

Optimal Design of Single-Cell Experiments within Temporally Fluctuating Environments

Zachary R Fox

Inria Saclay Ile-de-France, Palaiseau 91120, France

Institut Pasteur, USR 3756 IP CNRS Paris, 75015, France

School of Biomedical Engineering, Colorado State

University Fort Collins, CO 80523, USA and

zachrfox@gmail.com

Gregor Neuert

Department of Molecular Physiology and Biophysics,

School of Medicine, Vanderbilt University, Nashville, TN 37232, USA

Department of Biomedical Engineering, School of Engineering,

Vanderbilt University, Nashville, TN 37232, USA

Department of Pharmacology, School of Medicine,

Vanderbilt University, Nashville, TN 37232, USA and

gregor.neuert@vanderbilt.edu

1000 J. D. B. BROWN

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Central State University 1070 Morris, 33 30050, USA

School of Biomedical Engineering, Stevens Institute of Technology

University for Commons, 33 50020, USA and

oian.mansy@vuw.ac.nz

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24

Abstract

25 Modern biological experiments are becoming increasingly complex, and designing these experi-
26 ments to yield the greatest possible quantitative insight is an open challenge. Increasingly, compu-
27 tational models of complex stochastic biological systems are being used to understand and predict
28 biological behaviors or to infer biological parameters. Such quantitative analyses can also help
29 to improve experiment designs for particular goals, such as to learn more about specific model
30 mechanisms or to reduce prediction errors in certain situations. A classic approach to experiment
31 design is to use the Fisher information matrix (FIM), which quantifies the expected information a
32 particular experiment will reveal about model parameters. The Finite State Projection based FIM
33 (FSP-FIM) was recently developed to compute the FIM for discrete stochastic gene regulatory
34 systems, whose complex response distributions do not satisfy standard assumptions of Gaussian
35 variations. In this work, we develop the FSP-FIM analysis for a stochastic model of stress response
36 genes in *S. cerevisiae* under time-varying MAPK induction. We validate this FSP-FIM analysis
37 and use it to optimize the number of cells that should be quantified at particular times to learn as
38 much as possible about the model parameters. We then demonstrate how the FSP-FIM approach
39 can be extended to explore how different measurement times or genetic modifications can help to
40 minimize uncertainty in the sensing of extracellular environments, such as external salinity mod-
41 uations. This work demonstrates the potential of quantitative models to not only make sense of
42 modern biological data sets, but to close the loop between quantitative modeling and experimental
43 data collection.

44 INTRODUCTION

45 The standard approach to design experiments has been to rely entirely on expert knowl-
46 edge and intuition. However, as experimental investigations become more complex and
47 seek to examine systems with more subtle non-linear interactions, it becomes much harder
48 to improve experimental designs using intuition alone. This issue has become especially
49 relevant in modern single-cell-single-molecule investigations of gene regulatory processes.
50 Performing such powerful, yet complicated experiments involves the selection from among
51 a large number of possible experimental designs, and it is often not clear which designs
52 will provide the most relevant information. A systematic approach to solve this problem is
53 model-driven experiment design, in which one uses an assumed (and potentially incorrect)
54 mathematical model of the system to estimate and optimize the value of potential exper-
55 imental settings. In recent years, model-driven experiment design has gained traction for
56 biological models of gene expression, whether in the Bayesian setting [1] or using Fisher
57 information for deterministic models [2], and even in the stochastic, single-cell setting [3–
58 6]. Despite the promise and active development of model-driven experiment design from
59 the theoretical perspective, more general, yet biologically-inspired approaches are needed to
60 make these methods suitable for the experimental community at large. In this work, we
61 apply model-driven experiment design to an experimentally validated model of stochastic,
62 time-varying High Osmolarity Glycerol (HOG) Mitogen Activated Protein Kinase (MAPK)
63 induction of transcription during osmotic stress response in yeast [7–9]. To demonstrate a
64 concrete and practical application of model-driven experiment design, we find the optimal
65 *measurement schedule* (i.e., when measurements ought to be taken) and the appropriate
66 *number of individual cells* to be measured at each time point.

67 In our computational analyses, we consider the experimental technique of single-mRNA
68 Fluorescence *in situ* Hybridization (smFISH), where specific fluorescent oligonucleotide
69 probes are hybridized to mRNA of interest in fixed cells [10, 11]. Cells are then imaged
70 and the mRNA abundance in each cell can be counted, either by hand or using automated
71 software such as [12]. Such counting can be a cumbersome process, but little thought has
72 been given typically to how many cells should be measured and analyzed at each time.
73 Furthermore, when a dynamic response is under investigation, the specific times at which
74 measurements should be taken (i.e., the times after induction at which cells should be

75 fixed and analyzed) is also unclear. In this work, we use the newly developed finite state
76 projection based Fisher information matrix (FSP-FIM, [6]) to optimize these experimental
77 quantities for osmotic stress response genes in yeast.

78 The HOG-MAPK pathway in yeast is a model system to study dynamics of signal trans-
79 duction induced gene regulation in single cells [13–18] and stochastic models of HOG-MAPK
80 activated transcription have been used to predict adaptive transcription responses across
81 yeast cell populations [8, 9, 19]. In particular, previous studies have measured two stress
82 response genes, *STL1* and *CTT1*, and used them to infer the model depicted in Fig. 1a.
83 This calibration and uncertainty quantification process required intense experimental effort
84 to fix and image tens of thousands of cells at more than a dozen time points and for multi-
85 ple biological replicas as well as intense computational effort for both the processing of the
86 smFISH images and the fitting of stochastic kinetic models to the quantified experimental
87 data. In light of such expenses, we aim to develop methods that can specify experiments that
88 are equally or more informative, yet which could minimize experimental and computational
89 efforts.

90 Toward this goal, the first part of our current study demonstrates the use of FSP based
91 Fisher information to optimize experiments to minimize the uncertainty in stochastic model
92 parameters for the time varying MAPK-induced gene expression response. In the second
93 part of this study, we expand upon this result to find the optimal smFISH measurement
94 times and cell numbers to minimize uncertainty about unknown environmental inputs (e.g.,
95 salt concentrations) to which the cells are subjected. In this way, we are presenting a
96 new methodology by which one can optimally examine behaviors of natural cells to obtain
97 accurate estimations of environmental changes.

98 BACKGROUND

99 Finite State Projection models can predict osmotic stress responses in yeast.

100 Gene regulation is the process by which small molecules, chromatin regulators, and gen-
101 eral and gene-specific transcription factors interact to regulate the transcription of DNA into
102 RNA and the translation of mRNA into proteins. Even within populations of genetically
103 identical cells, these single-molecule processes are stochastic and give rise to cell-to-cell vari-

104 ability in gene expression levels. Adequate description of such variable responses can only
105 be achieved through the use of stochastic computational models [20–23].

106 In this work, we use the chemical master equation framework [24] of stochastic chemical
107 kinetics, which has been the workhorse of stochastic modeling of gene expression, whether
108 through simulated sample paths of its solution via the stochastic simulation algorithm [25],
109 moment approximations [7, 26], or finite state projections (FSP) [27]. Recently, it has come
110 to light that for some systems it is critical to consider the full distribution of biomolecules
111 across cellular populations when fitting CME-based models [6, 9], which can be done with
112 guaranteed errors using the FSP approach [27, 28]. This method truncates a CME into a
113 finite state, continuous time Markov chain, for which the set of ordinary differential equa-
114 tions, $\frac{dp}{dt} = \mathbf{A}(t)\mathbf{p}$ describes the flow of probability among all of the most likely observable
115 states for the system. Details of the FSP approach to solving chemical kinetic systems are
116 provided in Supplementary Note 1.

117 For signal-activated transcription in the HOG-MAPK stress response pathway in yeast,
118 an FSP model has been used to fit and predict mRNA distributions at a range of NaCl
119 concentrations [8, 9]. This model of osmotic stress response consists of transitions between
120 four different gene states, shown in Fig. 1a. The probability of a transition from the i^{th}
121 to the j^{th} gene state in the infinitesimal time dt is given by $k_{ij}dt$. Each i^{th} state also
122 has a corresponding mRNA transcription rate, k_{ri} , but the mRNA degrade with rate γ ,
123 independent of gene state. Further descriptions and validations of this model are given in
124 Supplementary Note 1 and in [8, 9, 19]. To accurately fit and predict mRNA levels across cell
125 populations, the authors in [8] cross-validated across a number of different potential models
126 with different numbers of gene states and time varying parameters. The most predictive of
127 these was the model shown in Fig. 1a, in which the transition rate from the second gene
128 activation state to the first gene activation state is a function of nuclear MAPK levels, $f(t)$.
129 The nuclear localization of MAPK affects this transition with a threshold function,

$$k_{21}(t) = \max[0, \alpha - \beta f(t)], \quad (1)$$

130 where α and β set the threshold for $k_{21}(t)$ activation/deactivation. Figure 1b (left) shows
131 the nuclear localization dynamics of MAPK (i.e. $f(t)$) for osmotic stress responses to 0.2M
132 and 0.4M NaCl, with simulated nuclear localization dynamics fit to a model (from [9],

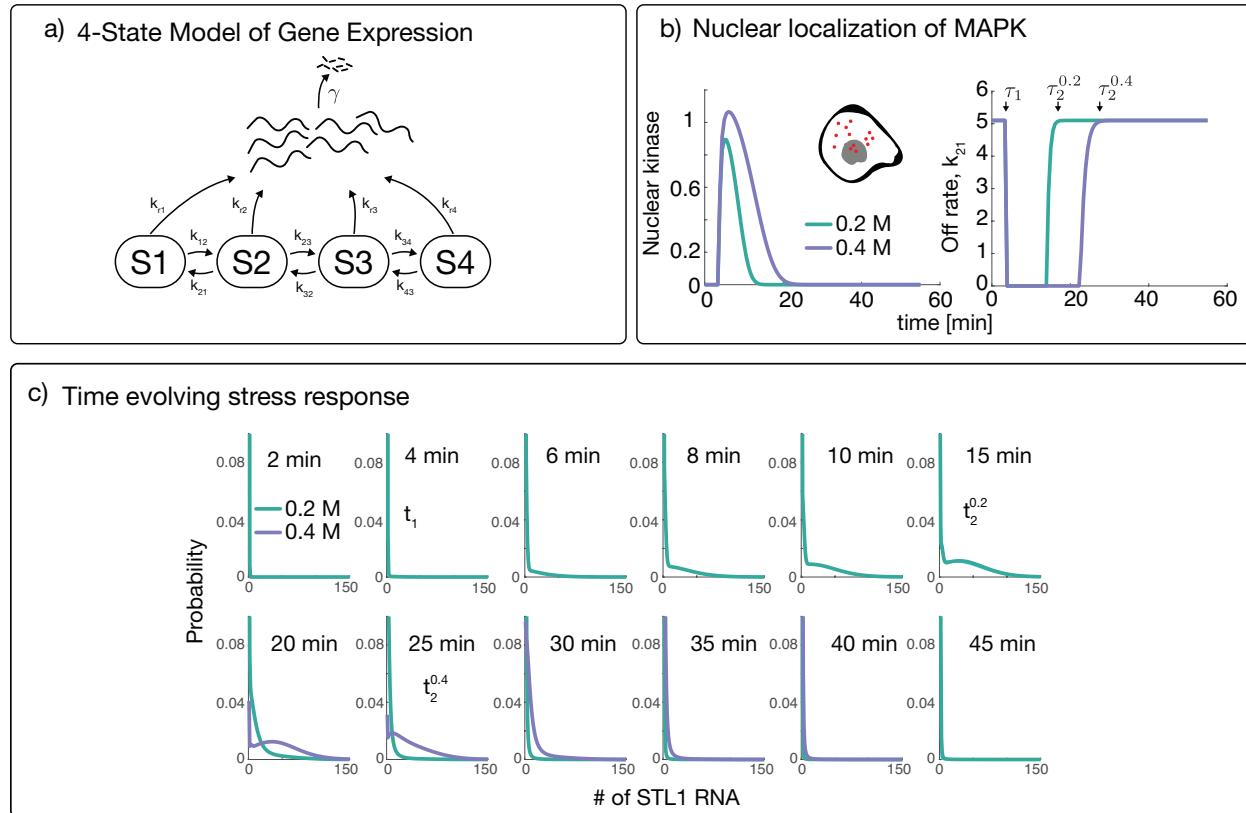


FIG. 1. *Stochastic modeling of osmotic stress response genes in yeast.* (a) Four-state model of gene expression, where each state transcribes mRNA at a different transcription rate, but all mRNA degrade at a single rate γ . (b) The effect of measured MAPK nuclear localization (depicted as red dots in the cell) (left) on the the rate of switching from gene activation state S2 to S1 (right) under 0.2M or 0.4M NaCl osmotic stress. The time at which k_{21} turns off is denoted with τ_1 and is independent of the NaCl level. The time at which k_{23} turns back on is given by τ_2^{NaCl} depending on the level of NaCl. (c) Time evolution of the *STL1* RNA in response to the 0.2M and 0.4M NaCl stress.

¹³³ Supplementary Note 2), and Fig. 1b (right) shows the value of $k_{21}(t)$ for each salinity level.
¹³⁴ This rate results in a time-varying generator $\mathbf{A}(t)$ for the master equation dynamics (See
¹³⁵ Supplementary Note 1).

¹³⁶ **LIKELIHOOD OF SMFISH DATA FOR FSP MODELS**

¹³⁷ To match FSP model solutions to single-cell data, one needs to compute and maximize
¹³⁸ the likelihood of the smFISH data given the FSP model [8, 9, 19, 28, 29]. We assume
¹³⁹ that measurements at each time point $\mathbf{t} \equiv [t_1, t_2, \dots, t_{N_t}]$ are independent, as justified by
¹⁴⁰ the fact that fixation of cells for measurement precludes temporal cell-to-cell correlations.
¹⁴¹ Measurements of N_c cells can be concatenated into a matrix $\mathbf{D}_t \equiv [\mathbf{d}_1, \mathbf{d}_2, \dots, \mathbf{d}_{N_c}]_t$ of the
¹⁴² observable mRNA species at each measurement time t .

¹⁴³ The likelihood of making the independent observations for all N_c measured cells is the
¹⁴⁴ product of the probabilities of observing each cell's measured state. For most gene expression
¹⁴⁵ models, however, states are only partially observable, and we define the observed state \mathbf{x}_i^L
¹⁴⁶ as the marginalization (or lumping) over all full states $\{\mathbf{x}_j\}_i$ that are indistinguishable from
¹⁴⁷ \mathbf{x}_i based on the observation. For example, the model of *STL1* transcription consists of four
¹⁴⁸ gene states (S1-S4, shown in Fig. 1a), which are unobserved, and the measured number of
¹⁴⁹ mRNA, which is observed. If we let index i denote the number of mRNA, then the observed
¹⁵⁰ state \mathbf{x}_i^L would lump together the full states (S1, i), (S2, i), (S3, i), and (S4, i). We next define
¹⁵¹ y_i as the number of experimental cells that match \mathbf{x}_i^L at time t . Under these definitions, the
¹⁵² likelihood of the observed data (and its logarithm) given the model can be written:

$$\ell(\mathbf{D}|\boldsymbol{\theta}) = M \prod_{t=t_1}^{t_{N_t}} \prod_{i \in \mathcal{J}_D} p(\mathbf{x}_i^L, t|\boldsymbol{\theta})^{y_i}$$

$$\log \ell(\mathbf{D}|\boldsymbol{\theta}) = \sum_{t=t_1}^{t_{N_t}} \sum_{i \in \mathcal{J}_D} y_i \log(p(\mathbf{x}_i^L, t|\boldsymbol{\theta})) + \log M, \quad (2)$$

¹⁵³ where \mathcal{J}_D is the set of states observed in the data, M is a combinatorial prefactor (i.e. from
¹⁵⁴ a multinomial distribution) that comes from the arbitrary reordering of measured data, and
¹⁵⁵ $p(\mathbf{x}_i^L)$ is the marginalized probability mass of the observable species,

$$p(\mathbf{x}_i^L) = \sum_{\mathbf{x}_j \in \mathbf{x}_i^L} p(\mathbf{x}_j).$$

¹⁵⁶ Neglecting the term $\log M$, which is independent of the model, the summation in Eq. 2 can
¹⁵⁷ be rewritten as a product $\mathbf{y} \log \mathbf{p}^L$, where $\mathbf{y} \equiv [y_0, y_1, \dots]$ is a vector of the binned data
¹⁵⁸ and $\mathbf{p}^L = [p(\mathbf{x}_0^L), p(\mathbf{x}_1^L), \dots]^T$ is the corresponding marginalized probability mass vector.

159 One may then maximize Eq. 2 with respect to $\boldsymbol{\theta}$ to find the *maximum likelihood estimates*
160 (MLE) of the parameters, $\hat{\boldsymbol{\theta}}$, which will vary depending on each new set of experimental
161 data. We next demonstrate how this likelihood function and the FSP model of the HOG-
162 MAPK system can be used to design optimal smFISH experiments using the FSP-based
163 FIM [6].

164 **The Finite State Projection based Fisher information for models of signal-activated
165 stochastic gene expression.**

166 The Fisher information matrix (FIM), is a common tool in engineering and statistics
167 to estimate parameter uncertainties prior to collecting data, and which allows one to find
168 experimental settings that can make these uncertainties as small as possible [3, 4, 30–33].
169 Recently, it has been applied to biological systems to estimate kinetic rate parameters in
170 stochastic gene expression systems [3–6, 34]. In general, the FIM for a single measurement
171 is defined:

$$\mathcal{I}(\boldsymbol{\theta}) = \mathbb{E} \left\{ (\nabla_{\boldsymbol{\theta}} \log \mathbf{p}(\boldsymbol{\theta}))^T (\nabla_{\boldsymbol{\theta}} \log \mathbf{p}(\boldsymbol{\theta})) \right\}, \quad (3)$$

172 where $\log \mathbf{p}(\boldsymbol{\theta})$ is the log-likelihood of observing that measurement, and the expectation is
173 taken across over the probability distribution of states $\mathbf{p}(\boldsymbol{\theta})$ assuming the specific parameter
174 set $\boldsymbol{\theta}$. As the number of measurements, N_c , is increased such that maximum likelihood
175 estimates (MLE) of parameters are unbiased, the distribution of MLE estimates is known
176 to approach a multivariate Gaussian distribution with a covariance given by the inverse of
177 the Fisher information matrix, i.e.,

$$\sqrt{N_c}(\hat{\boldsymbol{\theta}} - \boldsymbol{\theta}^*) \xrightarrow{\text{dist}} \mathcal{N}(0, \mathcal{I}(\boldsymbol{\theta}^*)^{-1}). \quad (4)$$

178 In [6], we developed the FSP-based Fisher information matrix (FSP-FIM), which allows one
179 to use the FSP solution, $\mathbf{p}(t)$, and the sensitivity matrix, $\mathbf{S}(t)$, to find the Fisher information
180 matrix for stochastic gene expression systems. The dynamics of the sensitivity of each state
181 in the process to the j^{th} kinetic parameter $\frac{d\mathbf{p}}{d\theta_j}$ is given by:

$$\frac{d}{dt} \begin{bmatrix} \mathbf{p} \\ \mathbf{s}_{\theta_j} \end{bmatrix} = \begin{bmatrix} \mathbf{A}(t) & \mathbf{0} \\ \mathbf{A}_{\theta_j}(t) & \mathbf{A}(t) \end{bmatrix} \begin{bmatrix} \mathbf{p} \\ \mathbf{s}_{\theta_j} \end{bmatrix}, \quad (5)$$

182 where $\mathbf{A}_j = \frac{\partial \mathbf{A}}{\partial \theta_j}$. The FSP-FIM at a single time t is then given by:

$$\mathbf{F}(\boldsymbol{\theta}, t)_{j,k} = \sum_i \frac{1}{p(\mathbf{x}_i; t, \boldsymbol{\theta})} \mathbf{s}_{\theta_j}^i(t) \mathbf{s}_{\theta_k}^i(t), \quad (6)$$

183 where the summation is taken over all states, $\{\mathbf{x}_i\}$, included in the FSP analysis (or over
 184 all observed states, $\{\mathbf{x}_i^L\}$, in the case of lumped observations). The FIM for a sequence of
 185 measurements taken independently (e.g., for smFISH data) at times $\mathbf{t} = [t_1, t_2, \dots, t_{N_t}]$ is
 186 then given by the sum across the measurement times:

$$\mathcal{I}(\boldsymbol{\theta}, \mathbf{t}, \mathbf{c}) = \sum_{l=1}^{N_t} c_l \mathbf{F}(\boldsymbol{\theta}, t = t_l), \quad (7)$$

187 where $\mathbf{c} = [c_1, c_2, \dots, c_{N_t}]$ is the number of cells measured at each l^{th} measurement time.
 188 For smFISH experiments, the vector \mathbf{c} plays an important role in the design of the study.
 189 By optimizing over all vectors \mathbf{c} that sum to N_{total} , one can find how many cells should be
 190 measured at each time point and which time points should be skipped entirely, (i.e., $c_l = 0$).
 191 We next verify the FSP-FIM for this stochastic model with a time-varying parameter, and
 192 later find the optimal \mathbf{c} for *STL1* mRNA in yeast cells.

193 RESULTS

194 The FSP-FIM can quantify experimental information for stochastic gene expression 195 under time-varying inputs

196 Our work in [6] was limited to models of stochastic gene expression that had piecewise
 197 constant reaction rates. Here, we extend this to time-varying reaction rates that affect the
 198 promoter switching in the system and which lead to time-varying $\mathbf{A}(t)$ in Eqn. 5. In our
 199 model, the temporal addition of osmotic shock causes nuclear translocation of HOG-MAPK,
 200 according to the time-varying function in Eq. 1.

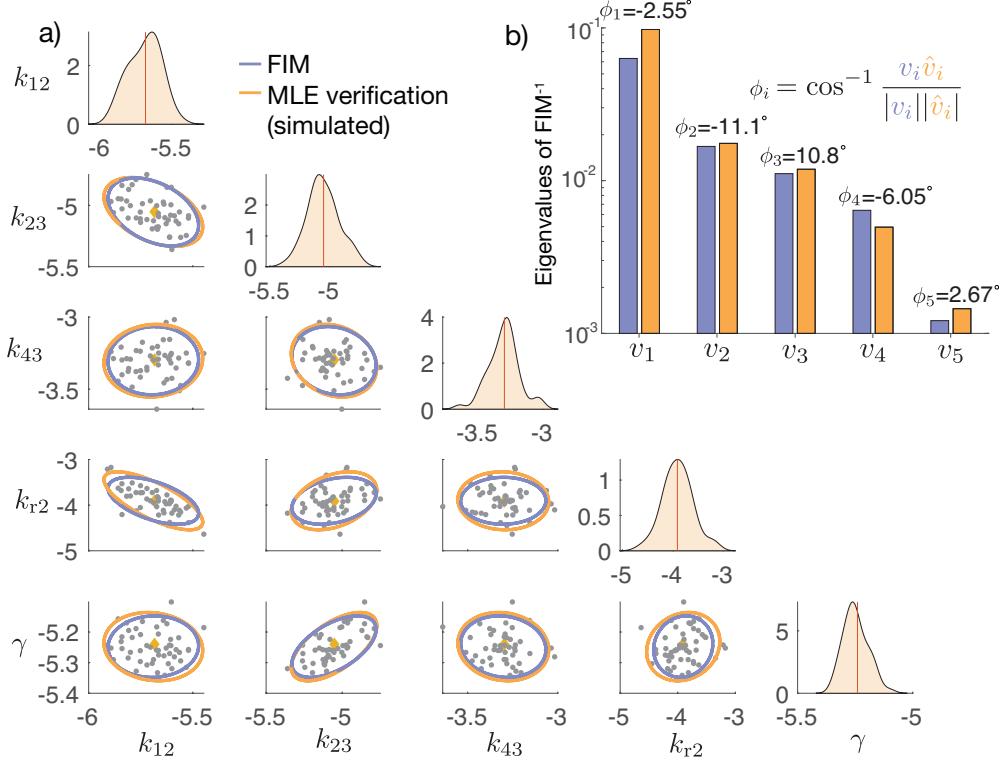


FIG. 2. *Verification of the FSP-FIM for the time-varying HOG-MAPK model.* (a) Scatter plots and density plots of the spread of MLE estimates for 50 simulated data sets for a subset of model parameters. All parameters are shown in logarithmic scale. The ellipses show the 95% CI for the inverse of the FIM (purple) and covariance of scatter plot (orange). The yellow dots indicate the parameters at which the FIM and simulated data sets were generated. (b) Rank-paired eigenvalues (v_i) for the covariance of MLE estimates (orange) and inverse of the FIM (blue). The angles between corresponding rank-paired eigenvectors (ϕ_i) are shown in degrees.

201 Model parameters simultaneously fit to experimentally measured 0.2M and 0.4M *STL1*
 202 mRNA were adopted from [9] and used as a reference set of parameters (yellow dots in Fig.
 203 2a and S1), which we define as θ^* . These reference parameters were used to generate 50
 204 unique and independent simulated data sets, and each n^{th} simulated data set was fit to
 205 find the parameter set, $\hat{\theta}_n$, that maximizes the likelihood for that simulated data set. This
 206 process was repeated for two different experiment designs, including the original intuitive
 207 design from [9] (results shown in Fig. 2) and an optimized design discussed below (results
 208 shown in Fig. S1). To ease the computational burden of this fitting, the four parameters
 209 with the smallest sensitivities and largest uncertainties (i.e., those parameters that had the

210 least effect on the model predictions and which were most difficult to identify) were fixed
211 at their baseline values. The resulting MLE estimates for the remaining five parameters
212 were collected into a set of $\{\hat{\theta}_n\}$ and are shown as yellow dots in Figs. 2 and S1. Using the
213 asymptotic normality of the maximum likelihood estimator and its relationship to the FIM
214 (Eq. 4), we then compared the 95% confidence intervals (CIs) of the inverse of the Fisher
215 information (i.e. the Cramér Rao bound) to those of the MLE estimates (compare the purple
216 and orange ellipses in Figs. 2a and S1a). We also compared the eigenvalues of the inverse
217 of the Fisher information, $\{v_i\}$, to the correspondingly ranked eigenvalues of the covariance
218 matrix of MLE estimates, Σ_{MLE} , in Figs. 2b and S1b. For further validation, we noted that
219 the principle directions of the ellipses in Figs. 2a and S1a also match for the FIM and MLE
220 analyses, as quantified by the angle between the paired FIM and Σ_{MLE} eigenvectors (Figs.
221 2b and S1b). For comparison, the angles between rank-matched eigenvectors of the FIM
222 and Σ_{MLE} were all less than 12° , whereas non rank-matched eigenvectors were all greater
223 than 79.9° . With the FSP-FIM verified for the HOG-MAPK model, we next explore how
224 the FIM can be used to optimally allocate the number of cells to measure at each time after
225 osmotic shock.

226 Designing optimal measurements for the HOG-MAPK pathway in *S. cerevisiae*

227 To explore the use of the FSP-FIM for experiment design in a realistic context of MAPK-
228 activated gene expression, we again utilize simulated time-course smFISH data for the os-
229 motic stress response in yeast.

230 We start with a known set of underlying model parameters that were taken from simulta-
231 neous fits to 0.2M and 0.4M data in [9] (non-spatial model) to establish a baseline parameter
232 set that is experimentally realistic. These baseline parameters are then used to optimize the
233 allocation of measurements at different time points $t = [1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55]$
234 minutes after NaCl induction. Specifically, we ask what fraction of the total number of cells
235 should be measured at each time to maximize the information about a specific subset of
236 important model parameters. We use a specific experiment design objective criteria referred
237 to as D_s -optimality, which corresponds to minimizing the expected volume of the param-
238 eter space uncertainty for the specific parameters of interest [34], and which is found by
239 maximizing the product of the eigenvalues of the FIM for those same parameters.

240 Mathematically, our goal is to find the optimal cell measurement allocation,

$$\mathbf{c}_{\text{opt}} = \arg \max_{\mathbf{c}} |\mathcal{I}(\mathbf{c}; \boldsymbol{\theta})|_{D_s} \text{ such that } \sum_{l=1}^{N_t} c_l = 1, \quad (8)$$

241 where c_l is the fraction of total measurements to be allocated at $t = t_l$, and the metric
242 $|\mathcal{I}(\mathbf{c}; \boldsymbol{\theta})|_{D_s}$ refers to the product of the eigenvalues for the total FIM (Eqn. 7). The fraction
243 of cells to be measured at each time point, \mathbf{c} was optimized using a greedy search, in which
244 single-cell measurements were chosen one at a time according to which time point predicted
245 the greatest improvement in the optimization criteria (see Supplementary Note 3 for more
246 information).

247 To illustrate our approach, we first allocated cell measurements according to D_s -
248 optimality as found through this greedy search. Figure 3 shows the optimal fraction of
249 cells to be measured at each time following a 0.2M NaCl input and compares these fractions
250 to the experimentally measured number of cells from [9]. While each available time point
251 was allocated a non-zero fraction of measurements, three time points at $t = [10, 15, 30]$
252 minutes were vastly more informative than the other potential time points. To verify this
253 result, we simulated 50 data sets of 1,000 cells each and found the MLE estimates for each
254 sub-sampled data set. We compared the spread of these MLE estimates to the inverse of
255 the optimized FIM, shown in Fig. S1.

256 Comparing Figs. S1 with Fig. 2 illustrates the increase in information of the optimal
257 0.2M experiment compared to the intuitively designed experiment from [9]. In addition to
258 providing much higher Fisher information, the optimal experiment requires measurement of
259 only three time points compared to the 16 time points that were measured in the original
260 experiment. Furthermore, we note that the FIM prediction of the MLE uncertainty is more
261 accurate for the simpler optimal design, which is likely related to our observation that MLE
262 estimates converge more easily for the optimized experiment design than they do for original
263 intuitive design.

264 Figure 4 next compares the D_s -optimality criteria for the optimal (solid horizontal lines)
265 and intuitive ([9], dashed horizontal lines) experiment designs to 1,000 randomly designed
266 experiments for the 0.2M (black) and 0.4M (gray) conditions. To generate these random
267 experiment designs, we selected a random subset of the measurement times, and allocated
268 the total 1,000 cells among chosen time points using multinomial distribution with equal

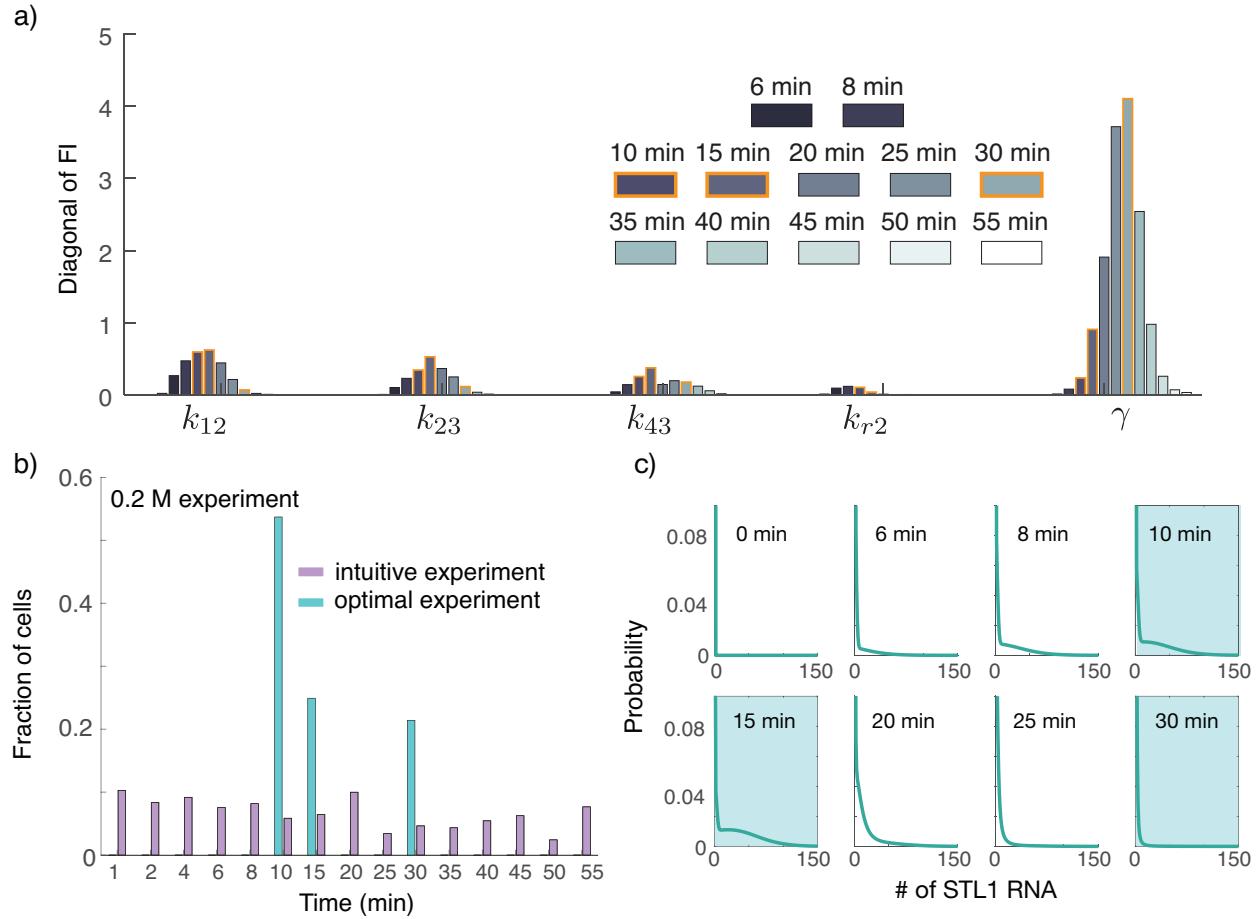


FIG. 3. *Optimizing the allocation of cell measurements at different time points.* (a) Diagonal entries of the Fisher information at different measurement times. The optimal measurement times $t = [10, 15, 30]$ minutes are highlighted in orange. (b) Comparison of optimal fractions cells to measure (blue) at different time points determined by the FSP-FIM compared to experimentally measured numbers of cells at 0.2 M NaCl (purple) from our work in [9]. (c) Probability distributions of *STL1* mRNA at several of measurement times. The blue boxes denote the time points of optimal measurements.

269 probability for each time point. Figures 4a-b show that the intuitive experiment is more
 270 informative than most random experiments, but is still substantially less informative than
 271 the optimal experiment. To explore the importance of knowing the exact process input
 272 dynamics prior to designing the experiment, we next asked how well an experiment design
 273 optimized for a 0.2M osmotic shock would do to estimate parameters using an 0.4M experi-

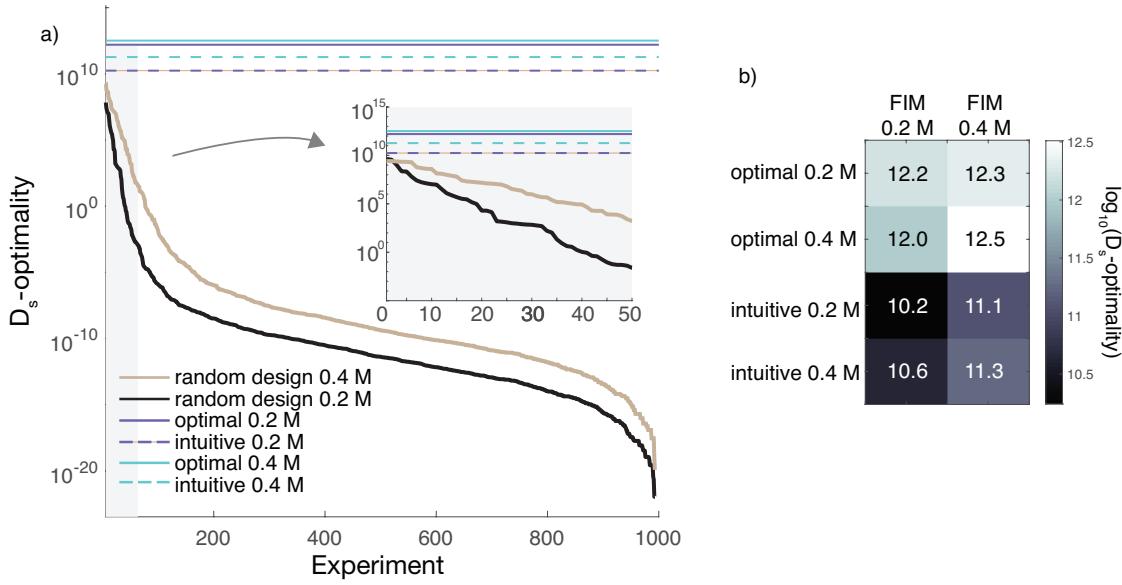


FIG. 4. *Information gained by performing optimal experiments compared to actual experiments*

(a) D_s -optimality for optimal design using three time points compared to the intuitive experiment design made using 16 time points (purple, 0.2M and blue, 0.4M). Dashed lines represent intuitive experiment designs. Randomly designed experiments with 0.2 M and 0.4 M NaCl are shown in black and gray. For the random experiments, the time points were selected by sampling them from the experimental measurement times, and then a random number of measurements were assigned to each selected time point. The inset shows the first 50 randomly designed experiments. (b) The D_s -optimality for different experiment designs (y-axis) computed using the Fisher information for either the 0.2 M perturbation or the 0.4 M NaCl perturbation.

274 ment and vice-versa. Figure 4b shows that the simpler optimal experiment designs perform
 275 better than the intuitive designs in all cases, even when the design was found assuming a
 276 different environmental condition.

277 **Using the FSP-FIM to design optimal biosensor measurements.**

278 Thus far, and throughout our previous work in [6], we have sought to find the optimal
 279 set of experiments to reduce uncertainty in the estimates of *model parameters*. In this
 280 section, we discuss how the FSP-FIM allows for the optimization of experiment designs to
 281 address a more general problem of inferring *environmental variables* from cellular responses.

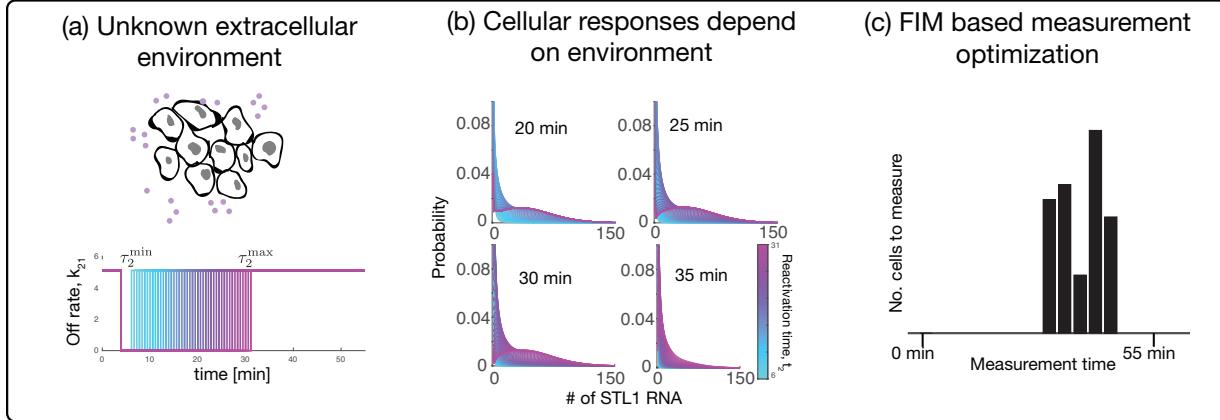


FIG. 5. *Overview of optimal design for biosensing experiments for the osmotic stress response in yeast.* (a) Unknown salt concentrations (purple dots) in the environment give rise to different reactivation times, τ_2 , which affect the gene expression in the model through the rate k_{21} . These different reactivation times cause downstream *STL1* expression dynamics to behave differently as shown in panel (b). (c) Different responses can be used to resolve experiments that reduce the uncertainty in τ_2 .

282 Toward this end, we assume a known and parametrized model (i.e., the model defined above,
 283 which was identified previously in [9]), but which is now subject to unknown environmental
 284 influences. We explore what would be the optimal experimental measurements to take to
 285 characterize these influences. Specifically, we ask how many cells should be measured using
 286 smFISH, and at what times, to determine the specific concentration of NaCl to which the
 287 cells have been subjected at $t = 0$ – or, equivalently, we ask what experiments would be
 288 best suited to measure the effective stress induction level caused by addition of an unknown
 289 solution to the cells.

290 In the HOG-MAPK transcription model, extracellular osmolarity ultimately affects stress
 291 response gene transcription levels through the time-varying parameter $k_{21}(t)$ in Eq. 1, and
 292 Fig. 1b shows the effect 0.2M and 0.4M salt concentrations on k_{21} activation. Higher salt
 293 concentrations delay the time at which $k_{21}(t)$ returns to its nonzero value, and the function
 294 in Eq. 1 is well-approximated by a the sum of three Heaviside step functions, $u(t - \tau_i)$ as:

$$k_{21}(t) = k_{21}^0 (u(t) - u(t - \tau_1) + u(t - \tau_2)), \quad (9)$$

295 where τ_1 is the fixed delay of the time it takes for nuclear kinase levels to reach the k_{21}

296 deactivation threshold (about 1 minute or less, [8, 9]), and τ_2 is the variable time it takes
 297 for the nuclear kinase to drop back below that threshold. In practice, the threshold-crossing
 298 time, τ_2 , is directly related to the salt concentration experienced by the cell under reasonable
 299 salinity levels. This relationship is shown in Fig. 1b and 5b, where a 0.2M NaCl input exhibits
 300 a shorter τ_2 than does a 0.4M input. For our analyses, we assume a prior uncertainty such
 301 that time τ_2 can be any value uniformly distributed between $\tau_2^{\min} = 6$ and $\tau_2^{\max} = 31$ minutes,
 302 and our goal is to find the experiment that best reduces the posterior uncertainty in τ_2 (and
 303 therefore the concentration of NaCl).

304 To reformulate the FSP-FIM to estimate uncertainty in τ_2 given our model, the first
 305 step is to compute the sensitivity of the distribution of mRNA abundance to changes in the
 306 variable τ_2 using Eqn. 5, in which $\mathbf{A}_{\theta_j}(t)$ is replaced with $\mathbf{A}_{\tau_2}(t) = \frac{\partial \mathbf{A}}{\partial \tau_2}$ as follows:

$$\frac{d}{dt} \begin{bmatrix} \mathbf{p} \\ \mathbf{s}_{\tau_2} \end{bmatrix} = \begin{bmatrix} \mathbf{A}(t) & \mathbf{0} \\ \mathbf{A}_{\tau_2}(t) & \mathbf{A}(t) \end{bmatrix} \begin{bmatrix} \mathbf{p} \\ \mathbf{s}_{\tau_2} \end{bmatrix}. \quad (10)$$

307 As $k_{21}(t)$ is the only parameter in \mathbf{A} that depends explicitly on τ_2 , all entries of $\frac{\partial \mathbf{A}}{\partial \tau_2}$ are zero
 308 except for those which depend on $k_{21}(t)$, and

$$\mathbf{A}_{\tau_2}(t) = \frac{\partial \mathbf{A}}{\partial k_{21}} \frac{\partial k_{21}}{\partial \tau_2} = \mathbf{A}_{k_{21}} k_{21}^0 \delta(\tau_2), \quad (11)$$

309 and therefore $\mathbf{A}_{\tau_2} = \frac{\partial \mathbf{A}}{\partial \tau_2}$ is non-zero only at $t = \tau_2$. Using this fact, the equation for the
 310 sensitivity dynamics is uncoupled from the FSP dynamics for $t \neq \tau_2$, and can be written
 311 simply as:

$$\frac{d}{dt} \mathbf{s}_{\tau_2} = \begin{cases} \mathbf{0} & \text{for } t < \tau_2 \text{ with } \mathbf{s}(0) = \mathbf{0} \\ \mathbf{A}(t) \mathbf{s}_{\tau_2} & \text{for } t > \tau_2 \text{ with } \mathbf{s}_{\tau_2}(\tau_2) = k_{21}^0 \mathbf{A}_{k_{21}} \mathbf{p}(\tau_2) \end{cases}. \quad (12)$$

312 If the Fisher information at each measurement time is written into a vector $\mathbf{f} =$
 313 $[f_1, f_2, \dots, f_{N_t}]$ (noting that the Fisher information at any time t_l is the scalar quantity,
 314 f_l), and the number of measurements per time point is the vector, $\mathbf{c} = [c_1, c_2, \dots, c_{N_t}]$, then
 315 the total information for a given value of τ_2 can be computed as the dot product of these
 316 two vectors,

$$\mathcal{I}(\tau_2) = \sum_{l=1}^{N_t} c_l f_l = \mathbf{c}^T \mathbf{f}. \quad (13)$$

317 Our goal is to find an experiment that is optimal to determine the value of τ_2 , given an
 318 assumed prior that τ_2 is sampled from a uniform distribution between τ_2^{\min} and τ_2^{\max} . To
 319 find the experiment \mathbf{c}_{opt} that will reduce our posterior uncertainty in τ_2 , we integrate the
 320 inverse of the FIM in Eq. 13 over the prior uncertainty in τ_2 ,

$$\mathbf{c}_{\text{opt}} = \arg \min_{\mathbf{c}, \sum c_l = 1} \int_{\tau_2^{\min}}^{\tau_2^{\max}} \frac{1}{\tau_2^{\max} - \tau_2^{\min}} \mathcal{I}^{-1}(\mathbf{c}; \tau_2 = \tau, \boldsymbol{\theta}) d\tau \quad (14)$$

$$= \arg \min_{\mathbf{c}, \sum c_l = 1} \int_{\tau_2^{\min}}^{\tau_2^{\max}} \mathcal{I}^{-1}(\mathbf{c}; \tau_2 = \tau, \boldsymbol{\theta}) d\tau. \quad (15)$$

321 For later convenience, we define the integral in Eq. 14 (i.e., the objective function of the
 322 minimization) by the symbol \mathcal{J} , which corresponds to the expected uncertainty about the
 323 value of τ_2 for a given \mathbf{c} .

324 Next, we apply the greedy search from above to solve the minimization problem in Eqn.
 325 15 to find the experiment design \mathbf{c}_{opt} that minimizes the estimation error of τ_2 . Figure 6
 326 shows examples of seven different experiments to accomplish this task, ranked according
 327 to the FSP-FIM value \mathcal{J} from most informative (top left) to least informative (bottom
 328 right), but all using the same number of measured cells. For each experiment, the FSP-FIM
 329 was used to estimate the posterior uncertainty (i.e., expected standard deviation) in the
 330 estimation of τ_2 , which is shown by the orange bars in Fig. 6. To verify these estimates, we
 331 then chose 64 uniformly spaced values of τ_2 , which we denote as the set $\{\tau_2^{\text{true}}\}$, and for each
 332 τ_2^{true} , we simulated 50 random data sets of 1,000 cells distributed according to the specified
 333 experiment designs. For each of the 64×50 simulated data sets, we then determined the value
 334 τ_2^{MLE} between τ_2^{\min} and τ_2^{\max} that maximized the likelihood of the simulated data according
 335 to Eq. 2. The root mean squared estimate (RMSE) error over all random values of τ_2^{true} and
 336 estimates, $\sqrt{\langle (\tau_2^{\text{MLE}} - \tau_2^{\text{true}})^2 \rangle}$, was then computed for each of the six different experiment
 337 designs. Figure 6 shows that the FIM-based estimation of uncertainty and the actual MLE-
 338 based uncertainty are in excellent agreement for all experiments (compare purple and orange
 339 bars). Moreover, it is clear that the optimal design selected by the FIM-analysis performed
 340 much better to estimate τ_2 than did the uniform or random experimental designs. A slightly

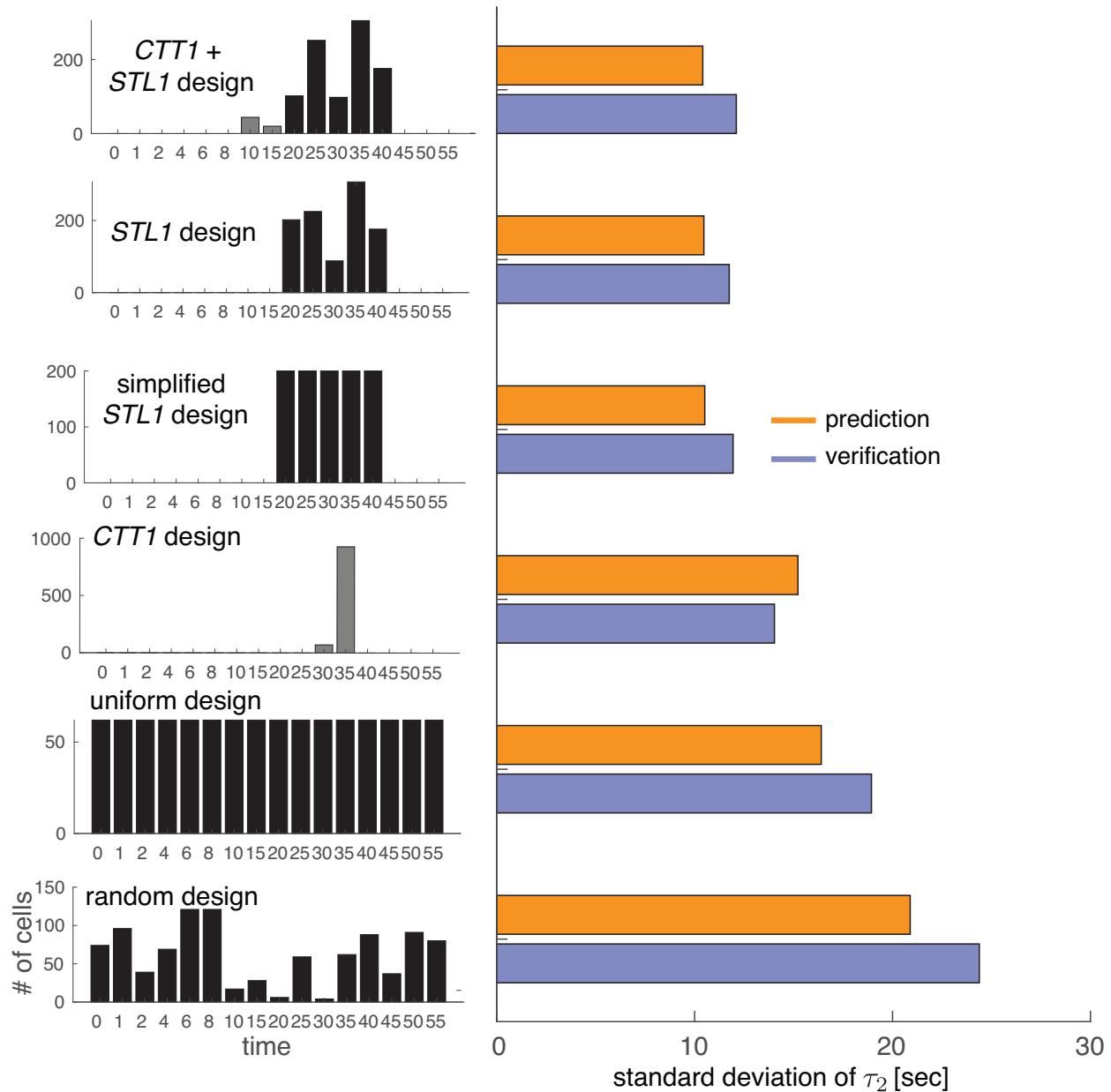


FIG. 6. *Verification of the uncertainty in τ_2 for different experiment designs.* The left panel shows various experiment designs, where the sum of the bars (i.e., the total number of measurements) is 1,000. Gray bars represent the measurements of *CTT1* and black bars *STL1*. The right panel shows the value of the objective function in Eq. 14 for each experiment design in orange, and the MSE values for verification are shown in purple.

³⁴¹ simplified design, which uses the same time points as the optimal, but with equal numbers
³⁴² of measurements at each time, performed nearly as well as the optimal design.

343 The set of experiment designs shown in Fig. 6 includes the best design that only uses
344 *STL1* (second from top), the best design that uses only *CTT1* (fourth from top), and the best
345 designs that uses some cells with *CTT1* and some with *STL1* (top design). To find the best
346 experiment design for measurement of two different genes, we assumed that at each time,
347 either *STL1* mRNA *or* *CTT1* mRNA (but not both) could be measured, corresponding to
348 using smFISH oligonucleotides for either *STL1* or *CTT1*. To determine which gene should
349 be measured at each time, we compute the Fisher information for *CTT1* and *STL1* for every
350 measurement time and averaged this value over the range of τ_2 . For each measurement time
351 t_l , the gene is selected that has the higher average Fisher information for τ_2 . The number
352 of cells per measurement time were then optimized as before, except the choice to measure
353 *CTT1* or *STL1* was based on which mRNA had the larger Fisher information (Eq. 13) at that
354 specific point in time. The best *STL1*-only experiment design was found to yield uncertainty
355 of 10.5 seconds (standard deviation); the best *CTT1*-only experiment was found to yield an
356 uncertainty of 15.2 seconds and the best mixed *STL1/CTT1* experiment design was found
357 to yield an uncertainty of 10.4 seconds. In other words, for this case the *STL1* gene was
358 found to be much more informative of the environmental condition than was *CTT1*, and the
359 use of both *STL1* and *CTT1* provides only minimal improvement beyond the use of *STL1*
360 alone. We note that although measurement times in the optimized experiment design were
361 restricted to a resolution of five minutes or more, the value of τ_2 could be estimated with
362 an error of only 10 seconds, corresponding to a roughly 30-fold improvement of temporal
363 resolution beyond the allowable sampling rate.

364 DISCUSSION

365 The methods developed in this work present a principled, model-driven approach to
366 allocate how many snapshot single-cell measurements should be taken at each time during
367 analysis of a time-varying stochastic gene regulation system. We demonstrate and verify
368 these theories on a well-established model of osmotic stress response in yeast cells, which
369 is activated upon the nuclear localization of phosphorylated HOG1 [8, 9]. For this system,
370 we showed how to optimally allocate the number of cells measured at each time so as to
371 maximize the information about a subset of model parameters. We found that the optimal
372 experiment design to estimate model parameters for the *STL1* gene only required three time

373 points. Moreover, these three time points ($t = [10, 15, 30]$ minutes, highlighted by blue in
374 Fig. 3b) are at biologically meaningful time points. At $t = 10$ and 15 minutes, the system is
375 increasing to maximal expression, and the probability of measuring a cell with elevated of
376 RNA is high, which helps reduce uncertainty about the parameters in the model that control
377 maximal expression. Similarly, at the final experiment time of $t = 30$ minutes, the system
378 is starting to shut down gene expression, and therefore this time is valuable to learn about
379 the time scale of deactivation in the system as well as the mRNA degradation rate. These
380 effects are clearly illustrated in Fig. 3a, which shows that times $t = 10$ and $t = 15$ minutes
381 provide the most information about parameters k_{12} , k_{23} and k_{43} , whereas measurements at
382 $t = 30$ minutes provide the most information about γ . Because γ is the easiest parameter to
383 estimate (e.g., its information is greater), not as many cells are needed at $t = 30$ minutes to
384 constrain that parameter. Similarly, because k_{r2} is the most difficult parameter to estimate
385 (e.g., it has the lowest information across all experiments), and because $t = 10$ minutes
386 is one of the few time points to provide information about k_{r2} , the optimal experimental
387 design selects a large number of cells at the time $t = 10$ minutes. This analysis demonstrates
388 that the optimal experiment design can change depending upon which parameters are most
389 important to determine (e.g., γ or k_{r2} in this case), a fact that we expect will be important
390 to consider in future experiment designs.

391 Because we constrained all potential experiment designs to be within the subset of ex-
392 periments performed in our previous work [9], we are able to compare the information of
393 optimal experiment designs to intuitive designs that have actually been performed. We
394 found that while the intuitive experiments performed were almost always better than could
395 be expected by random chance, they still provided several orders of magnitude lower Fisher
396 information than would be possible with optimal experiments (Fig. 4a). Moreover, in our
397 analyses, we found that optimal designs could require far fewer time points than those de-
398 signed by intuition (e.g., only three time points were needed in Fig. 3), and therefore these
399 designs can be much easier and less expensive to conduct. We also found that utility of
400 optimal experiment designs could be relatively insensitive to variation in the experimental
401 conditions compared to assumptions used in the experiments design (Fig. 4b), a fact that
402 allows for effective experiment designs despite inaccurate prior assumptions.

403 In addition to suggesting optimal experiments to identify model parameters, we showed
404 that the FSP-FIM combined with an existing model could be used to design optimal exper-

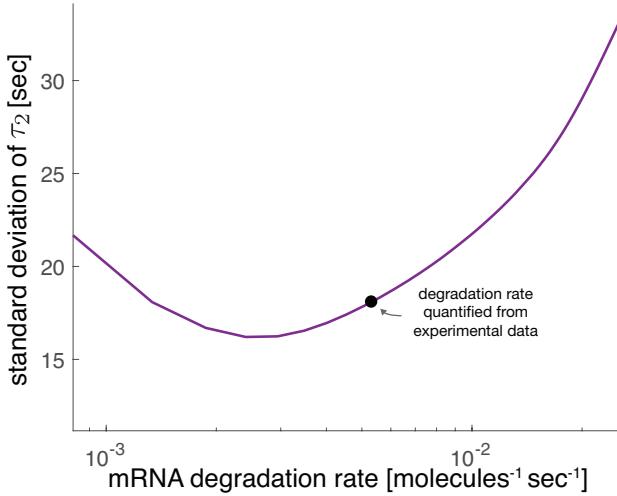


FIG. 7. *Optimal mRNA degradation rates to reduce uncertainty about the extracellular environment.* Uncertainty in the time at which the *STL1* gene turns off, τ_2 , as a function of mRNA degradation rate (purple). The black dot corresponds to the degradation rate that was quantified from experimental data.

405 iments to learn about fluctuating extracellular environments (Figs. 5 and 6). Along a very
 406 similar line of reasoning, one can also adapt the FSP-FIM analysis to learn what biological
 407 design parameters would be optimal to reduce uncertainty in the estimate of important envi-
 408 ronmental variables. For example, Fig. 7 shows the expected uncertainty in τ_2 as a function
 409 of the degradation rate of the *STL1* gene assuming that 50 cells could be measured at each
 410 experimental measurement time $t = [1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55]$ minutes
 411 using the smFISH approach. We found that the best choice for *STL1* degradation rate to
 412 most accurately determine the extracellular fluctuations would be 2.4×10^{-3} mRNA/min,
 413 which is about half of the experimentally determined value of $5.3 \times 10^{-3} \pm 5.9 \times 10^{-5}$ from
 414 [9]. This result is consistent with our earlier finding that the faster degrading *STL1* mRNA
 415 is a much better determinant of the HOG1 dynamics than is the slower-degrading *CTT1*
 416 mRNA, and suggests that other less stable mRNA could be more effective still. We ex-
 417 pect that similar, future applications of the FSP-based Fisher information to be valuable in
 418 other systems and synthetic biology contexts where scientists seek to explore how different
 419 cellular properties affect the transmission of information between cells or from cells to hu-
 420 man observers. Indeed, similar ideas have been explored recently using classical information
 421 theory in [35–37], and recent work in [38] has noted the close relationship between Fisher

422 information and the channel capacity of biochemical signaling networks.

423 We expect that computing optimal experiment designs for time-varying stochastic gene
424 expression creates opportunities that could extend well beyond the examples presented in
425 this work. Modern experimental systems are making it much easier for scientists and engi-
426 neers to precisely perturb cellular environments using chemical induction [39–41] or optoge-
427 netic control [42–44]. Many such experiments involve stochastic bursting behaviors at the
428 mRNA or protein level [7–9, 43], and precise optimal experiment design will be crucial to
429 understand the properties of stochastic variations in such systems. A related field that is
430 also likely to benefit from such approaches is biomolecular image processing and feedback
431 control, for which one may need to decide in real time which measurements to make and in
432 what conditions.

433 DATA AVAILABILITY

434 All data and codes associated with this article will be made available upon acceptance of
435 the article at: https://github.com/MunskyGroup/fox_et_al_complexity_2019.

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