

Quantification of Information Flow by Dual Reporter System and Its Application to Bacterial Chemotaxis

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Mutual information is a theoretically grounded metric for quantifying cellular signaling pathways. However, its measurement demands characterization of both input and output distributions, limiting practical applications. Here, we present alternative method that alleviates this requirement using dual reporter systems. By extending extrinsic-intrinsic noise analysis, we derive a mutual information estimator that eliminates the need to measure input distribution. We demonstrate our method by analyzing the bacterial chemotactic pathway, regarding multiple flagellar motors as natural dual reporters. We show the biological relevance of the measured information flow by comparing it with theoretical bounds on sensory information. This framework opens new directions for quantifying information flow in cellular signaling pathways.

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Introduction—Cells exhibit remarkable robustness in responding to changing environment despite the inherent stochasticity in sensing and signaling [1–3]. Such robustness has led to the hypothesis that cellular signaling systems may operate at theoretically optimal levels under sensory and signaling noise [4–15]. Supporting this idea, previous theoretical works have shown that biochemical reaction networks can implement information-theoretically optimal filtering [16,17], with the bacterial chemotactic network serving as an example of such possibility [18,19].

We can test these optimality theories from the actual information flow measured by mutual information [2,15,20–23]. Mutual information is a theoretically grounded metric that connects sensing and signaling to

decision-making and control, as exemplified by rate-distortion theory and stochastic thermodynamics [11,24,25]. For cellular systems, pioneering studies measured mutual information in the context of positional information during embryonic development of *Drosophila* [26,27]. Building on these studies, researchers have quantified mutual information and related information metrics across a range of cellular systems, including bacterial chemotaxis [11], yeast mating and stress responses [28,29], as well as mammalian and human immune signaling pathways, such as NF- κ B and ERK [30–36].

Despite technical developments, measuring mutual information in actual biological systems remains challenging. The difficulties arise primarily because mutual information demands characterization of the joint distribution of both input and output variables. This challenge becomes especially pronounced when analyzing high-dimensional data, such as complex environmental signals or temporal trajectories [29,32–35], where evaluating trajectory mutual information remains difficult even for analytically modeled processes [37–39]. Moreover, inferring the natural statistics of inputs is generally difficult, except in specific cases [26,27]. While previous studies have circumvented this issue by assuming uniform input distributions [29] or by optimizing the input distribution to estimate channel capacity [30–36], such assumptions may not represent the actual conditions under which cells operate.

To address these challenges, we propose an alternative method based on the dual reporter systems [40–43]. Dual reporter systems enable identification of extrinsic fluctuations conveyed by the upstream pathway without specifying the extrinsic noise sources. We extend this approach for

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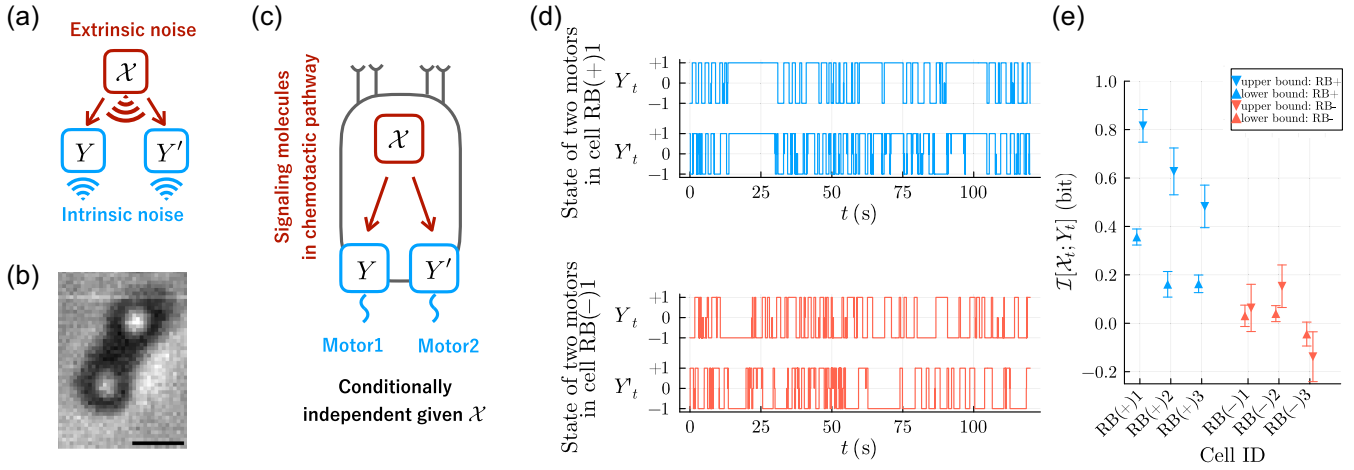


FIG. 1. Dual reporter systems and mutual information estimation using bacterial flagellar motors as dual reporters. (a) Schematic of dual reporter systems. The total variance of reporters Y decomposes into intrinsic noise σ_{int}^2 from the innate stochasticity of Y and extrinsic noise σ_{ext}^2 originating from input signal X . Dual reporters enable estimation of extrinsic noise σ_{ext}^2 without measuring input X . (b) Phase-contrast micrograph of an *E. coli* cell with beads attached to two flagellar motors. Scale bar: 1 μm . (c) Schematic of two flagellar motors within a single cell functioning as dual reporters. Motor states are denoted by Y_t and Y'_t , with input signal $\mathcal{X}_t$. (d) Representative time series of simultaneous measurements of two motors [48], showing CCW (+1), stop (0), and CW (-1) states. Data shown for cell RB(+)-1 [blue, functional chemotactic pathway, CheRB(+)] and cell RB(-)-1 [red, impaired pathway, CheRB(-)]. Complete data for all the cells are presented in SM Sec. S10 [49]. See Ref. [48] for detail of *E. coli* strains. (e) Mutual information estimates for six cells: cells RB(+)-1-3 [blue, CheRB(+), functional] and cells RB(-)-1-3 [red, CheRB(-), impaired]. Down- and up-pointing triangles show mean values of upper and lower bounds [Eqs. (6) and (7)] with error bars indicating ± 1 standard deviation (SD) (see SM Sec. S9 for robustness to a choice of lower bound estimator [49]). Statistics computed using stationary bootstrap sampling [67] (10^3 resamples, mean block length $10^{3.5}$; see Sec. S6 for detail). The negative upper bound in cell RB(-)-3 can be attributed to statistical variance of the estimator (see SM Sec. S7 for detail [49]). Motor states were binarized by replacing stop states $Y_t = 0$ independently for each motor and time point with $Y_t = \pm 1$ with equal probability (see Sec. S8 [49] for robustness to a choice of binarization method).

information flow estimation that eliminates the need for measuring information sources or inputs. We demonstrate the utility of our approach with the experimental data of the bacterial chemotactic pathway, where multiple flagellar motors function as natural dual reporters [44–46].

Dual reporter system—We formulate our approach using dual reporter systems, where the state of two reporter copies at time t , Y_t and Y'_t , are driven by a common input signal X [Fig. 1(a)]. In general, X is high dimensional and also Y_t and Y'_t are causally dependent on $\mathcal{X}_t := X_{0:t}$, the past trajectory of X up to t . The key physical properties underlying this system is that both reporters couple to the input identically while maintaining negligible direct interference between them. This design allows us to assume two useful properties: identical response statistics,

$$\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t) = \mathbb{P}_{Y'_t|\mathcal{X}_t}(y'|\mathcal{X}_t) \quad \text{for } y = y', \quad (1)$$

and conditional independence between reporters,

$$\mathbb{P}_{Y_t, Y'_t|\mathcal{X}_t}(y, y'|\mathcal{X}_t) = \mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t) \mathbb{P}_{Y'_t|\mathcal{X}_t}(y'|\mathcal{X}_t). \quad (2)$$

Based on these properties and the law of total variation, we can estimate the extrinsic and intrinsic fluctuations originating from the input trajectory, defined by

$\sigma_{\text{ext}}^2 := \mathbb{V}_{\mathcal{X}_t}[\mathbb{E}_{Y_t|\mathcal{X}_t}[Y_t|\mathcal{X}_t]]$, and from the innate stochasticity of Y_t , $\sigma_{\text{int}}^2 := \mathbb{E}_{\mathcal{X}_t}[\mathbb{V}_{Y_t|\mathcal{X}_t}[Y_t|\mathcal{X}_t]]$, where $\mathbb{V}[\cdot]$ represents the variance. These two fluctuations sum up to the total variation of Y_t : $\mathbb{V}_{Y_t}[Y_t] = \sigma_{\text{ext}}^2 + \sigma_{\text{int}}^2$. Notably, these quantities can be estimated without direct measurement of $\mathcal{X}_t$. Indeed, for a set of reporter measurements $\{Y_t, Y'_t\}_{i=1}^N$, the second moment of $\mathbb{E}_{Y_t|\mathcal{X}_t}[Y_t|\mathcal{X}_t]$ follows:

$$\begin{aligned} \mathbb{E}_{\mathcal{X}_t}[\mathbb{E}_{Y_t|\mathcal{X}_t}[Y_t|\mathcal{X}_t]^2] &= \mathbb{E}_{\mathcal{X}_t}[\mathbb{E}_{Y_t|\mathcal{X}_t}[Y_t|\mathcal{X}_t] \mathbb{E}_{Y'_t|\mathcal{X}_t}[Y'_t|\mathcal{X}_t]] \\ &= \mathbb{E}_{\mathcal{X}_t}[\mathbb{E}_{Y_t, Y'_t|\mathcal{X}_t}[Y_t Y'_t|\mathcal{X}_t]] \\ &= \mathbb{E}_{Y_t, Y'_t}[Y_t Y'_t] \approx \frac{1}{N} \sum_{i=1}^N Y_{t_i} Y'_{t_i}, \end{aligned} \quad (3)$$

where we sequentially apply Eqs. (1) and (2), and the tower property of conditional expectation. The final approximation holds for stationary and ergodic samples [47].

Information flow estimation—We extend this framework to estimate the mutual information between the input trajectory $\mathcal{X}_t$ and the instantaneous output Y_t :

$$\mathcal{I}[\mathcal{X}_t; Y_t] = \mathbb{E}_{\mathcal{X}_t} \left[\sum_y \mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t) \log \frac{\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)}{\mathbb{P}_{Y_t}(y)} \right].$$

Our strategy is to derive a quadratic approximation of the integrand $J := \mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t) \log[\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)/\mathbb{P}_{Y_t}(y)]$ with respect to $\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)$ and plug in estimators of its first and second moments. A second moment estimator is derived by replacing $\mathbb{E}_{Y_t|\mathcal{X}_t}[Y_t]$ in Eq. (3) with $\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)$ [see Supplemental Material (SM) Sec. S1 for derivation [49]]:

$$\begin{aligned} \alpha_2(y) &:= \mathbb{E}_{\mathcal{X}_t}[\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)^2] \\ &\approx \frac{1}{N} \sum_{i=1}^N \delta(Y_i - y) \delta(Y'_i - y) =: \hat{\alpha}_2(y). \end{aligned} \quad (4)$$

The first moment estimator follows:

$$\begin{aligned} \alpha_1(y) &:= \mathbb{E}_{\mathcal{X}_t}[\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)] = \mathbb{P}_{Y_t}(y) \\ &\approx \frac{1}{2N} \sum_{i=1}^N \{\delta(Y_i - y) + \delta(Y'_i - y)\} =: \hat{\alpha}_1(y). \end{aligned} \quad (5)$$

We derive two complementary quadratic approximations of the mutual information (see SM Sec. S1 for derivation [49]). First, using a tangent of the log function, we obtain an upper bound:

$$\mathcal{I}[\mathcal{X}_t; Y_t] \leq \mathcal{I}_{\text{UB}} := \sum_y \frac{\alpha_2(y)}{\alpha_1(y)} - 1. \quad (6)$$

Second, Taylor expansion around an arbitrary distribution P_0 yields

$$\mathcal{I}[\mathcal{X}_t; Y_t] \approx \mathcal{I}_{\text{TA}} := \mathcal{S}_Y - \mathcal{S}_0 + \mathcal{D} + \frac{1}{2} \left(\sum_y \frac{\alpha_2(y)}{P_0(y)} - 1 \right), \quad (7)$$

where $\mathcal{S}_Y := -\sum_y \alpha_1(y) \log \alpha_1(y)$, $\mathcal{S}_0 := -\sum_y P_0(y) \log P_0(y)$, $\mathcal{D} := \sum_y (\log P_0(y) + 1)(\alpha_1(y) - P_0(y))$. For binary state reporters $Y, Y' \in \{+1, -1\}$, this Taylor approximation $\mathcal{I}_{\text{TA}}$ with uniform probability $P_0(y) = 1/2$ coincides with a lower bound derived using the Pinsker inequality from the information theory [47] (see SM Sec. S2 for detail [49]):

$$\mathcal{I}[\mathcal{X}_t; Y_t] \geq \mathcal{I}_{\text{LB}} = \mathcal{I}_{\text{TA}}, \quad (8)$$

where $\mathcal{I}_{\text{LB}} := \mathcal{S}_Y - \mathcal{S}_0 + 2\{\alpha_2(y) - \alpha_1(y)\} + 1/2$ with y chosen arbitrary from either $+1$ or -1 . Beyond the binary reporter case, we can generally use this result to bound from below the mutual information because binarization of reporter values decreases the mutual information due to data processing inequality. We can now bound the mutual information from both above and below by substituting the estimators $\hat{\alpha}_1$ and $\hat{\alpha}_2$ given by Eqs. (4) and (5) into Eqs. (6) and (7), with reporter data binarized in advance when using Eq. (7) as a lower bound.

Application to bacterial chemotactic pathway—We demonstrate our approach in *Escherichia coli*'s chemotaxis

signaling pathway. *E. coli* cells perform chemotaxis by sensing ligand gradients and regulating multiple flagellar motors. The pathway accumulates the information of swimming direction, up or down the ligand gradient, obtained by sensory signal. Such information is stored and encoded in general into the trajectory of molecular states, $X_{0:t}$, in the pathway. The stored information in the trajectory should be decoded to efficiently control the current motor state Y_t as the output. We treat the multiple motors equipped with the cell as natural dual reporters to apply our method [Figs. 1(b) and 1(c)]. Previous studies established methods for simultaneous measurement of two motor copies, Y_t and Y'_t , within a single cell [48,68]. As shown in Fig. 1(d), each motor alternates primarily between CCW (counterclockwise; $+1$) and CW (clockwise; -1) states with the stop state (0) occurring infrequently.

Using this measurement data, we estimate the information flow from trajectory $\mathcal{X}_t := X_{0:t}$ of upstream pathway molecules to the current motor state Y_t , quantified by $\mathcal{I}[\mathcal{X}_t; Y_t]$. To evaluate the lower bound based on Eqs. (7) and (8), we binarize the motor states by replacing the infrequent stop state $Y_t = 0$ with $Y_t = \pm 1$ with equal probability independently for each motor and time point. To quantify the statistical uncertainty, we applied stationary bootstrap resampling [67], which preserves temporal correlations by sampling blocks of adjacent samples with lengths drawn from a geometric distribution (see SM Sec. S6 for detail [49]).

For cells with intact chemotactic signaling (CheRB+), we find lower and upper bounds ranging from 0.1 to 0.4 bit and 0.5 to 0.8 bit, respectively [Fig. 1(e)]. These values align with the fact that a cell primarily requires the binary information of whether it is swimming up or down the gradient to control the motors. However, these values may overestimate information transferred by the chemotactic signaling pathway, as upstream signal $\mathcal{X}_t$ could involve influence from other factors affecting both motors. Moreover, the values may reflect the interference between motors via the direct fluid dynamic intersection. To address this ambiguity and verify that our estimates primarily reflect chemotactic signaling rather than confounding factors, we analyzed mutants with impaired chemotactic pathways [CheRB-; Fig. 1(d)(bottom)]. As shown in Fig. 1(e), the upper bound of mutual information in these mutants drops below 0.2 bit, confirming that the higher information values observed in functional cells (CheRB+) predominantly result from chemotactic signaling.

Sufficiency of the quantified information for chemotaxis—We further evaluate the biological relevance of the estimated information $\mathcal{I}[\mathcal{X}_t; Y_t]$ in the context of bacterial chemotaxis. During chemotaxis, bacteria biases their random walk by regulating motors in response to sensed chemical gradients, which tells whether cells swim up or down the gradient. This control mechanism requires information flow from environmental signals E_t ,

representing swimming directions or chemical gradients, to motor states Y_t via signaling pathway molecules $\mathcal{X}_t$. We quantify this information flow using multivariate mutual information $\mathcal{I}[E_t; \mathcal{X}_t; Y_t] = \mathcal{I}[E_t; Y_t] - \mathcal{I}[E_t; Y_t | \mathcal{X}_t]$ [Fig. 2(a)]. We use $\mathcal{I}[E_t; \mathcal{X}_t; Y_t]$ rather than $\mathcal{I}[E_t; Y_t]$ to isolate information transmitted specifically through the

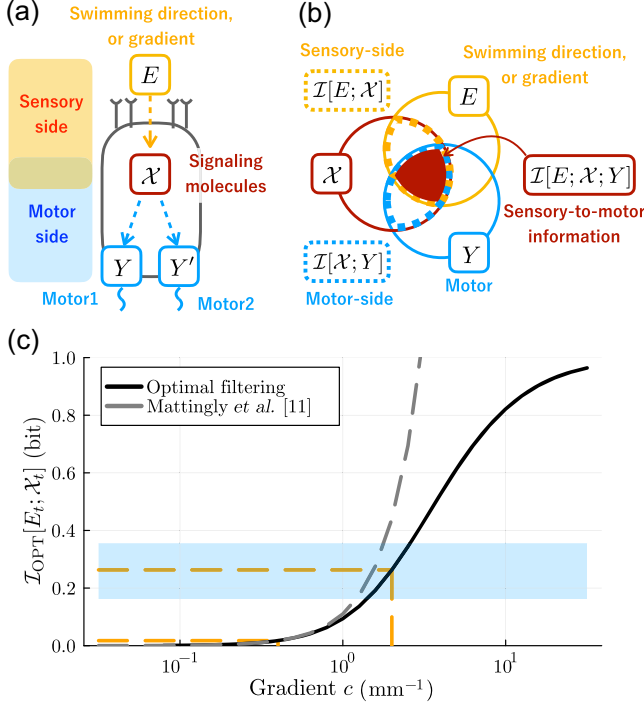


FIG. 2. Information flow in bacterial chemotaxis. (a) Schematic of the chemotactic signaling pathway comprising swimming direction or gradient signals E_t , signaling molecules $\mathcal{X}_t$, and two copies of motors Y_t, Y'_t . (b) Diagram illustrating the inequality relationships between sensory-side information $\mathcal{I}[E_t; \mathcal{X}_t]$, motor-side information $\mathcal{I}[\mathcal{X}_t; Y_t]$, and sensory-to-motor information $\mathcal{I}[E_t; \mathcal{X}_t; Y_t]$. (c) Optimal sensory-side information $\mathcal{I}_{\text{OPT}}[E_t; \mathcal{X}_t]$ as a function of gradient steepness c with noise strength σ fixed based on experimental information rate estimates. Gray dashed line shows experimental information rate estimates $\dot{I}_{s \rightarrow a} = \beta c^2$ ($\beta = 0.22 \text{ bits s}^{-1} \text{ mm}^2$) [11], shown after multiplying by the integration time $(2R)^{-1} = 0.5 \text{ s}$ (see Sec. S5 for detail). Black solid line shows our filtering model prediction fitted to the weak gradient regime ($c = 0.1\text{--}0.4 \text{ mm}^{-1}$) using noise strength σ as a single free parameter (see Sec. S4 for detail). The two lines diverge at strong gradients because the estimate by Mattingly *et al.* [11] is derived under a weak gradient approximation, whereas our filtering model remains valid across the full range of gradient strengths. With physiological parameters $v = 20 \mu\text{m s}^{-1}$ and $R = 1 \text{ s}^{-1}$ [44], the fit yields $\sigma = 6 \times 10^{-4} \text{ s}^{-1}$. Dashed vertical orange lines indicate representative gradients $c = 0.4$ and 2 mm^{-1} corresponding to weak and strong gradient regimes explored in previous experimental and theoretical studies [11,69–73]; horizontal dashed lines show the corresponding $\mathcal{I}_{\text{OPT}}[E_t; \mathcal{X}_t]$ values. The shaded blue region indicates the range of lower bound for the motor-side information $\mathcal{I}[\mathcal{X}_t; Y_t]$ in intact cells (ID RB(+)-1-3) estimated in Fig. 1(e).

chemotaxis signaling pathway $\mathcal{X}_t$ by excluding the pathway-independent correlations $\mathcal{I}[E_t; Y_t | \mathcal{X}_t]$. (See also SM Sec. S5 for the choice of information metric [49]). From the chain rule of mutual information and its nonnegativity, we derive the bounds $\mathcal{I}[E_t; \mathcal{X}_t; Y_t] \leq \mathcal{I}[\mathcal{X}_t; Y_t], \mathcal{I}[E_t; \mathcal{X}_t]$ [Fig. 2(b)]. These inequalities indicate that insufficient motor-side information flow $\mathcal{I}[\mathcal{X}_t; Y_t]$ could limit the overall information flow and chemotactic performance, even if sensory-side information $\mathcal{I}[E_t; \mathcal{X}_t]$ is sufficient. For optimal information transfer, the motor-side information should be comparable to the sensory-side information, minimizing information loss throughout the pathway [Fig. 2(a)].

To estimate the biologically relevant value of the sensory-side information, we draw on our previous work showing that bacterial sensory pathways likely implement Bayes filtering that maximizes mutual information by computing posterior probability [18,19]. This result enables us to infer the upper bound of sensory-side information $\mathcal{I}_{\text{OPT}}[E_t; \mathcal{X}_t] \geq \mathcal{I}[E_t; \mathcal{X}_t]$, which may be attained in actual sensory pathways. At steady state, $\mathcal{I}_{\text{OPT}}[E_t; \mathcal{X}_t]$ depends on a dimensionless signal-to-noise ratio (SNR) parameter $\lambda := 4c^2 v^2 / R\sigma$, which incorporates gradient steepness c , swimming speed v , tumble rate R , and noise strength σ . While v , R , and c are experimentally characterized, σ has not been measured directly. We therefore fix σ by requiring that the predicted information matches the recent experimental estimate of information rate $\dot{I}_{s \rightarrow a}$ in weak gradient regime ($c \leq 0.4 \text{ mm}^{-1}$) [11]. With this single fit, our filtering model reproduces the quadratic dependence of the information on c in Ref. [11] at weak gradients and yields predictions for stronger gradients [Figs. 2(c) and S3(a)]. We note that the discrepancy between the two information-theoretic measures at stronger gradients is expected because these are related but distinct quantities that coincide only in the weak gradient regime. Assumptions and derivations of these relationships are detailed in SM Secs. S4 and S5 [49].

We compare sensory-side information across physiologically relevant gradient steepness c with the lower bound of motor-side information estimated by our dual reporter method. In the weak gradient regime ($c \leq 0.4 \text{ mm}^{-1}$) [11], sensory-side information ranges up to 0.02 bits, value considerably below the motor information. This suggests that the motor side does not significantly limit information flow under weak gradient conditions. Beyond the weak gradient regime, sensory-side information increases to approximately 0.3 bits at steep gradient $c \sim 2 \text{ mm}^{-1}$ explored in previous experimental and theoretical studies [69–73]. This value is comparable to the lower bound of motor information, suggesting that motor side can be bottleneck in information transmission only at the upper range of physiologically relevant gradient steepness.

Conclusion—We introduced a new method for estimating mutual information that avoids direct input-output

measurement by leveraging the dual reporter framework. Applying this method to bacterial flagellar motors as natural dual reporters, we quantified information flow in the chemotaxis pathway. Combined with theoretical estimates of sensory information, our results indicate that motor pathway may be tuned to match the maximum sensory information that cells encounter under physiological conditions.

A key limitation of our method lies in its strength: by not measuring the input, we cannot directly attribute the observed information to specific upstream sources. Nevertheless, as demonstrated here, targeted disruptions—such as pathway knockouts or source removal—allow estimation of their contribution. This is especially useful when experimental manipulation is feasible but direct quantification is difficult. In addition, if combined with conventional input-output methods, our method can reveal hidden information pathways: when our estimates of information exceed those from conventional ones, it may indicate the presence of additional, unmeasured routes of information flow.

Our method relies on the assumptions of identical response statistics and conditional independence between reporters. These requirements can be satisfied in diverse biological systems through modern single-molecule and single-cell measurement techniques. Suitable reporter pairs include multiple copies of the same molecule species [74–76], homologous chromosomes for gene expression studies [77], neural populations with shared input or modulatory fluctuations [78,79], and developing tissues from bilaterally symmetric positions in multicellular organisms [80,81] (see SM Sec. S3 for detail [49]). Conditional independence can be validated experimentally by confirming near-zero information under knockout of the information source, as demonstrated in our chemotaxis example. Moreover, recent theoretical developments have extended dual reporter methods to relax these assumptions [43,82], further broadening the range of applicable systems.

In addition to relaxing these assumptions, this framework would be extended in several directions. One is the estimation of other information-theoretic quantities, such as transfer entropy or mutual information rates [7,11,83]. This could be achieved by considering dual-reporter statistics at two time points $\alpha(y_{t-1}, y_t)$, rather than the single-time point $\alpha(y_t)$ used in this Letter. Another is refinement via higher-order corrections using multiple reporters. With k reporter copies $Y_t^{(1)}, \dots, Y_t^{(k)}$ satisfying the dual reporter assumptions, we can estimate $\alpha_k(y) := \mathbb{E}_{\mathcal{X}_t}[\mathbb{P}_{Y_t|\mathcal{X}_t}(y|\mathcal{X}_t)^k]$, which enables higher-order corrections to our approximation (see SM Sec. S1 [49]). As single-molecule and single-cell measurement techniques advance, opportunities to observe multiple reporter copies increase, allowing such multi-reporter approaches.

Our approach and these extensions along with the conventional methodology will expand the analytical

toolkit for advancing our understanding of cellular information processing across diverse biological systems.

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Data availability—The data and code that support the findings of this article are openly available [84,85].

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