Cardiac cell as a calcium oscillator – theoretical model for enzyme-mediated noise reduction

To explain how changes in enzyme activity can regulate noise in a biochemical network, it is instructive to begin with a 'thought experiment'. Consider a reversible reaction between two states, for example the phosphorylated and dephosphorylated states of RyR. For simplicity, let us think about this reaction as a simple isomerization reaction, i.e., $RyR_{NP} \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} RyR_P$ (Fig. S8a). k_{on} and k_{off} are the rate constants for phosphorylation/dephosphorylation and will therefore depend on the concentration of kinase/phosphatase respectively.

Phosphorylation (same for dephosphorylation) is usually treated within the Michaelis-Menten framework, $\frac{d[RyR_P]}{dt} = k_{cat}[E]_0 \frac{[RyR_{NP}]}{K_M + [RyR_{NP}]}$, where $[E]_0$, k_{cat}, and K_M are the enzyme concentration, catalyst rate constant and Michaelis-Menten constant respectively. We can take the full dependence of the phosphorylation rate on the RyR channel concentration into account, but it is not necessary for our 'thought experiment'.

Increased activity of both the kinase and phosphatase will multiply k_{on} and k_{off} by an identical factor. Therefore, in this case, the ratio k_{on}/k_{off} will be the same and at equilibrium the fraction of phosphorylated *RyR*, $[RyR_P]/\left([RyR]_{NP} + [RyR]_P\right)$, as well as the probability distribution of the phosphorylated channels will not change. However, increased k_{on} and k_{off} will lead to faster isomerization kinetics. Fig.S7b shows the time evolution of the fraction of RyR_P, for two sets of kinetics constants ($k_{on} = k_{off} = 2.5\,\mathrm{sec}^{-1}$ for the 'slow' data set, and $k_{on} = k_{off} = 100\,\mathrm{sec}^{-1}$ for the 'fast' data set). The stochastic kinetics of the system was followed using the stochastic simulation algorithm (SSA) developed by Gillespie (Gillespie, 1976, Gillespie, 1977), which is described in

the next subsection. As clearly shown in Fig.S8, for the faster kinetics, the transition between the two states is accelerated. Suppose now that we measure the average fraction of phosphorylated channels in 'sampling windows' of size Δt (In Fig.S8b, Δt=1sec). If the kinetics is faster than Δt, ($1/k_{on}$, $1/k_{off}$ << Δt) , during the time interval, Δt, the channel will go back and forth between phosphorylated and dephosphorylated states, essentially sampling the full equilibrium distribution within each 'sampling window'. In this case, the average fraction measured in every 'sampling window' is nearly identical (Fig.S8b-c). In contrast, if the kinetics is slower than (or comparable to) Δt, the average fraction measured in each 'sampling window' will be different, as the time interval Δt does not allow enough time for the channels to convert between the two states. The width of the distribution of measured average fraction values (Fig.S8c) is the effective noise in the system. Faster kinetics, therefore, reduces system noise as measured by the average fraction of RyR_P at discrete sampling windows. The frequency of calcium oscillations is determined by the kinetics of RyR channels which depends on their phosphorylation state. The cell essentially 'samples' the RyR_P distribution and therefore faster kinetics which leads to low variability in the fraction of phosphorylated channels will reduce beat-to-beat variability. In order to demonstrate, that noise reduction by increased enzyme activity may occur through cardiac cells 'sampling' of phosphorylated RyR channel distribution, we conducted a stochastic kinetic simulation of a cardiac cell as described in detail below.

Simplified kinetic model for calcium oscillations

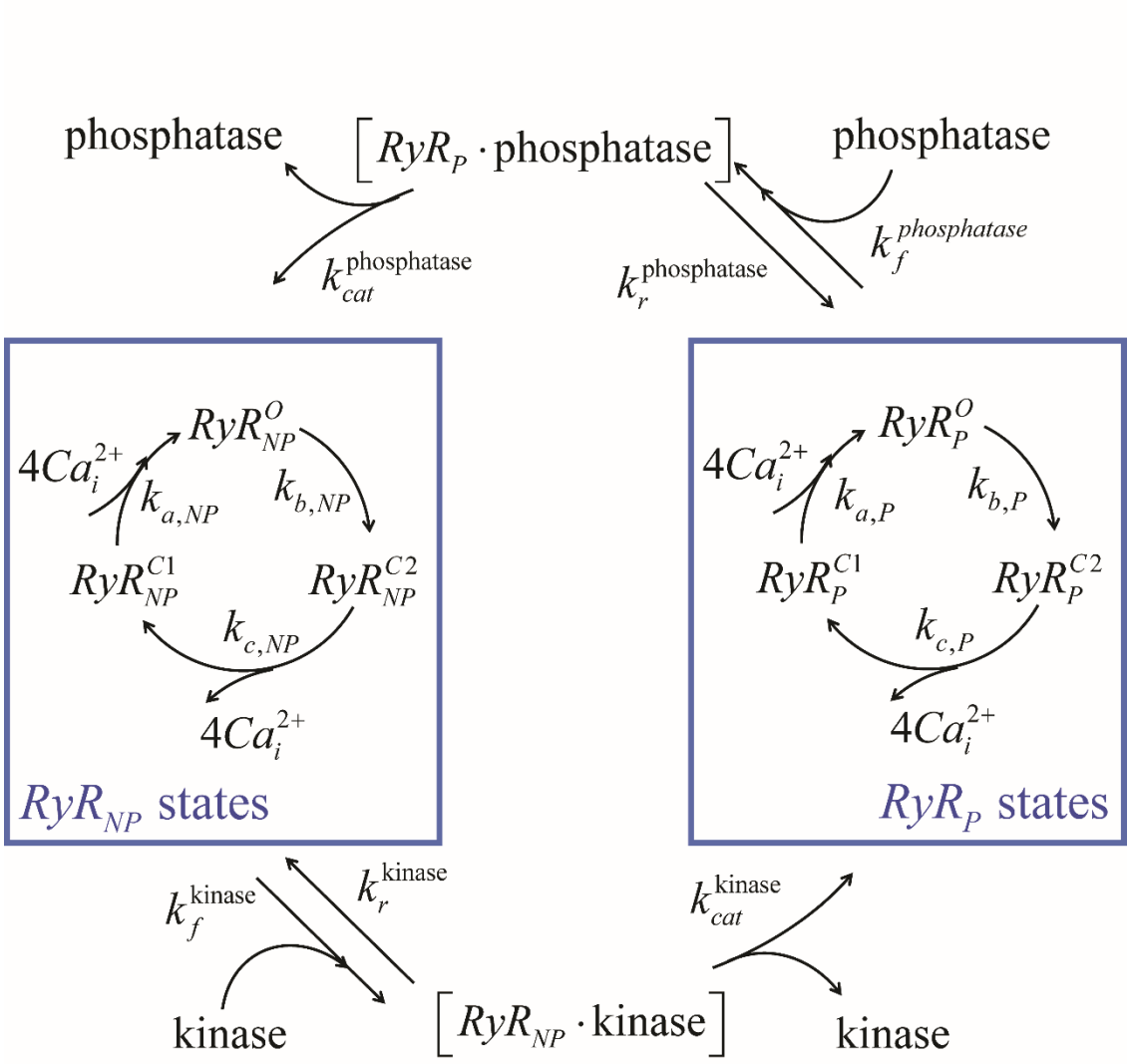
Cardiac cell spontaneous contractions arise from self-sustained oscillations of intracellular calcium concentration (Bers, 2002, Dupont et al., 2011). Initiation of which requires influx of calcium ions into the cytoplasm, which induce opening of RyR channels on an intracellular storage named the sarcoplasmic reticulum (SR) in a process known as Ca^{2+}-induced-Ca^{2+} release (CICR). After the

RyR channels open, Ca^{2+} diffuses to the cytosol according to the concentration gradient and the RyRs slowly become deactivated. Concomitantly, the The Sarco/endoplasmic reticulum Ca2+ATPase (SERCA) transports calcium ions back from the cytoplasm into the SR (using ATP). RyRs are packed in clusters found in specialized junctional domains of the SR called calcium release units (CRUs) (Franzini-Armstrong et al., 2006). Following previous work (Maltsev et al., 2011, Hake et al., 2012), we model calcium oscillations in a cardiomyocyte by a two dimensional lattice of CRUs, where different CRUs are coupled by diffusion of calcium ions through adjacent cytosolic domains. Each CRU is composed of two domains, cytosolic and SR, with local calcium concentrations denoted by, Ca_i^{2+} and Ca_{SR}^{2+} respectively (Fig. S9a). The kinetic scheme used is detailed below:

For the SERCA machinery, we consider a cooperative enzymatic reaction:

$$SERCA \; + \; 2Ca_i^{2+} \underset{k_r^{SERCA}}{\overset{k_f^{SERCA}}{\rightleftarrows}} \left[SERCA \cdot 2Ca_i^{2+} \right] \xrightarrow{k_{cat}^{SERCA}} SERCA \; + \; 2Ca_{SR}^{2+}$$

The RyR dynamics is described by a 3-state model, where the index *c* and *o* refer to close and open conformations. Each RyR can be either in a phosphorylated state or in a non-phosphorylated state as shown below. A general ‘kinase’ and ‘phosphatase’ can convert between the phosphorylated and non-phosphorylated species with a Michaelis-Menten kinetics. The identity of the kinase and phosphatase are not taken specifically in the model, but they may represent CaMKII and PP1 (protein phosphatase 1), that directly react with RyR (Wehrens et al., 2004, Marx et al., 2001). We have shown in the paper that CaMKII is involved in mechanically-induced noise reduction. PP1 has been shown to be activated by NO (Kohr et al., 2009, Price et al., 2017) that is often produced as a result of mechanical stimulation (Prosser and Ward, 2014, Jian et al., 2014).



The open state of RyR allows for diffusion of Ca^{2+} between the two domains, cytosol and SR, according to the concentration gradient. This is in addition to a basal Ca^{2+} leak between these domains through the SR membrane:

$$RyR^{O} + Ca_{SR}^{2+} \underset{}{\overset{k_{diff}^{RyR}}{\rightleftarrows}} RyR^{O} + Ca_i^{2+}$$

$$Ca_{SR}^{2+} + SR_{\text{membrane}} \underset{}{\overset{k_{leak}^{RyR}}{\rightleftarrows}} Ca_i^{2+} + SR_{\text{membrane}}$$

The kinetic rate constants for all of the reactions and simulation parameters are given in Tables S2-S3.

In principle, the reactions described above can be formulated as a system of coupled differential equations. Note that these equations describe the time evolution of the *average* number of species (e.g., channels, ions) in each state/domain. Such an approach does not monitor discrete events over time (e.g., channel conformational change, phosphorylation). Hence, in order to follow the stochastic fluctuations of the system (and therefore its noise), we use an alternative approach. We use the stochastic simulation algorithm (SSA) (Gillespie, 1976, Gillespie, 1977) that generates trajectories of the system (the average of which satisfies the differential equations). The SSA is a variation of kinetic Monte-Carlo algorithm. In kinetic Monte Carlo simulation, in every time step, a reaction occurs with a probability that is calculated according to its reaction rate. In short, the SSA method modifies this scheme to avoid steps where no reaction occurs by generating two random numbers at each step of the simulation, one that determines the time interval to the next reaction and a second that is used to choose the reaction that occurs.

Representative calcium oscillations are shown in Fig.S9b-c. As shown in Fig.S9d, the fluctuations in calcium oscillation frequency correlate with the fluctuations in the fraction of phosphorylated RyR channels. As predicted by the simple isomerization model presented in the beginning of this section, increased number of active enzymes reduce beating noise, ξ as quantified by the relative standard deviation of the frequency, $\xi_\omega = \sigma_\omega / < \omega >$, where $\langle \omega \rangle$ is the average frequency of calcium oscillations and σ_ω is the standard deviation (Fig.S9e).

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