

Improved identifiability of cardiac AP model parameters by physiological constraints

1

1

2

3

4 **Gradient-based parameter optimization to determine membrane ionic current composition of human**
5 **induced pluripotent stem cell-derived cardiomyocytes**

6

7 Hirohiko Kohjitani¹, Shigeeya Koda², Yukiko Himeno², Takeru Makiyama¹, Yuta Yamamoto¹, Daisuke

8 Yoshinaga³, Yimin Wuriyanghai¹, Asami Kashiwa¹, Futoshi Toyoda⁴, Yixin Zhang², Akira Amano², Akinori

9 Noma², Takeshi Kimura¹

10

11 ¹Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

12 ²Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Japan

13 ³Department Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

14 ⁴Department of Physiology, Shiga University of Medical Science, Otsu, Japan

15

16 * **Correspondence:**

17 Akira Amano

18

19 **Keywords: Parameter optimization method, hiPSC-CMs, Cardiac action potential, Mathematical**
20 **model, Computer simulation**

21

22 **Abbreviations**

23 hiPSC-CMs; human induced pluripotent stem cell-derived cardiomyocytes

24 AP; action potential

25 MDP; the maximum diastolic potential

26 SDD; slow diastolic depolarization

Improved identifiability of cardiac AP model parameters by physiological constraints

2

- 27 I_m ; membrane current
- 28 V_m ; membrane voltage
- 29 orp; optimization of randomized model parameters
- 30 PS; pattern search method
- 31 BP; base point for searching minimum MSE in the Pattern Search
- 32 NP; searching point in reference to BP in the Pattern Search
- 33 MSE; mean square error between two different V_m records
- 34 stp; step size to move NP
- 35 x; subscript to represent membrane current such as I_{Na} , I_{CaL} , I_{K1} , I_{hA} , I_{Kr} , I_{Kur} , I_{Ks} and I_{bNSC}
- 36

Improved identifiability of cardiac AP model parameters by physiological constraints

3

37 1. Abstract

38 Premature cardiac myocytes derived from human-induced pluripotent stem cells (hiPSC-CMs) show
39 heterogeneous action potentials (APs), most probably because of different expression patterns of membrane
40 ionic currents. We aim to develop a method of determining expression patterns of functional channels in terms
41 of the whole-cell ionic conductances (G_x) using individual spontaneous AP configurations. However, it has
42 been suggested that apparently identical AP configurations were obtained by different sets of ionic currents in
43 a mathematical model of cardiac membrane excitation. If so, the inverse problem of G_x estimation might not be
44 solved. We computationally tested the feasibility of the gradient-based optimization method. For realistic
45 examination, conventional 'cell-specific models' were prepared by superimposing the model output of AP on
46 each experimental AP record by the conventional manual adjustment of G_x s of the baseline model. Then, G_x s
47 of 4 ~ 6 major ionic currents of the 'cell-specific models' were randomized within a range of $\pm 5 \sim 15\%$ and
48 were used as initial parameter sets for the gradient-based automatic G_x s recovery by decreasing the mean
49 square error (MSE) between the target and model output. When plotted all data points of MSE - G_x relation
50 during the optimization, we found that the randomized population of G_x s progressively converged to the
51 original value of the cell-specific model with decreasing MSE. To confirm the absence of any other local
52 minimum in the global search space, we mapped the MSE by randomizing G_x s over a range of 0.1 ~ 10 times
53 the control. No additional local minimum of MSE was obvious in the whole parameter space besides the global
54 minimum of MSE at the default model parameter.

55

56 2. Introduction

57 During more than a half-century, the biophysical characteristics of ion transporting molecules
58 (channels and ion exchangers) have been extensively analyzed, and biophysical models of each functional
59 component have largely been detailed [1–4] (for human-induced pluripotent stem cells (hiPSC-CMs) see [5–
60 7]). In addition, various composite cell models, including the membrane excitation, cell contraction, and the
61 homeostasis of the intracellular ionic composition, have been developed by integrating mathematical models at
62 molecular levels into the cardiac cell models [8–11]. These models have already been quite useful in

Improved identifiability of cardiac AP model parameters by physiological constraints

4

63 visualizing individual currents underlying the action potential (AP) configuration under various experimental
64 conditions in matured cardiac myocytes. However, the utility of these mathematical cell models has been
65 limited because of the lack of extensive validation for the accuracy of the model output. This is the drawback
66 of the subjective manual fitting used in almost all mathematical cardiac cell models so far published. A new
67 challenge of such mechanistic models of cardiac membrane excitation might be an examination in a very
68 different paradigm to assess if the large but continuous variety of cardiac AP configurations, for example,
69 those recorded in the hiPSC-CMs, can be reconstructed by applying the automatic parameter optimization
70 method to the human cardiac cell models.

71 The automatic parameter optimization technique has been used to determine parameters objectively in
72 a wide range of various biological models (in cardiac electrophysiology; [12–15], in the systems
73 pharmacology; [16–20]). Because of this utility, a large variety of improvements have been made in the area of
74 information technology [21,22]. However, in electrophysiology, it has been suggested that different
75 combinations of model parameters can produce APs, which are very similar [23–25] (see also [13]). It has been
76 considered that the determination of current density at high fidelity and accuracy requires additional
77 improvements to the optimization method in the cardiac cell model because of complex interactions among
78 ionic currents underlying the membrane excitation (see [26], for review; [23]).

79 The final goal of our study is to develop an objective and accurate method of determining the current
80 profile (that is, the expression level of functional ionic currents) underlying individual AP configurations. As a
81 case study, we select a large variety of AP configurations in the hiPSC-CMs, which are difficult to classify into
82 the conventional nodal-, atrial- or ventricular-types. Nevertheless, it has been clarified that the molecular
83 bases of the ion channels expressed in the hiPSC-CMs well correspond to those in the adult cardiac
84 myocytes (GSE154580 [GEO Accession viewer \(nih.gov\)](#)). Thus, we use the human ventricular cell model
85 (hVC model, [11]) for the baseline model. In the present study, we computationally examine the feasibility of
86 the basic gradient-based optimization method, pattern search (PS) algorithm [21,27,28] in the model of cardiac
87 AP generation. We prepared a given AP configuration using each 'cell specific model', which was prepared by
88 the conventional manual fitting of the hVC model to the respective experimental recordings. To assess the

Improved identifiability of cardiac AP model parameters by physiological constraints

5

89 accuracy of the PS method of parameter optimization, this AP waveform generated by the cell-specific model
90 was used as a target of the optimization. Then, the initial set of parameters for the optimization was prepared
91 by uniform randomization centered around the model's default values. The PS algorithm should return the
92 original parameters by decreasing the error function (MSE) between the modified model output and target AP
93 waveforms. The accuracy of optimization was definitely judged by recovering of the original values of each
94 ionic current amplitude as the MSE progressively decreased toward zero.

95

96 3. Materials and Methods

97 3.1. The baseline model of hiPSC-CM membrane excitation

98 The baseline model of hiPSC-CMs was essentially the same as the human ventricular cell model (hVC
99 model), which has been fully described in references [10,11] and shares many comparable characteristics with
100 other human models so far published [8,9]. The model structure of the hVC model consists of the cell
101 membrane with a number of ionic channel species and a few ion transporters, the sarcoplasmic reticulum
102 equipped with the Ca²⁺ pump (SERCA), and the refined Ca²⁺ releasing units coupled with the L-type Ca²⁺
103 channels on the cell membrane at the nano-scale dyadic space, the contractile fibers, and the cytosolic three
104 Ca²⁺ diffusion spaces containing several Ca²⁺-binding proteins (Fig S1). All model equations and abbreviations
105 are in Supplemental Materials.

106 The source code of the present hiPSC-CM model was written in VB.Net and is available from our e-
107 Heart website (<http://www.eheartsim.com/en/downloads/>).

108

109 The kinetics of the ionic currents in the baseline model were readjusted according to new
110 experimental measurements if available in the hiPSC-CMs [29] (Fig S2). In the present study, the net
111 membrane current (I_{tot_cell}) is calculated as the sum of nine ion channel currents and two ion transporters (I_{NaK}
112 and I_{NCX}) (Eq 1).

$$113 \quad I_{tot_cell} = I_{Na} + I_{CaL} + I_{ha} + I_{K1} + I_{Kr} + I_{Ks} + I_{Kur} + I_{Kto} + I_{bNSC} + I_{NaK} + I_{NCX} \quad Eq \ 1$$

114

Improved identifiability of cardiac AP model parameters by physiological constraints

6

115 The membrane excitation of the model is generated by charging and discharging the membrane
116 capacitance (C_m) by the net ionic current (I_{tot_cell}) across the cell membrane (Eq 2). The driving force for the
117 ionic current is given by the potential difference between V_m and the equilibrium potential (E_x) (Eq 3). The net
118 electrical conductance of the channel is changed by the dynamic changes in the open probability (pO) of the
119 channel, which is mostly V_m -dependent through the V_m -dependent rate constants (α, β) of the opening and
120 closing conformation changes of the channel (Eqs 4 and 5).

$$121 \quad \frac{dV_m}{dt} = -\frac{I_{tot_cell}}{C_m} = -\frac{\sum I_x}{C_m} \quad \text{Eq 2}$$

$$122 \quad I_x = \bar{G}_x \cdot pO \cdot (V_m - E_x) \quad \text{Eq 3}$$

$$123 \quad \frac{dpO}{dt} = \alpha \cdot (1 - pO) - \beta \cdot pO \quad \text{Eq 4}$$

$$124 \quad [\alpha \ \beta]^T = \mathbf{f}(V_m) \quad \text{Eq 5}$$

125 The exchange of $3\text{Na}^+ / 2\text{K}^+$ by the Na/K pump and the $3\text{Na}^+ / 1\text{Ca}^{2+}$ exchange by the NCX also
126 generate sizeable fractions of membrane ionic current, I_{NaK} , and I_{NCX} , respectively. We excluded background
127 currents of much smaller amplitude, such as I_{KACH} , I_{KATP} , I_{LCCa} and I_{Cab} , from the parameter optimization and
128 adjusted only the non-selective background cation current (I_{bNSC}) of significant amplitude for the sake of
129 simplicity [30–32]. The I_{bNSC} is re-defined in the present study as a time-independent net current, which
130 remained after blocking all time-dependent currents.

131

132 3.2. The computational parameter optimization

133 The whole cell conductance G_x of a given current system (x) is modified by multiplying the limiting
134 conductance \bar{G}_x (Eq 3) of the baseline model by a scaling factor sf_x (Eq 6) and are used for the parameter
135 optimization.

$$136 \quad G_x = \bar{G}_x \cdot sf_x \quad \text{Eq 6}$$

137 The mean square error (MSE) function (Eq 7) was used in the parameter optimization, where $V_{m,a}$
138 represents adaptive V_m (the model output) generated by adjusting sf_x s of the baseline model. The target $V_{m,t}$
139 represents the AP of the intact baseline model.

Improved identifiability of cardiac AP model parameters by physiological constraints

7

140
$$MSE = \frac{\sum(V_{m,a} - V_{m,t})^2}{N}$$
 Eq 7

141 The MSE was stabilized by obtaining a quasi-stable rhythm of spontaneous APs through continuous
142 numerical integration of the model, usually 30 ~ 100 spontaneous cycles were calculated for a new set of sf_x s.
143 The MSE was calculated within a time window. The width of this time window was adjusted according to the
144 AP phase of interest. N is the number of digitized V_m points with a time interval of 0.1 ms.

145 In the usual parameter optimization, the $V_{m,a}$ is generated by modifying the baseline model for
146 comparison with the experimental record ($V_{m,t} = V_{m,rec}$). However, to evaluate the identifiability of the
147 parameter optimization, a simple approach was taken in the present study. Namely, we used the manually
148 adjusted 'cell-specific' model for the target ($V_{m,t}$), which was nearly identical to $V_{m,rec}$. More importantly, the
149 'cell-specific' V_m is totally free from extra-fluctuations (noise), which were observed in almost all AP
150 recordings in hiPSC-CMs. In the optimization process, the initial value of each optimization parameter was
151 prepared by randomizing the sf_x s of the cell-specific model by $\pm 5\sim 15\%$ at the beginning of each run of PS
152 ($V_{m,orp}$) in Eq 8 and the PS runs of several hundred were repeated. Thus, the error function is,

153
$$MSE = \frac{\sum(V_{m,orp} - V_{m,t})^2}{N}$$
 Eq 8

154 We call this optimization method 'orp test' in the present study.

155 The advantage of using a manually adjusted cell model for the optimization target is that the accuracy
156 of parameter optimization is proved by recovering all $sf_x = 1$ independently from the randomized initial
157 parameter set. Note the same approach was used in [23] in evaluating the accuracy of the parameter
158 optimization by applying the genetic algorithm (GA) to the TNNP model of the human ventricular cell [33].

159 The optimization of using the randomized initial model parameters were repeated for more than 200
160 runs. Thus, the orp test might be classified in a 'multi-run optimization'. The distribution of the sf_x data points
161 obtained during all test runs was plotted in a single sf_x -MSE coordinate to examine the convergence of
162 individual sf_x s with the progress of the orp test.

163

Improved identifiability of cardiac AP model parameters by physiological constraints

8

164 3.3. The pattern search method for the optimization

165 For a system showing the relatively simple gradient of MSE along the parameter axis, the gradient-
166 based optimization methods are more efficient in general than the stochastic methods for this kind of objective
167 function. We used one of the basic gradient-based optimization methods, the PS algorithm. The computer
168 program code of the pattern search [34] is simple (see Supplemental Materials) and does not require
169 derivatives of the objective function. We implemented the code into a homemade program for data analysis (in
170 VB) to improve the method for better resolution and to save computation time.

171 The primary PS method uses a base and new points [27]. In brief, sf_x is coded with symbols BP_x and
172 NP_x in the computer program, representing a base point (BP_x) and a new searching point (NP_x), respectively.
173 Namely, MSE is calculated on each movement of NP_x by adding or subtracting a given step size (stp) to the
174 BP_x , and the search direction is decided by the smaller MSE. Then, the whole mathematical model is
175 numerically integrated (Eqs 2, 3, 4, and 5) using NP_x to reconstruct the time course of AP ($V_{m,a}$). This
176 adjustment is conducted sequentially for each of the 4~6 selected currents in a single cycle of optimization.
177 The cycle is repeated until no improvement in MSE is gained by a new set of NP_x s. Then, the BP_x set is
178 renewed by the new set of NP_x for the subsequent series of optimization. Simultaneously, the stp is reduced by
179 a given reduction factor ($redFct$ of 1/4). The individual PS run is continued until the new stp becomes smaller
180 than the critical stp ($crtstp$), which is set to $2\sim 10 \times 10^{-5}$ in the present study.

181

182 3.4. Selection of ionic currents for the optimization

183 When we get a new experimental record of AP, we do not start the analysis with an automatic
184 optimization of G_x but first adjust the baseline model by conducting the conventional manual fitting. The nine
185 ionic currents in Eq 1 in the baseline model are adjusted bit by bit to superimpose the simulated AP on the
186 experimental one. During this step, it is important to pay attention to the influences of each sf_x adjustment on
187 the simulated AP configuration on the computer display. Thereby, one may find several key current
188 components which should be used in the automatic parameter optimization. Usually, currents showing a

Improved identifiability of cardiac AP model parameters by physiological constraints

9

189 relatively large magnitude of G_x were selected for the automatic optimization according to Eq 2, while those
190 which scarcely modified the simulated AP were left as default values in the baseline model.

191

192 3.5. Principal component analysis of the cell-specific models

193 When the orp test is conducted with p elements, it is possible to record the final point BP where the
194 MSE is improved in the p -dimensional space. Suppose we represent the matrix when n data points are acquired
195 as an $n \times p$ matrix X . In that case, we obtain a vector space based on the unit vector that maximizes the
196 variance (first principal component: PC1) and the p -dimensional unit vector orthogonal to it (loadings vector
197 $\mathbf{w}_{(k)} = (w_1, w_2 \dots, w_p)$). It is possible to convert each row, $\mathbf{x}_{(i)}$ of the data matrix X into a vector of principal
198 component scores, $\mathbf{t}_{(i)}$. The transformation is defined by

$$199 \quad \mathbf{t}_{k(i)} = \mathbf{x}_{(i)} \cdot \mathbf{w}_{(k)} \quad \text{for } i = 1, 2, \dots, n \quad k = 1, 2, \dots, p \quad \text{Eq 9}$$

200 In order to maximize variance, the first weight vector $\mathbf{w}_{(1)}$ corresponding to the first principal
201 component thus has to satisfy,

$$202 \quad \mathbf{w}_{(1)} = \arg \max_{\mathbf{w}} \left\{ \frac{\mathbf{w}^T \mathbf{X}^T \mathbf{X} \mathbf{w}}{\mathbf{w}^T \mathbf{w}} \right\} \quad \text{Eq 10}$$

203 The k -th component can be found by subtracting the first $(k-1)$ -th principal components from X

$$204 \quad \widehat{\mathbf{X}}_k = \mathbf{X} - \sum_{s=1}^{k-1} \mathbf{X} \mathbf{w}_{(s)} \mathbf{w}_{(s)}^T \quad \text{Eq 11}$$

205 Then the weight vector is given as a vector such that the variance of the principal component scores is
206 maximized for the new data matrix.

$$207 \quad \mathbf{w}_{(k)} = \arg \max_{\mathbf{w}} \left\{ \frac{\mathbf{w}^T \widehat{\mathbf{X}}_k^T \widehat{\mathbf{X}}_k \mathbf{w}}{\mathbf{w}^T \mathbf{w}} \right\} \quad \text{Eq 12}$$

208

209 3.6. Membrane excitation and its cooperativity with intracellular ionic dynamics

210 When any of $G_{x,s}$ is modified, the intracellular ion concentrations ($[\text{ion}]_i$) change, although the
211 variation is largely compensated for with time in intact cells through modification of the activities of both
212 $3\text{Na}^+/2\text{K}^+$ pump (NaK) and $3\text{Na}^+/1\text{Ca}^{2+}$ exchange (NCX). In the present study, we imitated this long-term

Improved identifiability of cardiac AP model parameters by physiological constraints

10

213 physiological homeostasis of $[ion]_i$ by introducing empirical Eqs 13 and 14. These equations induced 'negative
214 feedback' to the capacity ($maxI_{NaK}$ and $maxI_{NCX}$) of these ion transporters. Namely, each correcting factor (crf_x)
215 was continuously scaled to modify the limiting activity of the transporters to keep the $[Na^+]_i$ or the total
216 amount of Ca within the cell (Ca_{tot}) equal to their pre-set level ($stdNa_i$, $stdCa_{tot}$) with an appropriate delay
217 (coefficients 0.3 and 0.008 in Eqs 13 and 14, respectively).

218 For the control of $[Na^+]_i$,

$$\begin{aligned} \Delta crf_{NaK} &= -(stdNa_i - Na_i) \times 0.3, & stdNa_i &= 6.1mM, \\ I_{NaK} &= (crf_{NaK} \cdot maxI_{NaK}) \cdot vcy_{NaK} \end{aligned} \quad \text{Eq 13}$$

220 For the control of Ca_{tot} ,

$$\begin{aligned} \Delta crf_{NCX} &= -(stdCa_{tot} - Ca_{tot}) \times 0.008, & stdCa_{tot} &= 79amol, \\ I_{NCX} &= (crf_{NCX} \cdot maxI_{NCX}) \cdot (k_1 \cdot E_{1Na} \cdot E_{1NCX} - k_2 \cdot E_{2Na} \cdot E_{2NaCa}) \end{aligned} \quad \text{Eq 14}$$

222 The Ca_{tot} is given by $[Ca]_i$ included in the cytosolic three Ca-spaces *jnc*, *iz*, and *blk*, and in the
223 sarcoplasmic reticulum SR_{up} and SR_{rl} in the free or bound forms, respectively.

$$Ca_{tot} = [Ca_{tot}]_{jnc} \cdot vol_{jnc} + [Ca_{tot}]_{iz} \cdot vol_{iz} + [Ca_{tot}]_{blk} \cdot vol_{blk} + [Ca_{tot}]_{SR_{up}} \cdot vol_{SR_{up}} + [Ca_{tot}]_{SR_{rl}} \cdot vol_{SR_{rl}} \quad \text{Eq 15}$$

225 Here, the *vol* is the volume of the cellular Ca compartment (see more detail, [11]).

226

227 4. Results

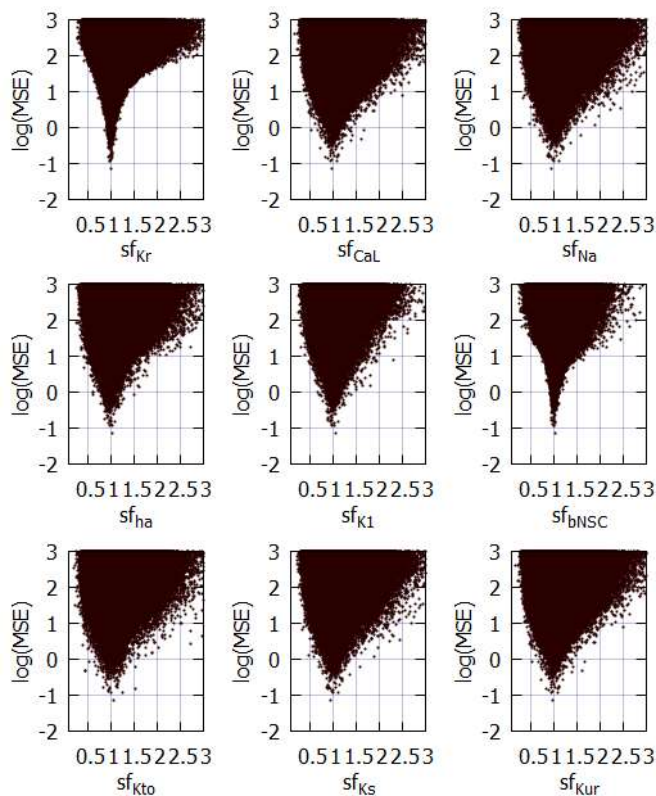
228 4.1. Mapping the magnitude of MSE over the nine global parameter space

229 Parameter identifiability has been one of the central issues in the parameter optimization of biological
230 models [14,20]. For confirmation of the identifiability of a unique set of solutions using the parameter
231 optimization method, mapping of the MSE distribution is required over an enlarged parameter space defined
232 by the sf_x of the nine ionic currents of the baseline model. The randomization of sf_x ranged from 1/10 to ~ 10
233 times the default values, and the calculation was performed for ~5,000,000 sets, as shown in Fig 1, where
234 magnitudes of $\log(MSE)$ were plotted against each sf_x on the abscissa.

Improved identifiability of cardiac AP model parameters by physiological constraints

11

235 The data points of MSE at a given sf_x include all variable combinations of the other eight sf_s . The
 236 algorithm of the PS method searches for a parameter set, which gives the minimum MSE at a given stp through
 237 the process of optimization. Although drawing a clear envelope curve by connecting the minimum MSEs at
 238 each sf_x was difficult because of the insufficient number of data points in these graphs (Fig 1), an approximate
 239 envelope of the minimum MSEs may indicate a single global minimum of MSE located at the control sf_x



equals one, as typically exemplified by I_{Kr} - and I_{bNSC} -MSE relations. On both sides of the minimum, steep slopes of MSE/sf_x are evident in all graphs. Outside this limited sf_x -MSE area, the global envelope showed a gentle and monotonic upward slope toward the limit on the right side. No local minimum was observed in all of the sf_x -MSE diagrams except the central sharp depression. It was concluded that the theoretical model of cardiac membrane excitation (hVC model) has only a single central sharp depression corresponding to the control model parameter.

253 Fig 1. Distribution of MSE calculated between the target and the simulated APs modified by randomizing the sf_x of 9 ionic
 254 currents in the coordinates of MSE- sf_x .
 255 All MSE data points were plotted on the logarithmic ordinate against the linear sf_x . A total of 5,141,382 points were calculated in
 256 cell model No.86 over the range of $1/10 \sim 10$ times the default sf_x . Since the configuration of V_m records were largely unrealistic
 257 at $sf_x > 3$, MSE points were cut out over $sf_x > 3.0$. To demonstrate the sharp decrease in MSE, the data points were densely
 258 populated near the default sf_x .

259 4.2. The prompt necessity for a method of parameter optimization as indicated by hiPSC-CM APs

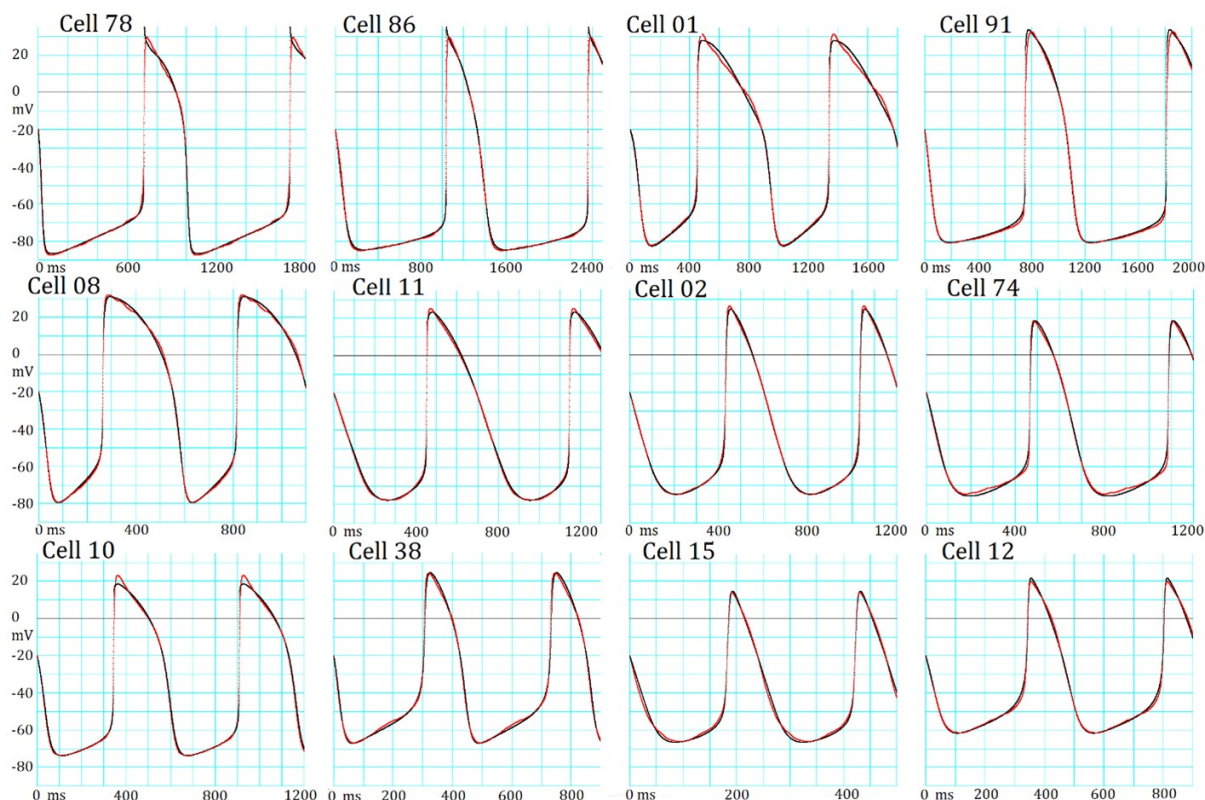
260 Fig 2 illustrates records of spontaneous APs (red traces) obtained in 12 experiments in the sequence of
 261 MDP (See Supplemental Materials for detail). All experimental records were superimposed with the simulated
 262 AP traces (black traces) obtained by the conventional manual fitting. In most cases, MSE of $1 \sim 6 \text{ mV}^2$
 263

Improved identifiability of cardiac AP model parameters by physiological constraints

12

264 remained (Eq 7) at the end of the manual fitting. This extra component of MSE might be largely attributed to
265 slow fluctuations of V_m of unknown origin in experimental recordings because the non-specific random
266 fluctuations were quite different from the exponential gating kinetics of ion channels calculated in
267 mathematical models. This extra-noise seriously interfered with the assessment of the accuracy of the
268 parameter optimization of G_x in the present study. Thus, APs produced by the manual adjustment ('cell
269 specific model') was used as the target AP, which were completely free from the extra noise when examining
270 the feasibility of the parameter optimization algorithm.

271 A comparison of AP configurations between these hiPSC-CMs clearly indicated that the classification
272 of these APs into atrial-, ventricular- and nodal-types was virtually impractical, as described in [7]. On the
273 other hand, if provided with the individual models fitted by an objective parameter optimizing tools using the
274 baseline model (black trace), the results should be fairly straightforward not only in estimating the functional
275 expression level of ion channels but also in clarifying the role of each current system or the ionic mechanisms
276 in generating the AP configuration in a quantitative manner. Thus, the objective parameter optimization of the
277 mathematical model is a vital requirement in cardiac electrophysiology.



Improved identifiability of cardiac AP model parameters by physiological constraints

13

278

279

280

281

282

283

284

Fig 2. The manual fitting of variable AP configurations in 12 different hiPSC-CMs.

Each panel shows the experimental record (red) superimposed by the model output (black) of the baseline model adjusted by the conventional manual fitting. At the top of each pair of AP records, the experimental cell number is presented. The extra fluctuations are obvious during the AP plateau in Cells 78, 08 and 01, while in Cells 15 and 74 during SDD. The length of abscissa is markedly different to illustrate the interval between two successive peaks of AP.

285

286

287

Table 1. AP metrics and MSE calculated after the manual fitting of varying AP configurations in 12 different hiPSC-CMs in Fig 2.

288

289

290

291

Table 1 indicates the AP metrics; the cycle length (CL), the peak potential of the plateau (OS), the maximum diastolic potential (MDP), and the AP duration measured at -20 mV in addition to the MSE between individual experimental record and the model output fitted by manual fitting. The CL, MDP and AP were very variable among different AP recordings of cells shown in Fig 2. The cells were arranged by the sequence of MDP.

	CL (ms)	OS (mV)	MDP (ms)	APD(ms) at -20mV	MSE (mV ²)
Cell 78	983.8	29.6	-87.4	271.7	5.8443
Cell 86	1326.0	29.7	-85.0	289.2	4.0554
Cell 01	887.4	31.2	-82.2	435.0	3.9330
Cell 91	1058.0	33.0	-80.4	308.6	7.2156
Cell 08	551.4	32.0	-79.5	287.6	1.4043
Cell 11	695.0	25.3	-77.6	243.5	2.6683
Cell 02	603.9	26.4	-74.9	173.4	1.0412
Cell 74	622.8	18.5	-74.8	157.0	2.2589
Cell 10	564.3	23.0	-73.7	220.9	3.2194
Cell 38	425.4	24.2	-66.8	123.4	3.6626
Cell 15	239.5	13.8	-66.1	57.1	2.8607
Cell 12	458.6	19.7	-61.5	119.0	1.3514

292

293

294

295

296

297

Table 1 indicates the AP metrics; the cycle length (CL), the peak potential of the plateau (OS), the maximum diastolic potential (MDP), and the AP duration measured at -20 mV in addition to the MSE between individual experimental record and the model output fitted by manual fitting. The CL, MDP and AP were very variable among different AP recordings of cells shown in Fig 2. The cells were arranged by the sequence of MDP.

298

299

The experimental study using the hiPSC-CMs was approved by the Kyoto University ethics review board (G259) and conformed to the principles of the Declaration of Helsinki.

300

Improved identifiability of cardiac AP model parameters by physiological constraints

14

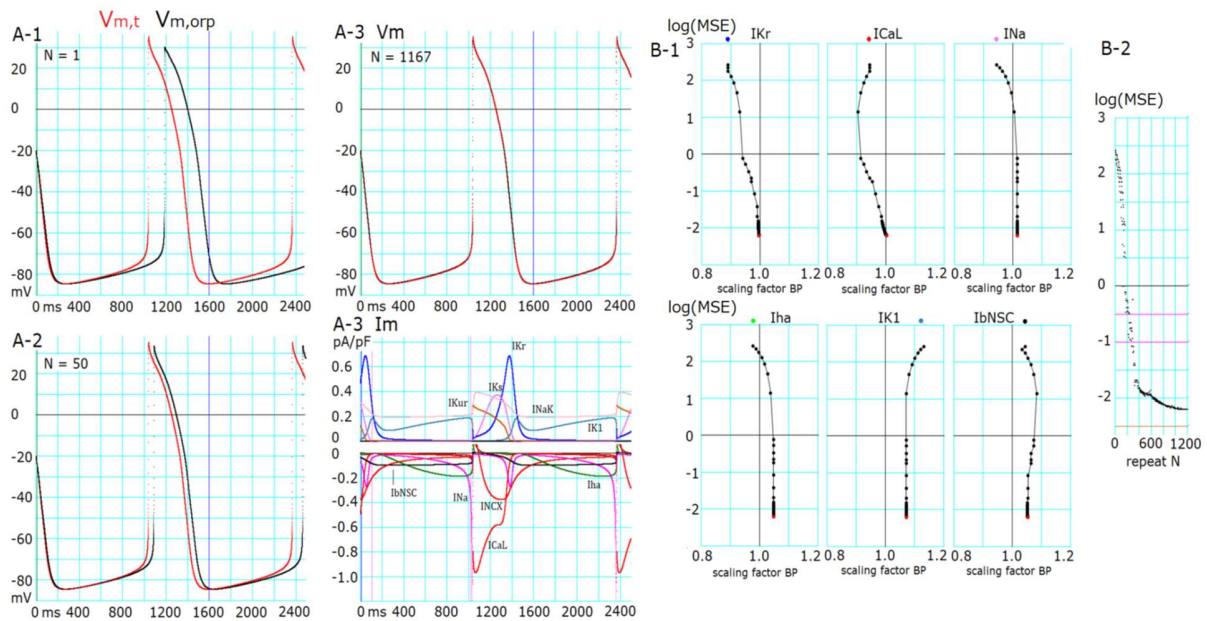
301 4.3. Feasibility of the PS algorithm for parameter optimization of membrane excitation models

302 The automatic parameter optimization was applied to the model of cardiac membrane excitation in a limited
303 number of studies (for review, see [23,26,35,36]) using various optimization methods, such as genetic
304 algorithms. To the best of our knowledge, the principle PS algorithm has not been successfully applied to the
305 detailed mathematic models of cardiac membrane excitation composed of both ionic channel and ion
306 transporters models, except for the pioneering work in [12], which applied more general gradient-based
307 optimization method to the simple ventricular cell model of Beeler and Reuter (BR model)[37].

308 Fig 3 shows a typical successful run of the new PS method in a hiPSC-CM, which showed an MDP
309 of ~ 85 mV. The PS parameter optimization was started after randomizing the sfs of the major six currents,
310 I_{Kr} , I_{CaL} , I_{Na} , I_{ha} , I_{K1} and I_{bNSC} , in the manual fit model within a range of $\pm 15\%$ around the default values
311 (normalized magnitude of 1). Fig 3A-1~3 compares the simulated $V_{m,orp}$ (black) with the target $V_{m,t}$ (red) at the
312 repeat number $N=1$, 50 and 1167, respectively (Eq 8). The OS, APD as well as the CL of spontaneous AP
313 were markedly different at the first cycle of AP reconstruction (Fig 3A-1). These deviations were largely
314 decreased at the PS cycle (Fig 3A-2 V_m , at $N = 50$), and became invisible in the final result (Fig 3A-3, $N =$
315 1167). The final individual current flow of nine current components are demonstrated in the lower panel of
316 Fig 3A-3 (I_m).

Improved identifiability of cardiac AP model parameters by physiological constraints

15



317

318 Fig 3. Results of the successful optimization in a cell (Cell86).

319 (A-1) Target AP ($V_{m,t}$, red) and AP generated by randomized initial sf_x s ($V_{m,orp}$, black). (A-2) $V_{m,t}$ (red) and $V_{m,orp}$ (black)
 320 generated after 50 cycles of adjusting BP. (A-3) V_m : $V_{m,t}$ (red) and $V_{m,orp}$ (black) generated by the final sf_x s. I_m : corresponding
 321 time courses of each current for the finalized AP shown in A-3 V_m . (B-1) Changes in sf_x s vs. $\log(MSE)$ during a successful
 322 optimization process of PS. (B-2) $\log(MSE)$ of all BP points during the search process in PS. The initial values of sf_x s are
 323 plotted by corresponding colors at the top of each sf_x - $\log(MSE)$ graph.

324

325 The time course of decreasing $\log(MSE)$ evoked by the multi-run PS optimization is plotted for each
 326 sf_x in Fig 3B-1 every time of resetting the set of base points. Fig 3B-2 shows all of the $\log(MSE)$ obtained at
 327 every adjustment by stepping individual BP points. The movement of all sf_x s were synchronized to decrease
 328 $\log(MSE)$ from ~ 2.4 to 1 during the initial 180 cycles of decreasing $\log(MSE)$, but the search directions of
 329 BP were quite variable. The detailed adjustment of sf_x s below $\log(MSE) < 0$ was driven by adjusting I_{Kr} , I_{CaL}
 330 and I_{bNSC} in this cell. The values of sf_{Kr} , sf_{CaL} and sf_{Na} approached the correct value of 1, while those for I_{ha} , I_{K1}
 331 and I_{bNSC} remained deviated from the unit by less than 10% of the value. The explanation for the deviation of
 332 these three sf_x s from the unit will be examined in the next section of the Results.

333

334

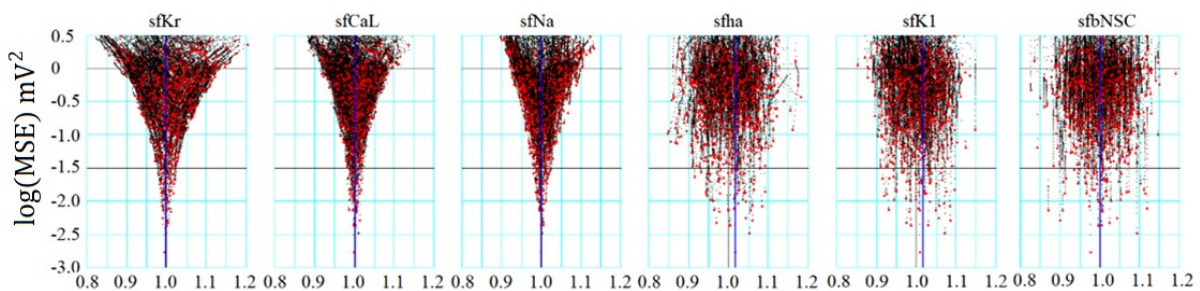
Improved identifiability of cardiac AP model parameters by physiological constraints

16

335 4.4. The six-parameter orp test successfully determined the conductance parameters of membrane 336 excitation models

337 In individual runs, the PS optimization was frequently interrupted at intermediate levels during the
338 progress of optimization and the probability of reaching $\log(MSE)$, for example, below -2, rapidly decreased
339 with increasing extent of the randomization of the initial set of parameters. Moreover, the complementary
340 relations between several ionic currents in determining dV_m/dt might have hampered the parameter
341 optimization. These facts indicate the requirement of statistical measures to improve the accuracy of the PS
342 method. Fig 4 shows the results of orp tests, in which the optimization shown in Fig 3 was repeated several
343 hundred times, and all results were plotted in a common coordinate of $\log(MSE)$ and individual sf_x s. The
344 population of sf_x correctly converged at a single peak point very close to 1 with increasing negativity of
345 $\log(MSE)$ for sf_{Kr} , sf_{CaL} , and sf_{Na} , while sf_{ha} , sf_{Kl} , and sf_{bNSC} showed obvious variance. Nevertheless, they also
346 showed a clear trend toward convergence to 1 in the average.

347



348

349 Fig 4. Convergence of sf_x in the orp test for Cell86.

350 The ordinate is the $\log(MSE)$ and the abscissa is the normalized amplitude of sf_x ; x stands for Kr , CaL , Na , ha , Kl , and $bNSC$.
351 Black points were obtained in the progress of optimization, and red ones are the final points in 829 runs of PS optimization.

352

353 Table 2 summarizes the mean of sf_x determined for the top 20 runs of the PS parameter optimization in
354 each of the 12 cells illustrated in Fig 2. The $[Na^+]_i$ as well as Ca_{tot} was well controlled to the reference levels
355 (std_{Nai} , and std_{Catot} in Eqs 16 and 17) of 6.1 mM and 79 amol, respectively, at the end of the parameter
356 optimization to ensure the constant $[Na^+]_i$ as well as Ca_{tot} . The mean of final $\log(MSE) = -2.74$ indicates
357 that the MSE was reduced by five orders of magnitude from the initial level just after the randomization by the
358 orp test, like in the successful example shown in Fig 3B. The mean of individual sf_x s were very close to 1 with

Improved identifiability of cardiac AP model parameters by physiological constraints

17

359 a minimum standard error (SE) of mean, which were less than 1% of the mean, even for I_{K1} , I_{bNSC} and I_{ha} ,
 360 which showed weak convergence against $\log(MSE)$. These results well validate the accuracy of the parameter
 361 optimization using the multi-run PS method in all of 12 cell-specific models, which showed the large variety of
 362 spontaneous AP recorded in the hiPSC-CMs.

363

364 Table 2. Measurements of sf_x s (mean + SE, n = 20), $[Na^+]_i$, mM and Ca_{tot} in amol in the 12 cells.

Cell No.	log(MSE)	sfKr	sfK1	sfCaL	sfbNSC	sfha	sfNa	sfKur	$[Na^+]_i$ (mM)	Ca_{tot} (amol)
78	-2.48321	1.00005	1.00157	1.00037	0.99460	1.00060	1.00134		6.10550	78.99979
91	-2.42008	0.99952	1.00644	1.00063	1.00280	1.00470	1.00068		6.09977	79.00044
86	-2.80257	1.00166	1.01394	1.00142	1.02670	1.00253	1.00702		6.09466	79.00008
01	-2.79709	0.99871	1.00157	0.99756	0.99692	1.00054	0.99779		6.08973	78.99984
08	-3.07432	0.00094	0.99982	1.00088	1.00041	0.99968	0.99985		6.12201	79.00056
11	-2.67641	1.00186	1.00686	1.00129	0.99768	1.00253	1.01028		6.10385	78.99995
10	-1.70278	1.00322	1.01081	1.00424	1.00396		0.99883		6.10968	79.00018
02	-2.35441	1.00161	1.02038	1.00341	0.99815	1.01324	1.00954		6.10184	79.00004
74	-2.43399	1.00126	1.01838	1.00308	0.99898	1.00004	1.00435		6.10118	79.99979
38	-3.01883	1.00075		1.00106	1.00061		0.98866	1.00151	6.10530	78.99969
15	-3.85992	1.00003		0.99894	0.99996		1.00015	0.98653	6.09902	79.00022
12	-3.33037	0.99978		1.00030	0.99990	0.97587		1.00188	6.10012	79.00007
Ave	-2.74617	0.99992	1.00886	1.00110	1.001723	0.99997	1.001681	0.99664	6.10272	79.08339
SE	0.07065	0.00093	0.010000	0.00164	0.00430	0.00729	0.00544	0.00690	0.00017	0.000345

365 The top 20 results obtained in the multi-run orp method were analyzed in each cell. Grand average (Ave) and
 366 SE are listed at the bottom rows.

367

368 4.5. Complementary relationship among I_{K1} , I_{ha} and I_{bNSC}

369 Fig 5A illustrates the distribution of sf_x s amplitude in the top 20 data points, where the final sf_x s in
 370 individual runs were connected with lines for each run of PS in Cell 86 (Fig 2). The values of standard error
 371 (SE) of mean were quite small in the sf_{Kr} and sf_{CaL} , less than 1%. In contrast, sf_{ha} , sf_{K1} and sf_{bNSC} showed
 372 evidently larger deviations. This finding is interesting since the former currents are mainly involved in
 373 determining the AP configuration and the latter group mainly in driving the relatively long-lasting SDD of
 374 approximately 1 sec in duration.

Improved identifiability of cardiac AP model parameters by physiological constraints

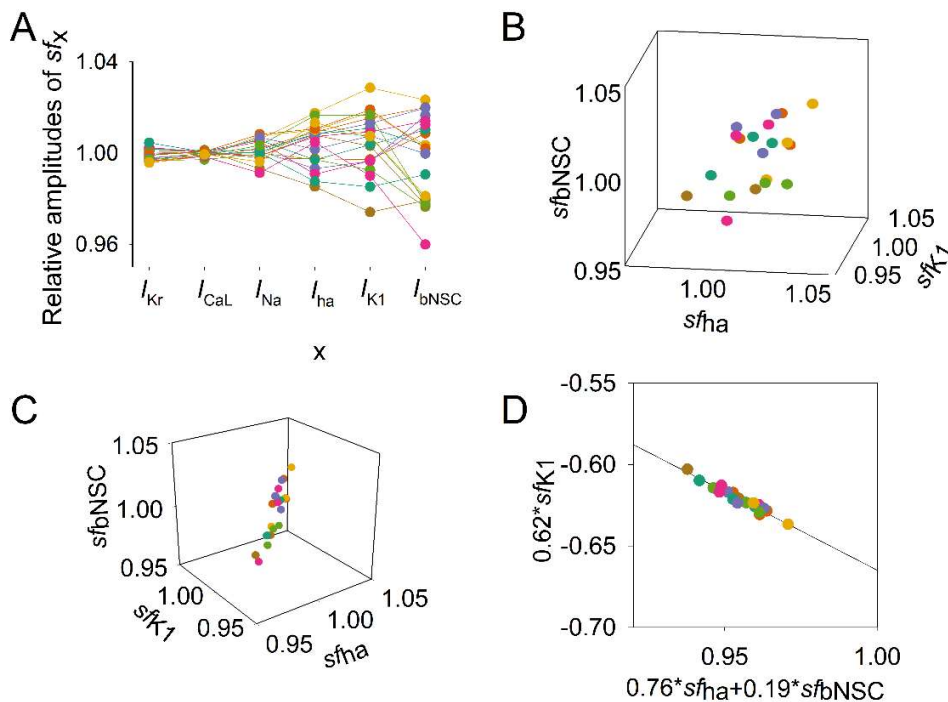
18

375 Thus, we analyzed the distribution of sf_{ha} , sf_{K1} and sf_{bNSC} within the top 20 MSE. Fig 5B and C show
 376 the distribution of sf_x points in the space of the three sf_x dimensions. In Fig 5B, the 20 data points seemed to be
 377 dispersed randomly in the parameter space, but when the space was rotated to a specific angle, a linear
 378 distribution was observed as in Fig 5C, indicating that the points are distributed approximately on a plane
 379 surface in the 3D space. Using the multiple regression analysis, we could obtain an equation that fits the 20
 380 data points as follows ($R^2=0.872$);

$$381 \quad 0.762 \cdot sf_{ha} - 0.619 \cdot sf_{K1} + 0.191 \cdot sf_{bNSC} = 0.333554 \quad Eq\ 16$$

382

383 By replotting the data points in the 2D space with the abscissa for the sum of two inward-going
 384 currents ($0.76 sf_{ha} + 0.19 sf_{bNSC}$) and the ordinate for the outward current $0.62 sf_{K1}$, we obtained a regression
 385 line as shown in Fig 5D. The close correlations among the three sf_x s were indicated with a quite large R^2 of
 386 0.941. This finding well confirms that the three currents have complementary relations with each other to give
 387 virtually identical configurations of spontaneous AP. In other words, $\log(MSE)$ remains nearly constant as far
 388 as the composition of the currents satisfies the relationship given by Eq 16.



389

Improved identifiability of cardiac AP model parameters by physiological constraints

19

390 Fig 5. Distribution of sf_x within the top 20 sets of sf_x s obtained from the multi-run orp test in Cell86 in Fig 2.
391 Data points of normalized sf_x in each set were depicted in a different color. (A) amplitudes of each sf_x (indicated on the abscissa)
392 were plotted. (B) Three parameters, sf_{ha} , sf_{K1} , and sf_{bNSC} were plotted in the 3D plot. (C) A different solid angle view of the 3D
393 plot showed a linear correlation; see text for the plot in (D)
394

395 The complementary relationship was further examined by conducting the orp test after fixing one of
396 the two factors, sf_{K1} or $(sf_{ha} + sf_{bNSC})$, illustrated in Fig 5B. Fig 6A shows the $\log(MSE)$ vs. sf_{K1} relation when
397 the $(sf_{ha} + sf_{bNSC})$ were fixed at the values obtained by the orp test. Indeed, the typical convergence of the sf_{K1}
398 was obtained. Alternatively, if the sf_{K1} was fixed, the convergence was obviously improved for both sf_{ha} and
399 sf_{bNSC} (Fig 6B-1, 2), but it was less sharp if compared to sf_{Kr} , sf_{CaL} and sf_{Na} (not shown, but refer to
400 corresponding results in Fig 4A). This finding was further explained by plotting the relationship between the
401 two inward currents, I_{ha} and I_{bNSC} , as illustrated in Fig 6C. The regression line for the data points was fitted by
402 Eq 17 with $R^2 = 0.86$, supporting the complementary relationship between the two inward currents, I_{ha} and I_{bNSC} .

$$403 \quad 0.9736 \cdot sf_{bNSC} + 0.2281 \cdot sf_{ha} = 1.2024 \quad Eq\ 17$$

404
405 The moderately high R^2 indicates that the SDD is determined not only by the major I_{ha} and I_{bNSC} but
406 also by other currents, such as I_{K1} , I_{Kr} , the delayed component of I_{Na} (I_{NaL}) and I_{CaL} , which were recorded
407 during the SDD as demonstrated in Fig 3.

408 Essentially the same results of complementary relationship among sf_{ha} , sf_{bNSC} and sf_{K1} were obtained in
409 Cell 91, which also showed the long-lasting SDD with the very negative MDP as in Cell 86, as shown in Fig 2
410 and Table 2. The regression relation for the data points was fitted by Eqs 18 and 19 with $R^2 = 0.656$ and
411 0.472 , respectively.

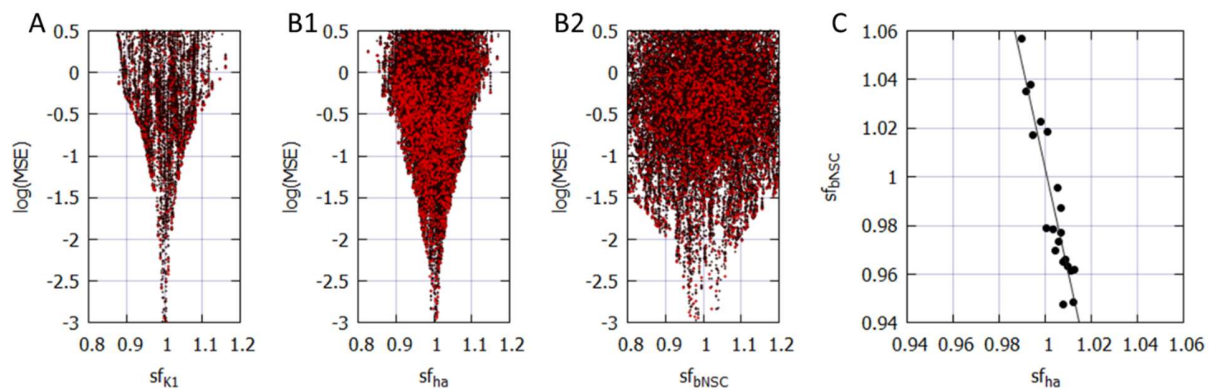
$$412 \quad 0.572 \cdot sf_{ha} - 0.132 \cdot sf_{K1} + 0.810 \cdot sf_{bNSC} = 1.25891 \quad Eq\ 18$$

$$413 \quad 0.9279 \cdot sf_{ha} + 0.3706 \cdot sf_{bNSC} = 1.30025 \quad Eq\ 19$$

Improved identifiability of cardiac AP model parameters by physiological constraints

20

414



415 Fig 6. The complementary relations among sf_{K1} , sf_{ha} and sf_{bNSC} .
416 (A) and (B) results of the multi-run orp test. A; the perfect convergence of sf_{K1} when sf_{ha} and sf_{bNSC} were fixed. (B1) improved
417 convergence of sf_{ha} and (B2) sf_{bNSC} when sf_{K1} was fixed. In these two orp tests, sf_x of other currents showed quite comparable
418 convergence as in Fig 4A. (C) the correlation between sf_{ha} and sf_{bNSC} .
419

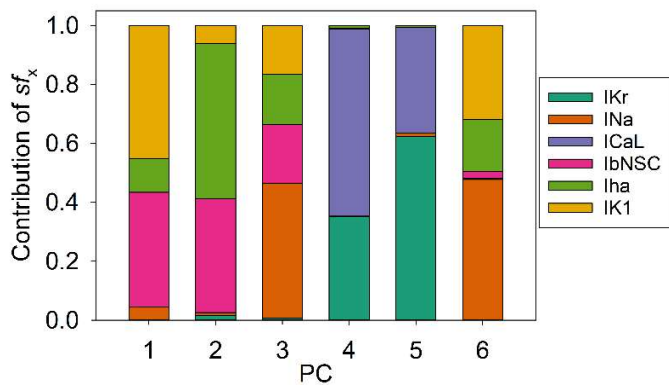
420 4.6. Principal components in the hiPSC-CM model

421 The PS frequently got stuck during the progress of parameter optimization and failed to reach the
422 global minimum in the present study (Figs 4 and 6). The major cause of this interruption may most probably be
423 attributed to the fact that sf_x s were used directly as the search vector of the PS. In principle, the algorithm of PS
424 parameter optimization gives the best performance when the parameters search is conducted in orthogonal
425 dimensions where each dimension does not affect the adjustment of other sf_x [28]. To get deeper insights, we
426 applied the principal component (PC) analysis to the set of 6 sf_x s selected in the baseline model. We performed
427 PC analysis on the data points recorded in the vicinity of the minima (using the top 20 data).

428 As illustrated in Fig 7, each of the 6 PCs was not composed of a single sf_x but mostly included
429 multiple sf_x sub-components. This finding indicates the inter-parameter interactions during the process of
430 parameter optimization. For example, the changes in sf_{K1} or sf_{bNSC} simultaneously affect PCNo.1, 3, 6 or 1, 2, 3
431 PCs, respectively. Both sf_{CaL} and sf_{Kr} affect PCNo.4, 5. It might be concluded that the frequent interruptions of
432 PS parameter optimization are most probably caused by the sporadic appearance of the local minima of MSE
433 through interactions among sf_x s.

Improved identifiability of cardiac AP model parameters by physiological constraints

21



434

435 Fig 7. PC1~6 to describe distribution of the 6 sf_x s. PC analysis was performed on the data population of the top 200 runs of the
436 orp test as in Fig 4, which showed good optimization results (Cell 86). Each magnitude of 6 PCs was normalized to give a unit
437 magnitude. Note each PC is composed of multiple components of ionic current, which are indicated in the Index with
438 corresponding colors.

439

440

441

442 5. Discussion

443

New findings in the present study are listed below.

444

(1) Mapping the MSE distribution over the enlarged parameter space was conducted by randomizing
445 the G_{xS} of the baseline model. It was confirmed that the baseline model has only a single sharp
446 depression of MSE at the default G_{xS} (Fig 1).

447

(2) The preliminary cell-specific models were firstly prepared by the conventional manual tuning of
448 G_{xS} to superimpose the model output on each of twelve experimental AP recordings (Fig 2).

449

Thereby, the parameter search space was restricted to a relatively small space to facilitate
450 parameter optimization.

451

(3) The sf_x s of 4 ~ 6 G_x parameters were initially assigned random values from a uniform distribution
452 ranging between $\pm 10\%$ of default values. The MSE was calculated between the randomized
453 model output and the intact model AP as the target of optimization (Fig 3).

454

(4) Plotting parameter sf_x in a common sf_x - MSE coordinates during each run of several hundred runs
455 of optimization (Fig 4), we found that the sf_x distribution of I_{Kr} , I_{CaL} , and I_{Na} converged sharply to

Improved identifiability of cardiac AP model parameters by physiological constraints

22

456 a single point with decreasing MSE, which exactly equaled the default ones. On the other hand,
457 estimates of sf_{K1} , sf_{ha} and sf_{bNSC} deviated slightly within a limited range around the default values
458 in cells showing long-lasting SDD (Fig 4).

459 (5) For statistical evaluation, the mean \pm SE of sf_x in the top 20 estimates of MSE was calculated in
460 individual cells (Table 2). The results of the parameter optimization in the 12 cells definitely
461 indicated that the means of sf_{xS} were very close to 1.00, with the SE less than 0.01 for all G_{xS} .

462 (6) A complementary relationship was found between sf_{K1} , sf_{ha} and sf_{bNSC} in determining the gentle
463 slope of long-lasting SDD in two representative cells (Fig 5). Supporting this view, the sf_{K1} clearly
464 focused on the unit provided that sf_{ha} and sf_{bNSC} were fixed and vice versa (Fig 6).

465 (7) The six search vectors of sf_x of the presented model could be replaced by the same number of
466 theoretical PCs, and each PC was mostly composed of multiple sf_{xS} (Fig 7). This finding
467 definitely supports the view [12] that the complex interactions among I_{xS} might interrupt the
468 progress of the parameter optimization when sf_{xS} were used as the search vector instead of using
469 theoretical orthogonal ones.

470

471 The use of an initial randomized set of parameters was crucial in examining if an optimization method
472 can determine unique estimates independent from the initial set of parameters, as used in the GA-based method
473 for determining the G_{xS} of the mathematical cardiac cell model [23]. The findings listed above well confirmed
474 the feasibility of the PS method. Most probably, the PS method is applicable to variable mathematical models
475 of other cell functions as well. See [26] for a more systematic review of the parameter optimization in the
476 cardiac model development.

477 It has been suggested that different combinations of parameters may generate simple outputs that are
478 very similar [12,23–25]. In the present study, this notion may be explained at least in part by the
479 complementary relationship, for example, between the I_{K1} , I_{ha} and I_{bNSC} in determining dV_m/dt of SDD, which
480 is a function of the total current (Eq 2, Figs 5 and 6). The gradient-based optimization method relies on the
481 precise variation in the time course of dV_m/dt induced by the time-dependent changes in individual sf_{xS} (Eq 2).

Improved identifiability of cardiac AP model parameters by physiological constraints

23

482 Therefore, the MSE was calculated over the whole time course of the spontaneous APs. Note, we did not use
483 the AP metrics, which reflect only indirectly the kinetic properties of individual currents. Even with this
484 measure of calculating the MSE, the time-dependent changes in pO (Eq 3) might be relatively small between
485 two major currents, I_{K1} and I_{ha} , in comparison to I_{bNSC} , which has no V_m -dependent gate during the SDD as
486 shown in the current profile Fig 3A-3. We assume that the gradient-based optimization method will be able to
487 determine different contributions of individual currents if the optimization is conducted only within a selected
488 time window of SDD. If MSE is calculated over multiple phases of the spontaneous AP, the influence of a
489 particular phase on the MSE should be diluted. In our preliminary parameter optimization, this problem was
490 partly solved by using a weighted sum for different phases of the spontaneous AP in summing up the MSE.

491 The small amplitude of a given current might be an additional factor in the weak convergence of sf_x
492 observed in the diagram of sf_x - MSE in the orp test of optimization. If the current amplitude was much
493 smaller in reference to the sum of all currents in determining dV_m/dt (Eq 2), the resolution of the PS method
494 would get lower. Sarkar et al. [24] demonstrated that the model output, for example, the AP plateau phase were
495 almost superimposable when the different ratio of G_{Kr} and G_{pK} were used in reconstructing the model output
496 (Figure 1 in [24]). They described that the AP metrics used for comparisons, such as APD, OS and APA
497 seemed quite similar. It should be noted, however, that the results were obtained by applying different
498 combinations of sf_x to the same TNNP model [33]. This means that the relative amplitudes of I_{Kr} and I_{pK} in the
499 TNNP model were much smaller than the major I_{CaL} during the AP plateau, even though I_{Kr} and I_{pK} have
500 totally different gating kinetics. Thus, the results of parameter optimization should be model-dependent. The
501 same arguments will also be applied to the use of FR guinea pig model [38] in the study by Groenendaal et al.
502 [23].

503 The gradient-based parameter optimization method was applied to the cardiac model of membrane
504 excitation in [12], which analyzed the classic BR model [37]. The whole cell current in the BR model was
505 composed of a minimum number of ionic currents, a background I_{K1} , and three time-dependent currents; I_{Na} , I_s ,
506 and I_{x1} , which were dissected from the voltage clamp experiments by applying the sucrose gap method to the
507 multicellular preparation of ventricular tissue. The gatings of the latter three currents were formulated
508 according to the Hodgkin-Huxley type gating kinetics, which was quite simple if compared with the recent

Improved identifiability of cardiac AP model parameters by physiological constraints

24

509 detailed description of the ionic currents. They described that the parameter optimization was difficult if the
510 AP configuration was used as the target of the parameter optimization, and they used the time course of the
511 whole cell current as of the target of parameter optimization. However, the number of parameters was quite
512 large, 63 in total, including limiting conductances and the gating kinetics. They suggested the feasibility of the
513 parameter optimization method will be improved if provided with additional experimental data.

514 In the modern mathematical cardiac cell models, most ionic currents were identified by the whole-cell
515 voltage clamp and single channel recordings in dissociated single myocyte [39] using the patch clamp
516 technique [40] and by identifying their molecular basis of membrane protein. It has been clarified that the
517 molecular basis of the ion channels expressed in the hiPSC-CMs is mostly identical to those in the adult
518 cardiac myocytes rather than in the fetal heart (GSE154580 GEO Accession viewer (nih.gov)). Moreover, the
519 gating kinetics have been much detailed to characterize the ionic currents within the cell model. In principle,
520 the detailed characterization of individual currents should facilitate the identifiability of the model parameter
521 but should not necessarily interfere with parameter optimization. We consider that the manual fitting of the
522 model parameters to the AP recording by using a priori knowledge of biophysical mechanisms should largely
523 facilitate the subsequent automatic parameter optimization. It might also be noted that the ionic currents left at
524 the default values work as a kind of constraint to improve the identifiability of the target parameters.

525 After validating the automatic parameter optimization method, the final goal of our study is to find the
526 principle of ionic mechanisms, which are applicable to the full range of variations of spontaneous AP records
527 in both hiPSC-CMs and matured cardiomyocytes. For this purpose, we will apply the multi-run PS method to
528 the experimental AP recordings using the initial parameter sets obtained by the conventional manual fit. The
529 protocol of measuring the G_{xS} will be the same as used in the present study except for the use of experimental
530 AP recordings in place of the output of the 'cell-specific model'. In our preliminary analysis, the magnitude of
531 individual model parameters obtained by the manual tuning was corrected by less than ~15% by the objective
532 parameter optimization. Finally, the ionic mechanisms underlying the SDD of variable time courses will be
533 analyzed in a quantitative manner, for example, by using the lead potential analysis [41], which explains
534 changes in V_m in terms of G_x of individual currents.

535

Improved identifiability of cardiac AP model parameters by physiological constraints

25

536 Limitations

537 In general, obvious limitations of the mathematical models of cardiac membrane excitation so far
538 published are caused by a shortage of functional components inherent in intact cells. For example, the $[ATP]_i$
539 controlled by energy metabolism is a vital factor in maintaining the physiological function of ion channels as
540 well as the active transport Na^+/K^+ pump [42]. Moreover, the followings are still not implemented in most
541 models; the modulation of the ion channel activity through phosphorylation of the channel proteins, detailed
542 modulation of the channel by the $[Ca^{2+}]_i$, the alterations of ion channel activity by PIP_2 [43,44] and by the
543 tension of the cell membrane through the cell volume change [45–48]. The detailed Ca^{2+} dynamics of the
544 $[Ca^{2+}]_i$ are still not implemented in most of the cardiac cell models; such as the Ca^{2+} release from SR activated
545 through the coupling of a few L-type Ca^{2+} channels with a cluster of RyRs at the dyadic junction [49], the Ca^{2+}
546 diffusion influenced by the Ca^{2+} -binding proteins [50]. To simulate the Ca^{2+} -binding to troponin during the
547 development of the contraction, it is necessary to include a dynamic model of contracting fibers [51–54].
548 These limitations should be thoroughly considered when pathophysiological phenomena, such as
549 arrhythmogenesis are concerned. The scope of the present study is limited to the AP configurations of hiPSC-
550 CMs, which were assumed to be 'healthy' with respect to the above concerns; for example, $[ATP]_i$, $[Na^+]_i$ and
551 Ca_{tot} were kept constant, and the standard contraction model was implemented as in the hVC model.

552 The parameter optimization presented in this study could be achieved in a practical way by limiting
553 the number of unknown parameters. We determined only G_x s based on the assumption that ion channel
554 kinetics are preserved as the same in the hiPSC-CMs as in the matured myocytes. Usually, 4~6 ionic currents
555 were selected for the optimization. We found that the orp method could be performed simultaneously for all
556 nine ionic currents described in Eq 1. However, the computation time was radically prolonged, and the
557 resolution was not as high as obtained by using the modest number of parameters. We consider that the
558 determination of the limited number of G_x s is quite relevant to solving physiological problems in terms of
559 detailed model equations for each current system.

560

Improved identifiability of cardiac AP model parameters by physiological constraints

26

561 Although I_{NCX} and I_{NaK} contribute sizeable fractions of the whole-cell outward and inward currents,
562 respectively (Fig 3A-3), we excluded the scaling factors, sf_{NaK} and sf_{NCX} from the parameter optimization for
563 the sake of simplicity. Instead, the possible drift of the intracellular ion concentrations was virtually fixed
564 during the repetitive adjustment of ionic fluxes by varying sf_x as shown in Table 2. The introduction of the
565 empirical equations (Eqs 13 and 14) was quite useful to adjust the $[Na^+]_i$ and Ca_{tot} (Table 2) so that the time
566 course as well as magnitude of I_{NCX} remained almost constant during the parameter optimization. When
567 influences of varying $[Na^+]_i$ and/or Ca_{tot} are examined under various experimental conditions in future, the
568 reference levels of $[Na^+]_i$ and/or Ca_{tot} ($stdNai$ and $stdCatot$ in Eqs 13 and 14) might be replaced by
569 experimental measurements.

570 The parameter optimization method was not applied to several ionic currents. For example, it was
571 difficult to determine the kinetics of T-type Ca^{2+} channel (I_{CaT} ; Cav 3.1) and excluded in the present study. A
572 very fast opening and inactivation rates described in [55] suggest a complete inactivation of I_{CaT} over the
573 voltage range of SDD, while a sizeable magnitude of window current described in [56] suggests a much larger
574 contribution to SDD. The kinetics of I_{CaT} still remain to be clarified in experimental examinations. The
575 sustained inward current, I_{st} , is recently attributed most probably to the Cav 1.3 [57], which is activated at a
576 more negative potential range than the activation of I_{CaL} (Cav 1.2) [58,59]. The I_{bNSC} was used to represent net
577 background conductance in the present study. However, several components of the background conductance
578 have been identified on the level of molecular basis in matured myocytes (see for review TRPM4, [60]).
579 Experimental measurements of the current magnitude for each component are also awaited.

580 Gábor and Banga indicated that the multi-run method had shown good performance in certain cases,
581 especially when high-quality first-order information is used and the parameter search space is restricted to a
582 relatively small domain [16] (see also [19]). Indeed, the manual fitting of the parameters (Fig 1) was required
583 to utilize the presented multi-run PS method over the restricted search space. One of the major difficulties in
584 the manual fitting of individual G_{xS} arose during the SDD, where I_{Kr} , I_{K1} , I_{bNSC} , and I_{ha} , in addition to I_{NaK} and
585 I_{NCX} constitute the whole-cell current (Fig 3A-3). Close inspection of the current components (Fig 3A-3),
586 however, suggests hints of how to do with the manual fit. The transient peak of I_{Kr} dominates the current
587 profile during the final repolarization phase from -20 to -60 mV in all 12 hiPSC-CMs [61], since I_{CaL} and I_{Ks}

Improved identifiability of cardiac AP model parameters by physiological constraints

27

588 rapidly deactivated before repolarizing to this voltage range. The I_{NaK} and I_{NCX} are well controlled by the
589 extrinsic regulation in Eqs 13 and 14. Thus, the manual fitting of sf_{Kr} is firstly applied to determine sf_{Kr} . The
590 MDP more negative than -70 mV is adjusted by the sum of time-dependent ($I_{Kr} + I_{K1}$) and the time-
591 independent I_{bNSC} . Then, I_{Kr} is deactivated when depolarization becomes obvious after the MDP, and gradual
592 activation of I_{ha} and the depolarization-dependent blocking of I_{K1} by the intracellular substances [62] take the
593 major role in promoting the initial linear phase of SDD. Thus, the amplitude of sf_{K1} and sf_{bNSC} might be
594 approximated during the initial half of SDD. The late half of SDD, including the foot of AP, namely the
595 exponential time course of depolarization toward the rapid rising phase of AP, is mainly determined by the
596 subthreshold V_m -dependent activation of I_{Na} (after MDP more negative than -70 mV) and/or I_{CaL} (after MDP
597 less negative than -65 mV). Thus, the sf_{Na} and sf_{CaL} are roughly determined by fitting the foot of AP and the
598 timing of the rapid rising phase of AP. The plateau time course of AP is determined by sf_{CaL} and the factor of
599 Ca^{2+} -mediated inactivation of I_{CaL} (the parameter KL , [4]). Since the kinetics of outward currents, I_{Kur} , I_{Kto}
600 (*endo-type*), and I_{Ks} are quite different from that of I_{Kr} , the plateau configuration is determined bit by bit by
601 adjusting these currents. We failed to observe the phase 1 rapid and transient repolarization in the hiPSC-CMs
602 (Fig 2), which is the typical sign of the absence of epicardial-type I_{Kto} .

603 In hiPSC-CMs showing less negative MDP than ~ -65 mV, the contribution of I_{K1} , I_{Na} and I_{ha} should
604 be negligibly small because I_{K1} is nearly completely blocked by the intracellular Mg^{2+} and polyamine, I_{Na} is
605 inactivated, and I_{ha} is deactivated during SDD, even if any expressed.

606 Nevertheless, parameter optimization might be laborious and time-consuming for those who are not
607 familiar with the electrophysiology of the cardiac myocyte. This difficulty might be largely eased by
608 accumulating both AP configurations and the underlying current profile obtained in the parameter optimization
609 into a database in the future. If this database becomes available, the computer may search for several candidate
610 APs for the initial parameter set, which is used for automatic parameter optimization.

611

612 6. Funding and financial conflicts of interest

613 The authors declare that the research was conducted without any commercial or financial relationships
614 that could be construed as a potential conflict of interest.

Improved identifiability of cardiac AP model parameters by physiological constraints

28

615 This work was supported by JSPS KAKENHI (Grant-in-Aid for Young Scientists) Grant Numbers
616 JP19K17560 for HK, 16K18996 for YH, and 21K06781 for FT.

617

618 7. Author Contributions

619 HK, YH, DY, YW, AK, and TM performed the wet experiments and analyzed them. HK, SK, YH,
620 YZ, FT, AA, and AN developed the simulation model and the parameter optimization method. HK, YH, AA
621 and AN wrote the manuscript. All authors reviewed the manuscript. AA and TK organized the research team.

622

623 8. Acknowledgments

624 The authors would like to thank our laboratory colleagues for their valuable comments and
625 discussions.

626

627 9. References

628

629 1. Noble D, Garny A, Noble PJ. How the Hodgkin-Huxley equations inspired the Cardiac Physiome Project:
630 Hodgkin-Huxley equations and the cardiac Physiome Project. *J Physiology*. 2012;590: 2613–2628.
631 doi:10.1113/jphysiol.2011.224238

632 2. Noble D, Rudy Y. Models of cardiac ventricular action potentials: iterative interaction between experiment
633 and simulation. *Philosophical Transactions Royal Soc Lond Ser Math Phys Eng Sci*. 2001;359: 1127–1142.
634 doi:10.1098/rsta.2001.0820

635 3. Winslow RL, Cortassa S, O'Rourke B, Hashambhoy YL, Rice JJ, Greenstein JL. Integrative modeling of the
636 cardiac ventricular myocyte. *Wiley Interdiscip Rev Syst Biology Medicine*. 2011;3: 392–413.
637 doi:10.1002/wsbm.122

638 4. Hinch R, Greenstein JL, Tanskanen AJ, Xu L, Winslow RL. A Simplified Local Control Model of Calcium-
639 Induced Calcium Release in Cardiac Ventricular Myocytes. *Biophys J*. 2004;87: 3723–3736.
640 doi:10.1529/biophysj.104.049973

641 5. Paci M, Hyttinen J, Aalto-Setälä K, Severi S. Computational models of ventricular- and atrial-like human
642 induced pluripotent stem cell derived cardiomyocytes. *Ann Biomed Eng*. 2013;41: 2334–2348.
643 doi:10.1007/s10439-013-0833-3

Improved identifiability of cardiac AP model parameters by physiological constraints

29

- 644 6. Paci M, Hyttinen J, Rodriguez B, Severi S. Human induced pluripotent stem cell-derived versus adult
645 cardiomyocytes: an in silico electrophysiological study on effects of ionic current block. *Brit J Pharmacol.*
646 2015;172: 5147–5160. doi:10.1111/bph.13282
- 647 7. Lei CL, Wang K, Clerx M, Johnstone RH, Hortigon-Vinagre MP, Zamora V, et al. Tailoring Mathematical
648 Models to Stem-Cell Derived Cardiomyocyte Lines Can Improve Predictions of Drug-Induced Changes to
649 Their Electrophysiology. *Front Physiol.* 2017;8: 986. doi:10.3389/fphys.2017.00986
- 650 8. Grandi E, Pasqualini FS, Bers DM. A novel computational model of the human ventricular action potential
651 and Ca transient. *Journal of molecular and cellular cardiology.* 2010;48: 112–121.
652 doi:10.1016/j.yjmcc.2009.09.019
- 653 9. O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the Undiseased Human Cardiac Ventricular Action
654 Potential: Model Formulation and Experimental Validation. McCulloch AD, editor. *Plos Comput Biol.* 2011;7:
655 e1002061-29. doi:10.1371/journal.pcbi.1002061
- 656 10. Asakura K, Cha CY, Yamaoka H, Horikawa Y, Memida H, Powell T, et al. EAD and DAD mechanisms
657 analyzed by developing a new human ventricular cell model. *Prog Biophysics Mol Biology.* 2014;116: 11–24.
658 doi:10.1016/j.pbiomolbio.2014.08.008
- 659 11. Himeno Y, Asakura K, Cha CY, Memida H, Powell T, Amano A, et al. A Human Ventricular Myocyte
660 Model with a Refined Representation of Excitation-Contraction Coupling. *Biophys J.* 2015;109: 415–427.
661 doi:10.1016/j.bpj.2015.06.017
- 662 12. Dokos S, Lovell NH. Parameter estimation in cardiac ionic models. *Prog Biophysics Mol Biology.*
663 2004;85: 407–431. doi:10.1016/j.pbiomolbio.2004.02.002
- 664 13. Dutta S, Chang KC, Beattie KA, Sheng J, Tran PN, Wu WW, et al. Optimization of an In silico Cardiac
665 Cell Model for Proarrhythmia Risk Assessment. *Front Physiol.* 2017;8: 616. doi:10.3389/fphys.2017.00616
- 666 14. Whittaker DG, Clerx M, Lei CL, Christini DJ, Mirams GR. Calibration of ionic and cellular cardiac
667 electrophysiology models. *Wiley Interdiscip Rev Syst Biology Medicine.* 2020;12: e1482.
668 doi:10.1002/wsbm.1482
- 669 15. Cairns DI, Fenton FH, Cherry EM. Efficient parameterization of cardiac action potential models using a
670 genetic algorithm. *Chaos Interdiscip J Nonlinear Sci.* 2017;27: 093922. doi:10.1063/1.5000354
- 671 16. Gábor A, Banga JR. Robust and efficient parameter estimation in dynamic models of biological systems.
672 *Bmc Syst Biol.* 2015;9: 74. doi:10.1186/s12918-015-0219-2
- 673 17. Degasperi A, Fey D, Kholodenko BN. Performance of objective functions and optimisation procedures for
674 parameter estimation in system biology models. *Npj Syst Biology Appl.* 2017;3: 20. doi:10.1038/s41540-017-
675 0023-2
- 676 18. Penas DR, González P, Egea JA, Doallo R, Banga JR. Parameter estimation in large-scale systems biology
677 models: a parallel and self-adaptive cooperative strategy. *Bmc Bioinformatics.* 2017;18: 52.
678 doi:10.1186/s12859-016-1452-4
- 679 19. Villaverde AF, Fröhlich F, Weindl D, Hasenauer J, Banga JR. Benchmarking optimization methods for
680 parameter estimation in large kinetic models. *Bioinformatics.* 2019;35: 830–838.
681 doi:10.1093/bioinformatics/bty736

Improved identifiability of cardiac AP model parameters by physiological constraints

30

- 682 20. Sher A, Niederer SA, Mirams GR, Kirpichnikova A, Allen R, Pathmanathan P, et al. A Quantitative
683 Systems Pharmacology Perspective on the Importance of Parameter Identifiability. *B Math Biol.* 2022;84: 39.
684 doi:10.1007/s11538-021-00982-5
- 685 21. Coope ID, Price CJ. A direct search conjugate directions algorithm for unconstrained minimization.
686 *Anziam J.* 2000;42: 478–498. doi:10.21914/anziamj.v42i0.609
- 687 22. Hough PD, Kolda TG, Torczon VJ. Asynchronous Parallel Pattern Search for Nonlinear Optimization.
688 *Siam J Sci Comput.* 2001;23: 134–156. doi:10.1137/s1064827599365823
- 689 23. Groenendaal W, Ortega FA, Kherlopian AR, Zygmunt AC, Krogh-Madsen T, Christini DJ. Cell-Specific
690 Cardiac Electrophysiology Models. *Plos Comput Biol.* 2015;11: e1004242. doi:10.1371/journal.pcbi.1004242
- 691 24. Sarkar AX, Sobie EA. Regression Analysis for Constraining Free Parameters in Electrophysiological
692 Models of Cardiac Cells. *Plos Comput Biol.* 2010;6: e1000914. doi:10.1371/journal.pcbi.1000914
- 693 25. Zaniboni M, Riva I, Cacciani F, Groppi M. How different two almost identical action potentials can be: A
694 model study on cardiac repolarization. *Math Biosci.* 2010;228: 56–70. doi:10.1016/j.mbs.2010.08.007
- 695 26. Krogh-Madsen T, Sobie EA, Christini DJ. Improving cardiomyocyte model fidelity and utility via dynamic
696 electrophysiology protocols and optimization algorithms. *J Physiology.* 2016;594: 2525–2536.
697 doi:10.1113/jp270618
- 698 27. Hooke R, Jeeves TA. “Direct Search” Solution of Numerical and Statistical Problems. *J Acm Jacm.*
699 1961;8: 212–229. doi:10.1145/321062.321069
- 700 28. Torczon V. On the Convergence of Pattern Search Algorithms. *Siam J Optimiz.* 1997;7: 1–25.
701 doi:10.1137/s1052623493250780
- 702 29. Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, et al. High purity human-induced pluripotent
703 stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. 2011.
- 704 30. Hagiwara N, IRISAWA H, Kasanuki H, Hosoda S. Background current in sino-atrial node cells of the
705 rabbit heart. *The Journal of physiology.* 1992;448: 53–72. doi:10.1113/jphysiol.1992.sp019029
- 706 31. Kiyosue T, Spindler AJ, Noble SJ, NOBLE D. Background inward current in ventricular and atrial cells of
707 the guinea-pig. *Proceedings Biological sciences.* 1993;252: 65–74. doi:10.1098/rspb.1993.0047
- 708 32. Cheng H, Li J, James AF, Inada S, Choisy SCM, Orchard CH, et al. Characterization and influence of
709 cardiac background sodium current in the atrioventricular node. *J Mol Cell Cardiol.* 2016;97: 114–24.
710 doi:10.1016/j.yjmcc.2016.04.014
- 711 33. Tusscher KHJ ten, Noble D, Noble PJ, Panfilov AV. A model for human ventricular tissue. *Am J*
712 *Physiol-heart C.* 2004;286: H1573–H1589. doi:10.1152/ajpheart.00794.2003
- 713 34. Ashford JR, Colquhoun D. Lectures on Biostatistics: An Introduction to Statistics with Applications in
714 Biology and Medicine. *J Royal Statistical Soc Ser Gen.* 1972;135: 606–606. doi:10.2307/2344687
- 715 35. Syed Z, Vigmond E, Nattel S, Leon LJ. Atrial cell action potential parameter fitting using genetic
716 algorithms. *Medical Biological Eng Comput.* 2005;43: 561–571. doi:10.1007/bf02351029

Improved identifiability of cardiac AP model parameters by physiological constraints

31

- 717 36. Guo T, Abed AA, Lovell NH, Dokos S. Optimisation of a Generic Ionic Model of Cardiac Myocyte
718 Electrical Activity. *Comput Math Method M.* 2013;2013: 706195. doi:10.1155/2013/706195
- 719 37. Beeler GW, Reuter H. Reconstruction of the action potential of ventricular myocardial fibres. *J Physiology.*
720 1977;268: 177–210. doi:10.1113/jphysiol.1977.sp011853
- 721 38. Faber GM, Rudy Y. Action Potential and Contractility Changes in $[Na^+]_i$ Overloaded Cardiac Myocytes:
722 A Simulation Study. *Biophys J.* 2000;78: 2392–2404. doi:10.1016/s0006-3495(00)76783-x
- 723 39. Powell T, Twist VW. A rapid technique for the isolation and purification of adult cardiac muscle cells
724 having respiratory control and a tolerance to calcium. *Biochem Bioph Res Co.* 1976;72: 327–333.
725 doi:10.1016/0006-291x(76)90997-9
- 726 40. Sakmann B, Neher E. Patch Clamp Techniques for Studying Ionic Channels in Excitable Membranes.
727 *Annu Rev Physiol.* 1984;46: 455–472. doi:10.1146/annurev.ph.46.030184.002323
- 728 41. Cha CY, Himeno Y, Shimayoshi T, Amano A, Noma A. A novel method to quantify contribution of
729 channels and transporters to membrane potential dynamics. *Biophys J.* 2009;97: 3086–3094.
730 doi:10.1016/j.bpj.2009.08.060
- 731 42. Winslow RL, Walker MA, Greenstein JL. Modeling calcium regulation of contraction, energetics,
732 signaling, and transcription in the cardiac myocyte. *Wiley Interdiscip Rev Syst Biology Medicine.* 2016;8: 37–
733 67. doi:10.1002/wsbm.1322
- 734 43. Hilgemann DW, Feng S, Nasuhoglu C. The Complex and Intriguing Lives of PIP2 with Ion Channels and
735 Transporters. *Sci Stke.* 2001;2001: re19. doi:10.1126/stke.2001.1111.re19
- 736 44. Suh B-C, Hille B. PIP2 Is a Necessary Cofactor for Ion Channel Function: How and Why? *Biophysics.*
737 2008;37: 175–195. doi:10.1146/annurev.biophys.37.032807.125859
- 738 45. Sasaki N, Mitsuiye T, Wang Z, Noma A. Increase of the delayed rectifier K^+ and Na^+ - K^+ pump currents
739 by hypotonic solutions in guinea pig cardiac myocytes. *Circ Res.* 2018;75: 887–895.
740 doi:10.1161/01.res.75.5.887
- 741 46. Hammami S, Willumsen NJ, Olsen HL, Morera FJ, Latorre R, Klaerke DA. Cell volume and membrane
742 stretch independently control K^+ channel activity: Cell volume, membrane stretch and K^+ channel activity. *J*
743 *Physiology.* 2009;587: 2225–2231. doi:10.1113/jphysiol.2008.163550
- 744 47. Peyronnet R, Nerbonne JM, Kohl P. Cardiac Mechano-Gated Ion Channels and Arrhythmias. *Circ Res.*
745 2016;118: 311–329. doi:10.1161/circresaha.115.305043
- 746 48. Gao J, Yun T, Xie X-L, Zhao J, Liu C, Sheng Y, et al. Losartan inhibits hyposmotic-induced increase of
747 IK_s current and shortening of action potential duration in guinea pig atrial myocytes. *Anatol J Cardiol.*
748 2020;23: 35–40. doi:10.14744/anatoljcardiol.2019.75332
- 749 49. Cannell MB, Kong CHT. Local control in cardiac E–C coupling. *J Mol Cell Cardiol.* 2012;52: 298–303.
750 doi:10.1016/j.yjmcc.2011.04.014
- 751 50. Bers DM. Calcium Cycling and Signaling in Cardiac Myocytes. *Annu Rev Physiol.* 2008;70: 23–49.
752 doi:10.1146/annurev.physiol.70.113006.100455

Improved identifiability of cardiac AP model parameters by physiological constraints

32

- 753 51. Greenstein JL, Hinch R, Winslow RL. Mechanisms of Excitation-Contraction Coupling in an Integrative
754 Model of the Cardiac Ventricular Myocyte. *Biophys J*. 2006;90: 77–91. doi:10.1529/biophysj.105.065169
- 755 52. Negroni JA, Lascano EC. Simulation of steady state and transient cardiac muscle response experiments
756 with a Huxley-based contraction model. *Journal of molecular and cellular cardiology*. 2008;45: 300–312.
757 doi:10.1016/j.yjmcc.2008.04.012
- 758 53. Timmermann V, Edwards AG, Wall ST, Sundnes J, McCulloch AD. Arrhythmogenic Current Generation
759 by Myofilament-Triggered Ca²⁺ Release and Sarcomere Heterogeneity. *Biophys J*. 2019;117: 2471–2485.
760 doi:10.1016/j.bpj.2019.11.009
- 761 54. Niederer SA, Campbell KS, Campbell SG. A short history of the development of mathematical models of
762 cardiac mechanics. *J Mol Cell Cardiol*. 2019;127: 11–19. doi:10.1016/j.yjmcc.2018.11.015
- 763 55. Hagiwara N, Irisawa H, Kameyama M. Contribution of two types of calcium currents to the pacemaker
764 potentials of rabbit sino-atrial node cells. *J Physiology*. 1988;395: 233–253.
765 doi:10.1113/jphysiol.1988.sp016916
- 766 56. Zhou Z, Lipsius SL. T-Type Calcium Current in Latent Pacemaker Cells Isolated from Cat Right Atrium. *J*
767 *Mol Cell Cardiol*. 1994;26: 1211–1219. doi:10.1006/jmcc.1994.1139
- 768 57. Guo J, Ono K, Noma A. A sustained inward current activated at the diastolic potential range in rabbit sino-
769 atrial node cells. *J Physiology*. 1995;483: 1–13. doi:10.1113/jphysiol.1995.sp020563
- 770 58. Toyoda F, Mesirca P, Dubel S, Ding W-G, Striessnig J, Mangoni ME, et al. CaV1.3 L-type Ca²⁺ channel
771 contributes to the heartbeat by generating a dihydropyridine-sensitive persistent Na⁺ current. *Sci Rep-uk*.
772 2017;7: 7869. doi:10.1038/s41598-017-08191-8
- 773 59. Toyoda F, Wei-Guang D, Matsuura H. Heterogeneous functional expression of the sustained inward Na⁺
774 current in guinea pig sinoatrial node cells. *Pflügers Archiv - European J Physiology*. 2018;470: 481–490.
775 doi:10.1007/s00424-017-2091-y
- 776 60. Guinamard R, Bouvagnet P, Hof T, Liu H, Simard C, Sallé L. TRPM4 in cardiac electrical activity.
777 *Cardiovasc Res*. 2015;108: 21–30. doi:10.1093/cvr/cvv213
- 778 61. Doss MX, Diego JMD, Goodrow RJ, Wu Y, Cordeiro JM, Nesterenko VV, et al. Maximum diastolic
779 potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on I(Kr). Barbuti
780 A, editor. *Plos One*. 2012;7: e40288. doi:10.1371/journal.pone.0040288
- 781 62. Ishihara K, Yan D, Yamamoto S, Ehara T. Inward rectifier K⁺ current under physiological cytoplasmic
782 conditions in guinea-pig cardiac ventricular cells. *J Physiology*. 2002;540: 831–841.
783 doi:10.1113/jphysiol.2001.013470

784

785

786