information by the same sensor. Taking a simple ligand-receptor as an example, we demonstrate that a single receptor with binary states (bound and unbound) can simultaneously measure tem-
perature and ligand concentration or even the media’s flow speed.

In information science, a sensor or information channel that can simultaneously pass on multiple channels of information is called multiplexing or muxing [21]. It has been recently pointed out by Minas et al. that NF-κB regulatory networks are capable of performing muxing [22]. Moreover, the concept of observing and even controlling high-dimensional non-linear dynamical systems by low dimension (reduced) observables have been constructed in the 1970s for deterministic non-linear systems [23][25]. In 2010, Preesé, Lee, and Dill have also demonstrated that detailed kinetic information of a Markov process can be inferred from a sequence of coarse-grained binary-state measurements [26].

In this paper, we provide a general theoretical description of the stochastic dynamics of a sensor-environment system and the sensor’s ability of muxing. Moreover, we obtain a theoretical upper bound of the number of independent variables that can be simultaneously sensed by a single sensor – upper bound for muxing. This theory and upper bound are verified by a simple Markov state model, where we found that the upper bound of muxing is typically saturated without any parameter-tuning. We then perform a detailed numerical simulation of a ligand-receptor sensor in a chemical bath, and demonstrate that a single receptor can simultaneously sense multiple environmental information such as bath temperature, concentration, and bath’s flow speed. The simulation also confirms that simple ligand receptor’s ability for muxing agrees with the theoretical upper bound and that the upper bound is saturated without carefully tuning the parameter or optimizing the design of the receptor.

II. THEORETICAL UPPER BOUND OF MUXING

Let us start with a general description of a sensor sensing information from its environment: a single microscopic sensor with internal degrees of freedom $x_s$ interacts with a stationary stochastic environment with degrees of freedom $x_e$. The microscopic states of the environment $x_e$ evolve erratically in time due to thermal fluctuations but is assumed to be at a macroscopic stationary state characterized by a few fixed macroscopic variables denoted by the $n$-dim vector $\theta$ (e.g. temperature, chemical concentration, pressure, flow speed, pH, etc.) The sensor, through its interaction with the environment, transduce environmental information $\theta$ to the downstream sensory network. In this picture, both the sensor and its environment evolve stochastically due to the thermal fluctuations; the state of the environment can impact the dynamics of the sensor, and the state change of the sensor can in turn perform a back action to the local environment. A general equation of motion of the composite system of the sensor and the environment can be written as

$$\frac{d(x_s, x_e)}{dt} = F(x_s, x_e; \theta)$$

where $F(\cdot; \theta)$ is a function of both $x_s$ and $x_e$ and is parameterized by the stationary environmental properties

$$\theta = (\theta_1, \cdots, \theta_n)$$

Here, $n$ denotes the number of independent environment properties needed to describe the environment. For biological sensing, the composite system of a sensor and environment is in contact with a larger background thermal environment, and thus one can interpret the above equation as Langevin dynamics where $F(x_s, x_e; \theta)$ contains both the deterministic dynamics and the stochastic terms due to the thermal fluctuations.

Discrete-State Sensor Assumption: Without presuming for what the sensor can measure, this work studies the sensor’s coarse-grained trajectory $s(t) = s(x_s(t))$ and estimates the number of independent environmental variables $\theta$ that can be inferred from $s(t)$. It is worth noting that $x_s$ represents the full microscopic state of the sensor (i.e., the locations and momentum of each atom in the receptor molecule), and it is unrealistic to expect the downstream bio-sensory network to observe or record a full micro-state trajectory of the sensor, $x_s$. Rather, the downstream sensory network may only read the sensor as in one of a few coarse-grained states. As a consequence, the coarse-grained state $s$ reduces the high-dimension micro-state space of $x_s$ into a lower-dimension or even discrete state space. For a simple ligand receptor, one can naturally assume that the sensor’s coarse-grained state is binary: $s \in \{0, 1\}$, which assumes state ‘0’ when the receptor is unbound from any ligand, and assumes state ‘1’ when the receptor is bound to a ligand. Given the thermal fluctuations and the stochastic dynamics of $x_s$, we take an assumption that $s(t)$ can be considered as a discrete-state stochastic process.

As we argued in the Introduction, merely obtaining the statistics of the sensor’s state by computing the average of $s(t)$ could result in a significant loss of the environmental information. Rather, the dynamic trajectories of the sensor $s(t)$ over a period of time contains abundant information of the environment which could allow us to infer multiple dimensional information even from a binary state sensor.

Discrete-Time Trajectory Approximation: It is worth noting that, in practice, recording a continuous-time trajectory $s(t)$ without error corresponds to infinitely high information and thus is associated with an infinitely high entropic and thermodynamic cost [27], which is unrealistic in a real biological sensory network. Rather, typical downstream sensory networks producing and accumulating downstream signaling molecules based on the sensor $s(t)$ can be considered as a noisy “kernel” with a finite time-resolution and finite memory lifespan, applied to the signal $s(t)$ over a long time. Even if the kernel has an infinitely high time-resolution but the memory
lifespan is much shorter than the transition time of the sensor, the accumulated result of the “kernel” is effectively single-time-point statistics, losing information on the time-correlation of \( s(t) \). On the other hand, if the kernel has a very long memory life-span but the time-resolution of the kernel is approximately equal to the memory life-span, then effectively, the kernel can at most encode information of two-time trajectories with time-lag equal to the memory life-span. Thus, we simplify the downstream reaction network, given its time-resolution and memory life-span, into a statistical estimator based only on the statistics of \( n_t \)-point discrete-time trajectories. In other words, only “visible” to the the downstream network is the statistics of an ensemble of discrete-time trajectories \( S \)'s acquired over a long period of time, where each \( S = (s(1), s(2), \cdots, s(n_t)) \) is a discrete-time trajectory of total time length limited by the memory life-span, and the \( n_t \) determined by the kernel’s time resolution. Notice that this argument is very general and the time-lag between \( s(i) \) and \( s(i+1) \), does not need to be equal to \( s(j) \) and \( s(j+1) \), for \( 1 \leq i, j \leq n_t - 1 \).

Under the “Discrete-State Sensor Assumption” and “Discrete-Time Trajectory Approximation”, the information visible to the downstream sensory network over a long period of time is reduced to an ensemble of discrete-state and discrete-time trajectories \( \{ S \} \). This allows us to obtain a theoretical upper bound of maximum. Such upper bound is defined as the maximum number of independent environmental variables from the \( n \)-dim \( \theta \)-space that a sensor could encode in the statistics of \( n_t \)-time trajectories \( \{ S \} \), where \( S = (s(1), s(2), \cdots, s(n_t)) \) and \( s(\cdot) \) assume \( n_s \) different values (sensor has \( n_s \) coarse-grained states).

Here the sensor establishes a map from the \( \theta \)-space into the probability distribution space consisting of \( P_\theta[S] \).

Illustrated in Fig. 1 a \( n \)-dim solid blob in \( \theta \) space is mapped to the dashed blob in the trajectory probability space. The probability space, by definition, is (subset of) a \( (n_{s_t}^{n_s} - 1) \)-simplex. The sensor’s ability of inferring \( \theta \) is thus limited by one’s ability to construct an inverse map from \( P_\theta[S] \)-space back to the \( \theta \)-space. Here we make one more assumption that the inverse maps are differentiable continuous or at least piece-wise differentiable continuous, ruling out the possibility of involving a space-filling-curve maps from low dimension to high dimension such as a Hilbert-curve [28]. Under this continuity assumption, if one can construct an injective map from \( P_\theta[S] \)-space to \( \theta \)-space, then one can completely infer \( \theta \) without any ambiguity. Furthermore, since \( P_\theta[S] \)-space has a finite-dimension, the injective continuous map can only be constructed to a space of similar or lower dimension. As a result, the maximum independent environmental degrees of freedom that one can infer from the sensor is bounded by the irreducible dimension of the \( P_\theta[S] \)-space, \( d(n_t, n_s) \leq n_s^{n_t} - 1 \).

As a result we arrive at a theoretical upper bound on the number of independent variables that the sensor can sense as

\[
r_s \leq d(n_t, n_s) \leq n_s^{n_t} - 1 \tag{3}
\]

where \( r_s \) is the upper bound of muxing, \( d(n_t, n_s) \) is the irreducible dimension of the trajectory probability space, which bounded by \( n_s^{n_t} - 1 \), the dimension of the probability simplex.

Notice that the second bound in Eq. 3 \( r_s \leq n_s^{n_t} - 1 \) is a loose bound because the actual probability distribution of \( S \) can be a subspace of \( (n_{s_t}^{n_s} - 1) \)-simplex due to certain symmetries or the statistical quality of the trajectory, explained below: First, the trajectory probability \( P_\theta[S] \) could contain symmetries. In the exam-
ple of \( n_t = 2 \) and \( n_s = 2 \), we find that the symmetry \( P[01] = P[10] \) further removes one degree of freedom (see SI.3) and thus the trajectory probability simplex, and thus the probability space is reduced to the 2-dim dashed triangle “ABC”. For \( n_t = 3 \) and \( n_s = 2 \), the 7-simplex is reduced to a 4-dim manifold due to 3 symmetries (see SI.3): \( P[001] = P[101] = P[110] \), and \( P[001] + P[101] = P[101] + P[110] \), as illustrated in the next section. Secondly, the actual dimension of the trajectory probability space could be reduced due to the imperfections of the sensor and/or downstream sensory network. Consider an example of \( n_t = 2 \) and \( n_s = 2 \), if the time-lag between the two points within the trajectory is much longer than the relaxation time of the composite system of sensor and environment (e.g., caused by a slow downstream network), then effectively the probability space is 1-dim since the two time-points \( s(1) \) and \( s(2) \) are totally uncorrelated and follow the same identical and independent distribution \( P[s] \) with \( s \in \{0, 1\} \).

In conclusion, the loose bound of \( r_s \) may seem to increase exponentially with the number of time steps \( n_t \), allowing for a single \( n_s \)-state sensor to easily sense multiple environmental information simultaneously. However, due to the symmetries and imperfections discussed above, tighter bound \( r_s \leq d(n_t, n_s) \) may not grow exponentially with time and may rely on the nature of a sensor, which will be discussed in a future work focusing on the optimal design of muxing into stochastic sensors.

III. MAXIMUM LIKELIHOOD ESTIMATION AND SIMPLE MARKOV MODEL SENSOR

To further illustrate the theory, below let us illustrate the upper bound of muxing \( r_s \) using Maximum Likelihood Estimation Theory [29]. We demonstrate below that \( r_s \) can also be considered as a sensory information rank, i.e., the rank of the log-likelihood function’s Hessian. Moreover, we construct a discrete time 6-state Markov model of a binary-state sensor coupled to a simple 3-state environment. This example demonstrates that even extremely simple sensors can perform muxing and saturate the tighter muxing upper bound (first inequality in Eq. 3) without tuning any parameters or optimizing the design.

Fig. 2 illustrates a simple toy model of a binary-state sensor coupled to a 3-state environment. The environment is simplified into a 3-states: \( E_1 \), \( E_2 \), and \( E_3 \). At each time-step, the environment can stay in the previous state (solid curved lines) or make a transition between state \( E_1 \) and \( E_2 \) or between \( E_1 \) and \( E_3 \) (solid straight lines), regardless of the state of the sensor. The 4 transitions, i.e., \( E_1 \rightarrow E_2 \), \( E_2 \rightarrow E_1 \), \( E_1 \rightarrow E_3 \), and \( E_3 \rightarrow E_1 \), are impacted by the nature of the environment, and their Markov transition probability are denoted by \( \theta_1 \), \( \theta_2 \), \( \theta_3 \), and \( \theta_4 \). These 4 parameters forms a basis of the environmental parameter space. The dynamics of the sensor and the environment can be captured by a 6-state discrete-

FIG. 2. The figure illustrates a simple six state Markov model constructed by combining a 3-state environment with a binary-state sensor. Each of the transparent planes denote a different state of the sensor (\( s = 0 \) or \( s \)) and each of the 3 spheres on a plane denote one possible environmental state. The 3 environmental states are denoted by \( E_1 \), \( E_2 \), and \( E_3 \). The transition between \( E_1 \), \( E_2 \), and between \( E_1 \) and \( E_3 \) correspond to the stochastic dynamics of the environment independent of the sensor. They are illustrated by the 2 solid lines with 4 directed transitions. Their transition rates are dictated by the environmental condition \( \theta \) and thus we represent these 4 transition probabilities by \( \theta = (\theta_1, \theta_2, \theta_3, \theta_4) \). Shown by the dashed lines are the transition between environmental states \( E_2 \) and \( E_3 \), which must be accompanied by the state change of the sensor. These 4 directed transitions for the two dashed lines are specified by the specific design of the sensor and the sensor’s interaction with the environment. Let us label these 4 transition probabilities as \( k = (k_1, k_2, k_3, k_4) \). At each time step, the environment and the sensor could remain in its previous state and this is characterized by curved loops starting and ending at the same sphere. The transition probability for each allowed transition is summarized into transition probability matrix in Eq. 4.
where each element of the matrix (transition probability) must take the value within \([0, 1]\). As a result the discrete-time evolution of the probability of the composite environment-sensor system \(p\) can be written as

\[
p(t = i + 1) = W(p(t = i))
\]

where \(p(t = i)\) is a 6-by-1 probability vector for states 1 to 6 at time \(t = i\). At anytime, we can write the probability of the sensor as \(P[0] = p_1 + p_2 + p_3\) and \(P[1] = p_4 + p_5 + p_6\).

If the sensor is left to sense the stationary environment (with a fixed \(\theta\)) for a long time, the composite system could reach thermal equilibrium (if \(W\) satisfies detailed balance condition) or reach a non-equilibrium steady state (if the dynamics is dissipative and \(W\) does not satisfy detailed balance). In the long time limit, we expect that the sensor with finite-time memory (of time length \(n_t\)) could accumulate statistics over all possible discrete-time trajectories of length \(n_t\). For illustrative purposes, let us assume that the binary sensor could obtain statistics of two-point trajectories (\(n_t = 2\)). There are 4 possible trajectories for a binary-state sensor; \(S \in \{00, 01, 10, 11\}\). Thus, given a fixed environmental condition \(\theta^*\), the probability density or the trajectories can be denoted by \(P_{\theta^*}[00], P_{\theta^*}[01], P_{\theta^*}[10],\) and \(P_{\theta^*}[11]\). At steady state for binary-state sensors, it is straightforward to show that \(P_{\theta^*}[01] = P_{\theta^*}[10]\) for any stationary \(\theta\). (See SI.3) In summary, the probability space is reduced from the 3-simplex into a 2-dim triangle ABC, i.e., \(d(n_t = 2, n_s = 2) = n_s^n_t - 2 = 2\).

With the exactly solvable Markov model, here we can treat the sensor as a Maximum Likelihood Estimator and analytically solve for the number of independent environmental information \(\theta\) that the binary sensor could infer from \(n_t = 2\)-point trajectories. We verify that indeed the upper bound for mixing for \(n_t = 2, n_s = 2\) is \(r = d(n_t = 2, n_s = 2) = 2\).

Given an example of \(n_t = 2\) and \(n_s = 2\) at a fixed environment \(\theta^*\), the probability to observe each possible trajectory is denoted by \(P_{\theta^*}[S]\). To estimate the environmental variables \(\theta^*\), let the sensor observe and accumulate \(m\) independent trajectories of the sensor’s state, \(\{S^1, S^2, \ldots S^m\}\), without knowing the values of \(\theta^*\). Let us perform a Maximum Likelihood estimation of \(\theta^*\) where the log likelihood function (llf) of environmental variables \(\theta\) is defined as

\[
l(\theta) = \frac{1}{m} \sum_{i=1}^m \ln P_\theta[S^{i*}]
\]

(6)

In the limit of abundant sampling, \(m \to \infty\), the llf reduces to a simple expression depending on the trajectory probabilities

\[
l(\theta) = \sum_{s \in \{S\}} P_{\theta^*}[S] \ln P_\theta[S]
\]

(7)

that is derived in detail in the SI.1. The MLE theory then states that the best estimation of the environmental variables is achieved at the maximum of the log likelihood function:

\[
\theta_{\text{est}} = \arg \max (l(\theta))
\]

(8)

In information theory, especially when the MLE is already known to be able to infer all degrees of freedoms of the unknown parameters, the inference accuracy is described by the Hessian Matrix of \(l(\theta)\). However, as stated above, it is possible that the single sensor can not simultaneously sense all of the parameters in \(\theta\), thus the llf may not be strictly concave, and the hessian may not be negative-definite. Here we argue that the maximum number of independent degrees of freedom that the sensor can sense is equal to the rank of the Hessian Matrix \(r_s\), where we have related the information rank to \(r_s\) in Eq.\[3\]

Without focusing on a specially chosen environment \(\theta\) or a carefully tuned design of the sensor \(k\), we can exactly solve for the steady-state Hessian Matrix of the llf for any \(\theta\) and any \(k\), where the \(\left\{i,j\right\}\)-th element of Hessian Matrix is (see SI.2 for derivation):

\[
H_{ij} = \sum_{S \in \{00,01,10,11\}} \frac{1}{P_\theta[S]} \frac{\partial P_\theta[S]}{\partial \theta_i} \frac{\partial P_\theta[S]}{\partial \theta_j}
\]

(9)

here for the simple example, \(i\) and \(j\) take the values from 1 to 4.

By randomly generating environmental variables and designs of sensors (\(\theta\) and \(k\)), we found that typically
FIG. 3. The probability density of (a) the two non-zero eigenvalues for the lfl’s Hessian matrix obtained from $n_t = 2$-point trajectories ($\lambda_3 = \lambda_4 = 0$) and (b) four non-zero eigenvalues for $n_t = 3$-point trajectories. Notice that there are 4 eigenvalues for both (a) and (b), and two eigenvalues for (a) are strictly zero.

(with probability 1), one can already infer 2 independent environmental variables from the two-point trajectories! In other words a randomly generated sensor can easily saturate the upper bound of muxing: $r_s = d(n_t = 2, n_s = 2) = 2$.

We have also performed similar analytical calculations for $n_t = 3$-point trajectories for the same binary-state sensor coupled to the 3-state environment. In this case, there are 8 possible trajectories: $P\theta^[000]$, $P\theta^[001]$, $P\theta^[010]$, $P\theta^[011]$, $P\theta^[100]$, $P\theta^[101]$, $P\theta^[110]$, $P\theta^[111]$. Notice that at the steady state the probability space cannot fully span the 7-dimensional simplex due to the following 3 symmetries (see SI.3): $P\theta^[001] = P\theta^[100]$, $P\theta^[011] = P\theta^[010] + P\theta^[001] + P\theta^[110]$, and $P\theta^[001] + P\theta^[101] = P\theta^[010] + P\theta^[001]$. As a consequence the predicted bound in Eq. 3 should read as $r_s \leq d(n_s = 2, n_t = 3) = 2^3 - 1 - 3 = 4$.

In Fig. 3(a) we demonstrate for $n_t = 2$ and in Fig. 3(b) for $n_t = 3$, the probability density functions of the non-zero eigenvalues of Hessian for randomly generated $\theta$ and k’s. For $n_t = 2$ we verify that almost all (with probability 1) randomly generated environment and sensors give us 2 non-zero eigenvalues, saturating the upper bound of muxing; and for $n_t = 3$ there are typically 4 non-zero eigenvalues, also saturating the upper bound of muxing.

As a brief summary, we have developed a general theory to predict the information upper bound of muxing for individual stochastic sensors, Eq. 3. With an exactly solvable model of a binary sensor coupled to an environment, we demonstrate using the MLE theory that our predicted bound $r_s \leq d(n_t, n_s)$ is easily saturated: $r_s = d(n_t, n_s)$.

IV. MUXING BY A LIGAND-RECEPTOR

The ligand-receptor sensor is typically believed to be a concentration sensor [20]. However, the environment contains more information than simply the ligand concentration. The binding/unbinding kinetics would be impacted by other environmental variables such as temperature, concentration, medium flow speed, etc. Here we numerically demonstrate that a single sensor has the ability to infer two independent dimensions of environmental information. In other words, a single ligand-receptor can perform muxing. This result also verifies that the tighter theoretical upper bound of muxing $r_s$ shown in Eq. 3 (left) is saturated by simple ligand receptor as well.

Here we simulate the dynamics of an individual ligand-receptor within a bath of ligands (within a tube, see Fig. 4). The bath is modeled by a pool of ligand
molecules at a fixed total concentration, fixed temperature, and fixed flow speed. The bath tube is modeled with a periodic boundary condition at the two ends. We allow the bath to have a stationary flow along the axis of the tube with flow speed \( v_x \) along the \( x \)-axis direction \( e_x \), while keeping the receptor fixed at the center. The bath is simulated by the Langevin dynamics of an ensemble of ligand molecules:

\[
\mathbf{r}_i = v_x \mathbf{e}_x + \frac{1}{m\gamma} \mathbf{F}(\mathbf{r}_i) + \eta(t)
\]

where \( \mathbf{r}_i \) is the position of the \( i \)-th ligand molecule, \( m \) is its mass, \( \gamma \) is the friction coefficient, \( v_x \mathbf{e}_x \) accounts for the background flow of the media, \( \mathbf{F}(\mathbf{r}) \) is the total deterministic force experienced by each ligand molecule (in a detailed simulation, this force accounts for the sum of pairwise interactions between ligand pairs and the ligand-receptor attraction), and \( \eta \) is the Gaussian noise characterized by \( \langle \eta(t)\eta(t') \rangle = 2\gamma k_B T \delta(t-t') \), where \( k_B \) is the Boltzmann constant. The ligand–ligand interaction can adopt a short range repulsive WCA potential \( [30] \) or can be modeled as free Brownian particles to reduce computational cost; this simplification does not qualitatively change the result (as shown in the SI.7). In brief, the simulated bath can be characterized by set of independent environmental variables \( \theta = (\mu, \beta, v_x) \):

\[
\mu = \ln c \quad \beta = (k_B T)^{-1}.
\]

The receptor located at the center of the tube can bind particles with a rate proportional to the local concentration \( c' \). Here the local concentration is computed at each instantaneous time as the the number of ligands within the cutoff distance 2.249 from the center of the receptor. At any given time, if the receptor is empty and the local concentration is \( c' \), then the receptor could bind with one ligand with rate

\[
R_{\text{on}} = c' e^{-\beta(E_b - E_{\text{off}})}
\]

where \( E_{\text{off}} \) is the energy of an empty receptor, and \( E_b \) is the energy barrier for the binding-unbinding transition. When the binding occurs, the number of particles in the local environment (and thus the simulation tube) reduces by 1. If the receptor is already bound with one ligand, then unbinding event could occur resulting in one ligand returning back into the bath. Considering the effect of the media flow speed \( v_x \) and the friction coefficient that each ligand experiences \( \gamma \), the unbinding occurs at the rate of

\[
R_{\text{off}} = e^{-\beta(E_b - E_{\text{on}} - \gamma a v_x)}
\]

where \( E_{\text{on}} \) is the energy of the receptor when it is bound with the ligand, and the term \( \gamma a v_x \) is the frictional work done by the flowing media to assist the ligand’s dissociation from the receptor. Here we assume that the ligand displaces from the receptor by a distance \( a \) to dissociate from the ligand.

By simulating the environment and receptor at a fixed condition \( \theta = (\mu, \beta, v_x) \), one can obtain stochastic trajectories of the state of the receptor \( s(t) \). The simulation parameters and details are listed in SI.4. Notice that the

\[
\begin{align*}
\langle \alpha \rangle & = \frac{1}{m\gamma} \mathbf{F}(\mathbf{r}_i) + \eta(t) \\
\langle E \rangle & = \frac{1}{m\gamma} \mathbf{F}(\mathbf{r}_i) + \eta(t) \\
\langle E_i \rangle & = \frac{1}{m\gamma} \mathbf{F}(\mathbf{r}_i) + \eta(t)
\end{align*}
\]
receptor is binary-state. Moreover, as stated in the Introduction, biological sensing should not be expected to accurately measure and store the trajectory with continuous time (infinite sampling frequency comes with infinite information entropy cost). Thus we consider that the receptor could only accumulate and pass on statistical information of discrete time binary-state trajectories such as “00”, “01”, “10”, and “11” for \( n_t = 2 \) and the time lag between the two time points is set to be 1 (where simulation time step \( dt = 0.01 \)). Below we numerically show that for ligand receptor, our theoretical bound of \( r_s \leq d(n_t, n_s) \) is valid. Moreover, this bound is sharp for binary state sensor \( n_s = 2 \) with two-time-point memory \( n_t = 2 \).

Our numerical simulation demonstrates that at a fixed environmental condition \((\mu, \beta, v_x)\), even for a simple binary-state sensor acquiring the statistics of merely two-time-point trajectories \((n_t = 2)\) one can infer 2 independent environmental variables. This is demonstrated here by obtaining the 3-by-3 Hessian matrix

\[
\begin{bmatrix}
(-2.247 \pm 0.003) \times 10^{-1} & (-3.6 \pm 0.1) \times 10^{-2} & (6.95 \pm 0.02) \times 10^{-1} \\
(-3.6 \pm 0.1) \times 10^{-2} & (-2.254 \pm 0.007) \times 10^{-1} & (-2.47 \pm 0.02) \times 10^{-1} \\
(6.95 \pm 0.02) \times 10^{-1} & (-2.254 \pm 0.007) \times 10^{-1} & (-2.22 \pm 0.03) \\
\end{bmatrix}
\]  

(15)

where the hessian is obtained around an arbitrarily chosen point in \( \theta \)-space: \( \theta^* = (\mu^*, \beta^*, v^*_x) = (\ln 100, 1.0, 0.2) \) with the numerical derivatives evaluated with finite increments \( \Delta \theta = (4.8459\% \mu^*, 5\% \beta^*, 5\% v^*_x) \). These results are obtained from trajectory statistics of \( 10^{10} \)-step simulations. The Hessian is verified to be rank-2 as it contains two negative eigenvalues and a zero eigenvalue:

\[
\begin{align*}
\lambda_1^{\mu, \beta, v_x} &= -2.48 \pm 0.03 \\
\lambda_2^{\mu, \beta, v_x} &= -2.060 \pm 0.008 \times 10^{-1} \\
\lambda_3^{\mu, \beta, v_x} &= (2 \pm 4) \times 10^{-3}
\end{align*}
\]

Thus, \( r_s = 2 \leq d(2, 2) \). A in-depth discussion of the Hessian and the eigen-analysis are listed in SI.5.

As shown above, a single receptor can infer 2 out of 3 environmental variables. If we assume that one of the 3 environmental variable is known, and instead compute the 2-by-2 Hessian Matrix for the remaining 2 variables, we verify that all 3 reduced Hessian Matrices are negative-definite. In other words, given the flow speed, a single ligand receptor could simultaneously sense both the temperature and the ligand concentration:

\[
\begin{align*}
\lambda_1^{\mu, \beta} &= -2.61 \pm 0.01 \times 10^{-1} \\
\lambda_2^{\mu, \beta} &= -1.89 \pm 0.01 \times 10^{-1}
\end{align*}
\]

Similarly given \( \beta \), the sensor can simultaneously sense both \( \mu \) and \( v_x \):

\[
\begin{align*}
\lambda_1^{\mu, v_x} &= -2.45 \pm 0.02 \\
\lambda_2^{\mu, v_x} &= (-7 \pm 4) \times 10^{-3}
\end{align*}
\]

and given \( \mu \), the sensor simultaneously senses both \( \beta \) and \( v_x \):

\[
\begin{align*}
\lambda_1^{\beta, v_x} &= -2.26 \pm 0.03 \\
\lambda_2^{\beta, v_x} &= (-1.95 \pm 0.01) \times 10^{-1}
\end{align*}
\]

The Hessian analysis above can only reflect the sensor’s performance around a single environment condition \( \theta^* \). To demonstrate that the ligand-receptor can indeed perform muxing \((r_s = 2)\) over a wide range of environmental conditions, here we demonstrate an alternative illustration of muxing within the range of \( \mu \in [\ln 25, \ln 398] \) and \( \beta \in [0.2, 1.8] \) at a fixed \( v_x = 0.2 \), shown in Fig. [4]. To demonstrate that one can simultaneously infer both concentration and temperature, let us introduce a contour-line crossing technique for functions \( s(\mu, \beta) \) (see Fig. [3](a)) and \( C_1(\mu, \beta) \) (see Fig. [3](b)), where the contour-line crossing is shown in Fig. [3](c). Here we have defined two independent functions that can be obtained from the statistics of the two-point trajectories:

\[
\overline{s} = \lim_{N \to \infty} \frac{1}{2} \sum_{i=1}^{N} \frac{s(t_i)}{t_i} = \frac{P_0[01] + P_0[10]}{2} + P_0[11]
\]

(25)

and

\[
C_1 = \frac{(s(t_i)s(t_{i+1}))}{(s(t_i)\overline{s})} = \frac{P_0[11]}{(P_0[01] + P_0[10])/2 + P_0[11]}
\]

(26)

where \( N \) is the total number of time steps in the performed simulation. Since the contour lines for the two functions are not parallel (similar) within the whole range of parameters, one can always simultaneously infer both \( \mu \) and \( \beta \) from the statistics of 2-point trajectories. In the SI.6, the contour-line crossing technique is also illustrated for other variable pairs of \((\mu, v_x)\) and \((\beta, v_x)\), demonstrating that statistics of only two-point trajectories out of a binary-state ligand receptor can simultaneously report 2 variables out of the \( \theta = (\mu, \beta, v_x) \).

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