## Review

# Quantifying information of intracellular signaling: progress with machine learning

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### Abstract
Cells convey information about their extracellular environment to their core functional machineries. Studying the capacity of intracellular signaling pathways to transmit information addresses fundamental questions about living systems. Here, we review how information-theoretic approaches have been used to quantify information transmission by signaling pathways that are functionally pleiotropic and subject to molecular stochasticity. We describe how recent advances in machine learning have been leveraged to address the challenges of complex temporal trajectory datasets and how these have contributed to our understanding of how cells employ temporal coding to appropriately adapt to environmental perturbations.

(Some figures may appear in colour only in the online journal)

## 1. Introduction

Cells are capable of sensing changes to their external environment and adapting their functions appropriately, a process known as cellular decision-making [1, 2]. This involves the transmission of information gathered by molecular sensors or receptors through biochemical signaling pathways that can be functionally pleiotropic [3, 4]. Signaling transduction is also subject to stochastic noise that affects molecular activities and mediates biological information transfer [5–9]. Cells may evolve to fine-tune noise levels to maximize information transmission [10], to distinguish stimulus conditions with

specificity [11, 12], or to allow for a degree of indeterminacy in decision-making within a population in a physiological strategy referred as bet-hedging [13]. An accurate quantification of the information flow within living systems is critical for characterizing such cellular behaviors and how their decision-making plays a role in physiology and pathology.

At a conceptual level, 'information' is a quantification on the amount of uncertainty. To formally quantify information, information theory was originally developed for the digital information transmitted through noisy channels [14]. Then, studies of electrical dynamics in neurons pioneered the application of information theory to biological systems [15–19]. Quantitative investigations addressed the information transmitted by neural spike trains elicited by the stimulus [20, 21] and the information encoded in temporal patterns of firing [22].

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Inspired by these studies, information-theoretic approaches have further been applied and adapted to study intracellular signaling processes [23, 24] of immune cells responding to noxious substances [25, 26].

For intracellular signaling, identifying signaling channels requires careful biological measurements to classify the groups of signaling molecules as pathways [4]. Thus, the term 'signaling channel' needs to be accompanied by particular signaling molecules or defined by having separate time scales. By viewing biochemical signaling as an information channel, the sender is usually regarded as an environmental stimulus that is perceived by a receptor or sensor molecule. What is considered as the receiver, however, varies among studies and depends on what the experimental approach actually measures. Some studies measure the activities or subcellular localization of major signaling molecules, others measure the stimulus-induced expression of genes, and others measure cellular scale responses such as growth, division, movement, or death. Recent progress in measuring gene expression and signaling activities in individual cells [27] enables a quantitative investigation of intracellular information transmission based on experimental data. Information-theoretic analysis of such data may be used to quantify the extent of stimulus discrimination by the cell [28], as a focused biological problem of this review.

Among the many information measures, mutual information (equation (3)) is a special one with important properties. It is a measure of correlation satisfying a set of requirements in Shannon's theory [29]. When the measured variables are nonlinearly correlated, computing mutual information is still convenient. In addition, mutual information provides a likelihood for model inference [30]. This is especially useful when writing a likelihood function is hard, as prevailing in biological systems, where the intervening steps between measured species typically do not have quantitative models. For the stimulus discrimination process, mutual information has a clear biological meaning (box 1); specifically, it indicates the amount of stimulus that cells can effectively discriminate by the intracellular signaling. Thus, mutual information is a major quantity to be reviewed.

Information-theoretic approaches are data-driven and involve a statistical estimation of the probability and entropy of the data. However, accurate estimation is hindered by some of the following properties of data. First, signaling-response data may be high dimensional, especially when based on imaging methods. Second, regulatory networks [31] of signaling molecules are capable of complex temporal patterns due to interdigitated feedback loops. Third, the data are affected by multiple sources that cause variability: preexisting biological heterogeneity within the population of cells, stochastic molecular noise that affects the signaling process, and technical imperfections in measurements. These make the estimation of the biologically relevant effective information capacity challenging.

Recent works have shown that these challenges can be approached by machine-learning approaches, where a class of models are trained by data to recognize patterns in the data, to infer probabilities and to inform the way unseen data are treated. In recent decades, machine learning has had great success in image classification, speech recognition, and more [32–35]. Machine-learning models use labeled data, known as training data, to learn the complex distribution of data. The model can also cluster training data into different categories, classify new data that are not seen during training to the corresponding category, and assign an accurate probability to new data when given a sufficient amount of training data. Among the many applications of machine learning, two specific tasks related to intracellular information processing include pattern classification and time series analysis.

First, for pattern classification where a specific number of potential answers exist and training data have been labeled, a machine-learning model can be trained to correctly classify unseen data. An example is the MNIST dataset of handwritten digits, where the trained model, such as a deep neural network [33], performs well on the task of recognizing new digits. Second, time series analysis aims to extract meaningful statistics from the time series data, including stock prices, climate change, and speech. Machine-learning models such as recurrent neural networks [36, 37], a class of neural networks where connections between nodes form a temporal sequence, are able to exhibit temporal dynamical behavior, learn the patterns in the time series data, and further predict future values, known as time series forecasting.

The machine-learning approaches for the above two tasks are applicable for evaluating the information content of single-cell signaling response data. The pattern classification may classify distinct single-cell signaling responses when cells encounter different ligands or concentrations of the same ligand [11]. The trained model is then used to classify the measured signaling responses from unknown stimulation conditions. The truth table of the classification enables us to estimate the intracellular information transmission. In addition, since the signaling processes happen in a time course, time series analysis is helpful to extract biologically meaningful statistics from the measured data and evaluate the information transmission over time.

Extending these approaches of machine learning to intracellular signaling, the past five years have seen new advances both in theory and in application. Although previous reviews have described studies of intracellular information processing [24, 30, 38, 39] and specific applications in immuno-oncology [40] or other biological problems [25, 41, 42], the new advances have yet to be summarized and put into context. Here, we summarize the foundational work on information-theoretic quantities and then describe recent advances in leveraging mathematical modeling and machine-learning approaches to quantify information transmission via biochemical signaling pathways (figure 1). A summary of data sources (table 2) and existing numerical packages (box 2) to estimate the information-theoretic quantities is provided to help interested readers learn more and contribute to this field.
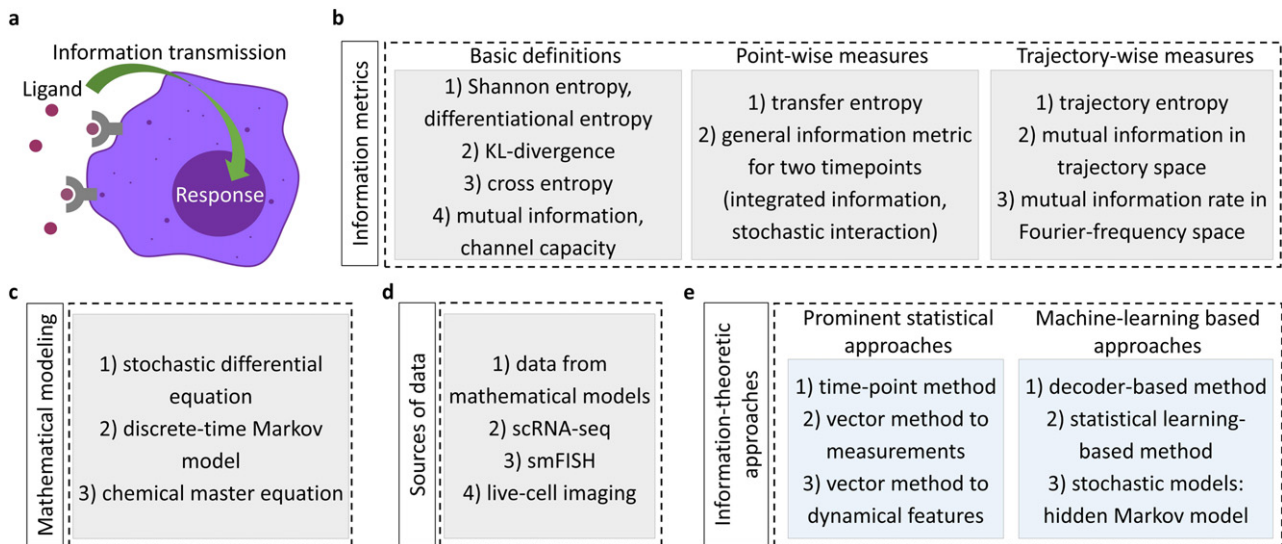
**Figure 1.** A summary on the major ingredients of information-theoretic approaches to be reviewed. (a) A schematic figure of the biological question of transmitting environmental information through intracellular signaling. (b)–(e) We summarize the ingredients of quantitatively studying information transmission: (b) the information metrics, (c) the mathematical models, (d) the data sources, and (e) the data-driven approaches, which are further categorized as traditional statistical approaches and more recent machine-learning based methods. Each of the topics will be covered in the following sections.

## 2. Foundational work on information theory for intracellular signaling

We begin with an overview of the fundamental information-theoretic quantities (table 1). First, we list the basic definitions in information theory [45], which have also been summarized in past reviews [40, 51]. We also review mathematical modeling to study intracellular information processing, where the model can also be used to generate data for the data-driven approaches of quantifying intracellular information transmission. Thus, the survey of this section on the basic qualities and mathematical modeling prepares us to review the data-driven approaches in the next sections.

### 2.1. Basic definitions of information quantities

*Shannon entropy.* Historically, four major types of entropy have been formulated, each of which provides a way of understanding the probabilistic nature of random variables. First, originating from the understanding of gas laws in the mid-1800s, Clausius introduced the concept entropy as the ratio between heat and temperature [52]. Second, based on the frequentist view of probabilities, Boltzmann formulated the entropy with maximum multiplicity of the macroscopic states [53] to obey the second law of thermodynamics at equilibrium, justifying the Maxwell–Boltzmann distribution [54]. Gibbs further developed this formulation as an ensemble of options [55]. Third, following Shannon's information theory [14], Jaynes reframed statistical thermodynamics as inferences with the least possible bias under limited data [29]. Fourth, Shore and Johnson proved the principle of entropy maximization as requirements to be satisfied by any distribution function [44]. We refer readers to [56, 57] for more detailed discussions.

We start with the formulation of entropy in Shannon's theory, as it is more appropriate to provide biologically sound interpretations for the major information measure of this review, the mutual information introduced below. The Shannon entropy [14] for a discrete random variable $x$ with possible states $X$ and a discrete probability distribution $P(x)$ is defined as:

$$H(X) = -\sum_{x \in X} P(x) \log_2 P(x). \tag{1}$$

Shannon entropy has a close connection to statistical physics [29], providing a likelihood for inference on models given data. Examples of such inferences in quantitative biology include the protein 3D structure from genomic sequences [58, 59], the prevalence landscape of mutated viral sequences [60], and the diversity of the antibody sequence repertoire [61].

*Differential entropy.* For a continuous probability density $p(x)$, the Shannon entropy is defined as differential entropy [45]:

$$H_{\text{diff}}(X) = -\int_{-\infty}^{+\infty} dx\, p(x) \log_2 p(x). \tag{2}$$

The estimate of entropy depends on estimating the probability distribution. One typically uses the frequency from finitely measured samples as an estimate of the probability to calculate the entropy of the probability distribution. Such an estimation of probabilities leads to an error in calculating the entropy, which is proportional to the number of states and scales as 1 over the sample size [30].

Given $N$ finite data, the cumulative probability distribution in the prefactor of equation (2) can be approximated by its sampling frequency [62]: $H_{\text{diff}}(X) = -\sum_{j=1}^{N} \delta_j \log_2 p(x_j)$, where $\delta_j$ is the frequency of observing the $j$th event. When the number of sampled data is infinitesimal compared with the number

**Table 1.** The fundamental information quantities useful for intracellular information processing. The three categories of information metrics: the basic definitions in information theory, the pointwise information measures, and the trajectory-wise measures.

| Information quantities | Mathematical definition | References |
|---|---|---|
| Shannon entropy, differentiational entropy | $H(X) = -\sum_{x \in X} P(x) \log_2 P(x),$ $H_{\mathrm{diff}}(X) = -\int_{-\infty}^{+\infty} \mathrm{d}x \, p(x) \log_2 p(x)$ | [14] |
| KL-divergence | $D_{\mathrm{KL}}(P \| Q) = \sum_{x \in X} P(x) \log_2[P(x)/Q(x)]$ | [43] |
| Cross entropy | $\mathrm{CE}(P \| Q) = D_{\mathrm{KL}}(P \| Q) + H(P)$ | [44] |
| Mutual information, channel capacity | $I(Y;X) = H(Y) - H(Y|X),$ $I_{\max}(Y;X) = \max_{P_X(x)} I(Y;X)$ | [45] |
| Transfer entropy | $T_{X \to Y} = H(y_n|y_{n-1}) - H(y_n|y_{n-1}, x_{n-1})$ | [46] |
| General information metric for two timepoints (integrated information, stochastic interaction) | $ci(x_i \to y_j) = \min_{q(x_{i,j}, y_{i,j})} D_{\mathrm{KL}}[p(x_{i,j}, y_{i,j}) \| q(x_{i,j}, y_{i,j})]$ | [47] |
| Trajectory entropy | $H(y_{1:n}) = -\log_2 p(y_{1:n})$ | [48] |
| Mutual information in trajectory space | $I(Y_{1:n};X) = H(Y_{1:n}) - H(Y_{1:n}|X)$ | [49] |
| Mutual information rate in Fourier-frequency space | $I_{\mathrm{R}}(x_{1:n}; y_{1:n}) = -\frac{1}{4\pi} \int_{-\infty}^{\infty} \mathrm{d}\omega \, \ln[1 - \frac{|S^{xy}(\omega)|^2}{S^{xx}(\omega)S^{yy}(\omega)}]$ | [50] |

of total configurations, the sampling frequency $\delta_j$ can be chosen as uniform for each sampled event, $\delta_j = 1/N$, giving an averaged entropy (Boltzmann entropy $-\log_2 p(x_j)$ for the configuration $x_j$) of the finite samples. When the sampled data are sufficient to cover the frequent configurations of the full probability distribution, the averaged entropy from the samples approximates the entropy of the full distribution. This approximation was found useful to produce an accurate estimation of the mutual information of intracellular signaling [62].

*Kullback–Leibler (KL)-divergence.* For two discrete probability distributions $P(x), Q(x)$, the KL-divergence [43] $D_{\mathrm{KL}}(P \| Q) = \sum_{x \in X} P(x) \log_2[P(x)/Q(x)]$ quantifies the dissimilarity between the two distributions. The KL divergence is also named the relative entropy. As a symmetrized KL divergence, the Jensen–Shannon divergence plays a similar role in measuring the similarity between two probability distributions. The divergence can be used to quantify the similarity between the data distribution and the distribution generated from the model in various scientific disciplines. In biology, it has been applied to quantify distributional dissimilarity, including that between genes in tumors and healthy samples [63] and that between transcriptional states of T lymphocytes [64].

*Cross entropy.* The cross entropy [44] for discrete probability distributions is $\mathrm{CE}(P \| Q) = D_{\mathrm{KL}}(P \| Q) + H(P)$, where $H(P)$ denotes the entropy of the probability distribution $P$ as in equation (1). It quantifies the information across the two probability distributions, which is extendable to the continuous case similarly to the differential entropy. Cross-entropy between distributions of data and models may serve as a loss function in machine learning. Its application in biology is similar to the KL divergence.

*Mutual information.* Given another random variable $y$ with possible states $Y$, the mutual information [45, 65, 66] between the two random variables is:

$$I(Y;X) = H(Y) - H(Y|X), \qquad (3)$$

where the conditional entropy $H(Y|X) = -\sum_{x \in X, y \in Y} P(x, y) \log_2[P(x, y)/P(x)]$. Mutual information quantifies the mutual dependence between the random variables, i.e., the amount of information about one random variable through observing the other. It being zero is equivalent to the two random variables being independent.

Mutual information is symmetric and represents the correlation of two variables, which is termed 'cooperativity' in physical biochemistry. In practice, one usually cares about the maximum mutual information (channel capacity), and maximization is conducted for only one variable, such that the interpretation becomes asymmetrical. The maximization is done by inferring the probability distributions from limited data under certain constraints, such as the probability normalization condition. Thus, mutual information is closely related to the type of entropy formulated by Jaynes [14, 29].

In addition, mutual information can also be regarded as a KL divergence between the conditional distributions and the prior distribution $P_X$: $I(Y;X) = E_Y[D_{\mathrm{KL}}(P_{X|Y} \| P_X)]$, where $E_Y$ is the expected value over the random variable $Y$. That is, mutual information is the expectation of the KL divergence of the univariate distribution $P_X$ from the conditional distribution $P_{X|Y}$ given $Y$. The more different the two distributions are on average, the greater the information gain.

The mutual information is widely useful. It helps disentangle interactions between a system's internal variables and their coupling to changing environments [67]. It has also been extended to various contexts, for example, the renormalized mutual information for continuous variables with a deterministic dependence [68]. More importantly, it is the mutual

information rather than the entropy that is more often used as a likelihood for the model inference [30, 65].

*Channel capacity.* Channel capacity is obtained by maximizing mutual information between the input and output distributions $P_X(x)$ and $P_Y(y)$, which measures the rate at which information is transmitted over a communication channel. The maximum mutual information is formally obtained as:

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**Box 1. The formulation of mutual information applied to stimulus discrimination via intracellular signaling.**

*Mutual information for stimulus discrimination.* Despite the interest in noise [5–8], mutual information serves as a fundamental quantity to understand information processing in biology [69] and has been widely used in biological systems [24, 30, 38]. For example, mutual information leads to a method to cluster genes [70], reconstruct the network of gene interactions [71], and quantify strengths of the influence between proteins [72].

For the focus of this review, intracellular signaling process, mutual information can be employed to measure the stimulus discrimination by signaling responses, where one random variable is the categorical stimulus set and the other is a set of the signaling responses under each stimulus. Specifically, the mutual information between $M$ conditions chosen in an experiment as the stimuli set ($S$) and the signaling responses set ($R$) is:

$$I(R; S) = H(R) - H(R|S), \qquad (5)$$

where $H(R|S)$ and $H(R)$ are the conditional and unconditional entropy from the definition in equation (3). The mutual information between the extracellular stimulus conditions and the intracellular signaling responses quantifies the amount of information about the stimulus conditions (identity and dose).

*Channel capacity of intracellular signaling channels.* With the probability distribution of the $M$ stimulus conditions $\mathbf{q} = \{q_1, q_2, \ldots, q_M\}$, the maximum mutual information is obtained by maximization with respect to this probability distribution:

$$I_{\max}(R; S) = \max_{\mathbf{q}} I(R; S), \qquad (6)$$

under the constraint of $q_1 + q_2 + \cdots + q_M = 1$ and $q_i \geqslant 0$. The maximization is useful especially when the stimulus distribution is empirically unknown. This is a Bayesian approach [30], where the prior for the probability distribution is typically assumed uniform, as uniform priors seem especially effective [19, 73].

The maximum mutual information depends on the stimulus conditions under consideration: if $M$ distinct conditions were considered, perfectly transmitted information leads to $\log_2 M$ bits, corresponding to the prior of a uniform distribution. A smaller value implies that the cells cannot fully discriminate the stimuli via the signaling response. With increased stimulus conditions in an experiment, the maximum mutual information approximates to the channel capacity through the signaling molecules. In addition, mutual information in trajectory space $I(R_{1:n}; S)$ can be formulated similarly, where signaling responses are time series data. The maximization in $I_{\max}(R_{1:n}; S)$ can be conducted by using data up to the timepoint $n$, which quantifies the maximum extent of information transmission cumulatively.

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$$I_{\max}(Y; X) = \max_{P_X(x)} I(Y; X), \qquad (4)$$

where the maximization is with respect to the input marginal distribution $P_X(x)$.

Mutual information and channel capacity are essential to quantify the stimulus discrimination process by intracellular signaling (box 1). Therefore, estimating the probabilities from limited measurements requires dedicated approaches, which will be elaborated in the next sections.

### 2.2. Pointwise information measures

We now review the pointwise information measures and measures that consider two consecutive timepoints. We denote two time series (trajectories) by $x_{1:n}, y_{1:n}$, where the subscript represents the timepoints, 1 to $n$. The dynamics of the time series can be incorporated by the transition probabilities, i.e., the conditional probabilities of the time series. For clarity, we consider a system with the Markov property: the conditional probability $p(x_n|x_{1:n-1}) = p(x_n|x_{n-1})$. The information measures can be extended to the case of a stationary Markov process with higher order, i.e., longer memory.

*Transfer entropy.* Given the transition probabilities, the transfer entropy [46] measures the amount of directed transfer of information between two time series, which can distinguish the driving and responding elements. It is defined as follows:

$$T_{X \to Y} = H(y_n|y_{n-1}) - H(y_n|y_{n-1}, x_{n-1}), \qquad (7)$$

$T_{X \to Y} = H(y_n|y_{n-1}) - H(y_n|y_{n-1}, x_{n-1})$, where the conditional entropies are for the time series. Transfer entropy is a conditional mutual information $T_{X \to Y} = I(y_n; x_{n-1}|y_{n-1})$, which has the history of the variable $y_{n-1}$ in the condition. Transfer entropy is a finite version of directed information [74]. Restricted directed information was used to infer the causal relation between genes from single-cell RNA (scRNA) sequence data [75]. Similarly, for a single time series, the excess entropy [76] measures the amount of uncertainty in the future explained by the past information.

*General information metric for two timepoints.* The strength of causal influences for two timepoints $i, j$ between two time series $x_{1:n}, y_{1:n}$ can be demonstrated by a unified framework of information measures [47]. To derive the framework, the authors approximated the joint probability distribution $p(x_{i,j}, y_{i,j})$ by another probability distribution $q(x_{i,j}, y_{i,j})$. The causal influences between two time series $ci(x_i \to y_j)$ can be quantified by minimizing the KL divergence between the two probability distributions $p(X_{1:n}, Y_{1:n})$, $q(X_{1:n}, Y_{1:n})$:

$$ci(x_i \to y_j) = \min_{q(x_{i,j}, y_{i,j})} D_{\mathrm{KL}}[p(x_{i,j}, y_{i,j}) \| q(x_{i,j}, y_{i,j})], \qquad (8)$$

under the constraint of the Markov condition: $q(x_i, y_j|y_i) = q(x_i|y_i)q(y_j|y_i)$ [47]. Note that here the two timepoints are denoted by the subscript, whereas it is denoted by $x, y$ in [47]. This general information metric can be reduced to various information measures [77], including mutual information and transfer entropy. It also generates integrated information [78] that quantifies the extent of synergistic causal influences between the two series and the stochastic interaction [79] that measures the mixed strength of causal and simultaneous influences. We expect that it will have applications in understanding the full information transfer between two dynamical variables of biological systems.

### 2.3. Trajectory-wise information measures

The above measures do not estimate the mutual information from an entire trajectory. To this end, the trajectory-wise information measures will be covered as follows.

*Trajectory entropy.* Given the trajectory's probability $p(y_{1:n})$ for the observed trajectory $y_{1:n}$, the trajectory entropy for each single trajectory is given by [48]:

$$H(y_{1:n}) = -\log_2 p(y_{1:n}). \quad (9)$$

The trajectory entropy is for a single trajectory, rather than the average on the trajectory ensemble [80]. The trajectory entropy was originally formulated for mesoscopic nonequilibrium systems [81]. Based on the trajectory entropy, a set of thermodynamical quantities can be formulated [56, 82]. In addition, the principle of maximum caliber [56] extends the principle of entropy maximization to trajectories, and thus the maximization with respect to trajectories can be conducted in a similar procedure.

The trajectory entropy itself may be a stochastic quantity, and different experimental realizations lead to different distributions of the trajectory entropy. However, when the experimental condition is fixed and only repeated measurements are conducted, the entropy's distribution is fixed and should be fully determined by a fixed distribution of the trajectory probability. Each trajectory configuration has a probability and an entropy value. In this case, the trajectory entropy can be inferred in the same way as the entropy for static variables, and the concept of information is defined similarly.

The probability of trajectory is not well defined mathematically in continuous-time space because the trajectory configurations are infinite and the total probability volume is infinite. Thus, discrete time is required to rigorously define the probability space. In practice, one can use the frequency of the trajectory with discrete time and finite state as an estimate of the probability and employ the differential entropy equation (2) to approximate the averaged trajectory entropy for an ensemble of trajectories. For example, the probability can be calculated by inferring a stochastic model from the data of signaling responses and is useful to quantify the mutual information from the time series data of intracellular signaling responses [49].

*Mutual information in trajectory space.* In trajectory space, mutual information can be formulated as in [49, 83]. We consider the mutual information between the input set $(X)$ and the

output trajectory set $(Y_{1:n})$, where $n = 1, 2, 3, \ldots$ denotes the timepoint. Up to each timepoint $n$,

$$I(Y_{1:n}; X) = H(Y_{1:n}) - H(Y_{1:n}|X), \quad (10)$$

where $H(Y_{1:n}|X)$ and $H(Y_{1:n})$ are the conditional and unconditional trajectory entropy based on equation (9). When the trajectory probability is generated from a dynamical model, the probability depends on the dynamics. Then, the trajectory entropy and the mutual information also depend on the dynamics, such that the information embedded in dynamical patterns of trajectories can be revealed by this mutual information. The maximization for $I(Y_{1:n}; X)$ is done at each time point, which is a quantification of the maximum extent of distinguishing the stimuli cumulatively (see subsection 'the stochastic model-based method').

*Mutual information rate in Fourier-frequency space.* The mutual information rate at which the information between trajectories increases with time has been formulated in the Fourier-frequency space [50, 84]. The authors considered two ensembles of time series at steady state with each obeying Gaussian statistics. The coupling between ensembles can be linearized. Under the assumptions, the joint probability distribution of the two series fluctuates around the steady state mean values, and $x_{1:n}, y_{1:n}$ is given by $\rho(z) = \exp(-v^T Z^{-1} v/2)/[(2\pi)^N |Z|^{1/2}]$, where the vector $z \doteq (x_{1:n}, y_{1:n})$. The covariance matrix $Z$ has the matrix blocks $C^{xx}, C^{xy}, C^{yx}, C^{yy}$, where each is defined as $C^{xx}_{ij} = \langle x_i x_j \rangle$ with $\langle \rangle$ denoting the noise average.

In the continuous-time limit at a fixed time interval, the mutual information rate between the two trajectory ensembles $I_R(x_{1:n}; y_{1:n}) \doteq \lim_{n \to \infty} I(x_{1:n}; y_{1:n})/n$ is calculated as:

$$I_R(x_{1:n}; y_{1:n}) = -\frac{1}{4\pi} \int_{-\infty}^{\infty} d\omega \, \ln\left[1 - \frac{|S^{xy}(\omega)|^2}{S^{xx}(\omega)S^{yy}(\omega)}\right], \quad (11)$$

where $S^{xx}(\omega), S^{xy}(\omega), S^{yy}(\omega)$ is the power spectrum from the Fourier transform of $C^{xx}, C^{xy}, C^{yy}$. The mutual information rate reveals the information transmission from the ligand concentration to the flagellar motor in the chemotaxis network of *Escherichia coli* [50].

### 2.4. Information theory to intracellular signaling with mathematical modeling

With the above information quantities, information transmission through signaling networks has been characterized with the help of mathematical models. A number of mathematical models have been constructed to model signaling networks [31] and analyze the information flow in the networks [85]. More specifically, the information flow was estimated in models of gene regulation [26, 86–88]. Optimal information processing strategies have been studied in different network topologies of gene regulation [89–95] using the data on noise levels of gene expression [10]. Information transmission in the MAPK/ERK pathway [96] and in the bacterial quorum sensing signaling network [97] was analyzed. The channel capacity was calculated from a discrete-time Markov model on the

signaling transduction [98], and the mutual information was evaluated through chemical reaction networks [99–101].

In addition to quantifying information transmitted through one signaling molecule, the information flow through shared network components for multiple inputs and outputs was studied in interferon signaling [102] and with a Boolean network of fibroblast signal transduction [103]. The contribution of duplicated components in the signaling pathway to channel capacity was investigated [104]. Information transmission was found to be maximized by synergistic control in noisy gene regulatory networks [105]. The information transfer between dynamical system components was formulated for both continuous and discrete systems [106], as well as stochastic dynamical systems [107, 108], where noise was tuned to improve information transmission [109]. Furthermore, information theory was used in deterministic dynamical systems to infer the structure of signaling networks [110]. The decoding of signaling information to determine downstream gene expression was explored [111, 112].

## 3. First data-driven approaches of information theory to intracellular signaling

Henceforth, we focus on the mutual information between the extracellular stimulus conditions and the intracellular single-cell signaling responses, which provides an estimate of the amount of information about the stimulus identity and dose (box 1). We mainly review the methods using single-cell measurements of signaling molecules by live-cell imaging, as it provides real-time tracking of the signaling activities that are crucial to quantify information transmission.

In this section, we review the first set of data-driven approaches in historical developments. The prominent statistical approaches in quantifying information transmission from the live-cell imaging data are listed in figure 2. A pioneering work employed a single-timepoint measurement [113, 114] (time-point method). A second approach [62] evaluated the information encoded in the signaling time course from the multivariate measurement (vector method), including a further extension by considering dynamical features of the signaling responses [115]. Extracting information transmission from long time series of signaling responses requires alternative approaches, which will be reviewed in the next section.

### 3.1. The time-point method

As a pioneering work in quantifying information transmission from measured single-cell signaling activity, the authors in [113] estimated the mutual information and channel capacity at a single timepoint. At each timepoint, the data from single-cell measurement under one stimulus condition led to a distribution of signaling activity across cells, and the distributions under various stimulus conditions provided mutual information for stimulus discrimination.

The estimated mutual information is affected by noise [28, 116] and the feedback of regulators [114, 117–119]. The analysis has been extended to multiple signaling molecules,

enabling the noise decomposition of biochemical signaling networks [120]. For measurements at multiple timepoints, the method is applicable to each timepoint separately, without taking into account the time course of signaling responses. Thus, the information transmission over a time course through the signaling molecule may be lost.

### 3.2. The vector method applied to measurements

Remarkable progress was made in [62] to quantify the information transmission over the time course of signaling responses. The method treated the time series data from each single cell as a multivariate vector and used the k-nearest neighbor to estimate the probability of the time series [121, 122]. The performance of the $k$-nearest neighbor estimator depends on the metric of the distance and the value of $k$ [123], which may need to be fine-tuned for each dataset. The error bars and bias of this estimated mutual information were evaluated [124, 125], and the accuracy was improved by kernel estimation [126]. Furthermore, information was coded by a combination of time series and molecular species [127].

Although the method can evaluate the information of the time course, the current limitation on the number of cells from live-cell imaging data restricts the length of the time course for an accurate estimation. As sampling the vectorial distribution suffers from a combinatorial explosion, the estimation becomes inaccurate when the number of timepoints increases over ~10 timepoints [49]. In addition, treating the time series as vectors makes the density estimation independent of the ordering of timepoints and thus does not distinguish dynamical patterns encoded in the time series.

### 3.3. The vector method applied to dynamical features

The dynamical features of the temporal signaling responses transmit information [128], such as through amplitude and frequency regulation of transcription factor activity [129]. Information transmission via the dynamical features of signaling responses has been quantified [115] (figure 3). The effect of representative features has been analyzed by adding one or a few features [130]. By adding dynamical features simultaneously, more information encoded in the trajectories was extracted [115]. This analysis uncovers the most informative features that optimize stimulus discrimination. Note that calculating each dynamical feature is subject to noise, which may alter the estimation of mutual information.

## 4. Recent approaches for estimating information transmission

To extract the information transmission from the long time course of the signaling responses [131–134], recent works have employed machine-learning methods. Below, we review three representative examples. They include the decoding-based approach [130] that uses the machine-learning classifier, the statistical learning-based method using logistic regression, and the stochastic model-based method which
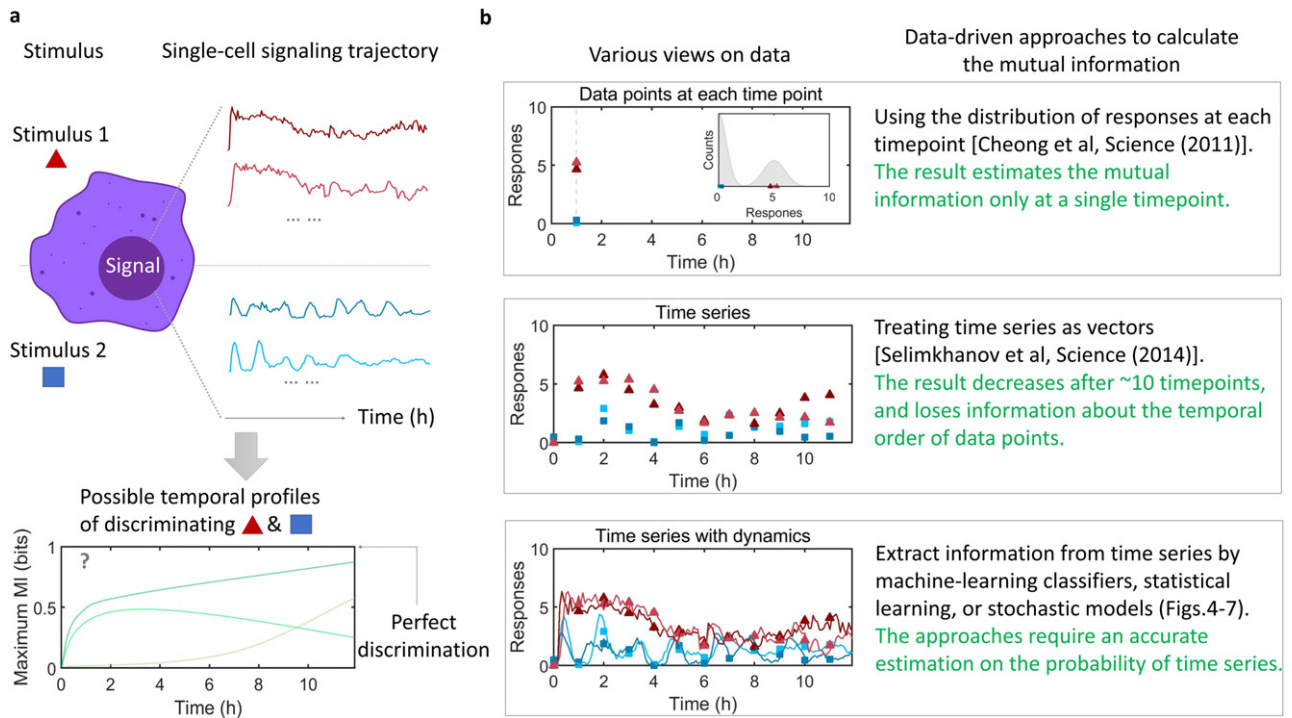
**Figure 2.** The approaches for estimating information transmission by using single-cell signaling responses. (a) A schematic figure on using the single-cell live imaging measurement on signaling responses to calculate mutual information, which quantifies the stimulus discrimination. (b) A schematic on the methods of using (upper) a single-timepoint data, (middle) a few timepoints, and (below) long time series. Reproduced from [49]. CC BY 4.0.
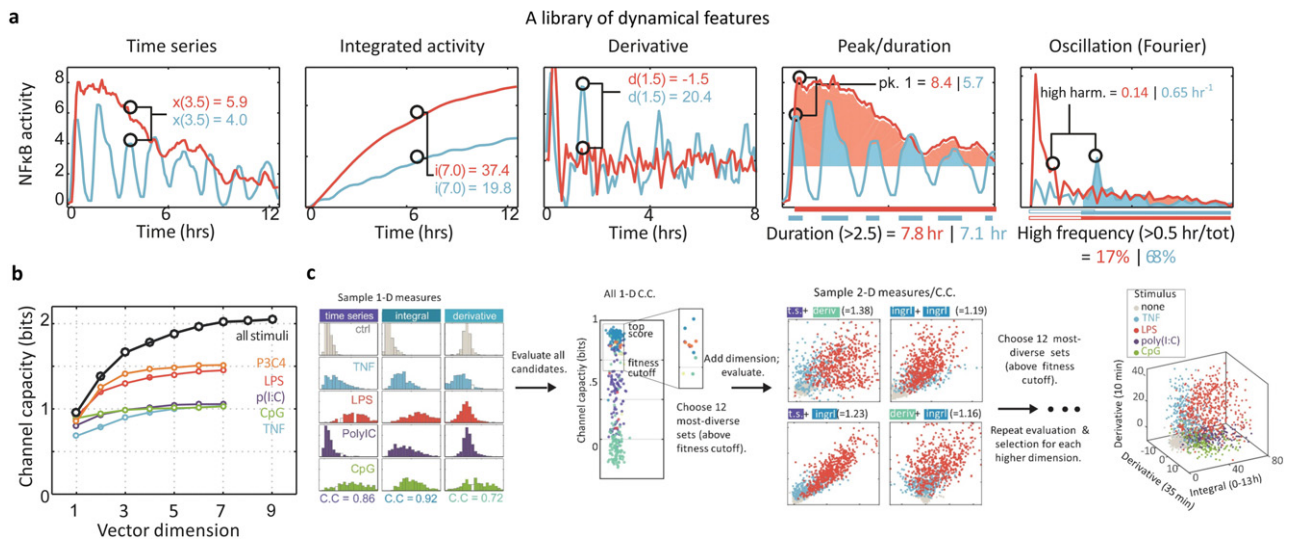


**Figure 3.** The vector method with dynamical features on estimating information transmission. (a) A library of dynamical features was calculated for the long time series data of NF$_\text{K}$B signaling responses. (b) The channel capacity was evaluated by the k-nearest neighbor estimation on the most informative dynamical features, for all stimuli and for different doses of one stimulus, as indicated. (c) The protocol of searching for the most informative combination of features. Reprinted from [115], Copyright (2021), with permission from Elsevier.

employs the hidden Markov model. In each case, the machine-learning methods help estimate the probability and information from the time series data. A comparison of the data-driven approaches is provided in the end.

### 4.1. The decoding-based approach

One approach used a machine learning decoder to calculate the mutual information [130] (figure 4). This method first trained a classifier given the time series of signaling responses under
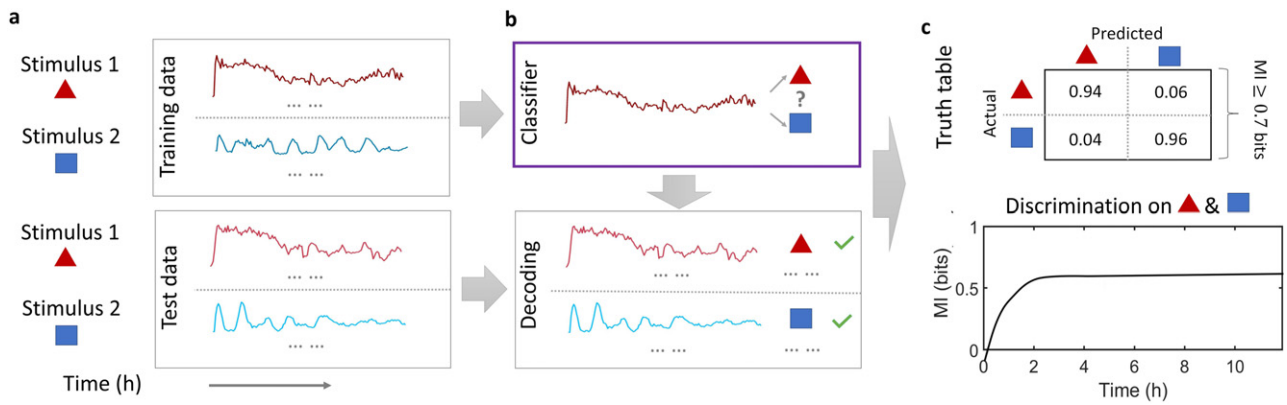
**Figure 4.** The decoding-based approaches to calculate the mutual information from time series of signaling responses. (a) The measured signaling responses under various stimulus conditions are used as the training data and the test data. (b) A classifier is trained by the training data and used to recognize the stimulus condition for the test data. (c) The truth table by the classifier gives an estimated lower bound on the mutual information for the stimulus discrimination. Reproduced with permission from [130]. © PNAS.
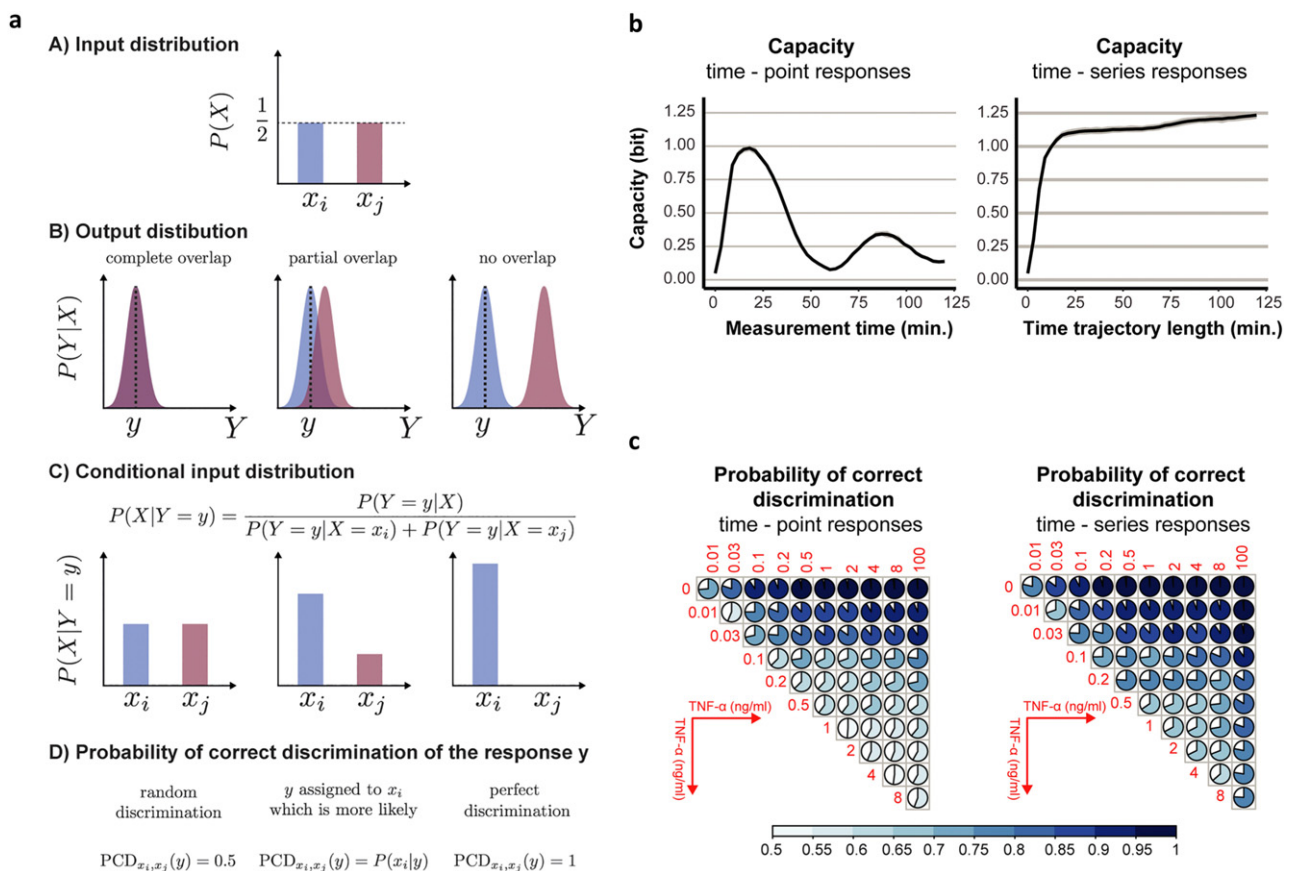


**Figure 5.** The SLEMI. (a) A schematic figure for the probabilities of discriminating two inputs. The input distribution $P(X)$ and the conditional output probabilities $P(Y|X)$ lead to the conditional input distributions $P(X|Y)$ by Bayes formula. (b) and (c) Information-theoretic analysis of NF$_\kappa$B signaling responses to the TNF$\alpha$ stimulus. (b) The channel capacity as a function of time by using a single-timepoint data individually and time series. (c) The probabilities (color filled fraction of the circle marks) of correct pairwise discrimination between TNF$\alpha$ concentrations for the 21 min responses and time series. See a full description on the figure and symbols in the original paper. Reproduced from [125]. CC BY 4.0.

each stimulus condition and used the classifier to separate new data of signaling responses into the group with the best match. It provided a lower bound on the mutual information, and the deviation depended on the accuracy of the classifier. When classifiers employ linear principal components, they may be inadequate for discriminating oscillatory and nonoscillatory trajectories. To overcome this issue, various machine-learning models, such as neural networks, can be used for classifiers to improve estimates [99]. In addition to the lower bound, an upper bound on the mutual information was derived [135].

The classifier can also use the most informative features to discriminate stimulus conditions, where the top-ranked features termed signaling codewords are identified by information-theoretic analysis [115]. The codewords were further used to construct a decision tree to classify the stimulus conditions binarily by specific dynamical features. In addition, the dynamical signaling patterns to realize the optimal transmission of information were obtained by optimal control theory [136].

### 4.2. The statistical learning-based method

As an efficient method, a statistical learning-based estimation of mutual information (SLEMI) was proposed in [125, 137] (figure 5). The method is applicable to high-dimensional time series of signaling responses, without restriction on the number of timepoints. The numerical package [125] enables a broader use to various datasets, generating mutual information, channel capacity and probabilities of correct pairwise discrimination. SLEMI used a Bayesian framework based on logistic regression to estimate the probabilities of stimuli given measured trajectories. As logistic regression assumes a linear fitting on the trajectories to calculate the ratio of the trajectory probabilities between stimulus conditions, it is not clear whether this approach can fully account for the complex dynamical patterns of observed signaling trajectories, such as oscillatory behavior [115, 138]. Thus, the estimated mutual information could be less accurate when applied to complex trajectories, where logistic regression may be replaced by a more advanced Bayesian classifier [125].

### 4.3. The stochastic model-based method

Inspired by the trajectory entropy defined along a single trajectory [81], the data can be viewed from the trajectory perspective. Then, stochastic dynamical models, such as the hidden Markov model that was used for speech recognition [139], can be applied to learn and reproduce the time course of the signaling responses [49] (figure 6). The hidden Markov model (or the time-inhomogeneous Markov model) captures the time-inhomogeneity of the trajectories and represents the trajectory ensemble with approximately 80% accuracy. The model further generates trajectory probabilities to calculate mutual information. The limited number of measured cells and timepoints in live-cell imaging may alter the accuracy of the model training and the subsequent mutual information estimation.

This framework provides an estimate of the information encoded in the signaling dynamics over time. The estimated information accumulation over time reveals the temporal ordering of the discriminating different stimuli and may decrease when the stimuli induce similar signaling responses in a certain time regime that diminish the extent of the stimulus discrimination. It also indicates the temporal phases of information transmission that can be mapped to the functionality of the regulatory circuit and the amount of information accumulation available to immune response genes [49].

### 4.4. A comparison of the data-driven approaches

Applying each of the approaches above to the $NF_KB$ signaling responses under 13 different immune stimulus conditions characterizes their properties (figure 7). All the methods give the maximum mutual information of approximately 1–2 bits, smaller than $\log_2 13 \approx 3.7$ bits under perfect transmission. The loss of information may be caused by molecular noise in signaling responses. For each method, the mutual information calculated from a single timepoint [113] ignores the information from time courses. The vector method [62] is ineffective when there are more than approximately 10 timepoints because it becomes inaccurate to sample the vectorial distribution from the measured data. Both methods do not distinguish the dynamical patterns with timepoints aligned properly.

The decoding-based method may not fully count the information over long time courses, as mutual information estimates are saturated after a handful of measurements. This can be improved with the performance of the classifier [99] and by using the optimal input distribution instead of the uniform distribution [130]. The random permutation of timepoints does not significantly alter the estimation, indicating an incomplete discrimination of the dynamical patterns of signaling responses and a lack of tracking information over time. Thus, the scope of applying the decoding-based method depends on the complexity of the time series and the quality of the accessible classifiers.

Both the SLEMI [125] and stochastic model-based method give increasing mutual information, implying distinct temporal patterns of signaling responses at all times, as consistently observed in the data [49]. After the random permutation of timepoints, the mutual information from the two methods decreases, corresponding to the information under the genuine order of timepoints where the distinct stimuli become less distinguishable. The stochastic model-based method provides continuously increasing mutual information over time, even after the random permutation of timepoints, as permuted signaling responses have persistent differences in response amplitudes [49]. However, mutual information, such as in the early time regime, may be underestimated if the searched optimal number of parameters has an underestimation or the stochastic model does not accurately learn the dynamics.

## 5. Outlook

We have reviewed studies on quantifying the information transmission of intracellular signaling with the aid of mathematical modeling and machine learning. Several outstanding questions are guiding current and future studies.

To improve the quantification of information transmission, advanced models of machine learning [32, 34] may be employed to learn the time course of signaling responses with higher performance. They may also enable the extraction of diverse useful information from the time series. Specifically, the recurrent neural network [36, 37, 140] has achieved great success in learning the dynamics of time series. The transformer with an attention-based architecture [141] performs well in learning complex time series because it can capture
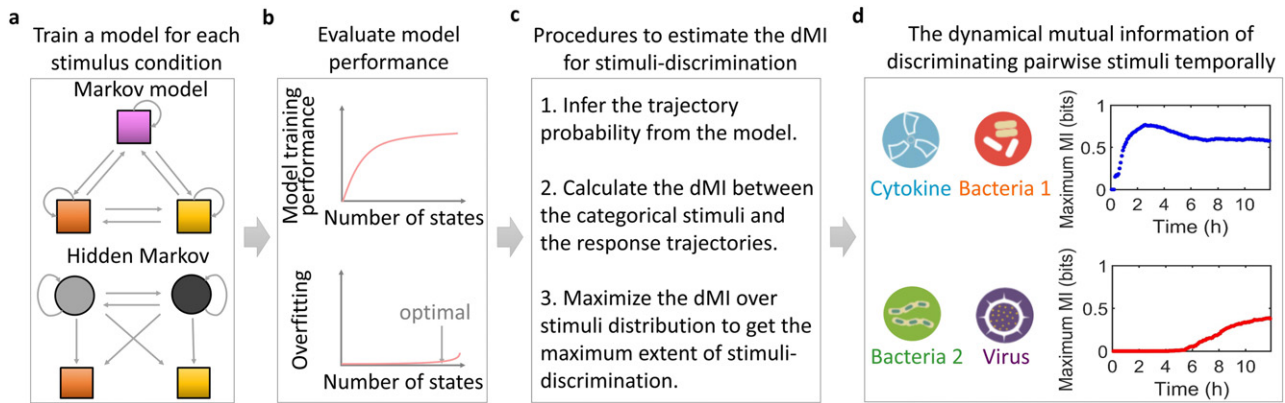
**Figure 6.** Quantifying the dynamical mutual information by using the stochastic models. Stochastic models such as the hidden Markov model can be used to learn the signaling dynamics, reproduce data, infer the trajectory probabilities, and evaluate the mutual information. (a) The stochastic models to learn the data. (b) The evaluation on the model performance when identifying the proper number of parameters. (c) The procedures on calculating the mutual information from the trained stochastic models. (d) The estimated mutual information encoded in dynamics reveal the temporal ordering of discriminating certain stimuli pairs. Reproduced from [49]. CC BY 4.0.
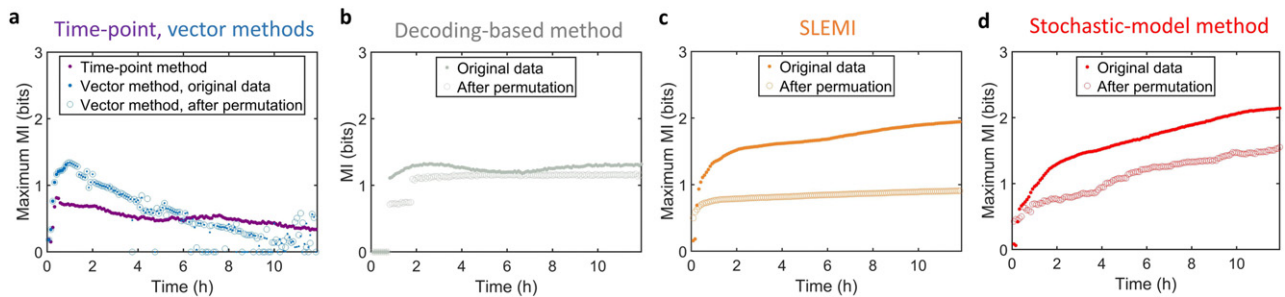


**Figure 7.** A comparison on the data-driven approaches for time series data. The data is the $NF_KB$ signaling responses under 13 different stimulus conditions (17 conditions in total with replicates) [49]. The $y$-axis is labeled as 'maximum MI', except for the decoding-based method providing a lower bound ($y$-axis is 'MI' without 'maximum') (a) The time-point method [113] and the vector method [62]. (b) The decoding-based method [130] by using the first 10 principle components and default parameters. (c) The SLEMI [125], with parameters 'boot_num' = 10, 'boot_prob' = 0.8, 'testing_cores' = 4 in the numerical package. (d) The stochastic model-based method [49] with 64 hidden states and 32 emission states for the hidden Markov model. The computational time of one bootstrap for the five methods is ∼10 min, ∼1 h, ∼10 min, ∼10 min, ∼10 h on personal desktop with Intel® Core™ i7-8700 CPU@3.7 GHz. Reproduced from [49]. CC BY 4.0.

long-range dependencies between input and output by designing neural networks with functional gates for memorizing and updating. The application of these models to single-cell signaling responses may have a better performance in reproducing data and predicting future responses.

In addition to information transmission by signaling molecules in single cells, the reliability of signal transduction is affected by cell populations [142, 143]. Cell subpopulations can independently transmit information that gives graded responses to stimuli [144]. Fractional response analysis by using Rényi information further reveals that changes in fractions of cells under various response levels scale linearly with the log of the cytokine dose [145]. It is also attractive to quantify the information content of the signaling process in more realistic contexts [146], such as under mixed natural signals. The mutual information estimates under time-varying signals reveal the information flow when cells are subject to environmental changes [99, 147]. The information flow can

be optimized by controlling the environment via reinforcement learning [148], which models how agents take action in an environment to maximize a cumulative reward, such as the information gain. In addition, the positional information from the spatially distributed signaling molecules has been evaluated by mathematical modeling [149, 150] and by constructing the decoder [151]. Quantifying the information transmission over large spatial and long time scales [152] awaits further developments, such as by convolution neural networks [153].

The increase of the single-cell data would continue to motivate future work of evaluating information content in a data-driven manner, and vice versa. In addition to live-cell imaging, applying machine-learning models to other single-cell data would reveal more insights into intracellular information processing. For example, the causal relation between genes has been inferred from scRNA sequence data by using restricted directed information [75]. Both scRNA and single-molecule

fluorescence *in situ* hybridization (smFISH) (table 2) data can measure downstream gene expression of the signaling molecule, providing a platform to test the hypothesis on conveying information of the signaling molecule to gene expression [49, 112]. The autoencoder [35] may help learn meaningful representations from these multigene data. Furthermore, predictions from information-theoretic approaches [112] can be tested experimentally by optogenetic approaches [131, 154, 155]. Such experimental setups avoid the coactivation of other, unknown factors involved in gene expression, providing an unambiguous way to measure information transmission by the signaling molecule to downstream genes. Exploring the decoding of the signaling information to responsive gene expression for cell fate decisions documents the actual physiological role of estimated information quantities and reveals evolutionary perspectives of cellular information processing and decision-making.

While machine-learning approaches show promising applications in understanding living information processing, we would like to remind ourselves that simply applying machine learning as a tool may have limitations. As quoted from Jaynes [156]: '*new data that we insist on analyzing in terms of old ideas* (*that is*, *old models which are not questioned*) *cannot lead us out of the old ideas. However*, *many data we record and analyze*, *we may just keep repeating the same old errors*, *and missing the same crucially important things that the experiment was competent to find. That is what ignoring prior information can do to us*; *no amount of analyzing coin tossing data by a stochastic model could have led us to discovery of Newtonian mechanics*, *which alone determines those data*[4]'. Therefore, machine learning and information theory should be taken as frameworks to help design the experiment, such as mutual information, which can frame an inference problem for modeling biological systems [30] and provide a new angle to understand and predict biological processes beyond existing data [157]. We anticipate that the cross-feeding between quantitative biology, information theory, and machine learning [158] will lead to significant advances in these areas.

### 5.1. Sources of single-cell data

We list sources of single-cell data useful for information-theoretic analysis, including mathematical model simulations, scRNA sequences [159], smFISH, and live-cell imaging [160] (table 2). In the main text, we have mainly reviewed the approaches using live-cell imaging, but other types of data may find increasingly important roles in future studies. See a complementary review on the data source for studying intracellular signaling [27].

*Data simulated from mathematical models.* To evaluate information transmission, the simulated data of signaling molecules can be generated from differential equations for modeling signaling transduction [62, 109, 112]. The trajectories of chemical species were also simulated from chemical

reaction networks [99, 100, 111] by the stochastic simulation algorithm (or the Gillespie algorithm) [161]. To generate trajectories that accurately simulate the real time-course of signaling activities, the mathematical model needs to be experimentally calibrated and verified, which may require massive measurements on the modeled molecules and exploration of the model parameters [162, 163].

*Single-cell RNA sequencing* (*scRNA-seq*). The development of scRNA-seq has increased in recent years, as it generates the sequence profiles of all transcripts with their relative abundances in single cells. However, scRNA-seq data from methodologies such as droplet sequencing are subject to nonnegligible noise, and accurately measured genes are sparse. Thus, the data typically need dimension reduction to generate useful statistics and do not meet the high resolution required for the information-theoretic approaches of quantifying intracellular information transmission. The specifically designed measurement, e.g., targeted scRNA-seq, may be more suitable, with a tradeoff between the number of measured genes and the control on the noise level.

In addition, scRNA-seq technologies initially measure gene expression at individual timepoints and do not track the transcriptome over time. To overcome this limitation, the pseudo-time can be inferred to map out the trajectories (e.g., developmental trajectories of gene expression) for single cells [164, 165], with multiple-timepoint measurements [166, 167]. However, the accuracy of trajectory inferences depends on the dynamics of gene expression [168] and needs to be verified, such as by real-time tracking. The underlying dynamical equations governing the cell state transition can be inferred [169], which may provide high-resolution augmentation of the noisy distribution of signaling molecules over time to estimate intracellular information transmission.

*Single molecule fluorescence in situ hybridization* (*smFISH*). As an imaging-based technique, smFISH enables the measurement of the expression of endogenous genes from ~10 000 cells. A recent technique (MERFISH) can simultaneously image 100 to 1000 RNA species in single cells [170]. Nevertheless, smFISH needs to fix the sample and only measure it at a single timepoint, which prohibits its usage in quantifying information transmission over time.

*Live-cell imaging.* Live-cell imaging is a direct method to measure the signaling activity of living cells in real time [62, 62, 115, 130, 133, 138, 171, 172]. The time resolution reaches the time scale of minutes, which can continue for days. Approximately one thousand cells were measured in each experiment. This technique has a limitation on the number of signaling molecules measured simultaneously, which typically allows one or two molecules to be probed to date. Live-cell imaging and smFISH are complementary based on their pros and cons.

### 5.2. Software packages

To calculate the information-theoretic quantities, a number of software packages are available (box 2). Some of these are also listed in [40].

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[4] We thank an anonymous reviewer for mentioning this quote.

**Table 2.** Sources of single-cell data. Reprinted from [27], Copyright (2018), with permission from Elsevier.

| Typical measurements | Data from mathematical models | scRNA-seq | smFISH | Live-cell imaging |
|---|---|---|---|---|
| # of cells | Model-specific | $\sim$100 000 | $\sim$10 000 | $\sim$1000 |
| # of molecules | Model-specific | $\sim$10 000 | $\sim$1000 | $\sim$1 or 2 |
| Timepoints vs time series | Time series | Timepoints | Timepoints | Time series |

---

**Box 2. Software packages of calculating information-theoretic quantities for intracellular signaling.**

The estimation of entropy, KL divergence, mutual Information and channel capacity can be found in the R package (http://strimmerlab.org/software/entropy) and the MATLAB package (https://github.com/maximumGain/information-theory-tool). Another Python and MATLAB packages for calculating entropy and mutual information can be found (https://github.com/robince). The MATLAB toolbox to evaluate the transfer entropy are provided by (https://figshare.com/articles/code/MuTE_toolbox_to_evaluate_Multivariate_Transfer_Entropy/1005245) and (https://github.com/trentool/TRENTOOL3) [173]. A Python package (https://github.com/wmayner/pyphi) computes the integrated information [174].

For evaluating the mutual information from the time series data, the k-nearest-neighbor approach [62, 66] was in a Python package (https://github.com/pawel-czyz/channel-capacityestimator). The decoding-based method [130] was implemented by a MATLAB package and a R package (https://github.com/swainlab/mi-by-decoding). The SLEMI [125, 137] has a R package in CRAN (https://github.com/sysbiosig/SLEMI). The approach by using stochastic dynamical models [49] has a MATLAB package (https://github.com/signalingsystemslab/dMI).

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## Data availability statement

No new data were created or analysed in this study.

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