Rapid Characterisation of hERG Kinetics Using Optimised Protocols on a High-Throughput System

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1. Introduction

- The KCNH2 gene (hERG) encodes the alpha subunit (Kv11.1) of the channel carrying I_{Kr}
- Blocking I_{Kr} prolongs the action potential (AP)
- Current regulatory guidelines (ICH-S7B) require evaluation of drug effects on the hERG channel during preclinical development
- The new cardiac safety pipeline (CiPA) encourages high-throuhput screening and in silico modelling
- Underlying cell-to-cell (and intersubject) variability of hERG remains unclear

2. Cardiac hERG Channel Model

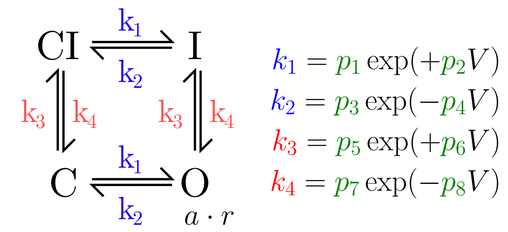
- Hodgkin-Huxley formulation

$$I_{Kr}(t, V|g_{Kr}, \{p_i\}) = g_{Kr} \cdot a \cdot r \cdot (V - E_K)$$

$$\frac{\mathrm{d}a}{\mathrm{d}t} = \frac{a_\infty - a}{\tau_a} \qquad \frac{\mathrm{d}r}{\mathrm{d}t} = \frac{r_\infty - r}{\tau_r}$$

$$a_\infty = \frac{k_1}{k_1 + k_2} \qquad r_\infty = \frac{k_4}{k_3 + k_4}$$

$$\tau_a = \frac{1}{k_1 + k_2} \qquad \tau_r = \frac{1}{k_3 + k_4}$$



$$k_1 = p_1 \exp(+p_2 V)$$
$$k_2 = p_3 \exp(-p_4 V)$$
$$k_3 = p_5 \exp(+p_6 V)$$
$$k_4 = p_7 \exp(-p_8 V)$$

Fig 1: A simple model of the current I_{Kr} shown in a Markov state diagram format, where t, V are time and voltage which are the control variables in the experiments, E_K is the reversal potential, and g_{Kr}, $\{p_i\}$ are parameters to be determined.

3.1 Experimental Design / Model Training/Fitting

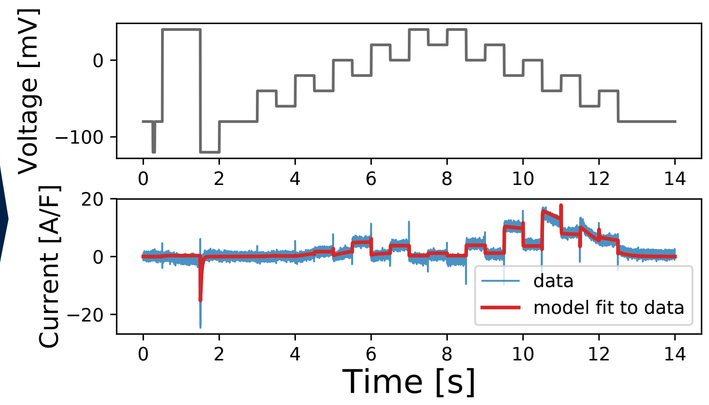


Fig 2: An optimised protocol that is designed for high throughput machine to recover model parameters. It shows the **actual experimental measurement** and the **fitted model**.

3.2 Model Validation

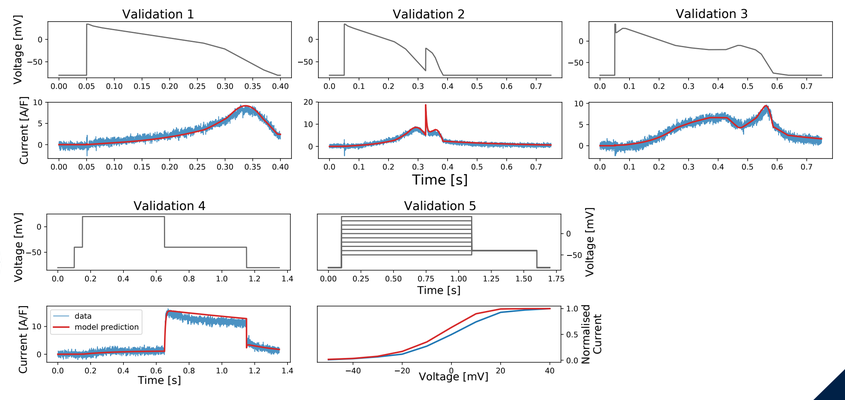


Fig 3: Independent experimental measurements of **the same cell** to use as model validation. Here we used both 'traditional' protocols (Validation 4, 5) and new physiologically inspired [1] protocols (Validation 1-3) which are the AP-like, EAD-like and DAD-like waveforms.

3.3 Variability Across Experiments

We repeated the same experiment on the same cell-line and recorded 65 individual cell measurements.

Can we capture experiment-to-experiment variability?

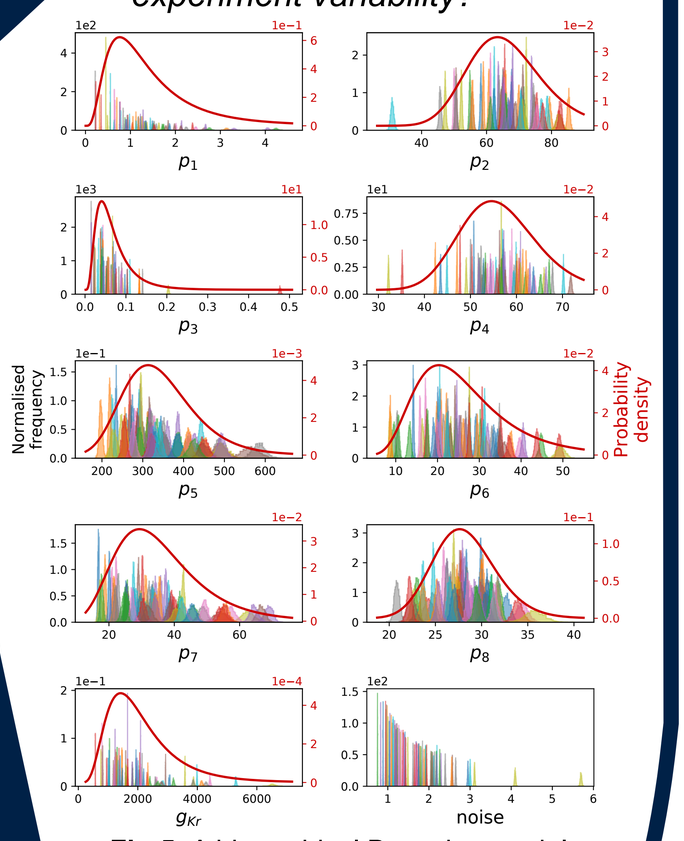


Fig 5: A hierarchical Bayesian model (HBM) was used to capture *experiment-to-experiment* variability. Marginal distributions of each experiment are shown, which reveal the variability between experiments. The **posterior predictive distributions** from the HBM are shown in red.

Variability in hERG Kinetics

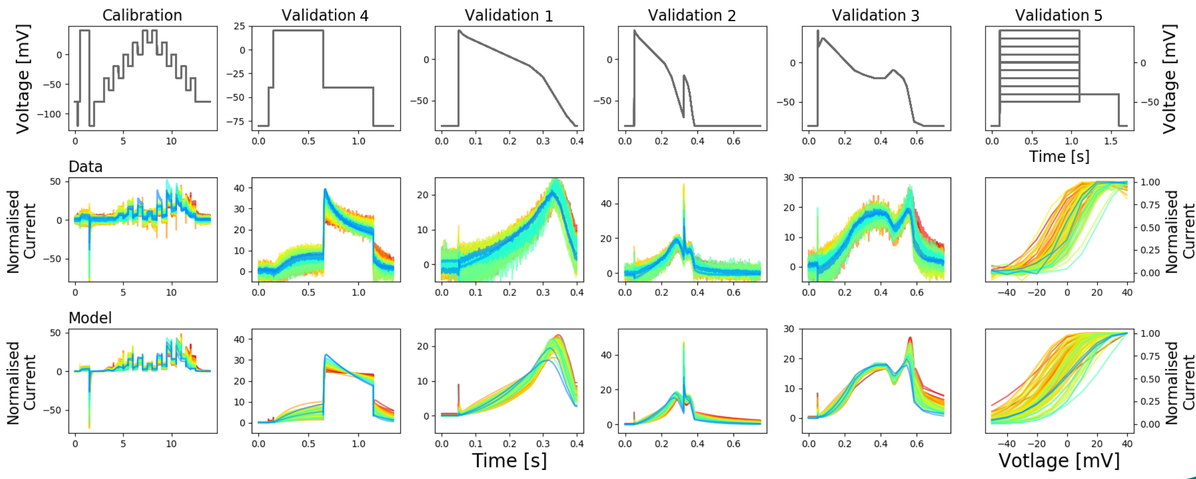


Fig 4: A total of 65 cells recordings under 6 different protocols reveal the variability in hERG kinetics. All currents are normalised to emphasise the differences in kinetics. The figure shows the recorded currents except Validation 5 where it shows the current-voltage (I-V) relation instead.

This is not the end!

3.5 Model Parameters Correlation

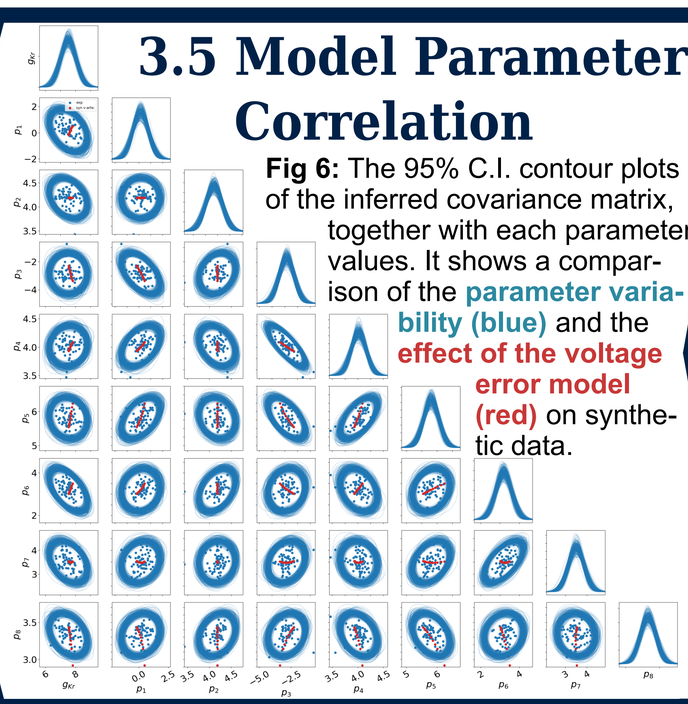


Fig 6: The 95% C.I. contour plots of the inferred covariance matrix, together with each parameter values. It shows a comparison of the **parameter variability (blue)** and the **effect of the voltage error model (red)** on synthetic data.

3.4 Consideration of Experimental Error Model

The above analyses have assumed experiments were done 'perfectly'. That is we have assumed our input of the command voltage V was what the cell experienced as the membrane voltage V_m during the experiment. However, this might not be exact [2, 3]. Therefore we have also considered the possible error source in the experiment, as error model, to give:

$$\text{Full model} = \text{Mechanistic model} + \text{Noise model} + \text{Error model}$$

where 'mechanistic model' is our I_{Kr} model and 'noise model' is a simple Gaussian/white noise model.

4. Experimental Methods

- Whole-cell patch-clamp voltage-clamp experiments were performed on CHO cells stably expressing hERG1a (Kv11.1).
- Experiments were perfromed at physiological temperature (36ºC).
- Electrophysiological recordings were made using the Nanion SyncroPatch 384PE high-throughput platform.
- A total of 6 voltage clamp protocols (see 3.1, 3.2) were used; one to fit the model, and five for validation.
- All measurements were leak corrected and E-4031 subtracted. All leak correction was performed offline and estimated using an initial step pulse.
- Selection criteria R_{seal} > 250 MΩ, C_m > 5 pF, and R_{series} < 25 MΩ were applied.

References

[1] Beattie et al. (2018) *J. Physiol.*

[2] Traynelis (1998) *J. Neurosci. Methods*

[3] Sherman et al. (1999) *Biophys. J.*

Acknowledgements

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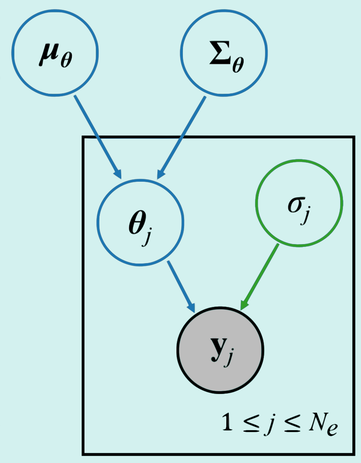
Hierarchical Model Structure

Fig 5: A schematic of the full hierarchical Bayesian model, which is a multi-level modelling technique that combines individual measures (lower level) as a group (top level). Prior distributions are specified for the μ_θ, Σ_θ, σ_j. This allows us to combine multiple experiments into one causal structure.



Notation:

$\boldsymbol{y}_j$: experimental observation of I_{Kr}
$\boldsymbol{\theta}_j$: model parameters, $\{g_{Kr}, \{p_i\}\}$
σ_j : noise model parameter
$\boldsymbol{\mu}_\theta$: hyper parameter, mean
$\boldsymbol{\Sigma}_\theta$: hyper parameter, covariance matrix
j : j^{th} experiment

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