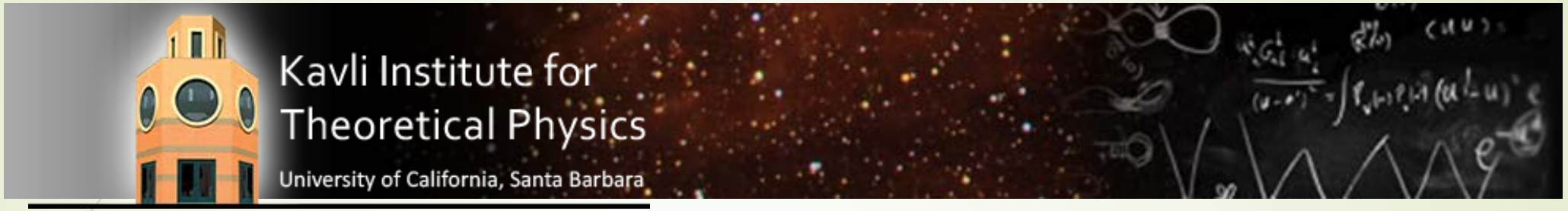


UNIVERSITAT POLITÈCNICA DE CATALUNYA BARCELONATECH

California State University Northridge

How to determine calcium homeostasis by analyzing the behavior of ion currents.

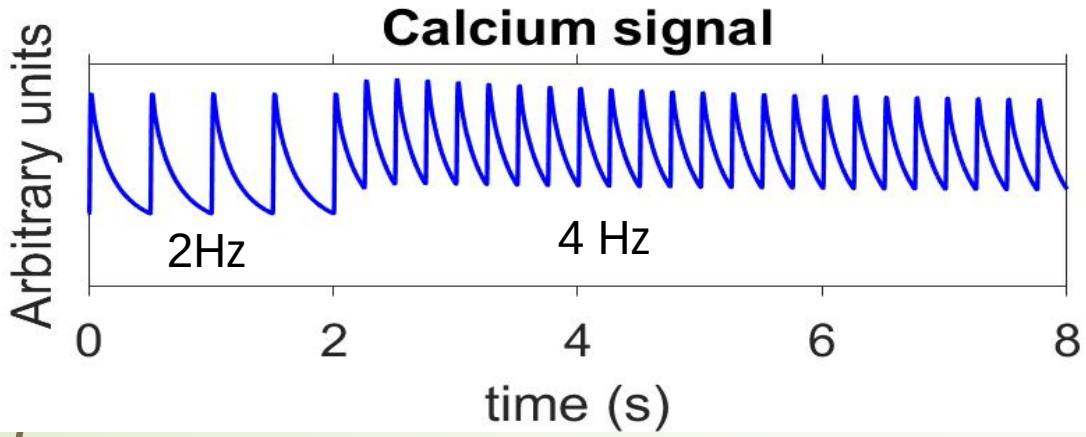
Enrique Alvarez-Lacalle

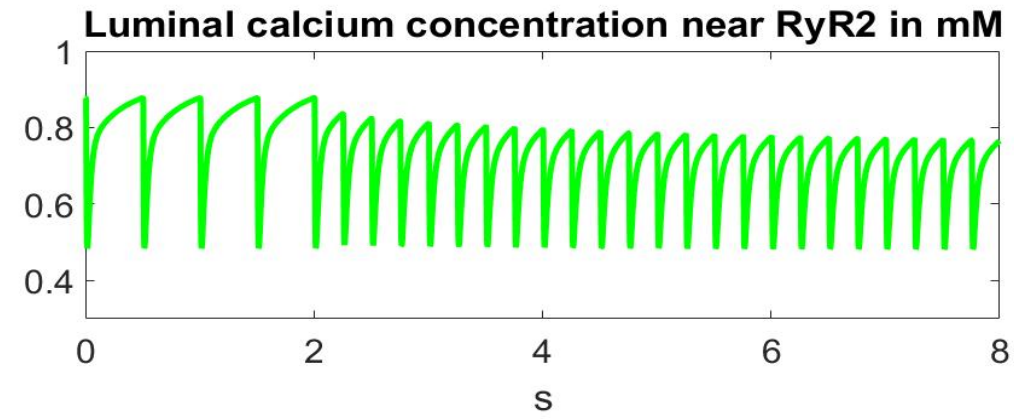
D.Conesa, I. Cantalapiedra.
A. Peñaranda, B.Echebarria

Y.Shiferaw

Calcium homeostasis

- A cardiomyocyte, upon changes in pacing, phosphorylation of proteins, external signals,… reaches a steady total where total calcium in the cell (Q_{TOT}) and total calcium (free and buffered) in the SR (QSR) at pre-systolic levels is constant.





Bondarenko, et al. mouse model

Relevance of understanding homeostasis: Force frequency relation

- The relation between pacing rate and contraction of the heart is species dependent. Most mammals increase calcium level.

M. Endo. European Journal Pharmacolgy 2004 (Rabbit data)

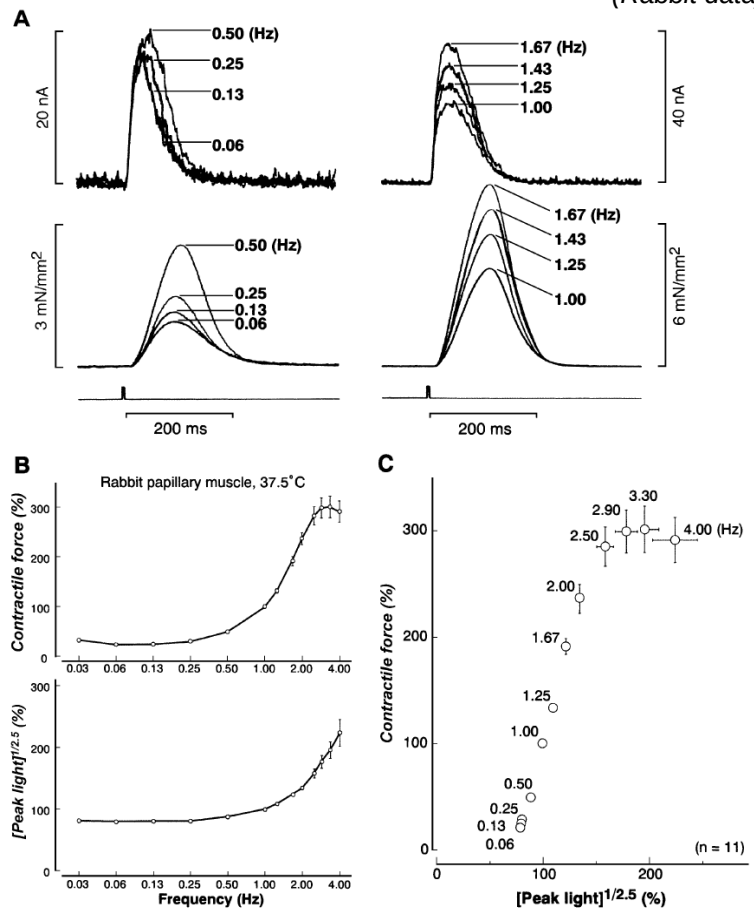
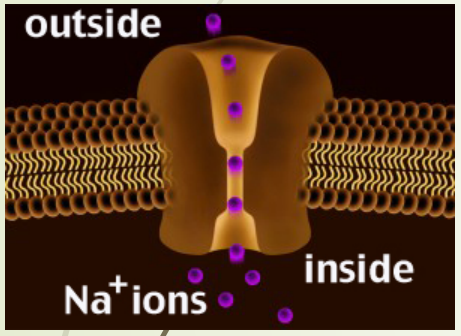


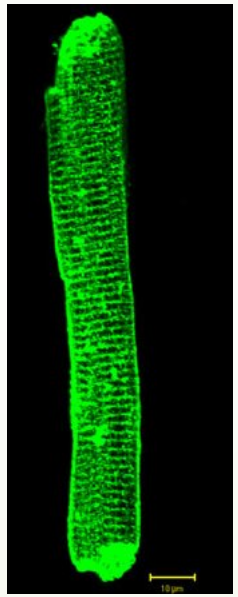
Fig. 1. Force–frequency relationship determined in isolated rabbit papillary muscles loaded with the Ca^{2+}-sensitive bioluminescent protein aequorin at 37.5 °C. (A) Actual representative tracings of aequorin light transients (upper tracings) and contractile force (lower tracings) over a range of lower frequencies (left-hand side panel) and of higher frequencies (right-hand side panel). (B) Force–frequency (upper panel) and Ca^{2+} transient–frequency (lower panel) relationships (n=11 each). (C) Steady-state relationship between the amplitude of Ca^{2+} transients and contractile force during alteration of the stimulation frequency.

Relevance: Going from channel to cell to tissue

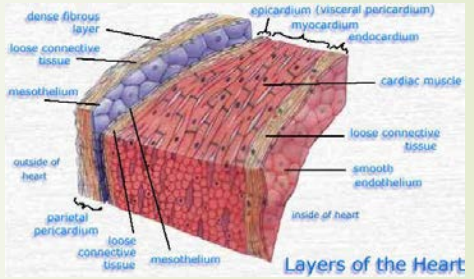


Doable experimentally

From desired
homeostatic level
to channel modification
requires understanding



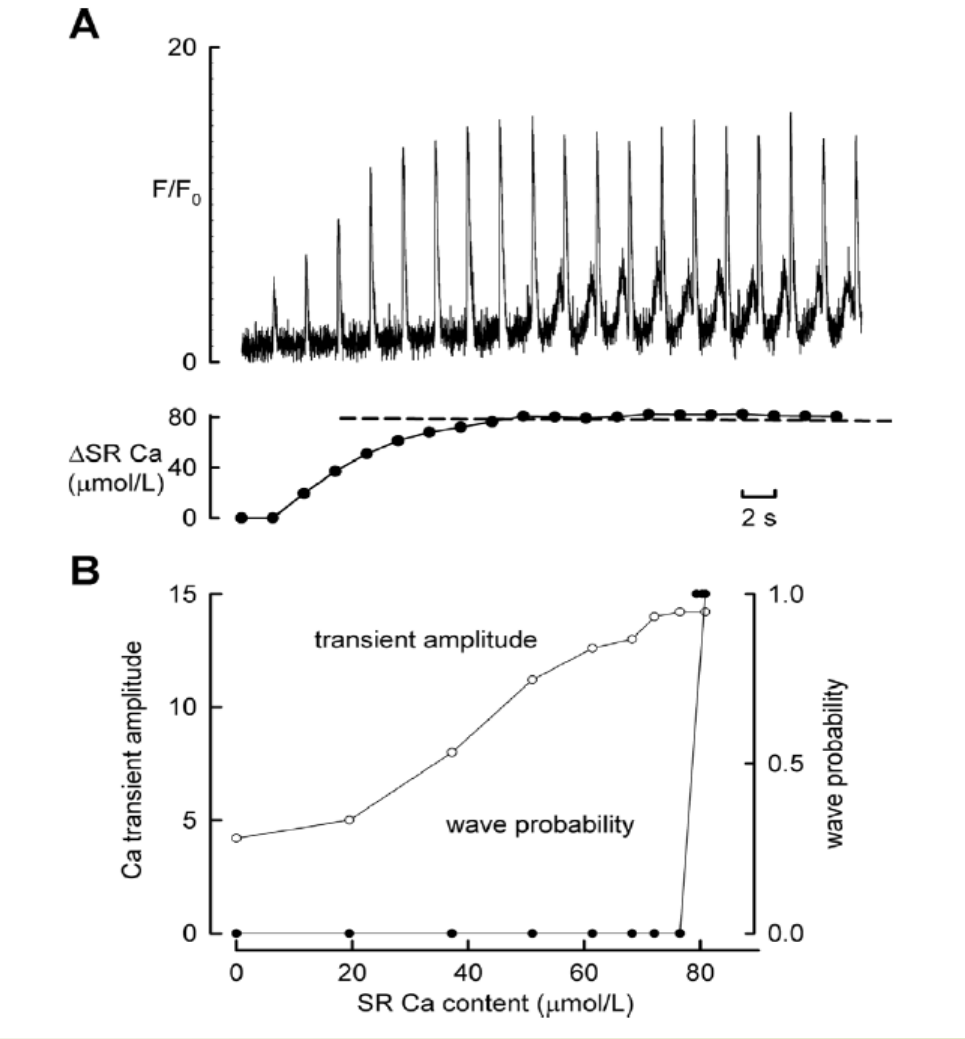
NO coordination problem



Relevance. Insights into arrhythmias.

- There is strong indications that arrhythmias related with calcium cycling might be related with high SR calcium overloads in the SR, which can only be reached if the homeostatic process allows it. Understanding how it works might help us eliminate it.

- There is also a general interest in developing tool to study control protocols of arrhythmias and know how to change the final state after the implementation of the protocol.

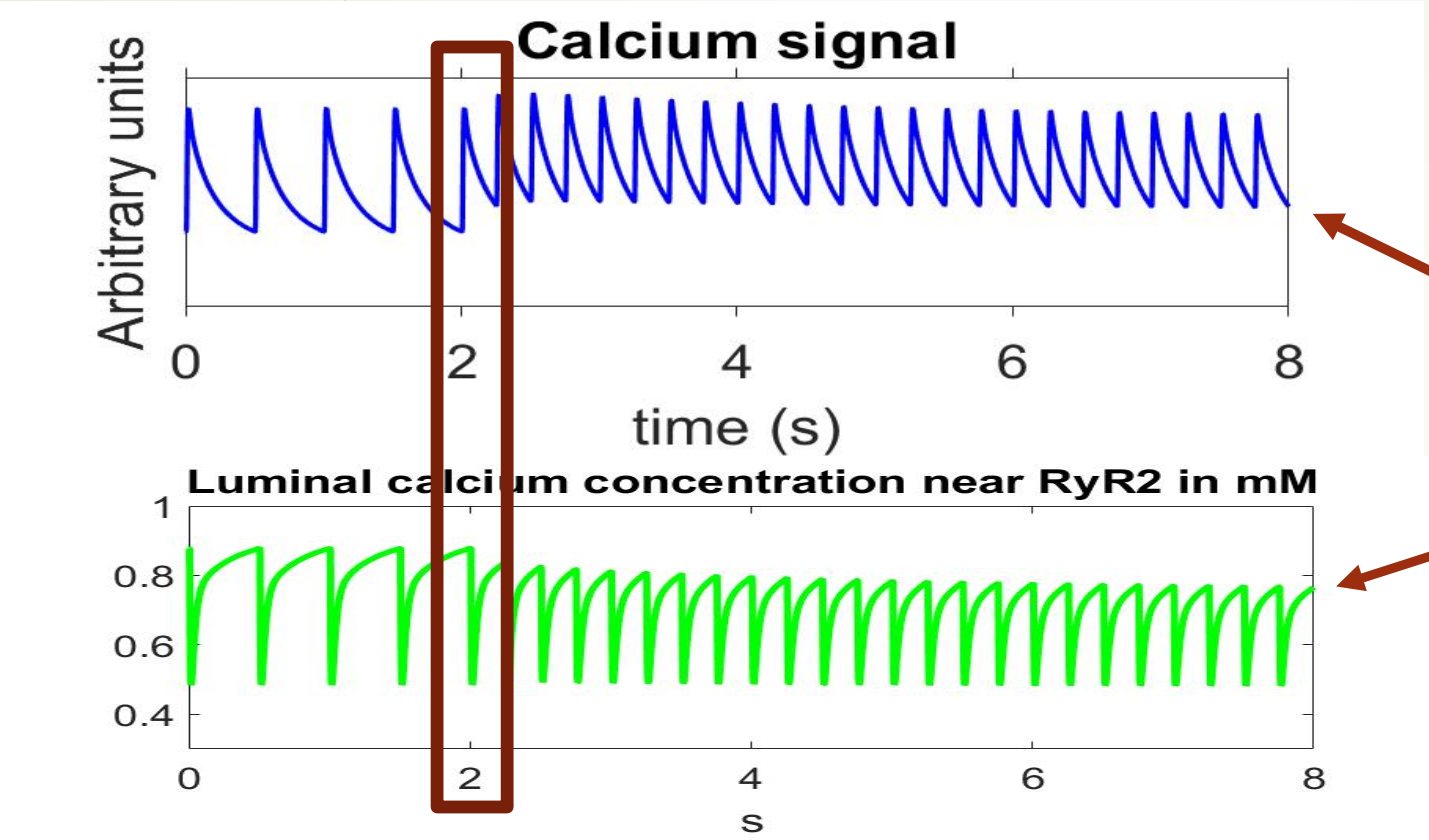


Eisner et al, Circ. J. 2009

Understanding homeostasis is predicting the equilibrium level from the first beat

We claim that to understand how homeostasis work you need to understand how the effects of a change in properties of the cell in the homeostatic level in the first beats of the application way before you have reached the steady state.

Poke and play with the cell during one beat

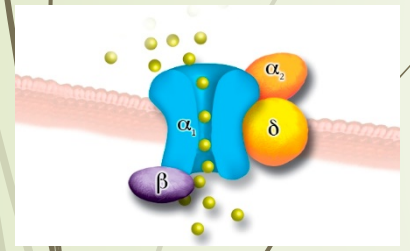


.....predict

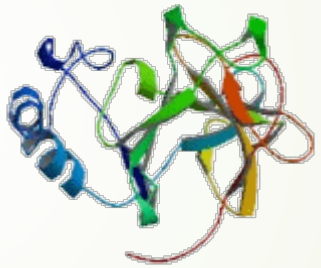
To understand homeostasis is studying what happens in one beat and then..

Basic nomenclature for one beat

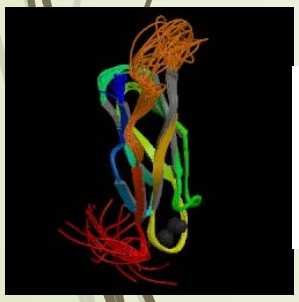
- In a particular cell with a particular pacing rate and environment we define the following nomenclature for the fluxes and calcium ion changes during one beat



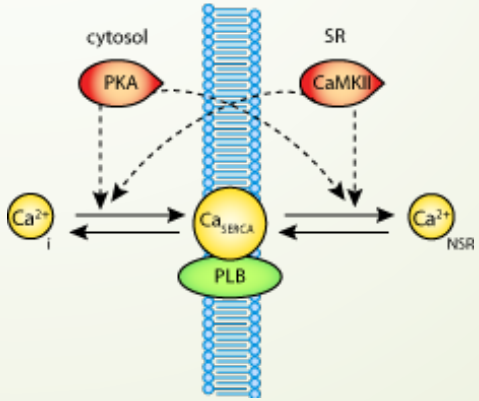
$$JQ_{CAL} = v_d \sum_i \int_0^T J^i_{CaL} dt$$



$$JQ_{REL} = v_d \sum_i \int_0^T J^i_{rel} dt$$



$$JQ_{NACA} = v_s \sum_i \int_0^T J^i_{NaCa} dt$$



$$JQ_{SERCA} = v_i \sum_i \int_0^T J^i_{up} dt$$

Our key claim

- Under general conditions in ventricle which do not involve very large pacing rate (time scale of the pacing larger than other time scales in the system (buffering, recovery of RyR2, LCC recovery dynamics), the above currents are basically a function of two pre-systolic values:

Pre-systolic total number of calcium ion in the whole cell Q_{TOT}

Pre-systolic total number of calcium ion in the SR: Q_{SR}

$$JQ_{CAL}(Q^n_{TOT}, Q^n_{SR})$$

$$JQ_{SERCA}(Q^n_{TOT}, Q^n_{SR})$$

$$JQ_{NACA}(Q^n_{TOT}, Q^n_{SR})$$

$$JQ_{REL}(Q^n_{TOT}, Q^n_{SR})$$

If this is true, we can iterate

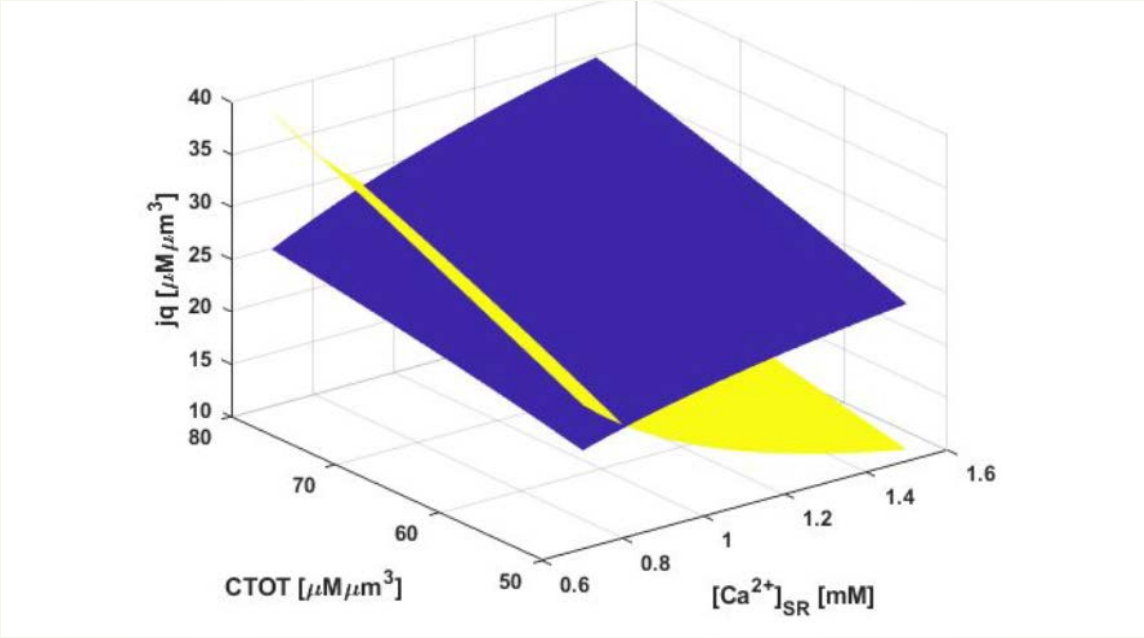
MAP WHICH IS HOPEFULLY EVIDENT

$$Q_{TOT}^{n+1} = Q_{TOT}^{n} + JQ_{CAL}(Q_{TOT}^{n}, Q_{SR}^{n}) - JQ_{NACA}(Q_{TOT}^{n}, Q_{SR}^{n})$$
$$Q_{SR}^{n+1} = Q_{SR}^{n} + JQ_{SERCA}(Q_{TOT}^{n}, Q_{SR}^{n}) - JQ_{REL}(Q_{TOT}^{n}, Q_{SR}^{n})$$

THE HOMOESTATIC LEVEL OR STEADY STATE IS REACHED WHEN

$$JQ_{CAL}(Q_{TOT}^{eq}, Q_{SR}^{eq}) = JQ_{NACA}(Q_{TOT}^{eq}, Q_{SR}^{eq})$$
$$JQ_{SERCA}(Q_{TOT}^{eq}, Q_{SR}^{eq}) = JQ_{REL}(Q_{TOT}^{eq}, Q_{SR}^{eq})$$

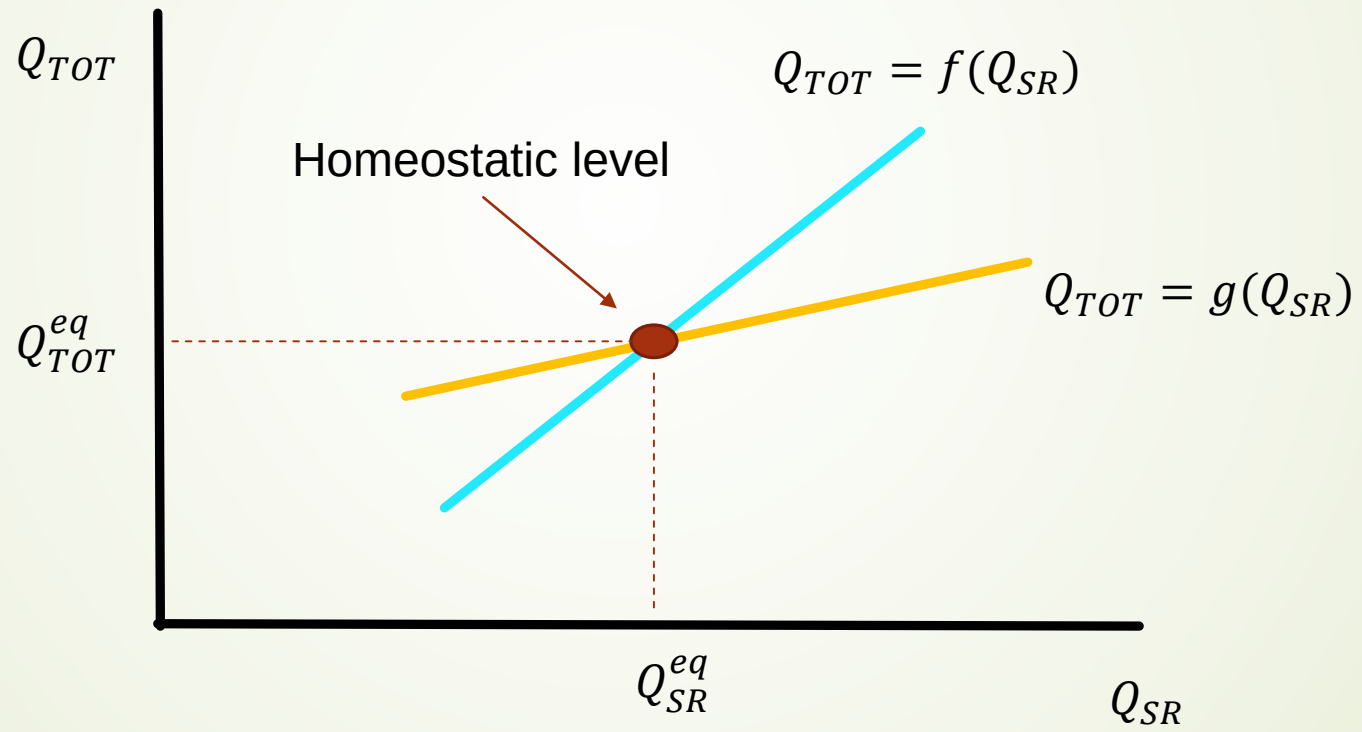
Steady state is generally not existent or where two nullclines intersect (I)



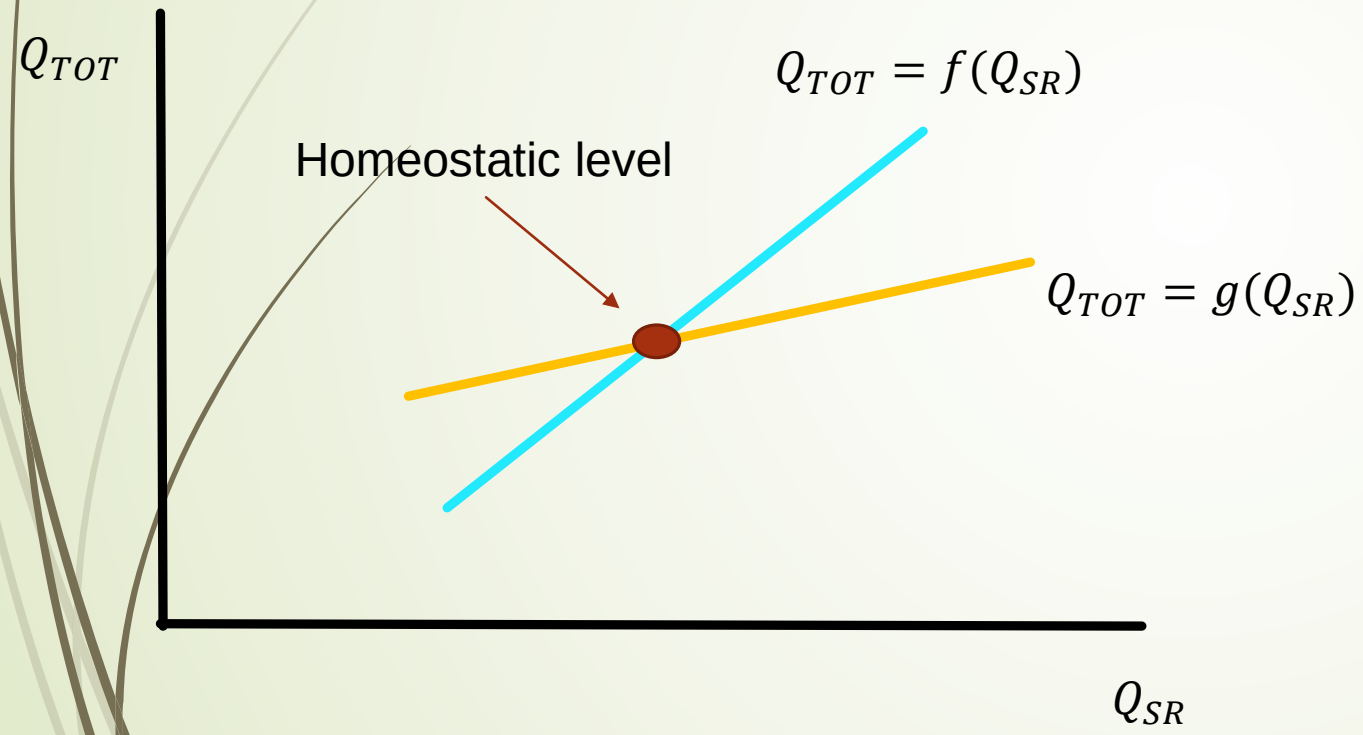
$$JQ_{CAL}(Q_{TOT}^{eq}, Q_{SR}^{eq}) = JQ_{NACA}(Q_{TOT}^{eq}, Q_{SR}^{eq}) \longrightarrow Q_{TOT} = f(Q_{SR})$$

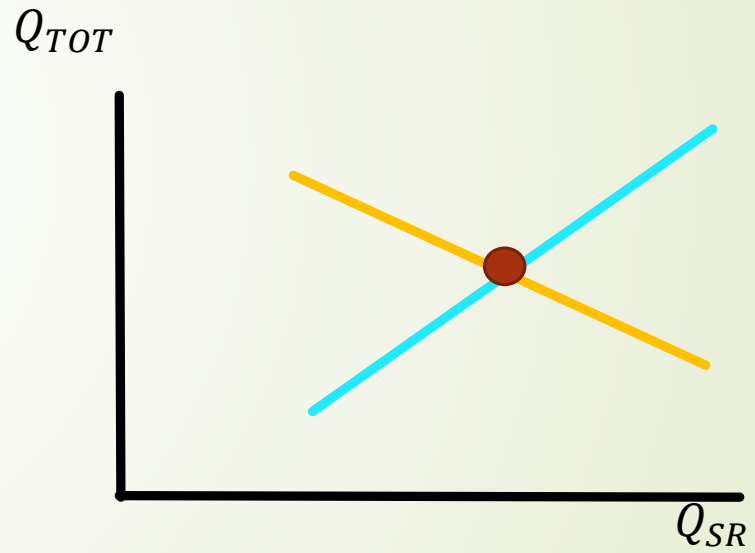
$$JQ_{SERCA}(Q_{TOT}^{eq}, Q_{SR}^{eq}) = JQ_{REL}(Q_{TOT}^{eq}, Q_{SR}^{eq}) \longrightarrow Q_{TOT} = g(Q_{SR})$$

Steady state is generally non existent or where two nullclines intersect (II)



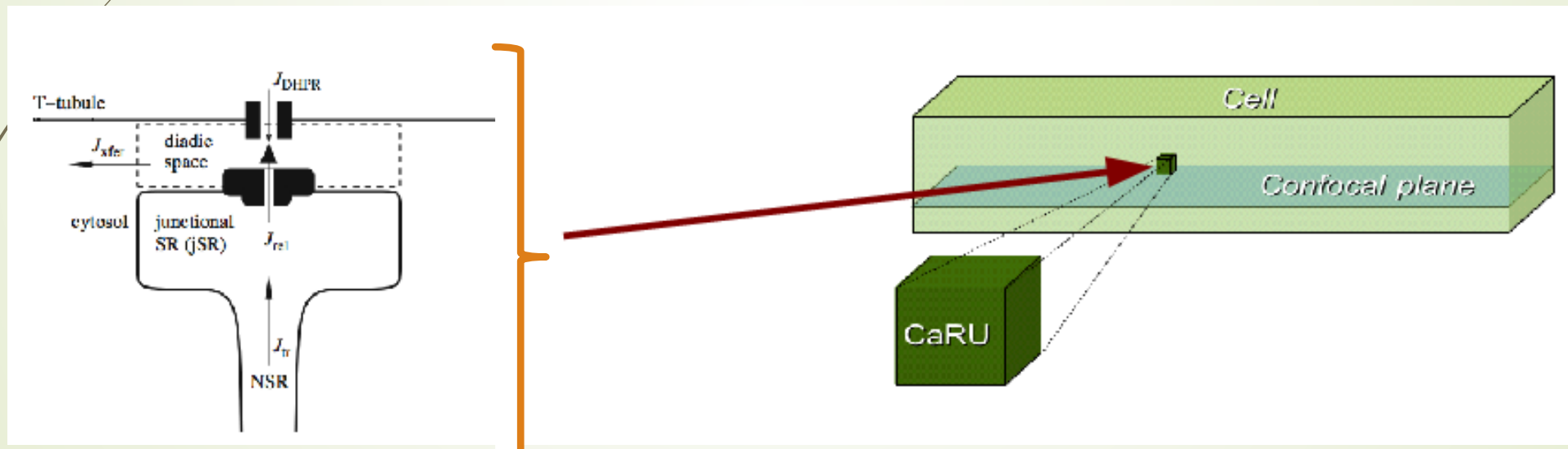
Steady state is generally non existent or where two nullclines intersect (III)





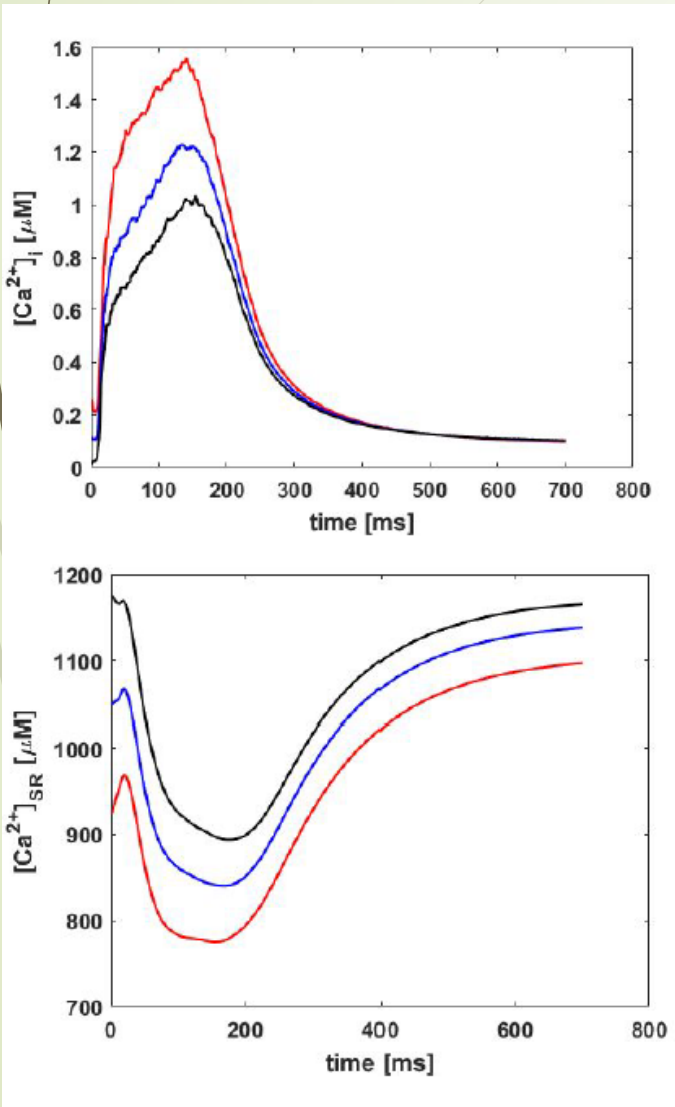
Our petri-dish ventricular cell is in-silico

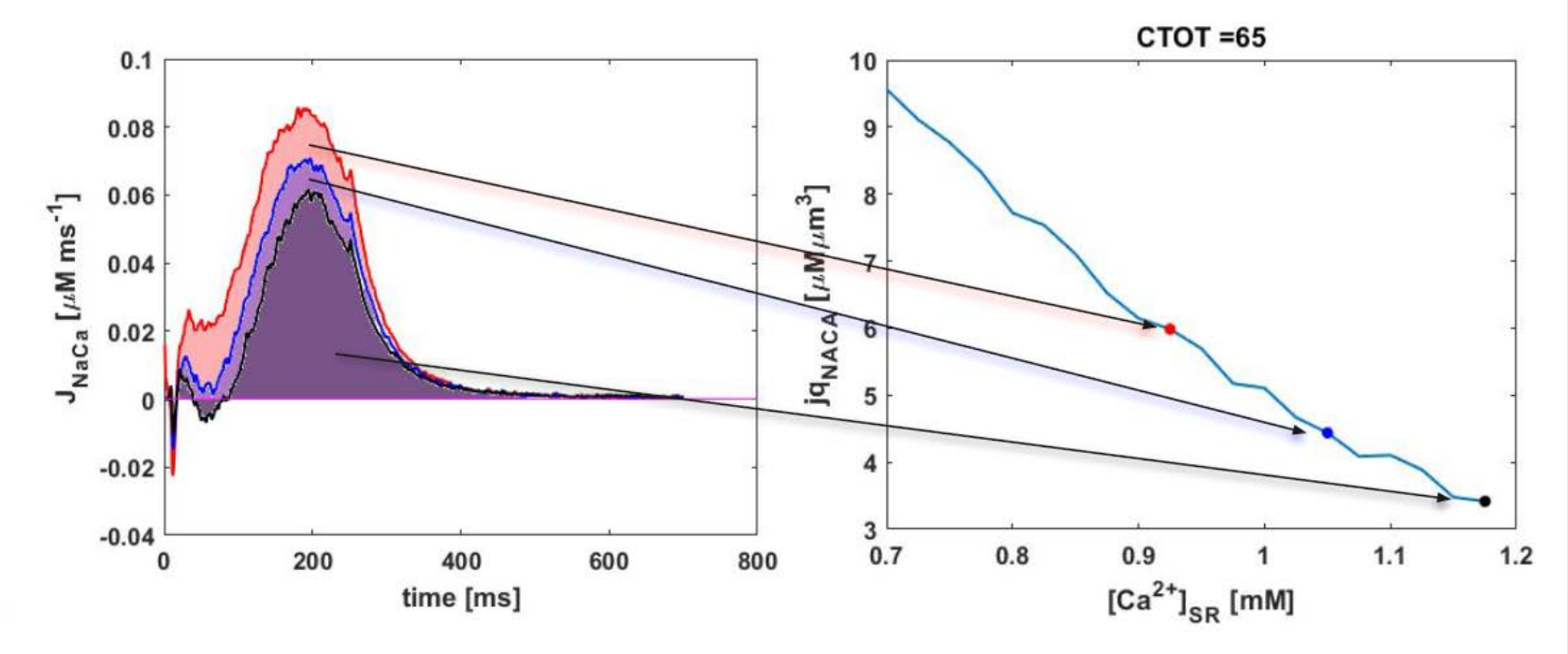
- In-silico cell are easy to poke and test. Restreppo et.al (2008) type model with Calcium release Units, different compartment, stochasticity in LCC and RyR2. Thousand of variables. Deterministic SERCA and EXCHANGER with expression from different models (a mix so that we re not guided). Voltage patched clamped at period of T=750 ms.



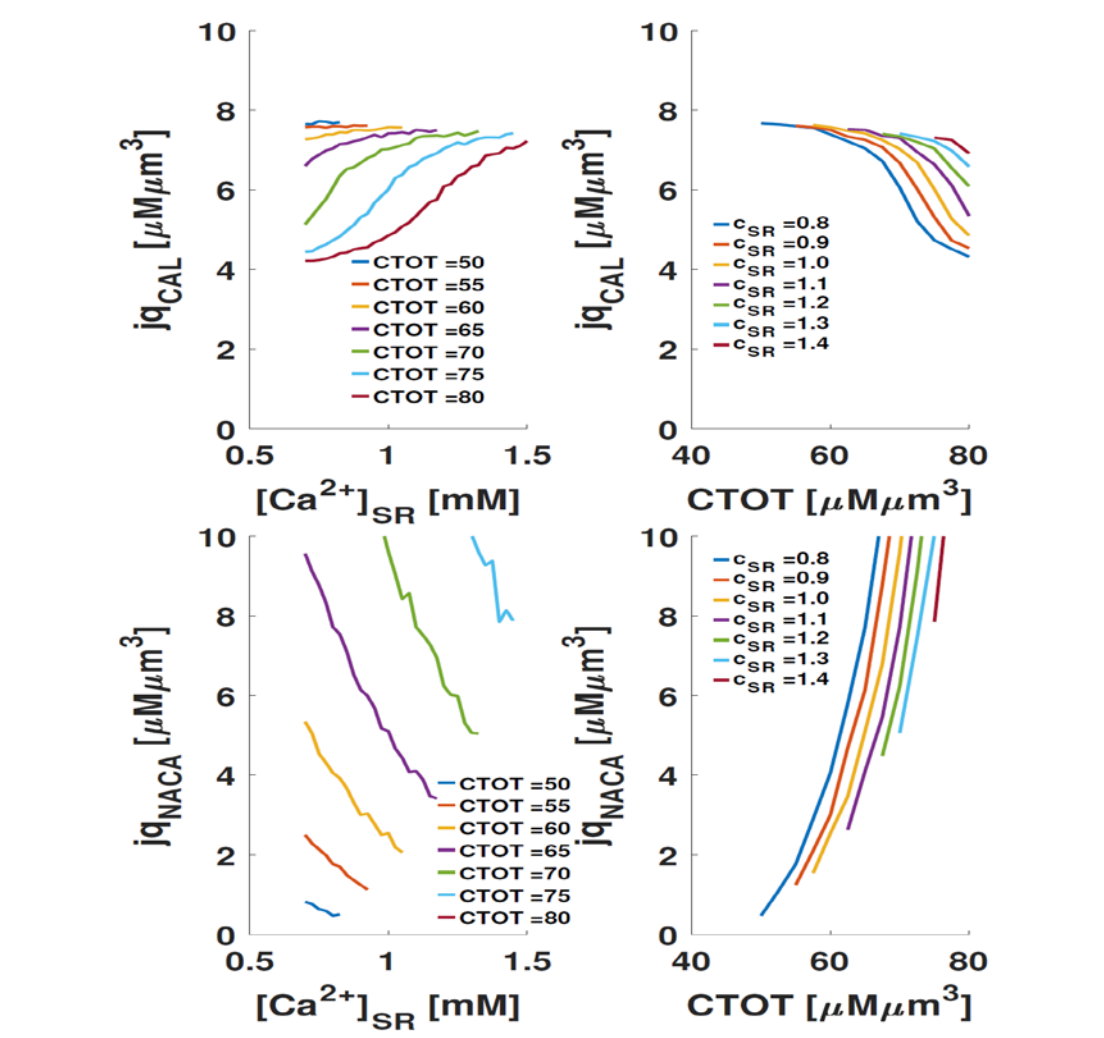
Thousands of variables (some stochastic) and we still want to predict homeostasis from analyzing one beat

We can measure the different current-surfaces using cuts of this surface



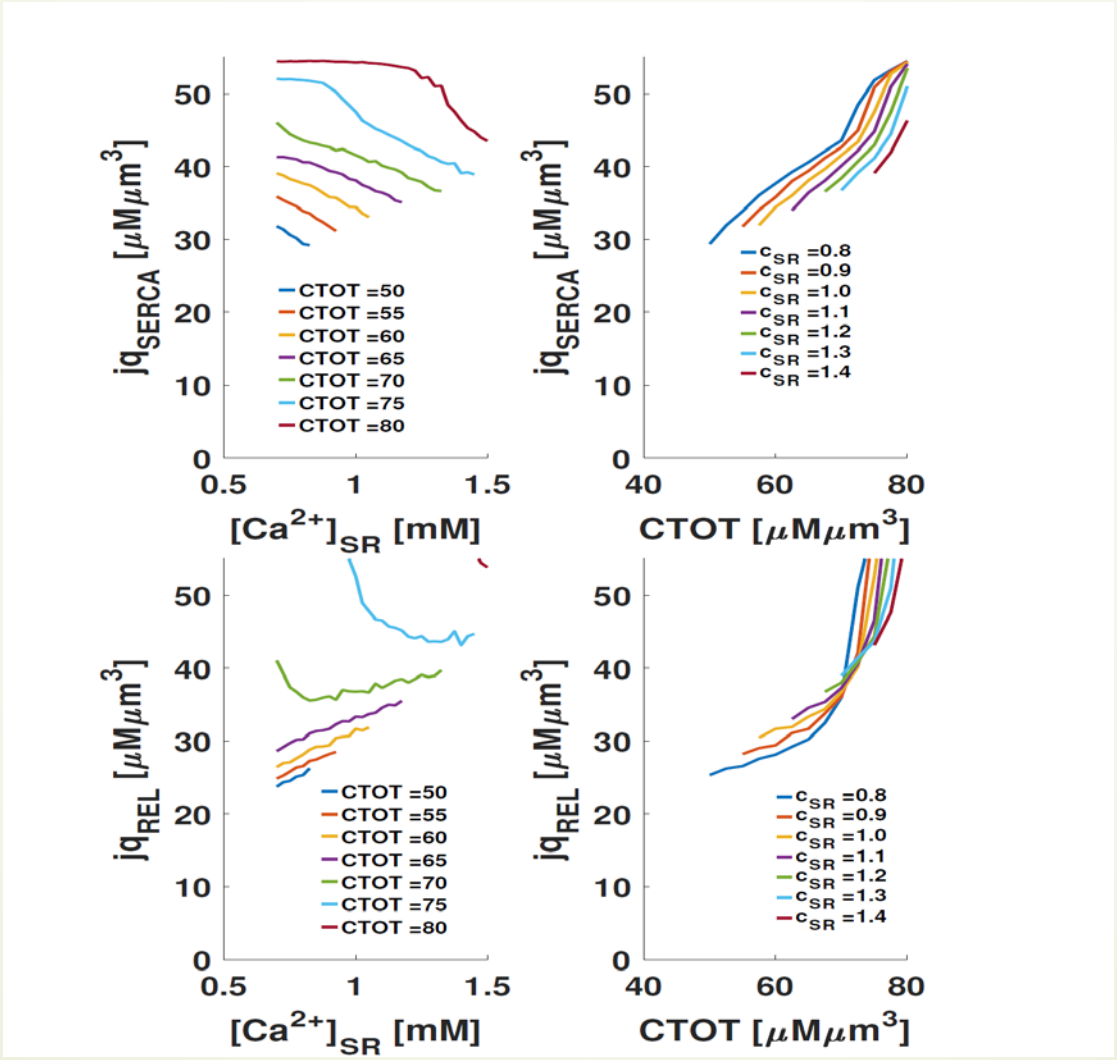


Current surface cuts in our cell (I)

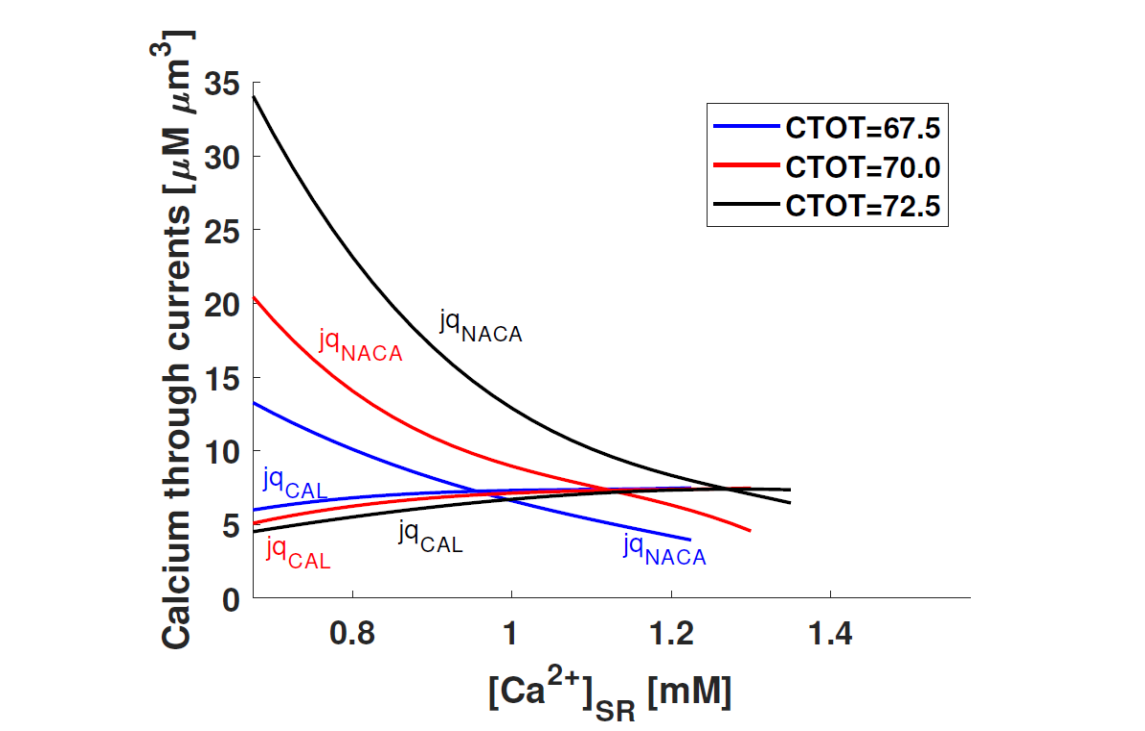


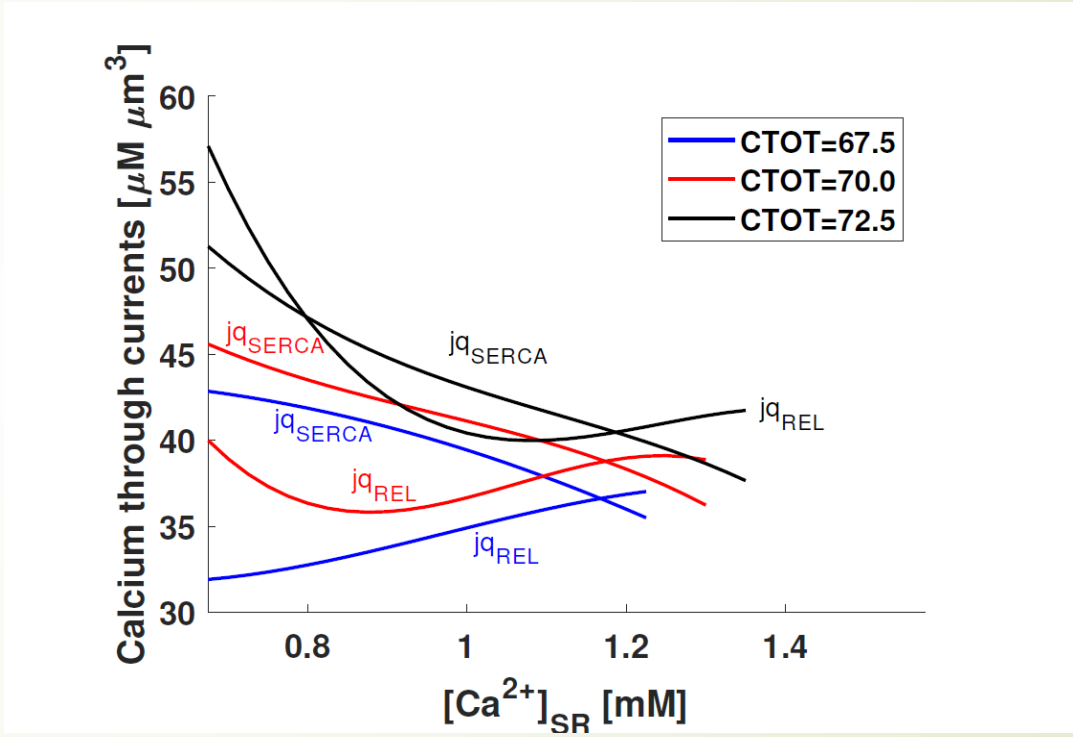
Here, shown in terms of free calcium in SR, and total calcium per CaRU

Current surface cuts in our cell (II)

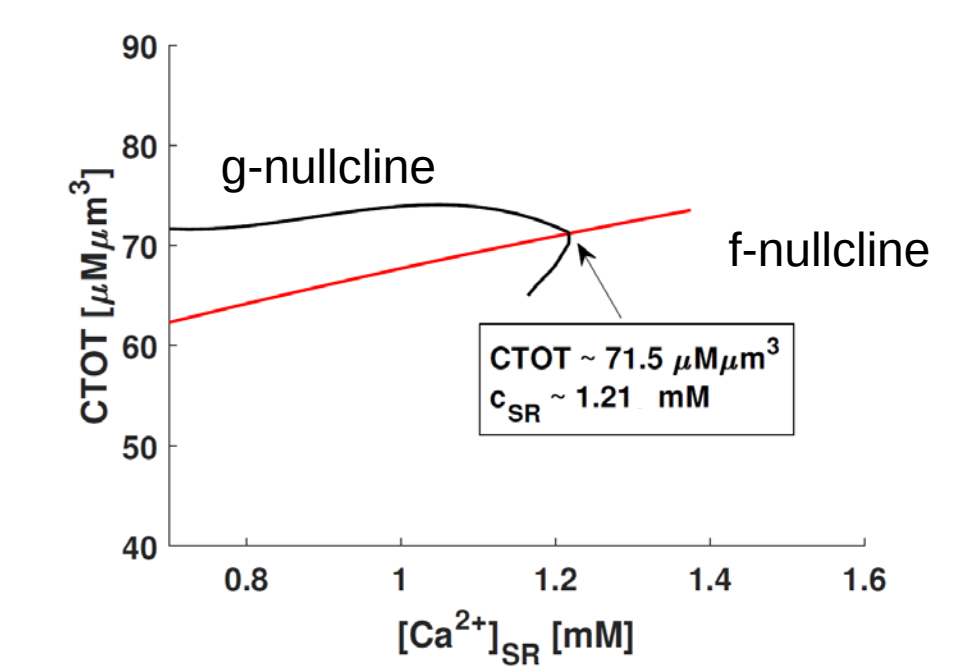


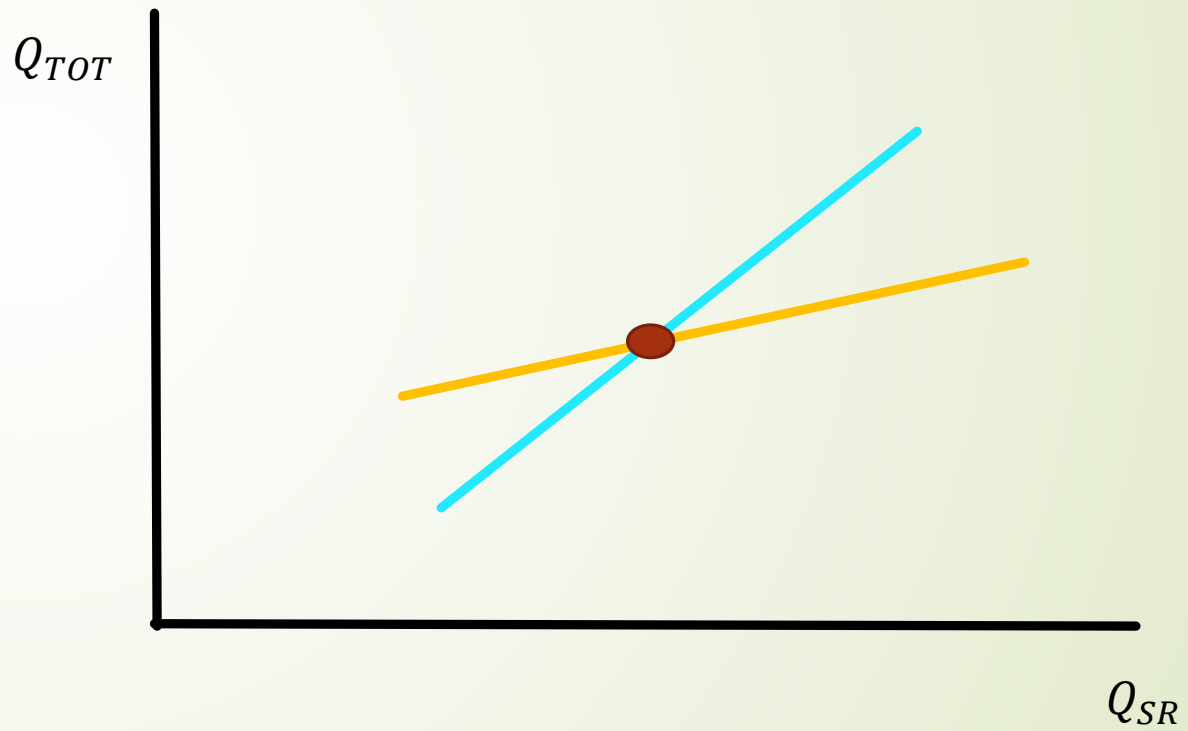
Slope of the nullclines in our in-silico cell



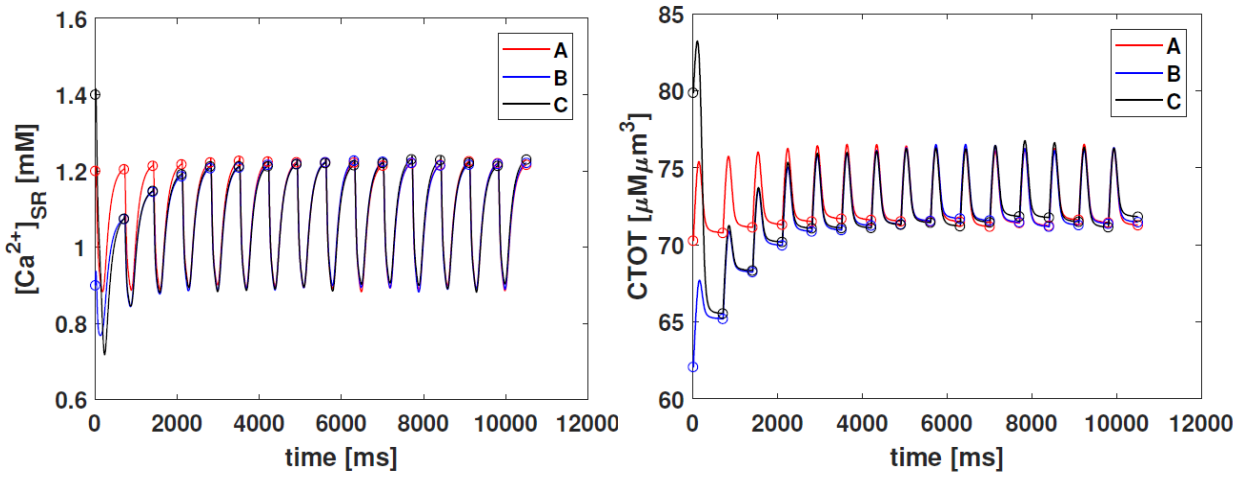


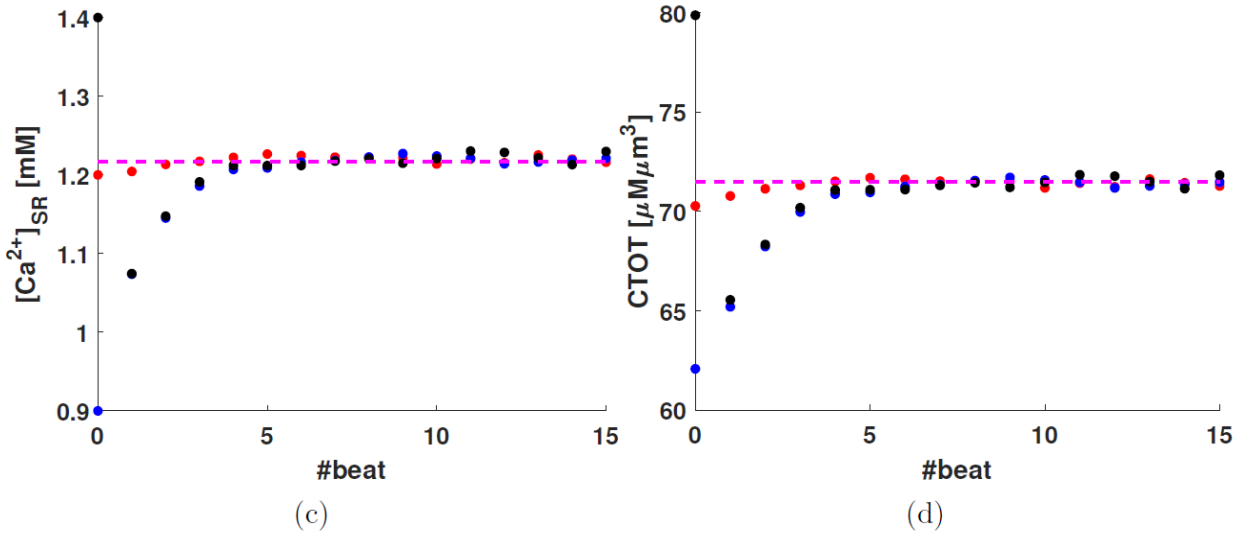
Slope of the nullclines in our in-silico cell





Successful prediction



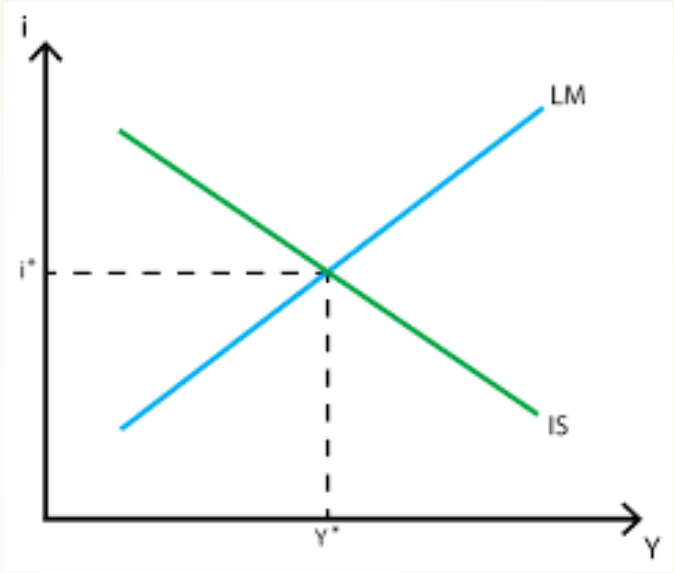


Prediction after poking one beat

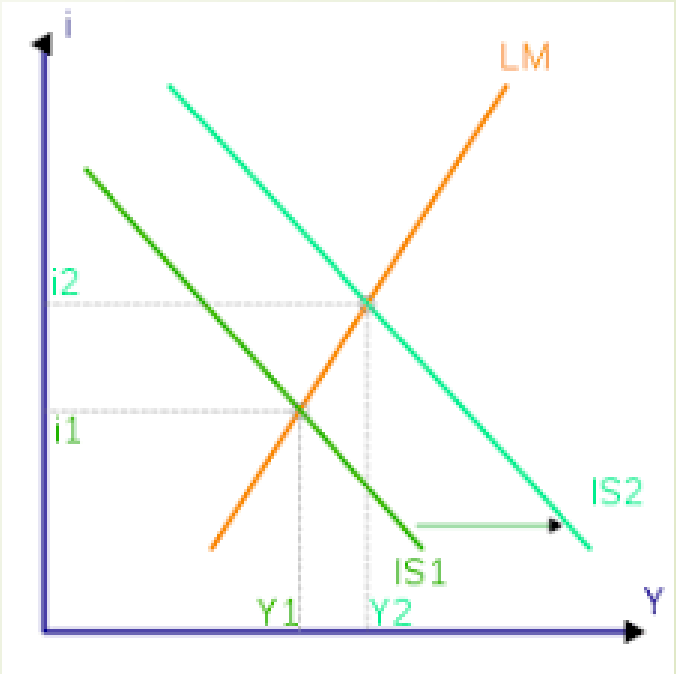
- Pre-systolic values at the corresponding beat for three runs with different initial conditions

This is basic.. macroeconomics

- IS-LM macroeconomic model:
- Variables are GDP and interest rates.
- Double global equilibrium is Investment-Saving and Loan preference-Money market



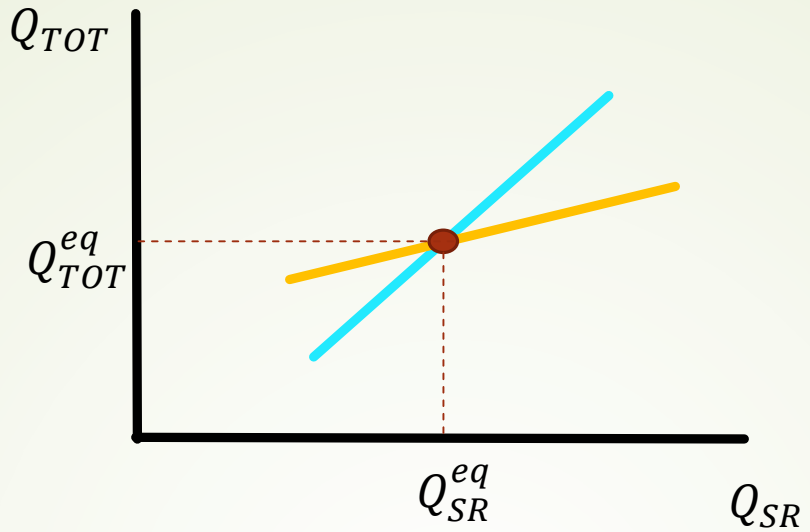
They talk about shocks!



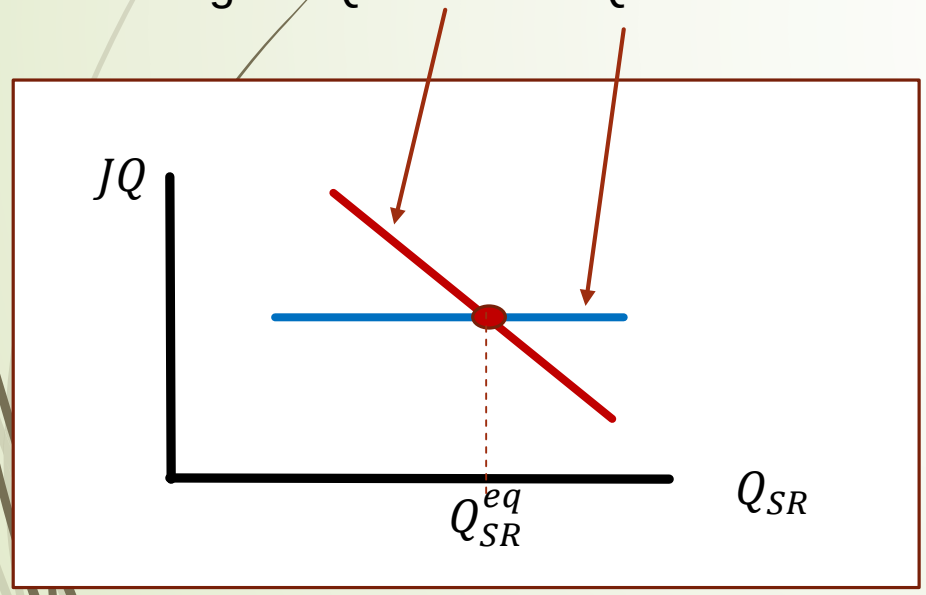
A shock is a change in pacing, a betablocker,…

- If the shock is such that all the surface currents are completely changed….you have a new problem…with a new fixed point that you have to analyze from scratch. NO LUCK

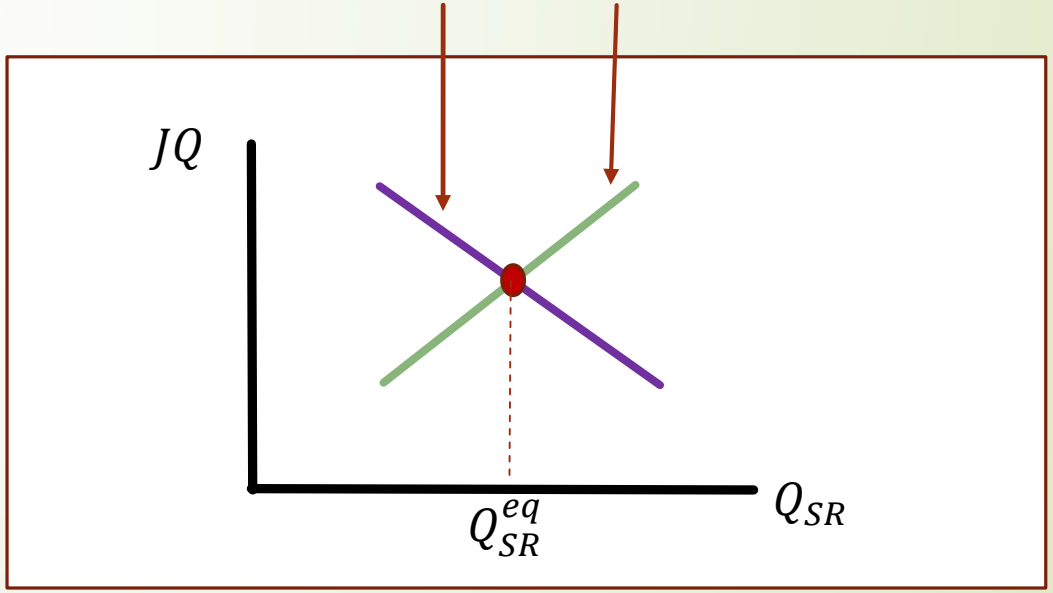
- But if you have a shock where two of the currents do not change much and two change a lot, or, better, the case where three do not change and one change….THEN YOU ARE LUCKY



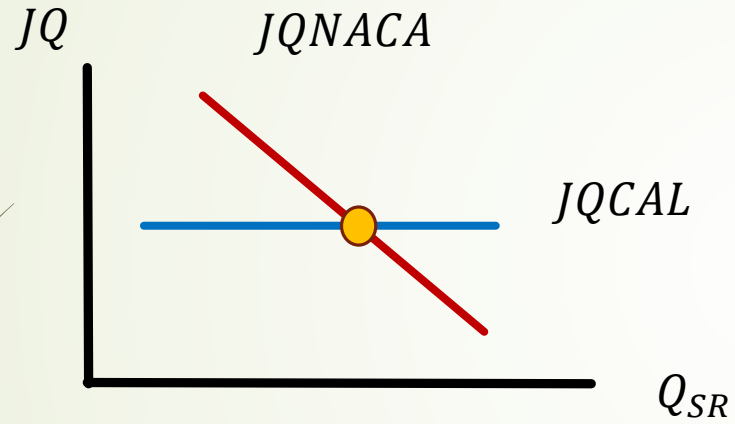
Type of crossing of JQNACA vs JQCAL for constant Q_{TOT}^{eq}

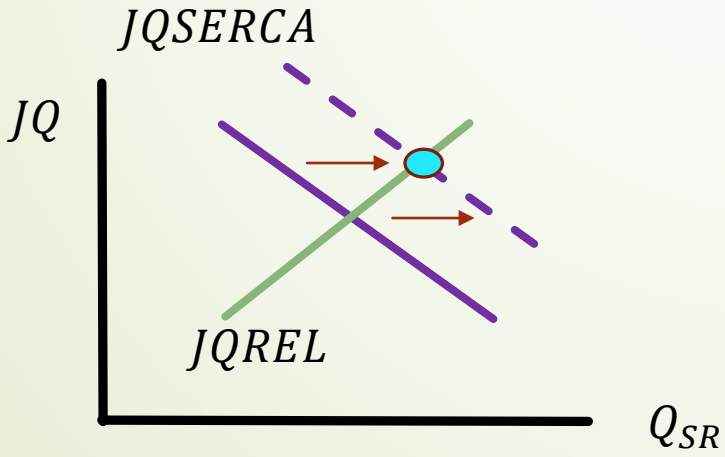


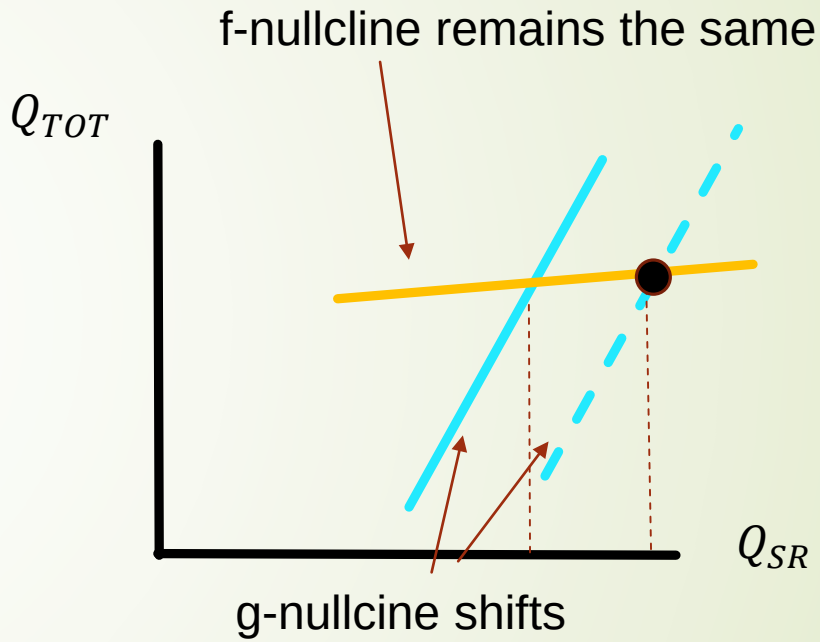
Type of crossing of JQSERCA vsJQRELfor constant Q_{TOT}^{eq}



Change in SERCA which increases across the board and does not affect other surfaces





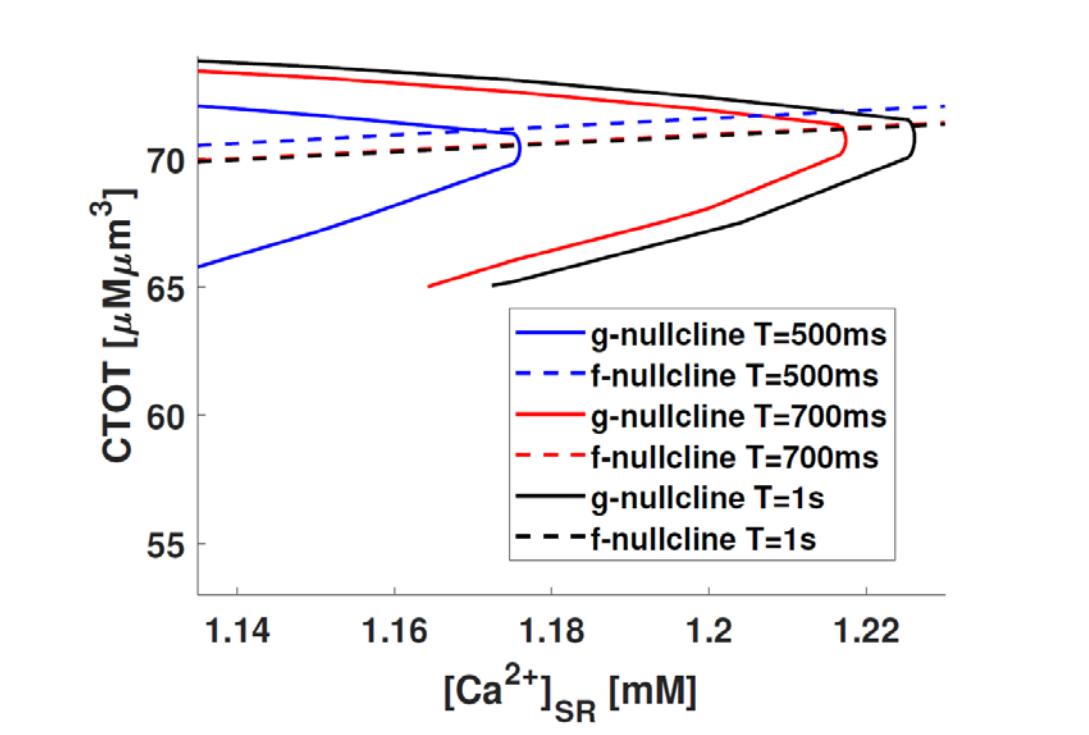


In one nullcline chnages, the slope of the other nullcline which remains unchanged has very important effects on the magnitude of the change.

Change in pacing rate

- When you increase or decrease pacing you can not affect almost at all the entrance of calcium by the LCC and the release which happen during the first hundred milliseconds. Changing the diastolic interval will change how SERCA and exchanger perform their work.

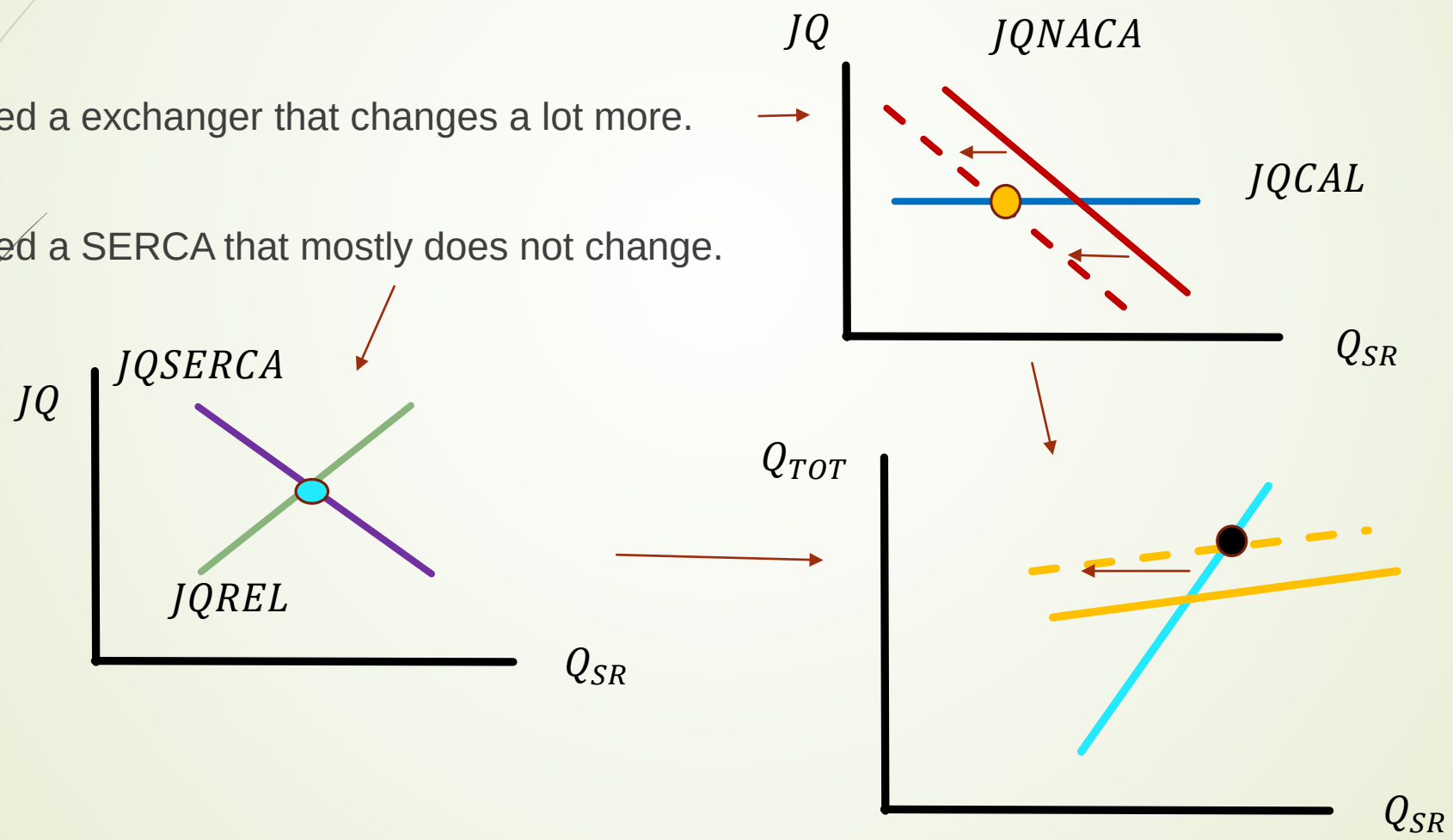
In our made-up cell, exchanger works mainly during transient and it is patch-clamped
So only SERCA changes…depletion of SR and slight reduction of total calcium



What if we want a positive force- frequency relation?

We need a exchanger that changes a lot more.

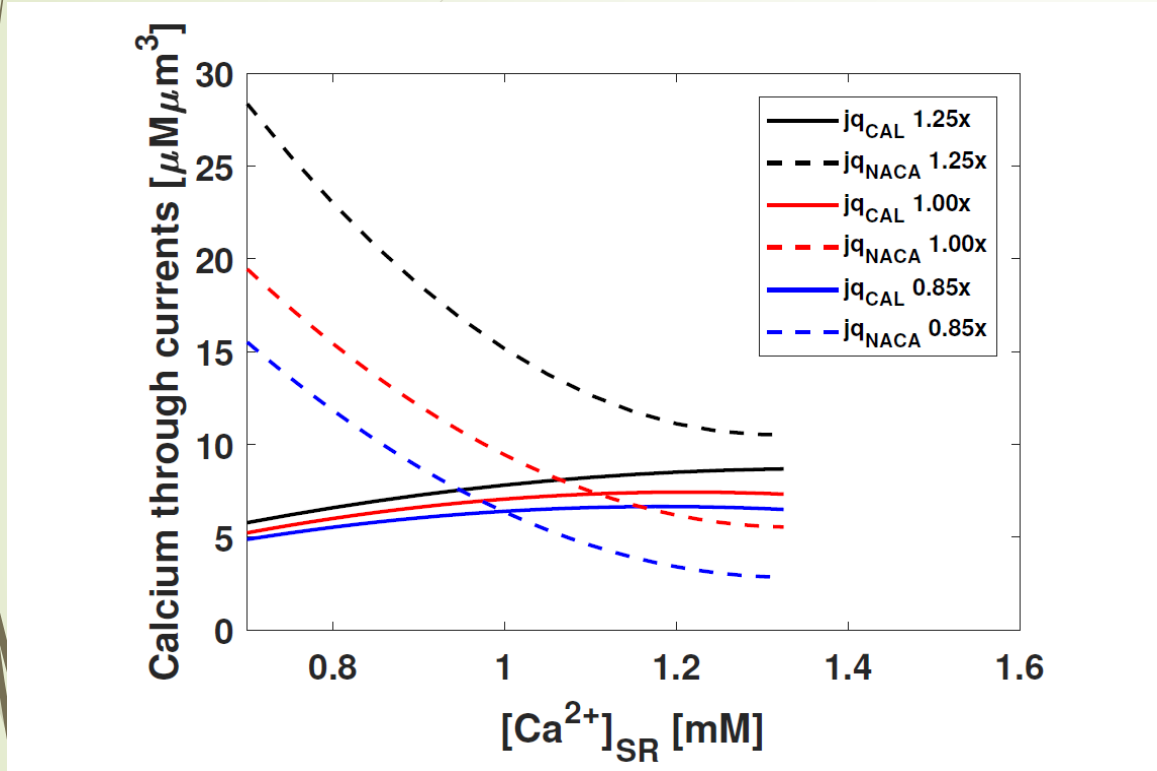
We need a SERCA that mostly does not change.

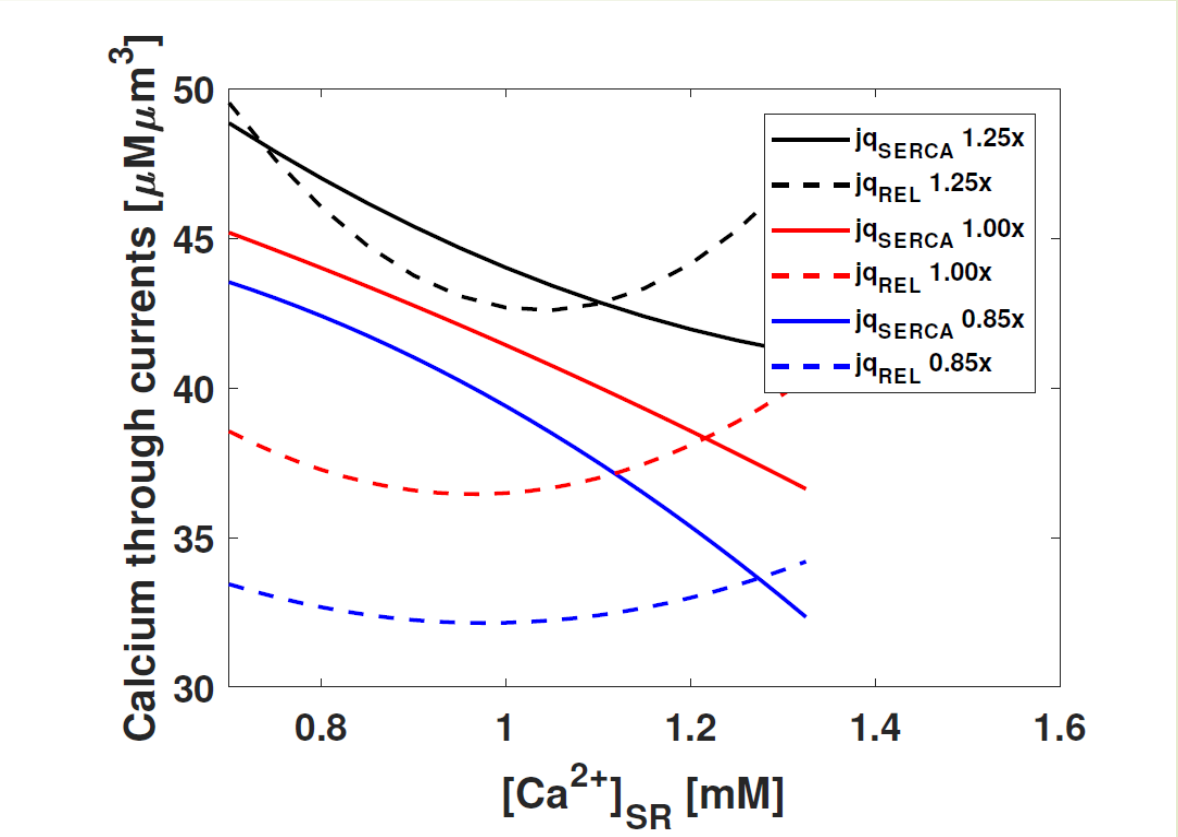


Examples of shocks with three and four shift effects

- Changes in the RyR2 are the typical examples of a three curve effect.
- Let me close with an example of a change that will affect all surface which still can be understood from this picture: A change in the conductivity of the LCC channel.

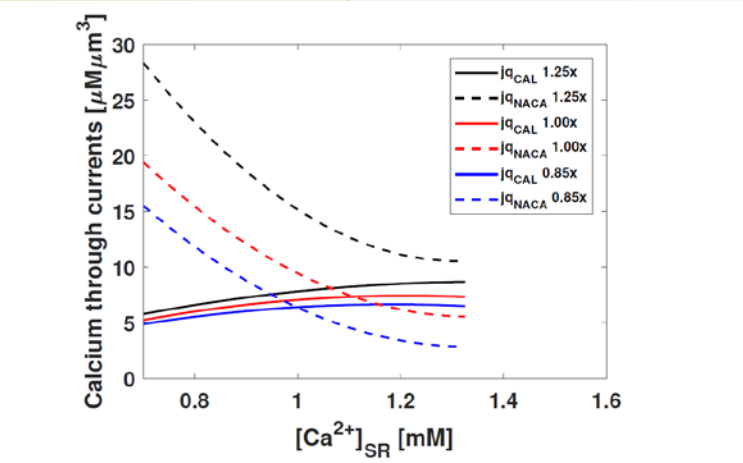
Changing LCC conductivity in our cell

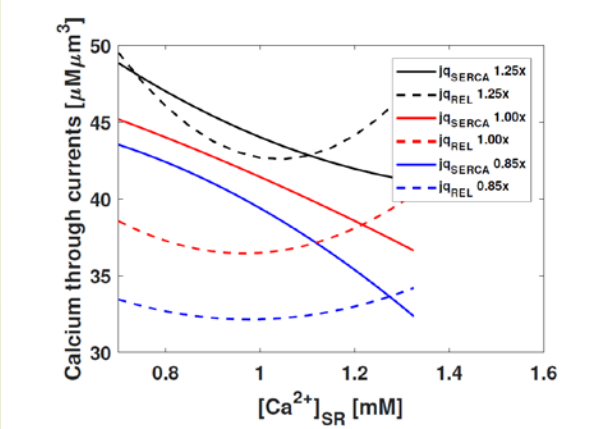


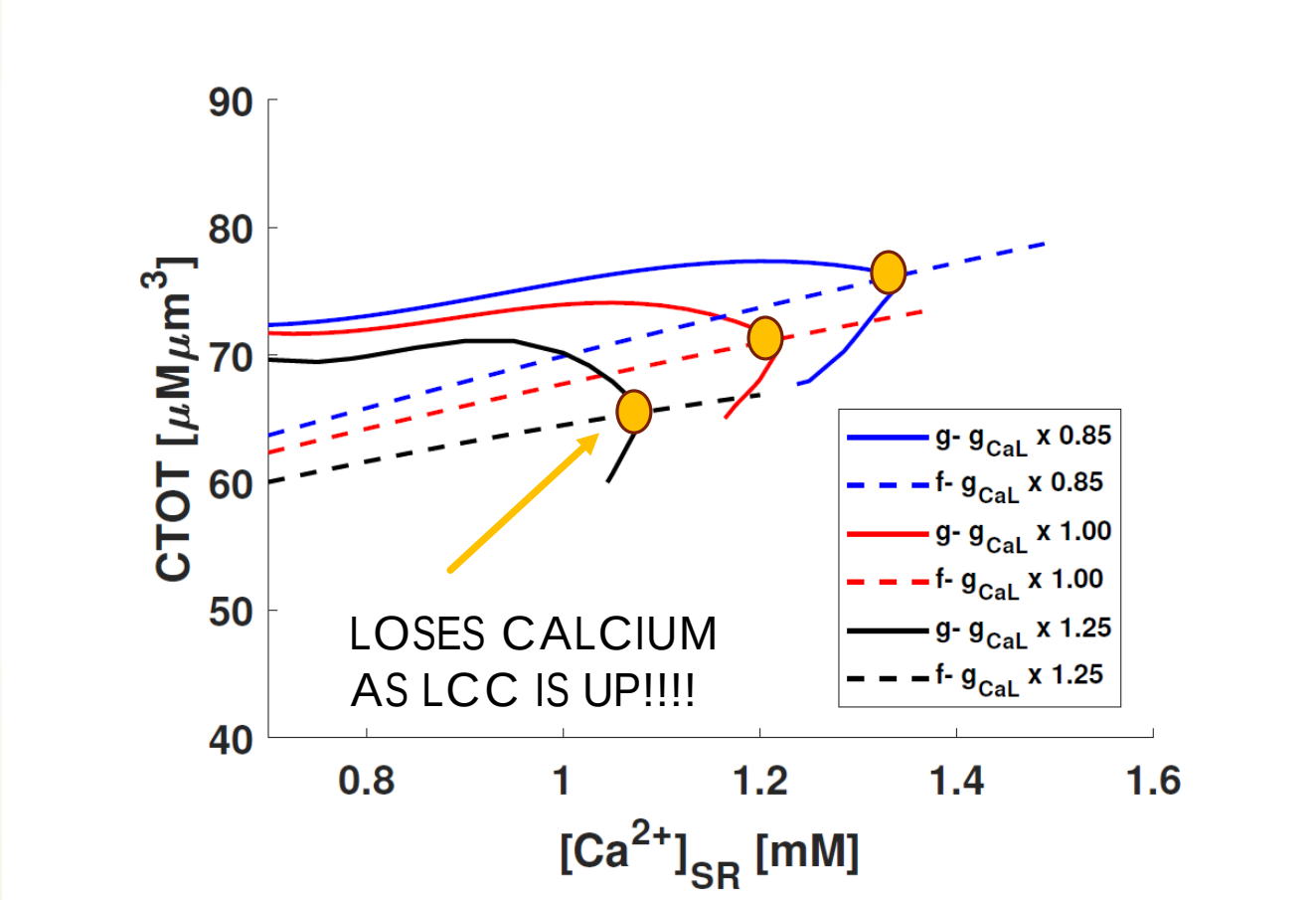


A relatively small change in LCC produces a huge change in release which has consequences in exchanger and SERCA

Changing LCC conductivity in our cell







Conclusion and discussion

- We have developed a framework to understand how calcium cycling homeostasis work.
- This framework leads to counterintuitive prediction about calcium cycling homeostasis. For example, increasing LCC open probability or conductivity can lead to a depletion of calcium of the cell.
- We would like to apply this framework to understand the different behavior in force-frequency relation among species and also in diseases or channelopathies.
- We would like to generalize this framework to situations where the voltage is not clamped in order to study the presence and control of arrhythmias.