Identifiability, reducibility, and adaptability in allosteric macromolecules

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The ability of macromolecules to transduce stimulus information at one site into conformational changes at a distant site, termed “allostery,” is vital for cellular signaling. Here, we propose a link between the sensitivity of allosteric macromolecules to their underlying biophysical parameters, the interrelationships between these parameters, and macromolecular adaptability. We demonstrate that the parameters of a canonical model of the mSlo large-conductance Ca2+-activated K+ (BK) ion channel are non-identifiable with respect to the equilibrium open probability-voltage relationship, a common functional assay. We construct a reduced model with emergent parameters that are identifiable and expressed as combinations of the original mechanistic parameters. These emergent parameters indicate which coordinated changes in mechanistic parameters can leave assay output unchanged. We predict that these coordinated changes are used by allosteric macromolecules to adapt, and we demonstrate how this prediction can be tested experimentally. We show that these predicted parameter compensations are used in the first reported allosteric phenomena: the Bohr effect, by which hemoglobin adapts to varying pH.

INTRODUCTION

Cellular signaling relies on macromolecules to transduce stimuli into information about conformational changes (Changeux and Edelstein, 2005). The mechanisms by which macromolecules accomplish this feat are often allosteric: a small stimulus applied at one area of the macromolecule regulates behavior at locations structurally distant from the active site of stimulation. A detailed and mechanistic understanding of allosteric regulation is a major goal of biophysics (Changeux, 2012, 2013). The Monod-Wyman-Changeux (MWC) model, which provides a physical-chemical interpretation of indirect regulation in terms of the geometry of the regulatory molecule (Monod et al., 1963, 1965; Marzen et al., 2013), has emerged as an essential tool in this effort. Operationally, any given MWC model represents a candidate hypothesis for how allosteric conformational change occurs. If a model is not able to quantitatively fit available data, it is rejected. For models that agree with the data, the model parameter values provide estimates of biophysically meaningful properties that cannot be measured directly. Tremendous effort has gone toward determining which mechanistically relevant parameters best fit available macromolecular data (Colequhoun and Hawkes, 1982, 1995; Horn and Lange, 1983; Blatz and Magleby, 1986; Ball and Sansom, 1989; Kienker, 1989; Ball and Rice, 1992; Colequhoun and Sigworth, 1995; Qin et al., 1996, 2000; Colquhoun et al., 2003; Celentano and Hawkes, 2004; Milescu et al., 2005; Moffatt, 2007). However, it has recently been observed that even simple MWC models suffer from parameter non-identifiability: the data in commonly used activity (or binding) curves do not provide sufficient constraining power to find unique values of the parameters, even if essentially noiseless (Hines et al., 2014; Middendorf and Aldrich, 2017a,b).

Essentially all work on parameter estimation in MWC models has treated non-identifiability as a hurdle to be overcome toward the estimation of individual MWC parameter values, which clearly confer mechanistically meaningful information about the macromolecule under study. Here, we argue that valuable mechanistic information may be lost by focusing on individual parameter values. We demonstrate that non-identifiable datasets admit identifiable “emergent” parameters and argue that these emergent parameters confer mechanistic information about macromolecular function not available from individual parameter values themselves, no matter how accurate.

We begin by studying the causes of parameter non-identifiability in a canonical MWC model of the mSlo large-conductance Ca2+-activated K+ (BK) ion channel (Horrigan and Aldrich, 2002; Yan and Aldrich, 2010), with respect to two common assays of functional activity. The non-identifiability is shown...
to arise because the parameters compensate for each other to produce similar model output, rather than because the model contains irrelevant parameters. In particular, we demonstrate that observed parameter non-identifiability is caused by “sloppy” sensitivity of parameters to model output: the model output is highly sensitive to particular nonlinear combinations of parameters and essentially insensitive to others (Brown and Sethna, 2003; Waterfall et al., 2006; Gutenkunst et al., 2007; Transtrum et al., 2010, 2011, 2015).

We address the issue of parameter non-identifiability by constructing “reduced” models of the channel for each of the two functional assays, using the recently developed manifold boundary approximation method (MBAM; Transtrum and Qiu, 2014; Transtrum, 2016 Preprint). Each reduced model has fewer parameters than the original model but describe its functional data equally well. Crucially, the parameters of these reduced models are (a) identifiable with respect to the model output and (b) explicitly expressed as emergent combinations of the original MWC parameters. Our reduced models therefore allow for quantitative estimates of biophysically relevant parameters, despite individual parameter non-identifiability.

The emergent parameters of our reduced models indicate which coordinated changes in biophysical parameters may preserve assay output. We therefore interpret our reduced models as predictions encoded in the original MWC model. These predictions confer information about the robustness of a macromolecule’s underlying biophysical parameters with respect to a functional output. We claim that these predictions have physiological and evolutionary relevance, so long as the model is mechanistically relevant and the functional assay adequately captures the macromolecule’s in vivo function.

It is difficult to determine confidently that any given assay or set of assays captures a macromolecule’s in vivo behavior. Here, we choose to study equilibrium binding curves, thereby adopting the hypothesis that in vivo purpose of a macromolecule is to act as a biological sensor that takes stimuli information as input and produces equilibrium activity as output; this hypothesis is common in information-theoretical studies of MWC models (Tkacik et al., 2008; Martins and Swain, 2011; Olsman and Goentoro, 2016).

Ultimately, the relevance of our predicted parameter compensations, and thereby our assay choice, must be determined experimentally. To this end, we demonstrate that a previous meta-analysis of hemoglobin oxygen-binding curves confirms the physiological relevance of parameter compensations in hemoglobin’s adaptation to varying pH, referred to as the Bohr effect and known to be important for the efficient transportation of oxygen through blood in vivo.

MATERIALS AND METHODS

The full code used to generate the synthetic data and all analyses is available on GitHub: https://github.com/gbohner/MBAM.

Synthetic data generation

Our model takes as input $N$ voltage and calcium pairs as an array $X$, and an $M \times 1$ vector of parameters $\theta$. The output of the model is an $N \times 1$ vector of open probabilities $y$. We represent the model as the function $f(\theta, X) = y$, defined as in the text. Our “base” parameters $\theta^*$ (Fig. 2 B) were chosen to match previous studies (Horrigan and Aldrich, 2002; Miranda et al., 2013).

Fitting noisy data

Given a $\theta$ to be estimated, noisy data $y'$ were generated from mode $f(\theta, X)$ via

$$y' = f(\theta, X) + \epsilon(\sigma),$$

where $\epsilon(\sigma)$ is drawn from the distribution Uniform([1 − $\sigma$, 1 + $\sigma$]). Additive and multiplicative Gaussian noise models were also tested.

We then used the Levenberg-Marquardt solver (Marquardt, 1963) to infer the best-fit parameters $\theta^*$ = argmin$\theta$ $\mathcal{C}(\theta, y')$, where $\mathcal{C}(\theta, y') = \|f(\theta, X) - y'\|$ is the norm of the discrepancy between data and model output. Each fit was performed from 24 different initial parameter vectors, from which the global best-fit parameters were selected.

Computing relative parameter error

$\Sigma_i$, a lower bound on the relative size of the 95% confidence interval for the $i$th parameter, was calculated (Gutenkunst et al., 2007; Apgar et al., 2010):

$$\Sigma_i = \exp\left(4 \times \left(\frac{\sigma^2}{N} (H_\theta^*)_{ii}\right)^{1/2}\right) - 1,$$

where

$$H_\theta = \frac{d^2}{d\log \theta^2} \mathcal{C}(\theta, y).$$

The log Hessian $H_\theta$ is used to estimate the widths of the constant cost ellipsoid (Fig. 3 B), and the diagonal elements of its inverse are used to place a lower bound on individual relative parameter errors, $\Sigma_i$. For all of our calculations, $N = 104$ and $\sigma = 10\%$.

MBAM

MBAM attempts to reduce the number of parameters in a model while quantitatively fitting a given dataset. This is accomplished by reducing the number of parameters in a model one at a time. Each single parameter reduction is the result of eliminating or combining diverging
parameters. These diverging parameters are found by searching through parameter space along a trajectory that minimizes the effect on model output. Such a trajectory is found by solving the following system of ordinary differential equations,

\[
\begin{align*}
\frac{d}{dt} \theta_t &= v_t \\
\frac{d^2}{dt^2} \theta_t &= \left[ \nabla r_t \left( \nabla r_t \right)^\top \right]^{-1} \frac{\nabla r_t v_t}{\|v_t\|^2} \left[ \nabla r_t \right] \left( \nabla r_t \right)^\top
\end{align*}
\]

where \( t \) denotes time spent following the trajectory, \( H_\theta \) refers to the log Hessian evaluated at \( \theta \), as defined above, \( r_t = f(\theta_t, X) - f(\theta^*, X) \) is the vector of output discrepancies for all inputs, and \( \nabla \) denotes the vector gradient operation with respect to the parameters.

The initial parameter vector \( \theta_0 \) is taken to be the vector of presumed best-fit parameters \( \theta_0 = \theta^* \), and the initial direction vector \( (v_0) \) is set to be the sloppiest direction of the constant cost ellipsoid. These initial conditions enforce that our search begins at the lowest point of our cost surface \( (\theta_0) \) and that we initially move in the direction that goes uphill as little as possible \( (v_0) \). This trajectory is followed until the diverging parameters are found \( (\theta_{t\text{end}}) \), or the cost becomes so large that the parameters no longer fit the data. The reader is referred to Transtrum (2016) (Preprint) and Transtrum and Qiu (2014, 2016) for full details.

**Online supplemental material**

Our supplemental text contains full algebraic details of the MBAM reductions presented in the paper. Fig. S1 shows RMS cost for each reduced model. Fig. S2 shows how parameter compensations explain the discrepancy between true and inferred values. Fig. S3 shows inferred parameters from the reduced \( \log(P_o) \) model.

**RESULTS**

The BK channel primarily senses two stimulus signals: membrane voltage and intracellular \( \text{Ca}^{2+} \) concentration (Horrigan and Aldrich, 2002; Latorre and Brauchi, 2006; Yan and Aldrich, 2010; Miranda et al., 2013). In response to these signals, BK opens its channel gate, allowing potassium ions to permeate. We consider a canonical model of BK gating (Horrigan and Aldrich, 2002; Yan and Aldrich, 2010), shown schematically in Fig. 1 A. The model consists of three functional domains: the channel gate, voltage-sensing domain, and \( \text{Ca}^{2+} \)-sensing domain. The channel gate is regulated by four identical and independent voltage and \( \text{Ca}^{2+} \) sensors. Consistent with the MWC framework, each domain can be in one of two conformations: C-O, R-A, X\cdot \text{Ca}^{2+}\cdot \text{X} for the gate, voltage, and \( \text{Ca}^{2+} \) subunits, respectively. The function of each domain is defined by an equilibrium constant \( (L, J, K) \), and the coupling between domains is mediated by allosteric factors \( (C, D, E) \). Formally, the model is given by

\[
P_i(\text{V}, [\text{Ca}^{2+}]) = \frac{L(1 + KC + JD + JKCD)}{L(1 + KC + JD + JKCD)^4 \times (1 + J + K + JK)^3},
\]

where \( i = 0, 1 \).
with equilibrium constants \( J, K, L \) given by

\[
L = L_0 \exp\left(\frac{-z_L V}{kT}\right); \quad J = J_0 \exp\left(\frac{-z_J V}{kT}\right); \quad K = \left[\frac{\text{Ca}^{2+}}{K_D}\right],
\]

where \( z_L \) and \( z_J \) are the partial charges associated with channel opening and voltage sensor activation, respectively. Synthetic data generated from this model is shown in Fig. 1 (B and C).

The biophysical parameters \( \{L_0, z_L, J_0, z_J, K_D, C, D, E\} \) in Eq. 1 have been estimated with a wide variety of experimental assays. We will focus on the identifiability of these parameters with respect to the steady-state open probabilities of BK at various voltages and Ca\(^{2+}\) concentrations. In the language of MWC models, this “\( P_o \)” curve corresponds to the ubiquitous activity or binding curve. We will also analyze a related common assay (Horrigan and Aldrich, 2002): the base 10 logarithms of the probabilities, \( \log(P_o) \), made possible by single-channel recordings that allow for the determination of very small steady-state open probabilities. We will see that the model exhibits non-identifiability with respect to both assays, but the non-identifiability is more severe for the \( P_o \) assay. We will demonstrate that this difference in non-identifiability implies that the \( \log(P_o) \) assay may be used to experimentally test for the presence of parameter compensations predicted to hold the \( P_o \) assay constant.

BK parameters are non-identifiable because of sloppiness

We begin by asking the following: how identifiable are the BK model parameters, with respect to each of the \( P_o \) and \( \log(P_o) \) assays? Fig. 2 illustrates the problem of non-identifiability via the \( P_o \) assay: noisy synthetic data generated from the BK model are well fit (within the 10% error bars) by parameter values far from the base values used to generate the data (Fig. 2, A and B). To get a sense of how widely the parameters inferred from noisy \( P_o \) data may vary, we fit the \( P_o \) model to 100 noisy synthetic \( P_o \) datasets (see Materials and methods) generated from the base parameters. We found that the best-fit parameters to noisy data spanned many orders of magnitude; the inferred best-fit parameters for \( L_0, D \) are shown in Fig. 2 (C and D). We investigated BK model identifiability systematically by computing lower bounds on the relative parameter error (see Materials and methods) around the base parameter values. Many parameters have significant errors with respect to each assay (Fig. 3 A). The model suffers from non-identifiability.

It has been argued that non-identifiability in scientific models arises because of sloppiness: the model output is extremely sensitive to some combinations of parameters but dramatically insensitive to other combinations (Gutenkunst et al., 2007; Machta et al., 2013). We there-
before ask: do either of the \( P_o, \log(P_o) \) assays exhibit sloppiness with respect to the underlying BK model parameters?

Formally, sloppiness is a feature of a model’s approximate “surface of constant cost.” To understand sloppiness, it is therefore useful to consider a surface of constant cost for a toy two-parameter model. The center point of the ellipsoid are the best-fit parameters. The parameters \( \theta_{1,2} \) are constrained in the stiff direction, but have large error regions \( \Sigma_{1,2} \) because of the presence of a large sloppy direction. \( w_i \) denotes the length of axis \( i \). (C) Calculated \( \log(1/w_i^2) \) values for the \( P_o \) assay (red) and \( \log(P_o) \) assay (black). Both exhibit a linear trend, the signature of a sloppy model. The dashed line is a visual aid aimed to draw attention to the gap in the data points.

Figure 3. The BK model is sloppy. (A) Lower bounds on parameter error (95% confidence interval) for each of the \( P_o, \log(P_o) \) assays. The \( P_o \) assay exhibits much worse identifiability than the \( \log(P_o) \) assay. (B) Ellipsoid of constant cost for a toy two-parameter model. The parameters \( \theta_{1,2} \) are constrained in the stiff direction, but have large error regions \( \Sigma_{1,2} \) because of the presence of a large sloppy direction. \( w_i \) denotes the length of axis \( i \). (C) Calculated \( \log(1/w_i^2) \) values for the \( P_o \) assay (red) and \( \log(P_o) \) assay (black). Both exhibit a linear trend, the signature of a sloppy model. The dashed line is a visual aid aimed to draw attention to the gap in the data points.
with the fact that the model reduction procedure lever-
ages the geometry of the model parameter space to re-
move existing sloppiness.

A reduced model for the $P_o$ assay admits identifiable
emergent parameters

Having shown that the BK model has non-identifi-
able parameters because of sloppiness, we now aim to
exploit the model’s sloppiness to construct new BK
models whose parameters are both identifiable and
of mechanistic interest. To construct such models, we
use the MBAM (Transtrum and Qiu, 2014; Transtrum,
2016 Preprint).

The MBAM algorithm takes three items as input:
data, a mathematical model thought to describe the
data, and the parameters of the model thought to
best fit the data. The goal of the algorithm is to find
a point in parameter space having two properties: (1)
the model output generated by the parameters must fit
the data well; and (2) one or more parameters must be
divergent, having values close to 0 or $\infty$. To find such a
point, the algorithm searches through parameter space,
along a trajectory that minimizes the effect on model
output. The search terminates once divergent parame-
ters are found, and the model is reparameterized such
that no parameters equal to 0 or $\infty$ remain. The algo-

\[
\begin{align*}
\phi_1 &= \sqrt[4]{C_0 D} \\
\phi_4 &= CE \\
X &= K_0 \\
\end{align*}
\]

The model produced by the third reduction step (Fig.
4 A) has eliminated five parameters and introduced two new emergent parame-
ters, $\phi_1 = \sqrt[4]{C_0 D}$, $\phi_4 = CE$. The model produced by the
fourth step (Fig. 4 B, left) does not fit the data well at
low $[\text{Ca}^{2+}]$. Synthetic data are labeled as in Fig. 1 (B and C).

Figure 4. Results of model reduction for the $P_o$ assay. (A, left) Schematic of
the model admitted by the third reduc-
tion; five parameters have been elimi-
nated from the original model (red crosses) and two new emergent parame-
ters have been added ($\phi_{1,4}$) for a
total reduction of three parameters. (A, right) This model fits the data (solid
lines) extremely well. (B, left) Schematic
of model admitted by the fourth reduc-
tion. (B, right) This model does not fit
the data well at low $[\text{Ca}^{2+}]$. Synthetic
data are labeled as in Fig. 1 (B and C).

same data with the same initial conditions, it will return
the same reductions.

An overview of our MBAM output with respect to the
$P_o$ assay is shown in Fig. 4. MBAM was run on a set of
synthetic data points generated from the BK model and
initialized with the “true” BK model parameters that
generated these data points. The algorithm was termi-
nated after five iterations, each of which reduced the
number of model parameters by one. The first three
MBAM iterations produced models that fit the data
essentially exactly (Fig. S1). The model produced by
the third reduction step (Fig. 4 A) has eliminated five parameters and introduced two new emergent parame-
ters, $\phi_1 = \sqrt[4]{C_0 D}$, $\phi_4 = CE$. The model produced by the
fourth step (Fig. 4 B, left) does not fit the data well at
low calcium concentrations (Fig. 4 B, right).

To better understand how the reduced models arise,
we plotted the numerical values of the model param-
eters during MBAM searches (Fig. 5, left) and the re-
sulting reduced models (Fig. 5, right). Because MBAM
searches along a trajectory of essentially equivalent
model behavior, each x-axis “time point” corresponds to
a set of parameter values that produce essentially equiv-
alent model output (Fig. 5, left). The algorithm con-

continues searching until one or more parameters diverge
to zero or infinity (Fig. 5, red lines). It is interesting to
note that as $z_L$ goes to zero (Fig. 5 A, red line), several
nondivergent parameters must compensate (Fig. 5 A, curved black lines) to fit the synthetic data. Subsequent
reductions (Fig. 5, B and C) take place without such significant compensation from nondiverging parameters.

The algebraic reparameterizations that give rise to the reduced models in Fig. 5 are worked out in full in the Supplemental text. To understand the emergent parameters biologically, it is instructive to consider the third and "final" reduced model, which is algebraically governed by

$$P_o(V, [Ca^{2+}]) = \frac{(J\phi_1)^4(1 + K\phi_1)^4}{(J\phi_1)^4(1 + K\phi_1)^4 + (1 + J\phi_2 + K\phi_3)^4},$$

with equilibrium constants \(J, K\) given by

$$J = J_0 \exp\left(-\frac{z_J V}{kT}\right); \quad K = \frac{[Ca^{2+}]}{K_D}.$$ 

The emergent parameter \(\phi_4 = CE\) represents the effect of \([Ca^{2+}]\) binding on the voltage-sensing process, and \(\phi_1 = \sqrt{L_0 D}\) represents the coupling of voltage sensing to the pore opening. Additionally, the remaining original parameter \(K_0\) is the binding constant of the ligand, \(J_0\) governs the midpoint voltage of the voltage-activation curve, and \(z_J\) controls its slope.

We next ask: do the reduced models have identifiable parameters? To address this issue, we calculated lower bounds on the relative error for each of the reduced models and found that each reduction step leads to a model with fewer unidentifiable parameters (Fig. 6). It is important to note that although each model reduction step leads to a smaller number of non-identifiable parameters than the one before it, it is not the case that each parameter becomes more identifiable after each reduction step. In the first reduced model, \(L_0, C, D, E\) and \(J\) all have much greater relative errors than in the original model (compare Fig. 6 A [table] with Fig. 3 A). Care must therefore be taken in interpreting these intermediate models (Fig. 6, A and B).

In contrast, interpreting the third reduced model (Fig. 6 C) is clear. The model fits the data extremely well.
well (Fig. 4 A) and has parameters that are identifiable and expressed in terms of mechanistically meaningful parameters (Fig. 6 C, table). Variations in underlying MWC parameters that keep these emergent parameters constant may, but do not necessarily, leave assay output unchanged. Conversely, any significant parameter variation that leaves the \( P_o \) assay unchanged must keep the emergent parameters constant. Observe that the differences in base and fit parameters that give rise to the same dataset in Fig. 2 B do indeed keep the emergent parameters essentially constant. Relative to the base parameter, the fit parameter \( C \) increased by a factor of \( 10^8 \); correspondingly, the parameter \( E \) decreased by a factor of \( 10^8 \), consistent with constant emergent parameter \( \phi_4 = CE \). Likewise, the value of \( \sqrt{L_0} \) decreased by a factor of \( 10^8 \), and correspondingly the value of \( D \) increased by a factor of \( 10^8 \), consistent with constant emergent parameter \( \phi_1 = \frac{CE}{\sqrt{L_0}} D \).

We interpret the emergent parameters as predictions of the original MWC model about potential compensatory mechanisms of the macromolecule. How can we test for the presence of these parameter compensations experimentally? By definition, the compensations are not discernible from the \( P_o \) assay. To address this issue, we therefore turn to the \( \log(P_o) \) assay.

The \( \log(P_o) \) model reduction reveals parameter differences unidentifiable by \( P_o \)

Having found a reduced, identifiable model for the \( P_o \) assay, we now aim to find such a model for the \( \log(P_o) \) assay. Because the \( \log(P_o) \) assay was observed to have fewer “very sloppy” directions than the \( P_o \) assay (Fig. 3 C), we expected to find an identifiable model after fewer reduction steps than needed for the \( P_o \) assay. Indeed, MBAM produces an identifiable model after only one reduction step. In this model, each of the orig-
inal parameters is identifiable except for $z_L$, which has been eliminated (Fig. 7).

Can we use the reduced log($P_o$) model to detect the presence of large compensatory parameter changes that give rise to identical $P_o$ datasets? To address this issue, we generated test and base parameters that produced equivalent $P_o$ data (up to 10% error), but contained the type of large compensatory changes shown in Fig. 2 B. The test and base parameters were then used to generate noisy synthetic log($P_o$) data, to which the reduced log($P_o$) model was fit. Values of the base parameters inferred by the reduced log($P_o$) model varied many orders of magnitude less than when inferred from noisy $P_o$ data generated from the same parameters (compare the black points in Fig. 8 [A and B] with Fig. 2 [C and D]), reflecting the greater identifiability of the reduced log($P_o$) model.

This increase in identifiability allows the reduced log($P_o$) model to discern large compensatory parameter changes that leave the $P_o$ assay constant (Fig. 8, red points). Note that the mean of the inferred parameter values (Fig. 8, solid lines) did not match the true values of the underlying parameters (Fig. 8, dashed lines). This is expected because of compensations from the other parameters (Fig. S2) and does not affect discernibility. Our analysis is limited in that we did not exhaustively sample the parameter space. Rather, we examined several sets of test parameters and found that large compensatory variations from the base parameters were discerned via the reduced log($P_o$) model in all cases, even when the test parameters were degenerate (Fig. S3). We wish here only to make the point that the log($P_o$) assay can elucidate parameter variations hidden by the $P_o$ assay in practice, not that it must in general.

**DISCUSSION**

Our results demonstrate that reduced models produced by MBAM allow for quantitatively accurate parameter information to be gleaned from models that are non-identifiable with respect to most or all individual parameters. Because the emergent parameters of our reduced models indicate which coordinated changes in parameter values are necessary to preserve the output for a given assay, they may be interpreted as predictions of the MWC model about which biophysical properties can compensate for each other to maintain a functional role. For what purposes would MBAM-elucidated compensatory effects be used by allosteric macromolecules? We propose experiments to test the role of parameter compensation in functional and evolutionary adaptation, both of which have been previously connected to sloppiness (Daniels et al., 2008).

**Emergent parameters may facilitate functional adaptation**

It is well known that macromolecules exhibit some amount of functional robustness with respect to experimental perturbations of temperature, pH, salt conditions, and sequence structure (Rennell et al., 1991; Somero, 1995; Suckow et al., 1996; Guo et al., 2004; Weber and Pande, 2012). We propose that this functional robustness may arise from compensatory mechanisms of the type identified here. That is, changes in environment or sequence structure that have little effect on assay output should be observed to have large but compensatory effects in the mechanistic parameters, such that the value of MBAM-identified emergent parameters remain constant. In BK, for example, we...
may expect that a change in temperature or sequence that marginally effects the $P_o$ data nonetheless largely increases $C$ but equivalently decreases $E$.

To test for the presence of a compensatory effect, an experimentalist generally needs at least two assays: a “physiologically relevant” assay whose output should not change upon environmental manipulation, from which compensatory effects may be predicted, and one or more secondary assays, from which the differences in individual parameters can be observed. Obvious candidates for physiologically relevant assays include binding curves (as analyzed here) and physiologically and thermodynamically relevant functions thereof (Wyman, 1967; Di Cera, 1995; Chowdhury and Chanda, 2012).

For BK, our results demonstrate that the log($P_o$) assay serves as a good secondary assay for testing compensatory mechanisms predicted from the $P_o$ assay.

Measurements of hemoglobin’s oxygen-binding curve confirms that parameter compensations facilitate functional adaptation

How speculative is this prediction? On one hand, the existence of MBAM-identified parameter compensations is general. Compensations depend only on the underlying sloppiness of the model and assay, which have been demonstrated to be ubiquitous in multiparameter models across systems biology (Gutenkunst et al., 2007). On the other hand, the relevance of MWC parameter compensations in allosteric macromolecules is ultimately a question for experiment. To that end, we note that an extensive meta-analysis of hemoglobin’s oxygen binding confirms the physiological relevance of MWC parameter compensations (Milo et al., 2007).

Milo et al. (2007) analyzed hemoglobin binding curves under varying physiological conditions and in different mammals. To quantitatively study these binding curves, Milo et al. (2007) used an MWC model that assumed the hemoglobin tetramer to be in one of two conformations: relaxed or tense. In each conformation, all four subunits have the same independent affinity for oxygen. The dissociation constants for the relaxed and tense states are $K_R$, $K_T$, respectively. The equilibrium constant between the fully deoxygenated tense and relaxed states is $L_0$. The three mechanistic parameters $K_R$, $K_T$, $L_0$ fully parameterize the model.

The authors found that this three-parameter model was non-identifiable with respect to the saturation curve. Using limit-style arguments that are possible for simple models and essentially equivalent to MBAM (Transtrum and Qiu, 2016), the model was reparameterized to have the emergent, identifiable parameters

$$L_4 = L_0 \cdot \frac{K^4}{K^3} \quad L_{MBR} = L_0 K_0.$$ (3)

The authors demonstrated analytically that each emergent parameter has a clear physiological interpretation: $L_{MBR}$ controls the half-saturation point of oxygen binding $p_{0.5}$; $L_4$ controls the cooperativity at this point.
Moreover, the authors found that \( n \) remained constant for human hemoglobin at varying pH (Fig. 9 A).

The results of Milo et al. (2007) therefore present an exceptionally simple framework in which to test our prediction. The \( n \) assay remains constant upon physiological variation and is controlled by the single emergent parameter \( L_4 \). Is this assay constancy the result of compensations of the underlying MWC parameters, as we predict?

Indeed it is. Across varying pH conditions, \( n \) stays constant while \( p_{50} \) varies. Correspondingly, \( L_4 \) stays constant as \( L_{70R4} \) varies (Fig. 9 C). It is immediate from the definitions of \( L_4 \) and \( L_{70R4} \) (Eq. 3) that in order for \( L_4 \) to remain constant as \( L_{70R4} \) varies, \( K_I^4 \) must compensate for changes in \( L_0 \cdot K_R^4 \).

**Emergent parameters may identify evolutionary relevant “neutral spaces”**

There is now significant theoretical (Draghi et al., 2010) and experimental (Hayden et al., 2011) evidence, including in allosteric proteins (Raman et al., 2016), that biological systems may evolve by drifting in genotype space such that their primary function remains unchanged. This is known as “neutral evolution” (Wagner, 2005, 2008). Neutral evolution proceeds via mutations that leave current function unchanged but may be advantageous in a subsequent environment. Because the utility of these mutations do not manifest until the correct environment arises, they are said to be cryptic. The spaces of sequences, parameters, or network topologies that give rise to equivalent behaviors are known as neutral spaces (Daniels et al., 2008).

Emergent parameters identified here may define neutral spaces. In particular, cryptic mutations in allosteric macromolecules may give rise to cryptic MWC parameter variations—large but compensatory effects in mechanistic parameters such that the value of MBAM-identified emergent parameters remain constant. This proposal can be tested by searching for cryptic MWC parameter changes between variants of an allosteric macromolecule within and across populations.

At first glance, the study of Milo et al. (2007) provides a way to interrogate this prediction as well. Milo et al. (2007) noted that the assay \( p_{50} \) stayed relatively constant upon physiological variation and is controlled by the single emergent parameter \( L_4 \). Is this assay constancy the result of compensations of the underlying MWC parameters, as we predict?

Indeed it is. Across varying pH conditions, \( n \) stays constant while \( p_{50} \) varies. Correspondingly, \( L_4 \) stays constant as \( L_{70R4} \) varies (Fig. 9 C). It is immediate from the definitions of \( L_4 \) and \( L_{70R4} \) (Eq. 3) that in order for \( L_4 \) to remain constant as \( L_{70R4} \) varies, \( K_I^4 \) must compensate for changes in \( L_0 \cdot K_R^4 \).

**Emergent parameters may identify evolutionary relevant “neutral spaces”**

There is now significant theoretical (Draghi et al., 2010) and experimental (Hayden et al., 2011) evidence, including in allosteric proteins (Raman et al., 2016), that biological systems may evolve by drifting in genotype space such that their primary function remains unchanged. This is known as “neutral evolution” (Wagner, 2005, 2008). Neutral evolution proceeds via mutations that leave current function unchanged but may be advantageous in a subsequent environment. Because the utility of these mutations do not manifest until the correct environment arises, they are said to be cryptic. The spaces of sequences, parameters, or network topologies that give rise to equivalent behaviors are known as neutral spaces (Daniels et al., 2008).

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constant across 25 different mammals, measured at the same physiological conditions (Fig. 9 B). So too did the emergent parameter that controls it: \( L_{\text{thr}} \) (Fig. 9 D). This is indeed what we would expect if the emergent parameter identified a neutral space.

Unfortunately, the study by Milo et al. (2007) cannot be used to confirm the presence of a neutral space. If the \( L_{\text{thr}} \) constancy reflected a neutral space, we would expect \( L_0 \) and \( K_R \) to vary cryptically between species. Because \( L_0 \) and \( K_R \) cannot be estimated from the available data, we cannot determine whether \( p_{20} \) ’s constancy across species results from this cryptic variation or from \( L_0 \) and \( K_R \) each remaining constant. The connection between emergent parameters and neutral spaces remains speculative, though we suspect it may prove an interesting source of future work.

Conclusions

We have argued that parameter compensation underlies both non-identifiability and adaptability in allosteric macromolecules. We therefore contend that non-identifiability should be expected in any adaptable allosteric macromolecule, so long as the model is mechanistically relevant and the functional assay adequately captures the macromolecule’s function.

Non-identifiability does not itself imply that a model or assay is physiologically relevant. Experimentally verifying that a predicted parameter compensation is actually used, as we have done with hemoglobin and its cooperativity at the half saturation point of oxygen binding, \( n \), is paramount. It must also be kept in mind that observed parameter compensations may be spurious rather than meaningful and must be assessed in context. In hemoglobin, the mechanistic relevance of the model parameters and clear physiological relevance of the half saturation point of oxygen binding support the relevance of the compensation we identified.

The enormous success of MWC models in describing allosteric macromolecules presents a wide array of opportunities for further experimentally testing the functional utility of parameter compensations. We anticipate that reduced models will prove essential for extracting mechanistically meaningful quantitative information from MWC models and provide a wealth of readily falsifiable predictions about the emergent properties of allosteric macromolecules.

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References


SUPPLEMENTAL MATERIAL

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In this Supplemental text, we demonstrate how the reduced MBAM models shown in Fig. 5 are derived algebraically. The Supplement should be read alongside Fig. 5.

Recall that our full model is given by

$$P(V, [Ca^{2+}]) = \frac{L(1 + J + K + JKCDE)^4}{L(1 + J + K + JKCDE)^4 + (1 + J + K + JKCDE)^4},$$

with equilibrium constants $(J, K, L)$ given by

$$L = L_0 \exp\left(-\frac{-2zLV}{kT}\right); J = J_0 \exp\left(-\frac{-zLV}{kT}\right); K = \frac{[Ca^{2+}]}{K_0}.$$

Our goal is to modify this model after each MBAM run such that no parameters equal to $\infty$ or zero remain. As we will see, our main two tools to accomplish this will be taking limits to zero and introducing new, finite parameters.

The first MBAM run sends $z_L \to 0$ (Fig. 5 A). The equation above shows that algebraically, $z_L \to 0$ sends $L \to L_0$. Taking this limit gives the equation for our once reduced model:

$$P(V, [Ca^{2+}]) = \frac{\left(\frac{\sqrt{L_0} + \sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}{\sqrt{L_0} + \sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}\right)^4}{\left(\frac{\sqrt{L_0} + \sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}{\sqrt{L_0} + \sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE} + 1 + J + K\right)^4},$$

with equilibrium constants $(J, K)$ given by

$$J = J_0 \exp\left(-\frac{-zLV}{kT}\right); K = \frac{[Ca^{2+}]}{K_0}.$$

Note that in Eq. S2 above, we have distributed the $L_0$ term inside of the brackets.

The second MBAM run sends $L_0 \to 0$, $E \to 0$, $C \to \infty$, $D \to \infty$ (Fig. 5 B). First, we take limits where we can, to remove parameters that have gone to zero. $L_0 \to 0$ and $E \to 0$ dictate that the lone $\sqrt{L_0} \to 0$ and $JKE \to 0$. This leaves

$$P(V, [Ca^{2+}]) = \frac{\left(\frac{\sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}{\sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}\right)^4}{\left(\frac{\sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE}{\sqrt{L_0}KC + \sqrt{L_0}JD + \sqrt{L_0}JKCDE} + 1 + J + K\right)^4},$$

(S3)

As written, this equation has infinite parameters $C, D$. The goal now is to introduce new finite parameters that can be used to create Eq. S3. What exactly do we mean by finite parameters? Fig. 5 B shows that $\sqrt{L_0}$ and $E$ go to zero at the same speed that $C$ and $D$ go to $\infty$. Because this same-speed divergence is happening in log space, we know that the product of $C$ or $D$ with either $\sqrt{L_0}$ or $E$ will be finite. If the divergence were happening in linear space, the corresponding sums (rather than products) would be finite.

It is also worth emphasizing that we work with $\sqrt{L_0}$ rather than $L_0$ is precisely because $\sqrt{L_0}$ diverges at the same speed as $C$ and $D$, whereas $L_0$ diverges four times faster than $C, D$.

We define the emergent parameters

$$\phi_1 = \sqrt{L_0}D, \phi_2 = \sqrt{L_0}C, \phi_3 = \frac{E}{\sqrt{L_0}},$$

which are all finite: $\phi_1, \phi_2$ for the reasons above and $\phi_3$ because $E$ and $\sqrt{L_0}$ moving to zero at the same rate in log space means that the ratio is always finite.

Crucially, these emergent parameters can be substituted into Eq. S3, leaving no infinite parameters remaining. The terms $\sqrt{L_0}C, \sqrt{L_0}D$ show up directly in Eq. S3, so the substitution is easy. Less trivially, the $JKE$ term in Eq. S3 can be written as a product of $J, K$ and our three finite, emergent parameters:

$$\sqrt{L_0}JKE = J \cdot K \cdot \frac{1}{\sqrt{L_0}} C \cdot \frac{1}{\sqrt{L_0}} D \cdot \frac{E}{\sqrt{L_0}}.$$

So Eq. S3 written in terms of our finite emergent parameters is

$$P(V, [Ca^{2+}]) = \frac{(K\phi_2 + J\phi_1 + JKC\phi_3)\phi_1^4}{(K\phi_2 + J\phi_1 + JKC\phi_3)\phi_1^4 + (1 + J + K)^4},$$

(S4)
The third and final MBAM run sends $\phi_2 \rightarrow 0$ and $\phi_3 \rightarrow \infty$ and (Fig. 5 C). Taking limits in Eq. S4, we have that $K\phi_2 \rightarrow 0$. We now need to get rid of the infinite term $\phi_3$. To do so, we note that the product $\phi_4 = \phi_2 \phi_3 = CE$ is finite and can be used to produce Eq. S4. This gives our final reduced model:

$$P_0(V, [Ca^{2+}]) = \frac{(J\phi_1 + JK\phi_4)^4}{(J\phi_1 + JK\phi_4)^4 + (1 + J + K)^4},$$

with equilibrium constants $(J, K)$ given by

$$J = J_0 \exp\left(-\frac{zJ V}{kT}\right); K = \frac{[Ca^{2+}]}{K_0},$$

from which Eq. 2 in the main text follows immediately.

Figure S1. RMS cost for each reduced model. Note a small uptick in error for the fourth reduction, consistent with the poor fit to low $[Ca^{2+}]$ in Fig. 4 B. Subsequent reductions do not fit the data well.

Figure S2. Parameter compensations explain discrepancy between true and inferred values. This figure shows parameter values during the first parameter reduction of the log($P_0$) assay, which results in the model with one reduced parameter ($z_L$ eliminated). The parameters that fit the original model at $t_{start}$ differ from the parameters that fit the data when $z_L \rightarrow 0$ ($t_{final}$). In particular: $L_0$ and $J_0$ must decrease, and $D$ must increase to compensate. We therefore expect that when the once-reduced model is fit to data generated from the full model, $L_0$, $J_0$ will be smaller than the true parameters, and $D$ will be larger than the true parameters. This is in quantitative agreement with Fig. 8.
Figure S3. **Inferred parameters from the reduced log(Po) model.** Values inferred by fitting the once-reduced log(Po) model to 100 noisy synthetic measurements generated from base (black) and test (red, blue) parameter sets. Test parameters are different from those in Fig. 8. Here, the inferred values of our compensatory parameters fall into two modes. This is because the model is degenerate at the true best-fit parameter values that the noisy synthetic data were generated from. Each set of inferred parameters was labeled either red or blue. The two clouds therefore show that the parameter compensation is consistent for all fits. Solid lines connect the means of the inferred base and test values; dotted lines connect the true generating base and test values. Each cloud is discernible from the base parameters, and the mean of the values is discernible from the base parameters.