METHOD ARTICLE

A range of voltage-clamp protocol designs for rapid capture of hERG kinetics

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Abstract

We provide details of a series of short voltage-clamp protocols designed for gathering a large amount of information on hERG (Kv11.1) ion channel gating. The protocols have a limited number of steps and consist only of steps and ramps, making them easy to implement on any patch clamp setup, including automated platforms. The primary objective is to assist with parameterisation, selection and refinement of mathematical models of hERG gating. We detail a series of manual and automated model-driven designs, together with an explanation of their rationale and design criteria. Although the protocols are intended to study hERG1a currents, the approaches could be easily extended and generalised to other ion channel currents.

Plain language summary

Ion channels are proteins that span the membranes of biological cells, and they allow certain ions to flow through them, to cross the membrane (e.g. K^+, Na^+ or Ca^{2+}). They are important in controlling concentrations of ions within the cell, and also used by the body to transmit electrical signals - controlling processes like nerve impulses or co-ordination of muscle contraction such as in the heart.

Many ion channels open and close in response to changes in the voltage across the cell membrane. Working out the intricate details of exactly how ion channels respond to voltage is difficult and often time consuming. A good method to use is voltage clamping, where some electronic apparatus is cleverly attached to a cell such that there is

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effectively one electrode inside the cell and one outside. By clamping the membrane voltage to carefully chosen waveforms, and recording the currents that flow, we can investigate how currents respond to voltage.

It is an active topic of research deciding what voltage waveform is most effective to use. One recently proposed route is to use comparatively short waveforms that rapidly perturb voltage, and use the resulting data to fit a mathematical model of the ion channel opening and closing. This article proposes a whole suite of this type of waveform, some designed manually and some designed by mathematical algorithms, which we expect to be valuable in characterising a particular heart ion channel current (hERG). The protocols should provide abundant data to train and test mathematical models of the current. These models can then be used to communicate how we think the channel works and to make predictions of its behaviour in new situations.

Keywords
experimental design, ion channel, mathematical model, computational model, patch clamp

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Introduction

This report describes a series of voltage-clamp protocol waveforms that were designed to explore the gating of cell lines expressing hERG1a / K_v11.1 channels, which are the primary subunit of the channels carrying the cardiac rapid delayed rectifier potassium current, I_{Kr} (Sanguinetti *et al.*, 1995; Vandenberg *et al.*, 2012).

The aim is to build on our previous studies that aimed to develop a range of short, information-rich voltage clamp protocols to use in experimental recordings to capture hERG gating behaviour (Beattie *et al.*, 2018; Lei *et al.*, 2019b). Here we extend these to a wide range of protocols to better parameterise, select and test mathematical models of hERG gating (Bett *et al.*, 2011) and in particular to gain a better understanding and quantification of model discrepancy — when models cannot correctly predict what happens in reality (Shuttleworth *et al.*, 2024). As a result, some protocols will focus on classic optimal experimental design in terms of reducing uncertainty / improving identifiability of model parameter estimates (Lei *et al.*, 2023). Whilst others focus on maximising differences between trained models to assist in model selection/discrimination.

All these protocols were designed during the Isaac Newton Institute's Fickle Heart programme in May–June 2019 (Mirams *et al.*, 2020). The protocols are all designed to be run on an automated patch platform, namely the Nanion SyncroPatch384PE (Obergrussberger *et al.*, 2016), which at the time had a restriction of only allowing up to 64 commands (steps or ramps) to define a single voltage-clamp protocol.

Models used in protocol design process

Our designs are model-driven akin to Lei *et al.* (2023), where mathematical models are used as part of automatic optimal design; even where our designs are manual they were done by visually examining the results of forward simulations.

The model structures that we used here are Beattie *et al.* (2018) and Wang *et al.* (1997) (also used in Fink *et al.* (2008)), with their Markov diagrams shown in Figure 1 and full equations reproduced below. The first model (Beattie *et al.*, 2018) is a Hodgkin-Huxley style model with two independent gates, which can be represented as a symmetric 4-state Markov model (see Fig. 4B of Rudy and Silva (2006)). The second model Wang *et al.* (1997) is a 5-state Markov model with 3 closed states, an open state, and an inactivated state connected sequentially.

Beattie model

In matrix/vector form, the Beattie *et al.* (2018) model can be written as,

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \begin{bmatrix} -k_1 - k_3 & 0 & k_4 & k_2 \\ 0 & -k_2 - k_4 & k_1 & k_3 \\ k_3 & k_2 & -k_1 - k_4 & 0 \\ k_1 & k_4 & 0 & -k_2 - k_3 \end{bmatrix} \mathbf{x},$$

where

$$\mathbf{x} = [C,\ I,\ IC,\ O]^T,$$

and

$$\begin{aligned} k_1 &= p_1 e^{p_2 V}, \\ k_2 &= p_3 e^{-p_4 V}, \\ k_3 &= p_5 e^{p_6 V}, \\ k_4 &= p_7 e^{-p_8 V}. \end{aligned}$$

This model is equivalent to a two gate Hodgkin-Huxley style gating model with open probability given by an "activation" a gate representing the 'right' transitions in Figure 1a multiplied by an "inactivation" r gate representing the 'down' transitions (Clerx *et al.*, 2019a; Mirams, 2023), so in the below designs

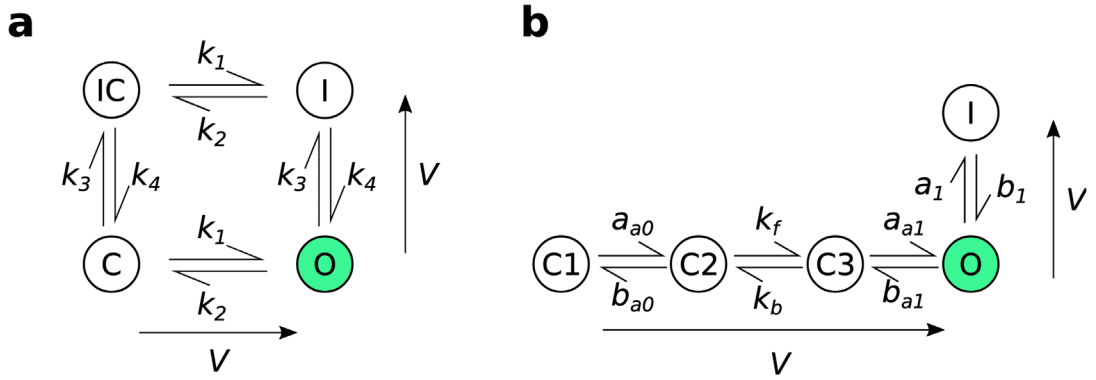

Figure 1. The model structures used for experimental design. (**a**): the four-state Beattie *et al.* (2018) model. (**b**): the five-state Wang *et al.* (1997) model. The arrows adjacent to each model structure indicate the direction in which rates increase as the voltage increases. Reproduced from Shuttleworth *et al.* (2024) under a CC-BY licence.

when we refer to "Hodgkin-Huxley" (HH) it is this interpretation of the model we are using.

Wang model

The Wang *et al.* (1997) model can be written as:

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \begin{bmatrix} -a_{a0} & b_{a0} & 0 & 0 & 0 \\ a_{a0} & -b_{a0} - k_f & k_b & 0 & 0 \\ 0 & k_f & -k_b - a_{a1} & b_{a1} & 0 \\ 0 & 0 & a_{a1} & -b_{a1} - a_1 & b_1 \\ 0 & 0 & 0 & a_1 & -b_1 \end{bmatrix} \mathbf{x},$$

where

$$\mathbf{x} = [C_1,\ C_2,\ C_3,\ O,\ I]^T,$$

and

$$\begin{aligned} a_1 &= q_1 e^{q_2 V}, \\ a_{a0} &= q_3 e^{q_4 V}, \\ a_{a1} &= q_5 e^{q_6 V}, \\ b_{a1} &= q_7 e^{-q_8 V}, \\ b_1 &= q_9 e^{-q_{10} V}, \\ b_{a0} &= q_{11} e^{-q_{12} V}. \end{aligned}$$

The default (room temperature) parameter values for both models are presented in Table 1. In practice we remove one state from the system and set it equal to "one minus the sum of the rest" to solve the ODE system, to improve numerical stability. All models are solved using a Python package Myokit (Clerx *et al.*, 2016) using SUNDIALS CVODE (Hindmarsh *et al.*, 2005).

Common protocol segments

As described in Mirams *et al.* (2024), all the protocols we have designed have common start and end sections, as defined in Table 2. The purposes of these sections are:

- Start — an 'activation step' to provoke a very large tail current and help with conductance estimation, as discussed in Beattie *et al.* (2018).
- End — a 'reversal ramp' to help assess whether the current is reversing at the expected Nernst potential, discussed in Lei *et al.* (2019b).
- both can also be used in quality control to check that these sections behave similarly over time when different protocols are applied to the same cell.

Manual protocol designs

The details of the protocols in this section are provided in Table 3.

Original staircase protocol

Figure 2 shows the original staircase protocol. It was manually designed to capture various dynamics of hERG (Lei *et al.*, 2019b;

Table 1. The default parameter sets we use for the Wang *et al.* (1997) and Beattie *et al.* (2018) models. The column 'Range' indicates the parameter range obtained from real data fitting results based on protocols staircaseramp, sis, hh3step, and wang3step, which is used for global sensitivity-based designs.

Wang model				Beattie Model			
	Value	Range	Units		Value	Range	Units
g	2.11	—	$\times 10^{-1}$ μS	g	2.44	—	$\times 10^{-1}$ μS
k_b	0.67	[0.67,99993]	$\times 10^{-2}$ ms^{-1}	p_1	1.68	[1.39,12.9]	$\times 10^{-4}$ ms^{-1}
k_f	1.31	[1.31,99550]	$\times 10^{-2}$ ms^{-1}	p_2	8.06	[1.08,8.49]	$\times 10^{-2}$ mV^{-1}
q_1	1.24	[1.24,1.81]	$\times 10^{-1}$ ms^{-1}	p_3	4.34	[2.77,32.3]	$\times 10^{-5}$ ms^{-1}
q_2	1.56	[1.55,2.06]	$\times 10^{-2}$ mV^{-1}	p_4	4.07	[2.48,4.56]	$\times 10^{-2}$ mV^{-1}
q_3	0.04	[0.03,1.02]	$\times 10^{-2}$ ms^{-1}	p_5	9.07	[6.40,19.9]	$\times 10^{-2}$ ms^{-1}
q_4	10.9	[0.0001,10.9]	$\times 10^{-2}$ mV^{-1}	p_6	2.67	[2.18,3.87]	$\times 10^{-2}$ mV^{-1}
q_5	0.24	[0.23,364]	$\times 10^{-2}$ ms^{-1}	p_7	7.32	[7.07,10.9]	$\times 10^{-3}$ ms^{-1}
q_6	0.0001	[0.0001,6.44]	$\times 10^{-2}$ mV^{-1}	p_8	3.22	[2.89,3.39]	$\times 10^{-2}$ mV^{-1}
q_7	3.15	[1.29,7.69]	$\times 10^{-4}$ ms^{-1}				
q_8	3.99	[2.97,3.99]	$\times 10^{-2}$ mV^{-1}				
q_9	5.75	[3.55,5.75]	$\times 10^{-3}$ ms^{-1}				
q_{10}	2.89	[2.89,3.34]	$\times 10^{-2}$ mV^{-1}				
q_{11}	0.28	[0.007,1458]	$\times 10^{-2}$ ms^{-1}				
q_{12}	10.7	[1.16,11.8]	$\times 10^{-2}$ mV^{-1}				

Table 2. Details of the Start and End clamp sections for all designs. 't' indicates the duration of the clamp section, and 'V' the relevant voltage(s) for this clamp. Where 'Ramp' is specified it is a linear ramp over time between the voltages shown, as opposed to a constant voltage clamp for a 'Step'. Reproduced from Mirams *et al.* (2024).

Clamp	Initial: for leak and conductance			End: reversal ramp sequence		
#	Step/Ramp	t (ms)	V (mV)	Step/Ramp	t (ms)	V (mV)
1	Step	250	–80	Step	1000	–80
2	Step	50	–120	Step	500	40
3	Ramp	400	–120 to –80	Step	10	–70
4	Step	200	–80	Ramp	100	–70 to –110
5	Step	1000	40	Step	390	–120
6	Step	500	–120	Step	500	–80
7	Step	1000	–80	—	—	—

Table 3. Details of the 5 protocols: staircase, sis, sisi, manualppx, and squarewave. All voltage values shown here are voltage steps to clamp to. These steps need to have the two 'bookend' sections added (see Table 2) which are identical for all designs.

Clamp	staircase		sis		sisi		manualppx		squarewave	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
1	-40	500	-40	500	40	500	60	200	60	24.9
2	-60	500	-60	500	0	500	-60	200	40	25
3	-20	500	-20	500	20	500	-100	200	60	25
4	-40	500	-40	500	-20	500	40	500	40	25
5	0	500	0	500	0	500	-90	200	60	25.1
6	-20	500	-20	500	-40	500	30	500	40	9.9
7	20	500	20	500	-20	500	-80	200	-12	15
8	0	500	0	500	-60	500	-100	200	8	25.1
9	40	500	40	225	-40	225	20	200	-12	24.9
10	20	500	-80	50	-80	50	-40	1000	8	25
11	40	500	-40	50	-40	50	60	200	-12	25.1
12	0	500	-60	50	-60	50	0	200	8	19.9
13	20	500	-20	50	-20	50	-50	1000	60	5
14	-20	500	-40	50	-40	50	-10	100	40	25
15	0	500	0	50	0	50	10	100	60	25.1
16	-40	500	-20	50	-20	50	-20	100	40	25
17	-20	500	20	50	20	50	-80	300	60	25

Clamp	staircase		sis		sisi		manualppx		squarewave	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
18	-60	500	0	50	0	50	0	100	40	24.9
19	-40	500	40	50	40	50	-20	100	60	5
20	—	—	20	50	20	50	-100	200	8	20.1
21			40	50	40	50	40	300	-12	24.9
22			0	50	0	50	-60	100	8	25
23			20	50	20	50	0	100	-12	25.1
24			-20	50	-20	50	-10	100	8	24.9
25			0	50	0	50	-20	100	-12	15
26			-40	50	-40	50	-30	100	40	10
27			-20	50	-20	50	-40	100	60	25.1
28			-60	50	-60	50	-80	100	40	24.9
29			-40	50	-40	50	30	100	60	25
30			-80	50	-80	50	60	100	40	25.1
31			40	225	-40	225	—	—	60	24.9
32			0	500	-60	500			-12	25.1
33			20	500	-20	500			-100	25
34			-20	500	-40	500			-120	25
35			0	500	0	500			-100	25
36			-40	500	-20	500			-120	24.9
37			-20	500	20	500			-100	10
38			-60	500	0	500			-48	15
39			-40	500	40	500			-68	25.1
40			—	—	—	—			-48	25
41									-68	24.9
42									-48	25
43									-68	20
44									-120	5
45									-100	25
46									-120	25.1
47									-100	25
48									-120	24.9
49									-100	25.1
50									-120	4.9
51									-68	20

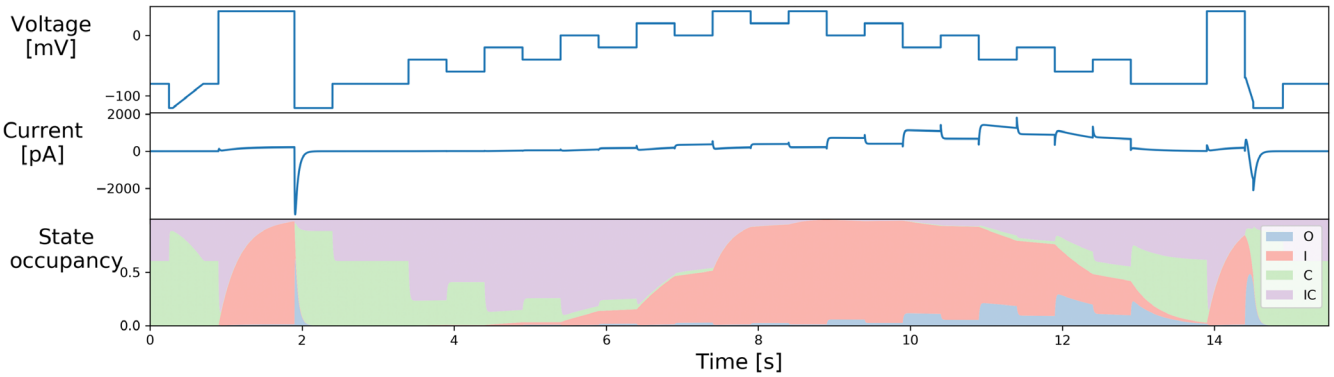

Figure 2. The manually-designed staircase protocol used in Lei, Clerx, Beattie, *et al.* (2019a; Lei *et al.*, 2019b) and its simulation, with state occupancy shown for the Beattie *et al.* (2018) model of Figure 1a. Reproduced from (Lei *et al.*, 2019b) under a CC-BY licence.

Lei *et al.*, 2019a), which has been used and tested on the Nanion SyncroPatch384PE. We have been using it as a quality control of the full run of the experiments when designing the protocols in the rest of this report.

Staircase-in-staircase protocol

The original staircase protocol provided a good foundation and motivation for improving experimental designs for characterisation of ion channel kinetics in high-throughput machines. We attempted to further improve this manual design by enhancing the exploration of inactivation processes of hERG. The original staircase protocol involves only voltage steps of 500 ms, which may not be able to explore fully the fast dynamics of hERG inactivation processes. Therefore, a shorter step duration version (50 ms) of the full staircase protocol is introduced at the middle of the staircase protocol, termed the staircase-in-staircase (sis) protocol (Figure 3, top). We also explored the possibility of inverting the order of the staircase as shown in Figure 3, bottom (sisi).

Phase-space filling protocol

The idea here is to have a protocol that fills up the phase-voltage space as much as possible. In brief, this design draws out the a, r, V three dimensional 'phase-voltage space' {[0,1],[0,1],[-120,60]} for the Beattie *et al.* (2018) model and subdivides it into 6 compartments in each dimension, giving a total of N = 6^3 = 216 boxes. Since the phase space defines all possible behaviours of a model, if a protocol forces the model to visit as many of these boxes as possible, then the observations should test model assumptions well and provide rich information to fit model parameters. We have published the rationale and details of the design process for these protocol separately in Mirams *et al.* (2024). Figure 4 (top) shows a manually-tuned phase space filling protocol (manualppx); no objective function *per se*.

A square-wave conversion of the sinusoidal protocol

In this design, we aim to design protocols based on sums of square waves, as inspired by Beattie *et al.* (2018). Such a protocol consists of a combination of N square waves, where each square wave i is defined by amplitude a_i, (angular) frequency ω_i, and phase lag ϕ_i. The protocol is defined by $3N$ parameters plus an extra parameter for an offset voltage, which can be expressed as:

$$V_{\text{square wave}}(t) = b + \sum_{i}^{N} a_i \ \text{sign}\left(\sin(\omega_i t + \phi_i)\right), \qquad (1)$$

where the function sign(·) takes a value +1 if its argument is positive, -1 if negative, or 0 if the argument is 0.

A direct conversion of the sine waves in the Beattie *et al.* (2018) protocol is performed, with the same amplitudes and frequencies, to square waves. It is a combination of three square waves (N = 3) with a_1 = 54 mV, a_2 = 26 mV, a_3 = 10 mV, ω_1 = 0.007 ms, ω_2 = 0.037 ms, ω_1 = 0.19 ms, and $\phi_1 = \phi_2 = \phi_3$ = 0, and an offset of b = −30 mV. The resulting protocol is called 'squarewave' and is shown in Figure 4 (middle).

Long action potential protocol

As a final 'manually-chosen' design, we also propose a lumped action potential protocol for validation purposes, as shown in Figure 4 (bottom). It consists of two action potential morphologies, an early after-depolarisation (EAD)-like action potential, and a delayed after-depolarisation (DAD)-like action potential. The details of this longap protocol are provided in Table 4.

Automated Iterative 3-step designs

Here we describe protocol design approaches that can be done objectively and automatically. With the same rationale as described in Mirams *et al.* (2024), we consider a protocol consists of $3N$ steps with $N \in \mathbb{N}$, and we split the protocol into N units with 3 consecutive voltage steps as a unit. For some designs, N is the number of model parameters, while for others, N is 17 to bring the total number of steps to 51 which is close to the 64 allowed by the Nanion SyncroPatch384PE when the start and end clamps are added (Table 2). For each unit i, we optimise the 3 voltage steps through an objective function S_i, with each step defined by two parameters:

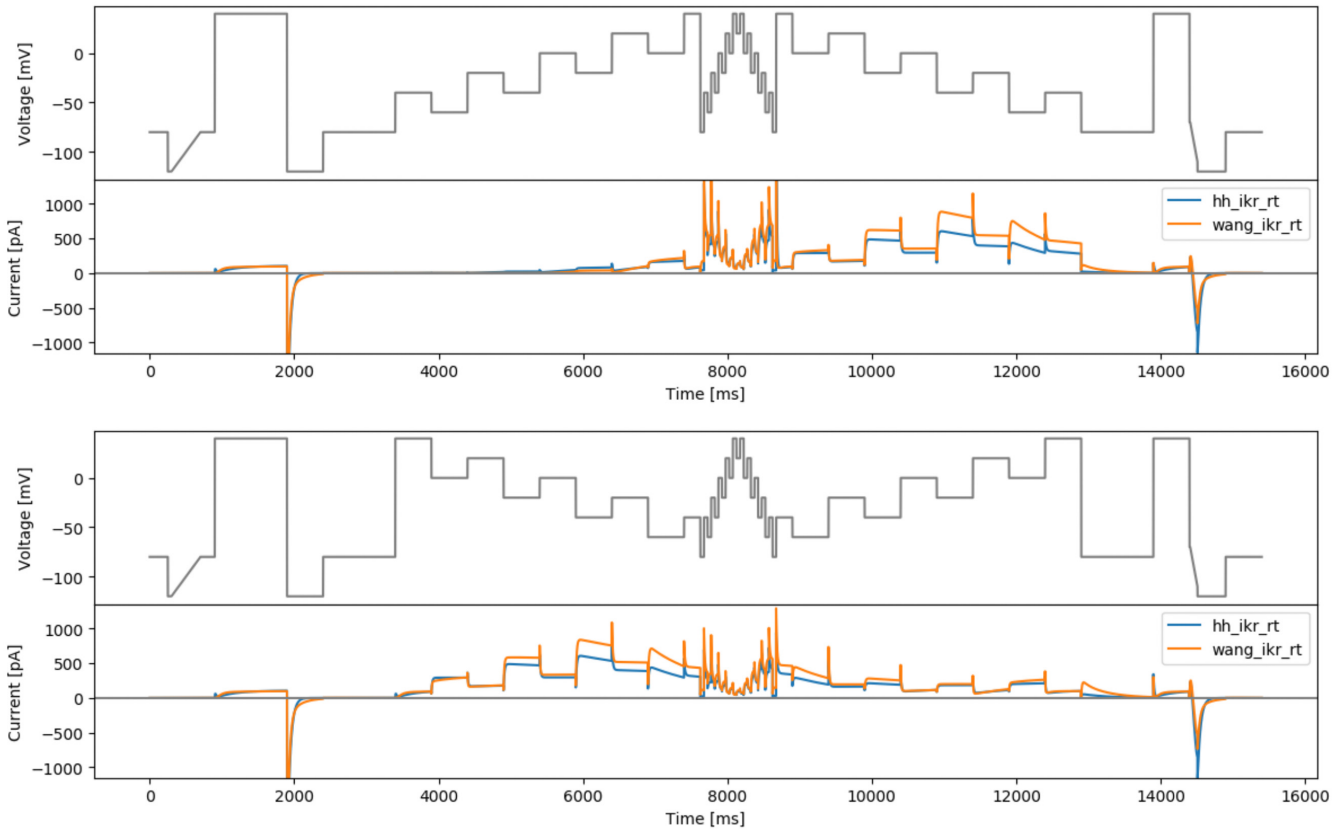

Figure 3. Manual designs. Top: the staircase-in-staircase (sis) protocol. Bottom: the 'inverted' staircase-in-staircase (sisi) protocol. Underneath each protocol are simulated currents from the two models (`hh_ikr_rt` is the Beattie *et al.* (2018) model of I_{Kr} and `wang_ikr_rt` is the Wang *et al.* (1997) model of I_{Kr}, both parameterised to room temperature data).

voltage V and duration Δt. Each objective function S_i (described in the sections below) aims to achieve a different purpose. We then iterate the process for all the objective functions $i = 1, 2, \ldots, N$, resulting in a $3N$ steps protocol.

The optimisation was performed using a global optimisation scheme, covariance matrix adaptation evolution strategy (CMA-ES, Hansen, 2006) implemented via a Python package PINTS (Clerx *et al.*, 2019b). All optimisation of the designs were repeated 10 times from different randomly varied initial starting points, and the best designs are presented here. Although we do not expect our design would reach the same global optimum as optimising all > 20 steps at once (Mirams *et al.*, 2024), our results still show promising protocol designs. We also tried to perform fitting 6-steps-at-once in Mirams *et al.* (2024) and showed that both resulted in similar performance. Finally, the presented results are the optimised results rounded to the nearest one decimal place in millisecond and millivolt for practical implementation (Mirams *et al.*, 2024).

The details of the protocols in this section are provided in Table 4, Table 5 and Table 6.

Sensitivity-based designs

Maximising approximated local sensitivity

For an ion channel current model I with N parameters p_1, p_2, ... p_N, we define an objective function for each 3-step unit i that maximises the absolute value of the sensitivity $\left|\frac{\partial I}{\partial p_i} p_i\right|$ of the model output I with respect to the parameter p_i while minimising all the absolute value of sensitivity of the rest of the parameters. This objective function can be mathematically expressed as

$$S_i\left(\{V_{i,j}, \Delta t_{i,j}\}_{j=1}^{3}\right) = \frac{\int_{\Delta t_{i,3}} \left|\frac{\partial I}{\partial p_i} p_i\right| dt}{\Sigma_k \int_{\Delta t_{i,3}} \left|\frac{\partial I}{\partial p_k} p_k\right| dt}.$$

The sensitivity was calculated using a first-order central difference scheme with δp_i being 0.1 % × p_i. Note that the integration is only over the last step of the 3 steps, the idea is to allow the first two steps to vary as much as it would need to be to

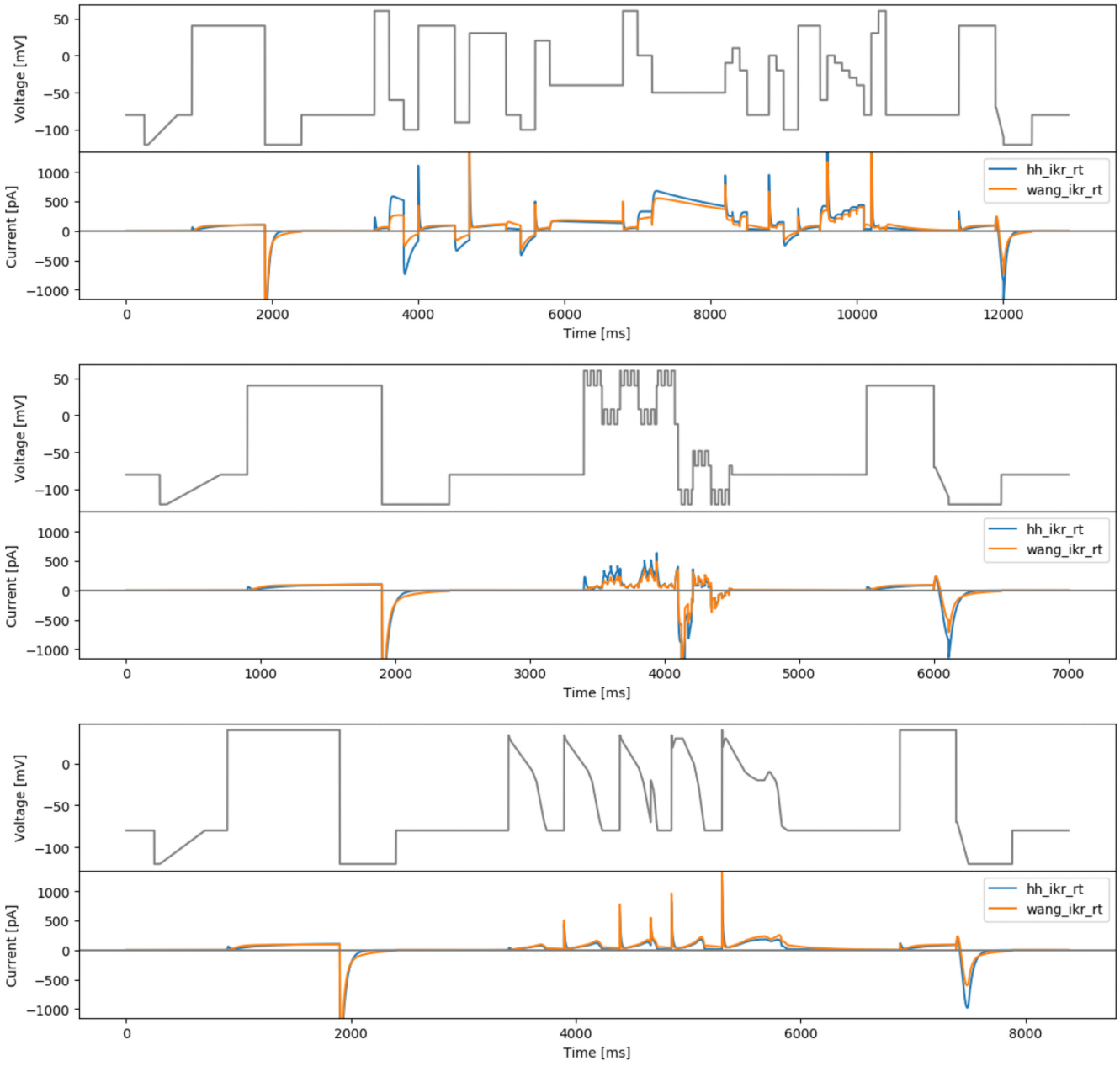

Figure 4. More manual designs. Top: the manual phase space protocol (manualppx). Middle: the square wave protocol of Beattie *et al.* (2018) (squarewave). Bottom: the lumped action potential protocol (longap). Beneath each protocol we show simulated currents from both the Beattie and Wang models.

maximise the approximated local sensitivity across the third step (it is fine if there is low sensitivity because of e.g. full inactivation in the first two steps). This has been repeated for both models and the results are shown in Figure 5.

Maximising Sobol sensitivity

Instead of the local sensitivity, we can also replace it with the first-order Sobol global sensitivity indices, given by

$$S_i\left(\{V_{i,j},\Delta t_{i,j}\}_{j=1}^{3}\right)=\frac{1}{\mathrm{Var}(I)}\mathrm{Var}_{p_i}\left(\mathrm{E}_{p_{\setminus i}}(I\mid p_i)\right).$$

Here the $p_{\setminus i}$ notation denotes the set of all parameters except p_i. This has been repeated for the Beattie & Wang models. The parameter range (Table 1) was taken from previous real data fits to staircaseramp, sis, hh3step and wang3step, using the approach from Lei, Clerx, Gavaghan, *et al.* (2019b) without

Table 4. Details of the 3 protocols: rtovmaxdiff, maxdiff, and longap. All voltage values for protocols rtovmaxdiff and maxdiff are voltage steps to clamp to. Protocol longap also indicates with 'Ramp' or 'Step'; 'Ramp' is specified it is a linear ramp over time between the voltages shown, as opposed to a constant voltage clamp for a 'Step'. These steps need to have the two 'bookend' sections added (see Table 2) which are identical for all designs.

Clamp	rtovmaxdiff		maxdiff		longap		
#	V (mV)	t (ms)	V (mV)	t (ms)	Step/Ramp	V (mV)	t (ms)
1	60	167	-120	12.5	Step	34	3
2	-65	516	60	12.5	Ramp	30	8
3	-49	861	-120	12.5	Ramp	26	15.2
4	-100	587	60	12.5	Ramp	-8	183.6
5	46	658	-120	12.5	Ramp	-21	39
6	-60	446	60	12.5	Ramp	-68	65.8
7	60	150	-120	12.5	Ramp	-80	25.2
8	4	185	60	12.5	Step	-80	155.6
9	-100	208	-120	12.5	Step	34	3
10	-74	935	60	12.5	Ramp	30	8
11	42	742	-120	12.5	Ramp	26	15.2
12	29	751	60	12.5	Ramp	-8	183.6
13	60	986	-120	12.5	Ramp	-21	39
14	-100	866	60	12.5	Ramp	-68	65.8
15	-2	797	-120	12.5	Ramp	-80	25.2
16	60	177	60	12.5	Step	-80	155.6
17	-84	79	-120	12.5	Step	34	3
18	60	943	60	12.5	Ramp	30	8
19	60	494	-120	12.5	Ramp	26	15.2
20	32	666	60	12.5	Ramp	-5	142.6
21	37	73	-120	12.5	Ramp	-21	38.4
22	-100	380	60	12.5	Ramp	-70	68.6
23	60	474	-120	12.5	Step	-20	2
24	12	101	60	12.5	Ramp	-30	20
25	-1	904	-120	12.5	Ramp	-40	10
26	60	162	60	12.5	Ramp	-65	15
27	9	989	-120	12.5	Ramp	-80	12
28	60	323	60	12.5	Step	-80	125
29	2	444	-120	12.5	Step	34	3
30	-50	492	60	12.5	Ramp	19	6
31	—	—	-120	12.5	Ramp	30	26.4
32			60	12.5	Step	30	65
33			-120	12.5	Ramp	0	99

Clamp	rtovmaxdiff		maxdiff		longap		
#	V (mV)	t (ms)	V (mV)	t (ms)	Step/Ramp	V (mV)	t (ms)
34			60	12.5	Ramp	-25	40
35			-120	12.5	Ramp	-80	55
36			60	12.5	Step	-80	155
37			-120	12.5	Step	40	3
38			60	12.5	Step	20	3
39			-120	12.5	Ramp	30	20
40			60	12.5	Step	30	10
41			-120	12.5	Ramp	-10	168
42			60	12.5	Ramp	-15.5	50.6
43			-120	12.5	Ramp	-20	61.2
44			60	12.5	Step	-20	60
45			-120	12.5	Ramp	-10	40
46			60	12.5	Step	-10	10
47			-120	12.5	Ramp	-20	50
48			60	12.5	Ramp	-30	20
49			-120	12.5	Ramp	-75	36
50			60	12.5	Ramp	-80	50

Table 5. Details of the 5 protocols: hh3step, wang3step, hhsobol3step, wangsobol3step, and spacefill26. All voltage values shown here are voltage steps to clamp to. These steps need to have the two 'bookend' sections added (see Table 2) which are identical for all designs.

Clamp	hh3step		wang3step		hhsobol3step		wangsobol3step		spacefill26	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
1	-96.7	983	59.8	1000	60	1000	-120	52.8	40	841
2	59.7	730	35.3	1000	60	1000	48.2	1000	-63	773
3	-50.9	266	-47.6	216	-69.4	54.9	-46.1	1000	-117.9	163
4	9.07	852	-107	341	-82.5	999	1.3	1000	59.8	174
5	45.8	621	-89.9	641	10.8	955	-4.03	1000	22.1	46
6	-45.5	360	-80.7	377	-51.1	219	-17.8	1000	-97	214
7	-120	999	-119	998	-81.8	999	-97.7	50.4	32.9	409
8	-120	1000	-74.6	281	60	51.5	-85	784	-106.1	29
9	-88	222	-60.4	54.4	-55.4	103	-85	232	25.1	20
10	30.2	388	59.8	1000	-80.2	488	-85.2	1000	-86.8	23
11	56.6	972	28.3	1000	60	1000	-89.8	711	59.9	56
12	-120	50.2	-47.8	233	-71.1	1000	-120	1000	-76.9	156
13	57.5	497	-111	61.2	60	1000	-82.4	195	-6	20
14	-120	1000	-99.2	398	60	1000	41.6	1000	-74.3	37

Clamp	hh3step		wang3step		hhsobol3step		wangsobol3step		spacefill26	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
15	-120	999	-78.7	116	-120	102	-57	108	-10.6	20
16	-96	642	-102	783	60	1000	-84.3	548	-75	164
17	59.8	806	-66.6	219	60	1000	5.02	261	55	160
18	-42.5	400	60	151	-7.19	269	51	129	-47.5	25
19	56	936	-97.6	443	-64.9	50	-99.5	1000	-7.8	38
20	-4.8	55.6	-97.6	784	-47.3	75.1	2.12	1000	-74.4	213
21	59.8	50	-107	317	-81.4	67.3	-41.7	187	-42.7	367
22	-53.1	488	-95.3	665	60	1000	-85.2	999	-52.5	483
23	59	989	-119	616	60	1000	41.9	50.2	-85	33
24	-42.8	321	-111	407	-1.7	1000	-85.1	650	5.5	20
25	-77.9	753	60	1000	-52.4	50	-69.6	1000	-105.5	27
26	46.5	911	-120	50	60	1000	-10.2	815	-58.6	32
27	-116	54.3	-120	50	-54.1	50	-71.5	1000	-114.2	20
28	—	—	59.6	1000	—	—	20.3	1000	14.1	108
29			30.5	1000			46.8	797	-90.5	20
30			-39.7	297			-120	663	-49.1	20
31			-120	725			-8.4	128	59.9	103
32			-106	225			36.1	374	-101.7	20
33			-108	568			53.8	999	15.1	20
34			59.6	1000			-85	949	-87.8	61
35			31.3	999			-84.9	423	15.4	272
36			-41.9	187			-111	129	-114	169
37			60	1000			-120	1000	34.7	892
38			60	1000			22	198	-83.5	87
39			60	1000			-88.9	1000	46.6	444
40			-66.1	727			-84.6	869	-100.2	23
41			-120	931			33.6	50	-3.3	23
42			0	50			27.3	99.3	21	26
43			60	159			-120	50.3	-55.8	421
44			-120	1000			-85	107	-95.3	29
45			-55.2	1000			-85	60.7	-8.4	32
46			—	—			—	—	-101.6	33
47									-20.7	20
48									-64.9	20
49									50.5	585
50									-97.4	115
51									3.7	658

Table 6. Details of the 5 protocols: spacefill10, spacefill19, hhbrute3gstep, wangbrute3gstep, and rvotmaxdiff. All voltage values shown here are voltage steps to clamp to. These steps need to have the two 'bookend' sections added (see Table 2) which are identical for all designs.

Clamp	spacefill10		spacefill19		hhbrute3gstep		wangbrute3gstep		rvotmaxdiff	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
1	50.5	336	60	142	37.7	795	60	837	19	500
2	-97.3	89	-69.5	844	-120	261	-43.7	506	-32	50
3	-12.7	20	-106.3	58	-36.6	735	-120	892	-31	50
4	-88.5	67	-11.6	33	41	231	-71.3	1000	-49	50
5	18.8	804	48.8	584	-45.3	815	-117	50	5	50
6	-114.3	166	-97	689	-65.4	50.2	-114	50	54	439
7	59	149	33.6	752	-120	530	57.9	169	22	499
8	-60.5	438	-79.1	398	60	459	-33.4	617	22	500
9	-97.5	120	-49.8	257	-120	714	-116	757	19	145
10	57.5	144	50.5	99	-19	1000	-51.9	50	-26	89
11	-52.4	496	-104.2	32	20.2	485	41.7	50	-66	50
12	-75	465	-33	35	-64.1	1000	57.2	50.4	-85	50
13	34.7	711	-106.3	62	50.4	947	12.8	446	7	50
14	-113.5	31	11.5	228	-34.1	362	-28.6	358	43	121
15	-9.7	299	-79.6	153	-36.5	991	-55.2	746	-17	53
16	-70	33	58.2	594	-47.3	1000	-15.6	1000	-13	50
17	12.2	79	-71.3	462	-30.8	1000	-82.6	50	9	500
18	-98.1	21	-24.4	110	-72.9	50.1	-94.9	152	-95	50
19	45.5	168	17.1	617	58.8	650	-48.9	339	-16	500
20	-85.9	59	-96.7	38	-120	471	60	293	-48	153
21	33.7	25	59.1	720	-41.7	762	-120	76.3	-13	500
22	-97.5	76	-47.8	351	-47	1000	10.8	363	-59	50
23	-42.7	32	-98.5	151	35.4	50	-27.8	50.5	-97	50
24	-109.7	21	-28.1	457	8.1	50	-43.6	1000	48	460
25	0.3	177	58.8	96	50.8	914	60	986	48	52
26	-86.8	144	-41.3	336	-32.1	376	-120	228	27	50
27	-23.3	455	-56.1	526	-120	251	60	672	-8	50
28	-106.3	33	58.4	144	-29.2	50	44.6	50	-64	50
29	54.6	20	-99.3	31	1.81	1000	49.8	50	-90	50
30	-60	169	59.8	382	-30.1	1000	-117	62.2	23	500
31	59.9	153	-28	886	-46.8	576	60	448	—	—
32	-74.2	29	-119.4	20	46.4	905	-44.1	817		
33	5.3	20	-16.4	221	-34.9	783	-120	561		
34	-29.5	933	-106.3	58	-17.8	1000	9.6	50		

Clamp	spacefill10		spacefill19		hhbrute3gstep		wangbrute3gstep		rvotmaxdiff	
#	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)	V (mV)	t (ms)
35	-105.9	35	54.5	586	-0.1	1000	28.6	50		
36	38	29	-107.9	146	15.3	50	43	50.4		
37	-91.2	80	59	123	50.4	913	60	153		
38	-19	493	-101	21	-34.9	835	-120	957		
39	-115.6	1007	37.2	20	-38.9	818	60	206		
40	59.9	218	-102.3	46	-116	50.5	32.1	50		
41	-99.5	54	37.7	182	50.5	115	-7.3	50.5		
42	-42.1	799	-27.8	849	-98.1	324	1.1	50		
43	-101.5	105	-43.9	44	60	512	58.9	200		
44	14.5	36	-93.5	37	-120	980	-45	947		
45	33.7	754	16.7	107	-37.1	98.9	-120	105		
46	56.3	45	-42.7	179	—	—	—	—		
47	-75.8	25	-97.3	102						
48	28.7	26	-8	250						
49	-20.5	364	26.4	93						
50	-98.9	26	-101.3	20						
51	13.1	21	26.8	27						

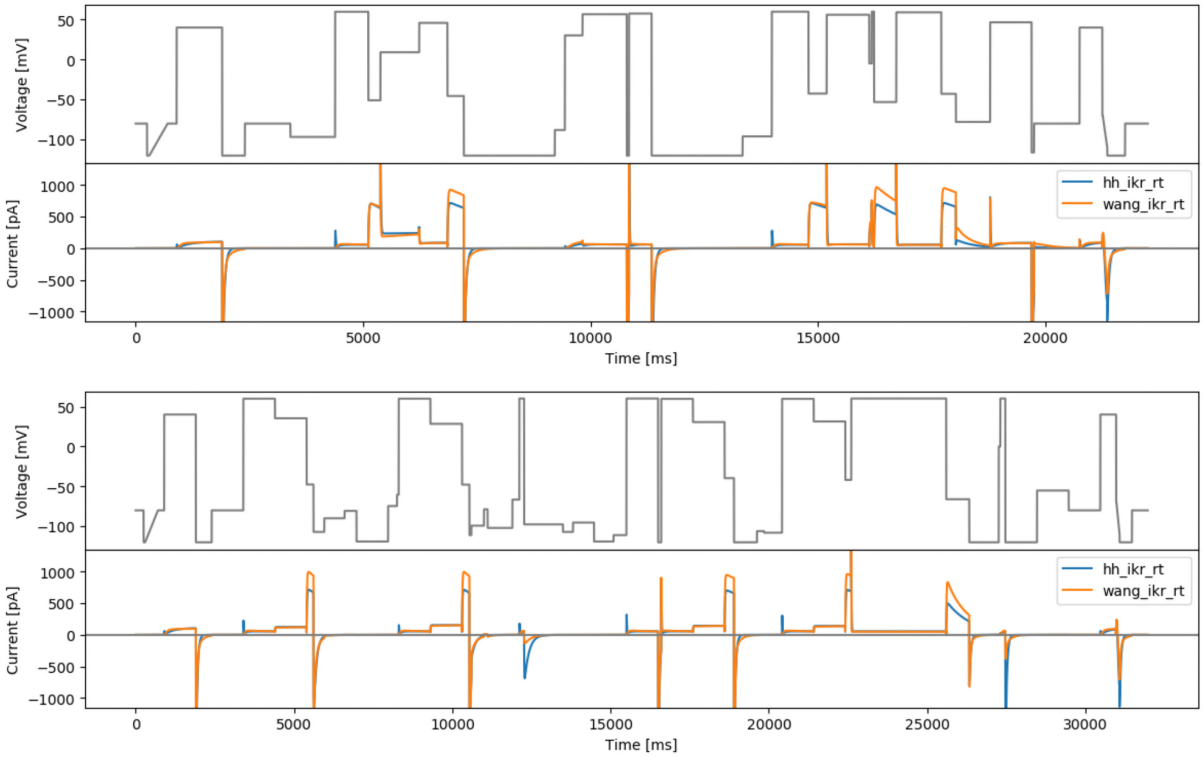

Figure 5. The 3-step local sensitivity designs. Top: protocol based on the Hodgkin-Huxley model (hh3step). Bottom: based on the Wang model (wang3step). With simulated currents from both models shown below the protocols.

accounting for experimental error (Lei *et al.*, 2020a; Lei *et al.*, 2020b).

To calculate Sobol sensitivities we used a modified version of the SA-lib library (Herman & Usher, 2017), to enable easier calculation of sensitivities over time series, which is included in our repository (see Data Availability). The results are shown in Figure 6.

Gibbs designs

We use the 3-step approach discussed above, but the difference here is that instead of defining each step by two parameters (voltage V and duration Δt), for each 3-step section we optimise only one of these parameters (either V or Δt) while randomly picking the other from a uniform distribution. This halves the number of parameters that are inferred to just 3 per 3-step section. However, since we have only the same objective function, all units would return the same optimum (or a few if multi-modal but very limited) which is not desired. Therefore we introduce some stochasticity to the protocol by randomly choosing one of the step parameters and optimising only the other one.

Maximising model output differences: a brute-force sampling approach

The approach taken in this design is similar to a global sensitivity analysis. For a given model I, we start with randomly picking M (ideally $\sim$ 1000s but practically $\sim$ 100s of) parameters from model parameter prior, then the objective function to be optimised is the sum of the root mean square deviation (RMSD) values between the model outputs from all combinations of the sampled parameter pairs. The model parameter prior could be an a-priori distribution of the parameters (for example those used in Beattie *et al.*, 2018; Lei *et al.*, 2019b), or based on previous fitting results (see below). The objective function for a 3-step unit i can be expressed as

$$S(\boldsymbol{\theta}_i) = \frac{2}{M^2} \sum_{j=1}^{M} \sum_{k>j}^{M} \mathrm{RMSD}(I_j, I_k), \tag{2}$$

where RMSD(x, y) denotes the RMSD between x and y, and I_j, I_k are the model output for the M parameter samples. We choose $\boldsymbol{\theta}_i = \{V_j\}_{j=1}^{3}$ with $\Delta t_j \sim$ Uniform(50,1000) ms for odd i, and $\boldsymbol{\theta}_i = \{\Delta t_j\}_{j=1}^{3}$ with $V_j \sim$ Uniform(–120,60) mV for even i.

This has been repeated for the Beattie and Wang models, with the parameter range (prior distribution) was taken from the extremes of the range defined by previous real data fits to staircsaeramp, sis, hh3step and wang3step, as provided in Table 1. The results are shown in Figure 7.

Maximising differences between two models

Unlike the previously defined approaches, where only one model was involved, this proposed approach aims to distinguish

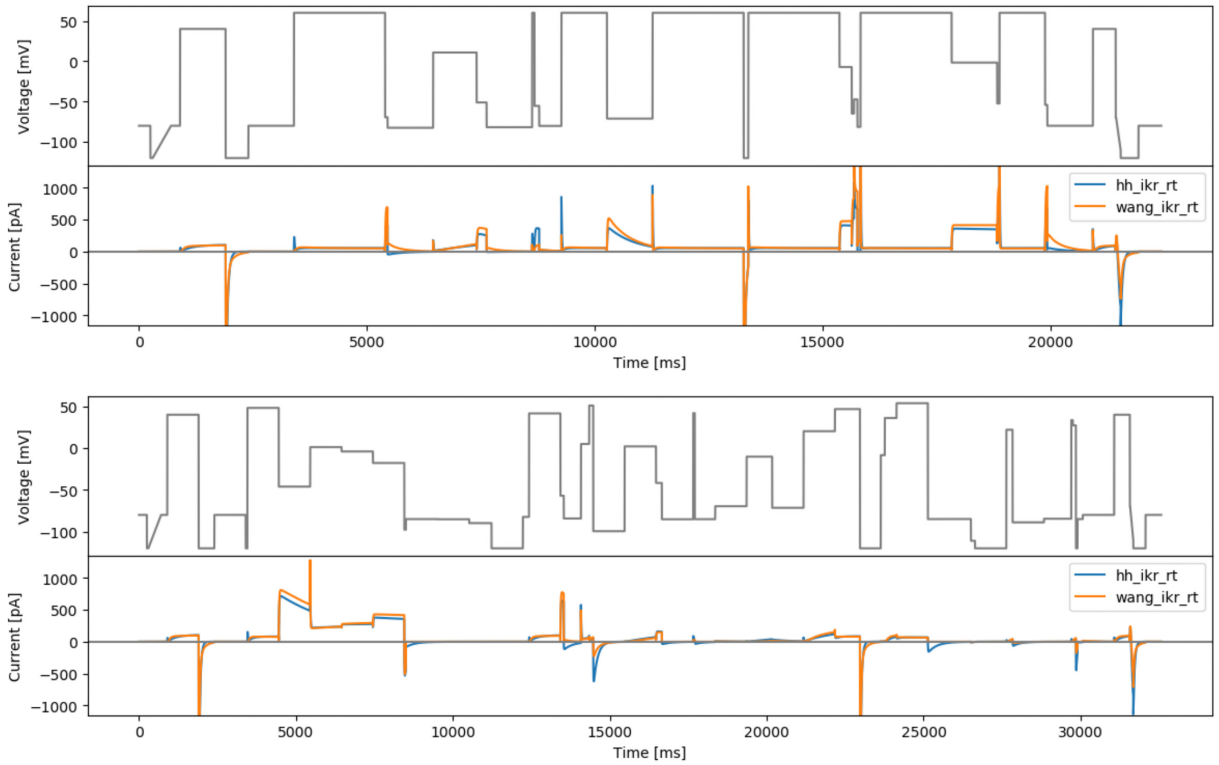

Figure 6. The 3-step Sobol sensitivity protocols. Top: based on the Hodgkin-Huxley model (hhsobol3step). Bottom: based on the Wang model (wangsobol3step). With simulated currents from both models shown below the protocols.

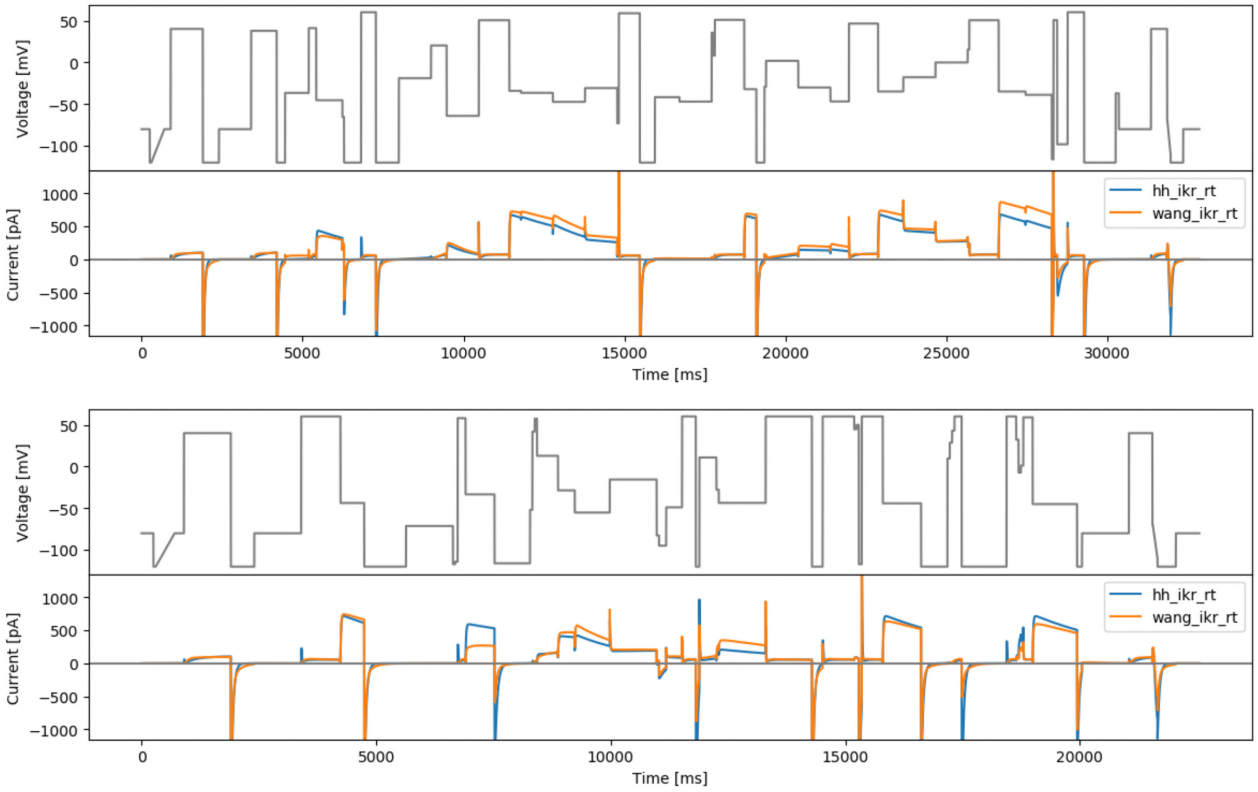

Figure 7. The brute-force sampling protocols. Top: based on the Hodgkin-Huxley model (hhbrute3gstep). Bottom: based on the Wang model (wangbrute3gstep). Simulated currents from both models are shown beneath each protocol.

between two candidate models. The objective function is defined as the RMSD value between two model currents, with a given set of model parameters (Table 1), so it is still a 'local' design with respect to model parameters. One protocol randomly picks time parameters for each 3-step unit, and optimises voltages ($\{V_j\}_{j=1}^{3}$ with $\Delta t_j \sim$ Uniform(50,500) ms and is termed 'rtovmaxdiff'); and the other method randomly picks voltages and optimises the step durations $\{\Delta t_j\}_{j=1}^{3}$ with $V_j \sim$ Uniform(–120,60) mV, and is known as 'rvotmaxdiff'. Applying this approach to the Beattie & Wang models results in Figure 8.

Phase-voltage space filling designs

For details of this approach, see Mirams *et al.* (2024). Briefly, an objective function tries to maximise the amount of new boxes that are visited by a model's trajectory for each new iterative '3 step' set of pulses (as described above) repeating sequentially until we have 17 sets of 3 steps. This approach has a stochastic optimisation step, and produces some protocols that appear to be challenging and information rich, where we appear to have a reasonable amount of current and interesting dynamics. After 30 optimisation runs with different random seeds and initial guesses, we selected the following 3 best protocols based on slightly different criteria:

- Figure 9, top — Number 26: the best space-filling objective function score (Mirams *et al.*, 2024).
- Figure 9, middle — Number 10: the largest RMSD value between the two models' simulated currents.
- Figure 9, bottom — Number 19: the best brute-force sampling score (Eq. (2)) for the Beattie *et al.* (2018) model.

All three protocols visit between 126–132 (58–61%) of the available 216 'boxes' in phase-voltage space. Note that this is a lower percentage than the protocols in Mirams *et al.* (2024) primarily due to 1 ms time samples being used in the 2019 optimisations presented here (see Discussion of Mirams *et al.* (2024)) along with extra initial guesses now being used in the Mirams *et al.* (2024) optimisation procedure to gain slightly higher coverage of the space.

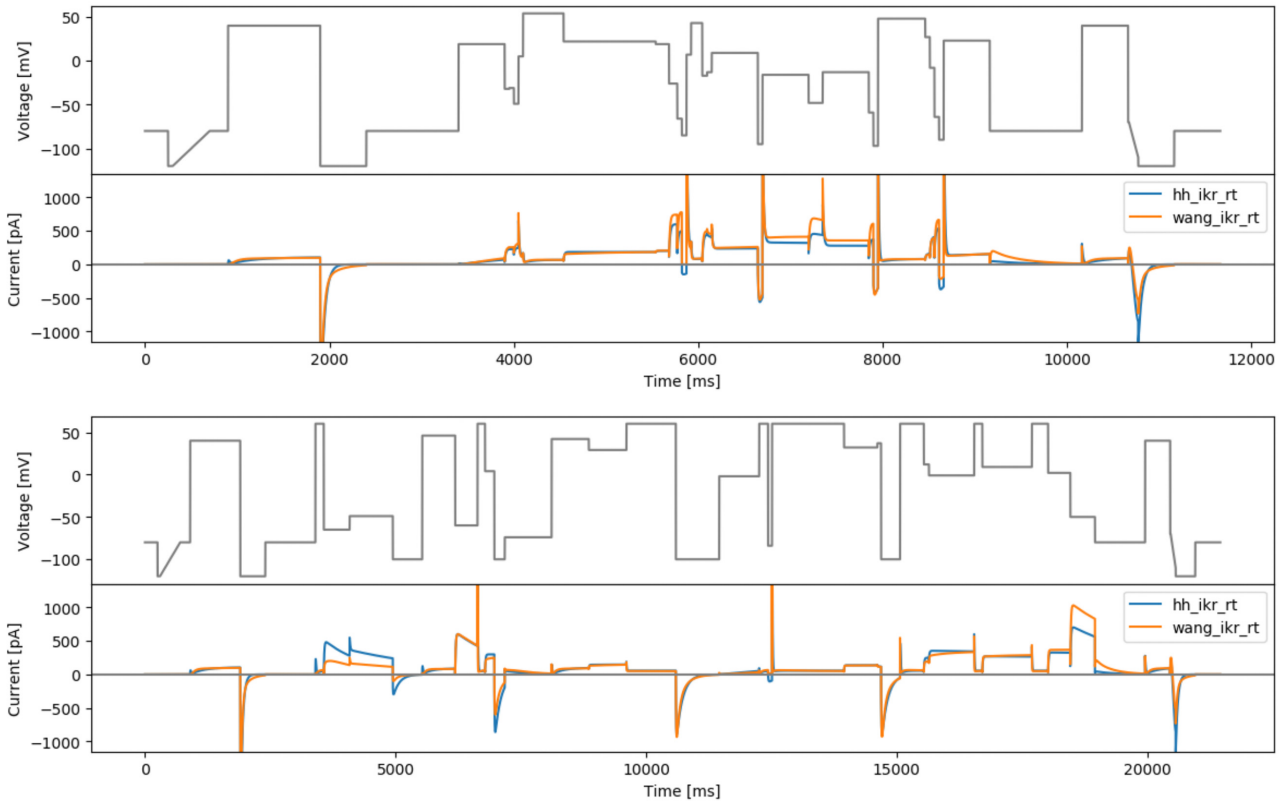

Figure 8. Protocols that maximise the difference between currents from the Beattie and Wang models. Top: based on randomised voltage and optimising time steps (rvotmaxdiff). Bottom: based on randomised time steps and optimised voltages (rtovmaxdiff). Simulated currents from both models are shown beneath each protocol.

Automated square waves

Following the same argument as in 'Maximising differences between two models' above, this design maximises the differences between two candidate models to aid model selection. Here we use $N = 3$ (as per Beattie *et al.*, 2018) which gives 9 parameters in total (see Equation (1)), with a fixed offset voltage of −30 mV. The square wave parameters are optimised based on an objective function that maximises the RMSD value between two model outputs. As above, the two models have a set of predefined model parameters, so it is still a 'local' model parameter method.

This approach was applied to the Beattie and Wang models using their original literature parameters. The resulting protocol (Figure 10) exhibits extremely high frequency and high amplitude (hitting the boundaries of the protocol parameters) behaviour. We believe these rapid changes of voltage tends to maximise the two model outputs, which is similar to the 'original sine wave #2' in Beattie (2015), and is likely to be impractical or uninformative for real experiments.

Discussion

Developing ion channel models remains a challenging task predominantly due to all the various sources of uncertainty and variability (Mirams *et al.*, 2016) — in terms of modelling approximations (Lei *et al.*, 2020c; Lei & Mirams, 2021) as well as experimental noise and artefacts (Lei *et al.*, 2020a). It is made more difficult due to the sparsity of available data for independent training and validation, with it still being common to calibrate models to all available data (Whittaker *et al.*, 2020). The protocols presented here encompass many design criteria, including parameterisation, model selection and rigorous testing of the underlying assumptions in hERG models (Fink & Noble, 2009; Lei *et al.*, 2019b; Mirams *et al.*, 2024). As such, we expect that this collection of voltage clamp protocols will be extremely useful for development of

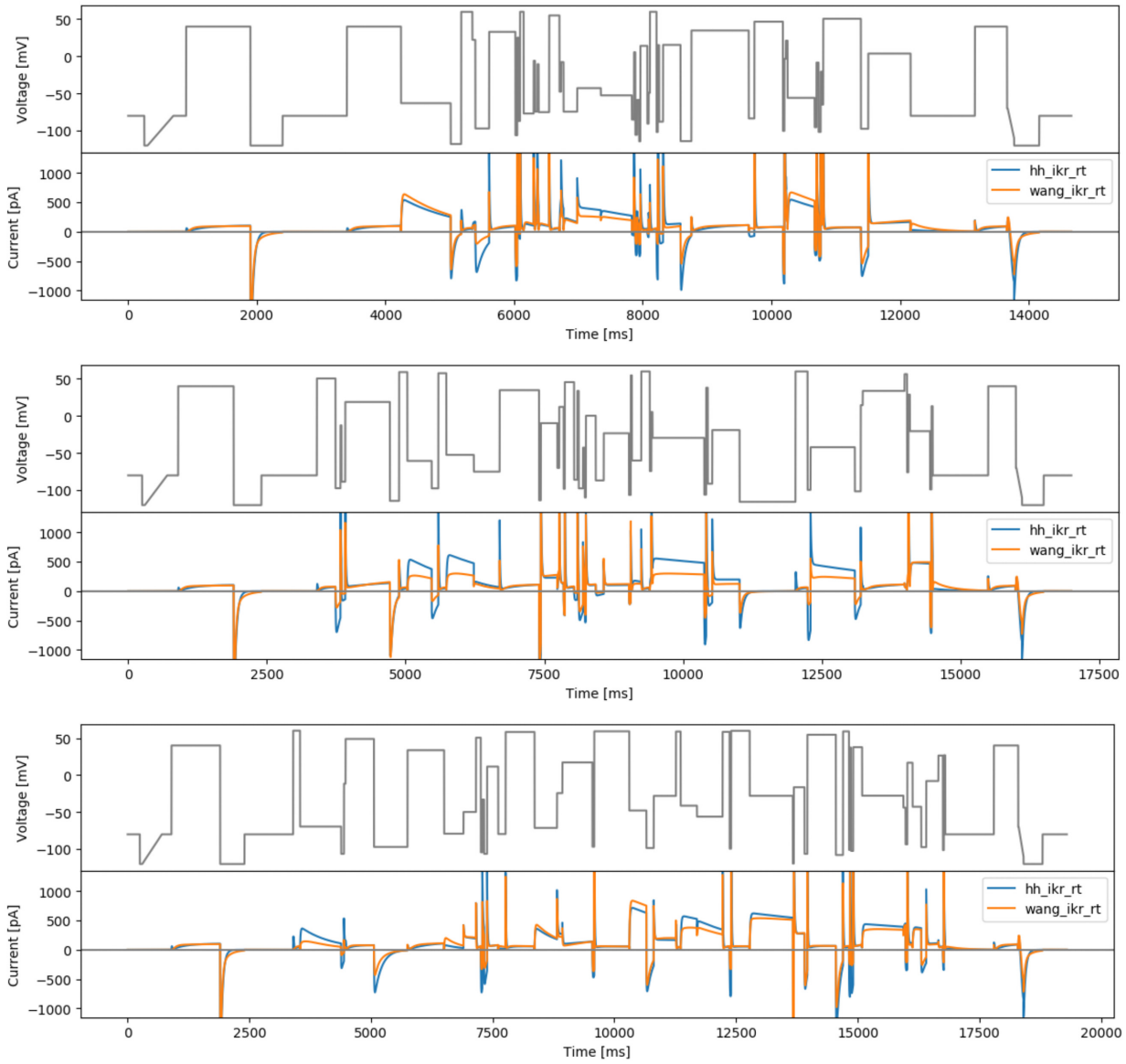

Figure 9. Phase-voltage space filling designs. Top: first phase-voltage space protocol (spacefill26). Middle: second phase-voltage space protocol (spacefill10). Bottom: third phase-voltage space protocol (spacefill19), with simulated currents from both models.

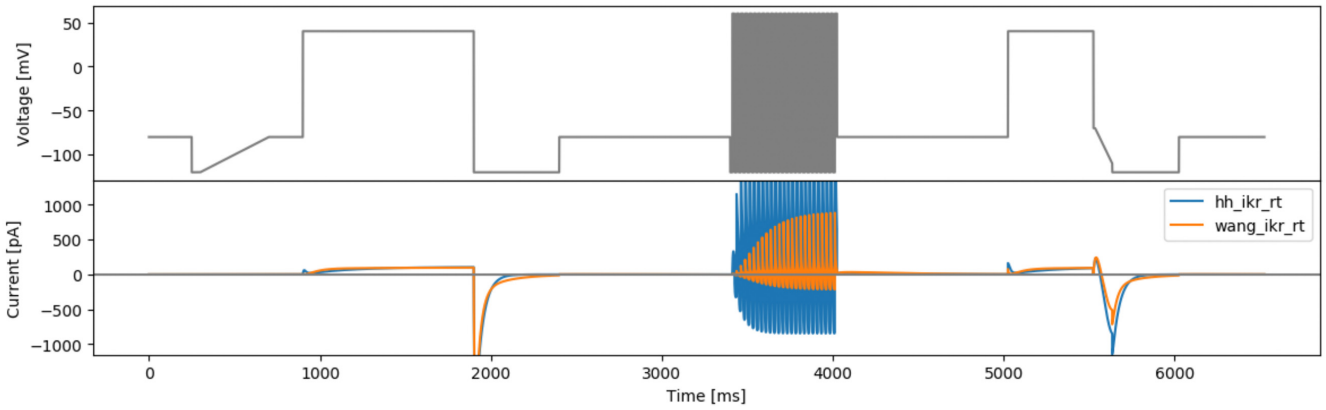

Figure 10. The square wave protocol for maximising two models' difference (maxdiff) and simulated currents from both models.

mathematical models for the physiological gating of the hERG potassium channel, and in particular by providing ample validation data for assessing their prediction errors due to model discrepancy (Shuttleworth *et al.*, 2024).

The same design criteria we have outlined here could easily be applied to other ion channels to create similar suites of protocols, using the provided open source codes.

Ethics and consent

Ethical approval and consent were not required.

Data availability

No data are associated with this article.

Software availability statement

- Source code available from: https://github.com/CardiacModelling/protocol-design-hERG
- Archived software available from: https://doi.org/10.5281/zenodo.13343099 (Lei *et al.*, 2024).
- License: BSD 3 clause license

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Version 1

Reviewer Report 10 February 2025

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Lucia Romero Perez
Universitat Politècnica de València, Valencia, Spain

This paper designs short voltage-clamp protocols to get data for hERG ion channel gating with the aim of producing better models of hERG. This is an important topic for cardiac modelling and simulation, and the methodology is sound.

The results are interesting, although they would be more exciting if there would be experimental data to compare the results provided by the simulations. This could be mentioned in the paper.

As it is stated “this collection of voltage clamp protocols will be extremely useful for development of mathematical models for the physiological gating of the hERG potassium channels…” However, I guess these protocols produce a lot of data. From all the simulated protocols in the paper, which ones would you choose if your could only choose two?

Based on the results produced by the simulations of the paper, what of the two model structures seems to produce more realistic currents?

I don´t understand the brute-force sampling approach, especially the sentence “For a given model I, we start with randomly picking M (ideally □ 1000s but practically □ 100s of) parameters from model parameter prior, then the objective function to be optimised is the sum of the root mean square deviation (RMSD) values between the model outputs from all combinations of the sampled parameter pairs.” Does that mean that you try to get a protocol that maximizes de differences in the currents predicted by M different models obtained by changing the values of the parameters buy maintaining the equations? In this case the objective function is maximized, isn`t it?

If I have understood well, the protocols obtained maximising approximated local sensitivity and Sobol sensitivity produce protocols that contain parts that art especially sensitive to one specific parameter. Could you please indicate what parts are especially sensitive to each parameter in the respective figures?

It would be better to put the tables with the details of the protocols in an appendix, as they are

also illustrated in the figures of the paper.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiac modelling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 26 December 2024

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Arpad Mike
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Understanding the kinetics of ion channel gating, how it is affected by temperature, and modified by various drugs, is a central question in the pharmacology of ion channels, whether the channel is the primary target, or it is involved in adverse effects. A thorough understanding often requires the construction of a mathematical model of the ion channel. What exact measurements are necessary to create a reliable model is not a trivial problem, and the researchers who created this manuscript are among the pioneers in addressing it. This manuscript investigates how different voltage protocols perform in helping researchers design, test, and compare models of the hERG

channel. The methods they propose for designing voltage protocols can be applied for studying other voltage-gated ion channels as well.

The methods are solid, and the message is interesting, although the way it is presented could be clearer.

I have one minor and one major concern with the manuscript.

The minor issue is the position of Tables 3 to 6.
The text discusses altogether 18 voltage protocols. The figures follow the order of the text (with the exception of “rtovmaxdiff” and “rvotmaxdiff” protocols, but they are both in Fig. 8, so there is no confusion). The tables, however, follow a rather arbitrary order:
1-2-3-4-5__13-18-6__7-8-9-10-15__16-17-11-12-14
What was the reason for this? The text is disrupted by several pages of large tables(they take up one-third of the manuscript), but they are not even in the right order. I suggest placing the tables describing all protocols (in the correct order) at the end of the text. I would even consider putting them in a single supplemental file.

My major concern regards the fundamental “So what?” question. Who will be the readers, and why should they be interested?
(By the way, the changes I suggest below are not intended as requirements for acceptance, but rather recommendations for improvement, which the authors may accept if they agree.)

I believe the primary audience of this particular paper would be pharmacologists, and the motivation for reading this manuscript would be, that one tries to reach a more profound understanding of the effect of a certain compound on a certain ion channel, or on a set of different ion channels.
Because the prospective readers are the type that seeks to understand things, I suggest that authors should discuss these results from a less technical and more practical point of view. It is nice that automatically designed protocols can differentiate between models, but which of the two models reproduced actual channel kinetics better? Does the topology of models tell something about which actual conformational transitions occur during the operation of the channel? Might it be necessary to design a model with a completely new topology? What would be the simplest (not the best) protocol that can differentiate between model topologies?

From a pharmacologist’s point of view, finding the model that best reproduces ion channel gating is only the first step, and it cannot be separated from the next one: finding a model that best reproduces drug effects on that specific ion channel. I understand that addressing this second question would exceed the limits of this manuscript, and I am aware that the authors have already dealt with the problem of modeling drug effects (*e.g.* in their BJP 2023 paper), but not from this aspect: What are the best protocols that can help determine drug mechanisms of action?
If they intend to pursue this goal, I am not sure that the “find the best protocol that can fit into 51 segments” approach is the best one. I would also suggest the “find the simplest protocol that still can differentiate between models” approach. The brute force, local or global sensitivity, space-filling, etc. designs are impressive, but they may not be as widely implemented by pharmacologist. In contrast, protocols like the “staircase”, or even something like the “sis” do make sense. Pharmacologists like to design their own protocols, and like to be able to justify why it looks the way it does.

In summary, the manuscript is a valuable collection of strategies for designing efficient voltage protocols for the study of hERG channels. I would personally prefer a more analytical, explanatory discussion of specific elements of the protocols, rather than focusing primarily on the results of automated processes.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes

Are sufficient details provided to allow replication of the method development and its use by others?
Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: pharmacology of ion channels

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.