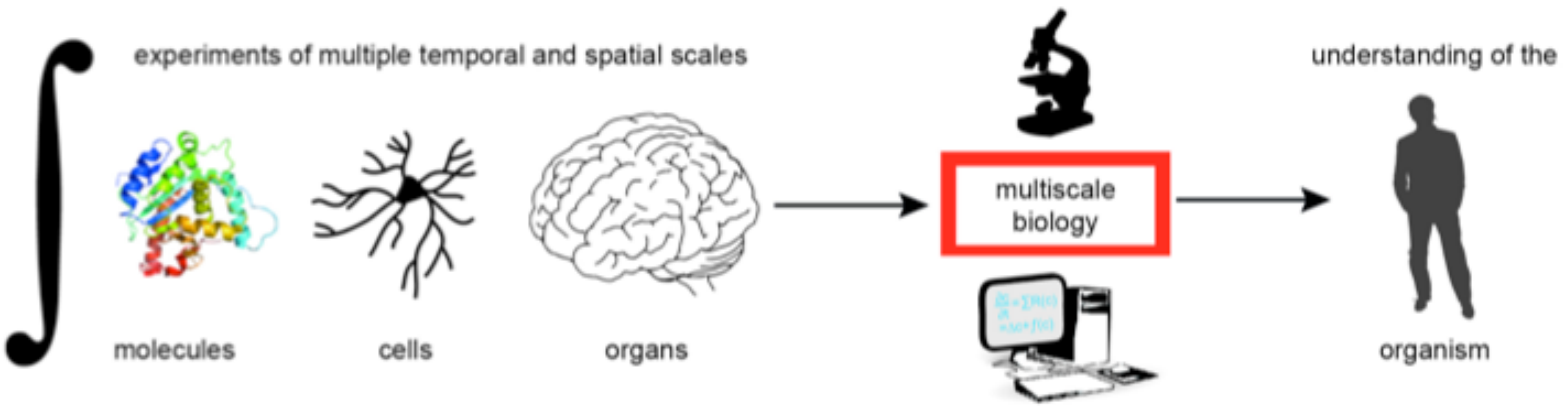
Calcium spiking as a mediator between metabolism and cell fate

– A life and death approach –



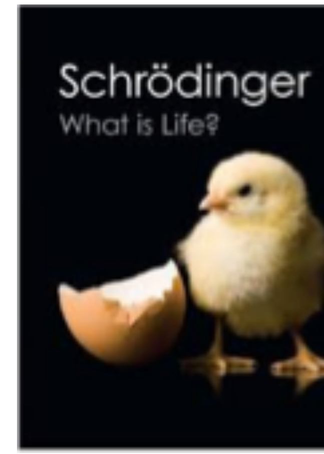
Alexander Skupin
Integrative Cell Signalling Group

Solvay Workshop
05/05/2018

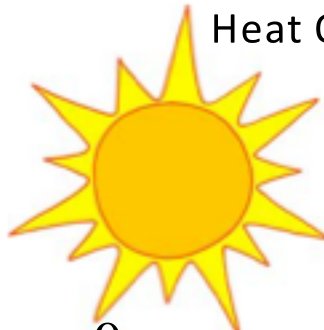
Luxembourg Centre for Systems Biomedicine

What is Life?

- **Complex self-organization**
- **“order from disorder”**
- **Robust information conservation and replication**
- **… the coolest thing on the planet**



Relies on non-equilibrium:



Heat Q and T=5000K

Q and T=300K

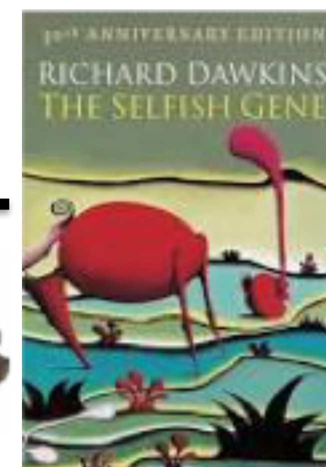
Order needs entropy export:

$$\Delta S = \frac{\delta Q}{5000K} - \frac{\delta Q}{300K} < 0$$

$$S = -k_B \sum x_k \ln x_k$$

$$\Rightarrow S_{long} < S_{short}$$

$$S_{long} = \Delta S_{cow} + S_{short} \Rightarrow \Delta S_{cow} < 0$$



What is Life?

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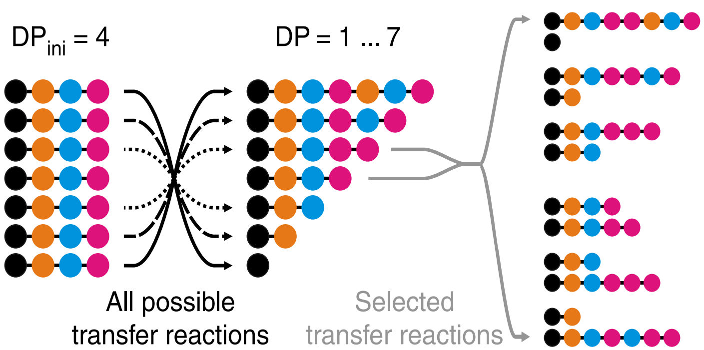
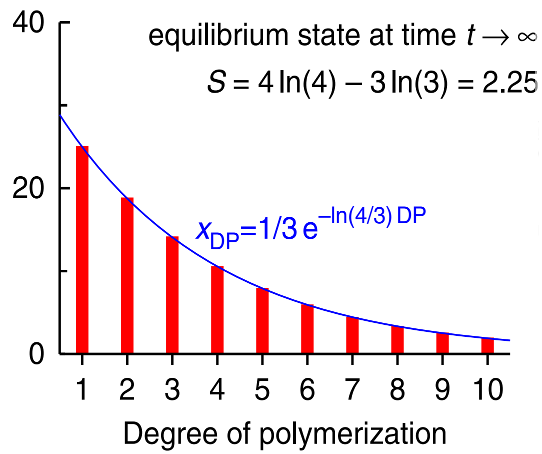
Only abstract framework? What's about real (molecular) life?

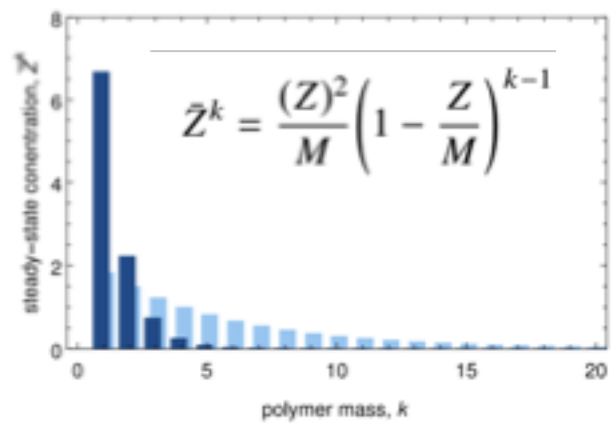
→ Disproportionating enzyms (DPE) use entropy for glycogen metabolism!

$$G_n + G_m \longleftrightarrow G_{n-q} + G_{m+q}$$

with $q = 1,2,3$







$$S_{ini} < S_{end}$$

$$x_{DP} = \left(e^{\beta} - 1\right) \cdot e^{-\beta \cdot DP} \quad \text{with} \quad \beta = \ln\left(\frac{DP_{ini}}{DP_{ini} - 1}\right)$$

$$\frac{S_{eq}}{R} = DP_{ini}\ln(DP_{ini}) - (DP_{ini} - 1)\ln(DP_{ini} - 1)$$

(Kartal, MSB 2011)

(Rao, J Chem Phys 2015)

What is Life?

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→ Disproportionating enzyms (DPE) use entropy for glycogen metabolism!

Life: smart way to use energy for information processing

(Kartal, MSB 2011)

(Rao, J Chem Phys 2015)

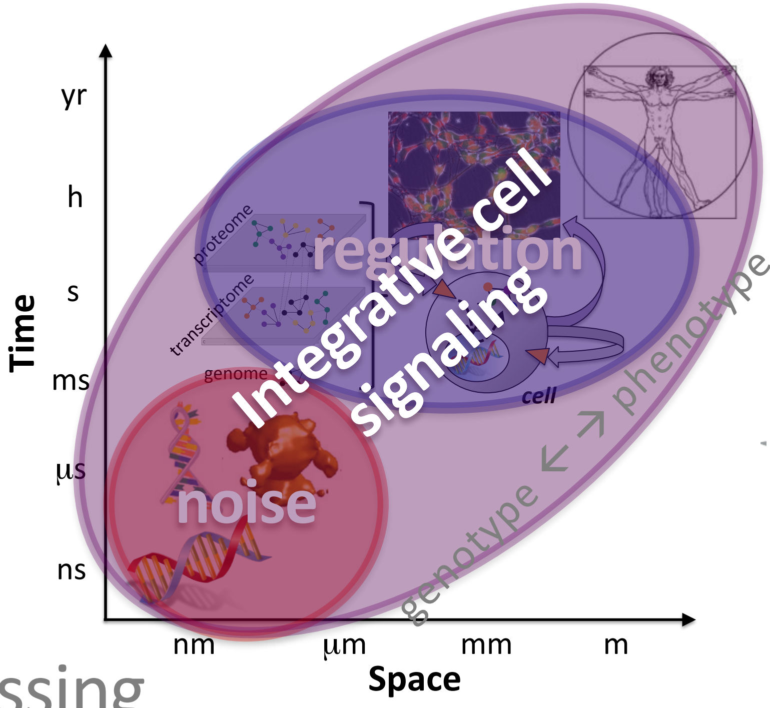
How Does (Multicellular) Life Work?

"Nothing in Biology Makes Sense Except in the Light of Evolution"

(Theodosius Dobzhansky 1973)

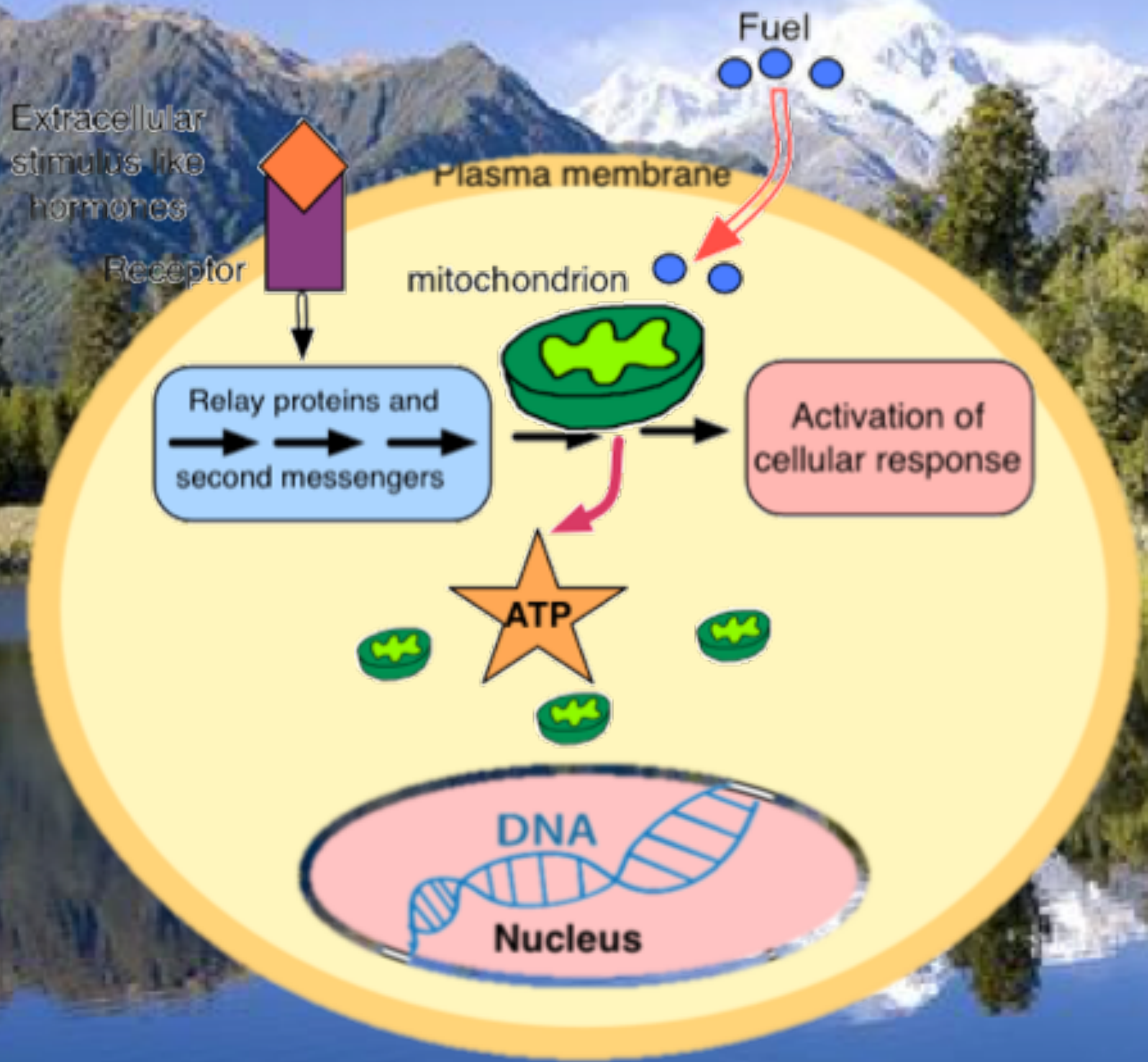
Evolution := mutation × selection
→ heterogeneity × dynamics

"Nothing in Biology Makes Sense Except in the Light of *Cellular Heterogeneity Dynamics* "



Life := noise × regulation
→ energy × information processing

Challenge of Life:
balance energy and information processing



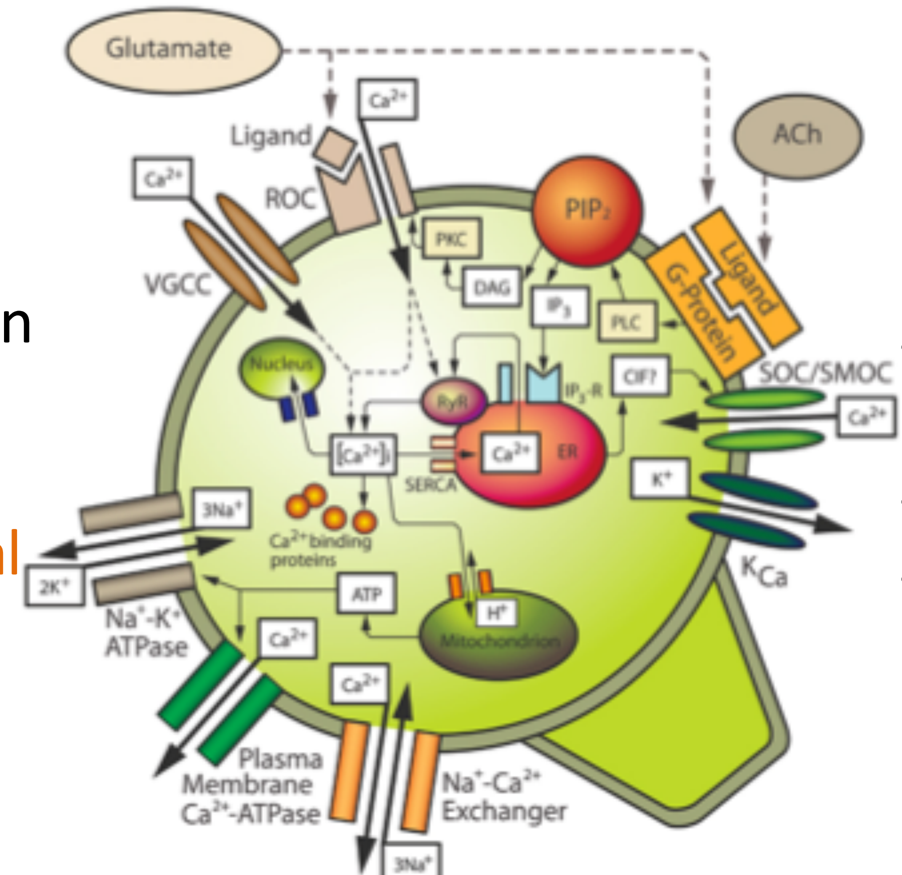
→ using energy **for gene-environment adaptation** away from equilibrium

The bright and dark side of Ca^{2+}

Life and death signal

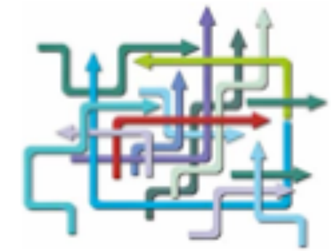
involved in:

- Fertilization
- Transcription
- Differentiation
- Cell Cycle
- Motility
- Mitochondrial activity
- …
- Apoptosis



(Weber 2012)

Complexity



- What is triggering what?
- Who comes first?
- How to dissect complex signaling mechanisms?

Complex development of Parkinson's Disease

Genotype

Parkin, PINK, DJ-1,
α-syn., **LRRK2**, …

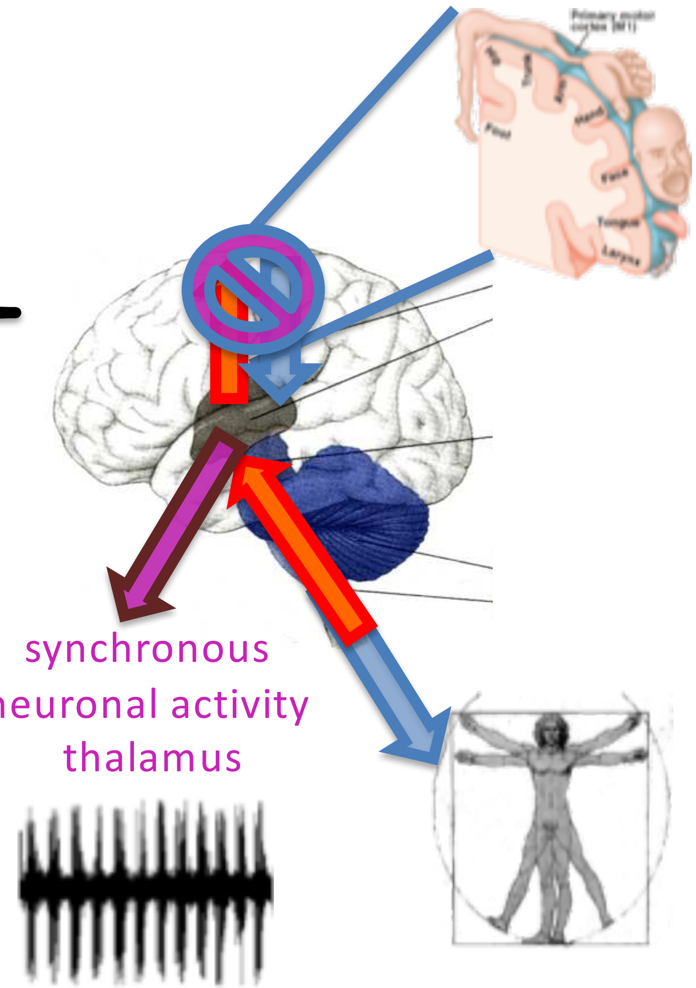
→ link to mitochondrial dysfunction

Environment/Lifestyle

Toxins accumulation, drug consume, food, exercise, …

Physiological Phenotype

→ death of dopaminergic in *substancia nigra*



Clinical Phenotype

tremor, rigidity, …

Complex development of Parkinson's Disease

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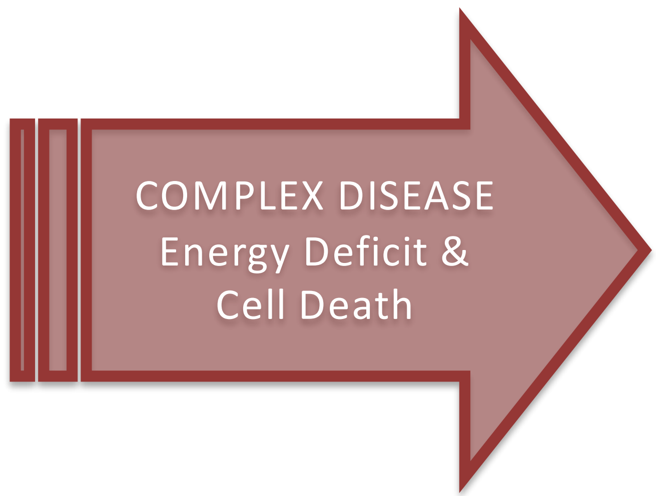
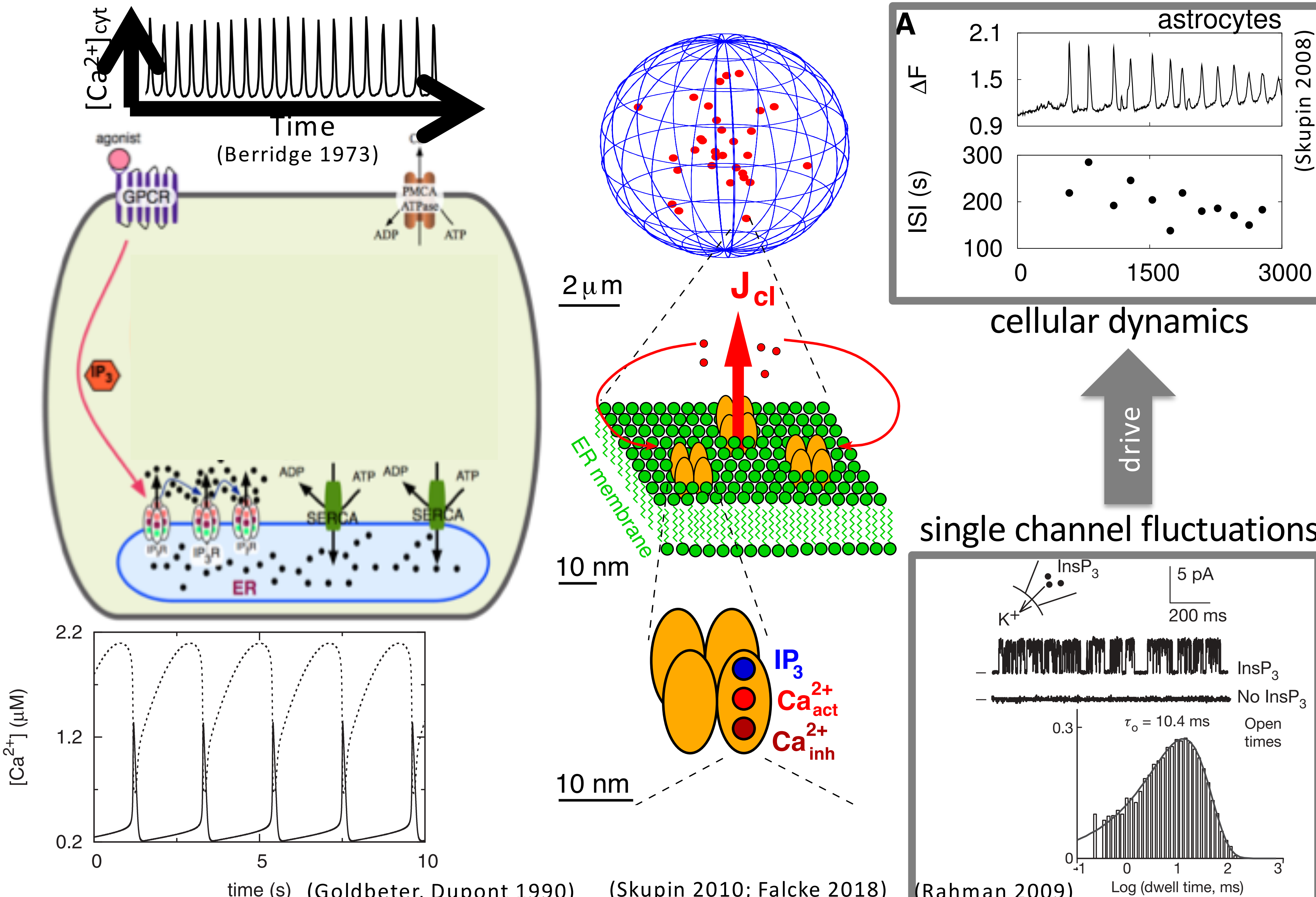


- Ca^{2+} as a mediator
- Surmeier hypothesis on selective vulnerability
- Brain energy metabolism as mechanism in neurodegeneration

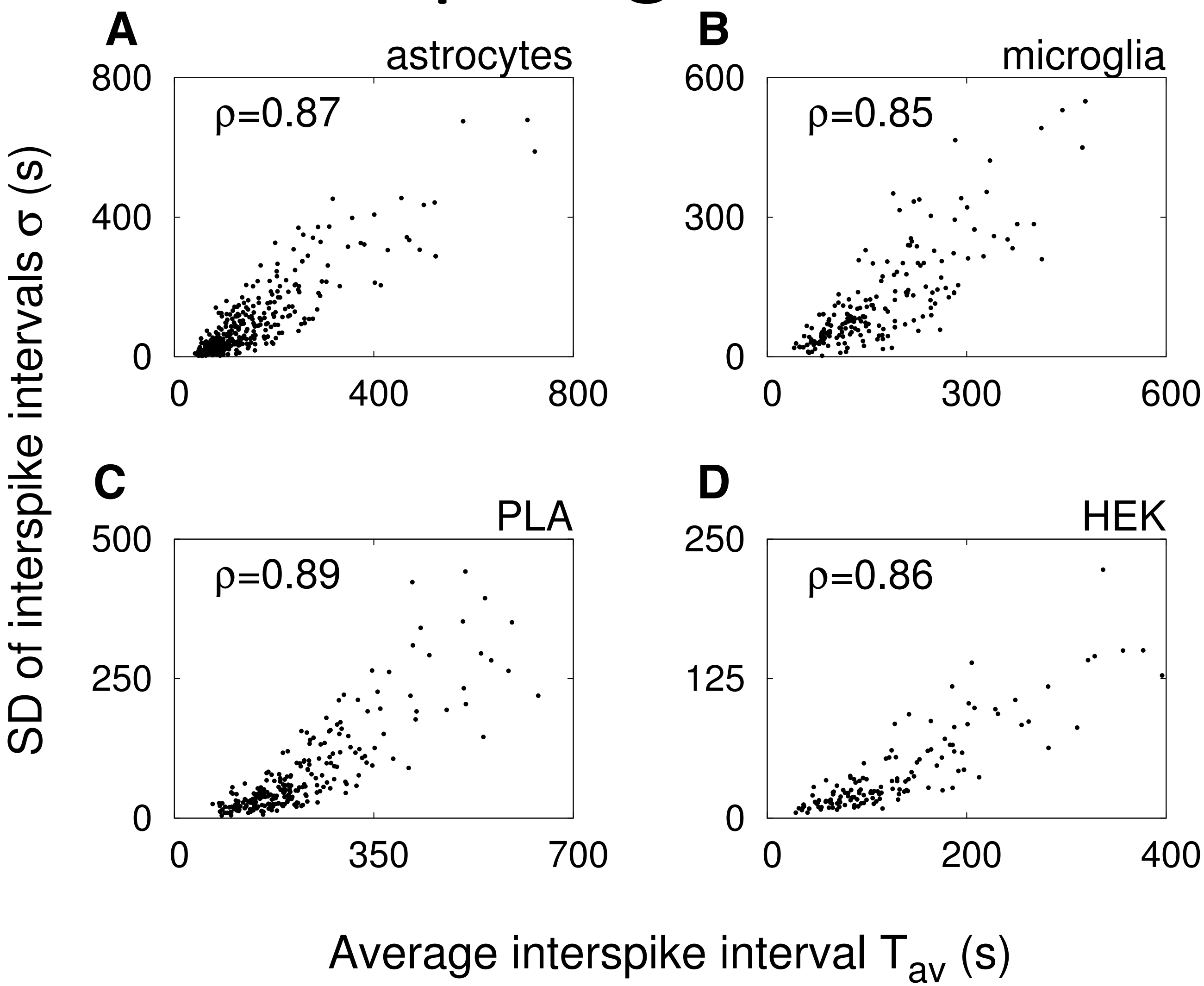
Clinical Phenotype

tremor, rigidity, …

Hierarchical implications of Ca^{2+} signals



Statistical spiking characteristics



- linear relation between σ and mean → evidence for stochastic process
- condition (cell type & stimulation) specific slope

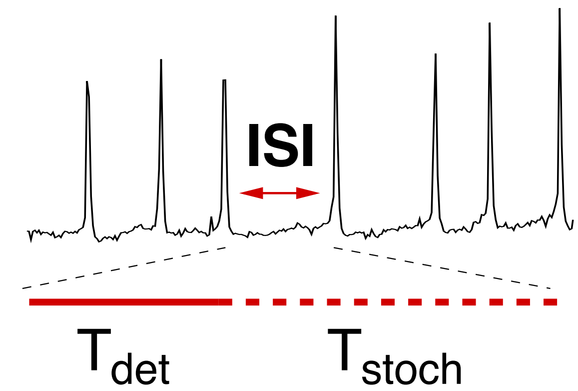
Probabilistic model

T_{stoch} described by:

- time-dependent Poisson process with rate $\Lambda(t) = \lambda\left(1 - e^{-\xi t}\right)$
  - $\lambda \Rightarrow$ nucleation rate
  - $\xi \Rightarrow$ recovery rate
- probability of a spike at time t:

$$P_\xi(t) = \lambda\left(1 - e^{-\xi t}\right) \exp\left[-\int_0^t \lambda\left(1 - e^{-\xi t'}\right) dt'\right]$$

ISI consists of 2 parts ...

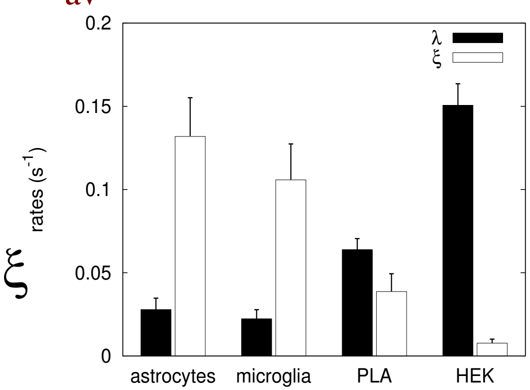


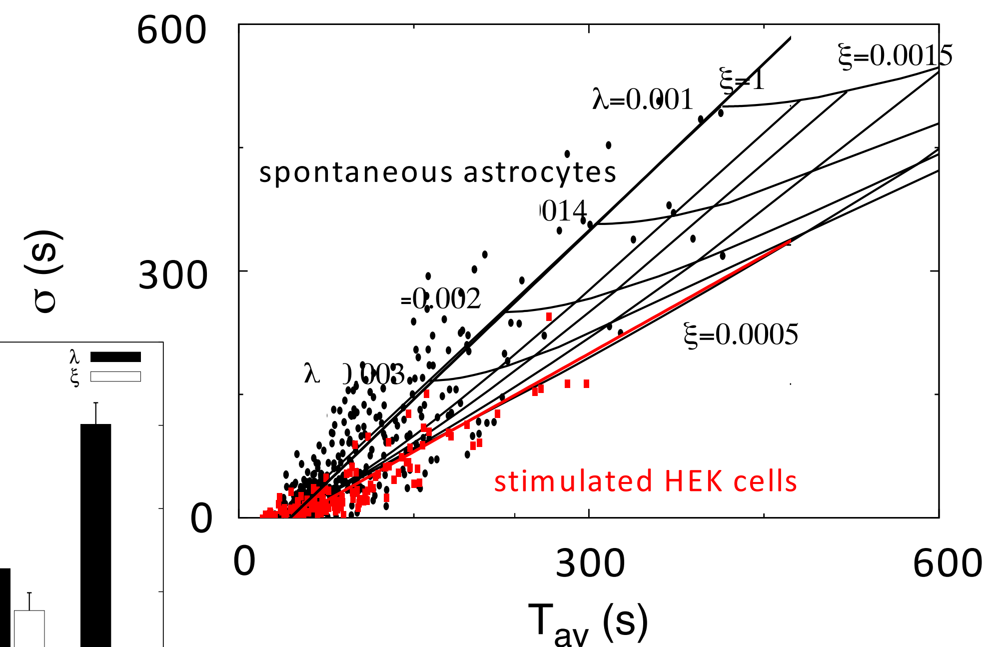
Features of statistical moments

- minimal ISI
- linear dependence of standard deviation σ on mean T_{av}

→ fitting gives:

- individuals cells: λ
- different cell types: ξ





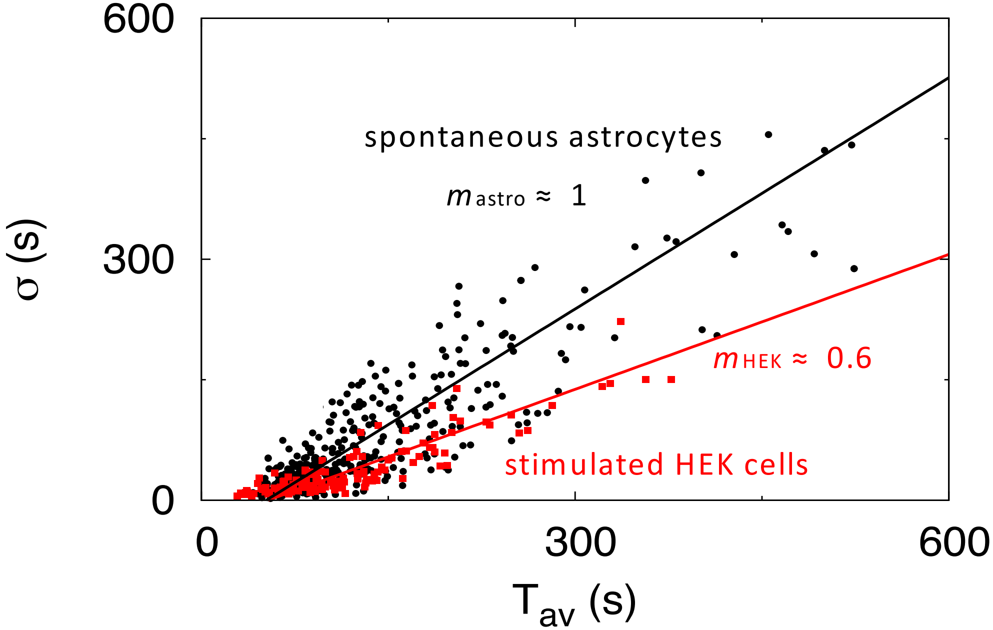
Information content of the environment

Information gain:

$$\mathcal{K}(p_1, p_2) = k \int_0^\infty p_1(t) \log \frac{p_1(t)}{p_2(t)} dt$$

For pure P_{poi} & recovery P_ξ Poisson

$$\begin{aligned}\mathcal{K}_\xi &= k\left[\mathcal{H}(\lambda/\xi) + \frac{1}{(1+\lambda/\xi)} - 1\right] \\ &= f(\lambda/\xi)\end{aligned}$$

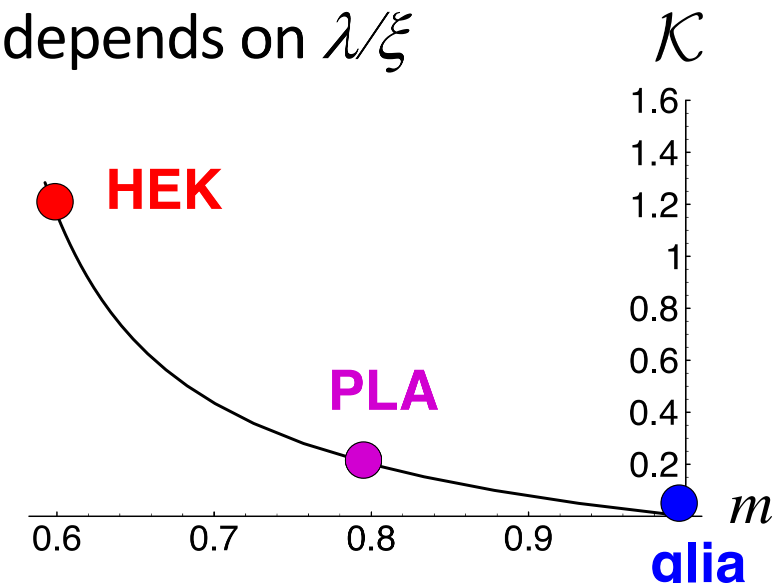


The σ-T_{av} slope $m = g(\lambda/\xi) = CV$ also only depends on λ/ξ

since P_ξ leads to first 2 moments:

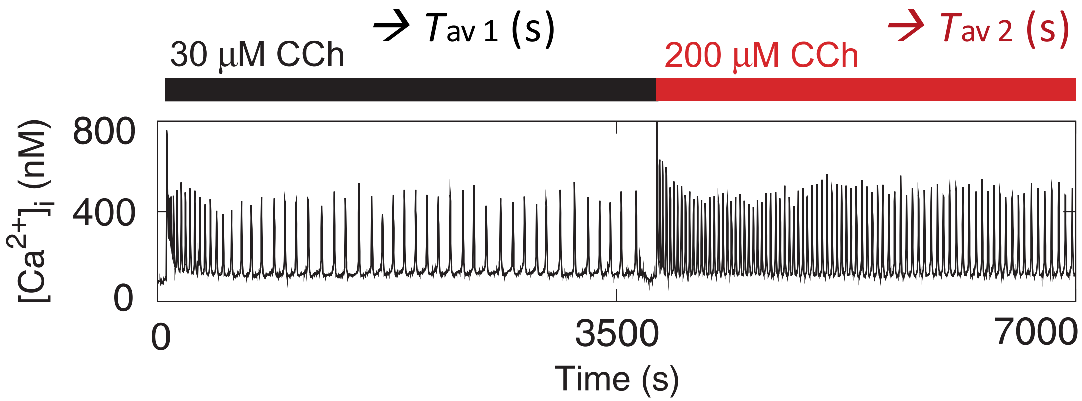
$$\mathrm{T}_{\mathrm{stoch}} = \frac{e^{\frac{\lambda}{\xi}} \left(\frac{\lambda}{\xi}\right)^{1-\frac{\lambda}{\xi}}}{\lambda}\left[\Gamma\left(\frac{\lambda}{\xi}\right) - \Gamma\left(\frac{\lambda}{\xi}, \frac{\lambda}{\xi}\right)\right]$$

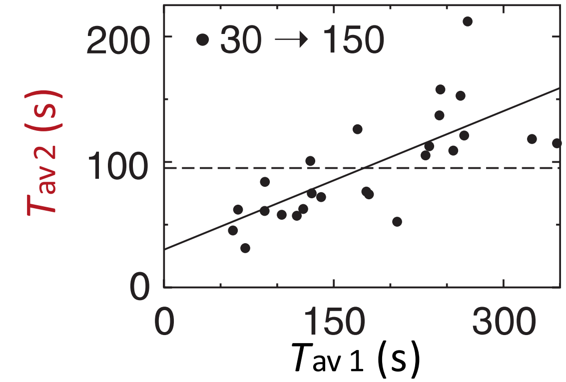
$$\langle \mathrm{T}^2 \rangle = \frac{2e^{\frac{\lambda}{\xi}}}{\lambda^2} {}_2\mathrm{F}_2\left[\left(\frac{\lambda}{\xi}, \frac{\lambda}{\xi}\right), \left(1+\frac{\lambda}{\xi}, 1+\frac{\lambda}{\xi}\right), -\frac{\lambda}{\xi}\right]$$



Encoding of information within cells

[Thurley 2014]

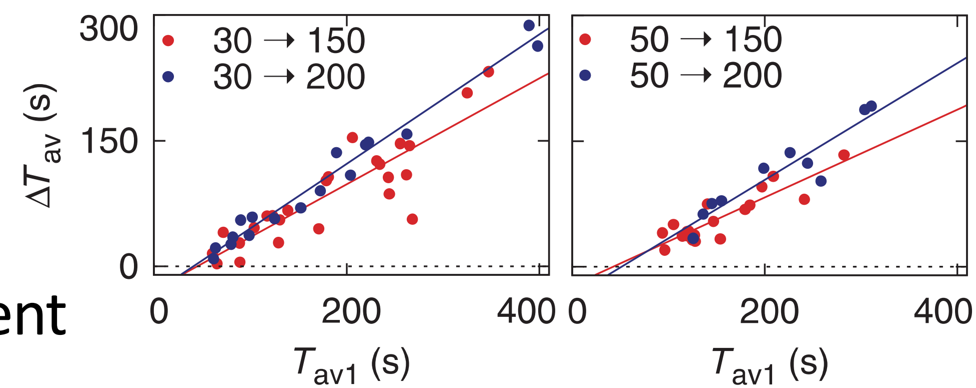




Indicating fold change behavior:

$$\beta = \frac{T_{av1} - T_{av2}}{T_{av1}} \Rightarrow \quad \Delta T_{av} = \beta \cdot T_{av1}$$

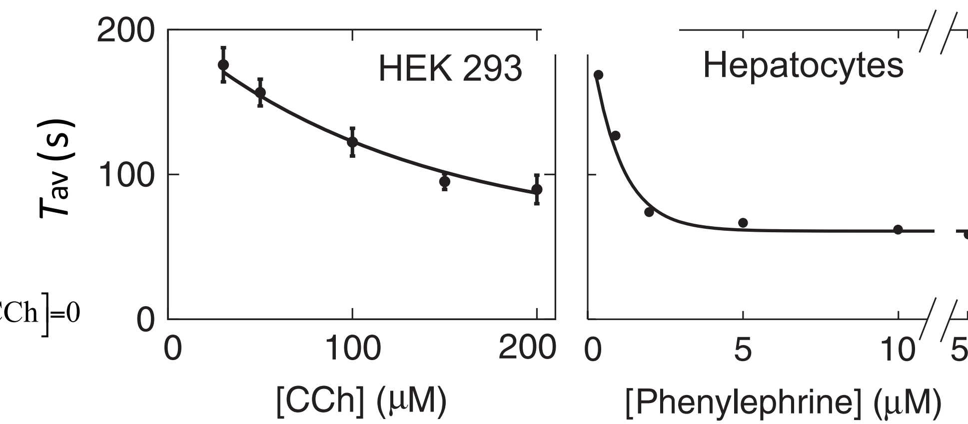
with Weber fraction β independent of reference concentration c_0



Encoding relation

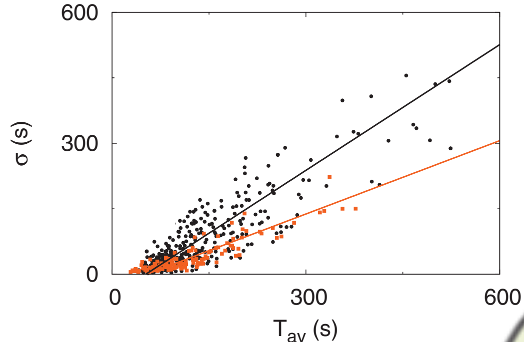
$$\frac{dT_{av}}{d\left[CCh\right]} = -\gamma \cdot T_{av}, \quad \text{with} \quad \gamma = \left.\frac{\partial \beta}{\partial \Delta\left[CCh\right]}\right|_{\Delta[CCh]=0}$$

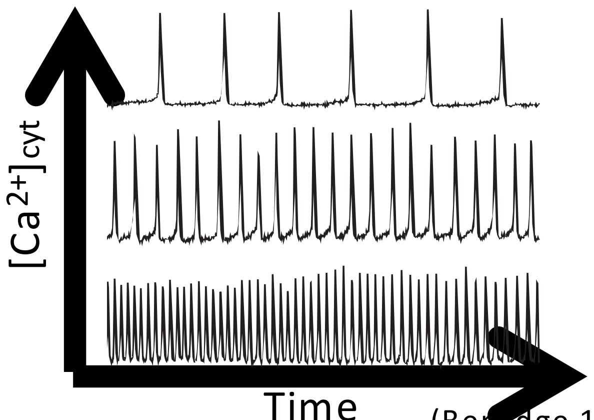
leading to $\beta = 1 - e^{-\gamma \Delta[CCh]}$



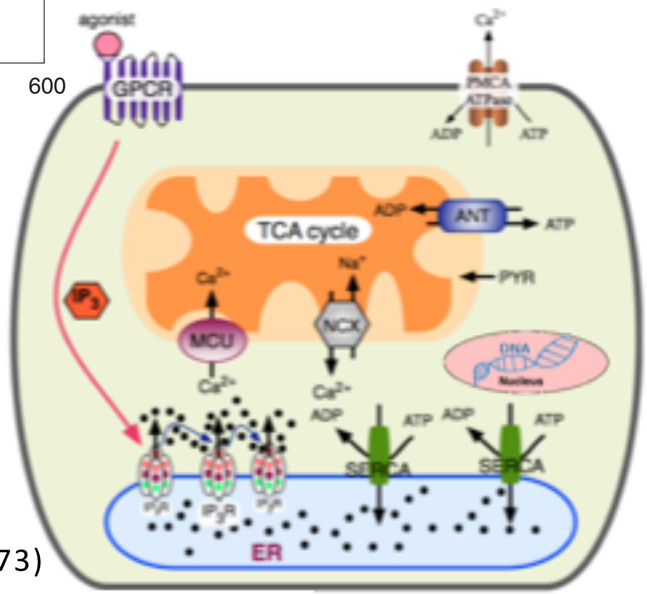
Implications for life (of cells)?

Stochasticity of CICR (Skupin 2008)



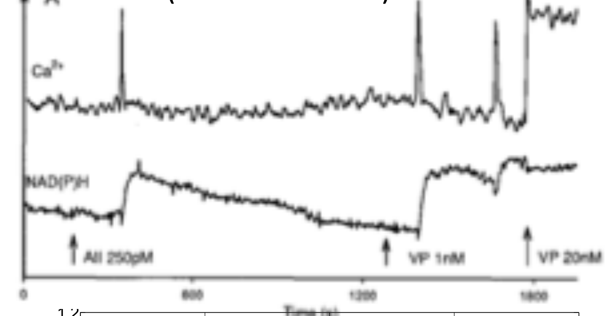


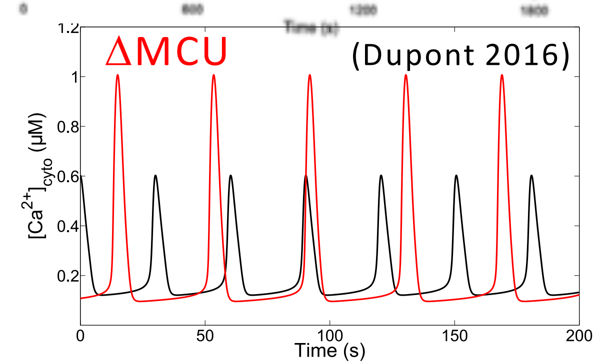
(Berridge 1973)



Mitochondria activation

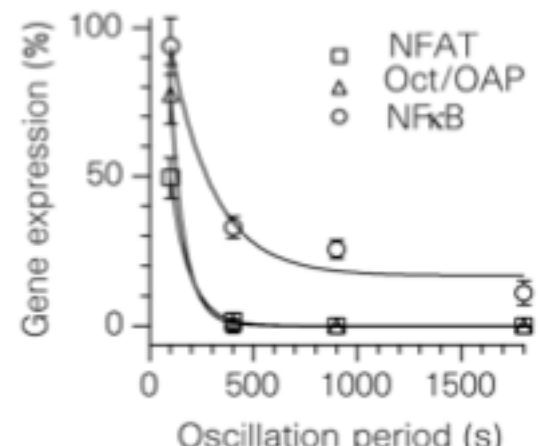
(Thomas 1995)





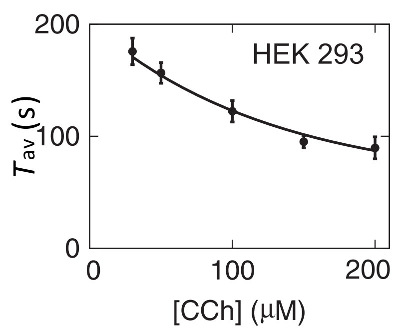
Gene Expression

(Dolmetsch 1999)

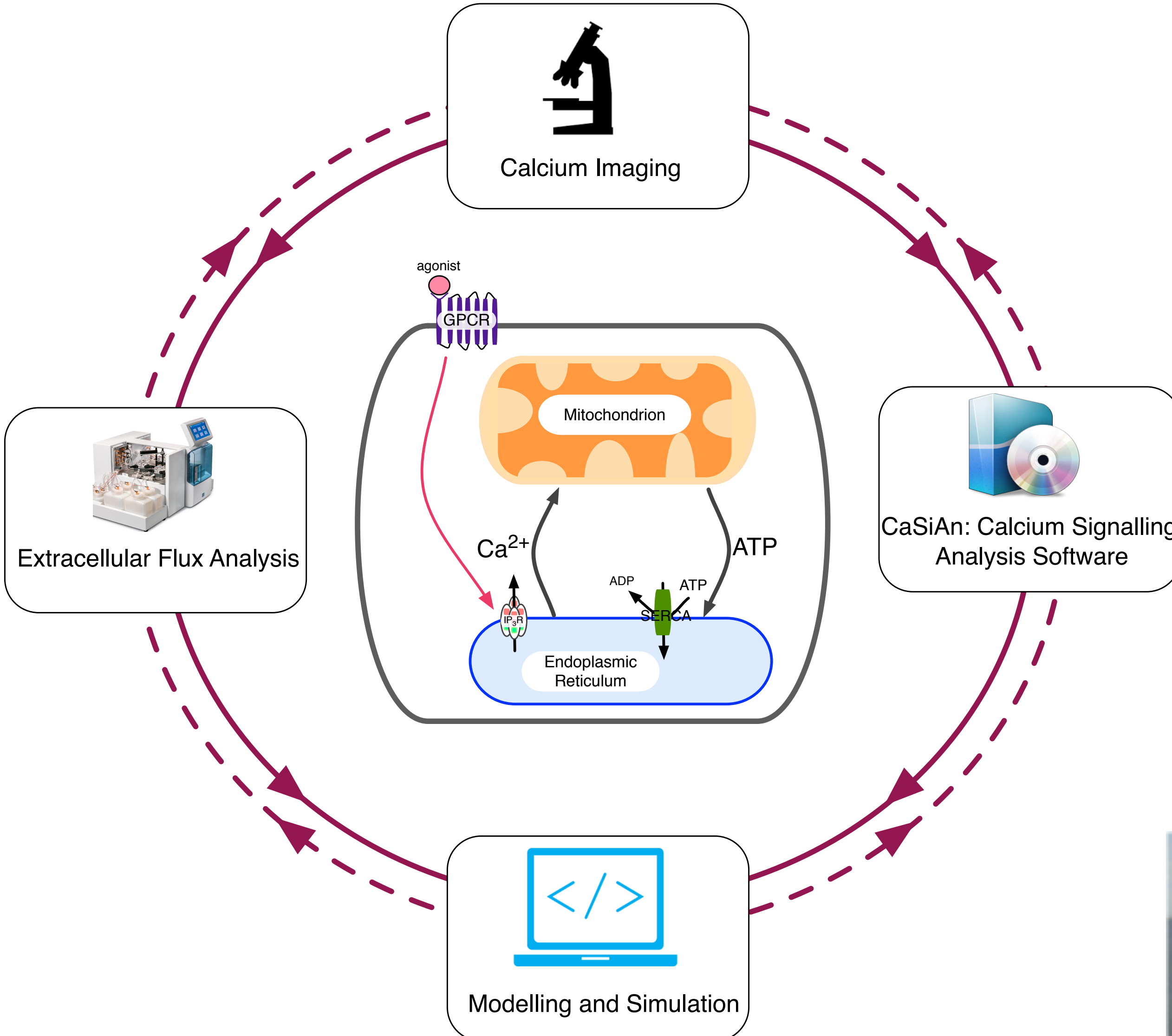


Encoding relation (Thurley 2014)

$$\frac{dT_{av}}{d[CCh]} = -\gamma \cdot T_{av}$$



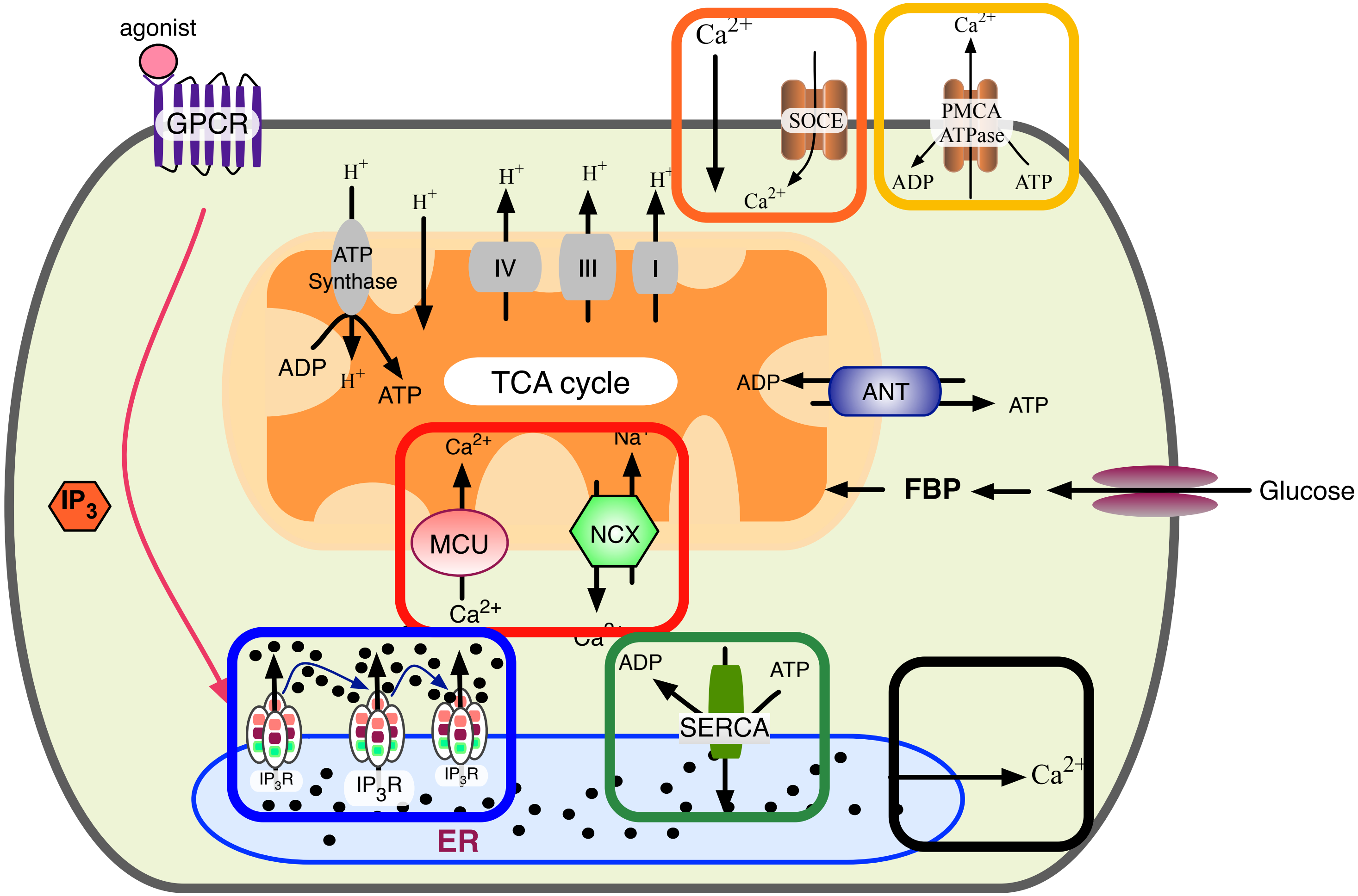
I. Dissecting crosstalk for metabolic decoding



by interdisciplinary approaches

Starting from computational model

$$\frac{\mathrm{d}Ca_c}{\mathrm{d}t} = f_{\mathrm{cyt}}\left(\boxed{J_{\mathrm{ext}}} + v_{\mathrm{m/c}}\left(\boxed{J_{\mathrm{NCX}} - J_{\mathrm{MCU}}}\right) - \boxed{J_{\mathrm{SERCA}}} + \boxed{J_{\mathrm{release}}} + \boxed{J_{\mathrm{leak,er}}} - \boxed{J_{\mathrm{PMCA}}}\right)$$



7 variables, 3 conservation laws, 16 flux expressions and 58 parameters.

Deterministic model based on previous work

Ca^{2+} Signaling Model

$$\frac{dCa_c}{dt} = f_{cyt}\left(J_{ext} + v_{m/c}\boxed{\left(J_{NCX} - J_{MCU}\right)} - J_{SERCA} + J_{release} + J_{leak,er} - J_{PMCA}\right)$$

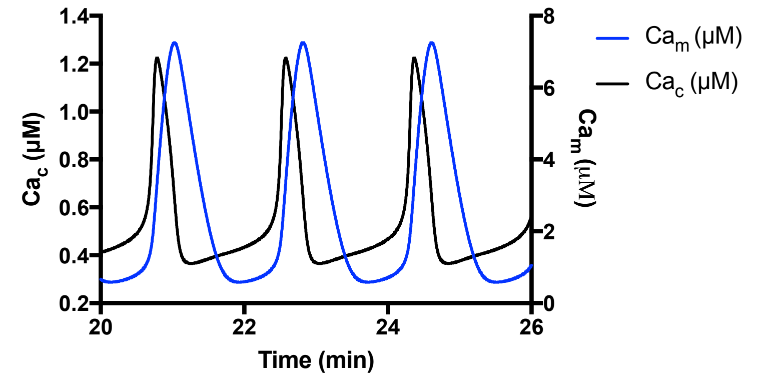
Mitochondrial calcium shape cytosolic calcium

$$\frac{dCa_{er}}{dt} = f_{er}v_{c/er}\left(J_{SERCA} - J_{release} - J_{leak,er}\right)$$

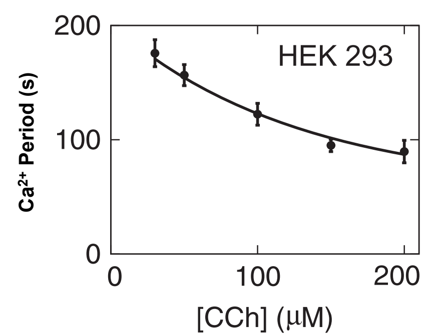
$$\frac{dCa_m}{dt} = f_m\left(J_{MCU} - J_{NCX}\right)$$

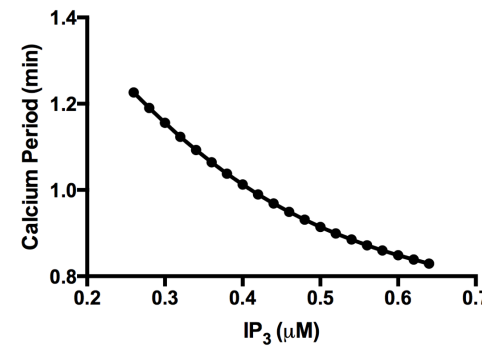
ATP is consumed by Ca^{2+} ATPase pumps

$$\frac{dADP_c}{dt} = -v_{m/c}J_{ANT} + J_{hyd,bas} + \boxed{2J_{PMCA}} + \boxed{J_{SERCA}}$$



Encoding check:





Bertram Mitochondrial Model

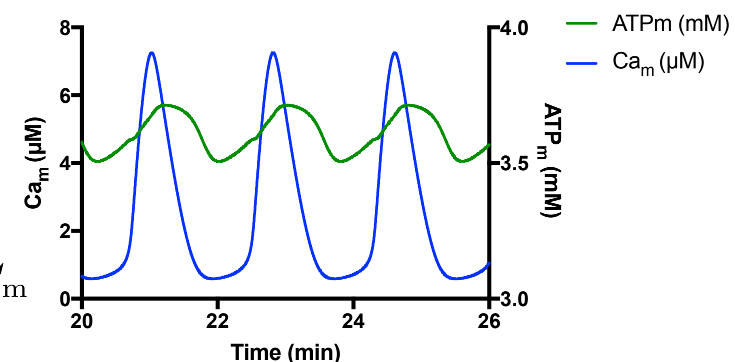
[Bertram 2006]

$$\frac{dNADH_m}{dt} = \gamma\left(J_{PDH} - J_o\right)$$

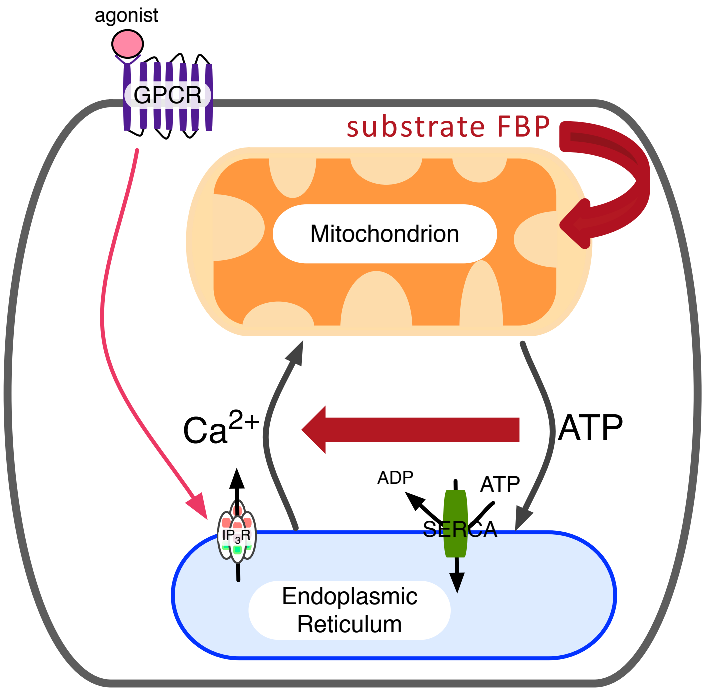
$$\frac{dADP_m}{dt} = \gamma\left(J_{ANT} - J_{F_1F_0}\right)$$

Ca^{2+} affects mitochondrial membrane potential

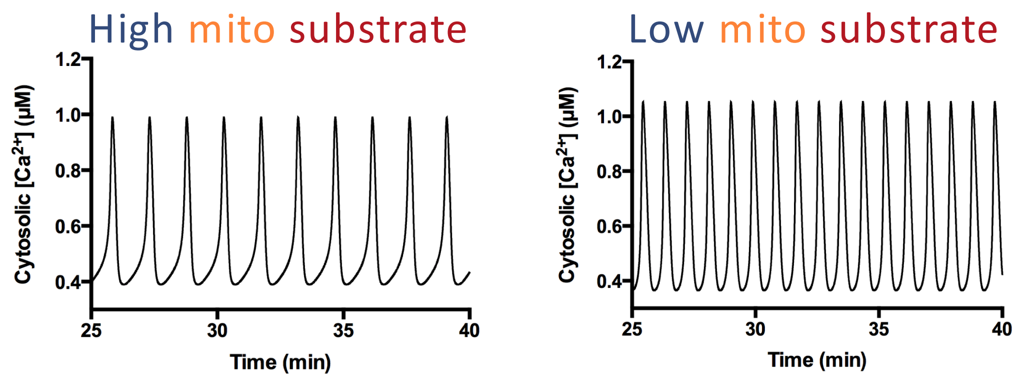
$$\frac{d\Delta\Psi}{dt} = \left(J_{H,res} - J_{H,atp} - J_{ANT} - J_{H,leak} - \boxed{J_{NaCa} - 2J_{uni}}\right)/C_m$$

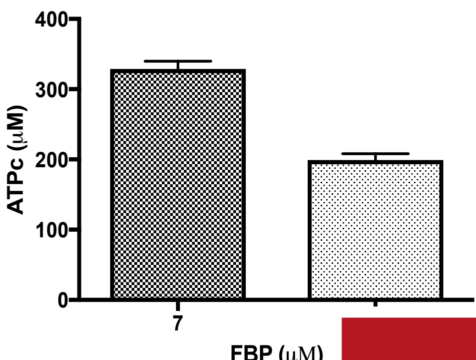


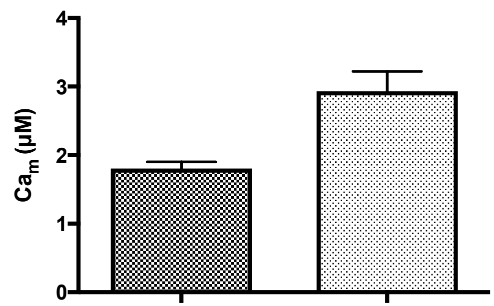
Impact of mitochondrial substrate on Ca^{2+}

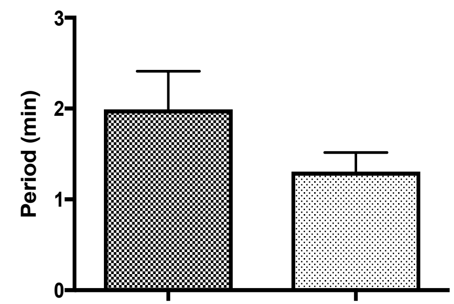


Exemplified model predictions





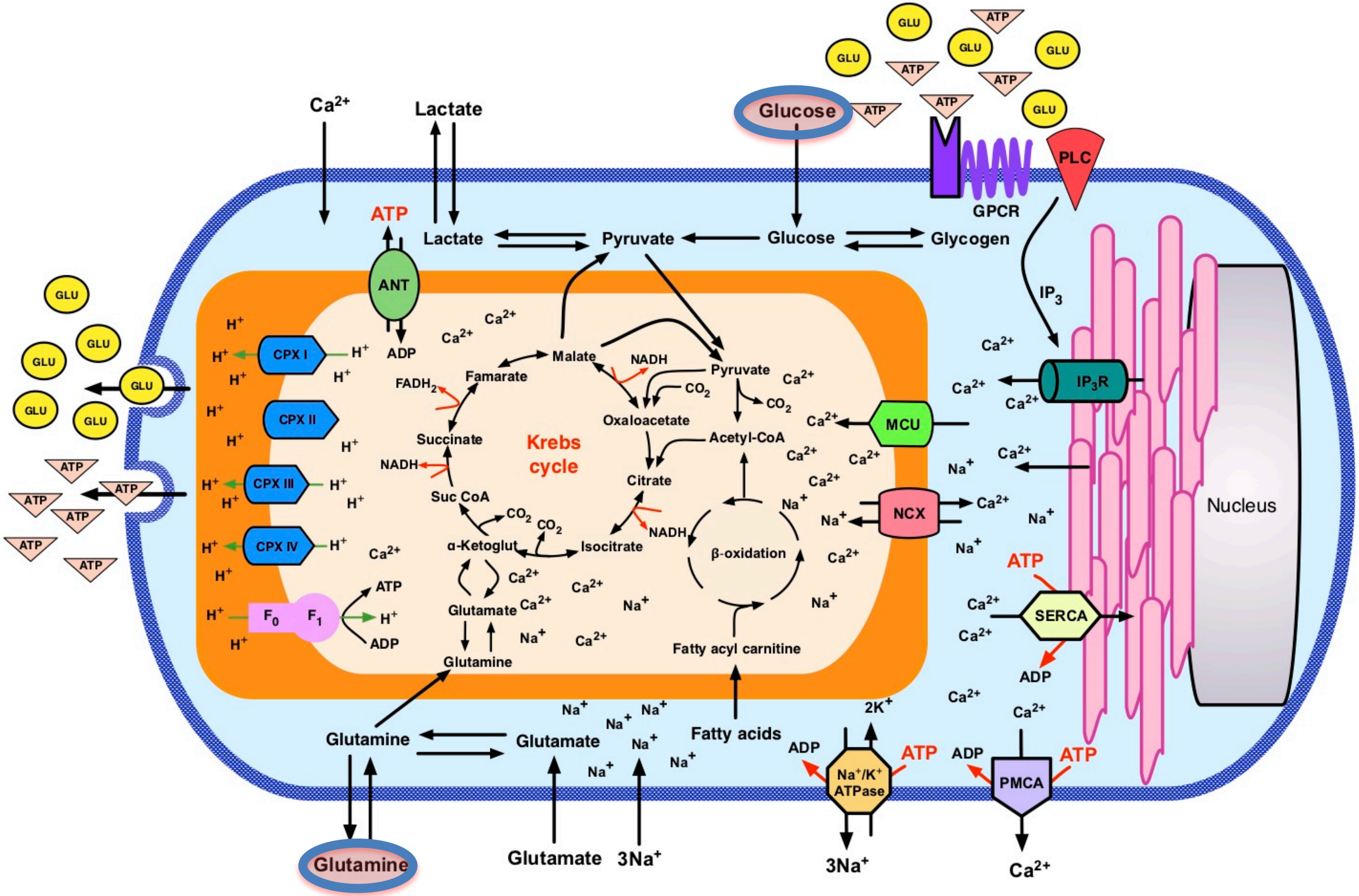




Reality check

Decreasing mitochondrial substrates leads to decreasing cytosolic ATP and increasing frequency of Ca^{2+} signals.

“Reality” in C8-D1A astrocytic cell line

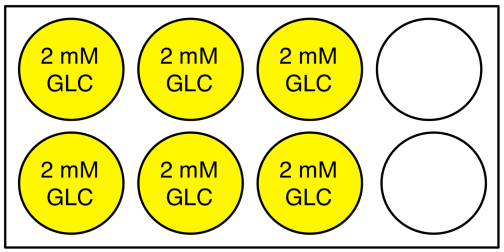


Ca^{2+} - Energy Metabolism Crosstalk

Effect of mitochondrial substrate limitations

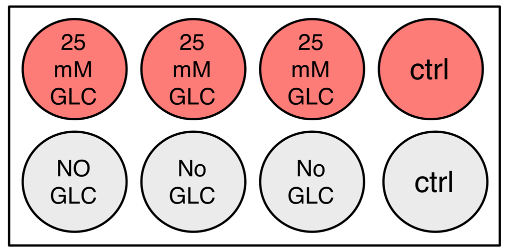
To observe effect "energy buffers" (like glycogen) have to be depleted

(i) "Starving" cells with 2mM glucose for 72h

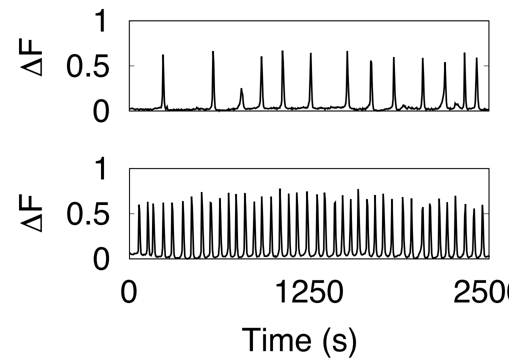


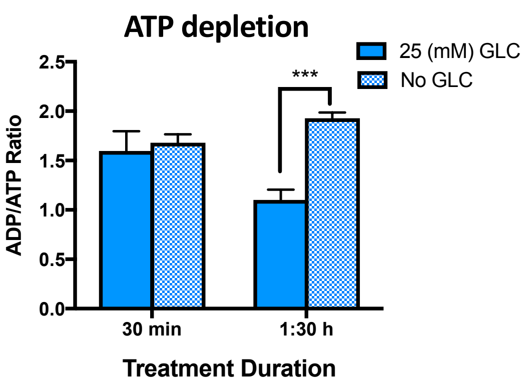
+ 4 mM glutamine

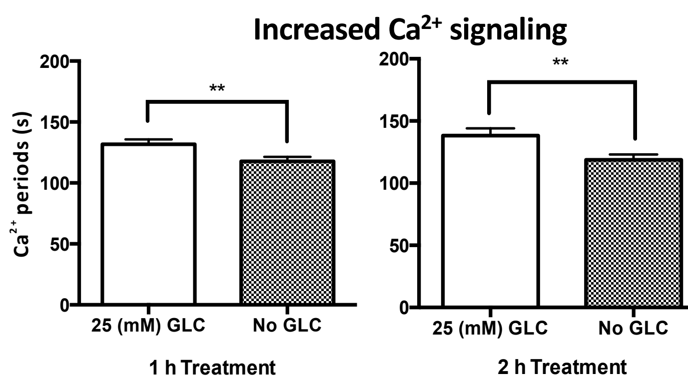
(ii) Changing medium and do measurement

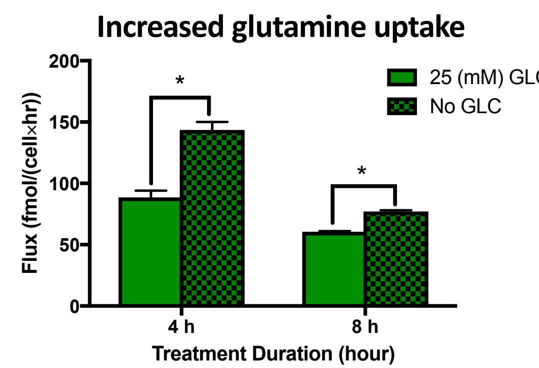


(iii) Equilibrating, stimulation and imaging



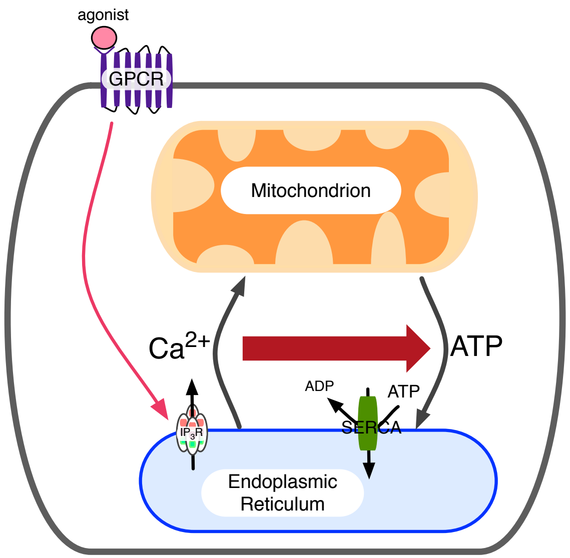




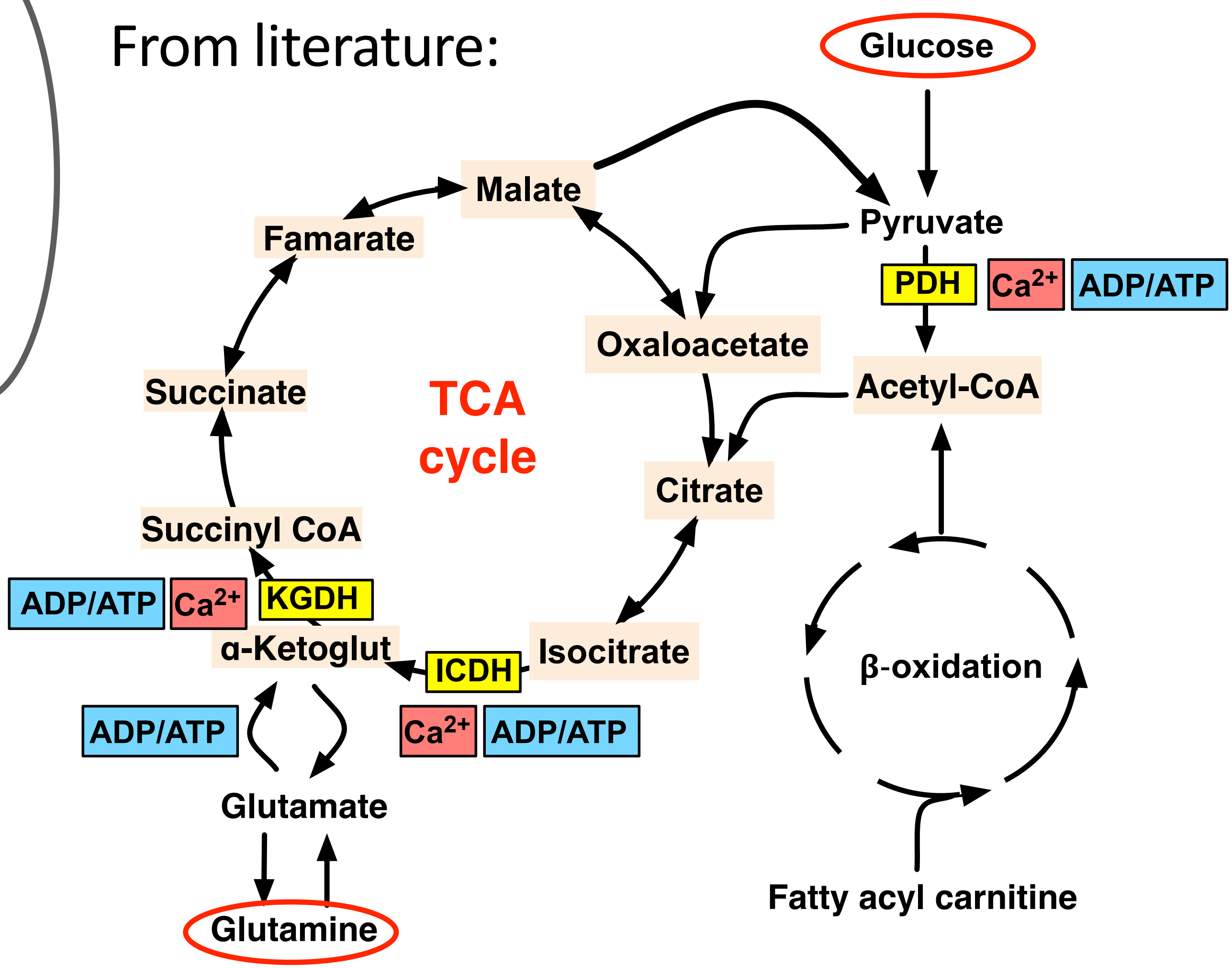


Decreasing mitochondrial carbon inputs decrease cellular ATP levels, increase Ca^{2+} frequency and glutamine uptake

Effect of Ca^{2+} frequency on energy metabolism



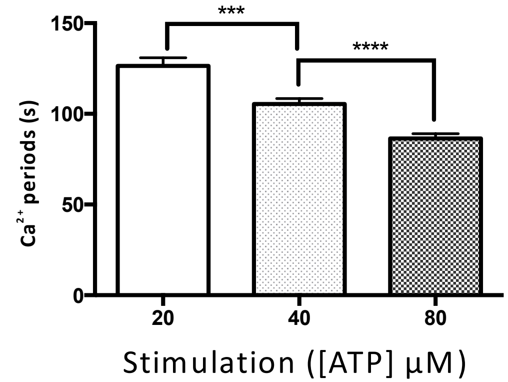
From literature:

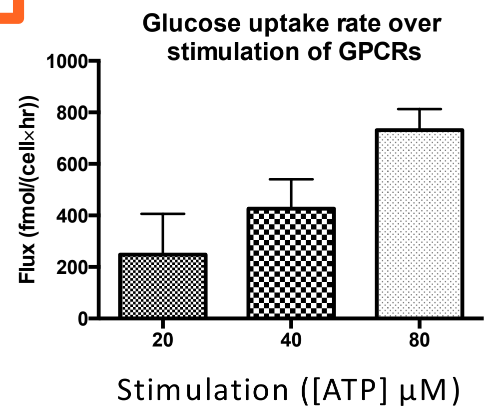


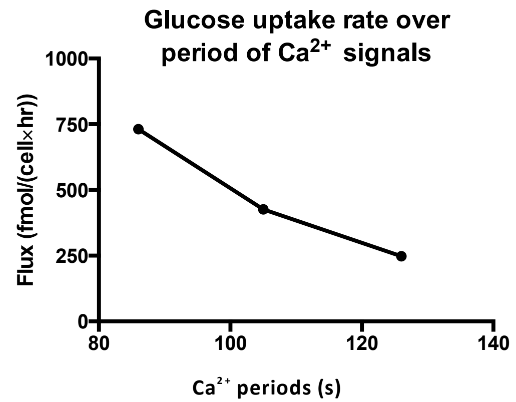
How does Ca^{2+} spiking affect enzyme activity and substrate uptake?

Impact of Ca^{2+} Periods on the Energy Metabolism

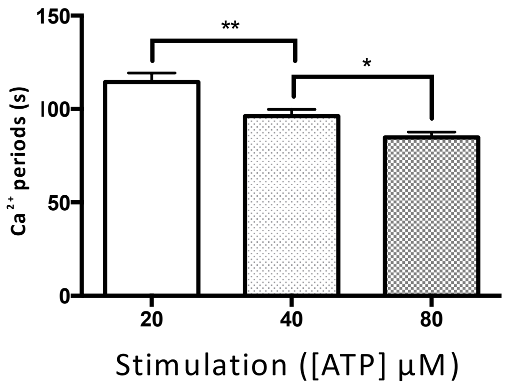
1 mM glucose, no glutamine

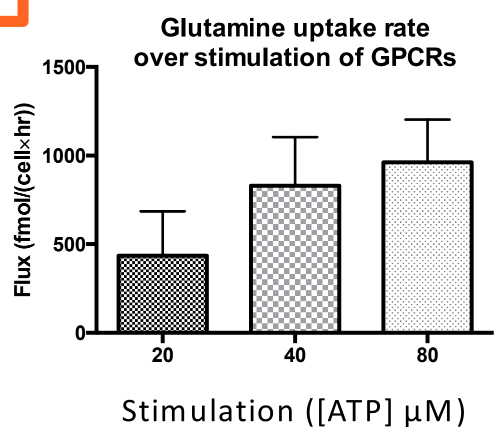


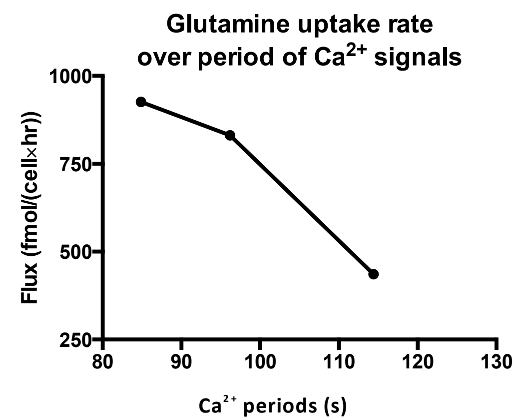




1 mM glutamine, no glucose



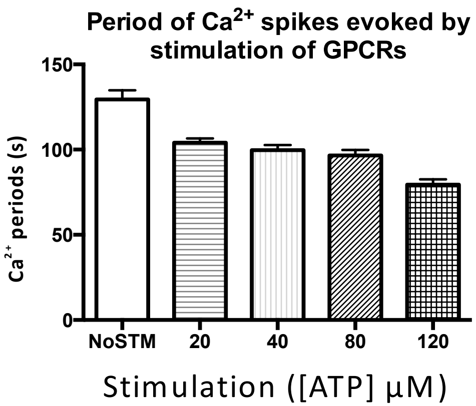


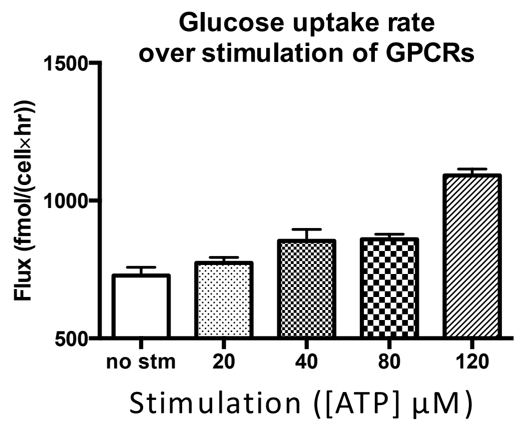


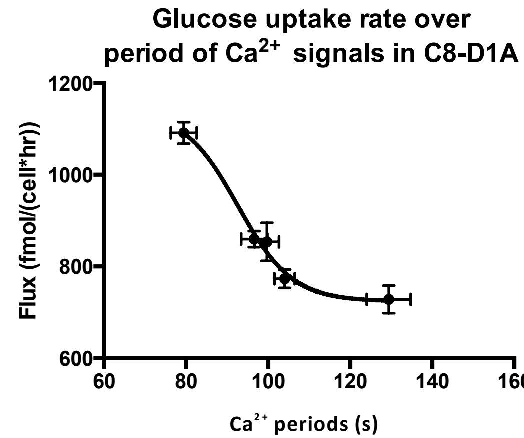
Glucose & glutamine uptake rates increase with decreasing Ca^{2+} periods

Metabolic Decoding of Ca^{2+} Spikes

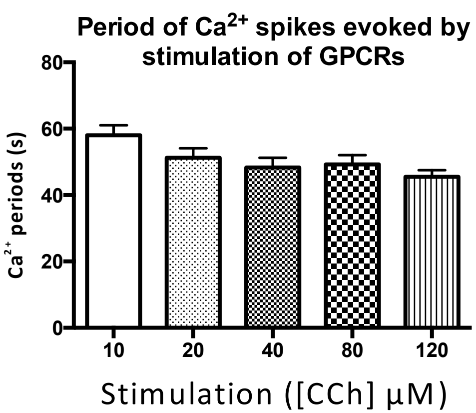
C8-D1A cells

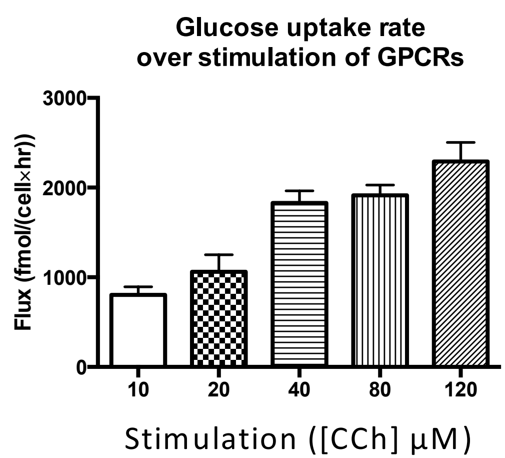


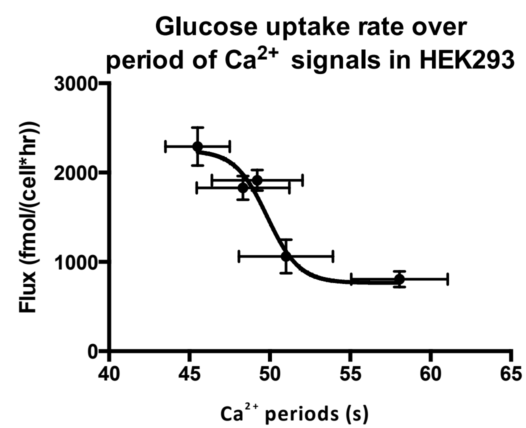




HEK293 cells



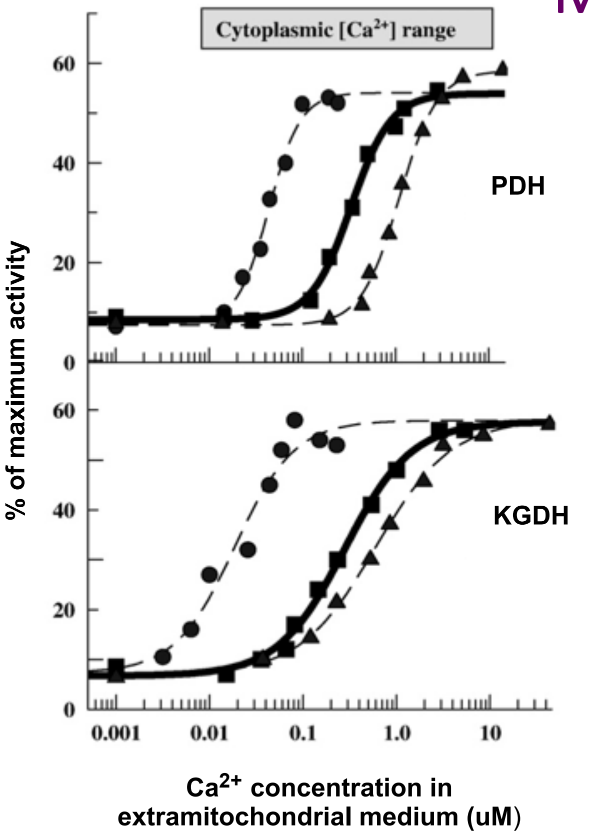




Extracellular stimuli → encoded → **Ca^{2+} signal periods** → decoded → **Metabolic Flux**

Integration of Ca^{2+} triggering activity

Enzyme Activity

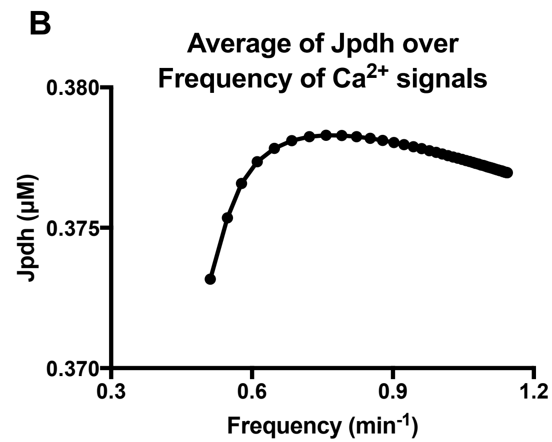


(●), no additions; (■), 15 mM NaCl and 0.5 mM Mg_2Cl;
(▲) uncoupler FCCP

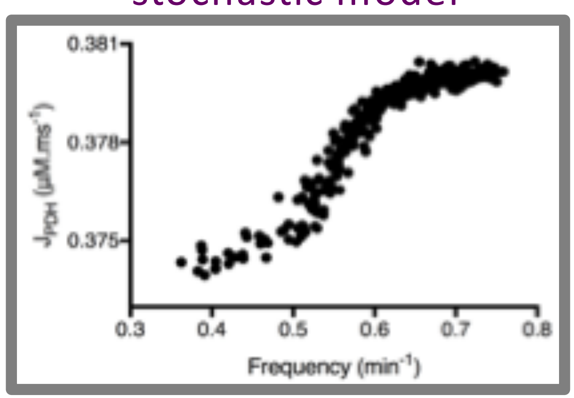
[Denton 2009]

Modeled Mitochondria Activity

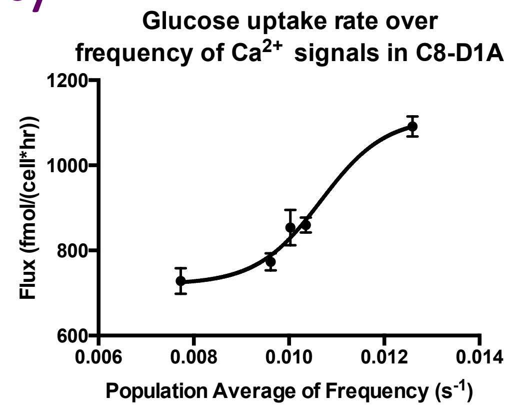
deterministic model

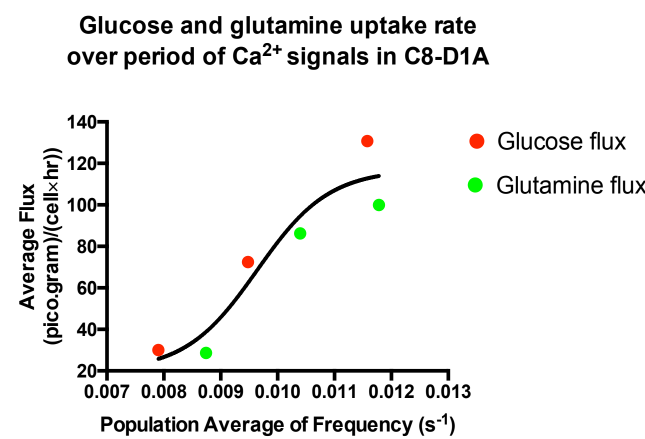


stochastic model



Metabolic fluxes





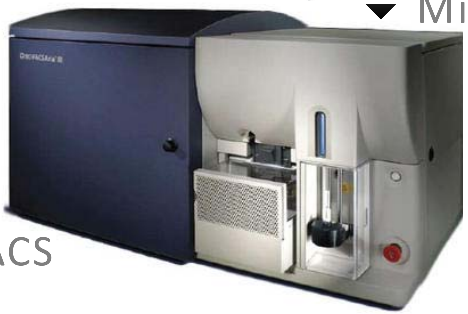
Indicates further importance of stochastic dynamics

II. Cell fate dynamics

Aymeric

Established platforms

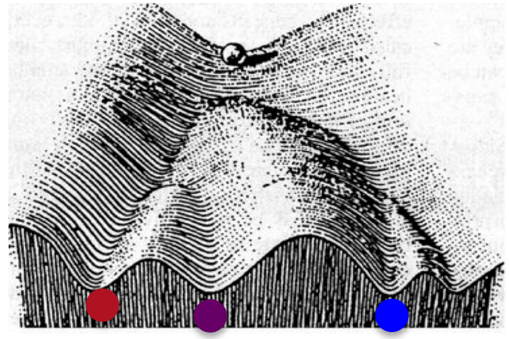
Microscopy

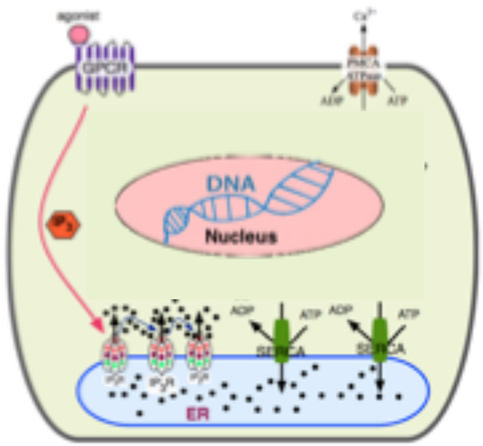


FACS

Fluidigm's C1

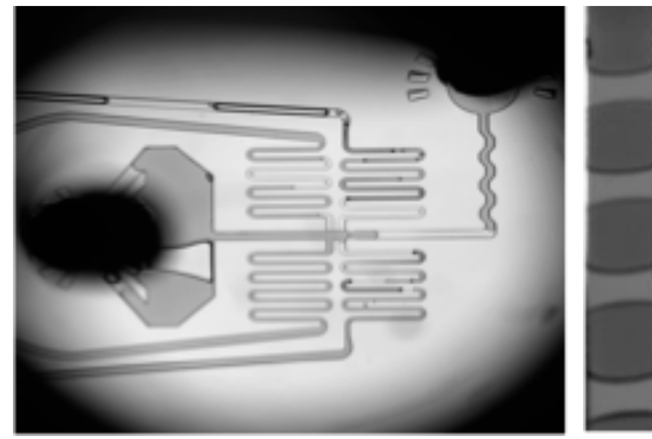
Waddington's vision





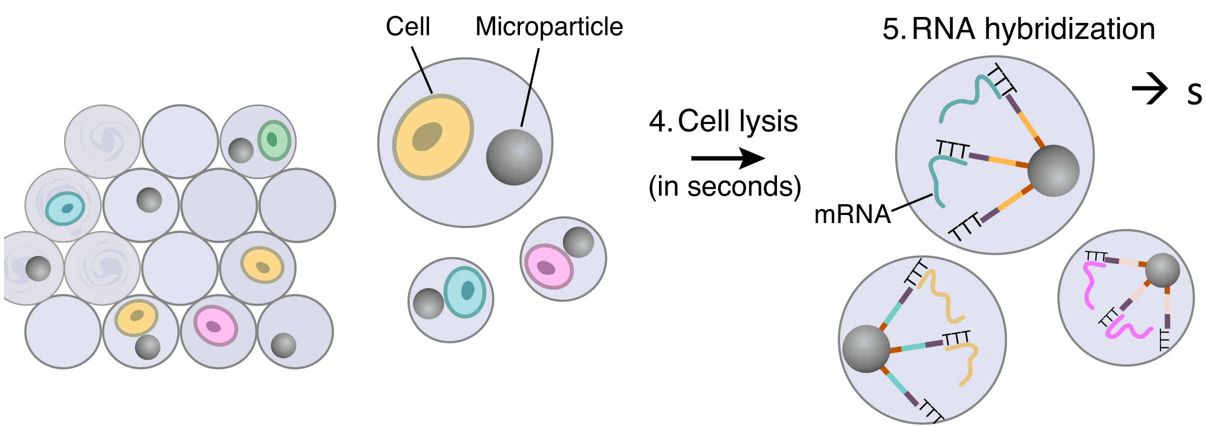
Kamil

Suresh



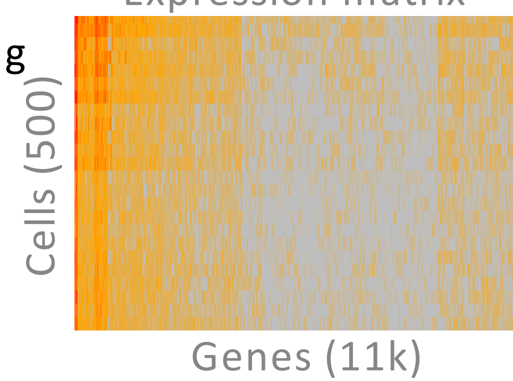
Customized microfluidics approaches

Drop-Seq approach

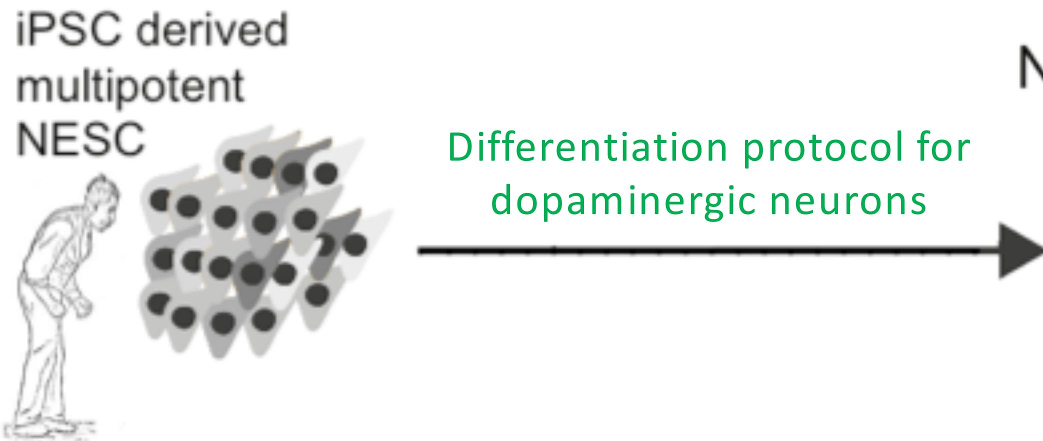


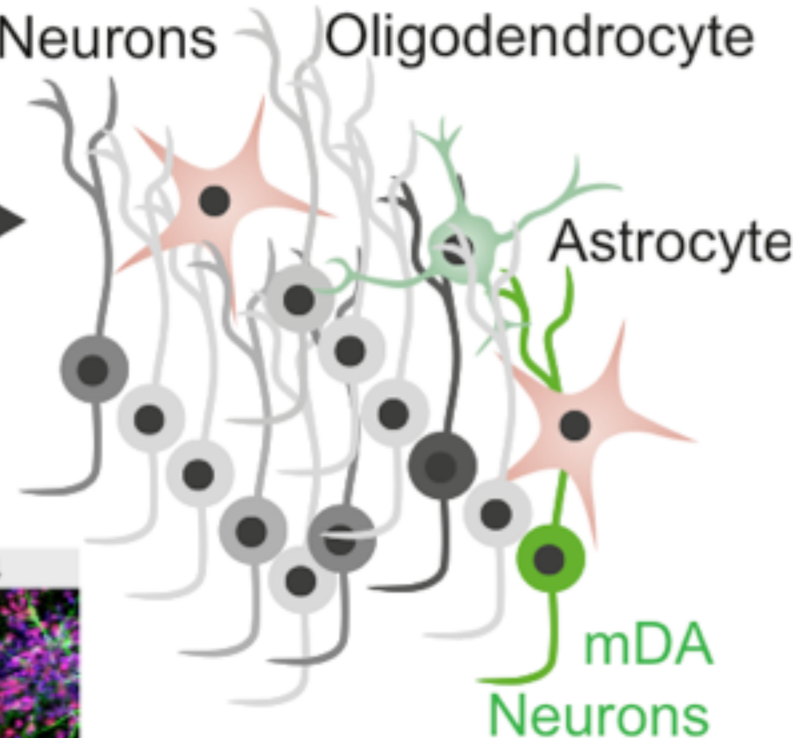
→ sequencing

Expression matrix

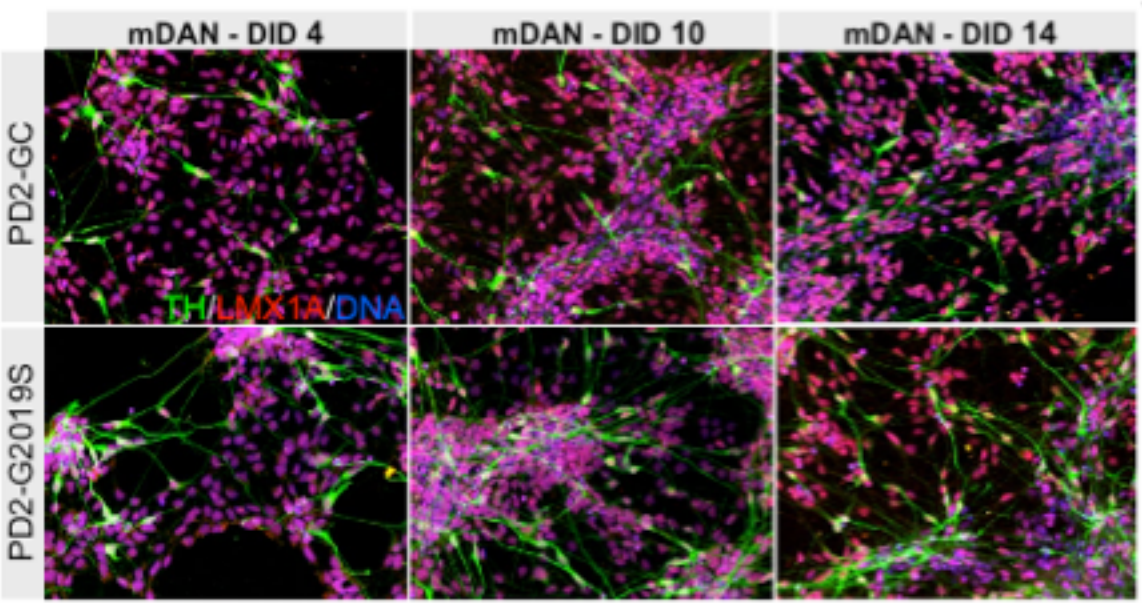


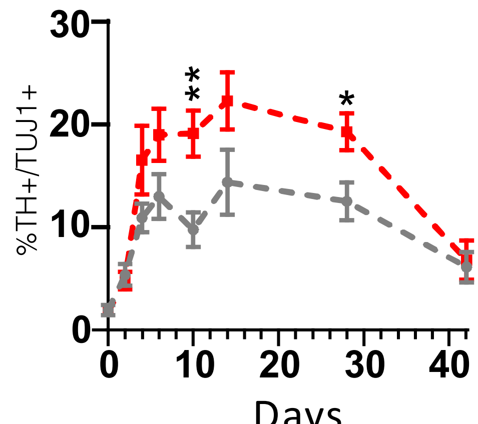
2. Cellular dynamics in PD





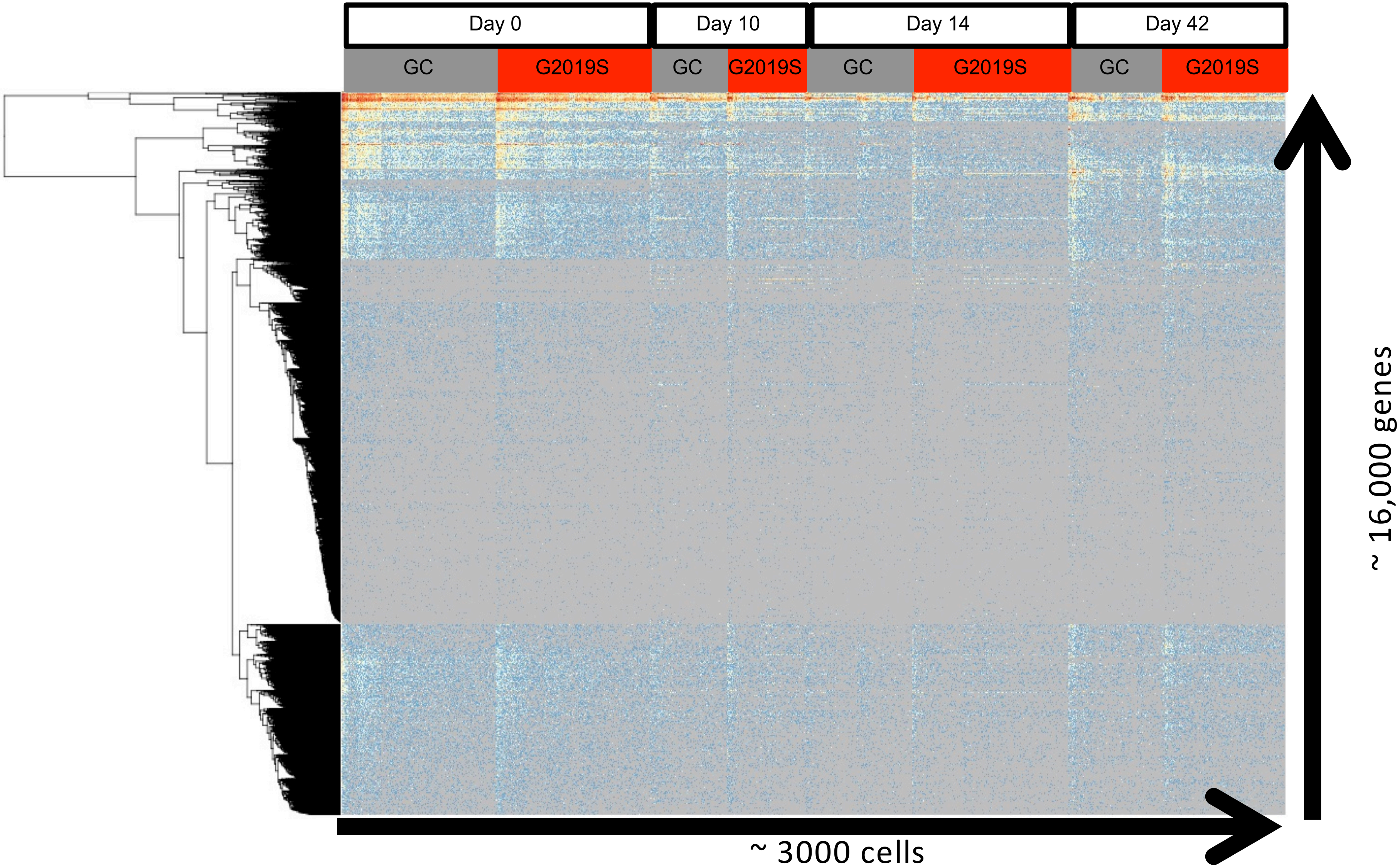
Effect of LRRK2 G2019S mutation?





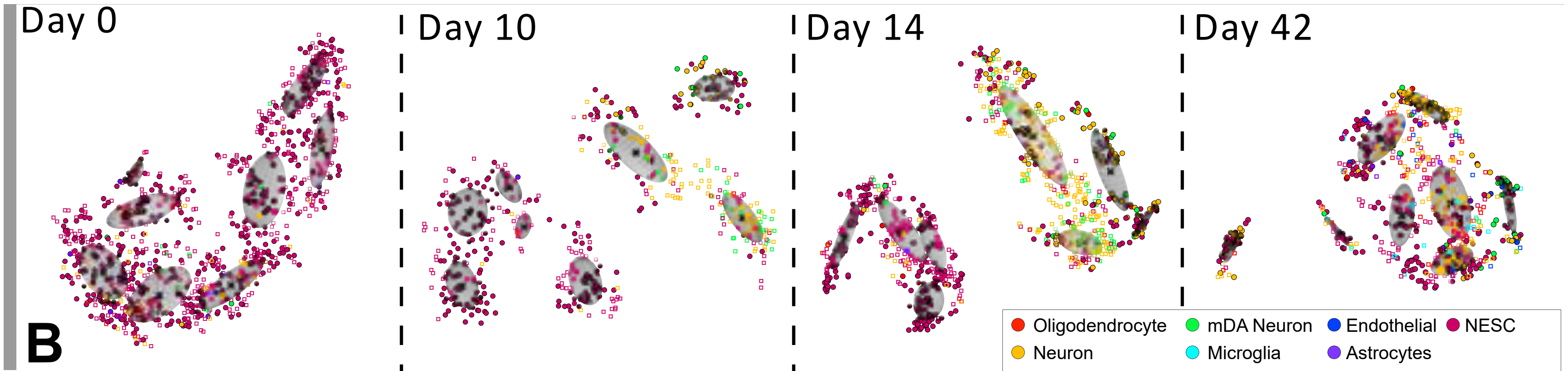
?

PD @ single cell RNAseq resolution



Cellular heterogeneity and gene characterization

Indicate faster dopaminergic (mDA) neuron development



Day 0

Percentage of total	0.7	5.1	1.8	0	0	1.3	91.1
PD/G2019S cells	100	88.2	77.7	-	-	46.1	46.9

Day 10

Percentage of total	0.8	21.6	8.6	0	0	0.2	68.8
PD/G2019S cells	75	76.8	74.4	-	-	0	38.3

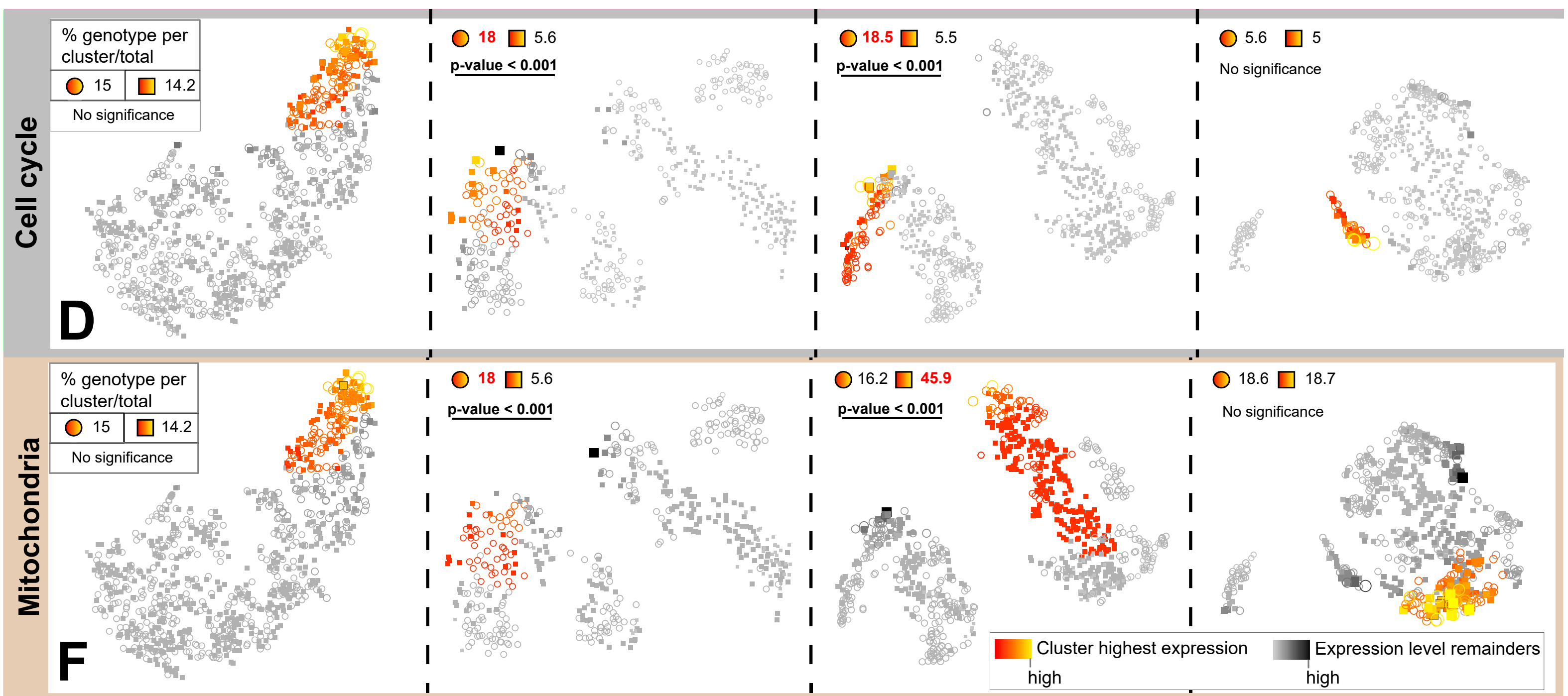
Day 14

Percentage of total	3	34	11	0.1	0	0.2	51.7
PD/G2019S cells	65.3	75.8	84	100	-	100	42

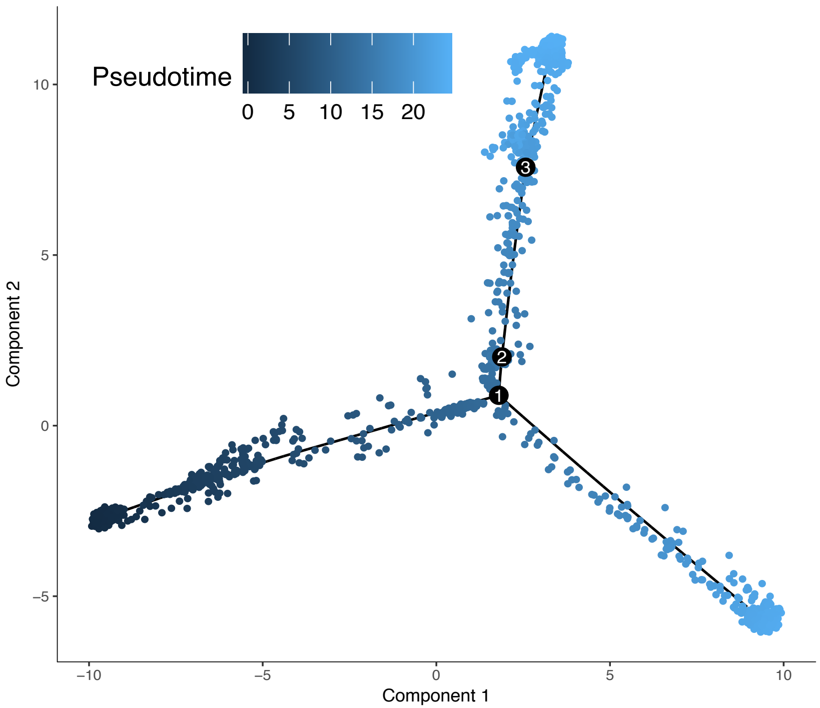
Day 42

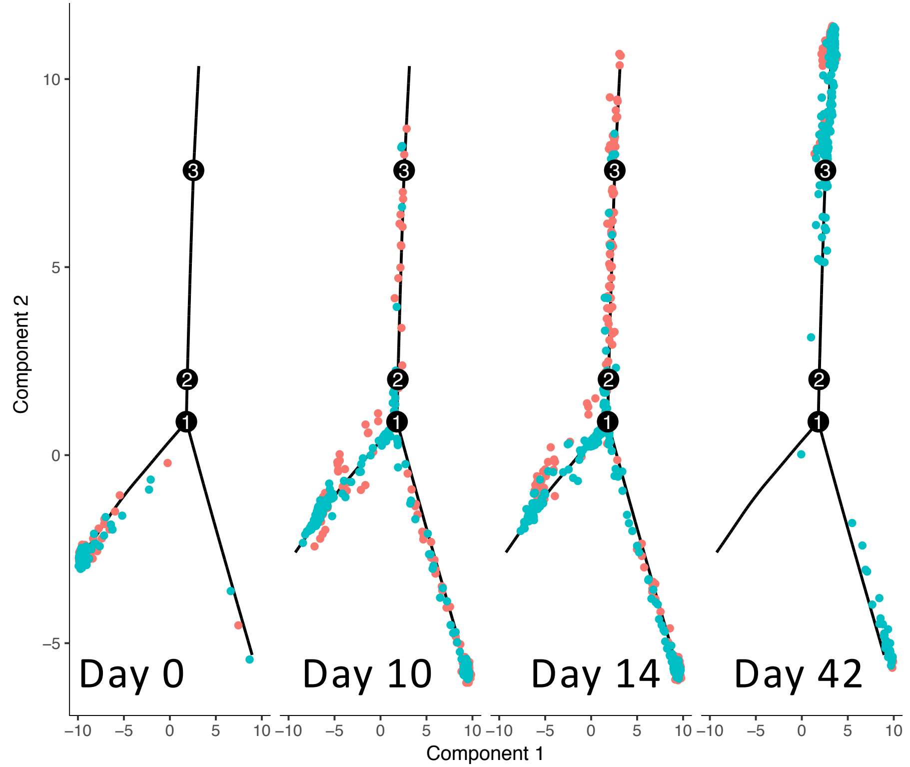
Percentage of total	9.6	28.4	5	4.1	3.6	2.2	47.1
PD/G2019S cells	82	70.8	22.8	93.1	64	66.6	43.3

Cell cycle (and stemness) and mito genes differ most at day 10 and 14

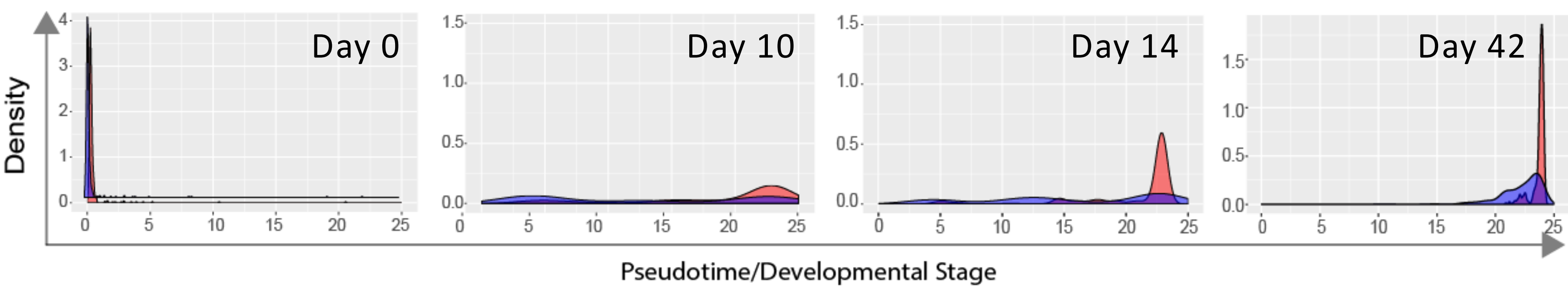


Branching analysis for development

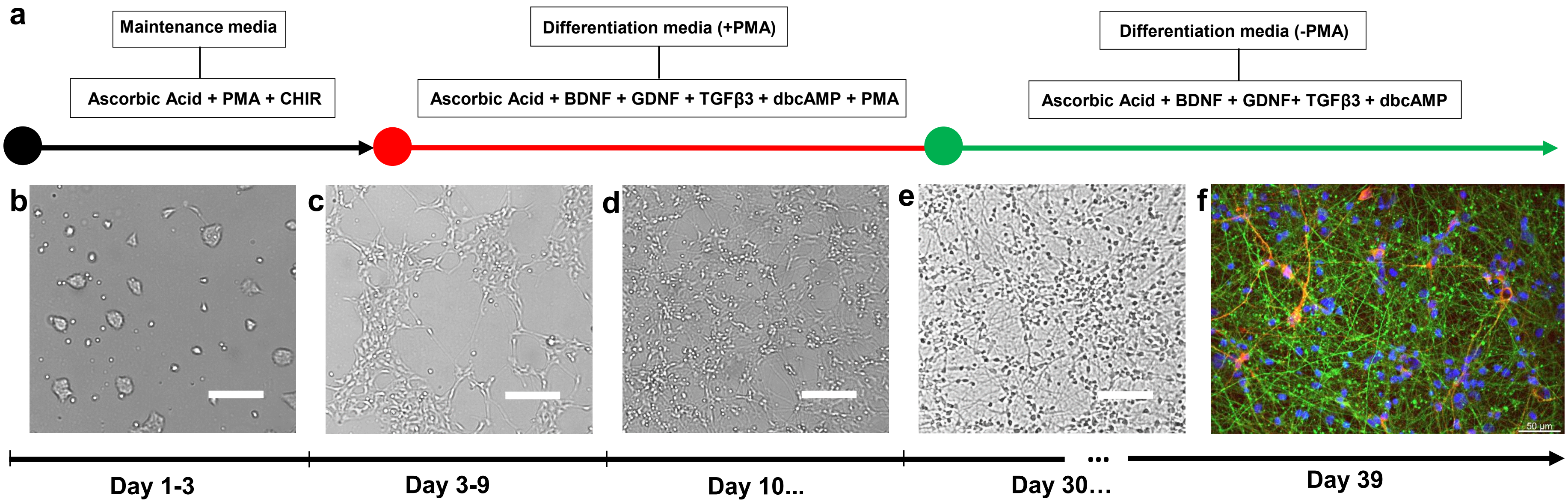




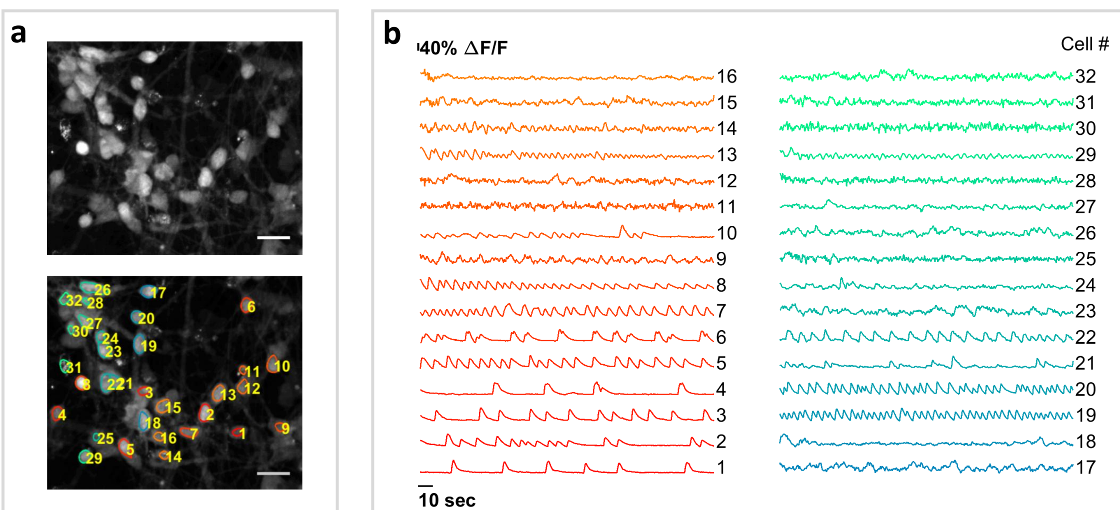
Ordering based pseudotime as a prior of developmental stage

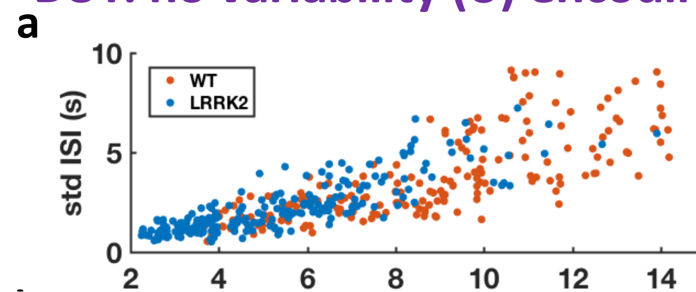


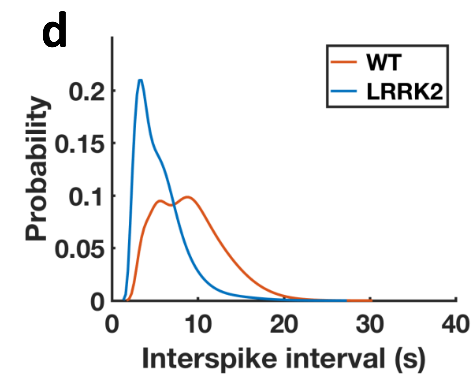
What's about calcium and LRRK2?



BUT: no variability (σ) encoding





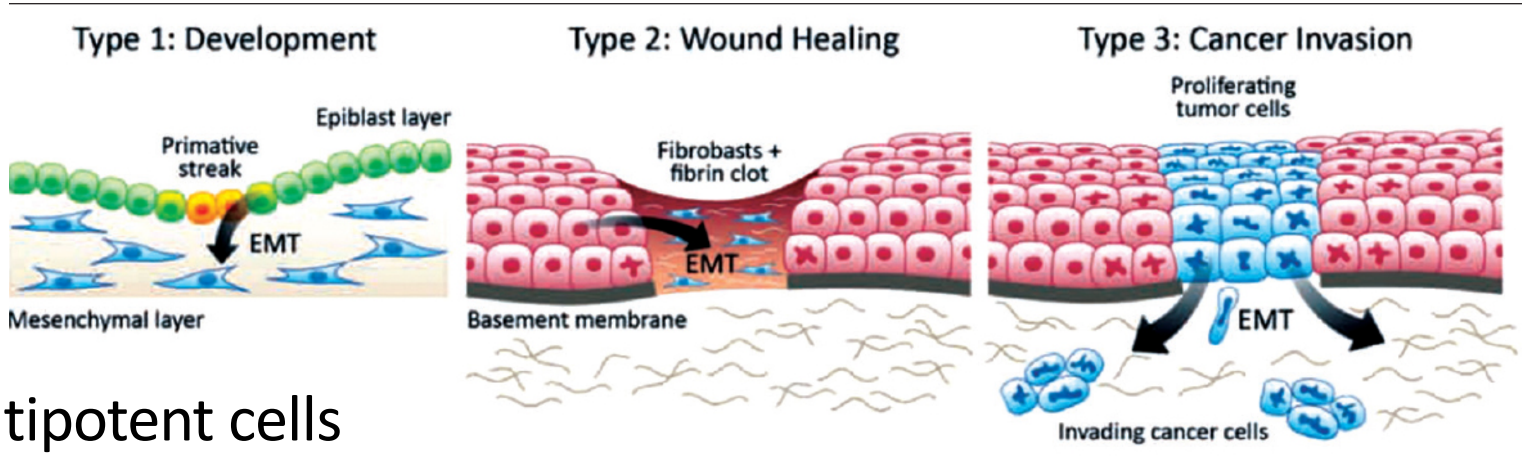


→ LRRK2 mutation induces hyperactivity that may increase mitochondrial activity and aging …

(Flemming lab)

3. Epithelial to mesenchymal transition

essential in:



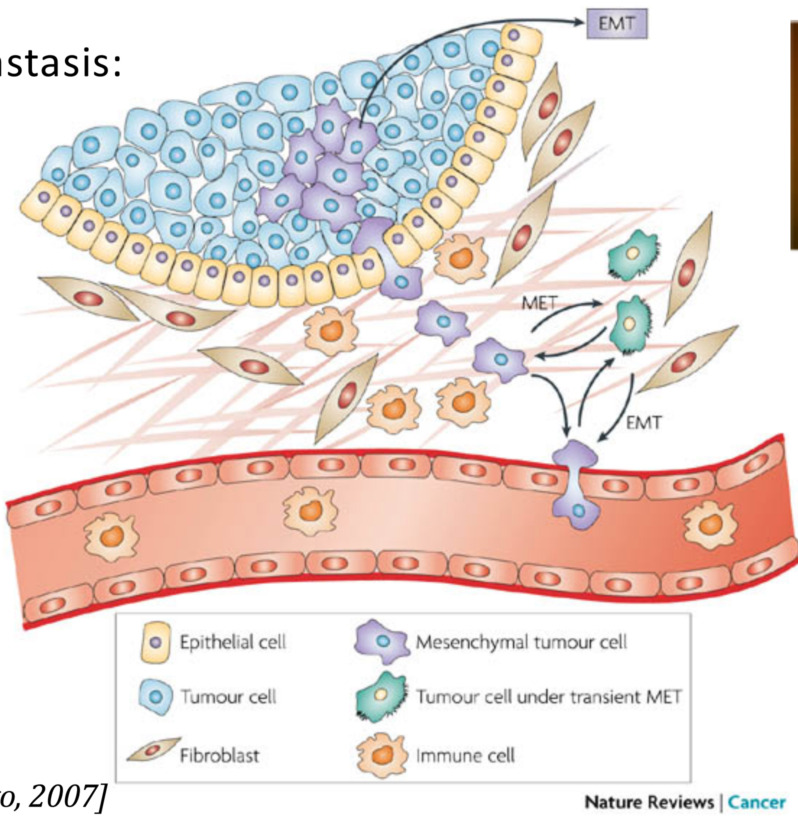
epithelial

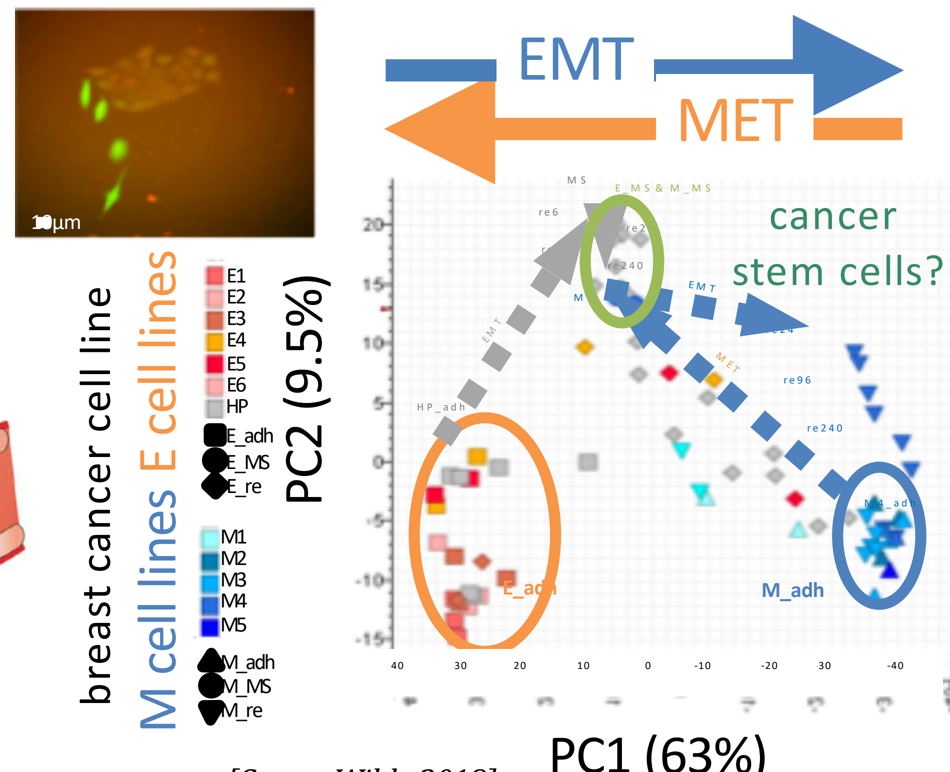
→ tissue cells

mesenchymal

→ mobile multipotent cells

metastasis:



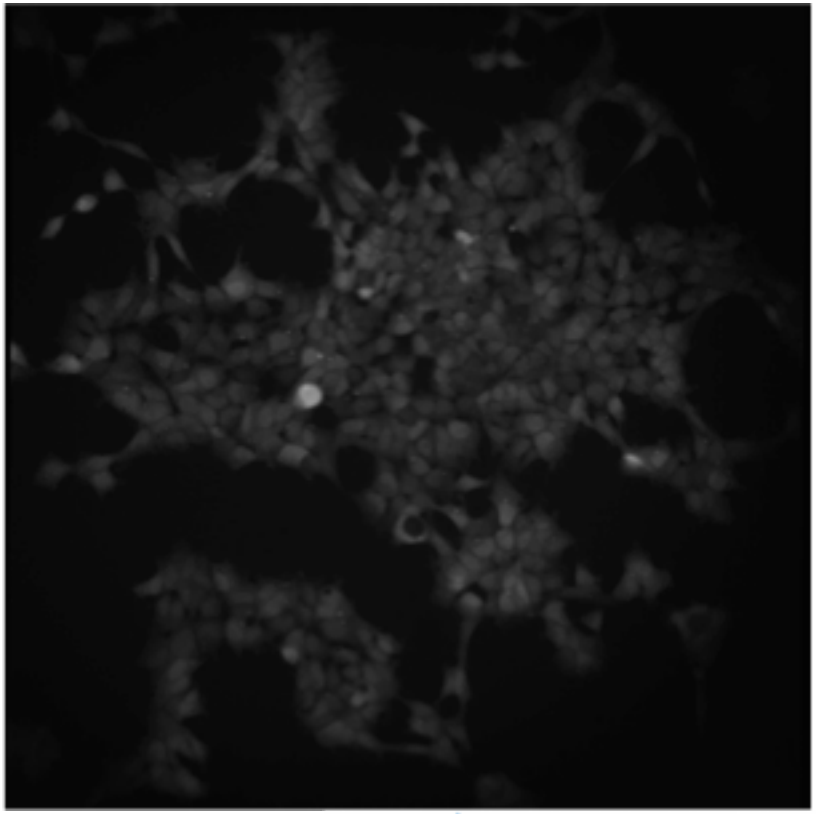


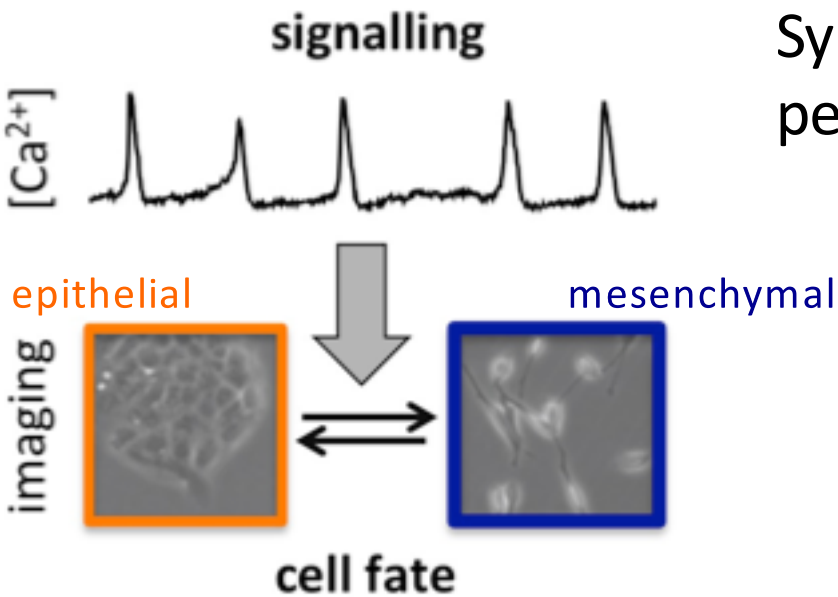
[Peinato, 2007]

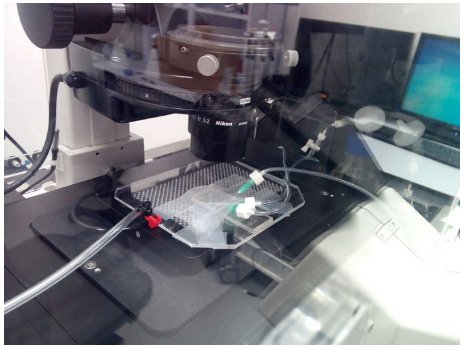
[Grosse-Wilde 2018]

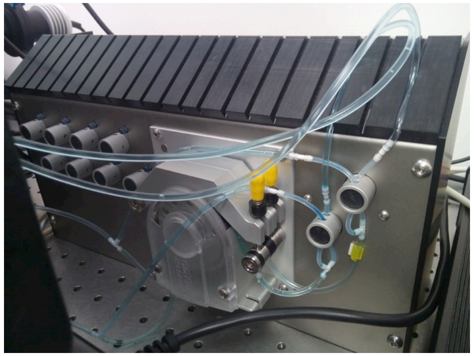
EMT triggered by Ca^{2+} dynamics

Synchronized cells by perfusion system

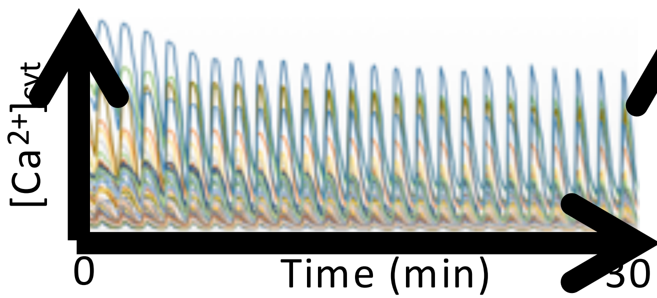


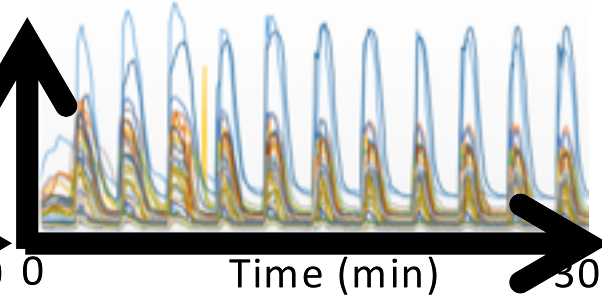




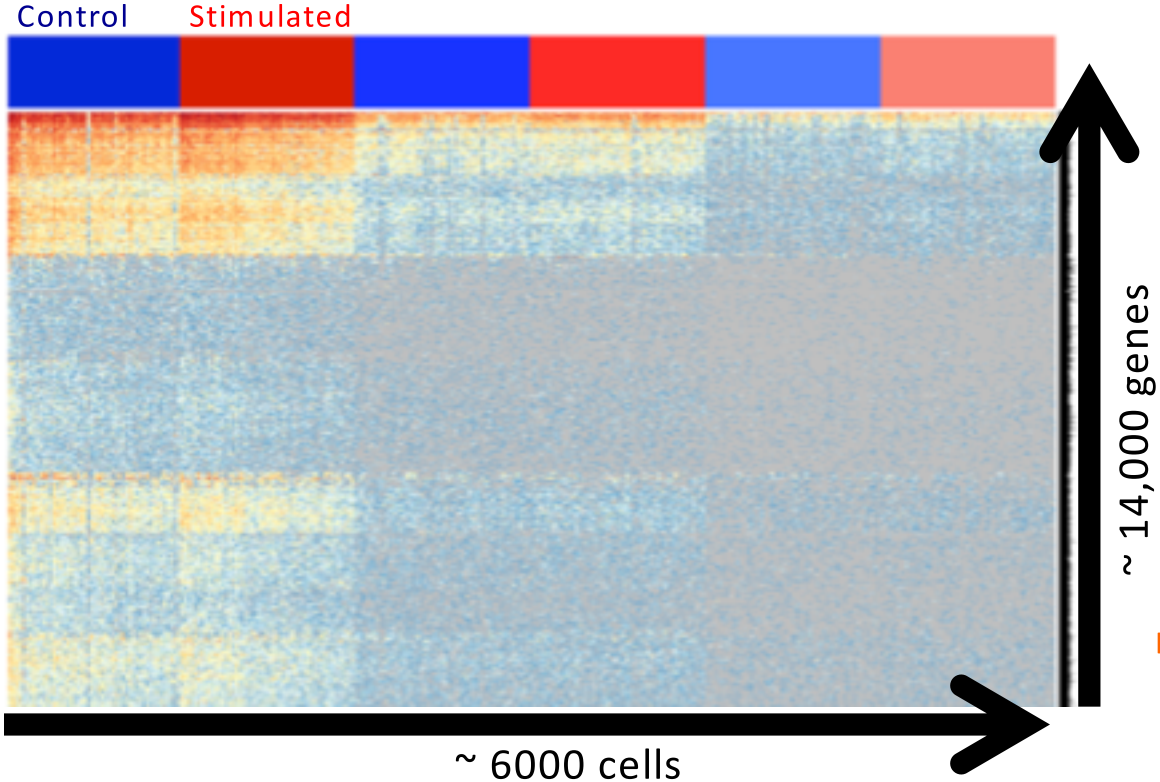


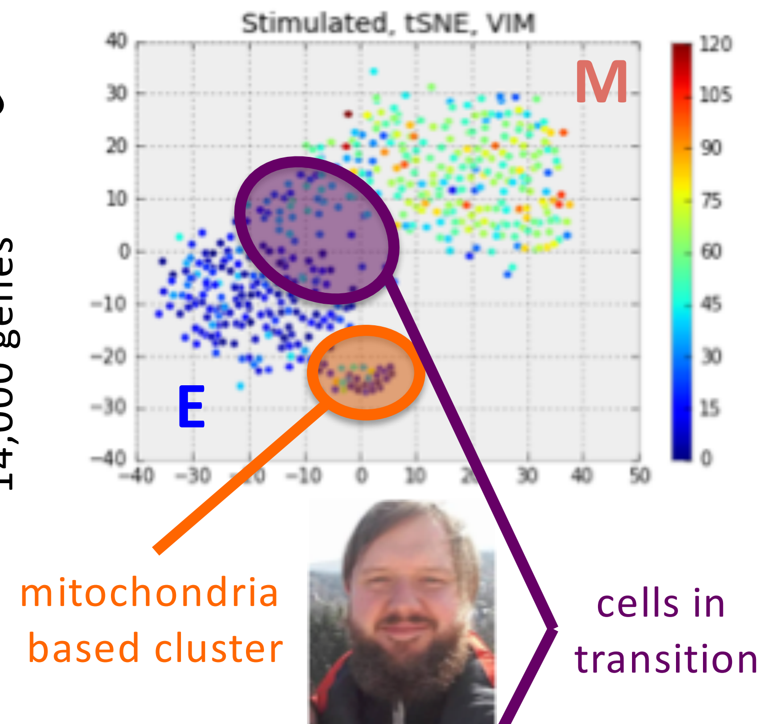
computer controlled perfusion system

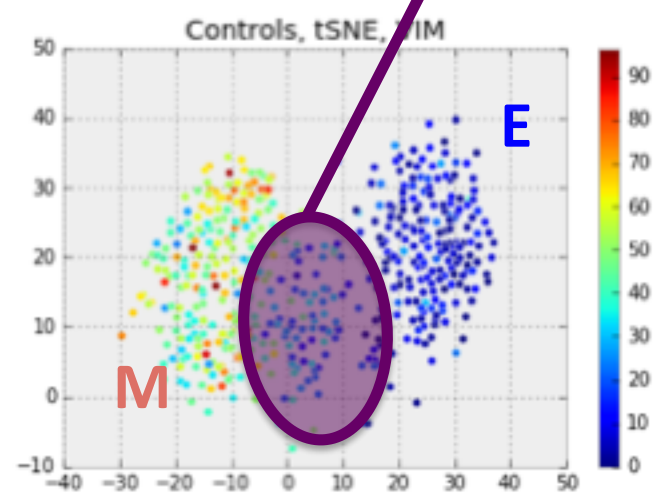




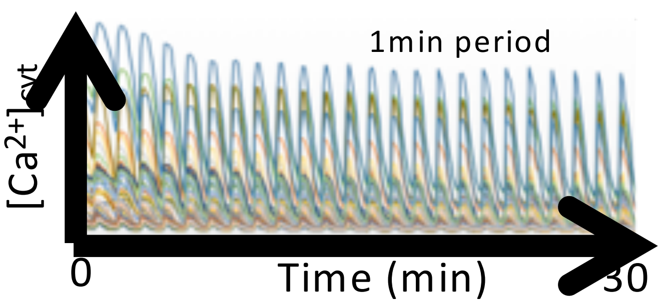
Downstream analysis @ single cell resolution







1h stimulation + 1h incubation



“Transition genes”

EMT	MET
CTNNB1	CDH11
ETV1	PERP
SNAI2	CADM1
HMGA2	COL17A1
TWIST1	PERP
SMAD3	VEGFC
FBLN5	ACVR1
SPINT2	
SPARC	

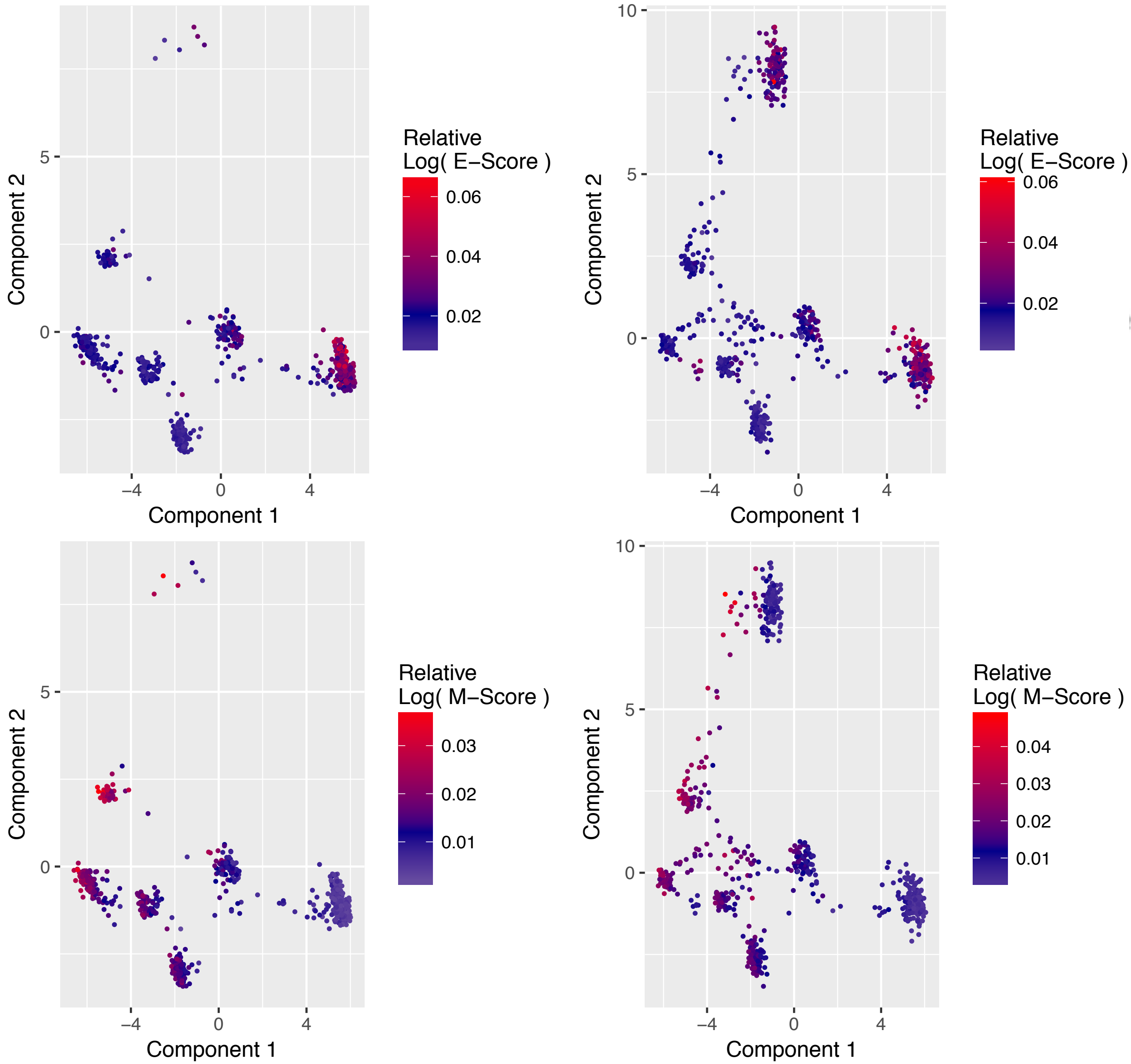
mesenchymal and epithelial cells

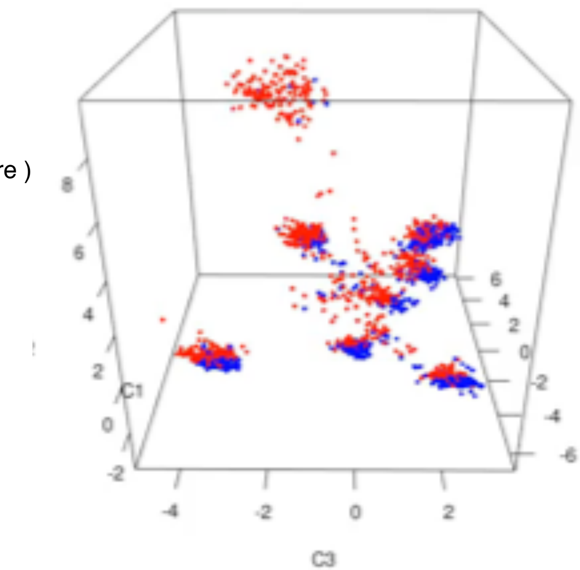
Branching analysis with dynamic clustering

Applying dynamic clustering (Monocle 2)

CTRL

Stimulated





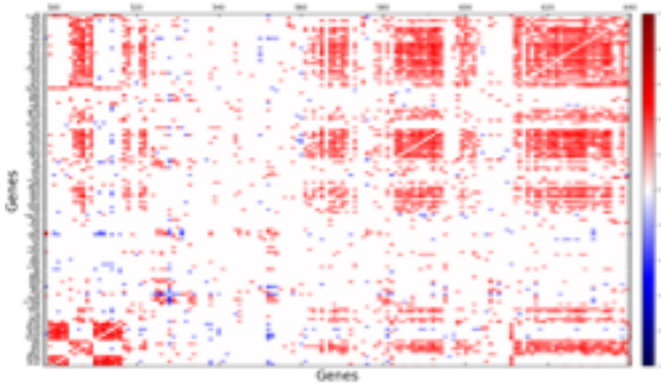
ATP induced Ca^{2+} spiking

→ MET in HMLER cells
→ E cells with changed mitochondrial expression
→ indicating apoptosis related genes

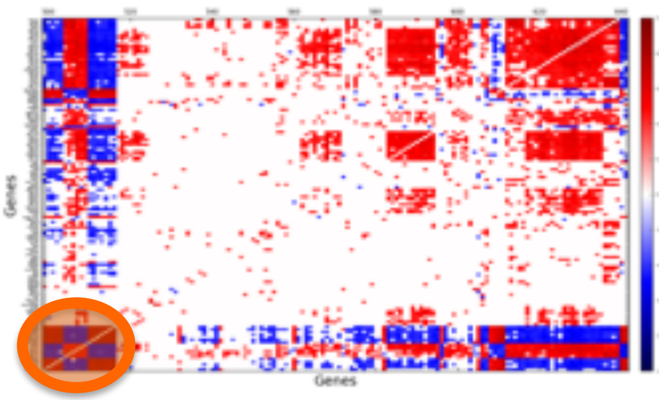
Core network insights

Most 200 correlated genes

CTRL

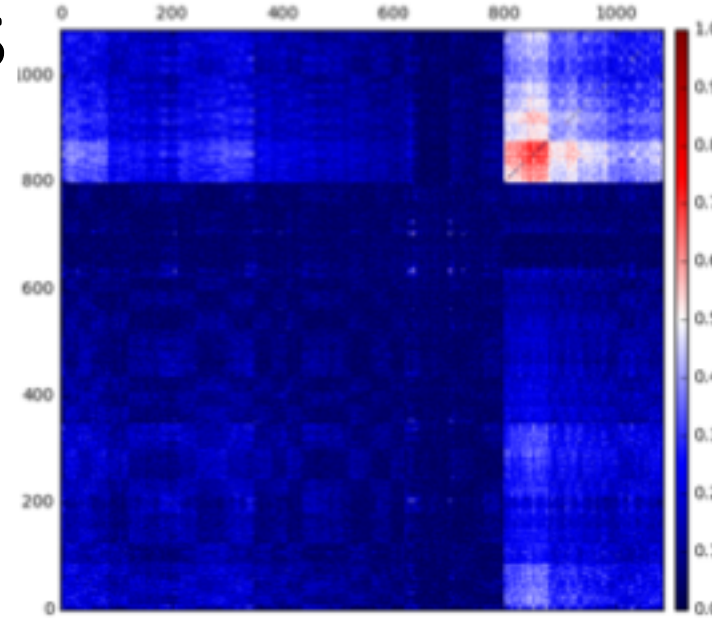


Stimulation



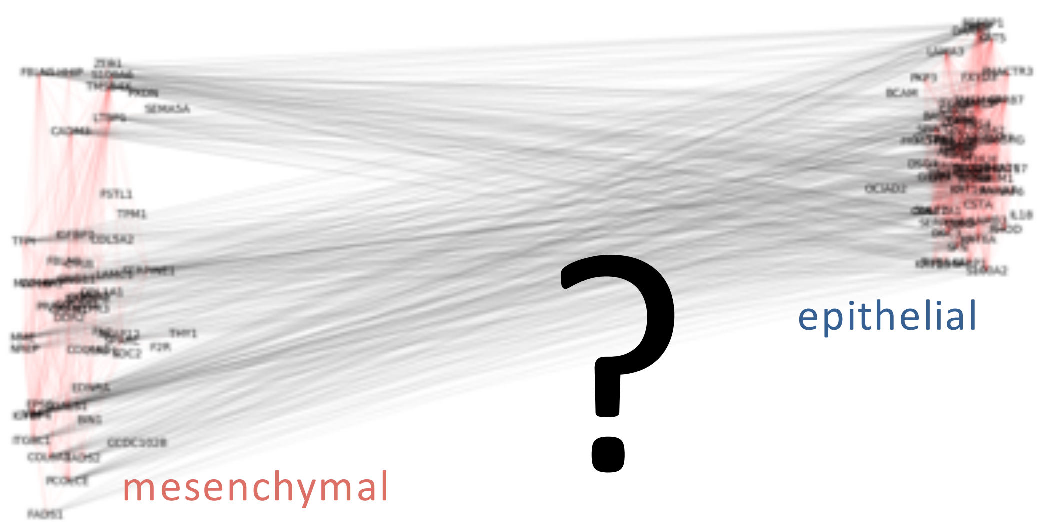
→ Regulation of mitochondria related genes

→ Apoptosis induction

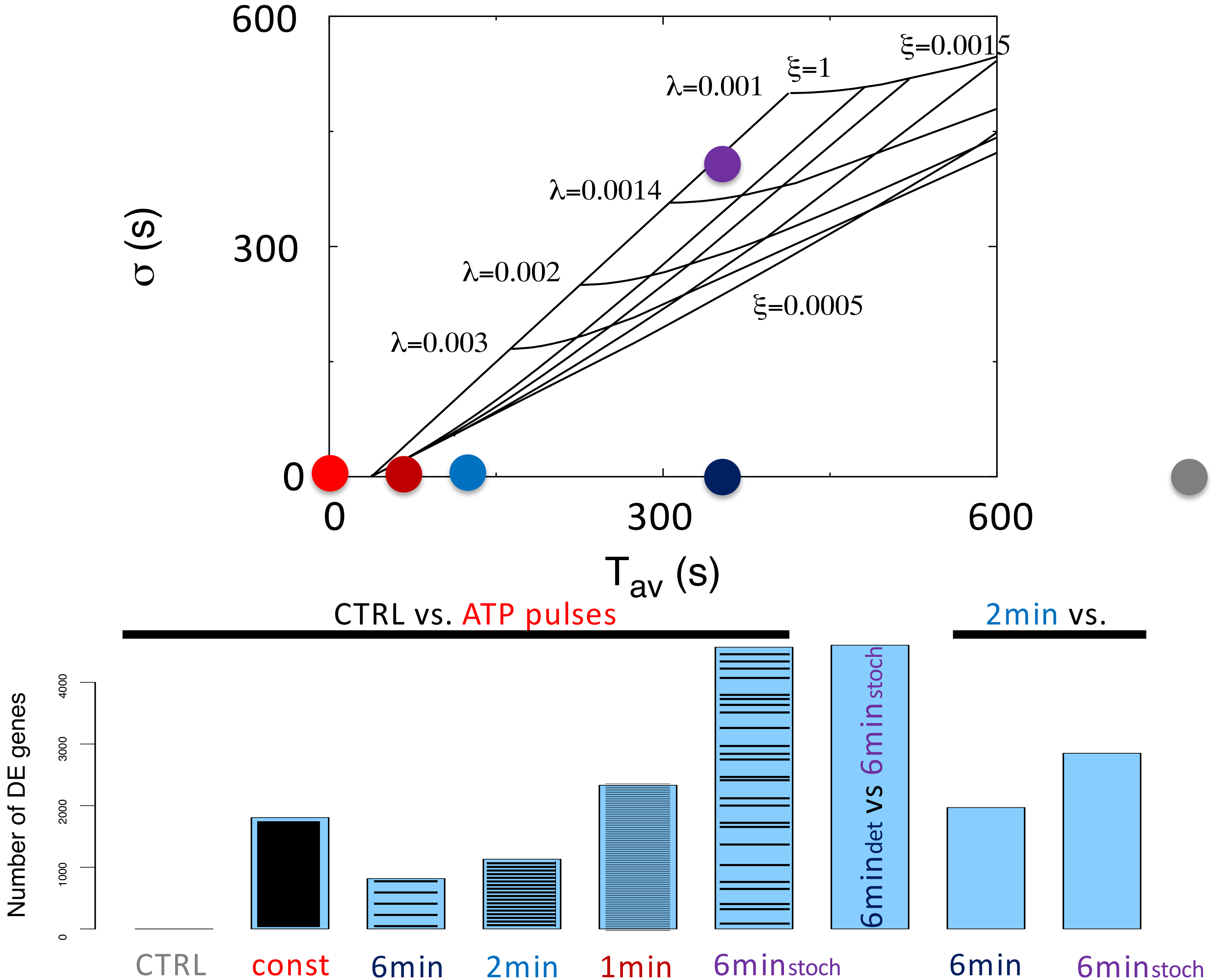


1200 most absolute correlated genes

Functional network



Moment based cell state decoding



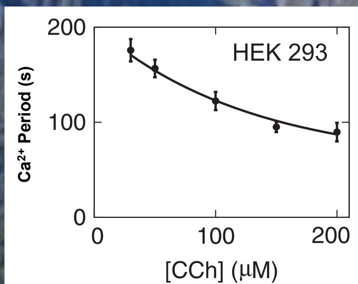
Stochastic spiking supports EMT

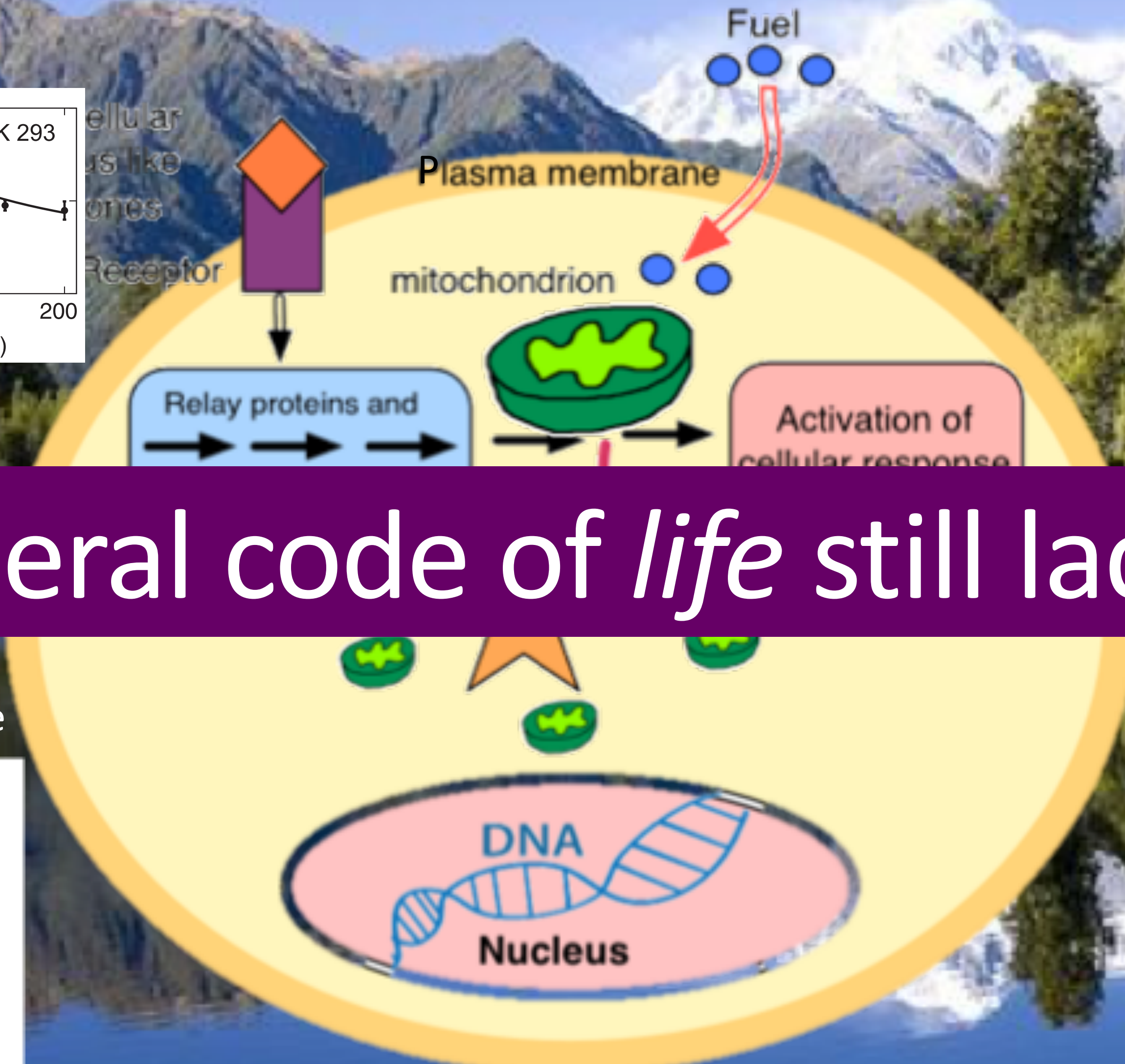
- CADM3↓ epithelial marker
- CDH1↓ epithelial marker
- CDH3↓ epithelial marker
- EPCAM↑ epithelial marker
- DSP↓ epithelial marker
- COL17A1↓ epithelial marker
- BCAM↓ epithelial marker
- CD63↑ mesenchymal marker
- FN1↓ mesenchymal marker
- FST↓ epithelial marker
- FXYD3↑ epithelial marker, insufficient for EMT(involved in E cell polarity)
- ITGB4↓ epithelial marker
- JUP↓ epithelial marker
- LAMB3↓ epithelial marker
- LAMC2↓ mesenchymal(EMT potential) in lung adenocarcinoma
- TMSB4X↑ facilitate cell motility(EMT)

… and increases cellular heterogeneity – to be followed up

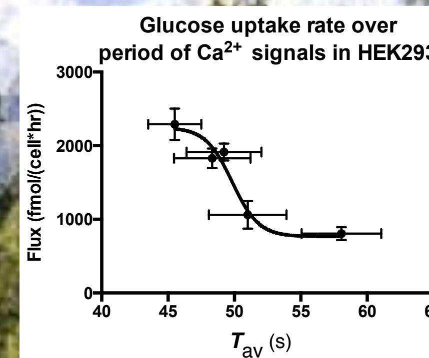
Ca^{2+} as the mediator of life: balance energy and information processing

encoding



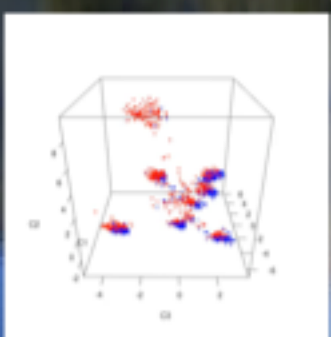


metabolic decoding

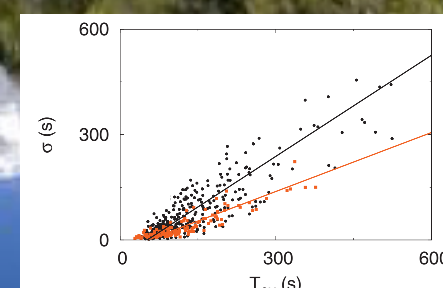


general code of *life* still lacking

various cell fate



ongoing:
variability encoding

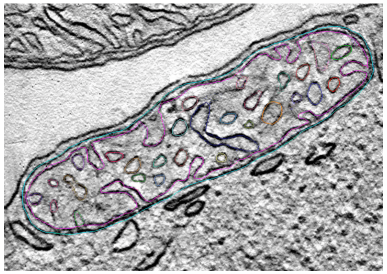


→ using energy for gene-environment adaptation away from equilibrium

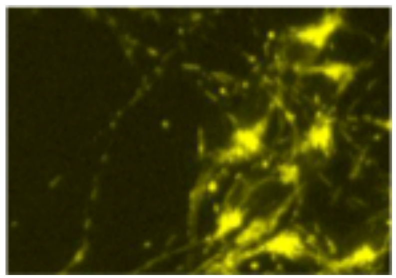
Integrative multiscale approaches

Experiment Energy ⟺ Information **Theory**

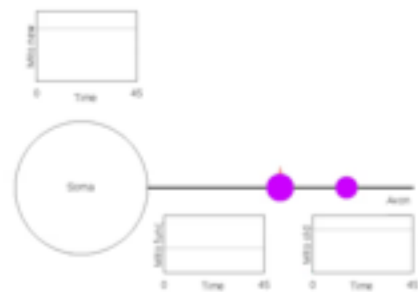
EM (with UCSD)



live cell imaging

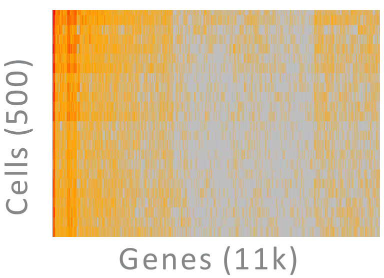


reaction diffusion



agent based

single cell RNAseq



post mortem

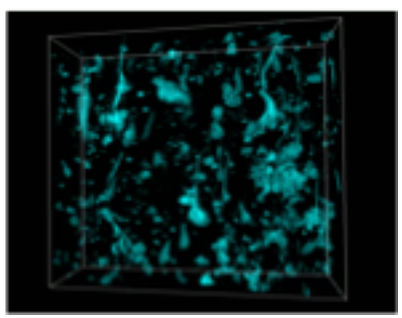
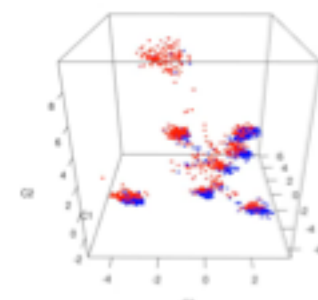
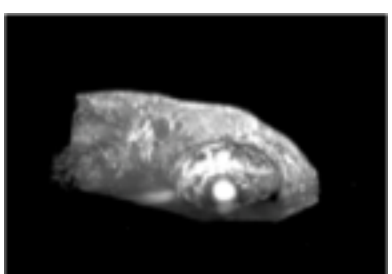


image analysis

bioinformatics

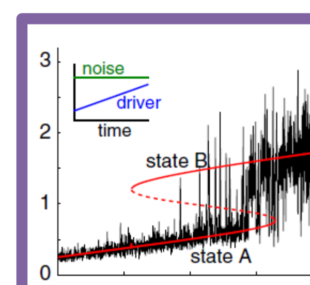


in vivo imaging

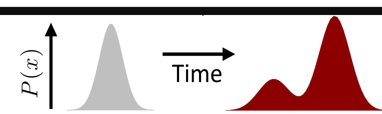


scale

phase transition



Distribution



Biology

Bioinformatics:
Systems Biology

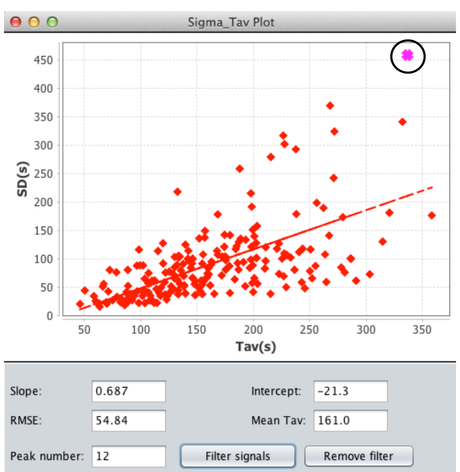
Final Commercials

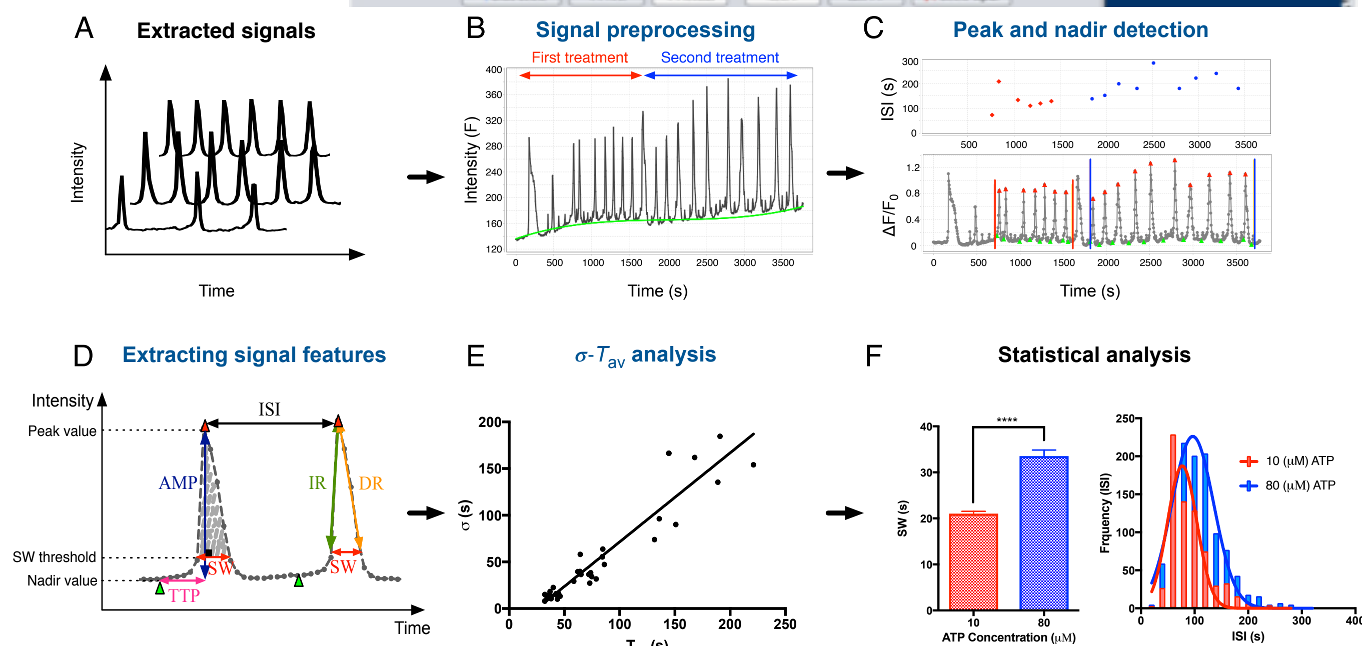
CaSiAn: a Calcium Signaling Analyzer tool

Mahsa Moein [1], Kamil Grzyb [1], Teresa Gonçalves Martins [1], Francesca Peri [3], Alexander Crawford [1], Aymeric Fouquier Alexander Skupin [1,4]*

Free, easy to use and GUI based analysis tool for (Ca^{2+}) spiking

- background removal
- spiking times
- amplitudes
- …





http://r3lab.uni.lu/web/casa

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