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Identifiability and uncertainty for ordinary differential equation models with qualitative or semiquantitative data

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The estimation of unknown parameters is a key step in the development of mechanistic dynamical models for biological processes. While quantitative measurements are typically used for model calibration, in many applications, only semi-quantitative or qualitative observations are available, posing unique challenges for parameter estimation.
Specialized approaches have been developed to integrate such data, offering trade-offs in bias, flexibility, and computational efficiency. Most of these approaches involve a recording function that maps the quantitative model onto nonabsolute data; however, this introduces additional degrees of freedom that can contribute to non-identifiability. Reliable calibration therefore requires structural and practical identifiability analysis, alongside robust uncertainty quantification.
In this work, we provide an overview of available methods, critically examine them with respect to identifiability and uncertainty considerations, identify methodological gaps, outline strategies to improve computational efficiency, and advocate for the development of standardized benchmarking frameworks to support informed method selection and best practices.

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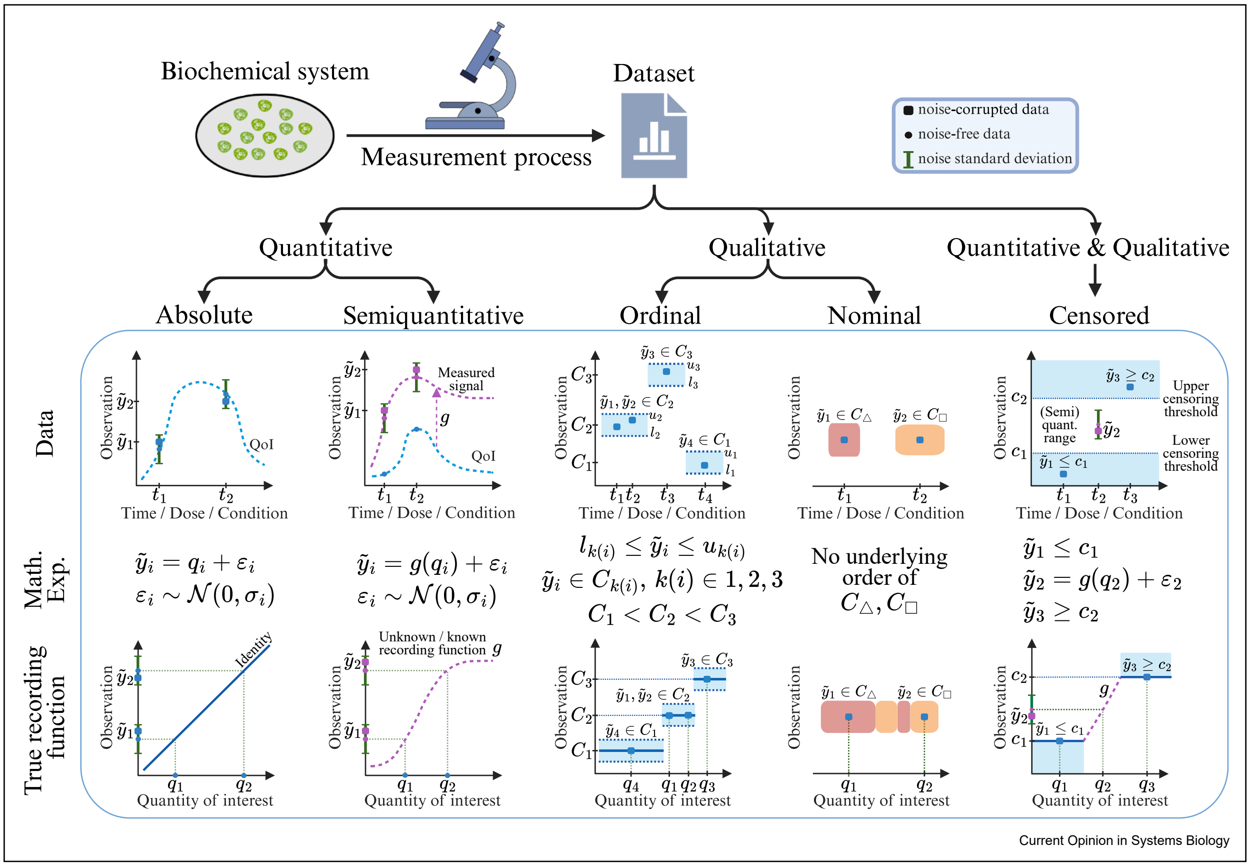
Introduction

Mechanistic mathematical modeling has become an essential tool in systems biology, enabling the quantitative analysis of complex cellular processes. These models are used across a broad spectrum of applications, including cellular signaling [1–3], cellular metabolism [4,5] and gene regulation [6,7]. Today, various modeling frameworks exist — from stochastic and agent-based formulations to constraint-based and logic-based methods — but ordinary differential equation (ODE) models remain among the most commonly applied approaches for capturing biochemical system dynamics. However, the utility of ODE models depends on accurate parameter estimation and reliable uncertainty quantification.

In systems biology, studies that employ ODE models typically use quantitative data for parameter estimation. **Absolute quantitative data** provide, for example, absolute measurements of biochemical species concentrations, but are often expensive and labor-intensive, as they require calibrated assays. Consequently, research that is not focused primarily on model development frequently relies on techniques that yield only semi-quantitative or qualitative data (Figure 1). **Semi-quantitative data** convey information about changes in the quantities of interest — such as concentrations — without providing absolute calibration. In some experimental setups, the observed signal is linearly related to the true quantity; in others, nonlinear effects (e.g., saturation) may arise. Typical sources of semiquantitative data include enzyme-linked immunosorbent assays (ELISA) [8,9], Förster resonance energy transfer (FRET) assays [10], and protein measurements via flow cytometry [11]. **Qualitative data** are descriptive — lacking numerical values — and subdivide into ordinal and nominal types. **Ordinal data** impose a rank order (e.g., low, medium, high abundance) but do not quantify differences, and are obtained from immunostaining assays, for example. The bounds defining ordinal categories may be known in some cases, while in others they remain unknown and must be inferred.

Figure 1



Types of experimental data and corresponding recording functions. Categorization of the main types of experimental data encountered in ODE model calibration — quantitative (absolute and semiquantitative), qualitative (ordinal and nominal), and censored — and their key characteristics. For each data type, a representative example is shown across three conceptual levels: (top) the data visualized as a time series, dose–response curve, or across other experimental conditions; (middle) the corresponding mathematical representation; and (bottom) the underlying recording function $g(\bullet)$, which maps the model's Quantity of Interest (QoI) q to the observed measurement. For quantitative data, the observed measurement is $\tilde{y}$ directly. While absolute data assume an identity recording function, semiquantitative data arise from unknown linear or nonlinear transformations. In the qualitative case, the observation is the membership of $\tilde{y}$ in its ordinal or nominal category. Ordinal and nominal data reflect categorical observations — ordered or unordered, respectively. Censored data often contain both qualitative and quantitative observations: qualitative inequality constraints relative to detection thresholds and (semi)quantitative data outside censored regions. These distinctions shape the modeling assumptions and influence the choice of estimation methods.

Nominal data provide purely categorical information (e.g., cell phenotype: viable vs. non-viable), routinely arising from microscopy or viability assays. Qualitative data may also come from prior knowledge rather than direct measurement, for example, expected thresholds or responses under specific experimental conditions (e.g., cell division is suppressed by a drug, or a marker remains below a detection threshold until a certain time). We refer to such data as **qualitative constraints**. Some datasets contain multiple data types — for example, quantitative measurements with censored values resulting in **censored data**, e.g., from detection limits in qPCR [12,13], with known censoring bounds.

Although semiquantitative, qualitative, and censored data are inherently less informative than absolute measurements, they nonetheless impose valuable constraints on parameter domains, thereby enhancing identifiability. By encoding bounds, ordinal relationships, or relative changes that reflect underlying biology, these data types may guard against nonphysical parameter estimates and help reduce parameter and prediction uncertainty. Thus, inclusion of any nonabsolute measurements in parameter estimation by themselves or alongside available absolute measurements will improve parameter identification in most cases. Over the past decade, substantial methodological advances — including optimal-scaling frameworks, ordinal-likelihood formulations, constraint-based approaches, and spline or Gaussian-process mappings — have broadened the toolkit for integrating nonabsolute observations into ODE model calibration. However, not all of these methods are compatible with standard identifiability analysis, which is essential for interpretability and reliable uncertainty quantification.

In this review, we provide a comprehensive survey of recent developments in methods for incorporating semiquantitative, qualitative, and censored data into parameter estimation workflows for ODE models. We introduce a consistent terminology and categorization scheme to unify disparate approaches and improve clarity across the field. For each class of methods, we evaluate compatibility with rigorous structural and practical identifiability analysis, assess computational efficiency, and highlight methodological gaps. Finally, we propose directions for establishing standardized benchmarking protocols and best practices to guide future research in the principled integration of nonabsolute data into mechanistic modeling.

Problem statement

In this manuscript, we consider parameter estimation problems for ODE models of biological processes. These models describe the time evolution of the process state $\mathbf{x}$,

$$\frac{\mathrm{d}\mathbf{x}(t, \boldsymbol{\varphi})}{\mathrm{d}t} = f(\mathbf{x}(t, \boldsymbol{\varphi}), \boldsymbol{\varphi}), \quad \mathbf{x}(0, \boldsymbol{\varphi}) = \mathbf{x}_0(\boldsymbol{\varphi}),$$

with vector field f and initial conditions $\mathbf{x}_0$ depending on the mechanistic parameters $\boldsymbol{\varphi}$. For example, f may encode a chemical reaction network, with $\mathbf{x}$ denoting species concentrations and $\boldsymbol{\varphi}$ reaction rate constants.

We define quantities of interest (QoIs) $\mathbf{q}$ as biological quantities intended to be measured. They are typically linked to the model state $\mathbf{x}$ and known or unknown parameters $\boldsymbol{\varphi}$ via a known function h:

$$\mathbf{q}(t, \boldsymbol{\varphi}) = h(\mathbf{x}(t, \boldsymbol{\varphi}), \boldsymbol{\varphi})$$

The QoIs can, for example, represent the total amount of some species across different compartments, $q(t, \boldsymbol{\varphi}) = h(\mathbf{x}(t, \boldsymbol{\varphi}), \boldsymbol{\varphi}) = x_1(t, \boldsymbol{\varphi}) + x_2(t, \boldsymbol{\varphi})$.

However, depending on the measurement technique, we may not always be able to directly observe the QoI, that is, obtain absolute measurements. Instead, our observables are the output of some recording function g:

$$\mathbf{y}(t, \boldsymbol{\theta}) = g(\mathbf{q}(t, \boldsymbol{\varphi}), \boldsymbol{\psi}).$$

For absolute measurements, g is the identity function and the observable is the QoI. For semiquantitative measurements (e.g., immunofluorescence or ELISA), g typically includes known or unknown linear scaling, additive offset, or even nonlinear saturating or sigmoidal characteristics (Figure 1). Depending on such characteristics, data integration methods may include method-specific parameters $\boldsymbol{\psi}$ (known or unknown); we denote the full parameter vector as $\boldsymbol{\theta} = (\boldsymbol{\varphi}, \boldsymbol{\psi})$, combining mechanistic $\boldsymbol{\varphi}$ and method-specific $\boldsymbol{\psi}$ parameters. Although the functions g and h are typically combined into one function and the line between the two is blurry, there is a clear difference: the function h depends on the model state and is commonly known, while g does not explicitly depend on the model state and is often partially or fully unknown. Because the data integration methods reviewed here model the recording function g (explicitly or implicitly), this separation is crucial to this article.

Once the model and its observables are defined, performing structural identifiability analysis prior to calibration can be valuable. It may enable model reformulations, reduce the parameter space, or reveal unidentifiable parameters [14]. A parameter is structurally unidentifiable if one can change it without necessarily having any influence on the trajectories of observed quantities y, meaning that its influence can be compensated for by other model parameters. Mathematically stated, a parameter θ_j, either mechanistic or method specific, is structurally identifiable if it can, in principle, be uniquely inferred from noise-free observables $\mathbf{y}(t, \boldsymbol{\theta})$:

$$\mathbf{y}(t, \boldsymbol{\theta}) = \mathbf{y}(t, \boldsymbol{\theta}') \;\; \forall t \quad \Rightarrow \quad \theta_j = \theta_j',$$

for all possible parameter values $\boldsymbol{\theta}$ and $\boldsymbol{\theta}'$.

Unknown mechanistic and method-specific parameters are inferred through the minimization of an objective function. The recording function g represents the deterministic, noise-free aspect of the measurement process. The whole measurement process also includes stochastic noise. For the commonly assumed additive normally-distributed noise model, measurements $\widetilde{\mathbf{y}} = (\widetilde{y}_i)_{i=1}^{n_t}$ taken at time points $(t_i)_{i=1}^{n_t}$ relate to the observable through:

$$\widetilde{y}_i = y(t_i, \boldsymbol{\theta}) + \varepsilon_i = g(\mathbf{q}(t_i, \boldsymbol{\varphi}), \boldsymbol{\psi}) + \varepsilon_i,$$

where $\varepsilon_i \sim \mathcal{N}(0, \sigma^2)$ represents the measurement noise. Most data integration methods assume noise is added at the observable level $\mathbf{y}$ in this way; i.e., after the recording function g has been applied. We do not consider the case where the noise is introduced at the level of the QoI; i.e. $\widetilde{\mathbf{y}} = g(h(\mathbf{x}(t, \boldsymbol{\varphi}), \boldsymbol{\varphi}) + \boldsymbol{\varepsilon}, \boldsymbol{\psi})$. Without loss of generality, for notational simplicity, we assume that there is only one observable. Minimizing the negative log-likelihood $(-\log \mathcal{L}_{\mathcal{D}})$ as the objective function, we obtain maximum likelihood estimates $\boldsymbol{\theta}^{MLE}$:

$$J(\boldsymbol{\theta}) = -\log\mathcal{L}_D(\boldsymbol{\theta}) = \frac{1}{2}\sum_{i=1}^{n_t}\log(2\pi\sigma^2) + \frac{1}{\sigma^2}(\widetilde{y}_i - y(t_i, \boldsymbol{\theta}))^2,$$

$$\boldsymbol{\theta}^{MLE} = \arg\min_{\boldsymbol{\theta}} J(\boldsymbol{\theta}).$$

After the model has been calibrated, it is important to quantify uncertainty in parameter estimates. Parameter uncertainty can be quantified using confidence sets [15]; a confidence set for a parameter is the set of all values that, given the available data, a statistical significance level and the noise model, produce data fits that are statistically indistinguishable from the best fit. Mathematically stated:

$$\mathrm{CS}_j^{\alpha} = \left\{\theta_j \mid \frac{\mathrm{PL}_j(\theta_j)}{\mathcal{L}_{\mathcal{D}}(\boldsymbol{\theta}^{MLE})} \geq \exp\left(-\frac{\Delta_\alpha}{2}\right)\right\}$$

$$\mathrm{PL}_j(p) = \max_{\boldsymbol{\theta}\in\{\boldsymbol{\theta}|\theta_j = p\}} \mathcal{L}_{\mathcal{D}}(\boldsymbol{\theta}),$$

where α is the significance level, Δ_α defines the χ^2-derived threshold for a $(1-\alpha)$ confidence set, and PL_j is the profile likelihood function for parameter θ_j. As profile likelihood functions $\{\mathrm{PL}_j(\bullet)\}_j$ can be multimodal, confidence sets of parameters can also be disconnected.

Another approach to uncertainty quantification, in a Bayesian formulation, is using $(1-\alpha)$ credible sets, which are similar in nature to confidence sets. A $(1-\alpha)$ credible set is the set of parameter values that, given the data and prior knowledge, contain the true parameter value with probability $(1-\alpha)$. Mathematically, a $(1-\alpha)$ credible set for θ_j is any set C with $\int_C p(\theta_j|\mathcal{D})\,\mathrm{d}\theta_j = 1-\alpha$, where $p(\boldsymbol{\theta}|\mathcal{D}) \propto \mathcal{L}_{\mathcal{D}}(\boldsymbol{\theta})\ \pi(\boldsymbol{\theta})$ is the posterior. In practice, the posterior $p(\theta_j|\mathcal{D})$ is estimated using MCMC samples and one of the two common types of credible sets are reported: equal-tailed percentile intervals or highest density sets. However, percentile intervals can underestimate joint uncertainty and coincide with highest density sets only for unimodal symmetric marginals [16].

Without a probabilistic observation model, uncertainty can be summarized via (i) nonparametric bootstrapping, (ii) local sensitivity diagnostics (e.g., Jacobian-based), and (iii) feasible parameter sets that reproduce the desired model behavior. These summaries are informative but offer no guarantees at a significance level α and depend on resampling heuristics and user-chosen tolerances.

Closely related to parameter uncertainty is the concept of practical identifiability analysis. Practical identifiability refers to whether, given noisy and limited data, a parameter can be estimated with sufficient precision to be useful. In practice, parameters are often considered practically identifiable if their confidence sets are finite [42].

However, this workflow of identifiability analysis, model calibration, and uncertainty quantification is only straightforward if the recording function, or at least its parameterization, is known. For semiquantitative data with unknown recording functions, methods capable of estimating these functions without predefined parameterizations are necessary. Qualitative data present further challenges due to their discontinuous, piecewise-constant recording functions (Figure 1). For qualitative data, the observable is not the (semi)quantitative value y_i, but its membership in a qualitative category $y_i \in C_k$. In our notation, y_i will always be a continuous value and hence not observable for qualitative data, unlike for (semi)quantitative data. Qualitative categories $\{C_k\}_{k=1}^{K}$ can be

- **ordinal** − $C_k = (l_k, u_k]$ with ordered thresholds $-\infty = l_0 < u_0 < \cdots < l_K < u_K = \infty$,
- **nominal** − disjoint but unordered C_k,
- or defined by inequalities such as $\widetilde{y}_i \leq c_{\text{lower}}, \widetilde{y}_i \geq c_{\text{upper}}$ for **qualitative constraints and censoring.**

The discrete membership assignment $y_i \in C_k$ makes the qualitative data recording function discontinuous. Although explicitly reconstructing these discontinuous recording functions could in principle be possible, existing integration methods generally circumvent this via by penalizing objective functions.

Furthermore, the compatibility of data integration methods with standard structural and practical identifiability analysis has remained unexamined. Evaluating these properties is essential but challenging, especially when the recording function g is unknown or discontinuous. Thus, a key question addressed in the following sections is to what extent existing methods support identifiability analysis and uncertainty quantification for both mechanistic $\boldsymbol{\varphi}$ and method-specific $\boldsymbol{\psi}$ parameters.

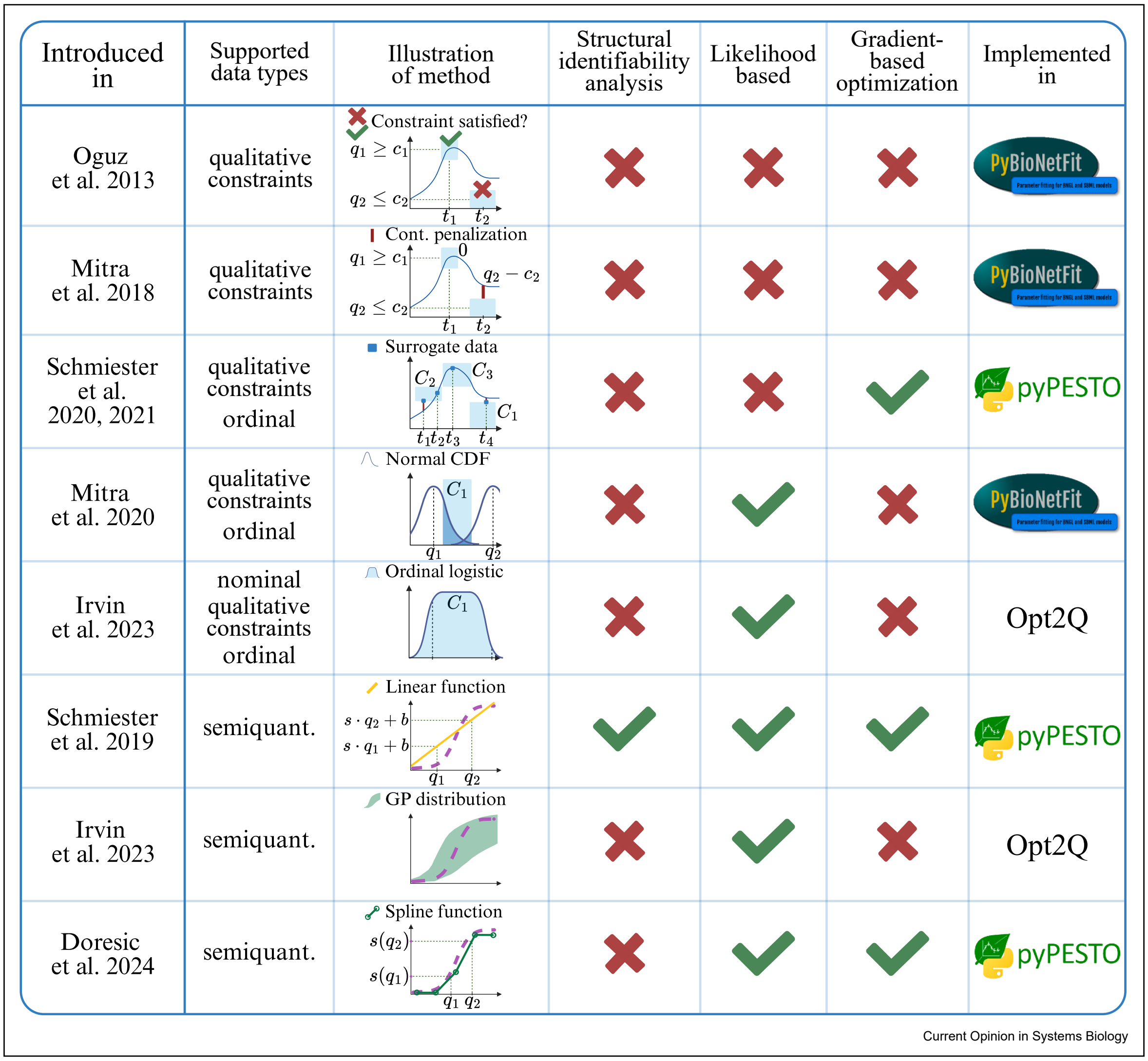
Integration methods for qualitative and semiquantitative data

In this section, we review available methods for estimating the parameters of dynamical systems from qualitative and semiquantitative data. A compact summary is provided in Figure 2.

Constraint-based methods

Constraint-based methods represent some of the first approaches to the integration of nonquantitative data. They enforce inequality constraints on QoIs predicted by the model:

Figure 2



Introduced in	Supported data types	Illustration of method	Structural identifiability analysis	Likelihood based	Gradient-based optimization	Implemented in
Oguz et al. 2013	qualitative constraints	Constraint satisfied?	✗	✗	✗	PyBioNetFit
Mitra et al. 2018	qualitative constraints	Cont. penalization	✗	✗	✗	PyBioNetFit
Schmiester et al. 2020, 2021	qualitative constraints ordinal	Surrogate data	✗	✗	✓	pyPESTO
Mitra et al. 2020	qualitative constraints ordinal	Normal CDF	✗	✓	✗	PyBioNetFit
Irvin et al. 2023	nominal qualitative constraints ordinal	Ordinal logistic	✗	✓	✗	Opt2Q
Schmiester et al. 2019	semiquant.	Linear function	✓	✓	✓	pyPESTO
Irvin et al. 2023	semiquant.	GP distribution	✗	✓	✗	Opt2Q
Doresic et al. 2024	semiquant.	Spline function	✗	✓	✓	pyPESTO

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Key features of published integration methods as implemented in the original publications or accompanying software. Each row corresponds to a specific method, with columns indicating supported data types, a conceptual illustration, and whether the approach enables structural identifiability analysis of method-specific parameters (e.g., spline coefficients), likelihood-based inference (and thus practical identifiability analysis), gradient-based optimization, and software availability. The figure reflects demonstrated capabilities, not theoretical extensions. For example, while most methods could in principle support gradient-based optimization, this is not available in all implementations.

$$y(t_i, \boldsymbol{\theta}) \geq c_{\text{lower},i} \quad \text{or} \quad y(t_i, \boldsymbol{\theta}) \leq c_{\text{upper},i},$$

where c are data-derived bounds. In theory, qualitative constraints of this type are equivalent to censored measurements — the exact value $\tilde{y}_i$ is unknown but is known to lie above or below a censoring bound, or within a censored interval. Constraint-based methods do not define an explicit recording function, but simply penalize the objective function when constraints are not met. For example, Oguz et al. [17] proposed an objective function that simply counts how many constraints are satisfied, but this objective function is discontinuous, which complicates optimization. To address this, Mitra et al. [18] introduced continuous penalty terms:

$$J(\boldsymbol{\theta}) = \sum_{i=1}^{n_t} w_i \max(0, c_i - y(t_i, \boldsymbol{\theta})),$$

where $\mathbf{w}$ are manually chosen weights to balance individual contributions. This objective function is not probabilistic, preventing uncertainty quantification based on confidence or credible sets. Furthermore, constraint-based methods are inherently limited to cases with known data-derived bounds c. They cannot handle ordinal data that include multiple ordinal categories with unknown or known upper and lower bounds.

Surrogate data methods

Surrogate data methods, such as optimal scaling, seek to relate ordinal observations to the continuous model output. Initially developed in statistics [19], these methods were used by Pargett and Umulis [20] to estimate surrogate variables $\widetilde{\mathbf{q}}$ by solving the optimal scaling optimization problem for given mechanistic parameters $\boldsymbol{\varphi}$ and the corresponding QoI $q(t_i, \boldsymbol{\varphi})$:

$$\min_{\widetilde{\mathbf{q}},\mathbf{l},\mathbf{u}} \quad J(\boldsymbol{\theta},\widetilde{\mathbf{q}},\mathbf{l},\mathbf{u}) := \sum_{i=1}^{n_t} w_i(\widetilde{q}_i - q(t_i,\boldsymbol{\varphi}))^2,$$

$$\text{s.t.} \, l_{k(i)} \leq \widetilde{q}_i \leq u_{k(i)}, \forall i \in \{1,\ldots,n_t\},$$

with heuristic weights $\mathbf{w} = \{w_i\}_{i=1}^{n_t}$, and category bounds $\mathbf{l} = \{l_k\}_{k=1}^{K}$ and $\mathbf{u} = \{u_k\}_{k=1}^{K}$. The resulting surrogate data do not necessarily represent the true quantitative underlying QoIs, but rather are the surrogate values that are closest to the simulation while preserving the observed measurement order [20]. The early implementations were computationally expensive and later improved through hierarchical optimization and gradient-based techniques [21,22]. However, like constraint-based methods, optimal scaling lacks a probabilistic foundation, preventing uncertainty quantification based on confidence or credible sets.

Probabilistic qualitative methods

Probabilistic qualitative methods leverage likelihood-based formulations to inherently enable uncertainty quantification. Mitra and Hlavacek [23] introduced a likelihood based on cumulative density functions (CDFs) for the integration of qualitative constraints and ordinal data. This approach assumes that the underlying quantitative data $\mathcal{D} = \{\widetilde{y}_i \sim \mathcal{N}(y(t_i,\boldsymbol{\theta}),\sigma_i^2)\}_{i=1}^{n_t}$ follow a normal distribution. The probability that an observation is above or below a bound is obtained by integrating its probability density over the corresponding interval, thus providing a likelihood function:

$$J(\boldsymbol{\theta}) = -\log\mathcal{L}_{\mathcal{D}}(\boldsymbol{\theta}) = \sum_{i=1}^{n_t} \mathbb{P}(\widetilde{y}_i \geq c_i|\boldsymbol{\theta}) = \sum_{i=1}^{n_t} \Phi\left(\frac{y(t_i,\boldsymbol{\theta}) - c_i}{\sigma_i}\right), \quad (1)$$

where $\Phi(\cdot)$ is the standard normal CDF and c_i is a predefined bound. Mitra and Hlavacek [23] assume a normal distribution; however, the method can be reformulated for other distributions. Furthermore, the method can be applied to ordinal data, but only if ordinal category bounds are known. The currently available implementation of this method does not support estimation of unknown ordinal category bounds [24].

Incorrect bounds in (1) could introduce bias if they do not reflect the true underlying ordinal category bounds. Irvin et al. [25] addressed this limitation by treating the ordinal category bounds as parameters to be estimated jointly with $\boldsymbol{\theta}$. In their implementation, they use an ordinal logistic regression-type formulation and optimize the objective function

$$J(\boldsymbol{\theta},\mathbf{a}) = -\log\mathcal{L}_{\mathcal{D}}(\boldsymbol{\theta},\mathbf{a}) = \sum_{i=1}^{n_t} \mathbb{P}(\widetilde{y}_i \in C_{k(i)}|\boldsymbol{\theta})$$

$$= \sum_{i=1}^{n_t} \varphi(a_{k(i)} - y(t_i,\boldsymbol{\theta})) - \varphi(a_{k(i)-1} - y(t_i,\boldsymbol{\theta})),$$

where $C_{k(i)}$ for $k(i) \in \{1, \ldots, K\}$ are the ordinal categories, $\mathbf{a} = (a_k)_{k=1}^{K}$ are estimated ordinal category bounds, and $\varphi(\cdot)$ is the logistic function. These likelihood-based formulations facilitate practical identifiability and uncertainty quantification but lack explicit continuous outputs, limiting structural identifiability analysis.

Recording function methods

Recording function methods estimate unknown transformations between QoIs and measurements, allowing semiquantitative data integration without predefined recording functions. For assays such as well-calibrated Western blots, a linear recording function can often be assumed:

$$g(q(t_i,\boldsymbol{\varphi}),\boldsymbol{\psi}) = s \cdot q(t_i,\boldsymbol{\varphi}) + b, \quad i = 1,\ldots,n_t \quad \boldsymbol{\psi} = (s,b)$$

with scaling and offset parameters (s, b). Weber et al. [26] introduced analytical solutions for the estimation of scaling factors in a hierarchical fashion, later extended by Loos et al. [27] to incorporate noise models. Schmiester et al. [28] further refined the approach with closed-form solutions for joint estimation of scaling, offset, and noise parameters, enabling efficient optimization even in large-scale models.

However, in cases where unknown systematic measurement effects could introduce saturation, thresholding, or other nonlinear distortions, linear mappings become insufficient, necessitating more flexible approaches. Irvin et al. [25] proposed placing a Gaussian process (GP) prior over the space of possible recording functions:

$$g(\mathbf{q},\boldsymbol{\psi}) \sim \mathcal{GP}(\mu(\mathbf{q}),\kappa(\mathbf{q},\mathbf{q}';\boldsymbol{\psi})),$$

where μ is the mean function and κ is typically a radial-basis kernel with unknown hyperparameters $\boldsymbol{\psi}$, such as length-scale and signal variance. Conditioning on observed data yields a posterior distribution over recording functions, from which a likelihood can be derived for measured values $\{\tilde{y}_i\}_{i=1}^{n_t}$ given model outputs $\{q(t_i, \boldsymbol{\varphi})\}_{i=1}^{n_t}$. GPs offer high flexibility and are compatible with uncertainty quantification due to their likelihood-based formulation. However, their implicit functional form limits interpretability, and their high flexibility can lead to overfitting, particularly with limited data. Computational costs also scale cubically with the number of time points due to kernel matrix inversion. Alternatively, Dorešić et al. [29] proposed a method using monotone piecewise linear splines:

$$g(q(t_i, \boldsymbol{\varphi}), \boldsymbol{\psi}) = s(q(t_i, \boldsymbol{\varphi}), \boldsymbol{\xi}), \quad i = 1, \ldots, n_t, \quad \boldsymbol{\psi} = \boldsymbol{\xi},$$

where the spline parameters ξ control the shape of the mapping. By explicitly parameterizing the recording function and imposing a monotonicity constraint, this approach ensures interpretability and limits unrealistic transformations. Moreover, the approach is scalable, as the implementation by Dorešić et al. [29] includes hierarchical and gradient-based optimization. However, overfitting can still occur with limited data, and the number of spline knots must be chosen carefully. The choice of recording function impacts not only interpretability and efficiency, but also whether structural identifiability analysis can be applied, as discussed in the following section.

Structural and practical identifiability analysis of data integration methods

All the aforementioned methods allow for the estimation of model parameters from semiquantitative and qualitative data. Yet, these parameter estimates are subject to uncertainty. Therefore, structural and practical identifiability analysis and uncertainty quantification are essential.

Structural identifiability analysis should ideally be the first step before model fitting [14]. Yet, most available methods for structural identifiability analysis fundamentally require smooth model observables y, including the recording function g and the QoI function h. In more detail, differential algebra methods [30–33] require polynomial or rational vector fields f and model observables y to eliminate state variables and derive input–output equations. The Exact Arithmetic Rank (EAR) method uses truncated power series expansions under similar assumptions [34,35]. The observability rank condition (ORC) methods [36–38] rely on Lie derivatives, requiring smooth model observables y. Similarly, series expansion methods [39,40] require analytically differentiable relationships.

For absolute data, the prerequisites for using structural identifiability analysis methods — namely, differentiable model observables — are usually met. In contrast, some qualitative and semiquantitative data integration methods — such as optimal scaling, ordinal likelihoods, and GPs — do not explicitly define model observables but only integrate recording function information into objective functions implicitly, through penalization, surrogate data, or probabilistic models. Thus, structural identifiability analysis cannot be applied. The same is true for the spline-based approach [29] as — while using an explicitly-defined recording function — the mappings from state to observable are only piecewise continuous. Constraint-based methods produce explicit but discrete outputs — binary constraint outcomes. A continuous approximation of these discontinuous or nondifferentiable mappings could enable identifiability analysis of the unknown parameters. Nevertheless, structural identifiability analysis can still be applied to mechanistic parameters $\boldsymbol{\theta}$ using QoIs $\mathbf{q}$ as outputs, assuming idealized quantitative data. This remains valuable as parameters that are unidentifiable under idealized absolute quantitative data will remain so when using less informative data types.

Practical identifiability and parameter uncertainty are commonly assessed using profile likelihood analysis [41,42] or Markov chain Monte Carlo (MCMC) sampling [43]. Profile likelihood methods, available in software such as Data2Dynamics [44], dMod [45] or pyPESTO [46], compute maximum projections of the likelihood function. MCMC, including the commonly used Metropolis–Hastings algorithm [47,48], generates posterior distributions through sampling, and thus credible sets of estimated parameters. Both approaches require a proper likelihood function, making them directly applicable in combination with approaches that provide a likelihood function, including probabilistic qualitative methods, GPs, splines, and linear scaling approaches.

In summary, structural identifiability is limited to integration methods with explicit, smooth, and differentiable outputs. Practical identifiability, by contrast, is broadly applicable when likelihoods are defined. These observations underscore a methodological gap and emphasize the need for a flexible yet explicit analytic method for the integration of semiquantitative data compatible with structural identifiability analysis techniques.

Efficiency of parameter inference

Integrating qualitative and semiquantitative data introduces additional parameters and nonlinearities that complicate optimization, thus requiring dedicated computational strategies. One of the most effective strategies is hierarchical estimation, which decomposes parameter inference into nested subproblems, improving convergence and efficiency [21,26–29]. A related approach is posterior marginalization, used by Raimúndez et al. [49], which was shown to substantially accelerate Bayesian inference for models with linear semiquantitative data, but it has not been yet explored for other types of recording functions.

Local and global optimization approaches exploiting gradient information have been shown to outperform gradient-free approaches [22,29]. Adjoint sensitivity analysis can further accelerate gradient computations [50], though applying adjoint sensitivity analysis to flexible recording functions remains unexplored. Yet, a promising alternative to the analytical derivation and numerical evaluation of sensitivity equations is the use of automatic differentiation, implemented, e.g., in JAX [51,52] and in Julia's Optimization.jl package [53].

Implementation and benchmarking

The diversity of methods for integrating qualitative and semiquantitative data has resulted in a fragmented landscape, limiting comparability. Most approaches have been tested on distinct datasets, and only a subset has been implemented in actively maintained general-use software.

Constraint-based and CDF-based ordinal methods are implemented in PyBioNetFit [24], which supports qualitative constraints and Bayesian ordinal likelihoods. Although optimization is limited to gradient-free methods, it remains useful for rule-based models or qualitative constraint specification. The approaches of Irvin et al. [25] are available through the Opt2Q framework with support for Bayesian inference; however, the package appears tailored to reproducing their study, with limited documentation and no updates since early 2024. Lastly, the Parameter EStimation TOolbox for Python (pyPESTO) [46] supports the integration of ordinal and semiquantitative data using hierarchical optimization and efficient gradient-based methods. Implemented approaches include optimal scaling [21], linear [28], and spline-based [29] recording function methods, with built-in uncertainty quantification via profile likelihood and sampling.

Despite these implementations, no benchmarking framework exists to compare methods systematically. Benchmarks like those of Hass et al. [54] or DREAM challenges [55] could help evaluate trade-offs — e.g., flexible recording functions that preserve quantitative structure but risk overfitting [29], versus ordinal simplifications that reduce complexity but lose information [56,18].

Systematically collecting benchmark problems and establishing an interoperable format for problem specification are key steps toward broader adoption and method selection grounded in performance comparisons.

Discussion

Integrating qualitative and semiquantitative data into the calibration of the ODE model expands the range of usable experimental data, allowing for informed parameter estimation when quantitative measurements are limited. However, this presents challenges in identifiability analysis, uncertainty analysis, and computational efficiency.

Most qualitative integration methods, such as constraint-based and ordinal approaches, lack explicit recording functions, making structural identifiability analysis infeasible. For semiquantitative data, only simple linear models are compatible with standard techniques. This highlights a key methodological gap: the need for a flexible, explicit, and analytic recording function that supports structural identifiability analysis. At the same time, likelihood-based formulations remain essential for practical identifiability analysis and uncertainty quantification. Methods based on CDF-based likelihoods, ordinal logistic regression, splines, and GPs enable profile likelihood and MCMC-based analyses, even when structural identifiability analysis is not directly applicable. However, their increased flexibility also adds a computational burden, necessitating scalable approaches such as hierarchical optimization, posterior marginalization, and gradient-based optimization. Furthermore, these flexible approaches often include additional hyperparameters that need to be chosen (e.g., number of spline parameters, GP length scales). For this, depending on the available data and the purpose of the model, one can employ either model selection or cross-validation. Experimental design, closely related to identifiability and uncertainty analysis, is beyond our scope. Applying information-optimal criteria (e.g., A/D/E-optimality; cf. [57]) in this context remains, to our knowledge, underexplored.

In summary, the current fragmentation of methods and the absence of systematic benchmarks hinder meaningful comparison and broader adoption. Future work could focus on standardized benchmarking and efficient implementations to enable generalizable and reusable data integration strategies. These steps are essential to reliably incorporate diverse experimental data into model calibration.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this article.

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Data availability

No data was used for the research described in the article.

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