A Likelihood-Based Framework for Analysing Sarcomeric Protein Machinery in Cardiac Myocyte Models

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Abstract

Cardiac contraction relies on the precise coordination of sarcomeric protein interactions. Yet the molecular dynamics remain challenging to quantify due to complex cross-bridge interactions and experimental variability.

We present a likelihood-based framework for parameter estimation and identifiability analysis in cardiac myocyte models. Combining maximum likelihood estimation with profile likelihood, we demonstrate a workflow to identify parameters controlling sarcomeric kinetics in engineered cardiac tissues derived from filamin C-mutant and CRISPR-corrected pseudo-wild-type lines. The approach identifies key transition-rate parameters, quantifies practical identifiability, and supports experiment-specific uncertainty-aware model reduction. This framework provides a reproducible route to calibrate and validate mechanistic models of cardiac contraction and biological interpretation.

1. Introduction

Mechanistic models of sarcomeric function are indispensable tools for linking molecular kinetics to cellular-scale contractility. However, such models are often subject to overparameterisation, correlated parameters, and non-identifiable transitions during calibration. Traditional fitting approaches rarely provide quantitative confidence intervals or formal identifiability checks, which limits reproducibility and interpretability.

Likelihood-based parameter estimation provides a rigorous statistical foundation for fitting mechanistic models to experimental data, addressing these challenges. In this framework, model parameters are treated as unknown quantities inferred by maximising the likelihood that the model predictions explain the observed measurements, given an explicit noise model. Maximum likelihood estimation (MLE) thus yields both optimal parameter values and a quantitative measure of goodness-of-fit under well-defined statistical assumptions. Importantly, the likelihood surface encodes rich information about parameter uncertainty and interdependence, which can be exploited to evaluate model identifiability [1–4].

Profile likelihood (PL) analysis extends MLE by systematically exploring the likelihood landscape around the optimum. The likelihood is recomputed for each parameter at a series of fixed values, with all the other parameters being reoptimised at each step. This process produces a profile showing how the likelihood varies as the parameter is changed while the other parameters are reoptimised, thereby visualising the uncertainty in the parameter estimate. This enables a distinction between structural identifiability, i.e., whether a parameter can be uniquely determined given the model structure and ideal data, and practical identifiability, which assesses whether the available experimental data are sufficient to constrain parameter estimates in practice. Parameters with non-flat profiles and unbounded confidence intervals or flat profiles are considered non-identifiable, which highlights either redundancy in the model or insufficient information content in the data. In contrast, well-defined, bounded profiles indicate reliable parameter estimation[2–4].

In the context of cardiac myocyte models, combining MLE and PL analysis allows a quantitative evaluation of how experimental design, observation noise, and model structure influence parameter certainty. This methodological integration supports uncertainty-aware model reduction, in which only identifiable parameters are retained for biological interpretation or subsequent predictive use. The result is a reproducible workflow that couples mechanistic interpretability with statistical rigour.

We adopted the myofilament kinetics model developed by Rice *et al.* (2008) [5], which integrates calcium-dependent thin filament activation with cross-bridge cycling to reproduce sarcomeric force generation. This model links molecular transition rates to emergent tension and shortening transients, with applications including the study of genotype-phenotype relationships, pharmacological interventions, and excitation-contraction coupling in cardiac muscle [6–8].

Recently, Wang *et al.* (2023) published engineered cardiac tissue (ECT) data of restrictive cardiomyopathy (RCM). They compared tissues derived from a Filamin-C (FLNc)-mutant (FLNc$_{mut}$) patient line and its CRISPR-corrected pseudo-wild-type (pWT) counterpart [9]. This system provides an opportunity to illustrate how our likelihood methodology can estimate parameters and identify uncertainty in a physiologically relevant setting. While we demonstrate our approach to compare contractile alterations caused by FLNc$_{mut}$ in an isogenic background for quantitative model calibration, the approach is generalisable to other experimental and model contexts.

In this study, we combine these ECT experiments with the Rice *et al.* (2008) [5] myofilament model to investigate whether contractile abnormalities in FLNc$_{mut}$ can be explained by altered cross-bridge kinetics. We calibrate the computational model to experimental contraction traces using maximum likelihood estimation (MLE) and evaluate parameter identifiability using profile likelihood (PL). This approach allows us to quantify experiment-specific parameter differences, assess model adequacy, and establish the basis for model reduction and uncertainty analysis in the context of mutation-specific cardiomyopathy.

2. Methods

2.1. Experimental Data Analysis

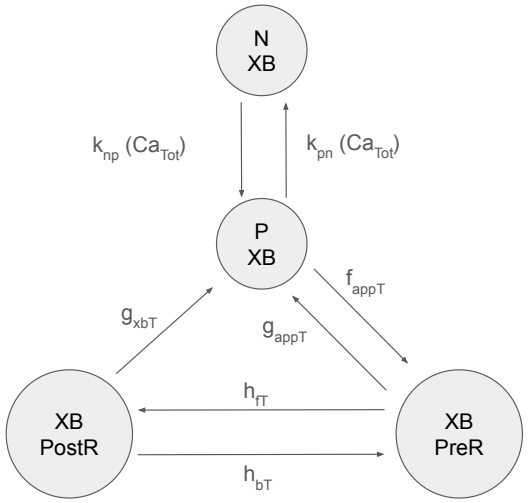
We digitalised and analysed contraction traces (normalised sarcomere length vs time) from two ECT types: (i) FLNc-mutant line from an RCM patient and (ii) CRISPR-corrected pWT line provided in Wang *et al.* (2023) [9] for shared genetic background and controlled comparisons.

2.2. Dynamical Model

We used the myofilament kinetics model of Rice *et al.* (2008) [5], which integrates regulatory-unit activation and cross-bridge cycling to predict sarcomere-level mechanics. The transition-rate expressions of the model were calibrated to the published data from Wang *et al.* (2023) [9] with simplified transition rates. Mathematically, this forms a system of ordinary differential equations (ODEs)

$$\dot{x} = f(x, \theta_{\mathrm{dyn}}) \,, \tag{1}$$

where the parameter vector θ_{dyn} includes the initial values of the depicted variables, as well as the transition rates. The observation function $g(t, \theta) = g\big(x(t, \theta_{\mathrm{dyn}}), \theta_{\mathrm{obs}}\big)$, with θ_{obs} denoting additional scaling parameters, estimates the measurable output. The argument x is the solution of Eq. (1) with $\theta = (\theta_{\mathrm{dyn}}, \theta_{\mathrm{obs}})$ the vector of all model parameters.



Taken from Rice et. al., 2008 p.2372

Figure 1. Schematic of the crossbridge model adapted from Rice *et al.* (2008, Figure 2, p. 2372)[5]. The model includes transitions between non-permissive ($\mathrm{N_{XB}}$), permissive ($\mathrm{P_{XB}}$), and crossbridge states ($\mathrm{XB_{PreR}}$, $\mathrm{XB_{PostR}}$). Transition rates are controlled by calcium concentration and mechanical interactions.

2.3. Parameter Estimation

Let the observed data be $y = (y_1, \ldots, y_N)$ at times $t_1, \ldots, t_N$. Assuming Gaussian noise, the objective function to be minimised is

$$\chi^2(\theta) = \sum_{i=1}^{N} \frac{\big(y_i - g(t_i, \theta)\big)^2}{\sigma_i^2} \,. \tag{2}$$

Minimisation of χ^2 provides the MLE

$$\hat{\theta} = \arg\min_{\theta} \chi^2(\theta) \,. \tag{3}$$

2.4. Identifiability Analysis by Exploiting the Profile Likelihood

An informative approach to assessing parameter identifiability is the calculation and analysis of likelihood profiles $\mathrm{PL}(\theta_{\mathrm{i}})$, obtained pointwise for each parameter θ_i by varying and fixing θ_i around the MLE $\hat{\theta}$ and reoptimising the remaining parameters [2–4]:

$$\mathrm{PL}(\theta_{\mathrm{i}}) = \min_{\theta_{\mathrm{j}\neq\mathrm{i}}} \chi^2(\theta) \tag{4}$$

Confidence intervals follow from the likelihood-ratio test principle:

$$\mathrm{C}_{1-\alpha}(\theta_{\mathrm{i}}) = \Big\{ \theta_{\mathrm{i}} \;\Big|\; 2\big(\mathrm{PL}(\theta_{\mathrm{i}}) - \chi^2(\hat{\theta})\big) \leq \chi^2_{1;\,1-\alpha} \Big\} \,. \tag{5}$$

A flat profile indicates structural non-identifiability. Parameters with non-flat profiles but unbounded confidence intervals are considered practically non-identifiable, whereas parameters with bounded confidence intervals are identifiable [2–4].

3. Workflow for Likelihood-Based Calibration and Assessment

The overall workflow for likelihood-based model calibration comprises four stages: parameter estimation, convergence assessment, profile construction, and visual diagnostics. Each step provides complementary information about model adequacy, parameter certainty, and numerical robustness.

3.1. Parameter Estimation and Optimisation Strategy

Parameter estimation is performed by minimising χ^2 (Eq. 3) through repeated optimisation from different initial conditions (the 'multistart' strategy). This strategy reduces the risk of convergence to local minima, which commonly arise in nonlinear dynamical systems. The ensemble of optimisation runs allows detection of the global optimum and quantification of solution robustness. Parallel execution and fixed random seeds ensure reproducible inference across repeated analyses [1].

3.2. Convergence and Fit Diagnostics

The quality of convergence is assessed by comparing the final objective values across all starts. A concentrated set of minima at low objective values signifies a distinct global optimum. Graphical diagnostics, visualise optimisation trajectories and convergence density to assess numerical robustness and reproducibility. Complementary residual analysis highlights structured mismatches between data and model predictions, informing targeted model adjustments.

3.3. Profile Likelihood Computation and Evaluation

Once the best-fit parameter set has been established, likelihood profiles are computed for all or selected parameters. For each profile, the parameter of interest is systematically scanned and varied while the remaining parameters are reoptimised. The resulting likelihood curve quantifies the information content of the data with respect to that parameter. Flat or unbounded profiles indicate non-identifiability or redundancy, whereas sharply curved profiles with well-defined intersections at a likelihood-ratio threshold correspond to identifiable, well-constrained parameters. Profiles can be computed in parallel using warm-starts, where the optimiser is initialised from the neighbouring solution obtained previously to enhance numerical efficiency.

3.4. Validation and Reproducibility

All intermediate results, including optimisation ensembles, profiles, residuals, and fits, are stored to enable full reproducibility and independent verification. Comparisons between experimental data and model predictions assess descriptive adequacy, while profile shapes and convergence patterns inform model reduction. These steps form a closed loop of inference, diagnostics, and refinement, which supports uncertainty-aware mechanistic modelling.

4. Discussion

Our framework combines MLE with PL-based identifiability assessment for uncertainty-aware, data-based model development. The following sections summarise the principal outcomes, methodological insights, and future directions of this study.

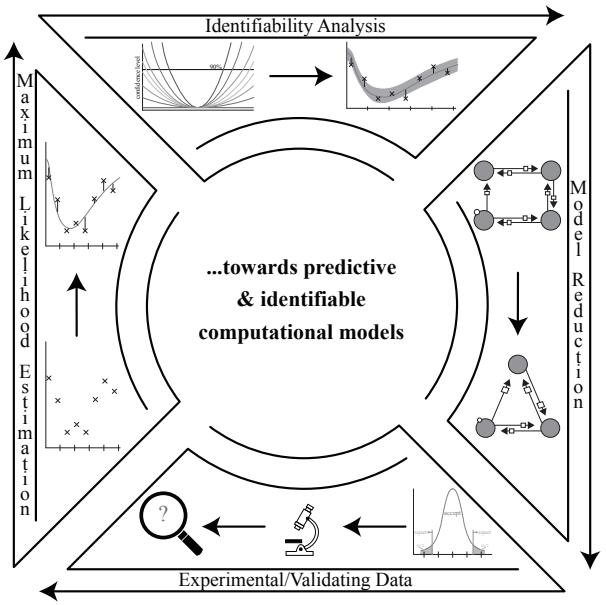


Figure 2. Schematic overview of the likelihood-based modelling workflow for developing predictive and identifiable computational models. Experimental data are used for data-driven best-fit parameters via maximum likelihood estimation. Profile likelihood analysis quantifies parameter uncertainty as well as structural and practical identifiability, informing targeted model reduction. The refined model is then re-evaluated against data, closing the loop towards reproducible and uncertainty-aware inference.

4.1. Main Findings

(i) Under the full transition-rate model, parameter estimation was numerically stiff with no unique minimum; (ii) constant-rate simplification improved optimisation and enabled initial profile-likelihood computation; (iii) best-fit rates suggest increased cross-bridge attachment propensity in FLNc$_{mut}$; (iv) parameter degeneracy and shape mismatches argue for identifiability-guided model reduction before biological interpretation.

4.2. Interpretation and Limitations

Increased transition from the permissive state to the pre-rotated state in the crossbridge cycle in FLNc$_{mut}$ is consistent with a higher effective attachment tendency or altered regulatory kinetics, but the observed degeneracy cautions against over-interpretation: multiple parameter combinations fit the data comparably well, especially in FLNc$_{mut}$. The constant-rate surrogate is a pragmatic device for exploration rather than a mechanistic claim; future work should restore biophysically grounded dependencies after identifiability-guided simplification.

4.3. Methodological Implications

We emphasise maximum-likelihood estimation under an explicit noise model, profile-likelihood-based confidence intervals, and using profile geometry to diagnose practical/structural non-identifiability and to inform model reduction and subsequent uncertainty propagation.

4.4. Next Steps

(i) Complete profiles for targeted parameters with warm-starts and parallelisation; (ii) reduce/regularise weakly informed parameters; (iii) re-introduce mechanistic rate dependencies post-reduction; (iv) propagate residual uncertainty in the identified subspace; (v) test sensitivity to the calcium transient and observation model; (vi) validate against additional ECT replicates/perturbations.

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