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Molecular motions that shape the cardiac action potential: Insights from voltage clamp fluorometry

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ABSTRACT

Very recently, voltage-clamp fluorometry (VCF) protocols have been developed to observe the membrane proteins responsible for carrying the ventricular ionic currents that form the action potential (AP), including those carried by the cardiac Na^+ channel, $Na_V1.5$, the L-type Ca^{2+} channel, $Ca_V1.2$, the Na^+/K^+ ATPase, and the rapid and slow components of the delayed rectifier, $K_V11.1$ and $K_V7.1$. This development is significant, because VCF enables simultaneous observation of ionic current kinetics with conformational changes occurring within specific channel domains. The ability gained from VCF, to connect nanoscale molecular movement to ion channel function has revealed how the voltage-sensing domains (VSDs) control ion flux through channel pores, mechanisms of post-translational regulation and the molecular pathology of inherited mutations. In the future, we expect that this data will be of great use for the creation of multi-scale computational AP models that explicitly represent ion channel conformations, connecting molecular, cell and tissue electrophysiology. Here, we review the VCF protocol, recent results, and discuss potential future developments, including potential use of these experimental findings to create novel computational models.

1. Introduction to voltage-clamp fluorometry

Nanoscale movements within cardiac ion channels fundamentally determine cardiac action potential (AP) dynamics (Hille, 2001). Genetic mutations (Shah et al., 2005), post-translational modification (Hund and Mohler, 2014), and drug binding (Heist and Ruskin, 2010) perturb these movements to alter channel function, causing or preventing deadly cardiac arrhythmias. Despite much progress in understanding how channels sense voltage and selectively allow ions to cross into and out of the cell, the conformational changes that determine AP dynamics are not well-defined. A method known as voltage-clamp fluorometry (VCF) allows simultaneous observation of changes in channel conformation and ionic current kinetics, and very recently, VCF protocols have been established for major cardiac ionic currents including the cardiac Na^+ current (I_{Na}), the L-type Ca^{2+} current ($I_{Ca,L}$), the rapid and slow components of the delayed rectifier K^+ currents (I_{Kr} and I_{Ks}), and the Na^+/K^+ ATPase (I_{NaK}). Here, we will review the VCF protocol, recent results, and how these findings might be incorporated into computational models to better understand how the nanoscale movements of cardiac ion channels determine the dynamics of myocardial excitation.

1.1. The VCF protocol

Cardiac ion channels are typically formed by homologous subunits or domains that comprise six transmembrane-spanning segments (S1–S6). The first four segments (S1–S4) form the voltage-sensing domain (VSD), and segments S5–S6 form the channel pore (Ganetzky et al., 1999; Gellens et al., 1992; Itoh et al., 1998; Long et al., 2005; Qin et al., 2002). Within the VSD, the S4 segment contains positively charged amino acids that respond to changes in the membrane potential to control pore conformation through the S4–S5 linker (Aggarwal and MacKinnon, 1996; Bezanilla, 2008; Liman et al., 1991; Seoh et al., 1996). VCF was first used to observe the prototypical *Shaker* K^+ channel VSD with a fluorophore tethered to the S4 segment (Mannuzzu et al., 1996). As the environment surrounding the S4 was altered by VSD movement in response to changes in membrane potential, the fluorescence emission from the tethered fluorescent molecule was also altered

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(Fig. 1A). Thus, the change in fluorescence emission could be used to observe the voltage-dependent kinetics of the VSD.

Fluorophore tethering for VCF is typically accomplished by introducing a cysteine residue into the area of interest and labeling it with a thiol-reactive fluorophore. Native cysteines are often removed in order increase the specificity of labeling and reduce background fluorescence (Gandhi and Olcese, 2008). To track VSD conformation, the fluorophore is usually conjugated to a cysteine into the S3–S4 linker (Fig. 1A). The fluorescence signal created by the change in channel conformation is usually quite small, so large numbers of channels must be expressed to observe a useful signal. The *Xenopus* oocyte is often used as it is well-known to express large numbers of channels from mRNA that is injected into the cytoplasm. The oocyte also has a dark pigmented layer directly beneath the membrane of the animal pole, which eliminates most of auto-fluorescence from cell, allowing for resolvable fluorescence signals (Gandhi and Olcese, 2008). In mammalian cells, the whole-cell patch clamp configuration has been combined with semi-confocal epifluorescence microscopy to observe Shaker K^+ channel conformations (Blunck et al., 2004). However, cardiac channels have not been successfully studied using this method. One challenge to studying these channels is much lower channel density, in

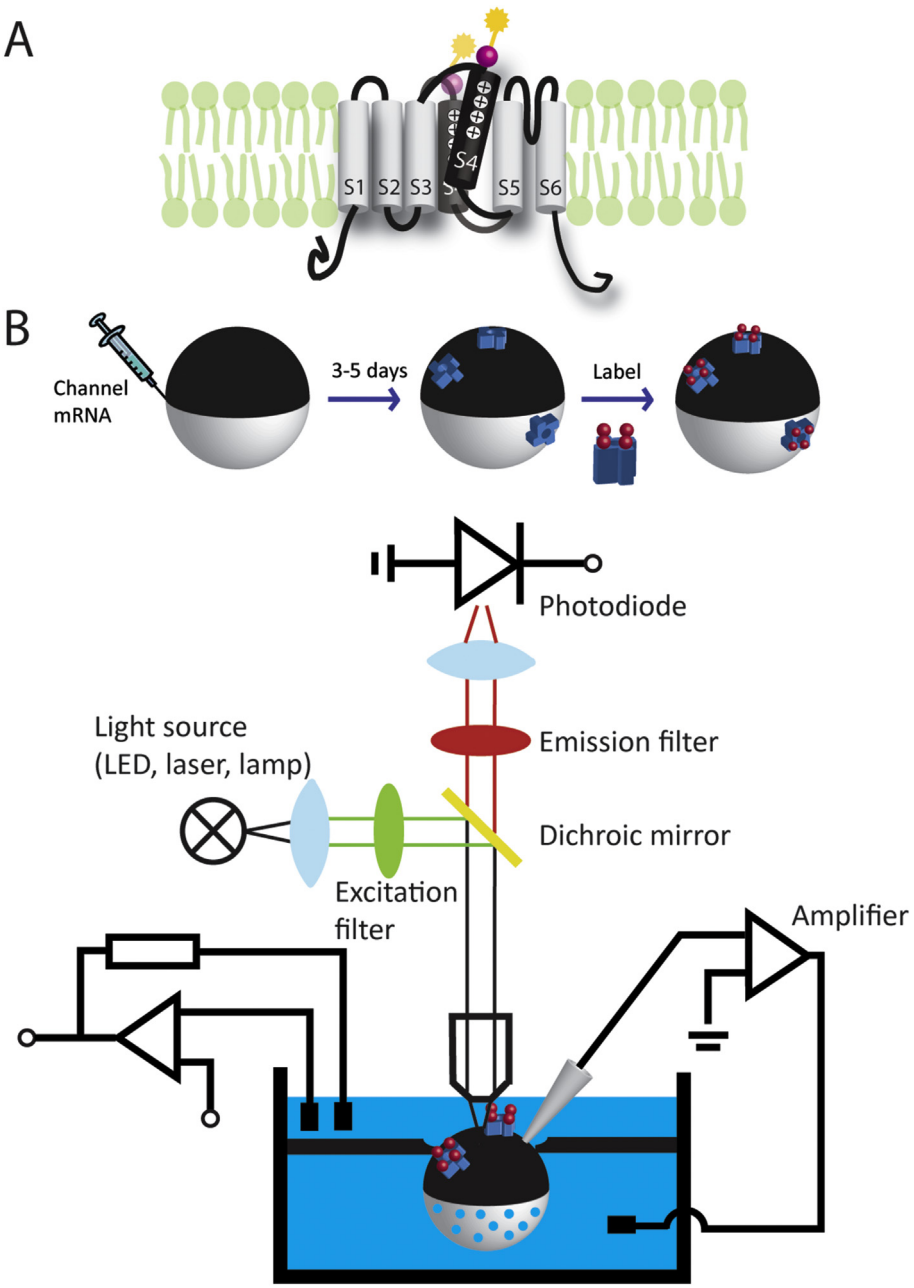

Fig. 1. A) K_v channel VCF construct under resting (transparent) and activated (solid) states. The pink dot represents the engineered cysteine residue and yellow star denotes the tethered fluorophore. The movement of the S4 segment causes fluorophore displacement. As a result of quenching by surrounding residues, and changes in fluorophore environment, fluorescence emission is altered. B) Above: mRNA encoding VCF channel constructs are injected into *Xenopus* oocytes. Channels express at high levels after several days. Channels are then labeled with a fluorophore, which conjugates to the introduced cysteine residues. Below: An example highlighting cut-open oocyte recording, which allows resolution of fast kinetics by clamping a small membrane patch. The upper chamber filled with external solution is clamped to the command voltages, while the bottom channel filled with internal solution is connected to ground. Membrane currents are measured using the electrode filled with 3 M KCl. Fluorescence emission is separated from excitation light by a dichroic mirror and filtered by the emission filter. Finally, it is collected with a photodiode that is connected to a low noise current amplifier.

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