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# Rapid Cellular Phenotyping of Human Pluripotent Stem Cell-Derived Cardiomyocytes using a Genetically Encoded Fluorescent Voltage Sensor

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## SUMMARY

In addition to their promise in regenerative medicine, pluripotent stem cells have proved to be faithful models of many human diseases. In particular, patient-specific stem cell-derived cardiomyocytes recapitulate key features of several life-threatening cardiac arrhythmia syndromes. For both modeling and regenerative approaches, phenotyping of stem cell-derived tissues is critical. Cellular phenotyping has largely relied upon expression of lineage markers rather than physiologic attributes. This is especially true for cardiomyocytes, in part because electrophysiological recordings are labor intensive. Likewise, most optical voltage indicators suffer from phototoxicity, which damages cells and degrades signal quality. Here we present the use of a genetically encoded fluorescent voltage indicator, ArcLight, which we demonstrate can faithfully report transmembrane potentials in human stem cell-derived cardiomyocytes. We demonstrate the application of this fluorescent sensor in high-throughput, serial phenotyping of differentiating cardiomyocyte populations and in screening for drug-induced cardiotoxicity.

## INTRODUCTION

A major advance in disease modeling has been the demonstration that human induced pluripotent stem cell technology can faithfully recapitulate many human diseases, including cardiac arrhythmia syndromes (Kim et al., 2013; Matsa et al., 2011; Roden and Hong, 2013). For cardiac diseases, there is interest in applying these models to large-scale screens for novel therapeutics or drug toxicities; however, efforts have been frustrated by the labor-intensive nature of conventional electrophysiological recordings (Mercola et al., 2013). Optical mapping with voltage indicators is an alternative, yet voltage-sensitive dyes, such as ANEPPS dyes, have inherent phototoxicity that limits the recording time and can degrade signal quality (Herron et al., 2012).

The field of genetically encoded voltage probes is rapidly evolving. Arch(D95N) (Kralj et al., 2012) is photostable and highly voltage sensitive, but in absolute terms is 100–1,000 times dimmer than GFP, necessitating the use of advanced imaging techniques. More recently, a novel voltage indicator, ArcLight, was developed by fusing the voltage-sensing domain of the *Ciona intestinalis* voltage-sensitive phosphatase to a super ecliptic pHluorin carrying the point mutation A227D (Cao et al., 2013; Jin et al., 2012).

Although ArcLight probes have limited frequency responses, they still may be suitable for monitoring cardiac action potentials (APs). Here, we demonstrate the use of A242-ArcLight (ArcLight) to noninvasively report APs from human embryonic stem cell-derived cardiomyocytes (hESC-CMs). We demonstrate the utility of ArcLight for characterizing hESC-CMs by reporting AP durations (APDs) over a broad range. In addition, ArcLight allows accurate recognition of early afterdepolarizations (EADs), as well as CM subtypes. Comparison with simultaneous patch-clamp recordings reveals consistent but relatively small errors due to the temporal response of this protein voltage reporter.

## RESULTS

### Fluorescence Measurement of APs in hESC-CMs

ArcLight expression in H7 hESC-CMs resulted in robust optical signals (Figure 1A) that were visible to the naked eye even in single isolated cells (Movie S1 available online). AP morphology, a functional marker of CM subtype, was readily recognized, permitting identification of atrial, nodal, and ventricular AP morphologies (Figure 1B). Fluorescent signals were remarkably photostable, permitting prolonged recording over minutes of continuous illumination (Figures S1A and S1B). In contrast, recordings from di-8-ANEPPS-loaded hESC-CMs displayed phototoxicity after several gated 6 s recordings (Figure S1C). Compared with patch-clamp electrophysiology, fluorescence data were rapidly acquired and APs from multiple cells in a single field could be simultaneously recorded (Figure 1C), permitting recordings from over 440 single hESC-CMs and small groups (<5 cells) in 1 day. This throughput allowed characterization of large cell populations by APD and morphology (Figures S1D–S1F).

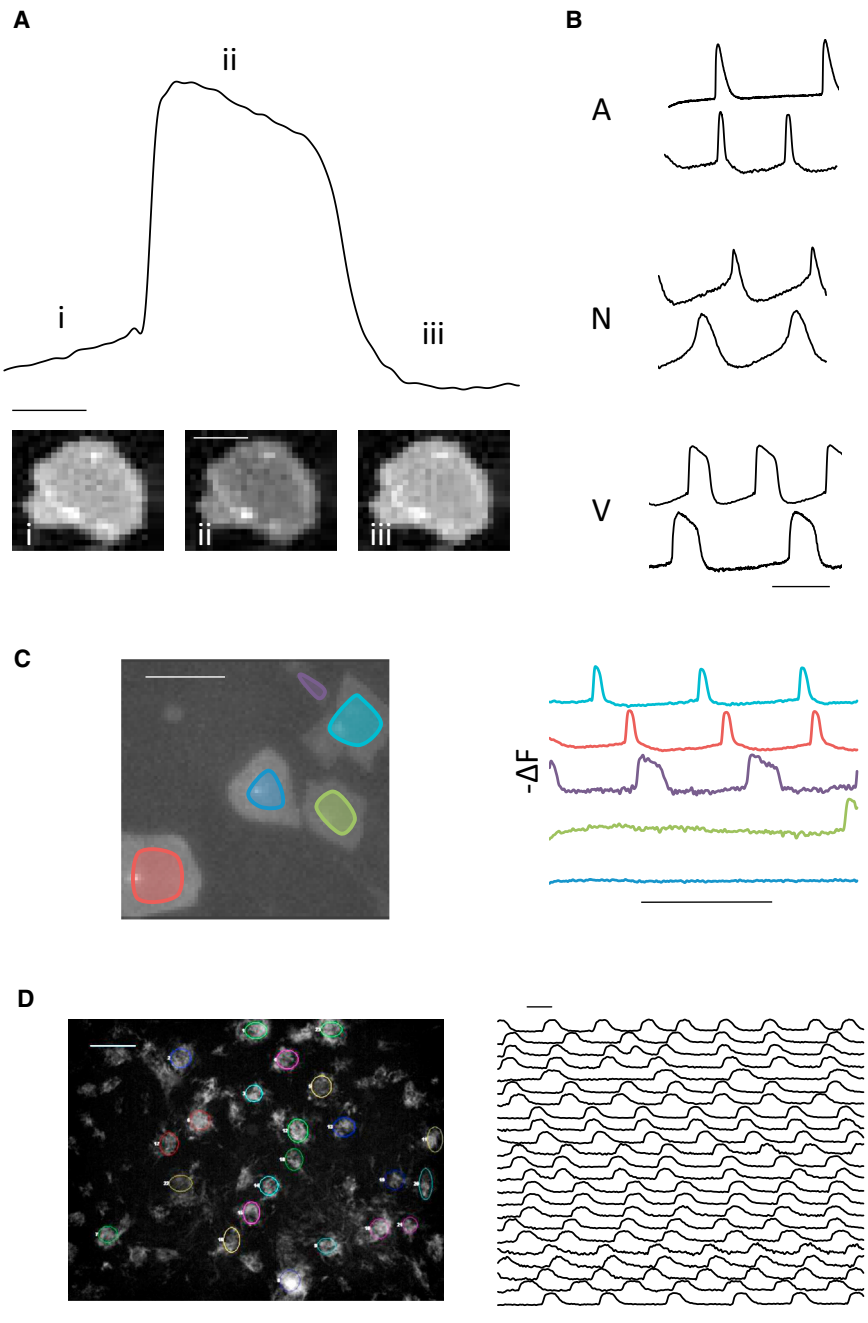


**Figure 1. Rapid and Simultaneous Recording of APs in hESC-CMs**

(A) Representative ArcLight fluorescence tracing from a single isolated hESC-CM with three images corresponding to (i) phase 4, (ii) plateau phase 2, and (iii) phase 4. Trace bar equals 200 ms. Scale bar, 50 μm (image).

(B) Representative fluorescence traces depicting typical atrial, nodal, and ventricular AP morphologies. Scale bar, 1 s.

(C) Still-frame image of several single ArcLight expressing hESC-CMs and corresponding simultaneous fluorescence traces. Scale bars, 100 μm (image) and 2 s (trace).

(D) Differentiating clusters of ArcLight-expressing hESC-CMs on day 19 with selected fluorescence recordings. Scale bars, 400 μm (image) and 500 ms (trace). See also Figure S1.

In addition to direct lentiviral transduction of differentiated CMs, we also introduced the ArcLight construct at the ESC stage, with a mean proviral integration of ~7.5 copies/cell. Under a CAG promoter, ArcLight expression rose upon cardiac differentiation within the transduced population as well as in subsequently derived clonal lines, and persisted through months of culture (Orbán et al., 2009). This enabled serial monitoring of composite APs during CM differentiation while preserving the integrity and sterility of the cell culture (Figure 1D; Movie S2).

## Accuracy in Reporting Transmembrane Potentials

We compared ArcLight fluorescence with simultaneous patch-clamp recordings in hESC-CMs (Figure 2A). The morphological characteristics of cardiac APs were well resolved, albeit with some loss of high-frequency components. Fluorescent measurements of $APD_{90}$ showed a linear correlation with simultaneous current-clamp values with a consistent overestimation of ~62 ms over a broad range of APDs ($R^2$ = 0.86; Figure 2B). Likewise, optical measurements of $APD_{90}$ also correlated closely to AP voltage-clamp