Title: The misleading certainty of uncertain data in biological network processes.
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Abstract
Mathematical models are often used to study the structure and dynamics of network-driven cellular processes. In cell biology, models representing biochemical reaction networks have provided significant insights but are often plagued by a dearth of available quantitative data necessary for simulation and analysis. This has in turn led to questions about the usefulness of biochemical network models with unidentifiable parameters and high-degree of parameter sloppiness. In response, approaches to incorporate highly-available non-quantitative data and use this data to improve model certainty have been undertaken with various degrees of success. Here we employ a Bayesian inference and Machine Learning approach to first explore how quantitative and non-quantitative data can constrain a mechanistic model of apoptosis execution, in which all models can be identified. We find that two orders of magnitude more ordinal data measurements than those typically collected are necessary to achieve the same accuracy as that obtained from a quantitative dataset. We also find that ordinal and nominal non-quantitative data on their own can be combined to reduce model uncertainty and thus improve model accuracy. Further analysis demonstrates that the accuracy and certainty of model predictions strongly depends on accurate formulations of the measurement as well as the size and make-up of the nonquantitative datasets. Finally, we demonstrate the potential of a data-driven Machine Learning measurement model to identify informative mechanistic features that predict or define nonquantitative cellular phenotypes, from a systems perspective.

Introduction
The combination of systems approaches and quantitative data promised a novel understanding of cellular mechanisms that would spur science-driven innovation in biology and medicine – as happened in physics, chemistry, and engineering. Despite massive research efforts and data accumulation, our understanding of cellular regulation, signaling and many other processes as biomolecular systems remains rudimentary. The systems and quantitative biology fields continue to employ strategies from physics and engineering to construct models of biological mechanism from first principles. However, these strategies are incompatible with the types of measurements and observations that predominate biological investigations. Observations from biological experiments investigating cell fate outcomes (apoptosis, necroptosis, etc.) are collected as categorical values, which are hard to define in terms of variables encoded in mathematical mechanistic models of biological processes. Therefore, the connection of mechanistic models to corresponding biological measurements is subject to practitioner interpretation. As a result, vast amounts of existing nonquantitative data in cell biology have led to mechanistic formulations based on simple inference and informal reasoning. Noise, complexity and the hierarchical organization of biology limits how we can experimentally perturb and measure biological systems. Therefore, a relative dearth of quantitative data exists that reveals itself in mechanistic models with poor parameter constraints. Unfortunately, both non-quantitative and quantitative data, collected in an unplanned manner, results in missed opportunities to quantitatively explain complex cellular mechanisms.

This data-to-knowledge problem in biology has prompted researchers to incorporate nonquantitative data as a complement or substitute for quantitative data in the development of mechanistic models. The traditional workflow employed to train mechanistic models to data comprises mechanistic models and experimental measurements linked through a calibration method (Box 1). Such workflows have been adapted to incorporate nonquantitative data into mechanistic models and have revealed their intrinsic value in mechanic hypothesis exploration. For example, pioneering work by Pargett and co-workers...
employed optimal scaling and multi-objective optimization for training mechanistic models to large
ordinal datasets. Schmiester et al. incorporated this strategy into PyPESTO, a model parameter
estimation framework. Their formulation imposes discrete boundaries on the mechanistic model to
reflect discrete ordinal values in the data, but this approach limits their ability to integrate multiple
datatypes or use Bayesian methods for training and uncertainty estimation of mechanistic models. More
recently, Mitra et al. applied predefined constraint-based models of categorical data and modified their
approach to allow definition of a likelihood function within a Bayesian formalism. However, the ad
hoc nature of their constraint models leaves room for biasing assumptions. Given the limited application
of Bayesian methods and biases introduced by ad hoc assumptions, the field still has a limited
understanding of the contribution of nonquantitative and quantitative data to mechanistic knowledge in
biological systems.

In this work, we tackle the data-to-knowledge challenge by introducing the concept of a measurement
model, a statistical construct, into systems modeling approaches, which aims to rigorously define
measurements and observations in terms of an underlying mechanisms. This definition entails
formulation of a function that maps variables encoded in a mechanistic model to values in the
nonquantitative data. Our approach departs from previous work in that it uses machine-learning based
classifiers whose free parameters are estimated to accomplish data-driven identification of measurement
model properties. It also uses a probabilistic formulation that lends itself to Bayesian methods and can
therefore provide an unbiased evaluation of the predictive power of models trained to nonquantitative
data. In what follows, we present our findings about common types of biological measurements, followed
by a presentation of our methodology. In this work we use a mechanistic model of apoptosis execution to
demonstrate how the amount and type of data applied to a mechanistic model can affect its predictive
power. It is well established that apoptosis signaling is involved in many cellular processes in health,
disease, and development. Its biological importance is further underscored by available quantitative and
nonquantitative empirical data. We also establish how an ad hoc formulation of a measurement model
can lead to spurious results and further show how these a priori assumptions can be examined within a
Bayesian, data-driven context. Finally, we demonstrate the potential of a machine learning measurement
model formulation to identify phenomenological links between features (e.g. predictors and drivers) of a
biomolecular mechanism and emergent biological phenotype. We expect our approach to improve our
understanding of the data-to-knowledge relationship in biological processes, leading to a probabilistic
understanding of biochemical mechanisms, and accelerated identification of systems-level interactions
that drive biological network dynamics.

Results
Contributions and biases from different data types to mechanistic models
We first explored how experimental data measurements are used to constrain mathematical models of
cellular processes. Mechanistic models typically employ physical chemistry formalisms comprised of
reaction rates and chemical species concentrations to represent networks of biochemical reactions. Direct
quantitative measurement of all chemical reactions and species would provide needed model parameters
to carry out simulations and in silico experiments. However, these measurements are typically not
available and likely untenable for real systems, thus leading to indirect measurements used to infer model
parameter values using an objective function (Eq. 7) or a likelihood function (Eq. 8). When these
functions are optimized, the resulting mathematical model can provide valuable new predictions and
insights about the cellular process. Measurements from cell biology experiments comprise four broad
types, namely, nominal, ordinal, semiquantitative, and quantitative (Figure 1); each data type reveals
different insights about the cellular process. In apoptosis signaling, for instance, nominal observations
supported early research where it helped identify key components in the apoptosis signaling pathway.
Apoptosis and survival outcomes – as indicated by nominal nuclear fragmentation data (Figure 1 top row)
– helped determine two parallel signaling arcs that proceed following initiator caspase activation:
mitochondria-dependent and -independent pathways. These pathways trigger apoptosis by activating
effector caspases. We built an abridged Extrinsic Apoptosis Reaction Model (aEARM)\(^\text{20}\), which represents these extrinsic apoptosis execution mechanisms as biomolecular reactions (Figure 2A).

Nominal observations do not provide a definitive estimation of their quantity of interest (i.e. their measurand) and instead, encode weak constraints on the measurand values (Eq. 1). They can guide mechanistic modeling by revealing salient structural elements of a cellular process but provide limited insight into the dynamics and complex regulatory cues of apoptosis signaling. Ordinal measurements have featured prominently in works investigating apoptosis signaling. They have uncovered clues about the dynamics and complex regulatory mechanisms of apoptosis. For instance, ordinal measurements of DISC (i.e. a ligand-dependent membrane bound ‘death inducing signaling complex’) components, initiator and effector caspases (Figure 1 second row), bID, etc. revealed how cells resist apoptosis by limiting (but not totally eliminating) pro-apoptotic cues\(^\text{21}\); the sub-maximal pro-apoptotic signaling presents as delay in the dynamics of caspase activation\(^\text{22}\). To better understand caspase activation dynamics and its effect on apoptosis and survival, we need mathematical models of the apoptosis signaling dynamics. Ordinal measurements, however, do not readily support a mathematical description of apoptosis signaling dynamics. Emerging work has leveraged ordinal and nominal measurements in the development of mathematical models of biological signaling but the weak constraints encoded by these measurements (Eq. 1 and Eq. 2) add uncertainty and bias to the modeling process.

Technical challenges confine our quantitative and semi-quantitative measurement to just a few apoptotic signaling proteins. Fluorescence indicators of caspase activity (and by proxy, caspase substrate cleavage) enabled time course measurements of Bid and PARP cleavage dynamics (Figure 1 third row)\(^\text{18}\). They revealed pro-apoptotic activation of Bid and PARP, in TRAIL induced apoptotic HeLa cells, follows sigmoidal dynamics with delays and switch times that are sensitive to various regulatory factors. These measurements provide the details necessary for a mathematical description of apoptosis signaling dynamics and complexity. Our mathematical model aEARM captures the events from initial death ligand cue, initiator caspase activation, BID truncation (tBID), mitochondrial outer membrane permeabilization (MOMP) and eventual PARP cleavage (cPARP), as shown schematically on Figure 2\(^\text{23,27}\). The model was calibrated to above fluorescence data, as described in Methods\(^\text{28}\). Semi-quantitative measurements like fluorescence, like non-quantitative measurements lack a definitive estimation of the measurand because their interpretation requires mathematical manipulation, typically through scaling (Eq. 3), which can also add uncertainty and bias. Quantitative measurements can be used directly in a model without further modifications (Figure 1, fourth row) thus minimizing the uncertainty and bias introduced in the model from measurement interpretation. Therefore, the specific type of measurement and its interpretation could add significant uncertainty and bias to the mechanistic explanation of a given process.

To study the bias and uncertainty originating from different types of measurements, we introduce a concept from statistics, and social sciences: the measurement model (Box 1)\(^\text{29}\). Briefly, a measurement model is a function (Eq. 6) that describes the relationship between the measurement and its measurand. This function maps variables from the mechanistic model \(\mathbf{x}\) to the values expressed in the data \(\mathbf{y}\). This function is often assumed or implied, particularly for semi-quantitative data that can more readily be applied to the model calibration. However, the application of nominal and ordinal datatypes to mechanistic models is not straightforward, because their interpretation (as we show in the following sections) can significantly bias model-derived insights. Consequently, modeling efforts have relied almost exclusively on quantitative and semi-quantitative data. By contrast, the much more abundant non-quantitative datatypes are often ignored or used inappropriately.

Early modeling efforts interpreted nonquantitative data as a series of arbitrary surrogate quantities for the ordinal or nominal values in a corresponding dataset\(^\text{14}\). More recently, discrete boundaries on the values of the measurand were imposed along with a distance metric to describe how well the mechanistic model satisfies nominal or ordinal constraints in the non-quantitative data\(^\text{10-13}\). These approaches reveal the value of nonquantitative data for mechanistic model calibration, but the often-ad hoc nature of these constraint-
based measurement models has been an overlooked source of model bias. To minimize biases from the interpretation of non-quantitative datatypes and apply Bayesian inference methods for model calibration, we developed a data-driven probabilistic measurement model (Box 2). Our measurement model is data-driven in that it possesses free parameters that are calibrated to match data; this lets us replace a priori assumptions about the measurement with a data-driven parametrization, and thereby calibrate mechanistic models whose accuracy and precision better reflect the information contained in the data. Our measurement model is probabilistic as it replaces discrete boundary-based measurement models and distance metrics with a probability (Box 2, Eq. 9) of the ordinal or nominal value, which enables easy formulation of a likelihood function and application of Bayesian optimization methods that utilize MCMC sampling. In our approach, the measurement model is a mathematical construct that represents the measurand through a Machine-Learning probabilistic classifier whose free parameters are simultaneously estimated with the free parameters of the mathematical model during calibration (Box 2). As a probabilistic classifier, the measurement model effectively describes the probability of the categories encoded in the non-quantitative data given values of the measurand (Eq. 9). The measurand, in our case, is encoded in the mechanistic model. For example, the measurement model (Eq. 9, Box 2) can use ordinal logistic classifiers to model the probability of a categorical value as a function of variable(s) encoded by the mechanistic model. Also, the probability that a cell death or survival observations represents a specific state of the mechanistic model. In the calibration process, the measurement model is an explicit intermediate step between simulation of the mechanistic model dynamics and calculation of the likelihood (Box 2). As described in the Methods section, this approach uses the Python based PySB models-as-programs framework and PyDREAM, a Python implementation of the DREAM$_{9S}$ algorithm to sample posterior values of models’ free parameters. However, other model building and parameter sampling (or optimization) algorithms could be employed by the user. In what follows, we examine the impact of different measurement modalities and interpretations on mechanistic model constraints in apoptosis execution. This work motivates an approach that could be generalized to any mathematical model to rigorously integrate quantitative and nonquantitative data types.

Uncertainty associated with different data types in model calibration

To date, molecular biology investigations of intracellular signaling processes and their mechanisms predominantly report nonquantitative measurements. However, it is unclear exactly how these measurements support the development of mechanistic models. We therefore asked how various measurement datatypes impact the certainty and accuracy of model calibrations. Specifically, we explored how to adjust the size and make-up of nonquantitative datasets to better support mechanistic inferences. The resulting posterior predictive region for tBID dynamics of aEARM calibrated to (semi-quantitative) fluorescence data is shown in Figure 2 (B, top row). As expected, the data can effectively constrain the model and the 95% credible region of posterior predictions for tBid dynamics falls within the data uncertainty region. We then extracted a parameter vector from the fluorescence optimized data and used it as a baseline (reference) to generate ordinal datasets for tBID and other aEARM variables as described in Methods and shown Figures 2 (B, bottom four rows). These synthetic datasets could be considered as numerical representations of a time-course western blot dataset. We then calibrated aEARM kinetic rate and measurement model parameters to the ordinal and nominal datasets.

As shown in Figure 2B, ordinal datasets accurately predicted quantitative predictions of “ground truth” dynamics for tBID. The 95% credible region of posterior predictions of tBID dynamics of aEARM trained to these ordinal datasets each contained “ground truth” dynamics for tBID. We also use the area bounded by the 95% credible region of posterior predictions of tBID as a measure of model certainty; with a smaller area indicating higher certainty. The ordinal dataset containing measurements at every 25-minute interval (i.e. typical of time-dependent western blot datasets), however, did not significantly constrain the posterior predictive regions of these dynamics (Figure 2D). Increasing the number of measurements, however, increases the certainty of the posterior predictions of tBID dynamics; this certainty approaches that of the typical semi-quantitative (fluorescence) dataset, which has an area of 2.7,
when then the number of ordinal measurements is increased threefold, which had an area of 6.2. The areas
bounded by the 95% credible region for each ordinal time-course dataset is described in the Figure 2B
(Bottom two rows).

To explore the impact of nominal data on model optimization, we again extracted a parameter vector from
the fluorescence optimized data and used it as a baseline (reference) to generate nominal datasets akin to
an apoptosis execution observation as described in Methods. Previous work has described how features of
apoptosis signaling dynamics can predict cell death vs survival\(^{18}\). The generated nominal dataset describes
binary cell-fate outcomes that emerge as a consequence of extrinsic apoptosis signaling dynamics. We
encode this information in a nominal measurement model as described in Methods. Parameters of
aEARM and the free-parameters encoded in the measurement model were jointly calibrated to a
synthetically generated dataset of 400 survival vs death outcomes as shown in Figure 3A (left). As shown
in Figure 3A (right), the binary cell-fate data minimally constrain the posterior predictive region of tBID
dynamics relative to the prior constraints on the model. This is expected as the binary cell-fate data-type
essentially condenses complex apoptotic signaling dynamics to a single categorical value.

In lieu of its limited ability to constrain mechanistic models, modeling efforts understandably disregard
nominal data. However, we hypothesized that combining nonquantitative datatypes and covering multiple
variables in the model could improve model certainty. To explore the effect of multiple data type
combinations on model calibration, we again optimized the aEARM model parameters, but this time to a
dataset containing nominal and ordinal measurements. As described in Methods, we added a synthetic
dataset containing 61 ordinal time-course measurements for the DISC complex to the nominal dataset
described above (Figure 3B (left)). We modeled the likelihood of this combined dataset as the product of
the likelihoods of the individual constituent datasets (see Methods for details). In Figure 3A and 3B
(right), we see the nominal and ordinal datasets yields larger 95% credible regions for the posterior
predictions of tBID dynamics. However, (in Figure 3C) the combined dataset better constrained the
posterior predictions of normalized tBID dynamics than either dataset alone, with a 95% credible region
area of 26.5 (compared to 55.0 and 56.4 for the ordinal and nominal datasets alone). Therefore, the model
uncertainty stemming from only using tBID nominal data was decreased by including more detailed
upstream measurements. However, the contribution of DISC ordinal data alone was comparable to that of
the tBID nominal data in isolation (Figure 3B (right)). This data suggests that distributed measurements
across multiple variables in a pathway yield synergistic effects on calibrated model accuracy and
certainty.

**Data-driven measurement model as an indicator of model bias**

Traditionally, applying quantitative or semi-quantitative data to a mechanistic model has been relatively
straightforward as they typically follow a well-establish and simple relationship between the measurement
and the measurand. However, for non-quantitative data, measurement uncertainty can prompt researchers
to make assumptions about the relationship between measurement and measurand, which may negatively
impact in the resulting mechanistic model. We therefore asked how the encoding of assumptions into our
models of non-quantitative measurements could impact mechanistic model calibrations. To attain this
goal, we calibrated aEARM kinetic rate parameters to the ordinal dataset, but this time we replaced the
free parameters in the measurement model fixed *a priori* parameterizations or we encoded our
assumptions as priors on the measurement model’s free-parameters. We tested four situations: (i) fixed
parameters, a case where the measurement model is pre-parameterized by the user, presumably reflecting
full confidence in their assumptions about the measurement; (ii) strong prior knowledge, a case where
there is strong belief in the assumed values of the measurement model parameters; (iii) weak prior
knowledge, a case where there is only weak belief in the assumed values of the measurement model
parameters; and (iv) no prior knowledge, that is no constraints on the measurement model parameters.
Mechanistic insights from data-driven measurement models

We have shown thus far how a machine-learning measurement model can reduce uncertainty and increase accuracy in model calibration. Through mechanistic model calibration to categorical data, we effectively employ machine-learning classifiers to constrain mechanistic model dynamics to a corresponding categorical phenotype. We can then employ the measurement model in reverse, to better understand how properties of a biological mechanism predict, drive and define a particular phenotype. This kind of knowledge would be essential for model-driven experimental data acquisition and model-guided validation.
To demonstrate this concept, we calibrated aEARM to nominal cell survival vs death data using a measurement model that estimated the contribution of variables in aEARM to the cell survival vs. death prediction. The survival vs death dataset was synthesized based on maximum log-rate of change of tBid and the time at which the rate of change maximized; these features were encoded into the measurement model, but their contribution was represented as a free parameter. In addition, the measurement model also considered the potential contribution of an unrelated variable (i.e. concentration of a reactants in reactions that occurred independently of the cell death ligand). Jointly calibrating aEARM and this measurement model to cell survival vs death data allowed data-driven predictions of how variables encoded in aEARM relate to cell survival vs death. Figure 5 shows posterior predictions of the values of potential predictors of cell survival vs death. The shade region marks the 95% credible interval for the line marking 50% cell survival probability. Figure 5 (bottom row) provides the posterior distribution of weight coefficients for each the features encoded in the measurement model. (Larger absolute values of the weight coefficient indicate greater importance of the feature.) The calibrated measurement model correctly identified time at maximum Bid truncation as the most important predictor of cell survival; and the unrelated variable as the least important predictor. Calibration of aEARM to the mixed dataset, described in the previous section, yielded a measurement model that equivalently predicted identified time at maximum Bid truncation as the most important predictor of cell survival; and the unrelated variable as the least important predictor. Calibration of a mechanistic model to categorical phenotype data, using data-driven measurement models, enabled correct identification of predictors (and potentially drivers or markers) of categorical phenotypes. The data-driven probabilistic measurement model we propose in this research was essential to this finding.

Discussion

We used data-driven probabilistic measurement models to calibrate, using Bayesian methods, a dynamical model of biological mechanism to quantitative and nonquantitative data. Our approach allowed us to estimate posterior predictive regions for the calibrated models and to observe how the size of a dataset, its different measurement types, and our assumptions about the measurements affect model accuracy and certainty. Our findings support results from previous studies that suggest nonquantitative data are valuable for mechanistic modeling efforts. For instance, a sufficiently large ordinal dataset can constrain the posterior predictions of a mechanistic model as much as quantitative dataset. However, we far more nonquantitative data than is typically generated would be necessary for nonquantitative assays to match the information content of quantitative assays. In Figure 2B (second row), fourteen ordinal measurements of tBid – typical of common immunoblot measurements of intracellular biology – did not constrain the model around an accurate prediction of tBid dynamics. Instead, it took 24x as many ordinal measurements of tBid (336 measurements) to constrain the mechanistic model of apoptosis as well as the fluorescence dataset (112 measurements). We also found that datasets that combined categorical measurements of multiple variables in aEARM out-perform the datasets with measurements of an individual variable. These findings suggest one could overcome challenges posed by a dearth of quantitative data by devising experiments that, while nonquantitative, produce a larger number of diverse measurements that can cover multiple variables.

We also found the posterior predictions of our mechanistic model were sensitive to the assumptions, we encode in the measurement model, about the relationship between measurement and measurand. All measurements possess uncertain (or unknown) properties, but this uncertainty has a pronounced presence in nonquantitative measurements. The limitations of nonquantitative data exist because they impose less informative constraints on models, and this leaves room for biasing assumptions and/or uncertainty. Uncertainty in nonquantitative measurements drives the, often unacknowledged and implicit, assumptions about the relationship between measurement and measurand (i.e. between data and model). With the proposed Bayesian calibration framework, we are able to observe how assumptions about measurement affected the uncertainty and accuracy of the posterior predictions, in essence providing a measurable quality of how well the model can make mechanistic predictions. We found that inaccurate ad hoc
assumptions about the measurement could produce models that suggested, with a higher degree certainty, an inaccurate prediction (Figure 4B). This finding suggests that ad hoc assumptions about measurements can lull practitioners into a false sense of confidence about the model and the data. This concern also motivated Schmiester and co-workers to avoid certain ad hoc assumption in their model calibration approach.

Having a measurement model whose attributes are determined by data creates an opportunity to learn new details about the relationship between a measurement and its measurand(s). For instance, could a model of biological mechanism plus cell phenotype observations data enable identification of cell phenotype predictors? To explored this, we encoded a small number of suspected cell-fate predictors into our measurement model and let the data (and the mechanistic model) determine, through model calibration, their respective contribution to phenotype. In doing so, model calibration using our data-driven measurement model performed feature selection to correctly identify the most important predictor of cell death. In general, this kind of measurement model, which relates mechanism to cellular phenotype, can be used to predict phenotype outcomes and identify potentially informative experimental conditions from in silico perturbation experiments.

The present work presents an analysis and a proof-of-concept that can be improved upon in future work. We chose linear logistic classifiers, as they enable easy formulation of a likelihood function and application of Bayesian calibration methods, but other probabilistic classifiers could be used. We constrained our measurement representation to small number of potential features to avoid complications of high dimensionality to our machine learned measurement model. However, dimensionality reduction and feature learning (e.g. PCA) can, in theory, be integrated into the measurement model’s preprocessing and/or model calibration workflow. Possibilities for integrating more complex machine learning into models of measurement will depend on dataset size, computational power, and modeling goals.

Our work introduces the concept of measurement models to the mechanistic modeling paradigm. Measurement models have their origin in social sciences and statistics. They also appear in more quantitative applications; some recent examples include management, manufacturing, and computer vision. These measurement models can take on more complexity than the examples we provided, depending on the unique needs of the problems in these areas. The use of measurement models in these areas is motivated by a desire to define and quantify observations of nuanced and/or subjective phenomena; and connect those observations to an underlying theory. Biology, being “harder” than social sciences, but arguably “softer” than physics will straddle the technical domains of both. As a field, we face the same challenge as these social sciences given that our mechanistic models are situated within a larger context of explaining nuanced and subjective biological phenomena (e.g. cell-fate, morphology, physiology and overall health vs. pathology). As practitioners, we never encode everything into our mechanistic models; instead there is always some aspect of the model (or its interpretation) that aims to connect back to these relevant biological phenomena. This fact ultimately motivates our application of data-driven probabilistic measurement models in our mechanistic models of intracellular biology.

Methods
Extrinsic Apoptosis Reaction Model
We built an abridged extrinsic apoptosis reaction model (aEARM) and trained it using PyDREAM to normalized fluorescence time-course data. We built this abridged version of EARM to simplify convergence of Bayesian calibration algorithms and thus make feasible probability-based predictions on the model-data relationship. The aEARM abstracts detailed mitochondrial reactions from the original model as two sequential mitochondrial outer membrane pore (MOMP) “signal” activation steps. In addition, apoptosome formation and effector caspase activation reactions take place in a single activation step. The aEARM does capture key dynamic characteristics, such as the snap-action delay dynamics of apoptotic effector molecules that is observed empirically. For this work, three additional non-apoptotic
species were encoded and linked via feedback activation and inactivation loops to test whether our data-driven measurement model could discriminate between drivers and non-drivers of apoptosis. 

(Supplemental Table 2). These additional species and reactions do not interact with any species or reaction in the aEARM model. The aEARM was encoded using rule-based modeling python package PySB. The aEARM parameters – initial conditions and rate coefficients – were adapted from the previously developed EARM and/or calibrated to fit available fluorescence data. Initial conditions parameters were lifted from the previously developed EARM (Supplemental Table 1). Previous work characterized extrinsic heterogeneity in the expression of proteins and its effect on apoptosis. To model extrinsic heterogeneity in apoptosis signaling, initial values of certain species (marked in table 1) were sampled from a log-normal distribution such that its mean equaled that in Supplemental Table 1 and coefficient of variation was 0.20. Rate coefficients were calibrated (described below) to fit normalized fluorescence time-course measurements of initiator and effector caspase reporter proteins (IC-RP and EC-RP respectively).

Integrating aEARM Dynamics

Snap-action delay dynamics present challenges for Ordinary Differential Equation (ODE)-based models, as they feature rapid non-stiff to stiff transitions during integration. For this work we employed the LSODA integrator (from scipy, via the PySB solver suite), suitable for non-stiff/stiff systems. However, we found that particularly poorly behaved parameter vectors could prolong integration evaluations in LSODA. Integrator settings were adjusted for efficiency and accuracy of integration as follows: mxstep (2^20), atol (1e-6 default), rtol (1e-3 default). The aEARM was integrated over a linear space of 100 time-points spanning 0 to 20160 seconds, in direct correspondence with the fluorescence time-course data. Additional time-points in the data were obtained via linear interpolation.

Measurement Models and Likelihood Functions

Likelihood formulations incorporated a measurement model and resulting distance metric for each datatype in the study: fluorescence time-course data, synthetic ordinal time-course data, and synthetic survival vs death binary data for a sample of 400 initial conditions. These likelihood functions were used to calibrate the models to each dataset. In addition to their use in the likelihood formulation, the measurement models, were also used to generate synthetic non-quantitative datasets.

We first trained the aEARM to normalized fluorescence time-course data for IC-RP and EC-RP, i.e. fluorescent proxies for substrates of initiator and effector caspase, respectively (i.e. Bid and PARP, respectively). Consistent with previous work, we defined a likelihood that assume an i.i.d. Gaussian-noise component \( \epsilon \sim N(0, \sigma^2) \) on normalized tBID and cPARP predictions of the aEARM; where \( \sigma^2 \) assumedly equals the variance of the data. This yields a log-likelihood function (Eq. 11) where data the, \( \hat{y} \), and normalized aEARM predictions, \( y \), are compared for each time-point, \( t \), and observable, \( i \) (i.e. tBID/IC-RP and cPARP/EC-RP). The aEARM trained to these fluorescence data served as the starting point in the synthesis of ordinal, nominal, mixed, etc. datasets, below.

\[
\log L(\hat{y}|\theta) = \sum_t^n \sum_i^t -1/2\sigma_i(t)^2 \times (\hat{y}_i(t) - y_i(t, \theta))^2 
\]

To train the aEARM to synthetic ordinal time-course data, a measurement model (i.e. that models the probability of each ordinal category as a function of an aEARM variable) was defined and applied in the formulation of a likelihood function. The ordinal logistic regression python package, MORD, applies empirical ordering constraints to Scikit-Learn’s logistic regression class; this class then calculates a probability for each ordinal category. The ordinal logistic model, encoded in MORD, defines ordinal constraints as a linear function of predicted values of an aEARM variable (e.g. \( p(y_{tBID} \geq c_j|x_{tBID}) \).
\[ \varphi(\alpha x_{t_{\text{BID}}} + \beta_j) \] for aEARM variable, \( x_{t_{\text{BID}}} \) where each ordinal constraint, \( j \), is a logistic function \( \varphi(z) \) with a different offset coefficient, \( \beta_j \), but shared slope coefficient, \( \alpha \), for each of the ordinal categories.

Each ordinal constraint function is combined, using the sequential model (i.e. the product of the logistic functions), to give a probability of each ordinal category, \( P(y_i(t) = c_j|x_i(t, \theta), \alpha, \beta_i, j) = 37, 38 \). These offset and slope coefficients are additional free parameters to be inferred in the model calibration. For example, a measurement model with \( K \) categories can be defined using \( K - 1 \) ordinal constraints and will therefore add a total of \( K \) free parameters (i.e. \( K - 1 \) offset coefficients and 1 shared slope coefficient) to the model. We also encoded error in our synthetic ordinal data by defining a 5\% misclassification probability; i.e. we assume 95\% probability the reported ordinal category, \( c_j = \hat{y} \), and 2.5\% probability of adjacent categories, \( c_j = \hat{y} \pm 1 \), (5\% for adjacent terminal categories). We model this by the marginal probability that the observation classified into the category predicted by the model: \( \Sigma_j^K P(\hat{y}_i(t)|y_i(t) = c_j)^{38} \).

Together, this yields a log-likelihood function (Eq. 12) where the probability of each category \( c_j \) is calculated for each time-point, \( t \), and observable, \( i \); and applied toward a likelihood of the data \( \hat{y} \) given the model. Where noted, we also trained the aEARM using measurement models with preset fixed parameters (Supplemental Table 3).

\[
\log L(\theta, \alpha, \beta) = \sum_i^N \sum_t^T \log \Sigma_j^K P(\hat{y}_i(t)|y_i(t) = c_j)P(y_i(t) = c_j|x_i(t, \theta), \alpha, \beta_i, j) \tag{12}
\]

We trained aEARM to synthetic binary (survival vs death) data by incorporating a measurement model (i.e. logistic model of the probability of each categorical outcome) similar to that used for the ordinal data. We used the Scikit-Learn logistic regression class to model the probability of a cell-death outcome, \( y = c_1 \), as a linear function of features, \( x_i \), derived from the aEARM simulation: \( p(y = c_1|x) = \varphi(\alpha(\beta + \Sigma_i^k \beta_i x_i)) \), where \( \alpha \) is a slope term, \( \beta \) is an intercept and \( \beta_i \) are weight coefficients for each of the \( L \) features\(^39\). Previous studies used a priori knowledge and assumptions about which features of a cell-fate marker’s dynamics to associate with the binary outcome. For instance, recent work delineates necrotic and survival cell fate outcomes using a threshold in the concentration of a known necroptosis marker (this assumption enabled models of necroptosis in the absence of an established relationship between the dynamics of the marker and commitment to necroptosis). Roux et al. investigated an empirical relationship between initiator caspase reporter protein (IC-RP), a fluorescent indicator of caspase activity or proxy for caspase substrate cleavage, and apoptosis in TRAIL stimulated HeLa cells\(^18\). They found, instead of concentration, the maximum rate of change in IC-RP and the time when that rate of change maximized better predicted the apoptosis-survival decision\(^18\). The features we use in our study are based on findings by Roux et al\(^18\). The features are derived from aEARM simulated tBID dynamics, \( x_{t_{\text{BID}}}(t, \theta): \) time at maximum rate of change, and log-maximum rate of change. To test the measurement model’s ability to discriminate between predictors and non-predictors of cell death, we encoded an additional feature: the concentration of an unrelated non-apoptotic species (USM2 in Table 2) when bid truncation maximizes. Together this totals three features. We interpret each observation in the dataset as an independent Bernoulli random variable. Each cell death vs survival observation is compared with these three features, \( x_{l,m} \), extracted from an aEARM trajectory that was simulated from a unique vector of initial conditions. There were 400 observations; 2 sets of 200 observations corresponding to 10 and 50ng/mL initial ligand concentration. Together, this yields a log-likelihood function (Eq. 13) where each, \( m \), of the \( M \) aEARM simulated trajectories corresponds to an observation \( \hat{y}_m \). Given the definitiveness of observed surviving vs dead outcomes, we considered the chance of misclassification to be zero (i.e. \( P(\hat{y}_m|y_m = c_1) = 0 \) when \( \hat{y}_m \neq c_1 \)).

\[
\log L(\hat{y}, \theta, \alpha, \beta) = \sum_m^M P(\hat{y}_m|y_m = c_1) \log \varphi(\alpha(\beta + \Sigma_i^l \beta_i x_{l,m})) \\
+ \sum_m^M (1 - P(\hat{y}_m|y_m = c_1)) \log [1 - \varphi(\alpha(\beta + \Sigma_i^l \beta_i x_{l,m}))] \tag{13}
\]
Generating Synthetic Datasets

The calibration of aEARM to IC-RP and EC-RP fluorescence time-course data provided an optimally fit vector of rate coefficient parameters, which served as the “ground truth” parameter vector in the synthesis of the nonquantitative datasets (Supplemental Table 5). These parameters were applied to aEARM, and the resulting aEARM was used simulate time-courses for variables to be indicated in the nonquantitative data: truncated BID (tBID), initiator caspase localization to the death inducing signaling complex (IC-DISC), and cleaved PARP (cPARP).

These time-courses were converted to ordinal time-course datasets. The effective bit resolution of a measurement technology dictates how many unique values it can distinguish\(^{40}\). The total number of ordinal categories, \(K\), was set such that resulting dataset had less than 70% of the effective bit resolution, \(EBR\), (Eq. 14) of the IC-RP of EC-RP data. The signal to noise ratio, \(SNR\), (Eq. 15) assumes the data, \(d\), were subject to Gaussian noise and a 0.10 misclassification rate between adjacent values; modeled as the 0.95 quantile of a unit normal distribution\(^{40}\). Therefore, the number of ordinal categories were 5 and 4 for tBID and cPARP, respectively. The number of ordinal categories for IC-DISC were arbitrarily set to 4.

Arbitrary values of slope and offset coefficients (Supplemental Table 3) were designated “ground truth” and applied to ordinal measurement models (described above). The resulting measurement models map the values in the aEARM simulated time-courses to probabilities of each ordinal category. These probabilities were used to simulate random class assignments for synthetic ordinal datasets (see Fig 2).

The aEARM was trained to time-course ordinal values of tBID and cPARP or time-course ordinal values of IC-DISC and nominal data described below.

\[
K \leq 0.7 \times 2^{EBR}, \quad EBR = -(SNR + 1.76)/6.02 \\
SNR = 20 \log_{10} q_{0.95} \text{rms}(d)/(\max d - \min d)
\]

To generate synthetic nominal (binary cell survival vs death) data, two heterogeneous populations of 200 aEARM tBID (and an unrelated non-apoptotic species, USM2) trajectories were simulated from ground truth parameters. The populations had distinct initial ligand concentrations (10 or 50 ng/mL).

Heterogeneity was modeled by a log-normal random sample of certain initial conditions (described above). These time-courses were preprocessed to yield values of the features encoded in nominal measurement model, above. This measurement model (which was encoded with preset “ground truth” values of slope, intercept and weight coefficients – See Supplemental Table 4) maps these features to probabilities of the binary outcomes. These probabilities were used to simulate random class assignments for synthetic nominal datasets (Fig 3b).

To generate a synthetic distribution of times at which Bid truncation was half-maximal, two heterogenous populations of 200 aEARM tBID time-courses, corresponding to 10 and 50ng/mL initial ligand concentrations, were simulated from ground truth parameters (as above). Time at half-maximal tBID was calculated via linear interpolation and rounded to the nearest 3-minute time-point (i.e. to reflect temporal resolution of common time-series intracellular experiments) (Fig 3a).

Model Calibration via Bayesian Inference

The aEARM was calibrated using DREAM(ZS) algorithm for all datasets\(^{41}\). Rate parameters in aEARM were given independent log-normal distribution prior probability functions with a location equal to the ground-truth parameter vector and a scale term of 1.5. The nominal (cell death vs survival) dataset features a heterogeneous population of values. We modeled this heterogeneity with a random sample of initial conditions (described above). This random sample was shifted and scaled according to inferred values of the model mean and variance. The mean (if estimated) was given a log-normal distribution prior probability function with a location equal to ground-truth and a scale term of 1.5. The extrinsic noise (or
Model Predictions

We simulated the equal-tailed 95% credible region of the posterior predictions of aEARM via samples of the model parameters posterior distribution. This was done by randomly generating 1000 parameter sets sub-sampled from the posterior sample of parameters generated via PyDREAM. For each parameter set, tBID time-courses (and/or cPARP, IC-DISC) were simulated from aEARM. The 95% credible region of the predictions was then determined via 0.025 and 0.975 quantile bounds on the tBID (or other variables) values for each time-point in the simulated time-course. The area bounded in the 95% posterior credible interval was determined by summing the difference between the 0.025 and 0.975 quantile bounds across 100 equally spaced time points on the trajectory. The 95% posterior credible intervals on the measurement model predictions were similarly described by calculating 0.025 and 0.975 quantile boundaries on the predictions of the measurement model parameterized via 1000 parameter set samples from a posterior. This includes the posterior probability distributions of the feature coefficients encoded in the nominal measurement model. To model predictions of the nominal dataset, however, we randomly generated 100 parameter sets via sub-sampling of the posterior parameter distribution. For each parameter set, we simulate tBID dynamics from the set of 400 initial conditions as described above; from that we compute maximum BID truncation rate and time at maximum BID truncation rate for each of the 400
trajectories. The 0.05 contour of the KDE of the resulting 400 values of maximum BID truncation rate and time at maximum BID truncation rate was plotted for each of the 100 parameter sets.

**Figure Legends:**

**Figure 1:** Measurements encountered in cell biology. *Nominal* measurements (top) can help understand intracellular signaling activity as it relates to broader cellular and physiological behaviors. With cellular phenotype markers or drivers, we can attribute different nominal observations to distinct (intra)cellular states. This is often modeled as in Eq. 1, where each observable measurement ($y_{obs}$) corresponds to a given state. *Ordinal* measurements (second row) can be graded cellular phenotype observations (e.g., cell state transitions in cellular differentiation) or measurements of intracellular contents where noise can obscure intervals between values (e.g. Western Blots). Ordinal measurements imply a relative ordering of quantities along an axis but not their relative distance; i.e. we may know $y_i \leq y_j$ without knowing $y_i - y_j$ (Eq. 2). *Semi-quantitative* measurements (third row) typically arise when an investigation has progress toward a more quantitative understanding of the intracellular signaling. Semi-quantitative measurements (e.g. fluorescent intracellular markers) imply a quantitative relationship but a scaling function is necessary for true quantitation (Eq. 3). *True quantitative* measurements (bottom row) do not imply assumptions and the quantity measured can be used directly in the model (Eq. 4), such as mass-spectrometry protein concentration measurements. As shown schematically on the left triangle schematic, ordinal and nominal measurements are more abundant in biology due to their ease of production but are more difficult to interpret, whereas semiquantitative and quantitative measurements are less common but have a more straightforward interpretation.

**Figure 2.** Predicted Bid truncation dynamics of aEARM trained to different sized ordinal datasets. Multiple Bayesian optimizations were run on the A.) abridged Extrinsic Apoptosis Reaction Model (aEARM) using different sized ordinal dataset to probe how dataset size influenced certainty of aEARM predictions. B.) Initiator caspase reporter (IC-RP) fluorescence time-course measurements (at 180s intervals) were measured (top left) as a proxy for truncated tBid (data from Albeck et al28). The plot shows the mean (dotted line) +/- 1 standard deviation (shaded region) for each time point. The 95% credible region (top right) of posterior predictions (shaded region) for tBID concentration in aEARM, calibrated to fluorescence measurements of IC-RP and EC-RP (See also supplemental figure 3). The median prediction (solid-line) and true (dotted line) tBID concentration trajectories are shown. In the next four rows (from top to bottom), Ordinal measurements of tBID (left) at every 1500, 300, 180 and 60s interval, respectively. The 95% credible region of predictions (shaded region), median prediction (solid line) and true (dotted line) tBID dynamics for aEARM calibrated to ordinal measurements of tBID and cPARP occurring at every 1500, 300, 180 and 60s timepoint are plotted in plots on the right. The plots for cPARP ordinal measurements and predictions are found in Supplemental Figure 3.

**Figure 3.** Predicted Bid truncation dynamics of aEARM trained to nominal and ordinal datasets. A.) Nominal cell death (x) vs survival (o) outcomes data for cells treated with 10ng/mL (orange) and 50ng/mL (grey) of TRAIL and with known relative values of DISC formation (x-axis). The 95% credible region (shaded region) of posterior predictions of tBID dynamics of aEARM calibrated to nominal data (right plot). The median prediction (solid-line) and true
Ordinal measurements for initiator caspase-DISC colocalization (IC-DISC) at 300s intervals (left plot). The 95% credible region (shaded region) of posterior predictions of tBID dynamics of aEARM calibrated to ordinal IC-DISC data (right plot), and C.) of aEARM calibrated to nominal and ordinal IC-DISC data. The median prediction (solid-line) and true (dotted line) were also plotted. The fit to IC-DISC data are shown in Supplemental Figure 9.

Figure 4: Predicted Bid truncation dynamics of aEARM trained to ordinal data using different measurement model parameterizations. A.) and B.) The 95% credible region of posterior predictions (shaded region) of tBID dynamics for aEARM calibrated to ordinal measurements two fixed parameterizations for the measurement model (see Supplemental Table 3). The adjacent panels plot the measurement models predicted probability of class membership (x-axis) as a function of normalized tBID concentration (y-axis). C.) D.) and E.) The 95% credible region of posterior predictions (shaded region) of tBID dynamics of aEARM calibrated to ordinal measurements uniform, Cauchy (scale=0.05) and Cauchy (scale=0.005) prior distributions for the parameterizations for the measurement model, respectively. In each, the median prediction (solid line) and true (dotted line) tBID dynamics are also shown. The adjacent panels give the 95% credible region of posterior predictions of the probability of class membership (x-axis) as a function of normalized tBID concentration (y-axis). Four accompanying plots show the prior (blue), posterior (orange) and true (dashed line) values of measurement model parameters.

Figure 5: Measurement model predicts features of cell death vs. survival using aEARM calibrated to cell death datasets.

Normalized predicted values of the features used in the cell death vs. survival measurement model – the x-axis is the maximum Bid truncation rate, and the y-axis is the time at maximum Bid truncation rate (top row) or an unrelated non-apoptotic signal (middle row) – for corresponding to observed cell death (x) and survival (o) outcomes. These feature values are modeled by aEARM parameterized by 100 parameter vectors randomly drawn from the posterior; for each parameterization, 5 out of the total simulated population of 400 cells were plotted. The grey and orange curves, in these plots, are 0.05 contours for the estimated density of simulated cell populations produced for each of the 100 parameter vectors – grey and orange correspond to 50 and 10ng/ml TRAIL treatments, respectively. The measurement model predicts a probability of cell death vs survival based on simulated values of the above features. The lower right region of the plots in the top row. (i.e., early maximization of Bid truncation and higher maximal Bid truncation rates) is associated with higher probability of cell death. The shaded region is the 95% credible region of the posterior prediction of the line marking 50% probability of cell death or survival. The black and blue lines are the median predicted and true 50% probability lines, respectively. The bottom row plots the posterior distributions of the weight for each feature (i.e. the product of the slope term and feature coefficient encoded in the measurement model): maximum Bid truncation rate (green), time at maximum Bid truncation (orange) and unrelated non-apoptotic signal (blue). Plots in the left column are predictions of aEARM calibrated to the cell death vs. survival dataset. Plots right column were those of aEARM calibrated to the cell death vs survival + ordinal IC-DISC combined dataset.
Box 1: Objective functions and the role of a measurement model. Mechanistic models of biological processes are typically encoded as systems of (ordinary) differential equations (Eq. 5).

Model calibration relies on an objective function (Eq. 7) -- or in a Bayesian setting, a likelihood function (Eq. 8) -- quantifies the degree of dissimilarity or similarity between model variables and corresponding measurements. Note, the objective or likelihood function uses measurement model (Eq. 6) which converts modeled variables \( x(t) \) to a quantity \( y(t_i, \theta) \) that can be compared to data \( \hat{y}(t_i) \). In physics and engineering, where measurements are typically quantitative, the measurement model can be neglected. For nonquantitative measurements and observations, the measurement model takes more consideration.

Box 2: Model calibration with the data-driven probabilistic measurement model. A.) The measurement model is an intermediate step between the mechanistic model and likelihood function of the measurement/observations. It receives variables from the mechanistic model and transforms for use in the likelihood function. This probabilistic machine-learning measurement model estimates probabilities of class membership as a function of the mechanistic model variables (Eq. 9). This measurement model is data-driven in that it contains free-parameters that are evaluated via the likelihood function (Eq. 10). B.) The measurement model uses values of e.g. tBID (grey curve) to estimate the probability of membership in an ordinal category (dotted data). C.) Plots a posterior ensemble of estimates of the probability of membership into 5 ordinal categories (x-axis) as a function of normalized tBID concentration (y-axis). The plot shows the median (solid line) and 95% credible region (shaded region) of the predictions (Category colors match data plotted in B). Algorithm) The mechanistic model and measurement model are calibrated simultaneously using Bayesian sampling methods through stepwise operations as described in each numeral.
References:


### Nominal
- e.g. cell staining.
- Early biomolecular investigation.
- Pathway features.
- Identify phenotype drivers.
- Difficult to characterize complex mechanisms.

\[ y_{\text{obs}} \in \{0, 1, \ldots, f\} \]
\[ y_{\text{obs},i} \neq y_{\text{obs},j} \Rightarrow y_i \neq y_j \]  \hspace{1cm} (1)

### Ordinal
- e.g. Western Blot / Immunoblot
- Reveals mechanistic details.
- Identify features and complexity in signaling pathways.
- Not quantitative.

\[ y_{\text{obs}} \in \{0, 1, \ldots, f\} \]
\[ y_{\text{obs},i} \leq y_{\text{obs},j} \Rightarrow y_i \leq y_j \]  \hspace{1cm} (2)

### Semi-quantitative
- e.g. molecular fluorescence reporters.
- Direct process measurement.
- Clear connection between mechanistic models and data.
- Requires mathematical manipulation for use.

\[ y_{\text{obs}} = f_M(y, \mathcal{E}) \]  \hspace{1cm} (3)

### Quantitative
- e.g. absolute quantification mass spectrometry
- Can use in mechanistic models directly.
- Difficult to obtain.

\[ y_{\text{obs}} = y \]  \hspace{1cm} (4)

Easier to Generate

Easier to Interpret
**Figure 2**

A. Diagram showing the complex interactions involving TRAIL, DISC, iCaspases, MOMP Signals, tBid, eCaspases, and PARP. The diagram illustrates the temporal and cellular levels of tBid fluctuations, with different intervals (1500, 300, 180, 60) depicted in separate sections.

B. Graphical representation of Reference Data and Calibrated Posterior for the normalized fluorescence. The graphs show the time (s) on the x-axis and normalized fluorescence (a.u.) on the y-axis, with experiment data and calibrated posterior curves for different intervals.
Figure 3

A. Nominal data

B. Ordinal data

C. Nominal and ordinal data
Figure 4

A. Ad hoc (case 1)

B. Ad hoc (case 2)

C. Uniform prior

D. Cauchy prior (s=0.05)

E. Cauchy prior (s=0.005)
Figure 5

Nominal vs Nominal + Ordinal

Death-related signal

Unrelated signal

Posterior distribution coefficients for features
Box 1

**Implicit measurement model**
Useful for quantitative and semiquantitative measurements. Can be an overlooked source of bias for non-quantitative measurements.

**Explicit measurement model**
Enable use of a wider range of measurements across multiple data types. Can be formulated to avoid measurement interpretation bias.

---

**Mechanistic Models** of dynamic cellular processes are often encoded as systems of ordinary differential equations (eq. 5). Models are typically calibrated to one or many observables.

\[ x(t) = f(t, \theta). \]  

**The Measurement Model** translates measurements and observations into mechanistic model variables. The measurement model encodes understanding of the measurement as it relates to mechanistic knowledge. The measurement model is most often implied (left box), which can introduce significant biases for non-quantitative measurements. An explicit, adaptive measurement model (right box) can significantly alleviate this potential bias.

\[ y(t_i, \theta) = f_M(x(t)) \]  

**Calibration** minimizes the distance \( d \) between observed data \( \mathcal{Y} \) and related model predictions. Bayesian methods maximize likelihood functions \( \log \mathcal{L} (\mathcal{Y} | \theta) \) to optimize this distance. Calibration can include a measurement model to minimize bias between measurements/observations and the model measurands.

\[ d = \sum_{i=1}^{n} w_i (\hat{y}(t_i) - y(t_i, \theta))^2 \]  

\[ \log \mathcal{L} (\mathcal{Y} | \theta) = c + \sum_{i=1}^{n} \frac{-1}{2\sigma^2} (\hat{y}(t_i) - y(t_i, \theta))^2 \]
**Algorithm**

1. Draw dynamical and measurement model parameters, $\theta$ and $\theta_M$, from their respective priors.
2. Simulate the dynamics $x(t) = f(t, \theta)$ using a numerical solver.
3. Predict the probability of each of the measurement categories using the measurement model.
   \[ P(y_i = c_1|x_i(t)), P(y_i = c_2|x_i(t)), \ldots, P(y_i = c_K|x_i(t)) = f_M(\theta_M, x_i(t)) \]  
4. Evaluate the Likelihood.
   \[ P(\mathcal{Y}|\theta, \theta_M) = \prod_{i=1}^{N} \sum_{j=1}^{K} P(\mathcal{Y}_i = c_j) P(y_i = c_j|x_i(t)) \]  
5. Use MCMC-MH sampling to draw new $\theta$ and $\theta_M$ from their respective priors.
6. Repeat 2-5 until convergence criteria are met.