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# *In vivo* cardiac nano-imaging: A new technology for high-precision analyses of sarcomere dynamics in the heart

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ABSTRACT

The cardiac pump function is a result of a rise in intracellular $Ca^{2+}$ and the ensuing sarcomeric contractions [i.e., excitation-contraction (EC) coupling] in myocytes in various locations of the heart. In order to elucidate the heart's mechanical properties under various settings, cardiac imaging is widely performed in today's clinical as well as experimental cardiology by using echocardiogram, magnetic resonance imaging and computed tomography. However, because these common techniques detect local myocardial movements at a spatial resolution of ~100 μm, our knowledge on the sub-cellular mechanisms of the physiology and pathophysiology of the heart *in vivo* is limited. This is because (1) EC coupling occurs in the μm partition in a myocyte and (2) cardiac sarcomeres generate active force upon a length change of ~100 nm on a beat-to-beat basis. Recent advances in optical technologies have enabled measurements of intracellular $Ca^{2+}$ dynamics and sarcomere length displacements at high spatial and temporal resolution in the beating heart of living rodents. Future studies with these technologies are warranted to open a new era in cardiac research.



**Contents**



*Abbreviations:* $[Ca^{2+}]_i$, Intracellular $Ca^{2+}$ concentration; CaT, Calcium transient; EC coupling, Excitation-contraction coupling; ECG, Electrocardiogram; EMCCD, Electron-multiplying charge-coupled device; LV, Left ventricle; LVP, Left ventricular pressure; RyR, Ryanodine receptor; SL, Sarcomere length.

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## 1. Introduction

A change in the length of myocardial sarcomeres of merely ~100 nm dramatically changes the heart's pump functions (the *Frank-Starling Law*; Allen and Kentish, 1985; Kentish et al., 1986; Katz, 2002 and references therein). Though numerous studies have been conducted to determine how the cardiac pump function is fine-tuned via modulation of single sarcomere dynamics in cardiomyocytes, in response to intracellular $Ca^{2+}$ transients (CaT), the mechanism is not fully understood at the sub-cellular level.

Since the birth of single molecular biophysics, optical fluorescence technologies have greatly advanced, and various imaging systems have been developed and applied for visualization of movements of various proteins *in vitro* (Funatsu et al., 1995; Sase et al., 1995; Vale et al., 1996). Given, however, that the chemical conditions markedly differ between the experimental solution and cytosol (e.g., ionic strength, temperature and viscosity), the properties of proteins obtained *in vitro* may not directly reflect the molecular functions in living cells. To our knowledge, Sako and colleagues (Sako et al., 2000) were the first to perform single molecular imaging in living cells (i.e., epidermal growth factor receptor signaling). Following their publication, various groups conducted studies on receptor functions in cultured cells.

The optical fluorescence technologies, based on single molecular biophysics, have been applied to cardiac physiology and indeed have enabled measurements of sarcomere length (SL), as well as the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), at high spatial and temporal resolution in isolated/cultured cardiomyocytes and in the heart, dramatically enhancing our understanding of excitation-contraction (EC) coupling under true physiologic conditions. In this article, we review recent advances in *in vivo* cardiac nano-imaging technologies.

## 2. Intracellular $Ca^{2+}$ regulation and the thin filament "*on-off*" switching mechanism

First, we briefly summarize the mechanisms of contraction and relaxation in cardiac muscle. Contraction/relaxation is regulated by micromolar concentrations of intracellular $Ca^{2+}$ (Bers, 2001, 2002; Prosser et al., 2010 and references therein). When the sarcolemma is depolarized via opening of $Na^+$ channels, $Ca^{2+}$ enters the myocyte through L-type $Ca^{2+}$ channels (Fig. 1). The $Ca^{2+}$ does not directly activate myofilaments, but $Ca^{2+}$ is effectively released from the $Ca^{2+}$ release channels (i.e., ryanodine receptors, RyR) of the sarcoplasmic reticulum (SR). Therefore, the $Ca^{2+}$ concentration is inhomogeneous in the cytosol in that it is highest at the T-tubules (at which the SR $Ca^{2+}$ release channels are present) as well as at the Z-disks, and lowest in the middle of the thick filament (Bers, 2001, 2002; Prosser et al., 2010 and references therein). On average, $[Ca^{2+}]_i$ is partially increased even at the peak of systole in the cytosol of cardiomyocytes.

The troponin (Tn)-tropomyosin (Tm) complex on the thin filament plays a key role in determining the state of the cardiac sarcomere, depending on $[Ca^{2+}]_i$ around the thin filament. Tn is composed of troponin C (TnC), troponin I (TnI) and troponin T (TnT) (Solaro and Rarick, 1998; Fukuda et al., 2009; Kobirumaki-Shimozawa et al., 2014 and references therein). Two metal binding sites exist in the C-terminal domain of TnC that bind both $Mg^{2+}$ and $Ca^{2+}$ with relatively high affinity. Because $Mg^{2+}$ exists at ~1 mM (Solaro and Rarick, 1998; Fukuda et al., 2009; Kobirumaki-Shimozawa et al., 2014 and references therein), these sites are normally occupied by $Mg^{2+}$ under normal physiological conditions. In contrast to fast skeletal TnC that has two regulatory $Ca^{2+}$-binding sites with relatively low affinity in the N-terminal domain of TnC, cardiac TnC has actually only one $Ca^{2+}$-binding site. When $[Ca^{2+}]_i$ increases from diastolic ~0.1 μM to systolic ~1.0 μM, $Ca^{2+}$ binds to the single regulatory $Ca^{2+}$-binding site, resulting in the onset of the conformational change of the thin filament (Takeda et al., 2003). During diastole, the C-terminal domain of TnI binds to actin, and Tm blocks the actomyosin interaction (i.e., "*off*" state). When $Ca^{2+}$ binds to the regulatory $Ca^{2+}$-binding site of TnC during systole, the C-terminal domain of TnI is dissociated from actin, and, then, binds to the N-terminal domain of TnC, as a result of the enhanced TnC-TnI interaction (i.e., "*on*" state).

## 3. Significance of SL in cardiac contractility

In 1982, Allen and Kurihara reported that the length dependence of isometric twitch force of mammalian cardiac muscle is composed of two phases (Allen and Kurihara, 1982); viz., the rapid, first phase that occurs independent of $[Ca^{2+}]_i$ based on sarcomeric activation, and the second phase that is coupled with a rise of $[Ca^{2+}]_i$ via $Ca^{2+}$ release from the SR. Provided that the *Law* operates on a beat-to-beat basis, the first phase is likely to underlie physiologic length-dependent activation. Later, Kentish et al. (1986) demonstrated that the production of active force of the cardiac sarcomere is highly dependent on its length, with or without the skinning treatment, in which a change in SL of only ~100 nm enhances active force production, especially at physiological partial activation states (Fig. 2A; see also SL-active force curves in Fukuda et al., 2001).

Length-dependent activation is most pronounced at physiologic partial activation (Kobirumaki-Shimozawa et al., 2014); viz., the phenomenon is dependent on the "*on-off*" equilibrium of the thin filament state. For instance, when the thin filament "*on-off*" equilibrium is shifted toward the "*on*" state upon an increase in the fraction of strongly bound cross-bridges, the length-dependent activation becomes attenuated as a result of a decrease in the fraction of "*recruitable*" cross-bridges that have ATP (Fitzsimons and Moss, 1998; Terui et al., 2008, 2010). Conversely, when the thin filament "*on-off*" equilibrium is shifted toward the "*off*" state, length-dependent activation becomes attenuated, because myosin attachment is depressed due to reduced thin filament cooperative activation (Fig. 2B; see also Inoue et al., 2013).

Then, a question arises as to the triggering factor that induces length-dependent activation at the sarcomere level. Currently, the general consensus has been that cardiac length-dependent activation results from an increase in the fraction of cross-bridges coupled with a reduction in interfilament lattice spacing (i.e., the spacing between the thick and thin filaments; Fig. 2C) (e.g., McDonald and Moss, 1995; Fuchs and Wang, 1996; Fukuda et al., 2000, 2003, 2005; Cazorla et al., 2001). Indeed, osmotic compression caused by dextran (MW: 500,000) reduces the lattice spacing and increases contractile force ($Ca^{2+}$ sensitivity: e.g., McDonald and Moss, 1995; Fuchs and Wang, 1996; Fukuda et al., 2000), and the