Supplementary Material
Optimal experiment design for practical parameter identifiability and model discrimination

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S.1 Forward-backward sweep (FBS) algorithm

In this section, we summarise the FBS algorithm used in Sec. 3. This algorithm numerically finds solutions to the PMP problem. We adapt the version found in [9], adding a rule for adaptively reducing the update rate, ω, during the iterations. More details on PMP and the FBS algorithm can be found in [7].

Algorithm 1 Forward-backward sweep with adaptive update rate

(I) Initialise $\boldsymbol{u}^0(t)$

(II) Set $\omega \leftarrow \omega^0, J[\boldsymbol{u}^0] \leftarrow \infty$

(III) For $i = 1, 2, \ldots$:

(1) Solve the state equation, Eq. (18), with $\boldsymbol{u} = \boldsymbol{u}^i$ and $\boldsymbol{x}(0) = \boldsymbol{x}_0$, forward in time to obtain $\boldsymbol{x}^i(t)$

(2) Solve the adjoint equation,

$$\frac{\mathrm{d}\lambda_i}{\mathrm{d}t} = (-1)^i 2 g_1(\boldsymbol{x}_1(t; \boldsymbol{u}(t))) - g_2(\boldsymbol{x}_2(t; \boldsymbol{u}(t))) g_i'(x_i) - \lambda_i \frac{\partial f_i}{\partial x_i}, \tag{S.1}$$

with $\boldsymbol{u} = \boldsymbol{u}^i$, $\boldsymbol{\lambda}(T) = 0$, backward in time to obtain $\boldsymbol{\lambda}^i(t)$.

(3) Compute the cost functional, $J[\boldsymbol{u}^i]$, Eq. (19), using $\boldsymbol{u}^i, \boldsymbol{x}^i, \boldsymbol{\lambda}^i$

(4) Check termination criterion: if $|J[\boldsymbol{u}^i] - J[\boldsymbol{u}^{i-1}]| < \epsilon$: return $\boldsymbol{u}^i$ as the optimal control

(5) Set

$$\boldsymbol{u}^{\text{new}}(t) \leftarrow \underset{\boldsymbol{u}' \in \mathcal{U}}{\operatorname{argmax}}\, H(\boldsymbol{x}^i(t), \boldsymbol{\lambda}^i(t); \boldsymbol{u}'), \tag{S.2}$$

where $H(\boldsymbol{x}, \boldsymbol{\lambda}; \lambda_0, \boldsymbol{u}) = -\mathcal{L}(\boldsymbol{x}, \boldsymbol{u}) + \boldsymbol{\lambda} \cdot \boldsymbol{f}(\boldsymbol{x}, \boldsymbol{u})$

(6) Adapt update rate: if $J[\boldsymbol{u}^i] > J[\boldsymbol{u}^{i-1}]$: set $\omega \leftarrow \omega/2$

(7) Set $\boldsymbol{u}^{i+1} \leftarrow (1-\omega)\boldsymbol{u}^i + \omega \boldsymbol{u}^{\text{new}}$

S.2 Additional example for optimising parameter identifiability

In this section, we provide an additional example for designing an experiment to optimise for parameter identifiability, using the same approach as in Sec. 2. This is to demonstrate the flexibility of the methodology. We consider a model of a hypothetical signalling pathway, which can also be interpreted as a regulatory network, consisting of three signalling proteins, named A, B, and C. In this model, A promotes both B and C directly, and B promotes C. This motif has been identified in several instances in biology, usually as a part of a more complex system, such as in the regulation of cancer-associated genes [1], transcriptional regulation in *E. coli* [10], and cytokine signalling [5].

The model equations are

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix}A\\B\\C\end{bmatrix} = \begin{bmatrix}-a_1 & 0 & 0\\ b_1 & -b_2 & 0\\ c_1 & c_2 & -c_3\end{bmatrix}\begin{bmatrix}A\\B\\C\end{bmatrix} + \begin{bmatrix}a_0\\b_0\\0\end{bmatrix} + \begin{bmatrix}u_a(t)\\u_b(t)\\u_c(t)\end{bmatrix}, \quad \begin{bmatrix}A(0)\\B(0)\\C(0)\end{bmatrix} = \begin{bmatrix}A_0\\B_0\\C_0.\end{bmatrix} \tag{S.3}$$

The state variables are $\boldsymbol{x} = (A, B, C)$, the control inputs are $\boldsymbol{u} = (u_a, u_b, u_c)$, and the model parameters are

$$\boldsymbol{\theta} = (a_0, a_1, b_0, b_1, b_2, c_1, c_2, c_3, A_0, B_0, C_0).$$

All parameters are non-negative. We assume that A and C, but not B, can be directly measured, giving the observation model

$$g(\boldsymbol{x}; \boldsymbol{\theta}) = \begin{bmatrix}A\\C\end{bmatrix}. \tag{S.4}$$

As this is a conceptual model, all quantities are considered to be non-dimensional. In the absence of control, this model is structurally non-identifiable, since A can impact C both directly, and indirectly through B. Without measurements of B, it is difficult to distinguish between the two effects. It can be shown that the parameters a_0, a_1, A_0, C_0 are structurally identifiable, but the model on the whole is not (see Supplementary Materials S.3). The analysis also shows that there are a further five structurally identifiable combinations of parameters, but they are too cumbersome to be of any practical use.

For experimental design, we use the following "ground truth" parameter values,

$$\begin{aligned}&(\hat{a}_0, \hat{a}_1, \hat{b}_0, \hat{b}_1, \hat{b}_2, \hat{c}_1, \hat{c}_2, \hat{c}_3, \hat{A}_0, \hat{B}_0, \hat{C}_0) = (1,\ 0.1,\ 0,\ 0.6,\ 0.4,\ 0.3,\ 0.4,\ 1,\ 1,\ 0,\ 0),\\ &T = 50, \quad \Delta t = 0.5, \quad \sigma = 0.3.\end{aligned} \tag{S.5}$$

Given the three possible control inputs u_a, u_b, u_c, for this simple demonstration, we consider applying one of them at a time, and we will consider only the case of continuous control. In Fig. S.1, we present the profile likelihoods of the model parameters of interest when these controls are applied. For this cursory exploration, we choose, more or less arbitrarily, $\tau_0 = 10$ and $\tau = 20$. We will discuss how to choose τ_0 and τ appropriately in the next example. The focus of this example is to demonstrate that the control inputs are not equally effective, and certain control inputs result in greater improvement in practical identifiability compared to others. We take $u_{a,\max} = 2, u_{b,\max} = 18, u_{c,\max} = 18$. Under these choices, the three possible control inputs elevate the equilibrium for C by the same amount, which makes for a fair comparison.

Fig. S.1 shows how each of these controls impacts the practical identifiability of the model. Observe that when no control inputs are applied (Fig. S.1(a)), the profile likelihood for c_2 appears perfectly flat. This indicates that this parameter is structurally non-identifiable, so a shift in the value of c_2 can be perfectly compensated for by an appropriate shift in the value of the other parameters, resulting in an identical model solution. Furthermore, while the parameters c_1 and c_3 appear to be practically identifiable, their confidence intervals are very wide. This would limit the usefulness of the estimates for these parameters. The MLE for c_1 is at zero, which misleadingly suggests that A does not influence C directly.

When u_a is applied (Fig. S.1(b)), the shape of the profile likelihood of c_1 changes so that it is now a roughly parabolic curve centered near $\hat{c}_1 = 0.3$. This parameter describes the rate at which A up-regulates C, so it is unsurprising that manipulating the level of A allows us to better pin down the value of c_1. The profile likelihoods of c_2 and c_3 remain qualitatively the same as in the no-control case (Fig. S.1(a)).

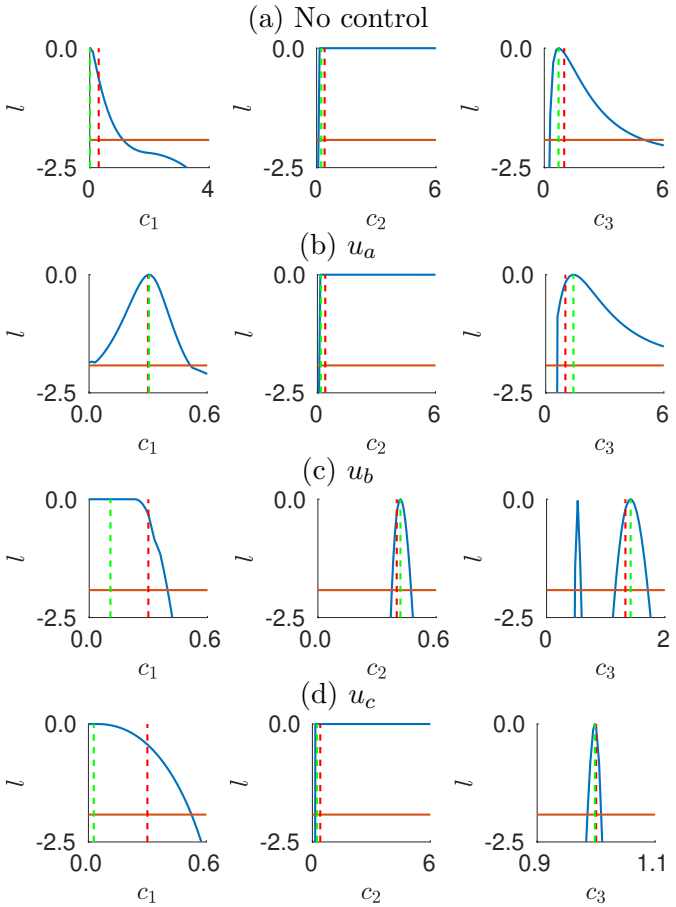

Figure S.1: Profile likelihoods for the parameters c_1, c_2 and c_3 in the signalling pathway model (Eq. (S.3), (S.4)), computed using synthetic datasets generated with the parameter values in Eq. (S.5). (a) No control is applied. (b-d) one of u_a, u_b or u_c is applied from $\tau_0 = 10$, for a period of $\tau = 20$. The magnitudes of the controls are $u_{a,\max} = 2, u_{b,\max} = 18, u_{c,\max} = 18$, respectively. The green dashed lines denote the MLE, and red dashed lines denote the "ground truth" parameter values, and the orange horizontal line indicates the cut-off for the 95% confidence interval. Note that the range of the x-axes varies between the plots.

When u_b is applied (Fig. S.1(c)), all three parameters are practically identifiable. However, some

identifiability issues remain. For example, the profile likelihood for c_1 is nearly perfectly flat for $0 \leq c_1 \leq 0.2$, so all values in that interval are equally valid as an estimate for c_1. Furthermore the profile likelihood for c_3 is bimodal, so the resulting confidence region is a union of two disjoint intervals, and we cannot tell which peak corresponds to the true value of the parameter. Despite these issues, the case of applying u_b is the only case where all three parameters have finite confidence regions.

When u_c is applied (Fig. S.1(d)), c_1 and c_3 are identifiable, while c_2 is not. The main improvement compared to the no-control case (Fig. S.1(d)) is that the confidence interval for c_3 is now much narrower. These results suggest that applying the control u_b is the most effective for improving the identifiability of the model, as it is the only control input that results in all three parameters being practically identifiable. Since the value of u_b is known to the experimenter, applying u_b is akin to an indirect measurement of B. Since the non-observability of B is the main source of non-identifiability in this model, it is not surprising that u_b is the best choice for the control input. However, u_a and u_c would be better choices for a study focused on measuring the values of c_1 and c_3, respectively.

S.3 Structural identifiability analysis

In this section, we review the definition of structural identifiability, and present the result of structural identifiability analysis for the models considered in this paper. The definitions given below follow [3, 4]. An ODE model (Eq. (1)) with an observation model (Eq. (2)) is called

- globally structurally identifiable, if for almost any $\boldsymbol{\theta} \in \Theta$,

$$\boldsymbol{y}(t;\boldsymbol{\theta}) = \boldsymbol{y}(t;\boldsymbol{\theta}') \;\forall\; t \Rightarrow \boldsymbol{\theta} = \boldsymbol{\theta}';$$

- locally structurally identifiable, if for almost any $\boldsymbol{\theta} \in \Theta$, there exists an $\epsilon > 0$ such that

$$\boldsymbol{y}(t;\boldsymbol{\theta}) = \boldsymbol{y}(t;\boldsymbol{\theta}') \;\forall\; t, \text{ and } ||\boldsymbol{\theta} - \boldsymbol{\theta}'|| < \epsilon \Rightarrow \boldsymbol{\theta} = \boldsymbol{\theta}';$$

- structurally non-identifiable, if it is not locally structurally identifiable.

Note that under this definition, globally structurally identifiable is a strictly stronger property than locally structurally identifiable.

S.3.1 Logistic and Richards models

We will use the Taylor series method [8] to perform structural identifiability analysis for the logistic and Richards models, which can be summarised as a single equation,

$$\frac{\mathrm{d}C}{\mathrm{d}t} = rC\left[1 - \left(\frac{C}{K}\right)^{\gamma}\right] - \delta C. \tag{S.6}$$

The logistic model has $\gamma = 1$, and the Richards model has $\gamma > 0$. We will use a prime ($(\cdot)'$) to denote a derivative with respect to time in this section. The Taylor series approach for structural identifiability analysis assumes that the solution is smooth and can be expressed as a Taylor series in terms of time,

and certain regularity conditions on the right-hand side of the ODE model hold. Under these assumptions, [8] showed that the model is structurally identifiable if the parameters can be uniquely determined if measurements of the system and all its derivatives in time at $t = 0$ are given, that is, $C(0)$ and $C^{(k)}(0)$ for $k \geq 1$. Knowing the values of these derivatives is equivalent to knowing the coefficients of the Taylor series of the model solution at $t = 0$. In practice, we attempt to write the model parameters (or their combinations) as functions of $C(0)$, $C'(0)$, and the higher order derivatives, which are considered known quantities. If a parameter, or a parameter combination, can be written entirely in terms of known quantities, then they are at least locally structurally identifiable. If a parameter or parameter combination cannot be expressed in terms of known quantities alone, then they are structurally non-identifiable.

The leading order derivatives of the model solution can be written as:

$$C(0) = C_0 \tag{S.7a}$$

$$C'(0) = rC_0(1 - (C_0/K)^\gamma) - \delta C_0 \tag{S.7b}$$

$$C''(0) = C'(0)[r - r(1+\gamma)(C_0/K)^\gamma - \delta] \tag{S.7c}$$

$$C'''(0) = C''(0)^2/C'(0) - C'(0)^2[r(\gamma+1)\gamma C_0^{\gamma-1}/K^\gamma] \tag{S.7d}$$

$$C''''(0) = \begin{cases} A - C'(0)^2[r(\gamma+1)\gamma(\gamma-1)C_0^{\gamma-2}/K^\gamma] & \text{if } \gamma \neq 1 \\ A & \text{if } \gamma = 1 \end{cases}, \tag{S.7e}$$

where A denotes terms that can be written exclusively using known quantities. For both the Richards and logistic models, Eq. (S.7a) shows that the initial condition, C_0, can be determined from data, if it was not already specified.

For the Richards model, Eq. (S.7d) and Eq. (S.7e) together provide two polynomial constraints on γ and the parameter combination r/K^γ, which means that in general, there are at most a finite number of solutions for both, so γ and r/K^γ are at least locally identifiable. Given values for γ and r/K^γ, Eq. (S.7c) allows $r - \delta$ to be uniquely identified, so $r - \delta$ is at least locally identifiable. However, the parameters r, K, δ cannot be identified individually, since r always appears in the combinations of either $r - \delta$ or r/K^γ in Eq. (S.7b) or the higher derivatives, so the Richards model is overall non-identifiable. If the explicit death term is not present, or equivalently, $\delta = 0$ is fixed, then all parameters are at least locally identifiable.

For the logistic model, which has $\gamma = 1$ fixed, Eq. (S.7d) shows that the parameter combination r/K can be determined from data, which together with Eq. (S.7c), allows $r - \delta$ to be uniquely determined. None of the three parameters can be uniquely determined individually, for the same reason as for the Richards model. Therefore, the logistic model is structurally non-identifiable. However, knowing the value of one of r, δ, K will allow the other two parameters to be uniquely identified. Therefore, the logistic model without the explicit death term (Eq. (S.6) with $\gamma = 1$ and $\delta = 0$ fixed) is globally identifiable.

S.3.2 Signalling pathway model

We again use the Taylor series method to perform structurally identifiability analysis for the signalling pathway model (Eq. (S.3), (S.4)). It has been shown that, for linear models such as the signalling pathway model, we need to compute the Taylor series to at most $2n_x - 1$ degrees to determine structural

identifiability [11], where n_x is the number of state variables. For the model at hand, this means we need to consider up to order 5.

The constant term in the Taylor series for the observable state variables A, C are A_0 and C_0, respectively, therefore these two parameters are structurally identifiable. The coefficients of the Taylor series for $A(t)$ are

$$A'(0) = -a_1 + a_0, \tag{S.8a}$$

$$A''(0) = a_1^2 - a_0 a_1, \tag{S.8b}$$

$$\frac{\mathrm{d}^k}{\mathrm{d}t^k} A(0) = (-1)^n \left[a_1^k - a_1^{(k-1)} a_0 \right]. \tag{S.8c}$$

Eqs. (S.8a) and (S.8b) imply that there are at most two possibilities for a_0 and a_1. Under conditions of genericity, one of these possibilities can be eliminated using Eq. (S.8c), therefore a_0 and a_1 are at least structurally locally identifiable, and are globally identifiable in generic cases.

The first two coefficients of the Taylor series for $C(t)$ are

$$C'(0) = c_2 B_0 + c_1, \tag{S.9a}$$

$$C''(0) = c_1(-a_1 + a_0 - c_3) + c_2(b_1 + b_0) - (b_2 + c_3) c_2 B_0, \tag{S.9b}$$

and the coefficients of the higher order terms are increasingly complicated. Each of these coefficients, up to the fifth one, represents a structurally identifiable combination of parameters. Since there are seven remaining parameters $(b_0, b_1, b_2, c_1, c_2, c_3, B_0)$, which is more than the number of constraints (five), the model is structurally non-identifiable.

S.4 Direct control as an alternative to PMP

In this section, we discuss direct control as an alternative to PMP for the purpose of optimally designing an experiment for discriminating between two models. Direct control, in this context, means solving an infinite-dimensional functional optimisation problem by applying a discretisation scheme, resulting in a discrete-time problem involving only finitely many variables, that approximates the original problem. This finite-dimensional problem is then solved using a general-purpose optimisation algorithm. We discretise the time interval $[0, T]$ into n_t evenly spaced points $t_i = i\Delta t$, $i = 1, \ldots, n_t$, where $\Delta t = T/n_t$. The control $u(t)$ is parameterised as its values at t_i, interpolated linearly between these values, where we choose $n_t = 100$. We use *fmincon* with the default interior point algorithm [2], with a termination criterion based on the Karush-Kuhn-Tucker (KKT) first-order optimality measure, which is akin to the first derivative condition in the presence of constraints. This is a reasonable choice, as the interior-point algorithm was found to be suitable for directly optimising a variety of control problems in [6]. We initialise the optimisation at $u(t) \equiv 0$ when applying direct optimisation alone.

The caveat of direct optimisation is that a general-purpose algorithm cannot exploit the structure of the original control problem. We also need a sufficiently large n_t for the discrete-time system to be able to approximate the original continuous-time system well, which means that the discrete-time optimisation problem is very high-dimensional, and we expect the optimisation algorithm to converge slowly, and to

be sensitive to the choice of initial point where we start our search. To partially mitigate these problems with direct optimisation, we also consider a hybrid method, where we initialise the aforementioned direct optimisation procedure at the output of the FBS algorithm.

To illustrate these approaches, we revisit the problem of discriminating between two logistic models in Eq. (21). The outputs of direct optimisation and the hybrid methods are given in Fig. S.2, along with the output of the FBS algorithm, originally given in Fig. 5. Notice that the optimal controls given by all three methods are very close. There are some noticeable numerical artifacts in the form of oscillations in the output of the direct optimisation procedure. The FBS algorithm terminated after 73 iterations, and produced a control with the objective value of $J = -784470$ (cells/mm^2)^2. Direct optimisation terminated after 991 iterations of *fmincon*, although each of its iterations, which only require evaluating the objective function J and its partial derivatives with respect to the discretised control variables, is much faster than one iteration of FBS. Its output achieved an objective value of $J = -782476$ (cells/mm^2)^2, which is close to, but a little worse, than that of the FBS algorithm. Finally, the hybrid method required 67 iterations of *fmincon* (in addition to the computation of the FBS algorithm), and achieved an objective value of $J = -785081$ (cells/mm^2)^2, the best out of the three methods.

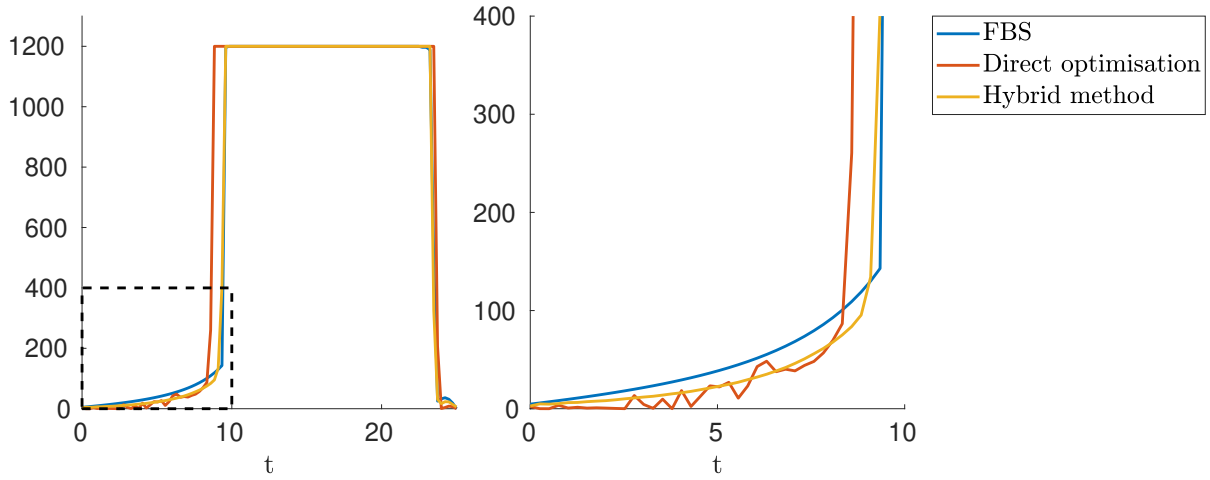

Figure S.2: Results from applying the FBS algorithm, direct optimisation, and the hybrid method to the optimal control problem in Eq. (19). The plot on the right is a zoomed-in version of the area inside the dashed rectangle in the plot on the left, where the three solutions noticeably differ. The control found by the FBS algorithm is the same as the one presented in Fig. 5. The units are $[t] = \text{h}, [u_K] = \text{cells/mm}^2$.

This example show that the FBS algorithm should be preferred over the direct optimisation algorithm with naive or uninformed initialisation. However, direct optimisation can be initialised at the output of the FBS algorithm to further refine the control if desired.

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