

Multiscale Computational Models for Optogenetic Control of Cardiac Function

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ABSTRACT The ability to stimulate mammalian cells with light has significantly changed our understanding of electrically excitable tissues in health and disease, paving the way toward various novel therapeutic applications. Here, we demonstrate the potential of optogenetic control in cardiac cells using a hybrid experimental/computational technique. Experimentally, we introduced channelrhodopsin-2 into undifferentiated human embryonic stem cells via a lentiviral vector, and sorted and expanded the genetically engineered cells. Via directed differentiation, we created channelrhodopsin-expressing cardiomyocytes, which we subjected to optical stimulation. To quantify the impact of photostimulation, we assessed electrical, biochemical, and mechanical signals using patch-clamping, multielectrode array recordings, and video microscopy. Computationally, we introduced channelrhodopsin-2 into a classic autorhythmic cardiac cell model via an additional photocurrent governed by a light-sensitive gating variable. Upon optical stimulation, the channel opens and allows sodium ions to enter the cell, inducing a fast upstroke of the transmembrane potential. We calibrated the channelrhodopsin-expressing cell model using single action potential readings for different photostimulation amplitudes, pulse widths, and frequencies. To illustrate the potential of the proposed approach, we virtually injected channelrhodopsin-expressing cells into different locations of a human heart, and explored its activation sequences upon optical stimulation. Our experimentally calibrated computational toolbox allows us to virtually probe landscapes of process parameters, and identify optimal photostimulation sequences toward pacing hearts with light.

INTRODUCTION

For more than 40 years, biologists have studied microorganisms that produce proteins to directly regulate the flow of charged ions across their plasma membrane in response to light. The first identified protein of this kind was the light-gated ion pump bacteriorhodopsin, transporting positively charged hydrogen ions across the cell membrane (1). While bacteriorhodopsin naturally acts as an on-switch for electrically active cells, the second reported light-gated ion pump, halorhodopsin, transports negatively charged chloride ions, thereby acting as an off-switch (2). The first reported light-gated ionic channel illustrated in Fig. 1, channelrhodopsin, was only identified 30 years later (3,4), but has since revolutionized neuroscience.

Since the early 1990s, we have known that phototaxis and photophobic responses in the green alga *Chlamydomonas reinhardtii* are mediated by rhodopsins with a microbial-type all-*trans* retinal chromophore (5,6). The photochemical isomerization of this all-*trans* retinal to 13-*cis* retinal is illustrated in Fig. 2. It occurs at peak absorption wavelengths of 470 nm, opening the channel to sodium, potassium, and calcium cations in response to blue light. In the dark, the covalently bound retinal spontaneously relaxes to

all-*trans*, providing closure of the channel and regeneration of the chromophore.

A breakthrough-enabling technology was reported in 2005, when the light-sensitive target was first introduced genetically using engineered viruses (7,8), a technique that is now known as optogenetics (9,10). Since then, optical tools for controlling the electrical activity of neurons have rapidly evolved, and are now gaining widespread use in neuronal research and medicine (11,12). Although initial applications of optogenetics have been restricted exclusively to the neuronal system, optogenetic tools have now advanced to a level of maturity, where they can confidently be applied to other cells and organs (9). Natural first candidates of choice are stem cells, and electrically active glial cells, muscle cells, and cardiac cells (O. Abilez, J. A. Baugh, M. L. Gorrepati, R. Prakash, C. Lee-Messer, M. Huang, F. Jia, J. Yu, K. D. Wilson, J. C. Wu, K. Deisseroth, and C. K. Zarins, unpublished; and (14)).

The objective of this study is to demonstrate the potential of optogenetic control of the cardiac system using a hybrid experimental/computational technique. We demonstrate that channelrhodopsin-2 (ChR2) can be expressed stably and safely in human embryonic stem cells (hESC), which can then be differentiated into cardiomyocytes (hESC^{ChR2}-CM). Upon photostimulation, ChR2 opens rapidly and allows sodium ions to enter the cell, inducing a defined transmembrane potential, commonly known as the action potential. We illustrate how ChR2 can be introduced into a computational autorhythmic cell model via an additional photocurrent governed by a light-sensitive gating variable to simulate this

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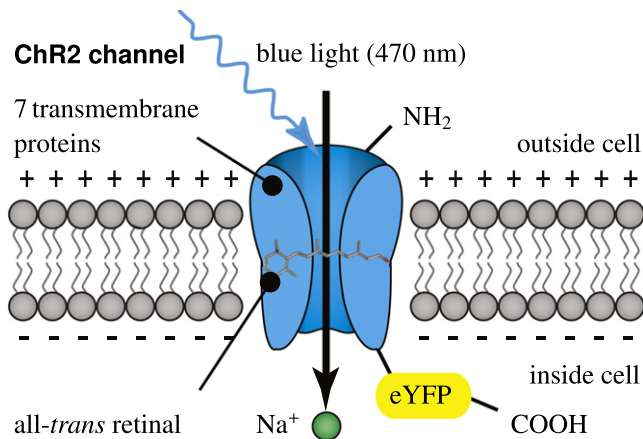


FIGURE 1 Channelrhodopsin-2 (ChR2) is a light-gated cation channel native to the green alga *C. reinhardtii*. It consists of seven transmembrane proteins and absorbs light through its interaction with retinal. Here, we induce channelrhodopsin coupled to enhanced yellow fluorescent protein (eYFP) into undifferentiated human embryonic stem cells via a lentiviral vector and differentiate these cells into cardiomyocytes.

effect. The calibrated cell model is capable of reliably reproducing photostimulation amplitudes, pulse widths, and frequencies from single action potential readings. Using a custom-designed finite element model, we virtually inject our calibrated model cells into different locations of a human heart to illustrate the potential of the proposed approach toward pacing hearts with light.

MATERIALS AND METHODS

All experiments, methods, and protocols for this study were approved by the Stanford University Stem Cell Research Oversight committee.

Opsin sources and lentiviral vector

The channelrhodopsin-2 (ChR2) variant described here was optimized for mammalian expression by truncating the native sequence from 2241 base-pair (bp) to 933 bp, by changing the native histidine (H) codon (CAC) to the arginine (R) codon (CGC) coding for protein residue 134 of ChR2 (H134R), and by changing its gene's codon usage to conform to human codon usage distribution (15,16). The lentiviral vector pLenti-EF1 α -ChR2-eYFP-WPRE (pLECYT) was constructed as previously described (7,16). The pLET plasmid contains the ubiquitously expressed elongation factor 1- α (EF1 α), to obtain high levels of ChR2-eYFP expression in

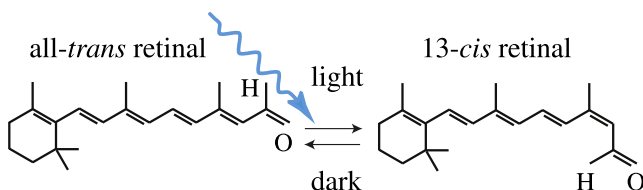


FIGURE 2 Channelrhodopsin-2 (ChR2) is activated by photoisomerization of all-*trans* retinal to 13-*cis* retinal at wavelengths of 470 nm. After photoisomerization, the covalently bound retinal spontaneously relaxes to all-*trans* in the dark, providing closure of the ion channel and regeneration of the chromophore.

a mammalian system ((8), O. Abilez, J. A. Baugh, M. L. Gorrepati, R. Prakash, C. Lee-Messer, M. Huang, F. Jia, J. Yu, K. D. Wilson, J. C. Wu, K. Deisseroth, and C. K. Zarins, unpublished). All constructs have been fully sequenced previously for accuracy of cloning (7,15). High-titer lentivirus was produced using a second-generation lentiviral system by cotransfection of 293FT cells (Invitrogen, Carlsbad, CA), the pLECYT viral vector described above, pCMVR Δ 8.74 (containing GAG and POL), pMD2.G (containing VSVg), and calcium phosphate as previously described (16,17).

Stem cell culture and differentiation

Channelrhodopsin-expressing human embryonic stem cells (hESC^{ChR2+}) were grown as monolayers (O. Abilez, J. A. Baugh, M. L. Gorrepati, R. Prakash, C. Lee-Messer, M. Huang, F. Jia, J. Yu, K. D. Wilson, J. C. Wu, K. Deisseroth, and C. K. Zarins, unpublished) on hESC-qualified Matrigel (BD Biosciences, San Jose, CA) and maintained in the pluripotent state through daily feeding with mTeSR1 media (StemCell Technologies, Vancouver, British Columbia, Canada) (18), supplemented with 1 \times penicillin/streptomycin (Invitrogen). Cardiomyocyte differentiation was usually begun 2–5 days after initially seeding hESC^{ChR2+} on Matrigel (BD Biosciences). At this time, the cells were transferred to RPMI-1640 media supplemented with B27, 1 \times nonessential amino acids, 1 \times penicillin/streptomycin, and 0.1 mM β -mercaptoethanol (all Invitrogen) and our differentiation method was begun using aspects of other methods previously described (19,20).

On the first day of differentiation, Day 0, RPMI media with 50 ng/mL of Activin A (R&D Systems, Minneapolis, MN) was added to each well. On the subsequent day, Day 1, 5 ng/mL of BMP-4 (R&D Systems) was added to each well. On Day 3, fresh RPMI media was added to each well and was replaced every 48 h until Day 11, when the cells were transferred to Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) supplemented with 5% fetal bovine serum (Invitrogen), 1 \times nonessential amino acids, 1 \times penicillin/streptomycin, and 0.1 mM β -mercaptoethanol. This DMEM was then replaced approximately every 48 h. Cardiomyocytes generally began spontaneously beating between Days 9 and 20.

Fluorescence-activated cell sorting

Fluorescence-activated cell sorting (FACS) was performed with a BD FACSAria instrument equipped with BD FACSDiva 6.0 software (BD Biosciences). Up to 1 \times 10⁶ cells transduced with the ChR2-eYFP lentivirus were sorted. Sorted cells were then resuspended in mTeSR1 media and replated on Matrigel-coated wells. After 2–3 days in culture, enhanced yellow fluorescent protein (eYFP) signal was confirmed via fluorescence microscopy. Analysis of FACS data was performed offline with FlowJo 7.6.1 software (Tree Star, Ashland, OR).

Polymerase chain reaction

For undifferentiated hESC, polymerase chain reaction (PCR) primers with gene product length for the following genes were used: GAPDH (152 bp), Oct-4 (169 bp), Nanog (154 bp), a region within eYFP (187 bp), a region spanning eYFP-ChR2 (197 bp), and a region within ChR2 (174 bp). Total RNA was isolated and RNA yield was then quantified using a Quant-iT kit (Invitrogen) and Qubit fluorometer (Invitrogen) per the manufacturer's instructions. For cDNA synthesis, 1 μ g total RNA, random hexamers, annealing buffer, 2 \times First-Strand Reaction Mix (Invitrogen) and SuperScript III/RNase OUT Enzyme Mix (Invitrogen), and RNase/DNase-free water were combined, then incubated per the manufacturer's instructions. For PCR amplification, AccuPrime Pfx SuperMix (Invitrogen), custom primers for pluripotency markers as described above, and cDNA were combined. Nontemplate control reactions were prepared by substituting cDNA with distilled water. Samples were transferred to a thermal cycler and the following cycling program was used: 1), initial denaturation at 95°C for

2 min; 2), 30 cycles of 95°C for 30 s, 60°C for 30 s, and 68°C for 1 min; and 3), final extension at 68°C for 5 min.

Finally, PCR products, a 100-bp ladder (Invitrogen), and nontemplate control reactions were loaded in separate wells of a 2% agarose E-gel with SYBR-Safe (Invitrogen) and run for 30 min. Bands were then visualized with an E-gel iBase blue-light transilluminator (Invitrogen).

Immunocytochemistry

Human-embryonic-stem-cell-derived cardiomyocytes (hESC-CMs) were labeled with primary antibodies for the cardiac markers α -actinin (IgG, 1:500; Sigma, St. Louis, MO) and troponin I (TnI) (IgG, 1:200; Millipore, Billerica, MA). The secondary antibody used was goat anti-mouse IgG-Alexa 594 (1:1000; Invitrogen) for both α -actinin and TnI. Cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Sigma) for 10 min. An AxioObserver Z1 (Carl Zeiss, Göttingen, Germany) inverted microscope was used to visualize hESC-CMs. The Zeiss microscope was equipped with a Lambda DG-4 300 W Xenon light source (Sutter Instruments, Novato, CA), an ORCA-ER charge-coupled device camera (Hamamatsu, Bridgewater, NJ), and AxioVision 4.7 software (Zeiss).

Optical stimulation

Optical stimulation was delivered to hESC-CMs via a Lambda DG-4 300 W Xenon light source (Sutter Instruments) or with a 470 nm LED at 7 mW/mm² (Thorlabs, Newton, NJ). For multielectrode array (MEA) electrophysiology, optical stimulation consisted of a monophasic waveform with peak amplitude of 0, 33, 75, or 100% of maximum power (10 mW/mm² for 40× objective), pulse width of 100 ms, and frequency of 0.5, 1.0, or 1.5 Hz. For whole-cell patch-clamp electrophysiology, optical stimulation consisted of a monophasic waveform with peak amplitude of 0, 12.5, 25, 50, or 100% of maximum power (10 mW/mm² for 40× objective) and a pulse width of 1000 ms. Optical power delivered to cells at each microscope objective was measured with a digital power meter (Thorlabs) at the focal plane of the objective.

Multielectrode array electrophysiology

Multielectrode arrays (MEA) with sixty 30- μ m titanium nitride electrodes equally spaced 200- μ m apart, with indium tin oxide leads, and with an internal reference (Thin MEA 200/30 iR indium tin oxide; MultiChannel Systems, Reutlingen, Germany) were coated with 25 μ g/mL fibronectin (Sigma). Desired cardiomyocyte (CM) colonies were then manually dissected off their plates, transferred to the MEAs, and allowed to attach. A single MEA containing cells and Tyrode's solution (Sigma) was then placed in the amplifier (MEA 1060-Inv-BC; MultiChannel Systems) for recordings. Signals were acquired at 1 kHz from a USB-6225 M Series DAQ (National Instruments, Austin, TX). Videos of contracting CM were captured at 30 fps for a duration of 1–30 s with a Retiga 2000R Color Cooled Camera (QImaging, Surrey, British Columbia, CA). The MEA amplifier was configured with MEA Select 1.1.0 software (MultiChannel Systems) and electrical and video signals were acquired and controlled with a custom program created with LabVIEW 2009 (National Instruments). To visualize hESC-CM contractions, a custom edge detection algorithm in LabVIEW 2009 (National Instruments) was used to detect rising and falling edge locations along a grayscale profile generated from a user-defined region of interest based on a user-defined threshold value.

Patch-clamp electrophysiology

hESC-CMs were recorded by means of whole-cell patch-clamp, using an Axon Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), an Axon Digidata 1440A data acquisition system (Molecular Devices),

and pClamp 10 software (Molecular Devices) as previously described (15,21). Cells were visualized and optically stimulated on an Olympus upright microscope equipped with a 470 nm LED (Thorlabs) and EXFO X-Cite halogen light source (Lumen Dynamics, Mississauga, Ontario, Canada) through a 40×/0.8 NA water immersion objective. When using the halogen light source coupled to a shutter (VCM-D1; Uniblitz, Rochester, NY), an excitation filter of HQ470/40, dichroic Q495LP (Chroma Technology, Bellows Falls, VT) was used for delivering blue light for ChR2 activation (10, 5, 2.5, 1.25 mW/mm²). eYFP was visualized with a standard eYFP filter set (excitation 500/20, dichroic 515LP, emission 535/30; Chroma Technology). Borosilicate glass (Sutter Instruments) pipette resistance ranged from 3 to 6 M Ω .

Whole-cell patch-clamp recordings were performed as previously described (15), using the intracellular solution: 129 mM K-gluconate, 10 mM HEPES, 10 mM KCl, 4 mM MgATP, 0.3 mM NaGTP, titrated to pH 7.2; and extracellular Tyrode's solution: 125 mM NaCl, 2 mM KCl, 3 mM CaCl₂, 1 mM MgCl₂, 30 mM glucose, and 25 mM HEPES, titrated to pH 7.3. For voltage-clamp recordings, cells were held at -70 mV. All experiments were performed at room temperature, 22–25°C. Fluorescent cells were patched immersed in Tyrode's solution containing 5–10 μ M blebbistatin to eliminate contractility while preserving electrical activity (22). Patch-clamp data were analyzed using ClampFit 10.2 (Molecular Devices).

Mathematical model of channelrhodopsin photocycle

To model the channelrhodopsin photocycle, we adopt a three-state photocycle model (23,24), which is characterized through an open, a closed but still recovering, and a fully closed state (4), as illustrated in Fig. 3. Upon photoabsorption, molecules that are in the closed state g_c undergo a fast transition into the open state g_{ChR2} . After being open for some time, molecules spontaneously transition into the recovering state g_r , where the ion channels are closed, but the molecules are not yet ready to photoswitch again. After a recovery period, the molecules finally return to the closed state g_c , ready to undergo a new photocycle when exposed to light (25). Fig. 3 suggests the following first-order model for the channelrhodopsin photocycle:

$$\begin{aligned} \dot{g}_{ChR2} &= \epsilon n_{pho} g_c - \Gamma_r g_{ChR2} \\ \dot{g}_r &= \Gamma_r g_{ChR2} - \Gamma_c g_r \\ \dot{g}_c &= \Gamma_c g_r - \epsilon n_{pho} g_c. \end{aligned} \quad (1)$$

Here Γ_r and Γ_c are the rates of recovery and full closure, ϵ is the quantum efficiency of the channelrhodopsin system, and n_{pho} is the number of

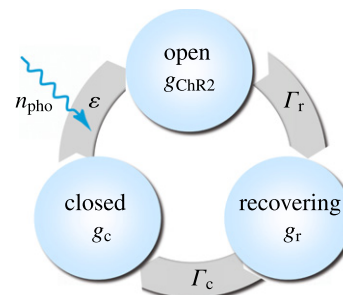


FIGURE 3 Three-state model for the channelrhodopsin photocycle. Upon photoabsorption, molecules in the closed state g_c undergo a fast transition into the open state g_{ChR2} . After for some time, molecules spontaneously turn into the recovering state g_r , where the ion channels are closed, but the molecules are not yet ready to photoswitch again. After a recovery period, the molecules finally return to the closed state g_c , ready to undergo a new photocycle when subjected to light.

photons hitting the cell per second. Herein, g_{ChR2} , g_r , and g_c define the fraction of molecules in the open, recovering, and closed states, scaled such that they sum up to one, $g_{\text{ChR2}} + g_r + g_c = 1$. This implies that the photocycle system (Eq. 1) can be characterized through two independent variables, e.g., the fraction of molecules in the open and in the recovering states:

$$\begin{aligned} \dot{g}_{\text{ChR2}} &= \epsilon n_{\text{pho}} - \left[\epsilon n_{\text{pho}} + \Gamma_r \right] g_{\text{ChR2}} - \epsilon n_{\text{pho}} g_r \\ \dot{g}_r &= \Gamma_r g_{\text{ChR2}} - \Gamma_c g_r \end{aligned} \quad (2)$$

We identify the state g_{ChR2} as the channelrhodopsin gating variable and integrate it into a well-defined autorhythmic cell model (26) characterized through $n_{\text{gate}} = 10$ gating variables in total as illustrated in Fig. 4:

$$g_{\text{gate}} = \left[g_m, g_h, g_{\text{ChR2}}, g_y, g_x, g_{\text{dL}}, g_{\text{fL}}, g_{\text{fCa}}, g_{\text{dT}}, g_{\text{fT}} \right]. \quad (3)$$

These are the fast sodium channel activation gate g_m , the fast sodium channel inactivation gate g_h , the channelrhodopsin activation gate g_{ChR2} , the hyperpolarization activated inward current activation gate g_y , the delayed rectifier current activation gate g_x , the long-lasting calcium channel activation gate g_{dL} , the voltage-dependent long-lasting calcium channel inactivation gate g_{fL} , the calcium-dependent long-lasting calcium channel inactivation gate g_{fCa} , the transient calcium channel activation gate g_{dT} , and the transient calcium channel inactivation gate g_{fT} . The gating variables are parameterized in terms of the transmembrane potential ϕ , the ionic concentrations c_{ion} , and the gating variables g_{gate} themselves. Their evolution is governed by classic Hodgkin-Huxley type equations,

$$\dot{g}_{\text{gate}} = \frac{1}{\tau_{\text{gate}}(\phi)} \left[g_{\text{gate}}^{\infty}(\phi, c_{\text{ion}}) - g_{\text{gate}} \right], \quad (4)$$

each characterized through a steady-state value g_{gate}^{∞} and a time constant τ_{gate} for reaching this steady state (27). Both are usually exponential functions of the transmembrane potential ϕ .

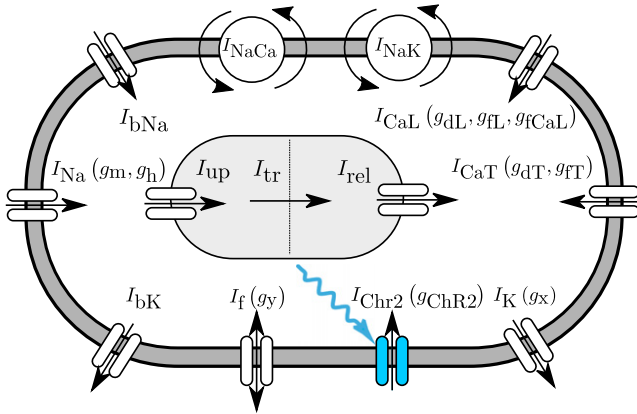


FIGURE 4 Ionic model of genetically engineered light sensitive cardiac cell. The electrochemical state of the cell is characterized in terms of $n_{\text{ion}} = 8$ ion concentrations, $c_{\text{ion}} = [c_{\text{Na}}^e, c_{\text{K}}^e, c_{\text{Ca}}^e, c_{\text{Na}}^i, c_{\text{K}}^i, c_{\text{Ca}}^i, c_{\text{Ca}}^{\text{up}}, c_{\text{Ca}}^{\text{rel}}]$, the extracellular and intracellular sodium, potassium, and calcium concentrations, and the sarcoplasmic reticulum calcium uptake and release. Ion concentrations are controlled through $n_{\text{crt}} = 12$ ionic currents, $I_{\text{crt}} = [I_{\text{Na}}, I_{\text{bNa}}, I_{\text{f}}, I_{\text{NaCa}}, I_{\text{NaK}}, I_{\text{ChR2}}, I_{\text{K}}, I_{\text{bK}}, I_{\text{CaL}}, I_{\text{CaT}}, I_{\text{up}}, I_{\text{rel}}]$, where the baseline autorhythmic cell model (26) has been enhanced with the channelrhodopsin current I_{ChR2} (shown in blue). The channels are governed by $n_{\text{gate}} = 10$ gating variables $g_{\text{gate}} = [g_m, g_h, g_{\text{ChR2}}, g_y, g_x, g_{\text{dL}}, g_{\text{fL}}, g_{\text{fCa}}, g_{\text{dT}}, g_{\text{fT}}]$, which may be functions of the current membrane potential ϕ .

Mathematical model of ionic currents

The channelrhodopsin gating variable g_{ChR2} introduced in the previous section governs the channelrhodopsin current I_{ChR2} , for which we make the following Ansatz:

$$I_{\text{ChR2}} = C_{\text{ChR2}} g_{\text{ChR2}} [\phi - \phi_{\text{ChR2}}]. \quad (5)$$

Here, C_{ChR2} is the channelrhodopsin conductance and ϕ_{ChR2} is the reversal potential of channelrhodopsin (see the Supporting Material). We integrate the channelrhodopsin current I_{ChR2} into an autorhythmic cardiac cell model (26,28), defined through a total of $n_{\text{crt}} = 12$ ionic currents,

$$I_{\text{crt}} = [I_{\text{Na}}, I_{\text{bNa}}, I_{\text{f}}, I_{\text{NaCa}}, I_{\text{NaK}}, I_{\text{ChR2}}, I_{\text{K}}, I_{\text{bK}}, I_{\text{CaL}}, I_{\text{CaT}}, I_{\text{up}}, I_{\text{rel}}], \quad (6)$$

which are, mathematically speaking, functions of the transmembrane potential ϕ , the individual gating variables g_{gate} , and the ion concentrations c_{ion} :

$$I_{\text{crt}} = I_{\text{crt}}(\phi, g_{\text{gate}}, c_{\text{ion}}). \quad (7)$$

These 12 ionic currents consist of 10 transmembrane currents, i.e., the fast sodium current I_{Na} , the background sodium current I_{bNa} , the hyperpolarization-activated sodium and potassium currents I_{f} , the sodium calcium exchanger current I_{NaCa} , the sodium potassium pump current I_{NaK} , the channelrhodopsin sodium current I_{ChR2} , the delayed rectifying potassium current I_{K} , the background potassium current I_{bK} , the long-lasting L-type calcium current I_{CaL} , and the transient T-type calcium current I_{CaT} , and two sarcoplasmic reticulum currents, i.e., the calcium uptake I_{up} and the calcium release I_{rel} .

Mathematical model of ionic concentrations

From a chemical point of view, light induces a channelrhodopsin current I_{ChR2} , which directly impacts the intracellular sodium concentrations c_{Na}^i ,

$$\dot{c}_{\text{Na}}^i = -\frac{1}{FV_i} [I_{\text{Na}} + I_{\text{bNa}} + I_{\text{fNa}} + 3I_{\text{NaCa}} + 3I_{\text{NaK}} + I_{\text{KNa}} + I_{\text{ChR2}}], \quad (8)$$

where V_i is the cytosolic volume and F is the Faraday constant. The sodium concentration will directly, and indirectly through the resulting changes in the transmembrane potential ϕ , affect all other ionic concentrations in the cell. The biochemistry of our cell model is characterized through $n_{\text{ion}} = 8$ ion concentrations,

$$c_{\text{ion}} = [c_{\text{Na}}^e, c_{\text{K}}^e, c_{\text{Ca}}^e, c_{\text{Na}}^i, c_{\text{K}}^i, c_{\text{Ca}}^i, c_{\text{Ca}}^{\text{up}}, c_{\text{Ca}}^{\text{rel}}], \quad (9)$$

defined in terms of their evolution equations,

$$\dot{c}_{\text{ion}} = \dot{c}_{\text{ion}}(\phi, g_{\text{gate}}, c_{\text{ion}}), \quad (10)$$

which are parameterized in terms of the transmembrane potential ϕ , the gating variables g_{gate} , and the ion concentrations c_{ion} themselves. In particular, c_{Na}^e , c_{K}^e , and c_{Ca}^e are the extracellular sodium, potassium, and calcium concentrations; c_{Na}^i , c_{K}^i , and c_{Ca}^i are the intracellular sodium, potassium, and calcium concentrations; and $c_{\text{Ca}}^{\text{up}}$ and $c_{\text{Ca}}^{\text{rel}}$ are the sarcoplasmic reticulum calcium uptake and release (see the Supporting Material for details about the cell model and its material parameters).

Mathematical model of action potential propagation

From an electrical point of view, light induces a channelrhodopsin current I_{ChR2} , which directly impacts the action potential propagation ϕ in the heart.

Unlike the local ion concentrations for single cells, however, the action potential is a global field variable (29). Its spatiotemporal evolution

$$\dot{\phi} = f^{\phi}(\phi, g_{\text{gate}}, c_{\text{ion}}) + \text{div } \mathbf{q}(\phi) \quad (11)$$

is driven by a local source term f^{ϕ} at the single cell level, and by a global flux term, $\text{div } \mathbf{q}$, the divergence of the propagation vector \mathbf{q} at the organ level. The local source term

$$f^{\phi} = -\frac{1}{C} [I_{\text{Na}} + I_{\text{bNa}} + I_{\text{f}} + I_{\text{NaCa}} + I_{\text{NaK}} + I_{\text{ChR2}} + I_{\text{K}} + I_{\text{bK}} + I_{\text{CaL}} + I_{\text{CaT}}] \quad (12)$$

is directly related to the negative sum of all transmembrane currents scaled by the individual cell membrane capacitance per unit surface area C . To account for the nonlocal nature of propagating excitation waves in the heart, we introduce the propagation vector

$$\mathbf{q} = \mathbf{D} \cdot \nabla \phi \quad (13)$$

through the second-order diffusion tensor \mathbf{D} scaling the gradient of the action potential field $\nabla \phi$ (see the Supporting Material for the finite-element-based solution of the action potential propagation problem, and see (27,29,30)).

RESULTS

Fig. 5 demonstrates our ability to stably transduce undifferentiated hESC with a ChR2-eYFP lentiviral vector. The resulting hESC^{ChR2+} remain pluripotent and can differentiate into hESC^{ChR2+}-CM. In Fig. 5 *a*, the PCR shows that hESC^{ChR2+} express the pluripotent Oct-4 gene (169 bp, lane 4) and Nanog gene (154 bp, lane 5) (blue box). In addition, amplification within the ChR2 gene (174 bp, lane 6), across the ChR2-eYFP gene (197 bp, lane 7), and within the eYFP gene (187 bp, lane 8), confirms stable transduction of the ChR2-eYFP lentivirus in undifferentiated hESC^{ChR2+} (yellow box). A ladder (100 bp, lane 1) confirms the predicted sizes of PCR products. Nontemplate control (lane 2) and GAPDH (152 bp, lane 3) serve as negative and positive controls, respectively. Fig. 5 *b* illustrates that pluripotent hESC^{ChR2+} is stain-positive for alkaline phosphatase (red). Fig. 5 *c* demonstrates the hESC^{ChR2+} positive eYFP signal upon fluorescence microscopy (green). Fig. 5 *d* confirms that hESC^{ChR2+}-CM have positive TnI signals (red), consistent with a CM phenotype. DAPI staining (blue), demonstrates the position of nuclei. Fig. 5 *e* illustrates transmission electron microscopy of hESC^{ChR2+}-CM showing sarcomeres with characteristic z-lines and mitochondria. Fig. 5 *f* shows the light microscopy of three hESC^{ChR2+}-CM colonies (dashed white circles) on a multi-electrode array.

Fig. 6 demonstrates the experimental and computational sensitivity of hESC^{ChR2}-CM with respect to different light intensities. With light on, the photocurrent I_{ChR2} increases rapidly, peaks, and decays toward a characteristic plateau value. With light off, the photocurrent I_{ChR2} drops rapidly and decays to zero. Fig. 6 (top) illustrates the varying light

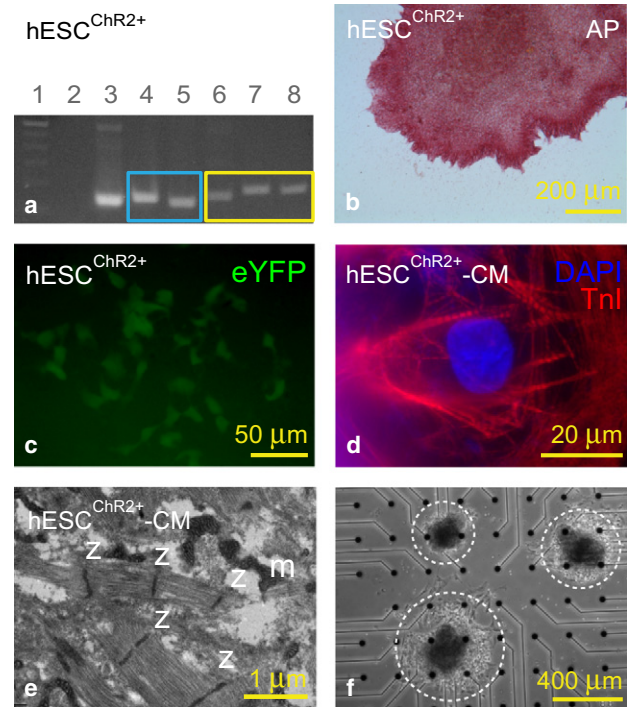


FIGURE 5 Undifferentiated human embryonic stem cells (hESC) stably transduced with a ChR2-eYFP lentiviral vector (hESC^{ChR2+}) remain pluripotent and can differentiate into cardiomyocytes (hESC^{ChR2+}-CM). (a) PCR shows that hESC^{ChR2+} express the pluripotent Oct-4 gene (169 bp, lane 4) and Nanog gene (154 bp, lane 5) (blue box). In addition, amplification within the ChR2 gene (174 bp, lane 6), across the ChR2-eYFP gene (197 bp, lane 7), and within the eYFP gene (187 bp, lane 8), confirms stable transduction of the ChR2-eYFP lentivirus in undifferentiated hESC^{ChR2+} (yellow box). A ladder (100 bp, lane 1) confirms the predicted sizes of PCR products. Nontemplate control (lane 2) and GAPDH (152 bp, lane 3) serve as negative and positive controls, respectively. (b) Pluripotent hESC^{ChR2+} stain is positive for alkaline phosphatase (red). (c) Fluorescence microscopy shows hESC^{ChR2+} has a positive eYFP signal (green). (d) hESC^{ChR2+}-CM have positive TnI signals (red), consistent with a CM phenotype. DAPI staining (blue) demonstrates the position of nuclei. (e) Transmission electron microscopy shows sarcomeres with associated z-lines (z) and mitochondria (m) in hESC^{ChR2+}-CM. (f) Light microscopy shows three hESC^{ChR2+}-CM colonies (dashed white circles) on a multi-electrode array.

intensities from 12.5% to 25%, 50%, and 100% for which we measure the photocurrent I_{ChR2} upon whole-cell voltage-clamp. Fig. 6 (middle) demonstrates the experimentally measured photocurrent I_{ChR2} , which increases with increasing light intensity. Fig. 6 (bottom) shows the calibrated computational cell model that captures the characteristic light sensitivity, displaying increased photocurrents I_{ChR2} with increased light intensity (see the Supporting Material).

Fig. 7 demonstrates the experimental and computational sensitivity of hESC^{ChR2}-CM with respect to different stimulation frequencies. Applied light stimulation at 100% intensity is illustrated (blue lines) at 100-ms pulse width. Experimentally measured electrical field potentials and mechanical contractions (black and red lines) and computationally predicted electrical transmembrane potentials are indicated

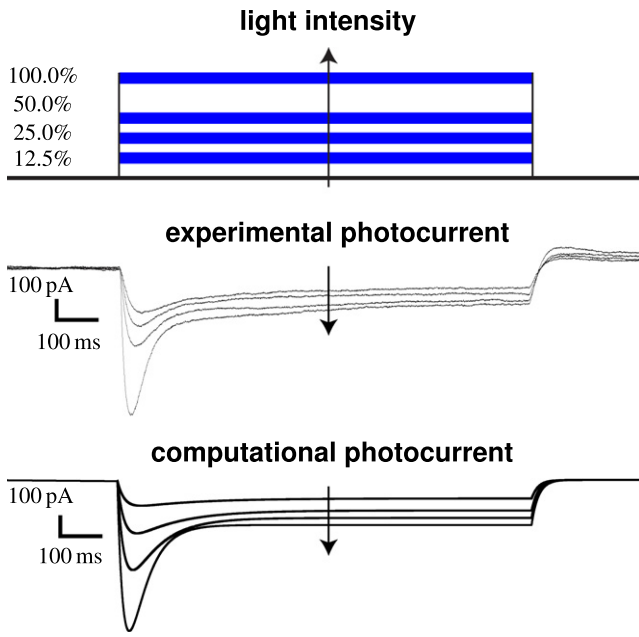


FIGURE 6 Experimental and computational sensitivity of hESC^{ChR2}-CM with respect to light intensity. With light on, the photocurrent I_{ChR2} increases rapidly, peaks, and decays toward a characteristic plateau value. With light off, the photocurrent I_{ChR2} drops rapidly and decays to zero. Light intensity is varied from 12.5% to 25%, 50%, and 100% (top). Whole-cell voltage-clamp reveals an increased photocurrent I_{ChR2} as the light intensity increases (middle). The computational hESC^{ChR2}-CM model captures the light sensitivity and displays increased photocurrents I_{ChR2} with increased light intensity (bottom).

(green lines). We probe the cells with light stimulation at 0.5 Hz (Fig. 7, top), 1.0 Hz (Fig. 7, middle), and 1.5 Hz (Fig. 7, bottom). Electrical and mechanical signals during light stimulation, shown in the center, are significantly different from pre- and poststimulation signals at all frequencies, shown at the beginning and end of the readings. The computational hESC^{ChR2}-CM model excellently captures the electrical signal both during light stimulation, and pre- and poststimulation. The two initial double spikes (see green curves) of the computational model paced at 0.5 Hz are caused by an interference of the photostimulation with the cell's natural frequency of 0.7 Hz. This interference is only present when cells are paced below their natural frequency, and does not occur during photostimulation at 1.0 Hz and 1.5 Hz.

Fig. 8 illustrates the potential of the proposed technology to virtually pace a human heart with light. The finite element model of the heart created from magnetic resonance images consists of 3129 nodes and 11,347 three-dimensional tetrahedral elements (31) (see the Supporting Material). Fig. 8 (top) shows the effects of atrioventricular (AV) node photostimulation initiated through hESC^{ChR2}-CM, which are virtually injected into the basal region of the septum, whereas all other regions are modeled as standard ventricular CM. For AV node pacing, the depolarization wave is initiated at the AV node, travels down the septum,

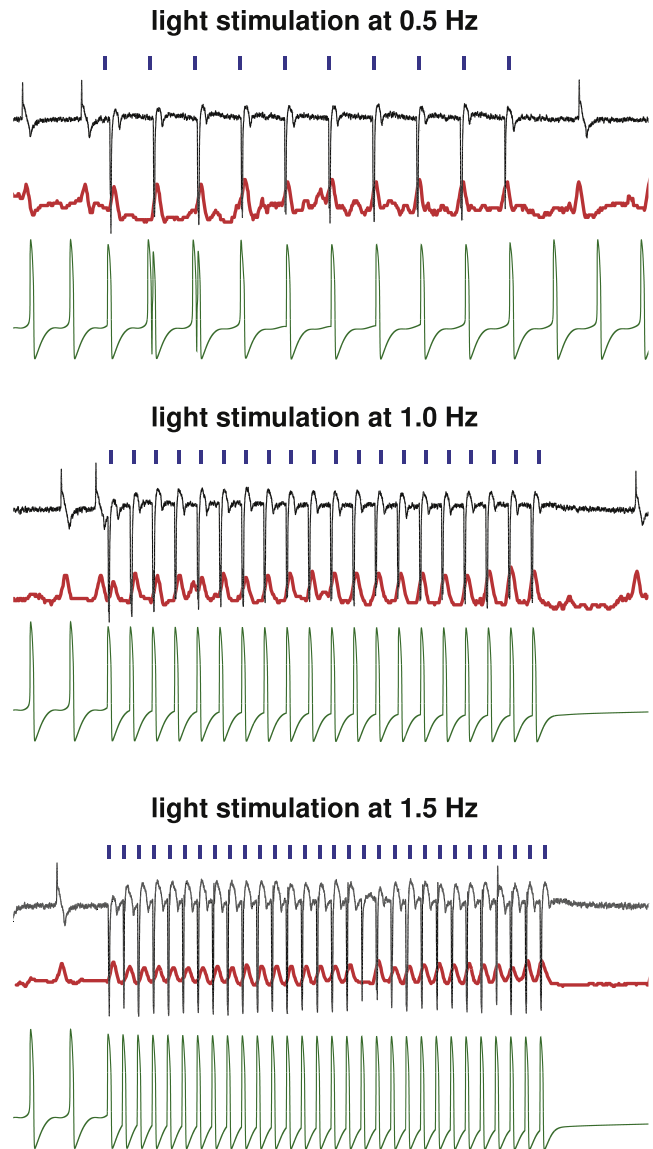
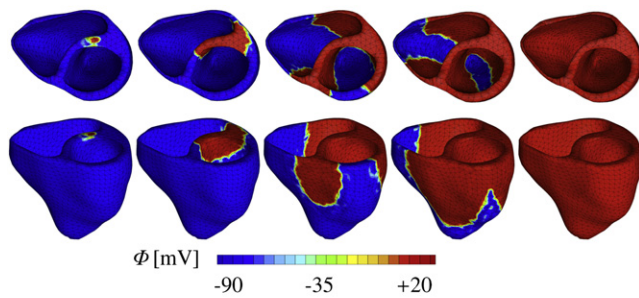


FIGURE 7 Experimental and computational sensitivity of hESC^{ChR2}-CM with respect to stimulation frequency. Light stimulation (blue) evokes field potentials (black) that translate into mechanical contractions (red). Light stimulation at 100% intensity is performed at 0.5 Hz (top), 1.0 Hz (middle), and 1.5 Hz (bottom). Evoked signals during light stimulation (center) are markedly different from pre- and poststimulation signals at all frequencies (left and right). The computational hESC^{ChR2}-CM model (green) captures the electrical signal at all frequencies, both during light stimulation (center), and pre- and poststimulation (left and right).

and then activates the left and right ventricles. Fig. 8 (bottom) shows the effects of biventricular photostimulation, initiated through hESC^{ChR2}-CM, which are virtually injected into the lateral wall of the left and right ventricles, whereas all other regions are modeled as standard ventricular CM. For biventricular photostimulation, the depolarization wave is initiated at the lateral left and right ventricular walls, travels along the ventricles, and then activates the apex and the septum. The color code indicates the

AV-node photostimulation of through hESC^{ChR2}-CM



bi-VP photostimulation through hESC^{ChR2}-CM

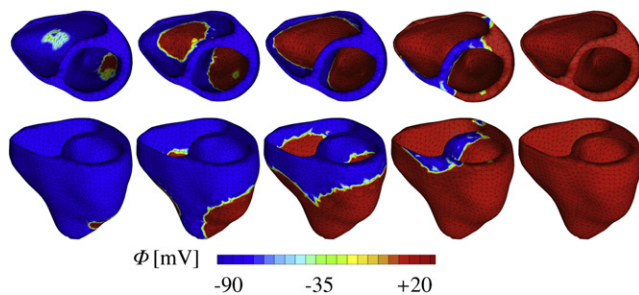


FIGURE 8 Virtual activation sequences of light-paced hearts. Atrioventricular node (*top*) and biventricular (*bottom*) photosimulations are initiated through hESC^{ChR2}-CM, virtually injected into the basal septum and into both lateral walls, respectively. All other regions are modeled as standard ventricular CM. The color code indicates the magnitude of the transmembrane potential ϕ varying from -90 mV (*blue*) to $+20$ mV (*red*).

magnitude of the transmembrane potential varying from -90 mV (*blue*) to $+20$ mV (*red*).

DISCUSSION

In Fig. 5, we have shown that ChR2 can be expressed stably and safely in hESC-CM to drive CM depolarization via photostimulation. Using a lentiviral vector (7,16), we have introduced ChR2 coupled to YFP into undifferentiated hESC. After confirming expression via immunocytochemistry, we have expanded these ChR2-expressing cells and demonstrated their pluripotency using PCR. Via directed differentiation, we have created hESC^{ChR2}-CM, which we have then subjected to optical stimulation. We have successfully recorded their electrical, biochemical, and mechanical signals using patch-clamping, MEA recordings, and video microscopy. These data have allowed us to calibrate our computational hESC^{ChR2}-CM model.

In Fig. 6, we have demonstrated the sensitivity of both experimental and computational photocurrents with respect to the stimulating light intensity (25). Both graphs illustrate the characteristic rapid increase to the intensity-dependent peak current, followed by a slower decrease toward the intensity-dependent asymptotic plateau value (32,33).

Although the translation of optogenetic techniques into clinical practice may still have many technical hurdles to pass, the technology itself can already serve as a valuable research tool in cardiac electrophysiology. Traditional tools based on the simultaneous use of electrical stimulation and electrical recording typically suffer from unavoidable artifacts (34). The inherent orthogonality of optical and electrical techniques allows us to significantly reduce these spurious errors when using optical stimulation combined with electrical recordings, as shown in Fig. 7.

A tremendous potential of opsin-based systems for optical manipulation lies in their inherent ability to not only turn-cells-on using channelrhodopsin as a blue-light-gated ion channel transporting positively charged cations along their concentration gradients (9), but also to turn-cells-off using halorhodopsin as a yellow-light-driving ion pump transporting negatively charged chloride ions against their concentration gradients (10). A promising first study in zebrafish has shown that a combination of channelrhodopsin and halorhodopsin allows for optically controlling heart rate, reversing cardiac conduction, and inducing disease-like arrhythmias (14).

Computational modeling allows us to predict the response of living cells, both in isolation and in interaction with their environment. In Fig. 8, we have demonstrated our very first proof-of-principle, using finite element modeling in an attempt to bridge the scales from cells to systems (35). Finite-element-based models allow us to combine virtually any cell type (36) on virtually any geometry (37). Here, they allow us to predict the activation sequences in the human heart for different pacing sites (38).

Pacing hearts in silico by means of photostimulation will allow us to virtually probe different scenarios (39,40) toward our ultimate goal of light pacing hearts in vivo (14). In contrast to electrical pacemakers, light delivery is minimally invasive, genetically targeted, and temporally precise. Most importantly, light can be delivered at a distance. Unlike pacing leads for electrical stimulation, which are known to have a high failure rate due to mechanical fatigue (41), the light source for optical stimulation does not have to sit directly on the continuously moving heart muscle. Light pacing might therefore be an attractive remote, less invasive, and more durable alternative to current electrical pacing leads (42).

Limitations

This study presents our first attempts toward pacing the heart with light using a multiscale approach. On the photocycle level (4), we have adapted a classic three-state model for the ChR2 photocycle, which was initially proposed for ChR2-expressing neurons. This conceptually elegant model has allowed us to reliably reproduce the characteristic features of a light-evoked response in ChR2-expressing hESC-CM. However, the extension to a four-state (24) or

five-state (43) model, either of which is known to reproduce the biexponential decay of the light-off current more accurately (23), is conceptually straightforward. In terms of photostimulation, we have applied pulse widths of 100 and 1000 ms. These relatively long durations of channel opening may increase sodium overload and adversely influence action potential profiles. Because the rapid upstroke of the action potential takes place in the first 10 ms (26), the stimulation pulse width could potentially be decreased to 10 ms or less (O. Abilez, J. A. Baugh, M. L. Gorrepati, R. Prakash, C. Lee-Messer, M. Huang, F. Jia, J. Yu, K. D. Wilson, J. C. Wu, K. Deisseroth, and C. K. Zarins, unpublished), which would favorably limit light exposure time (see the [Supporting Material](#)). Novel developments in ultrafast optogenetics suggest using the rationally engineered channelrhodopsin ChETA, which provides high-fidelity optical control of spiking at high frequencies and eliminates plateau potentials during continued stimulation (44).

On the ion channel level, we have assumed that the ChR2 current is driven exclusively by concentration gradients in the sodium concentration (24). Accordingly, we have modeled ChR2 to be selectively permeable to sodium ions only. Although ChR2 is known to be a general cation channel (12), its effects on potassium and calcium remain poorly characterized (45). However, making the channel permeable to other monovalent and divalent cations (4), would require only modular changes in the mathematical model.

On the cellular level, due to the lack of mathematical models for stem cell-derived cardiac cells, we have adopted a widely used and well-characterized model for mature cardiac cells (26,28). A recent study confirmed that ChR2 expresses its characteristic features independent of the particular expression system (45). Therefore, we have modified the mature cell model via an additional photocurrent governed by a light-sensitive gating variable. A thorough identification of the individual channel characteristics of hESC-CM and their quantitative comparison with mature CM and hESC^{ChR2+}-CM remain to be addressed to fully validate our conceptual approach (21).

On the whole-heart level, the simulation of the light-paced heart is admittedly relatively simplistic. Although our algorithm can, in principle, handle arbitrary mixtures of different cell types (36), here, we have assumed that the injected cells are pure hESC^{ChR2+}-CM. We have adopted a common procedure to model cell injection (37), which does not address additional obstacles commonly associated with cell delivery such as cell migration away from the injection site or cell survival in the myocardial wall. At this stage, our model also fails to appropriately predict the effects of light scattering by tissue and absorption by blood. Light scattering is a general barrier to in vivo translation, and efforts are underway by our groups and others to red-shift the opsins and maximize the efficiency of light delivery through tissues (15,46).

CONCLUSION

At an unprecedented temporal and spatial precision, optogenetic tools now enable us to manipulate electrically active cells. This study capitalizes on recent developments in optics and genetics, supplemented by novel technologies in stem cell biology, electrophysiology, and computational mechanics. It documents our first attempts to introduce a light-sensitive ion channel in human-embryonic-stem-cell-derived cardiomyocytes, with the ultimate goal to control the cardiac system by means of photostimulation. Unlike traditional electrical stimulation, optogenetics allows us to precisely control the selective permeability of the plasma membrane, its conductivity with respect to different ions, its sensitivity to light of different wavelengths, and the spatiotemporal evolution of different opening and closing profiles. Given this incredible freedom, we need to establish an economical strategy to optimize the matrix of input variables. Predictive computational models allow us to virtually probe landscapes of process parameters and identify optimal photostimulation sequences in various different tissues and organs. Here, as a very first proof-of-principle, we virtually inject photosensitive cells into different locations of a human heart model, and pace the heart with light. We believe that this concept will be widely applicable to systematically manipulate electrically active cells and, ultimately, support the design of novel therapies for various types of neuronal, musculoskeletal, pancreatic, and cardiac disorders such as depression, schizophrenia, cerebral palsy, paralysis, diabetes, pain syndromes, and cardiac arrhythmias.

SUPPORTING MATERIAL

Supporting equations, two tables, and three figures are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(11\)00943-X](http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)00943-X).

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