Bioelectricity of non-excitable cells: modeling of instructive multicellular patterns

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Allen Discovery Center at Tufts University, MA, USA

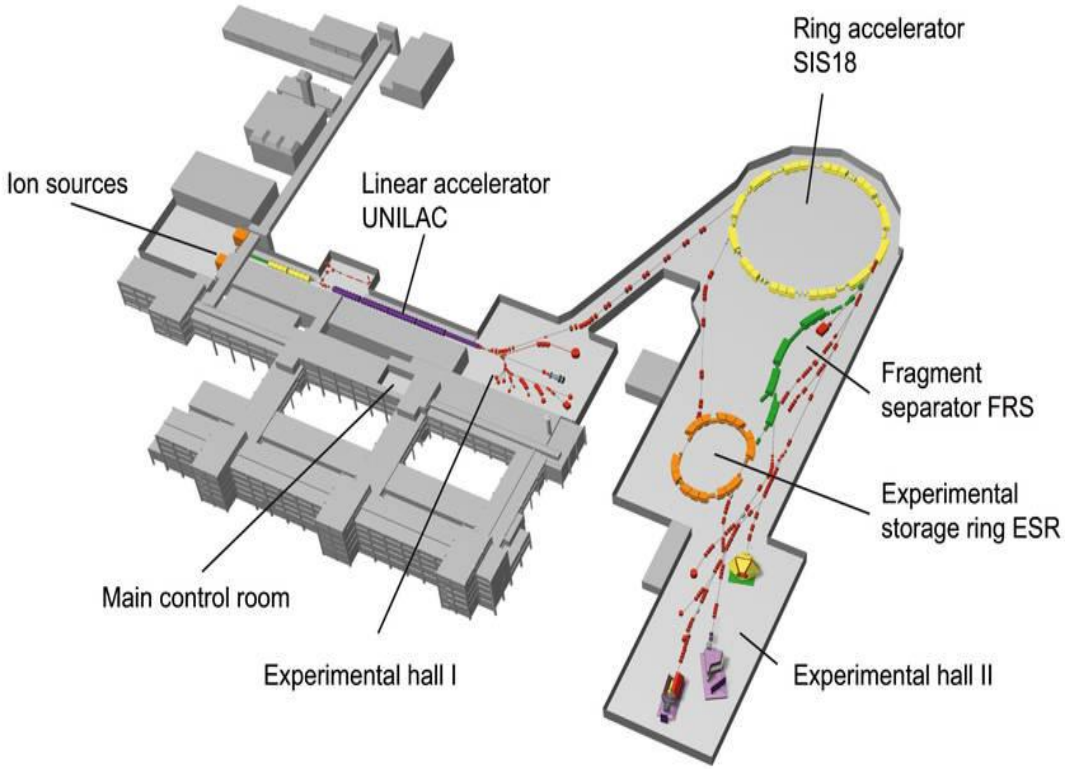
1. *Where we (Javier and Salvador) come from*
2. Multicellular organization: bioeng., biochem., and bioelec. views
3. Some experimental facts of bioelectricity + molecular biology
4. Bioelectrical model assumptions and equations
5. Theoretical results of qualitative relevance
6. *Where we go*: identifying key bioelectrical steps in biological complexity

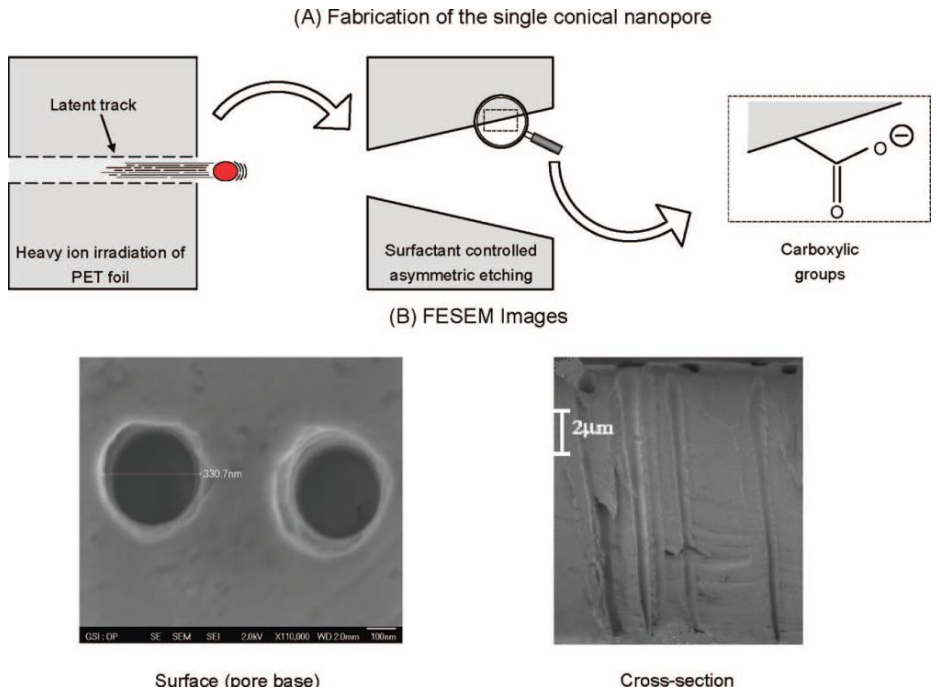
1. Where we (Javier and Salvador) come from

ACS Nano 2009
10.1021/nn900039f
Adv Funct Mat 2012
10.1002/adfm.201102146

*1a Applied Physics/Nanoscience/Biomimetic nanopores (with **Patricio Ramirez**)*

Biomimetic nanopores with voltage-gated conductances and memory effects provide *qualitative insights* on biological channels. A single conical nanopore fabricated by irradiation of a polymer foil (polyethylene terephthalate or polyimide) at the linear accelerator *UNILAC* with single swift heavy ions (Pb, U, and Au) of energy 11.4 MeV per nucleon and subsequent chemical etching.

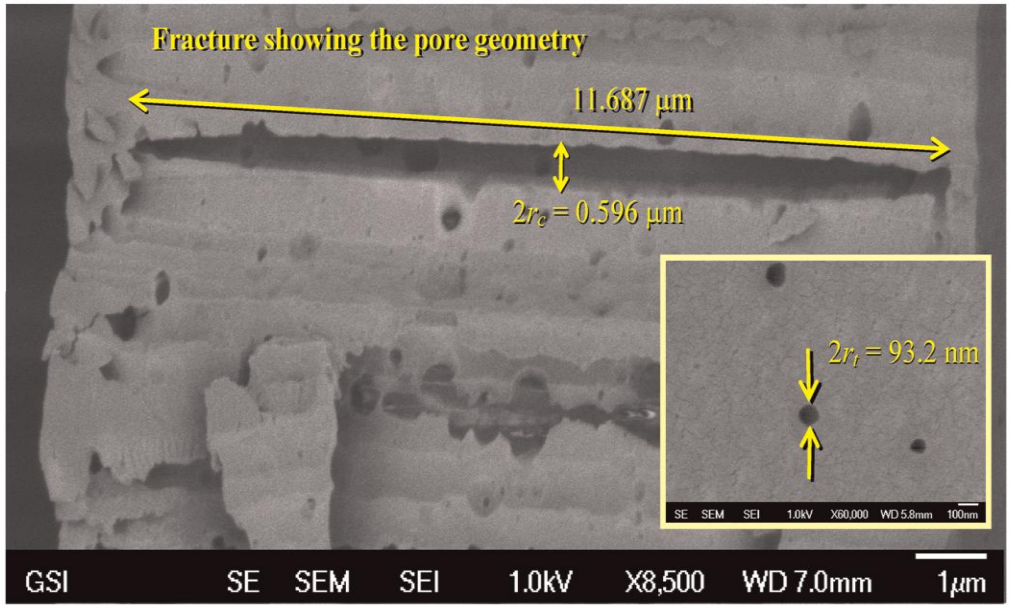




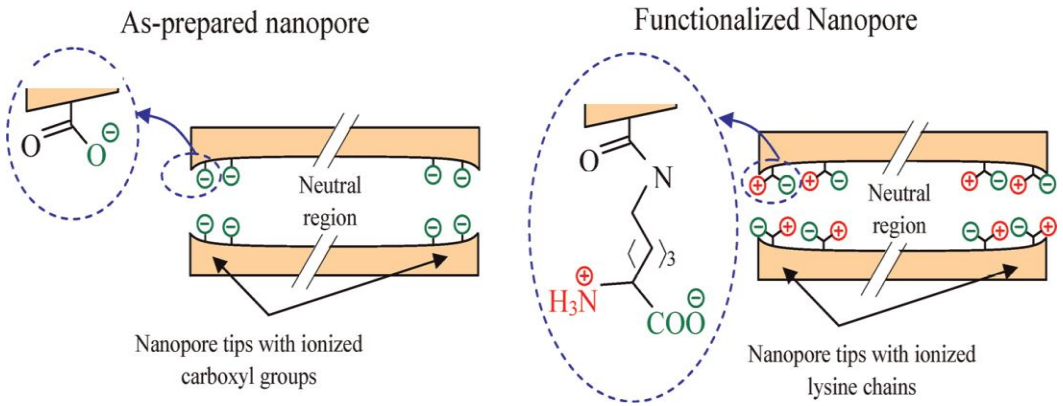
nanopores as chemically-gated transistors (with M. Ali and W. Ensinger)

ACS Nano 2012 10.1021/nn3010119
ACS Nano 2012 10.1021/nn303669g

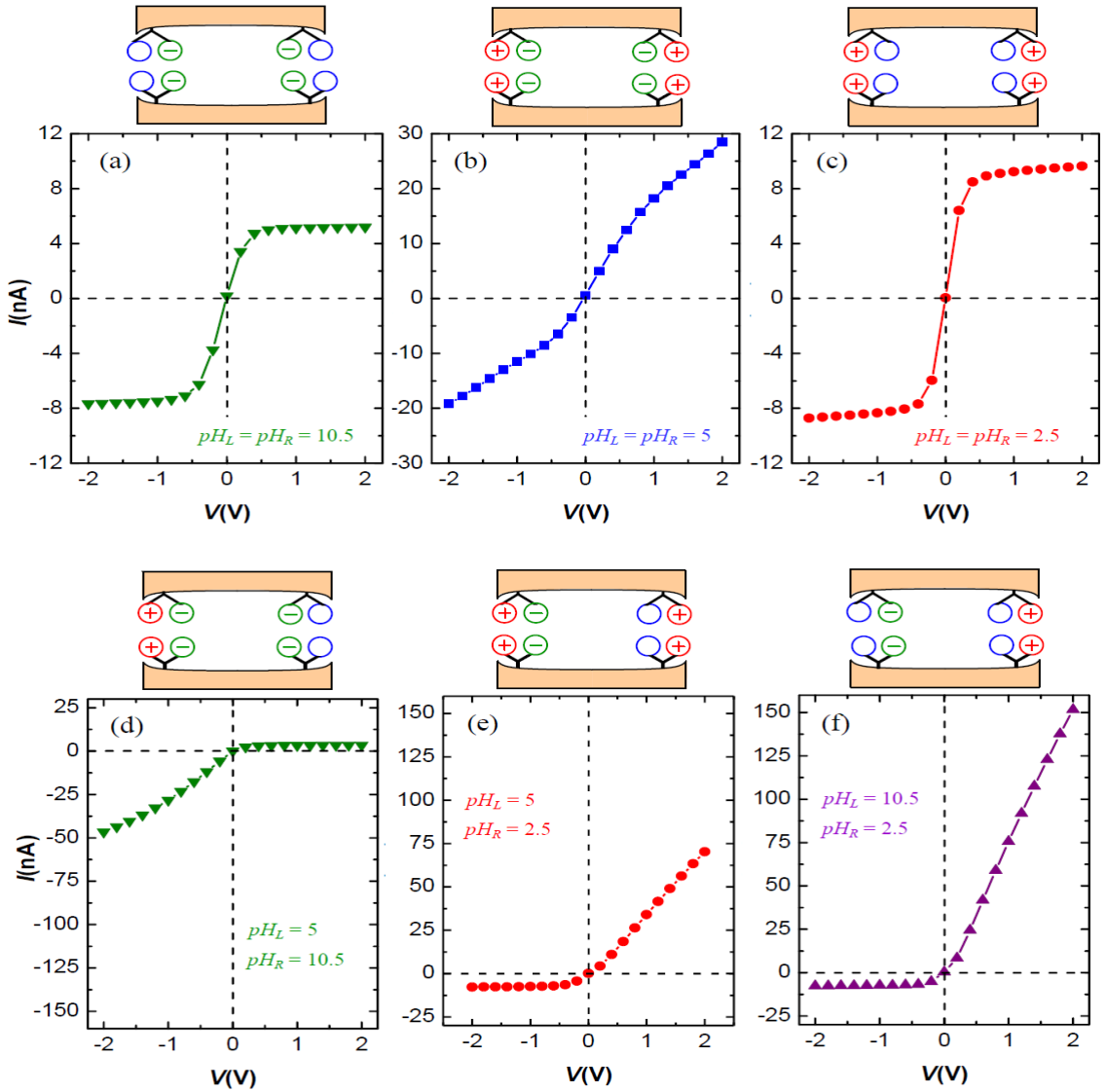
(a) Pore geometry



(b) Cigar-shaped pores after functionalization

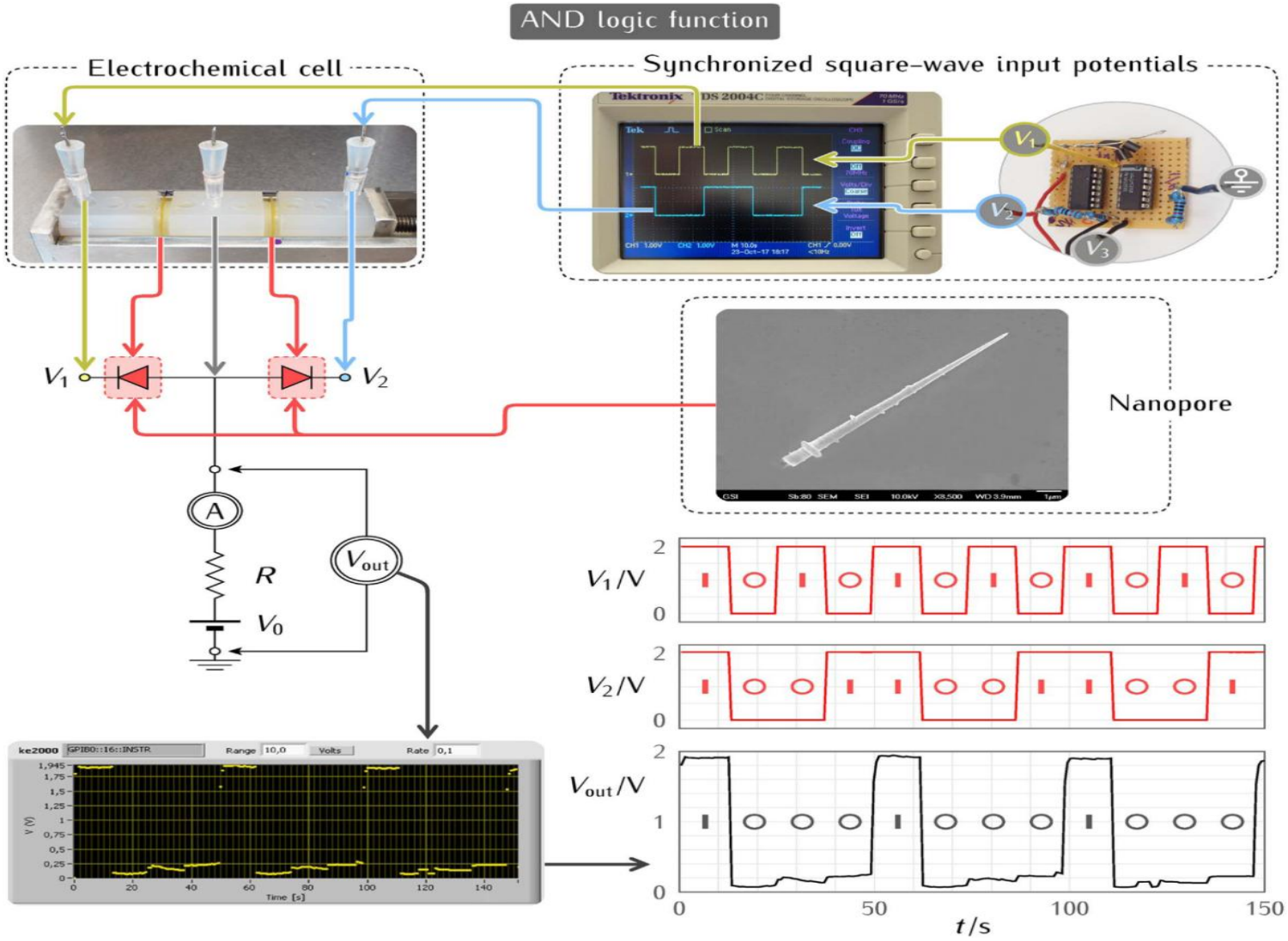


I-V curves of cigar-shaped nanopores functionalized with lysine chains



nanopore logical functions (with Vicente Gómez)

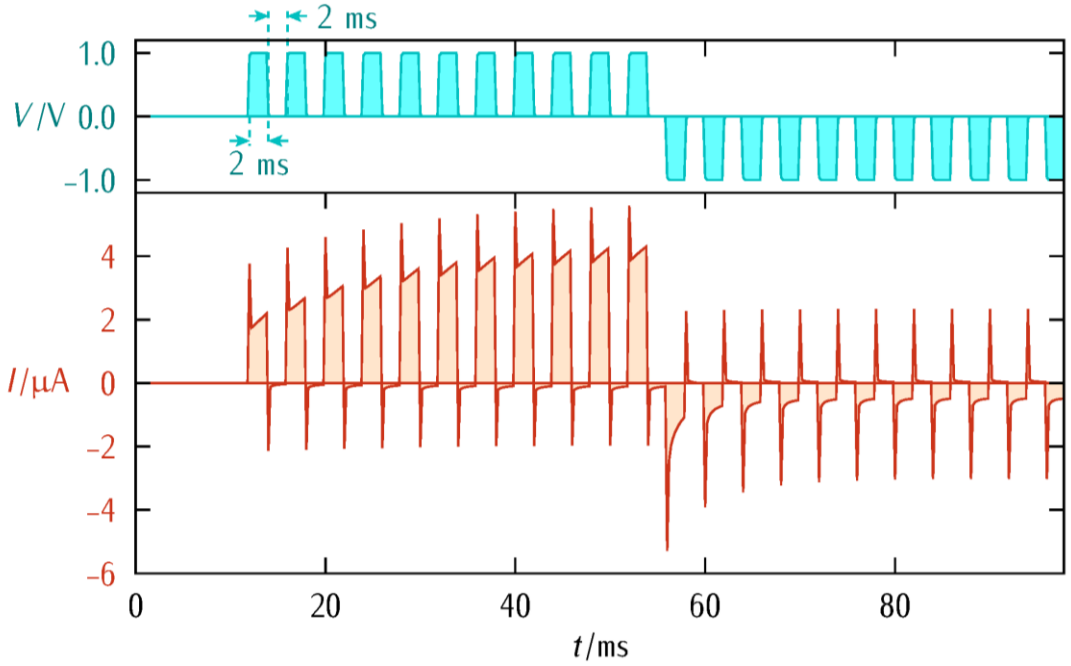
Phys. Rev. Applied 2017
10.1103/PhysRevApplied.7.064035
Appl. Phys. Lett. 2012, 2016
10.1063/1.4754845
10.1063/1.4954764
Electrochem. Commun. 2018
10.1016/j.elecom.2018.01.016

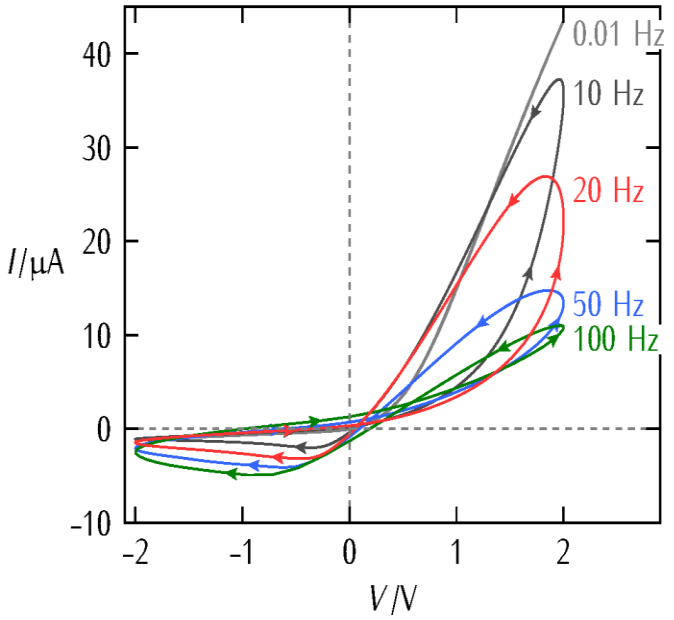


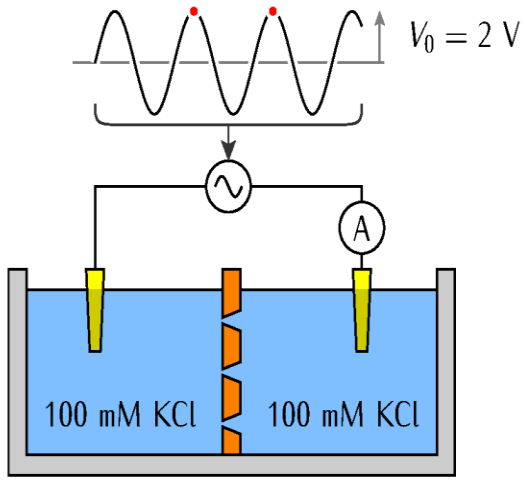
nanopores as rectifying neuromorphic memristors (with Juan Bisquert and Sergio Portillo)

J. Phys. Chem. Lett. 2023
10.1021/acs.jpclett.3c02796
Phys. Fluids 2024
10.1063/5.0204219

frequency-dependent ionic currents
and *voltage-gated* pore conductances





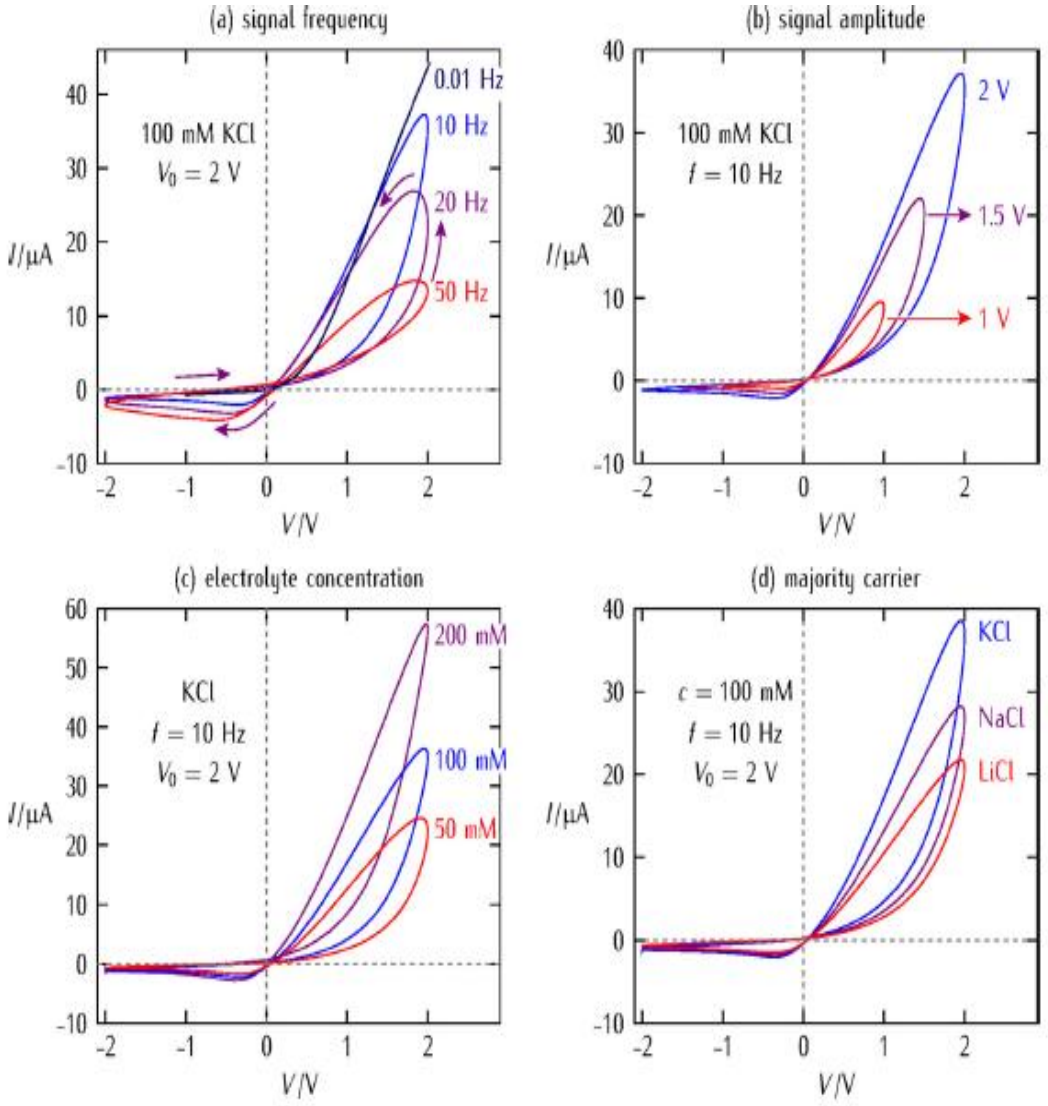


conductance *potentiation/depression*

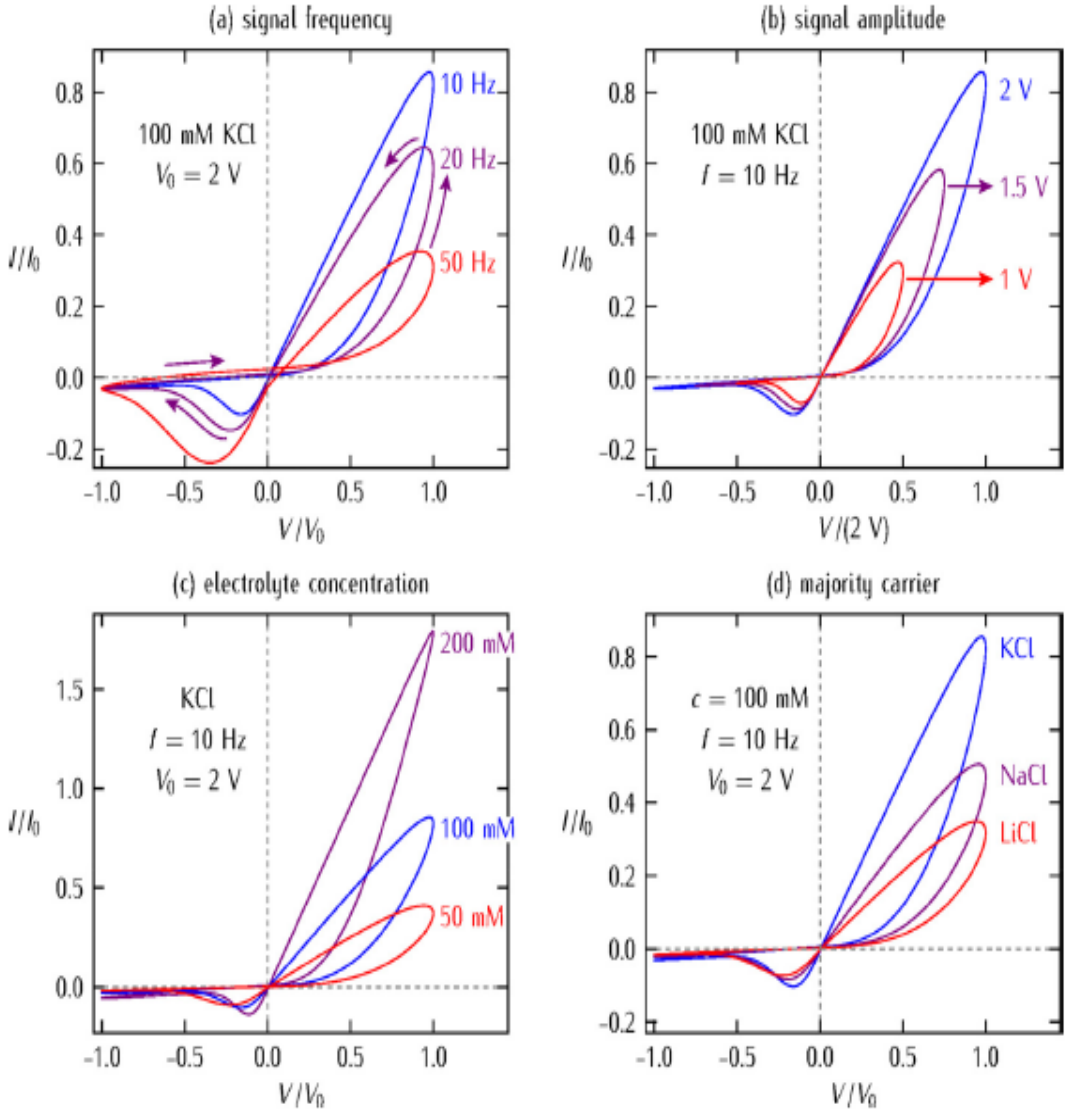
J. Phys. Chem. Lett. 2023
10.1021/acs.jpclett.3c02796

Phys.Fluids 2024
10.1063/5.0204219

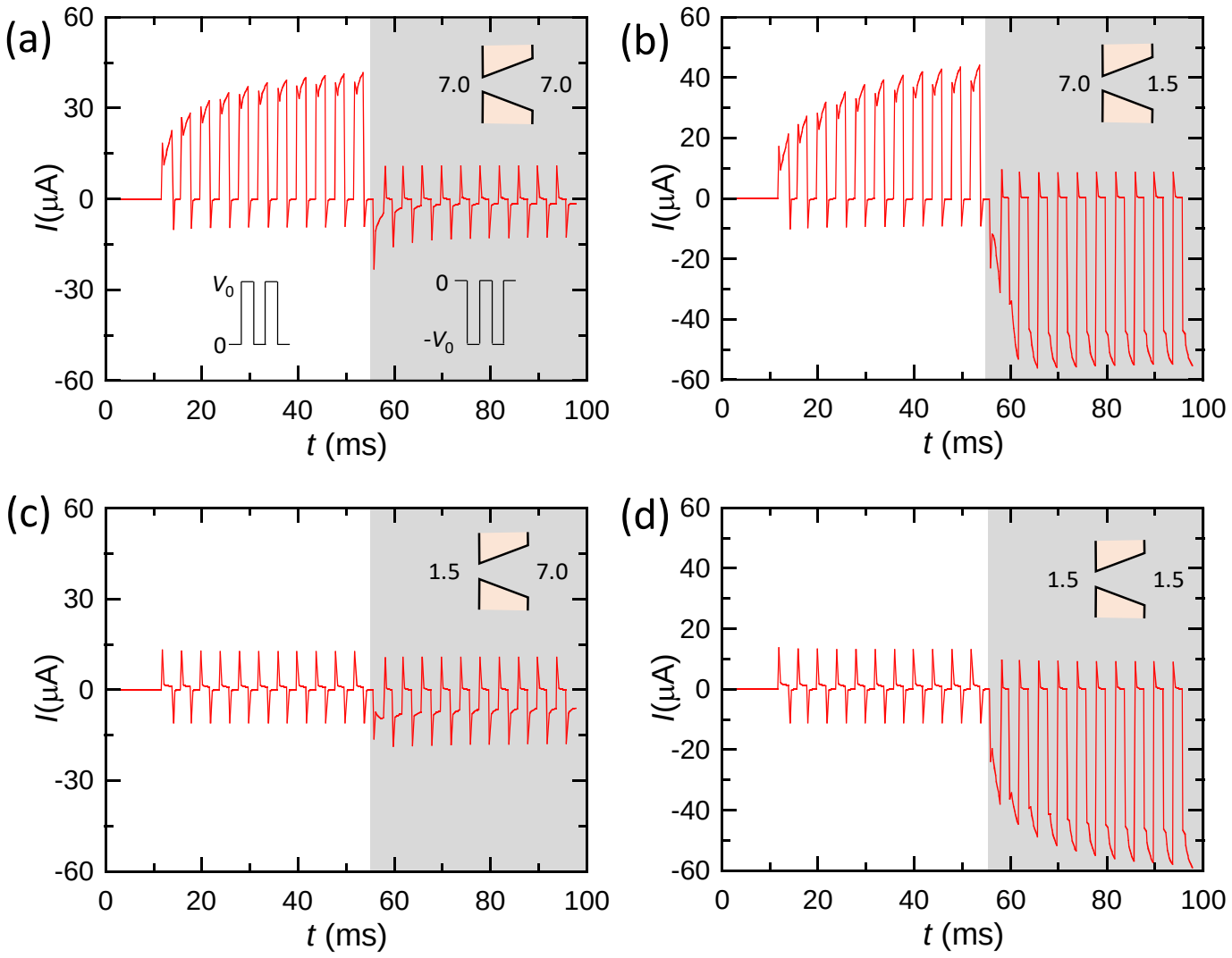
Experimental data



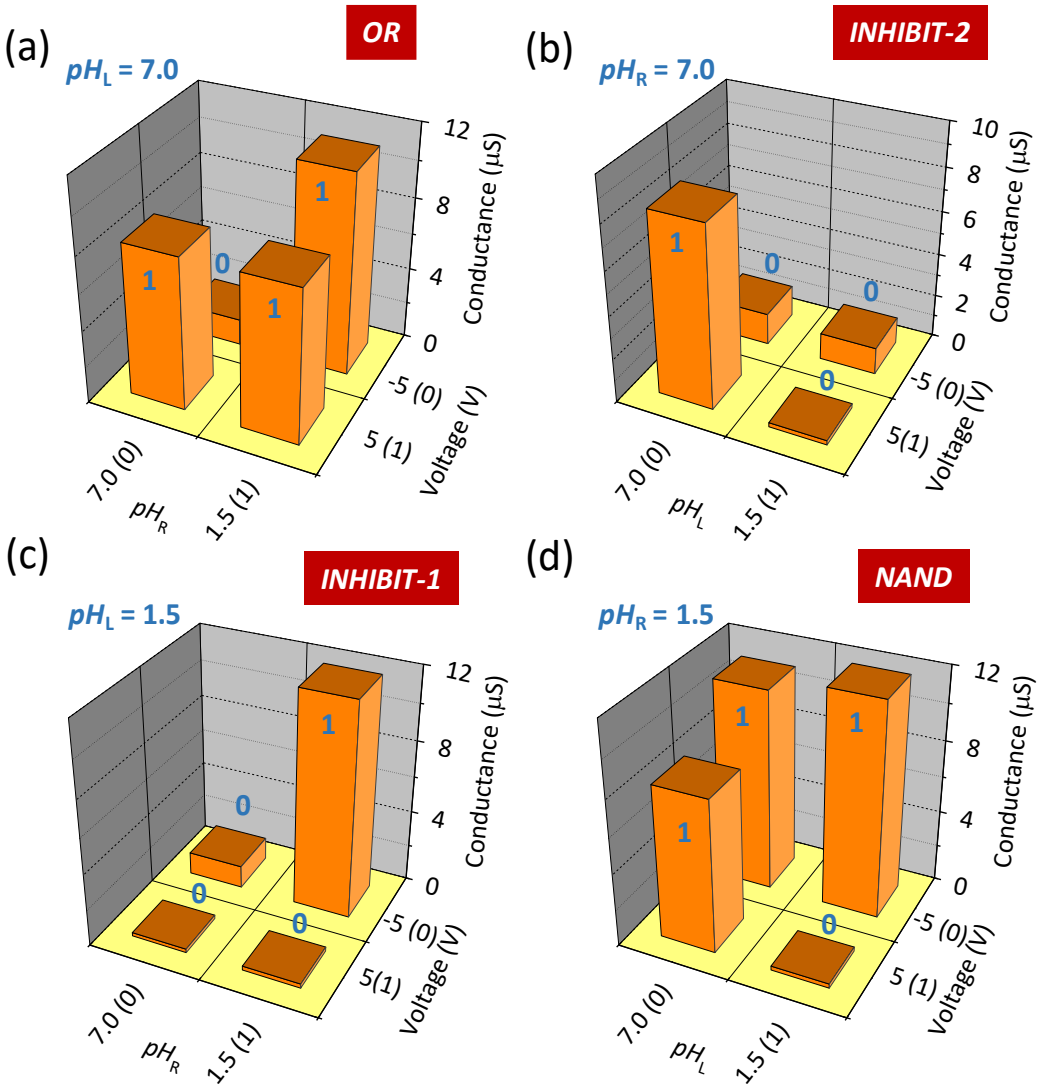
Model results



J. Phys. Chem. Lett. 2024
10.1021/acs.jpclett.4c01610

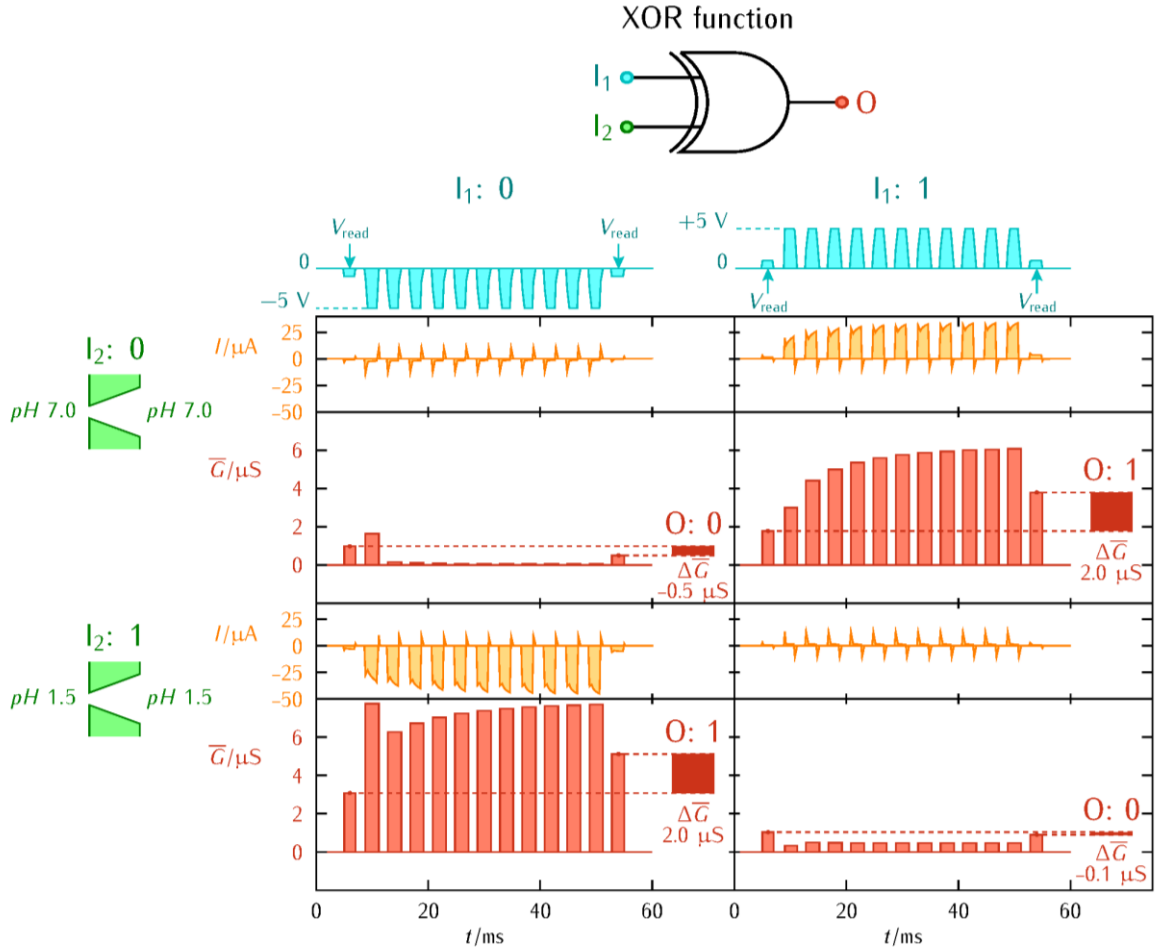


Current vs. time traces for different *pH tip/base pore configurations* obtained with sequences of voltage pulses.

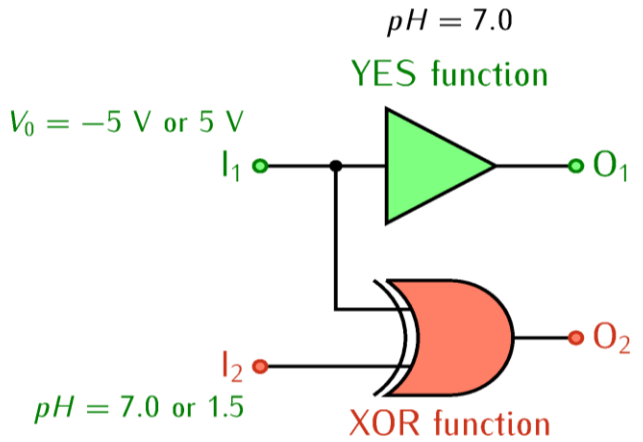


Logic functions. Inputs *applied voltage V* and *pH*. Output membrane *conductance G = I/V*.

Phys. Rev. E 2024
10.1103/PhysRevE.110.065101

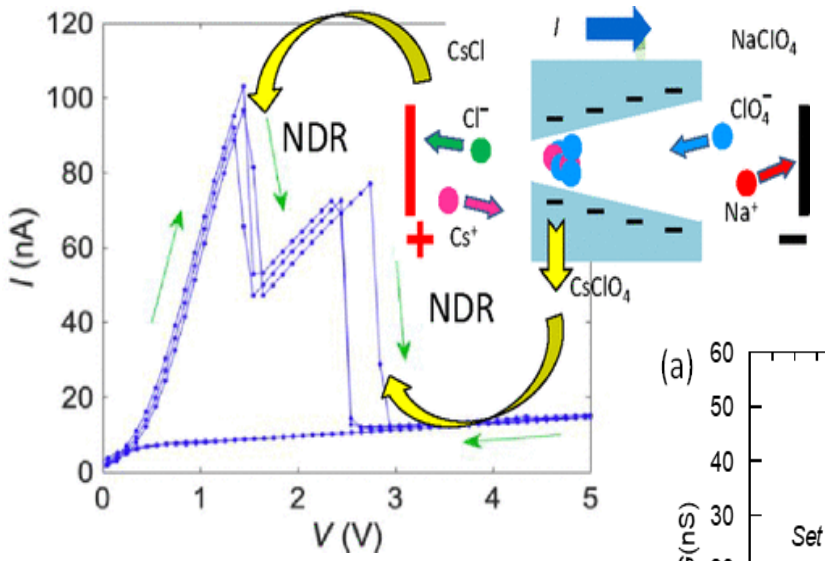


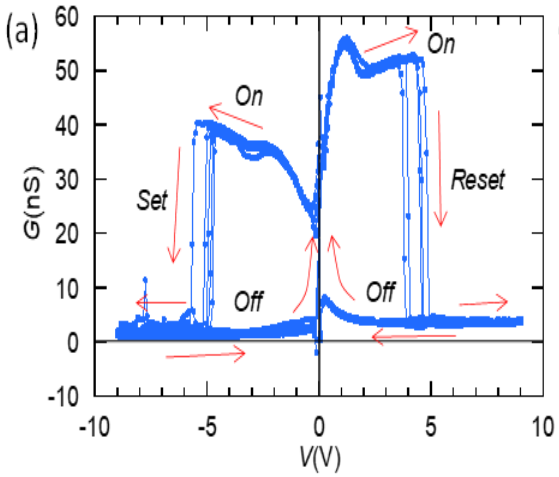
Voltage sign and the *pH solution* (*inputs*) give a *XOR* logics for *membrane conductance* (*output*) in a 100 mM KCl solution. Sequences of negative and positive voltage pulses give the average conductance difference that characterizes the conductance potentiations.

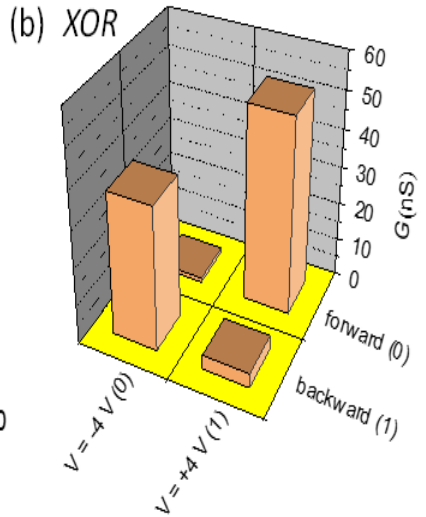


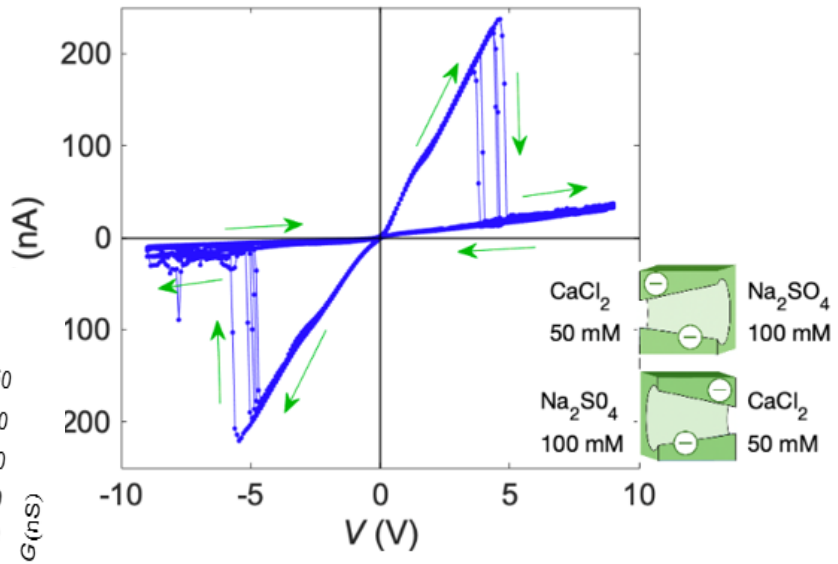
Input		Output: $\lvert\Delta\overline{G}\rvert$/μS	
$I_1 \equiv V_0$/V	$I_2 \equiv pH$	O_1	O_2
−5 (**0**)	7.0 (**0**)	0.5 (**0**)	0.5 (**0**)
+5 (**1**)	7.0 (**0**)	2.0 (**1**)	2.0 (**1**)
−5 (**0**)	1.5 (**1**)	0.5 (**0**)	2.0 (**1**)
+5 (**1**)	1.5 (**1**)	2.0 (**1**)	0.1 (**0**)

Feynman reversible logical function implemented by the combination of the *YES* and the *XOR* functions.







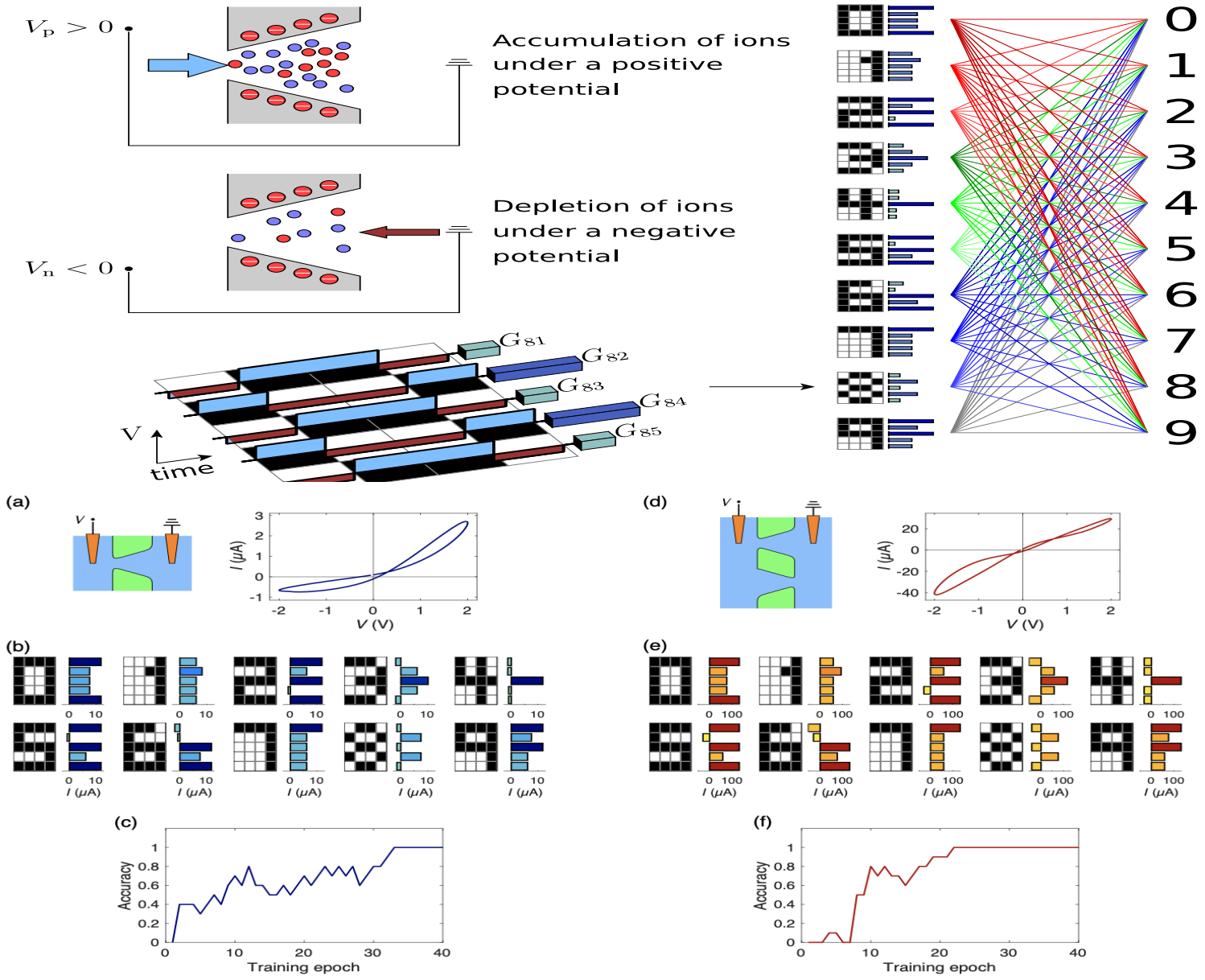


J. Colloid Interface Sci. 2026
10.1016/j.jcis.2025.138690
J. Phys. Chem. Lett 2025
10.1021/acs.jpclett.5c03327

Multi-threshold ionics in a membrane with *two parallel* conical nanopores (*top*, *left*). Ionic accumulation, pore blocking and *negative differential resistance* (NDR) occur for voltages $V > 0$ (forward polarization) leading to salt precipitation. The *I*–*V* curve obtained for two pores arranged in *antiparallel* configuration (*top*, *right*). In this case, precipitation occurs also at $V < 0$ (reverse polarization) and the two threshold voltages provide a digital-like response with a non-pinched behavior at zero voltage. The conductance-voltage *curve* resulting from the antiparallel arrangement under continuous set and reset cycling operation gives a *XOR logic response* (*bottom*). Ionic blocking and NDR effects are typical of voltage-gated calcium, sodium, and potassium ion channels and intercellular junctions.

Neuromorphic reservoir computing with memristive nanofluidic diodes

NanoLett 2025
10.1021/acs.nanolett.5c00853



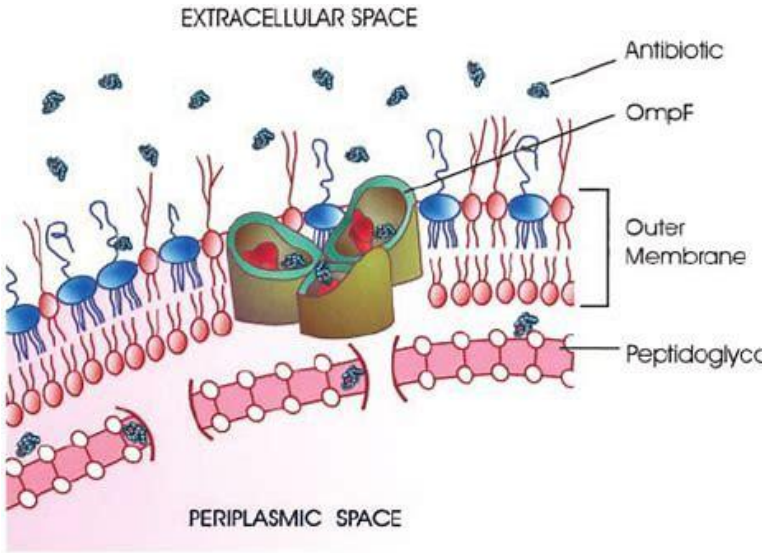
Mapping of *positive and negative voltage pulses* (input) into the distinct reservoir states (read-out) identifies *patterns of ten digits* (output). Ionic accumulation (voltage $V_p > 0$, high membrane conductance) and depletion (voltage $V_n < 0$, low membrane conductance) in the *nanofluidic conical pores* provide the *short-term plasticity* of the membrane conductance.

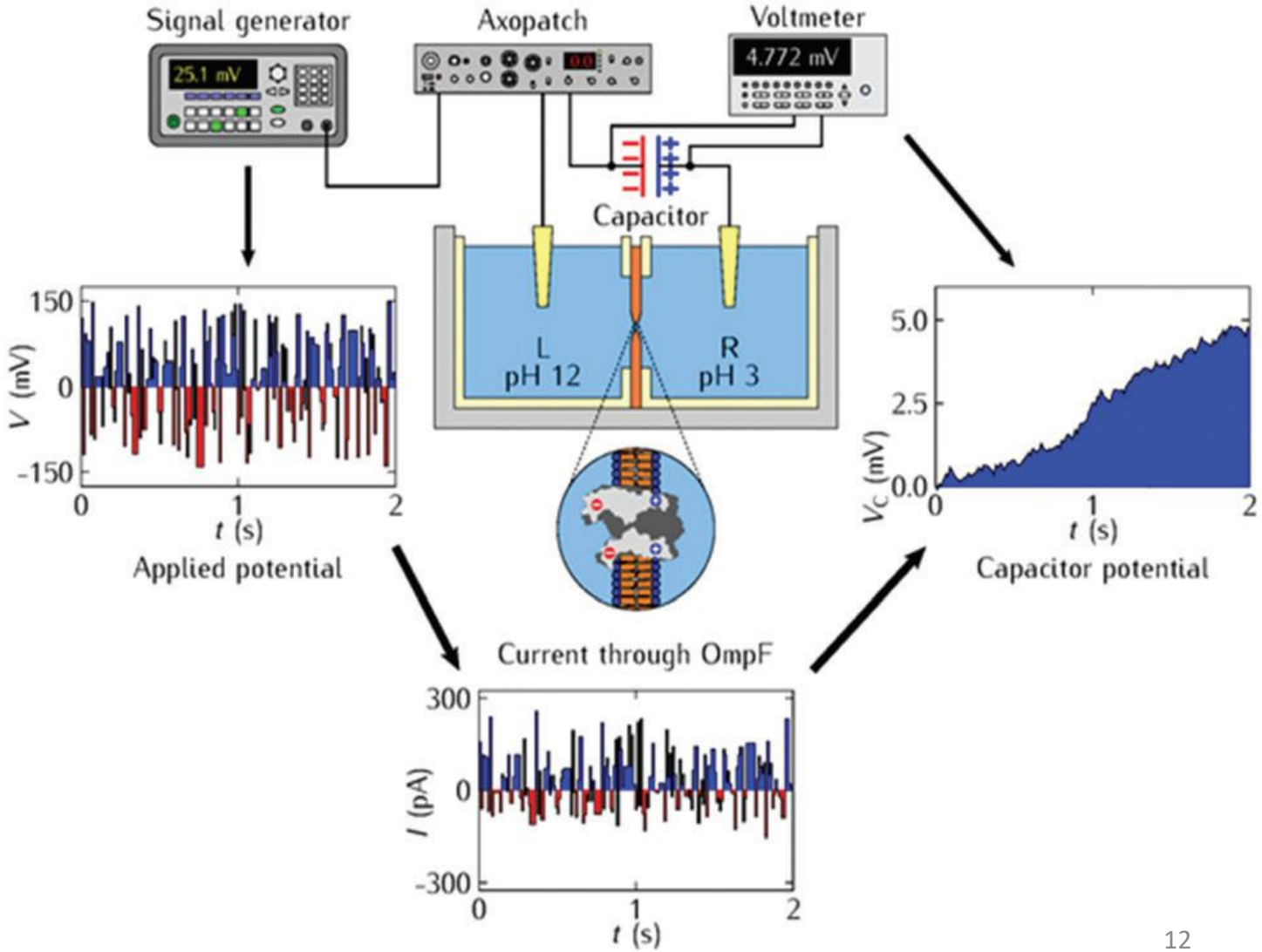
Final conductance states for the digits i = 0, 1, …, 9 and the membranes j = 1, 2, …, 5 are grouped in *conductance matrix* G_{ij}. Reservoir states are then processed by the read-out layer, which associate the different pulse inputs patterns to the distinct digit outputs. The *accuracy vs. training epoch* curves.

*1b Molecular biophysics: protein channels as nanofluidic diodes (with **Patricio Ramirez**)*

Small 2018
10.1002/smll.201702252
Phys. Chem. Chem. Phys. 2017
10.1039/c6cp06035h

Electrical rectification produces *cumulative effects* in the Outer Membrane Porin F of *E. Coli* inserted in an artificial lipid bilayer





2. Multicellular organization: bioeng., biochem., and bioelec. views

In *biological patterns*:

- individual cells can make decisions with *local info,* usually without having a highly precise knowledge of its particular position in a multicellular ensemble;

- *environmental changes* and *individual variability* may help *system exploration*. The result is a continuous interplay between *short-term* individual changes and *long-term* collective persistence; and

- traditional attempts to understand *instructive patterns* have favored spatio-temporal distributions of *biochemical* signals.

2.a Distributed epigenetic shape formation and regeneration algorithm for a swarm of robots

Algorithm 2 Algorithm for the shape regeneration by the swarm of robots

```
1:  while TRUE do
2:    if State == Danger then
3:      LId == LeaderElection(); {the robot has the lowest Id
        among all the Queued robots}
4:      if Id == LId then
5:        State ← Leader;
6:      else
7:        State ← Queued;
8:        Algorithm-1();
9:      end if
10:   end if
11:   if State == Leader then
12:     CountPopulation(); {Count the remaining population}
13:     GenerateScaledShape(); {Scale down the input target
        shape}
14:     Share the new shape with the remaining robot;
15:     State ← Active;
16:     Form new seed robots;
17:     Algorithm-1();
18:   end if
19: end while
```

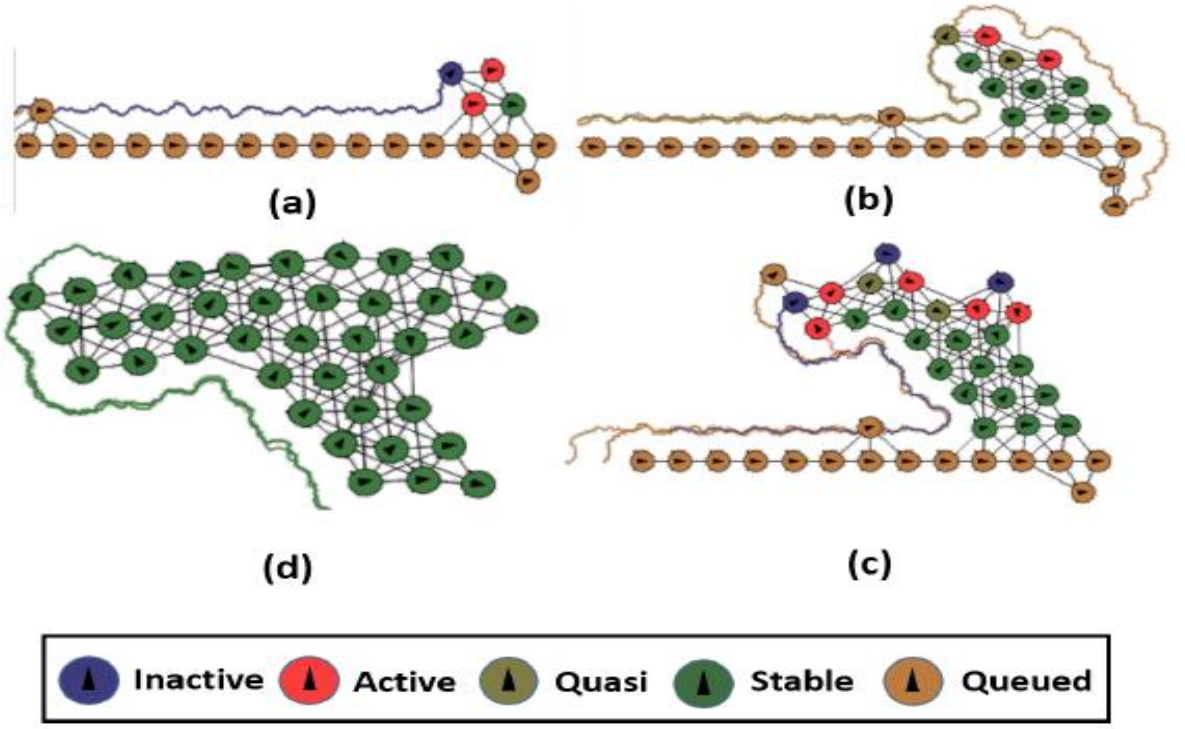


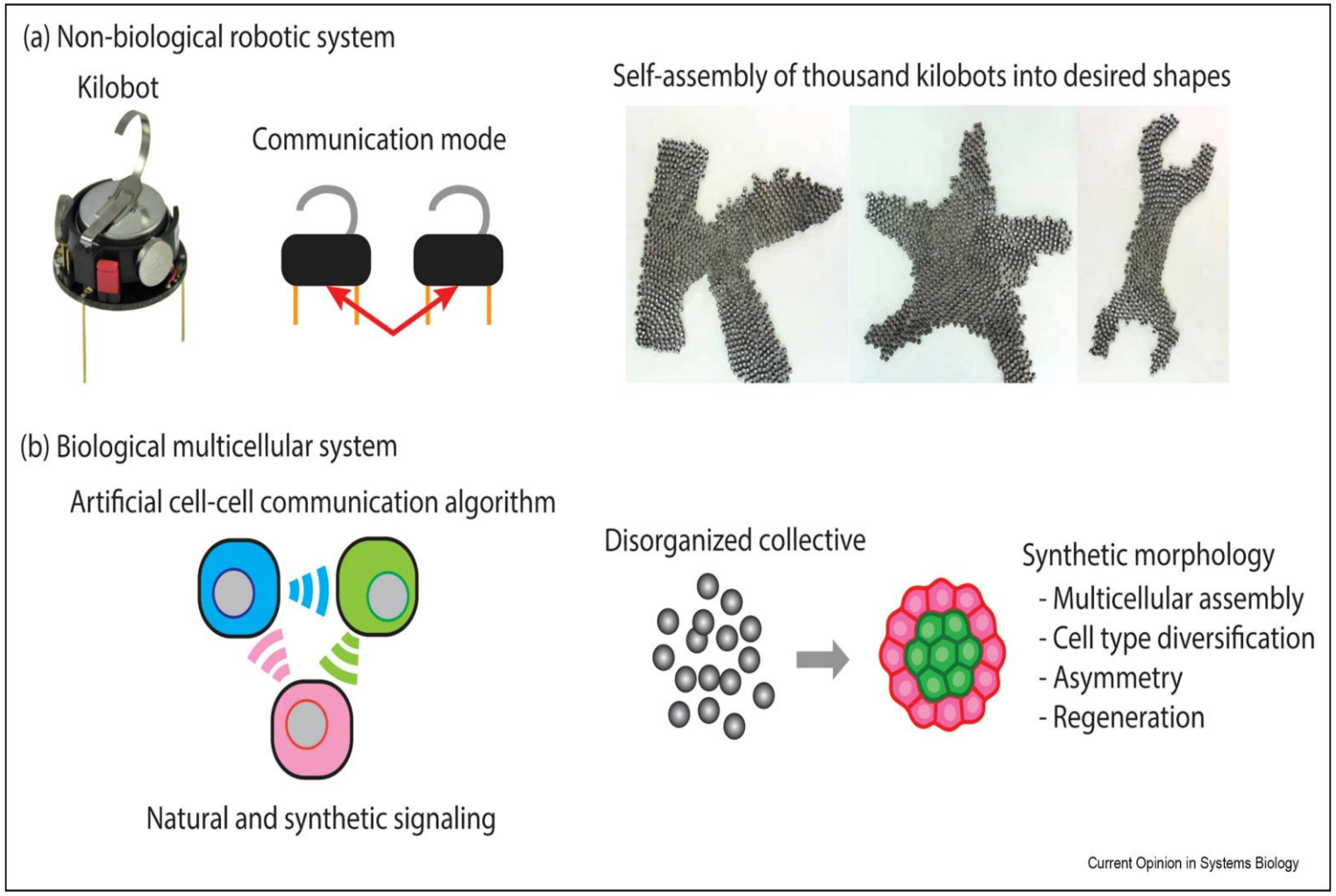
Figure 5: Generation of alphabet 'T' in 2 hours 50 minutes and 8 seconds

GECCO '18 Companion 2018 (ACM)
10.1145/3205651.3208300

Biologically inspired algorithm for shape formation and regeneration exhibited by a swarm of robots.. Here, a *gene* is similar to an *instruction manual* which aids cells to *reproduce and form* arbitrary shapes. Given an input binary image, the gene required for the shape formation is computed and transferred to *all the robots* constituting the swarm. The robots ("cells") then use this gene to form a shape in a distributed and decentralized manner.

2.b Forward engineering of collective systems

Curr. Opin. Syst. Biol. 2019
10.1016/j.coisb.2019.02.008

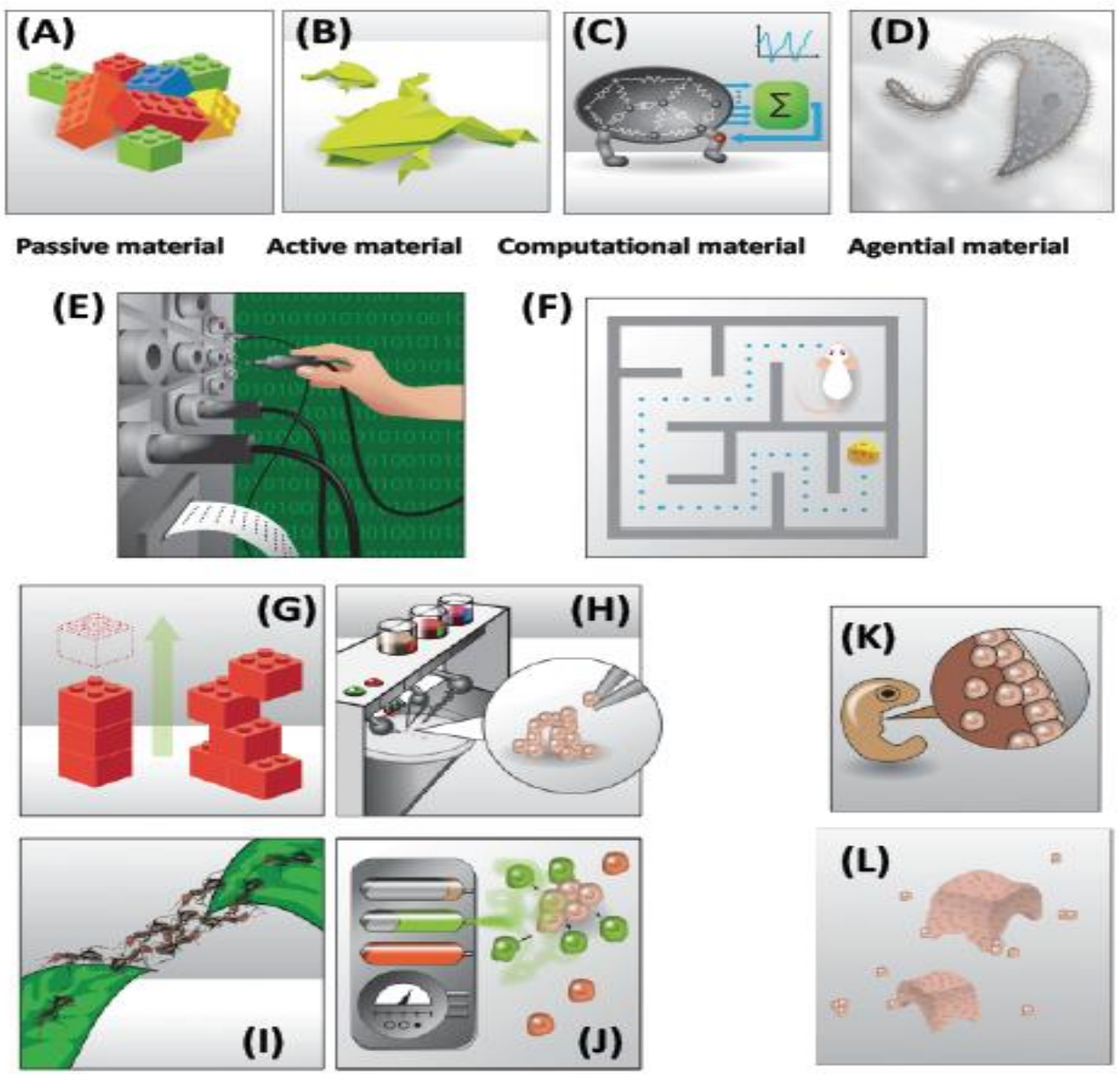


(a) *Robotic swarms of Kilobots* can communicate with neighbors by reflecting infrared light off the table below to decide how they move according to a *user-designed communication algorithm*. Examples of collective patterns.

(b) *Engineering of cell–cell interactions to drive multicellular self-organization*. Artificial algorithms can program disorganized cells to behave collectively and self-organize into particular structures. Features can mimic processes in natural developmental systems (self-assembly, cell type diversification, symmetry breaking, and regeneration).

2.c Engineering with agential materials

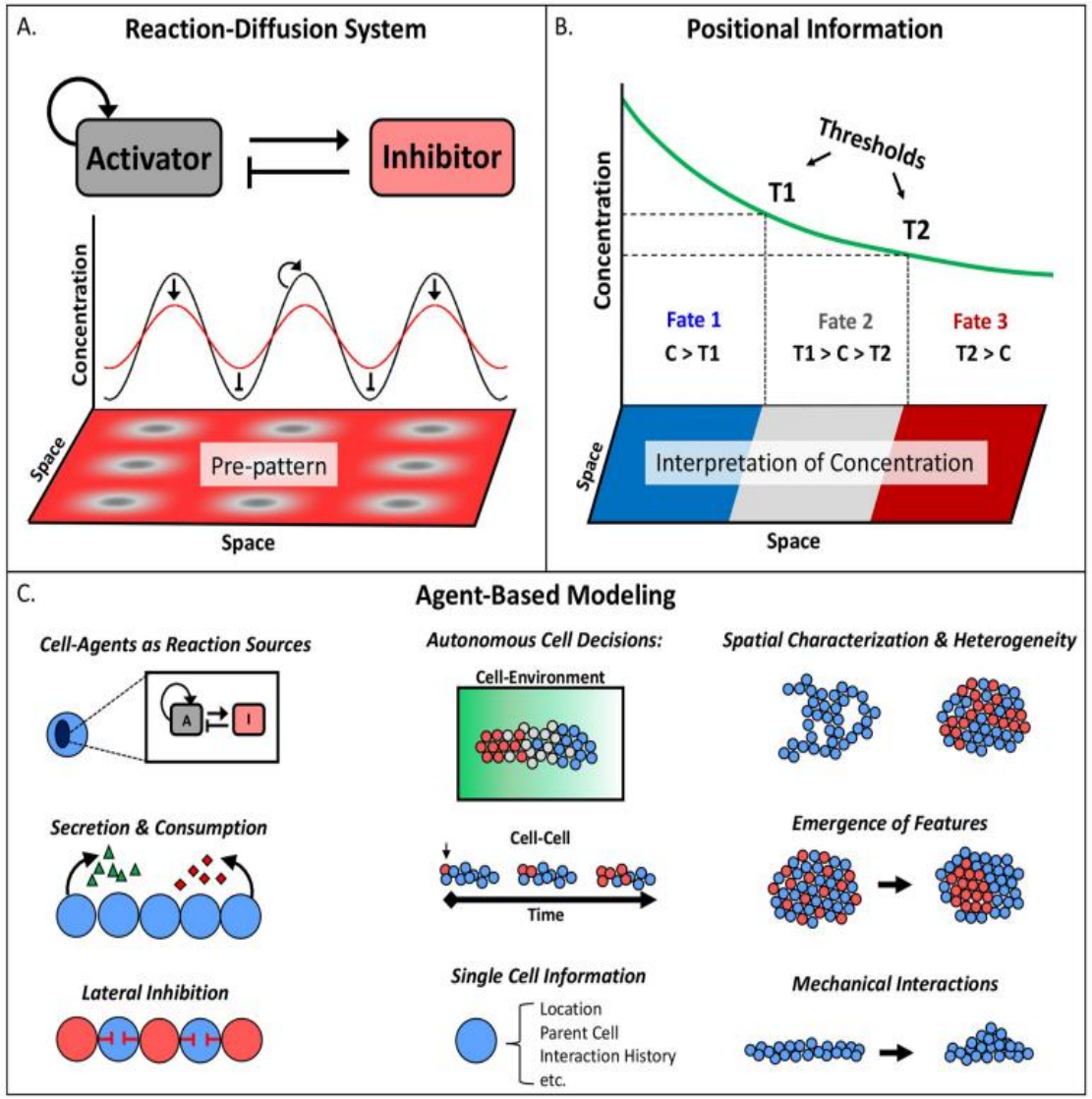
Soft Robot. 2023
10.1089/soro.2022.0142



Engineering with passive materials (A) managed for desired functionality has moved toward active matter (B) and computational media (C). Biorobotics considers material as *agential*, composed of subunits (D, living cells) that include *problem-solving* in *problem spaces*. They are not only subjected to rewiring (E) but also to behavior-shaping (F). In complement to 3D-printing designed for building with passive matter (G), which also works with cells (H), collective intelligence of living systems, e.g. ant swarm (I), allows manipulating the collective behavior of cells (J) in *anatomical morphospace*, as instructive signals from other cells cause frog ectodermal cells to be a 2-dimensional barrier in embryos (K) and stimuli can achieve guided self-assembly toward novel form and function (L).

2.d Biochemical multicellular patterns

Plos Biol. Comput. 2019
10.1371/journal.pcbi.1006577



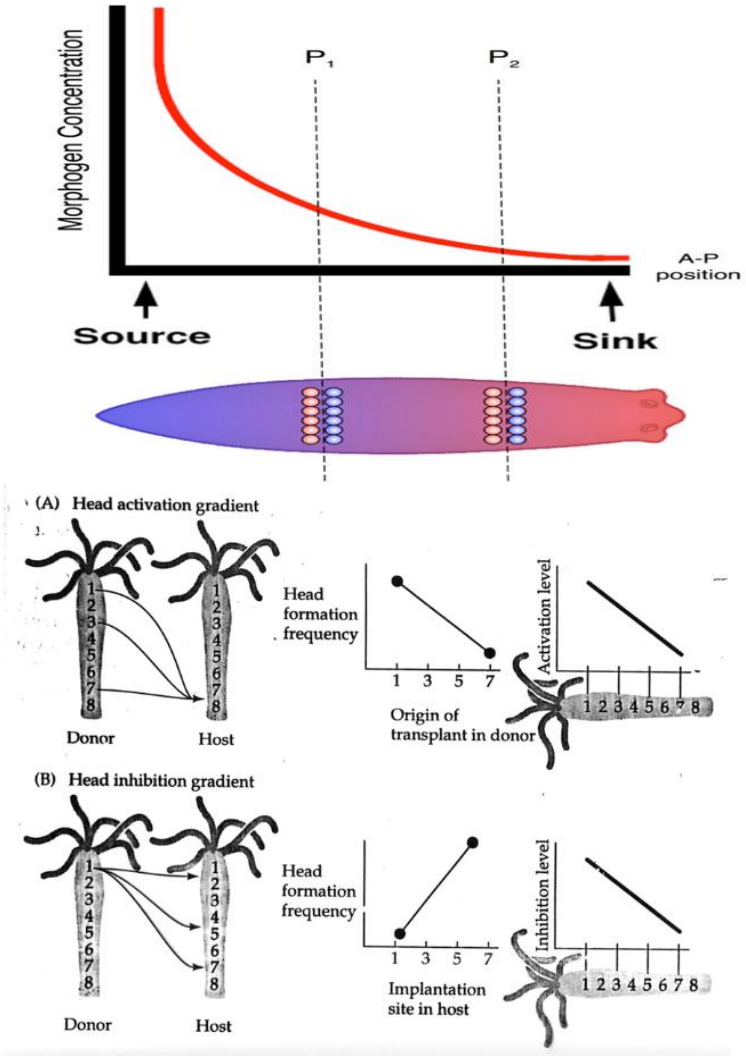
pattern formation during morphogenesis

(A) *Turing reaction–diffusion system* with an activator and an inhibitor: a chemical prepattern is established in advance of cell fate decision.

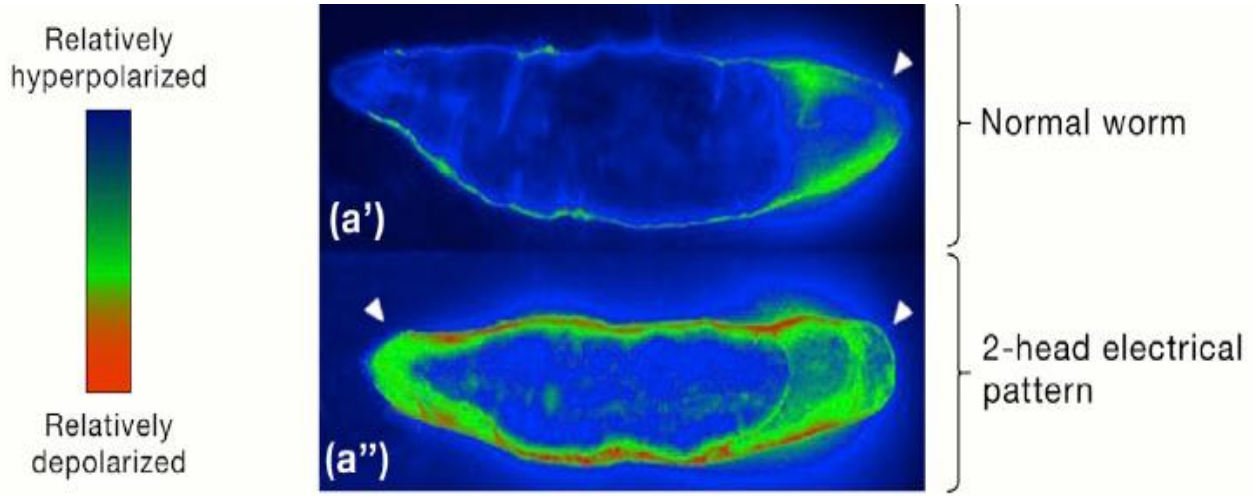
(B) *Wolpert's positional information:* concentration thresholds allow cell multiple fate decisions from a single molecular gradient.

(C) *Agent-based models* incorporate features from both theories. Cell agents are sources of activators and inhibitors, permit localized reactions, and make autonomous decisions in response to local environment. Static and dynamic spatio-temporal patterns can reproduce complex behaviors.

Biochemical gradient *vs.* *Bioelectrical* pattern



one (two?) *morphogen gradients* needed
(a head activator and a head inhibitor?)



biofield level –
(no head)

biofield level +
(head)

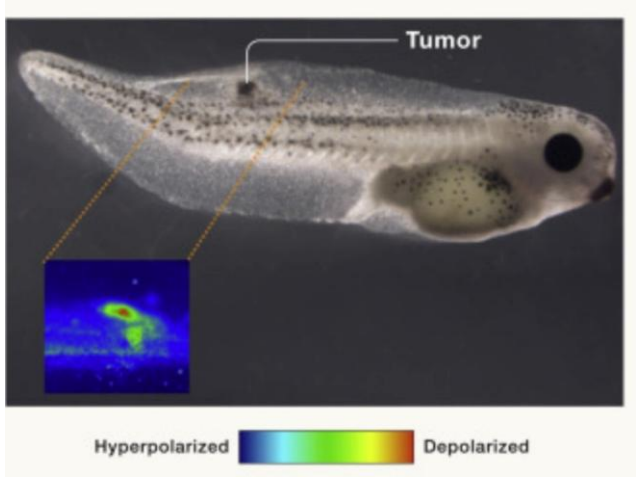
one *bioelectrical field*
influences the animal polarity

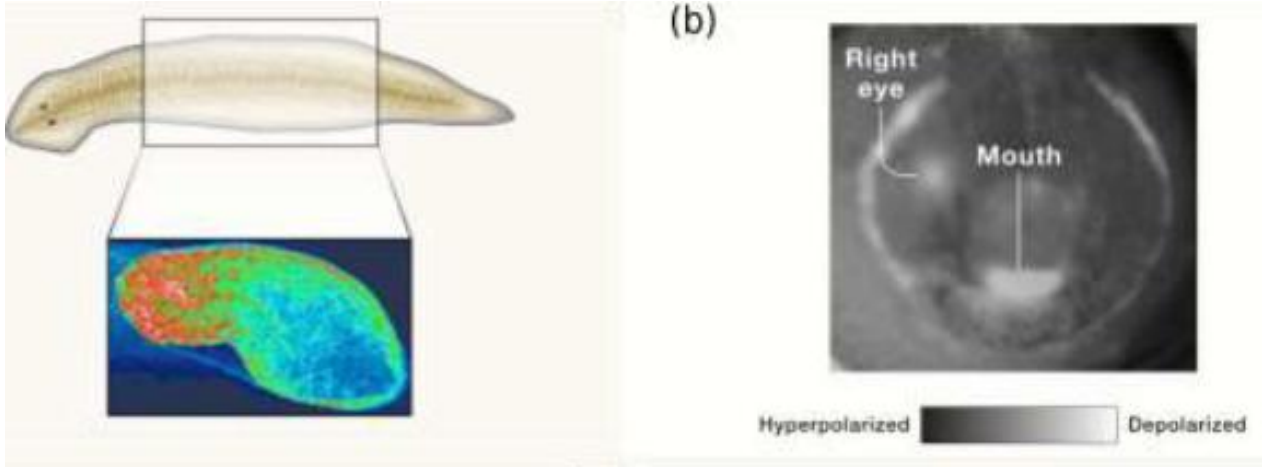
2.e Bioelectrical multicellular patterns

Cell 2021
10.1016/j.cell.2021.02.034

Phys. Rep. 2023
10.1016/j.physrep.2022.12.003

1) Cells are sensitive to *spatio-temporal bioelectrical patterns* in *developmental phases* and *regeneration stages.* Cell potential maps can be correlated with distributions of potassium and calcium ions, neurotransmitters, and other transcription activators, acting as *pre-patterns* for slow *biochemical signals*.





2) A *cell* needs both individual *independence* to respond to external stimuli and *coordination* with the multicellular aggregate for morphological outcomes. Information concerning the body plan must reside within the *DNA*, a *single-cell language* for making proteins. However, while morphology is data-driven, it is *not evident* only from the data. Here, we consider the *ion channels* and the *intercellular junctions* as the *computational* and *cognitive* layer needed to specify multicellular coordination.

3) We explore a *bioelectrical multicellular language* based on two assumptions:

3.1) *"hardware"*: *transcription* provides voltage-sensitive *ion channel* and intercellular *gap junction* proteins that regulate the *cell membrane potential*. In turn, the cell potential allows *feedback* mechanisms that modulate the *transcription* of these proteins;

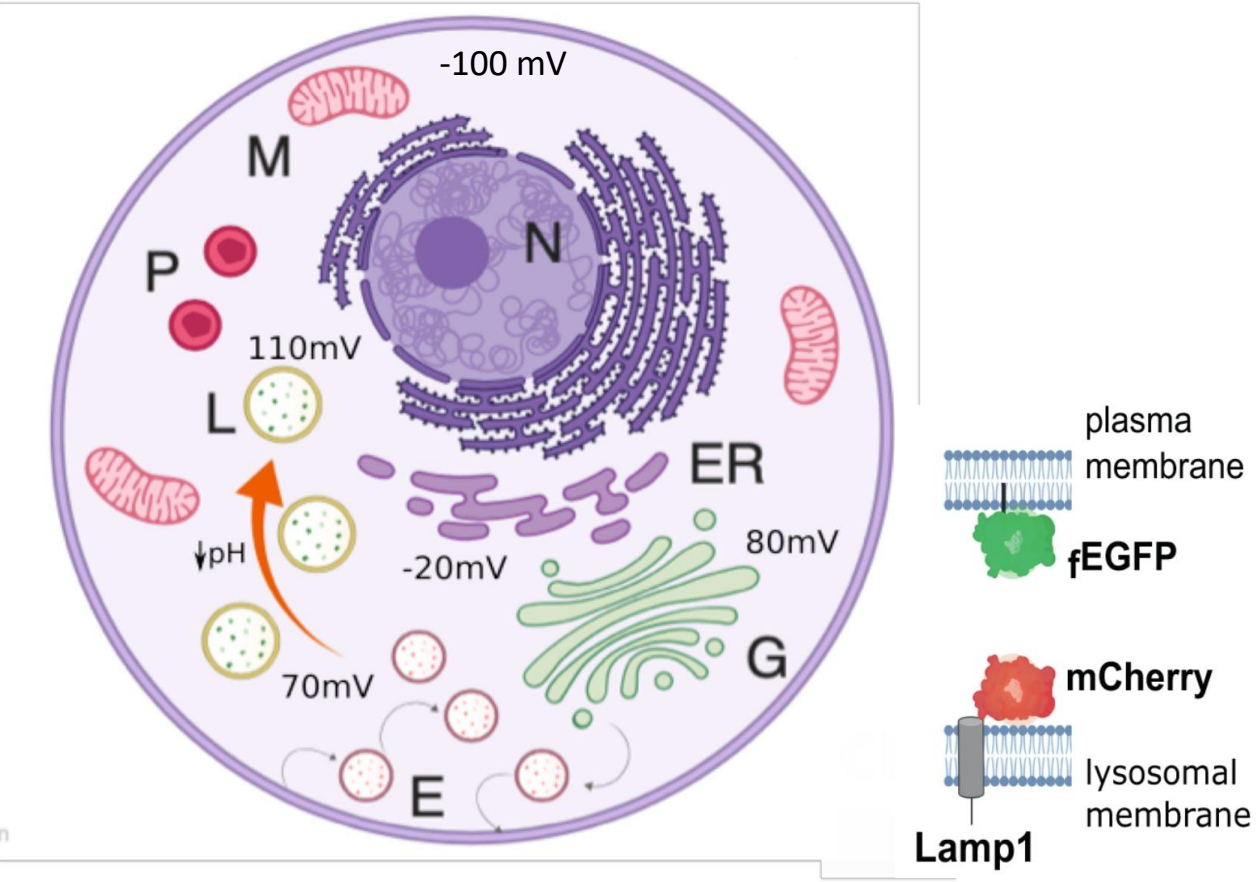
3.2) *"software"*: the spatio-temporal *pattern* of *membrane potentials* constitutes a *bioelectrical network* that contributes to the required *multicellular coordination*; and

4) we attempt to reduce biological complexity by identifying a small number of observable magnitudes that may control *crucial steps*, making emphasis on *bioelectrical mechanisms.* Then, we suggest *operational actions* based on *average multicellular potentials*. These *actions* are not only *system-* but also *context-dependent* because of multiple *single-cell* and *multicellular* bioelectrical feedbacks.

3. Some experimental facts of bioelectricity + molecular biology

bioRxiv 2019
10.1101/578765
Comm. Biol. 2021
10.1038/s42003-021-01916-6

Multiscale bioelectricity: single-cell *regionalization* of organelle *membrane potentials* as a low scale spatio-temporal integration: can *higher scales* be defined?

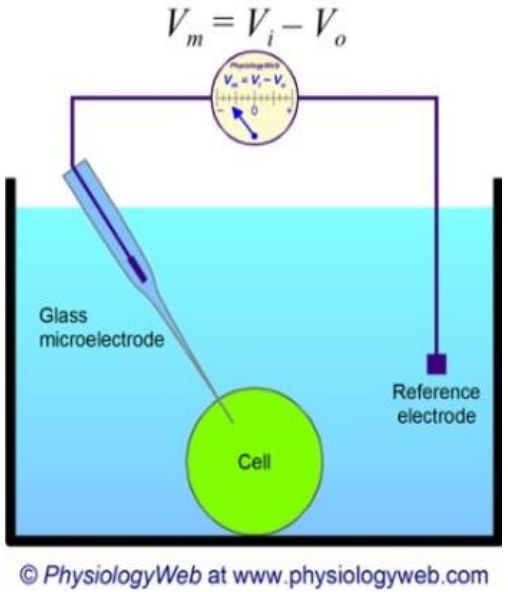


Organellar membrane potential compartmentalization. *Lysosomes* (L, yellow) and *golgi* (G, green) compartments may have a relatively large and positive resting potential in contrast with the modest and negative inside potential of *ER* (purple). Pumping of protons into the lysosomal lumen by the V-ATPase leads to acidification and a more hyperpolarized membrane potential in the mature lysosome (orange arrow). *Mitochondria* (M, red) have a relatively large and negative potential.

Optical multiplexing of organellar activity and topology of the probes.

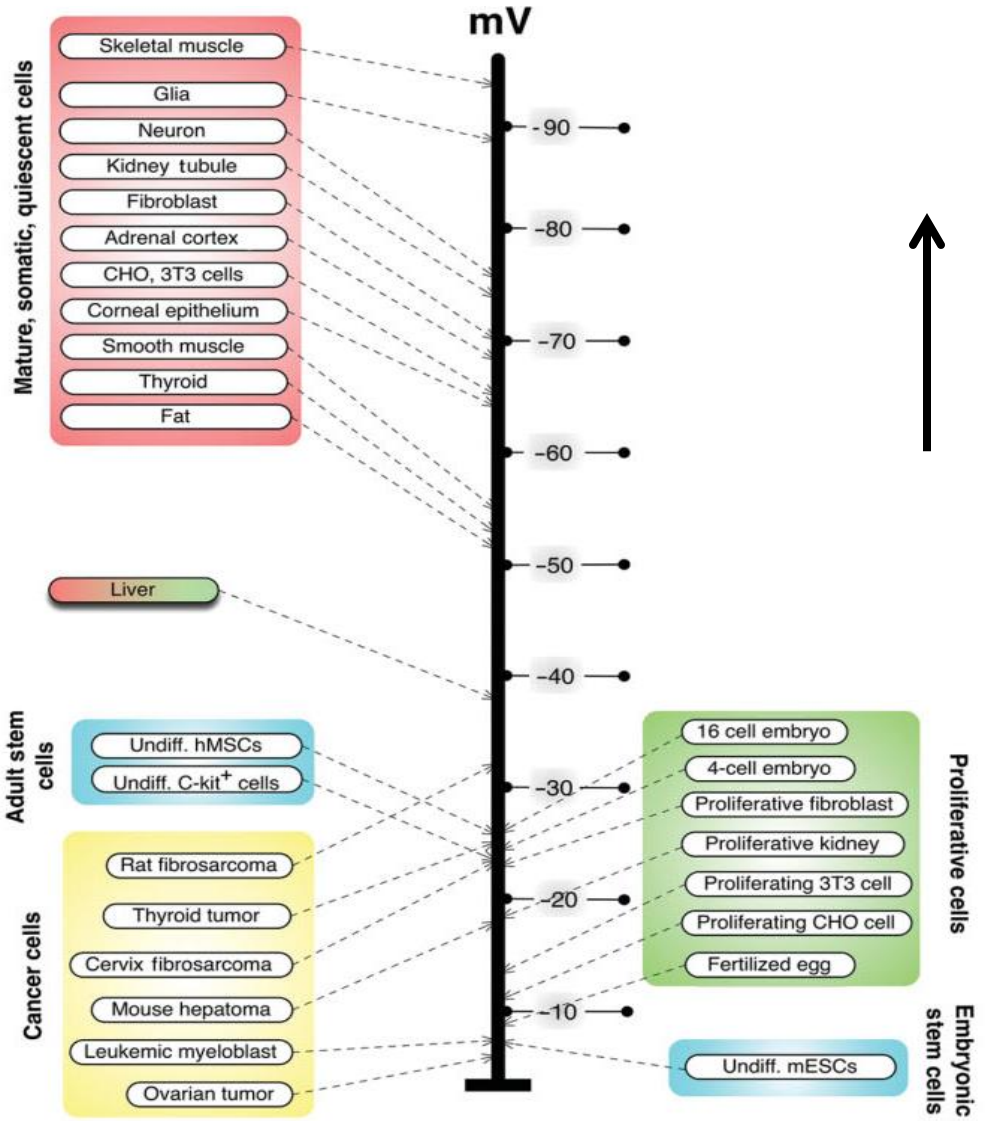
3.a Single-cell potentials: the membrane as a bioelectrical control surface

Wires. Sys. Biol. Med. 2013 10.1002/wsbm.1236
Prog Biophys Mol Biol. 2016 10.1016/j.pbiomolbio.2016.06.006
PLoS. Biol. 2025 10.1371/journal.pbio.3003052



Polarized and *depolarized* potential *windows* exist at the individual *cell scale*.

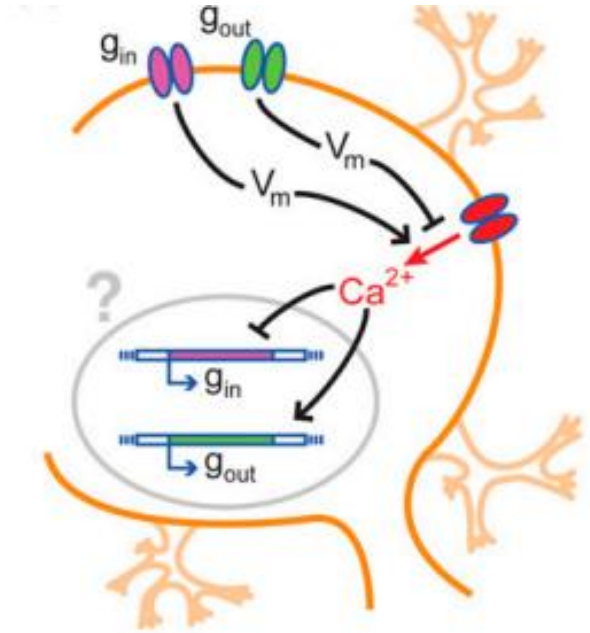
However, we are interested in *multicellular patterns* and *collective mechanisms* here.



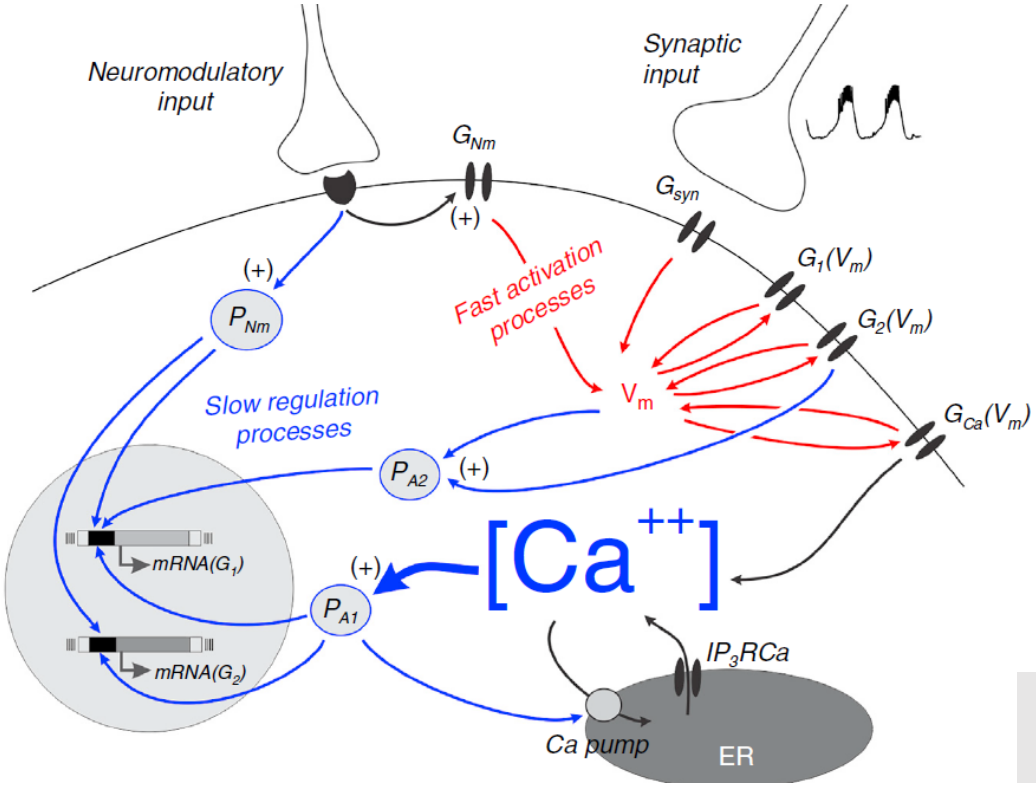
Mature cells are *polarized* (high $|V_m|$). The *default* mode?

Proliferative cells are *depolarized* (low $|V_m|$).

3.b A reduced number of counteracting voltage-gated channels influence protein transcription, regulating single-cell and intercellular potentials



PNAS 2013
10.1073/pnas.1309966110



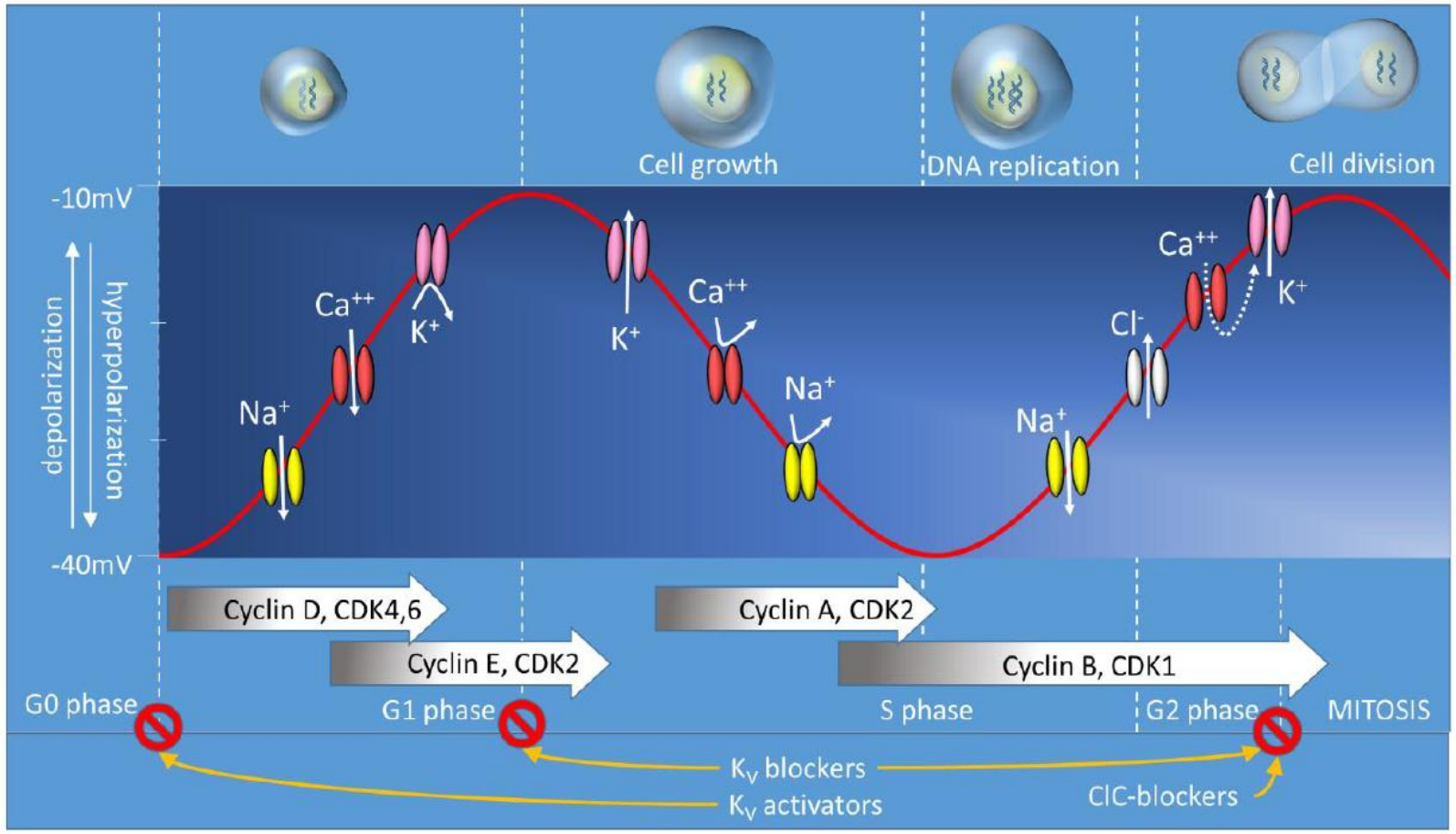
homeostatic channel regulation in neurons

Current Biol. 2019
10.1016/j.cub.2019.05.029

Interplay between *bioelectricity* and *transcription*: voltage-gated channel proteins have mRNA levels modulated by a *calcium-dependent transcription*. A neuron regulated by inward (g_{in}) and outward (g_{out}) conductances where the Ca^{2+} influx through voltage-gated calcium channels depends on the membrane potential, which is regulated in turn by the channel conductances.

cell cycle

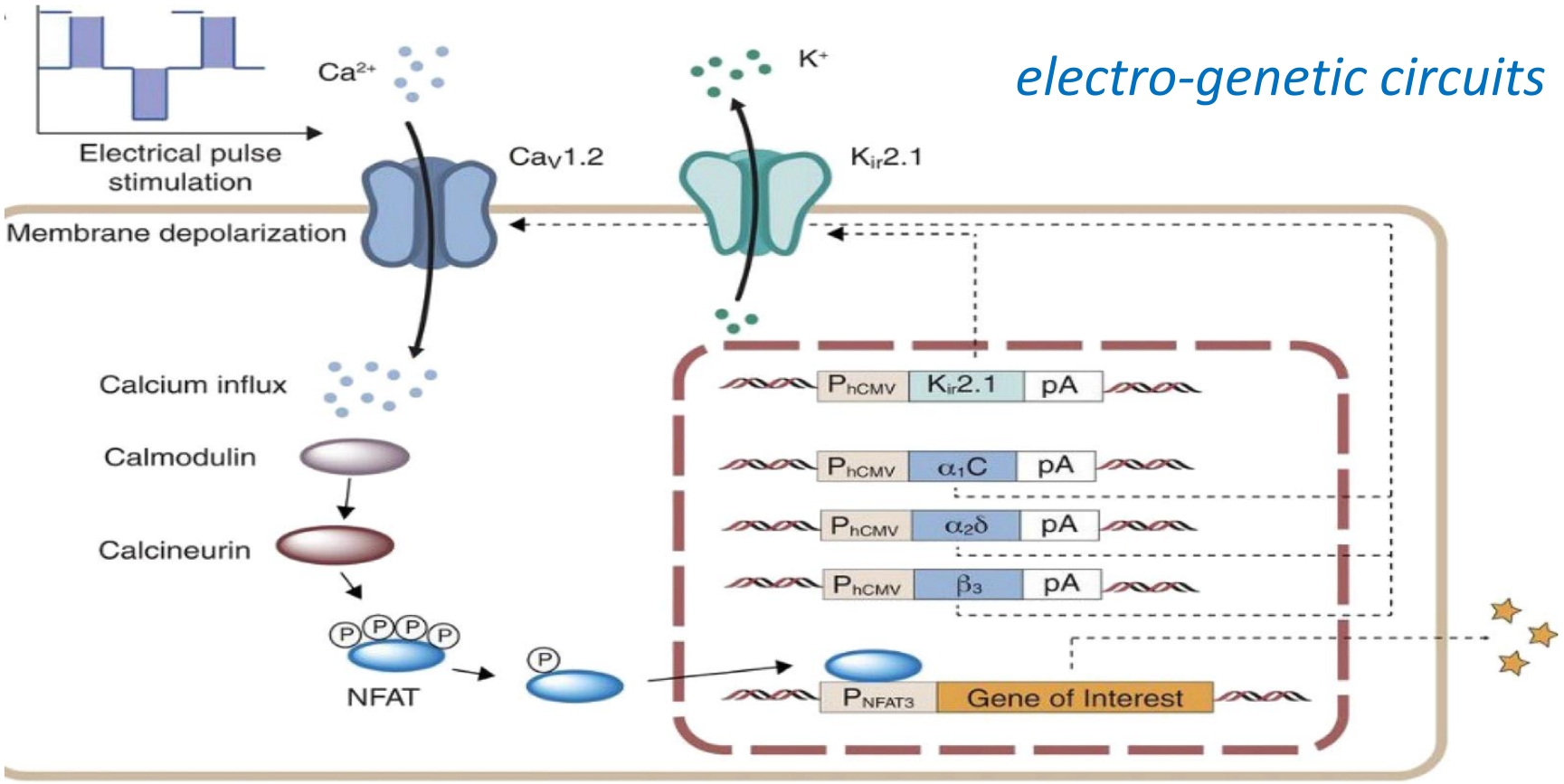
Cancers 2015
10.3390/cancers7020813



Proposed model of voltage-gated ion channel activity during the *cell cycle*. Note the *counteracting channel actions* based on their different expression levels. The oscillation of the membrane potential follows the progression of the cell cycle. Could this *bioelectrical process* be arrested at particular *polarized* or *depolarized* cell states by acting on specific channels?

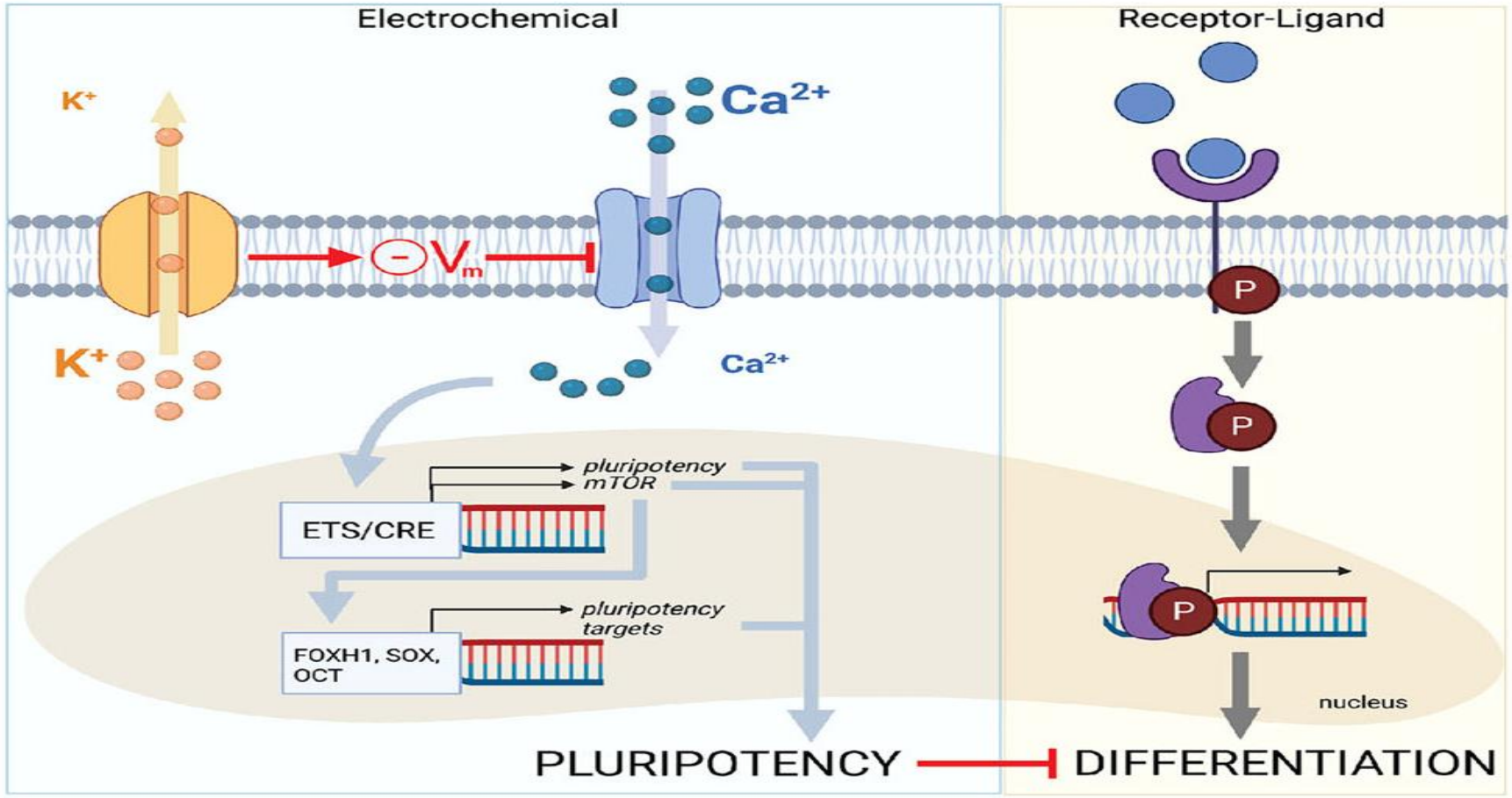
Science 2020
10.1126/science.aau7187

electro-genetic circuits



Transcriptional control of mammalian cells by membrane depolarization shows the *coupling* between *bioelectrical* and *biochemical signals*. In the electrogenetic circuit, an inward-rectifying potassium (*Kir*) channel sets initially the potential of human embryonic kidney (*HEK-293T*) cells. External pulses provoke the depolarization that opens the voltage-gated calcium channel (*Cav*). The increase in the calcium level yields *downstream processes* (activation of the calmodulin/calcineurin pathway, dephosphorylation of the nuclear factor of activated T cells (NFAT), and the NFAT-sensitive promoter) towards the *target transgene expression*.

Nat. Commun. 2022
10.1038/s41467-022-34363-w

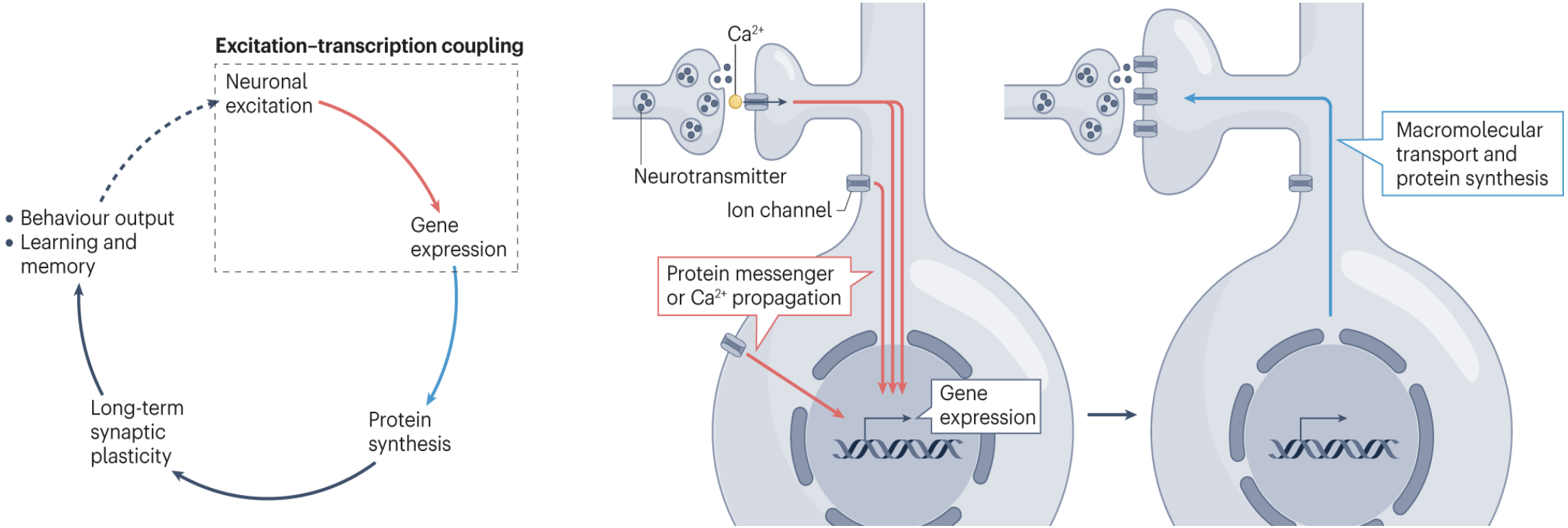


embryonic differentiation

Membrane potential (*left*) and *receptor-ligand* (*right*) *signaling* in the onset of *embryonic differentiation*. Potassium channels set the polarized cell potential, which keeps voltage-gated calcium channels inactivated, thus avoiding the high calcium levels required for downstream processes to pluripotency. While gene expression is also mediated by intracellular signal transducers and biochemical pathways essential to the expression of differentiation factors, *bioelectricity* may influence also the *timing of pluripotency genes*.

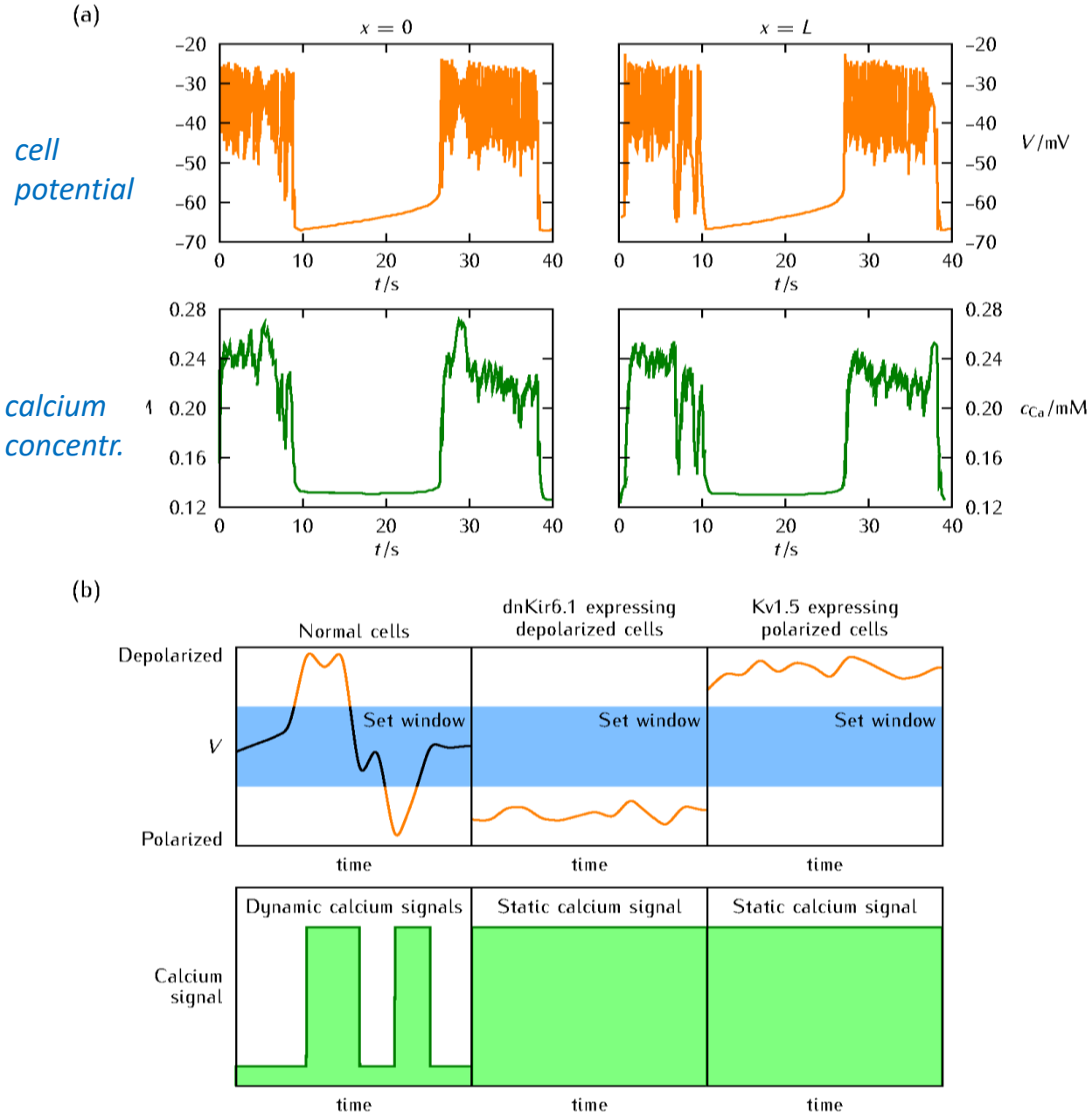
Nature Rev. Neurosci. 2023
10.1038/s41583-023-00742-5

excitation–transcription coupling in synaptic plasticity



A model of *lasting synaptic plasticity* relies on neuronal activity-induced transcription in the nucleus and transport of translated proteins back to the synapse. The conversion of neuronal excitation to gene expression involves an *excitation–transcription coupling* initiated by membrane depolarization at the synapse and the soma, which drives *Ca^{2+} entry via ion channels and activates Ca^{2+}-sensitive transcriptional signaling cascades* (*red arrows, left*). The gene expression yields macromolecules from the nucleus back to the synapse (*blue arrow, right*) to sustain long-term potentiation.

Phys. Rep. 2023
10.1016/j.physrep.2022.12.0030370-1573



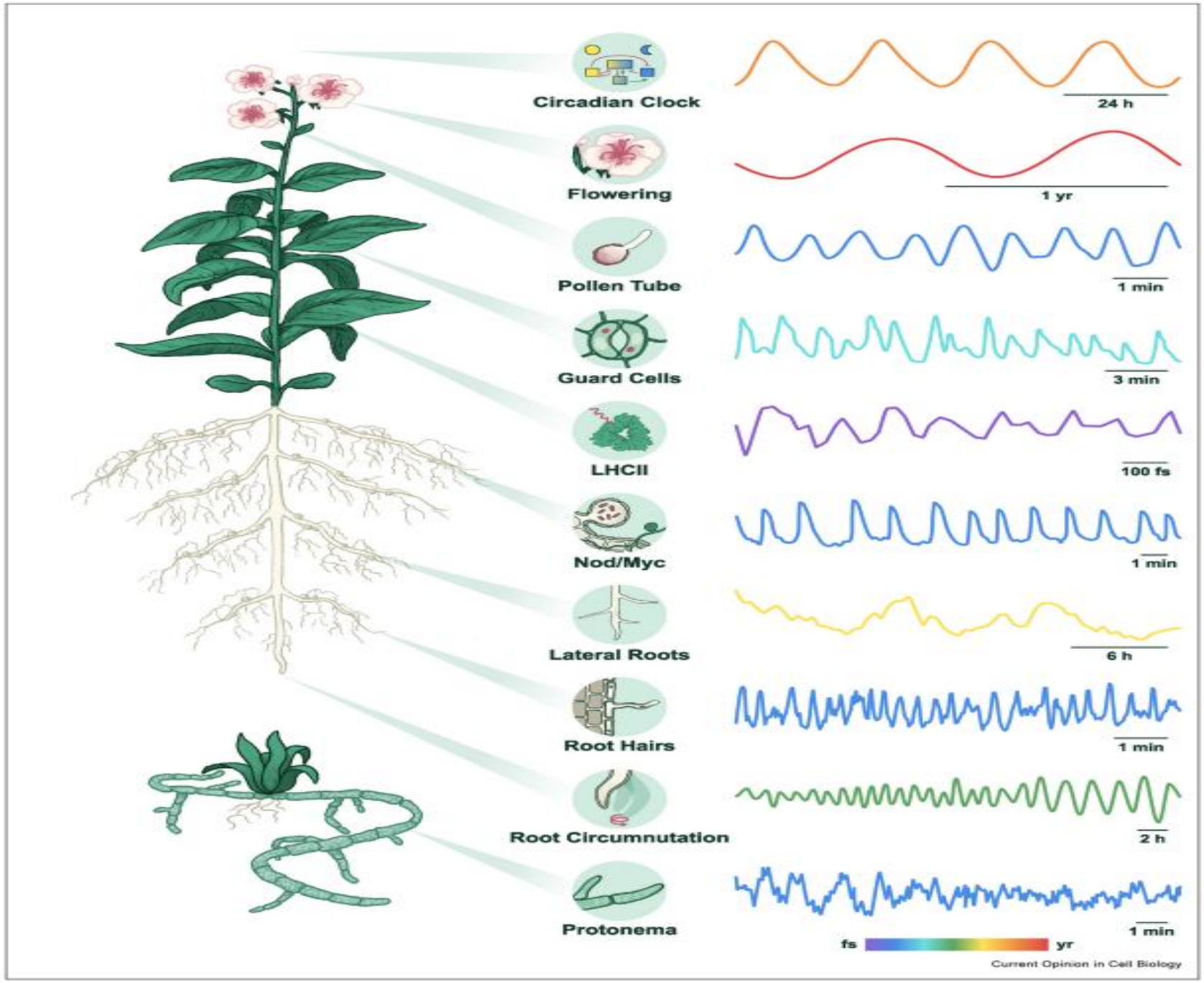
experimental coupling between bioelectrical and biochemical (Ca^{2+}) signals

(a) The propagation of a bioelectrical excitation wave in multicellular *pancreatic islets*. The activity time delay for the first ($x = 0$) and last ($x = L$) cells of a linear chain is shown. Note *correlation* between the *bi-stable* time traces of the *cell potential V* and the intracellular *calcium concentration cCa*.

(b) *Cell potentials* and *cytoplasmic calcium levels* are interrelated in *Xenopus embryogenesis*. Normally developing cells show a *potential window*. Polarization and depolarization phenomena associated with the expression of two potassium channels (inward-rectifying pore-mutant dnKir6.1 and Kv1.5) cause elevations of calcium levels that fade over developmental time.

These examples suggest that *ion channels, cell potentials,* and *signaling ions levels* can be *coupled* in *oscillatory* and *bi-stable memories*.

Curr. Opin. Cell Biol. 2022
10.1016/j.ceb.2022.102113



Plants also generate electric potential and Ca^{2+} level *oscillations* in many spatio-temporal scales of biological organization. Also, *calcium-binding proteins* influence key *transcriptional processes* here.

Ion channels are involved in most *oscillatory regulations*, as shown by optogenetic techniques, which allow direct manipulation of membrane potential and hypothesis testing.

3.d Experimental systems and facts

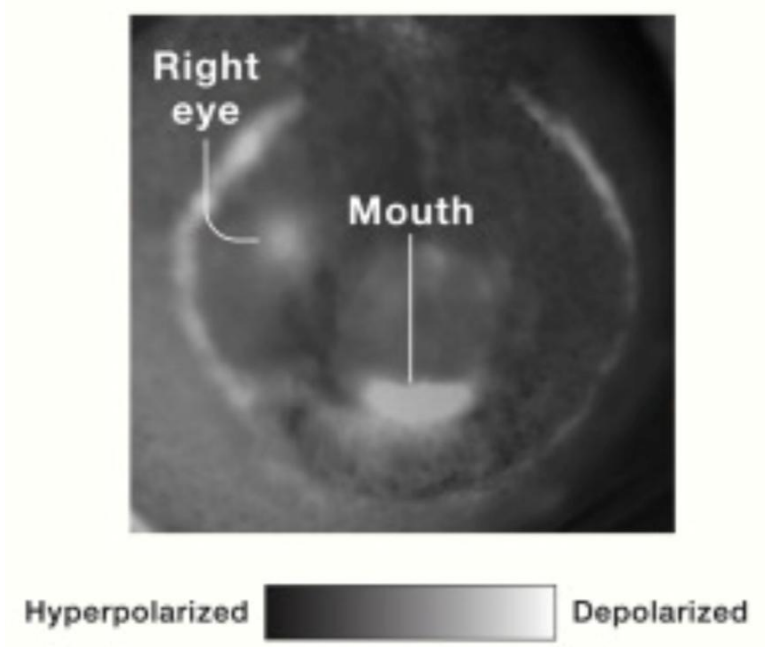
Are *ion channels, cell potentials,* and *signaling ions/molecules levels coupled* in *oscillatory* and *bi-stable memories*?

- *Homeostatic channel regulation in neurons*
- *Cell cycle*
- *Electro-genetic circuits*
- *Embryonic differentiation*
- *Excitation-transcription coupling in synaptic plasticity*
- *Developmental processes*
- *Calcium levels in animals and plants*

suggest that *bioelectricity* can influence *protein expression* through a *small number* of *counteracting voltage-gated channels*. We consider that *single-cell* and *multicellular potentials* can participate in transcription, together with other transporters such as *ligand-gated channels,* through complex downstream processes.

*3.c Multiscale bioelectricity: patterns of cell potentials as a real-time, high scale spatio-temporal integration (**Michael Levin**)*

Wires. Sys. Biol. Med. 2013
10.1002/wsbm.1236

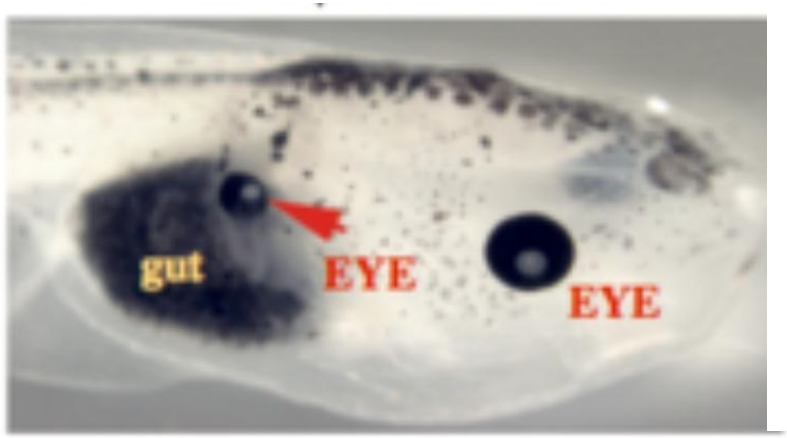


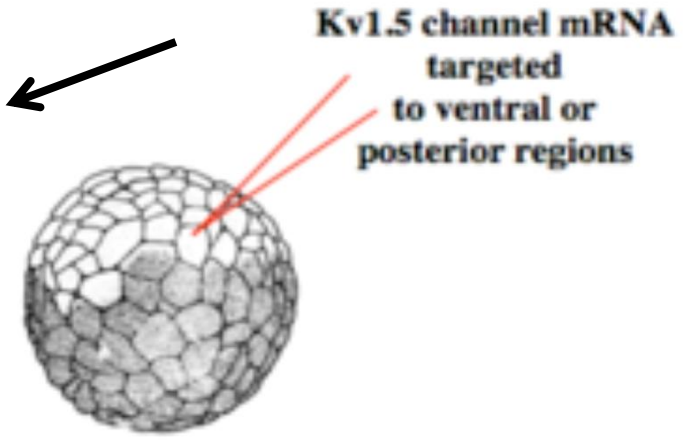
Voltage-sensitive fluorescent dyes non-invasively reveal bioelectric patterns.

Multicellular potentials act as functionally *instructive patterns*.

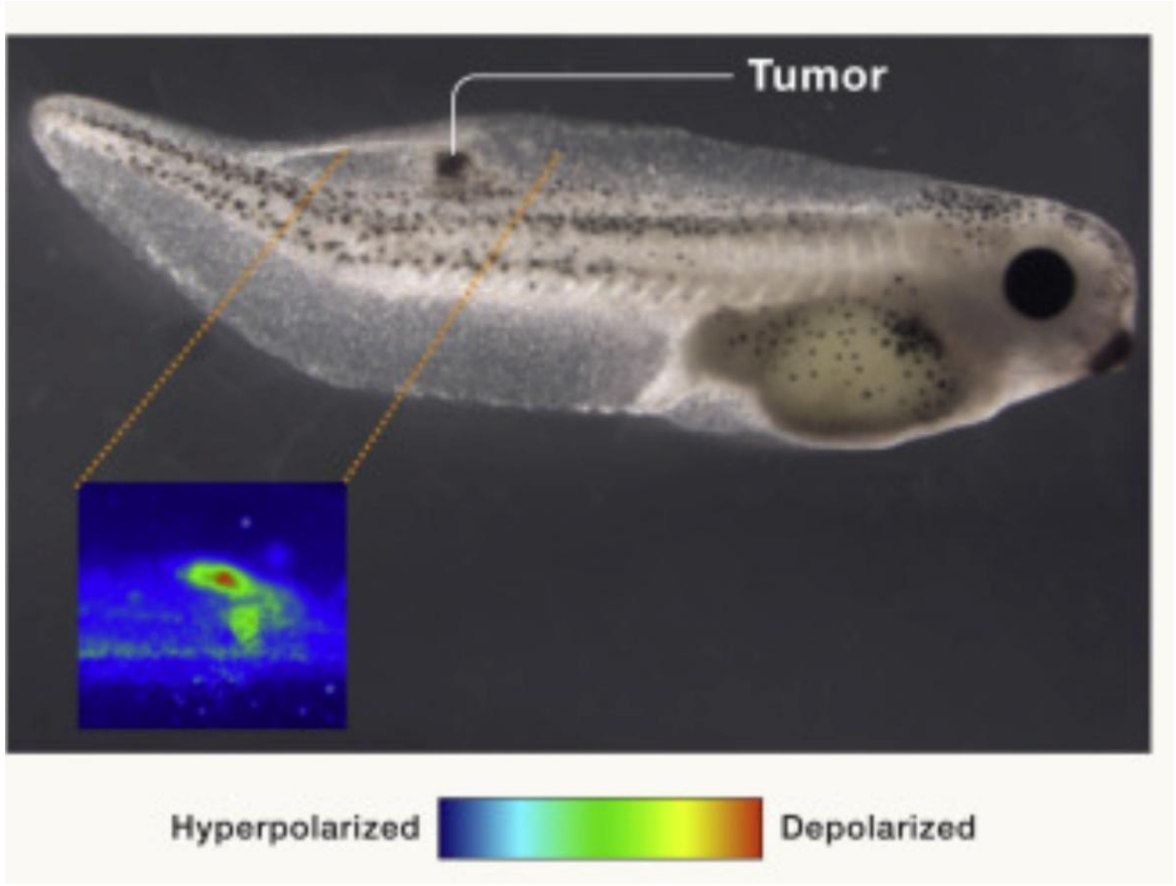
The *bioelectrical face*: prior to organ generation, fluorescence dyes show hyerpolarized regions where the face organs (eye, mouth) will develop.

Local *mRNA transfer* experiments may generate *ectopic eyes* by changing the multicellular potential of a localized region.





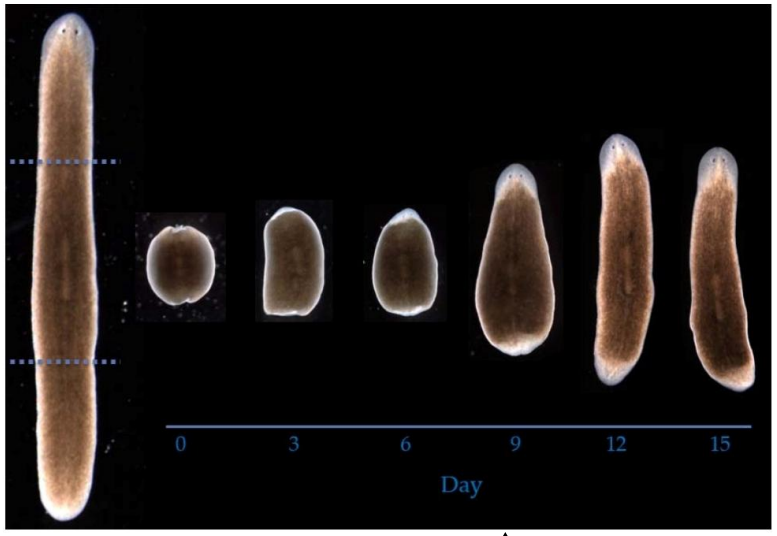
Cell 2021
10.1016/j.cell.2021.02.034

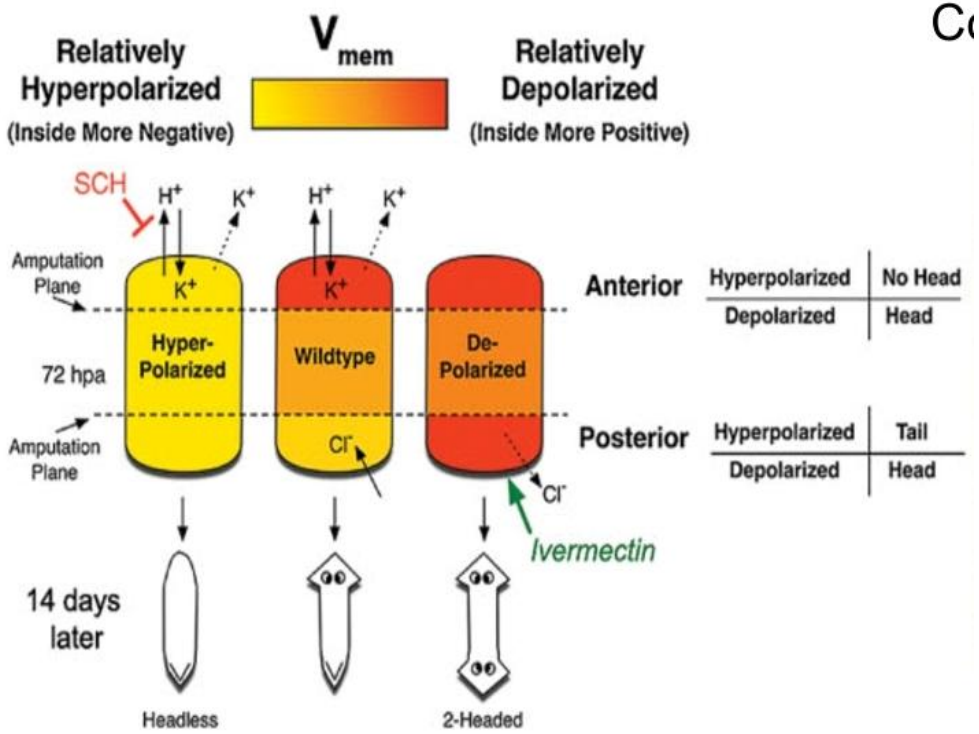


Experimental tools can *read* and *write* the *bioelectric patterns* of non-neural tissues using molecular-genetics, pharmacological, and optogenetical techniques to open and close ion channels and gap junctions.

A *zebrafish embryo* shows a *locally depolarized* region with a tumor, suggesting a possible role of multicellular *bioelectricity*.

Mol. Biol. Cell 2017
10.1091/mbc.e13-12-0708

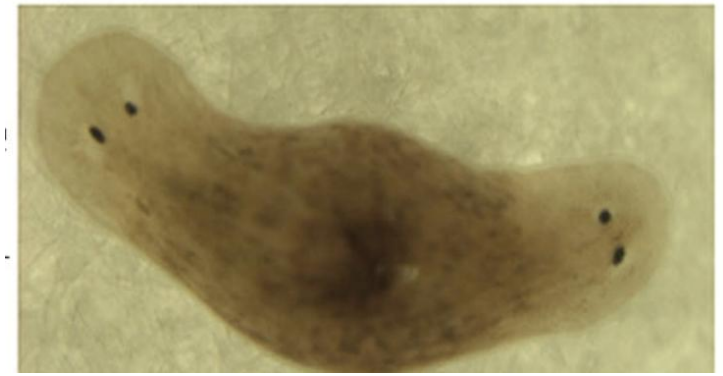




Pattern completion by *bioelectrical modules* may allow generating the entire anatomy from a small piece. The bioelectric network stores a pattern memory that controls individual cells to restore the whole organism.

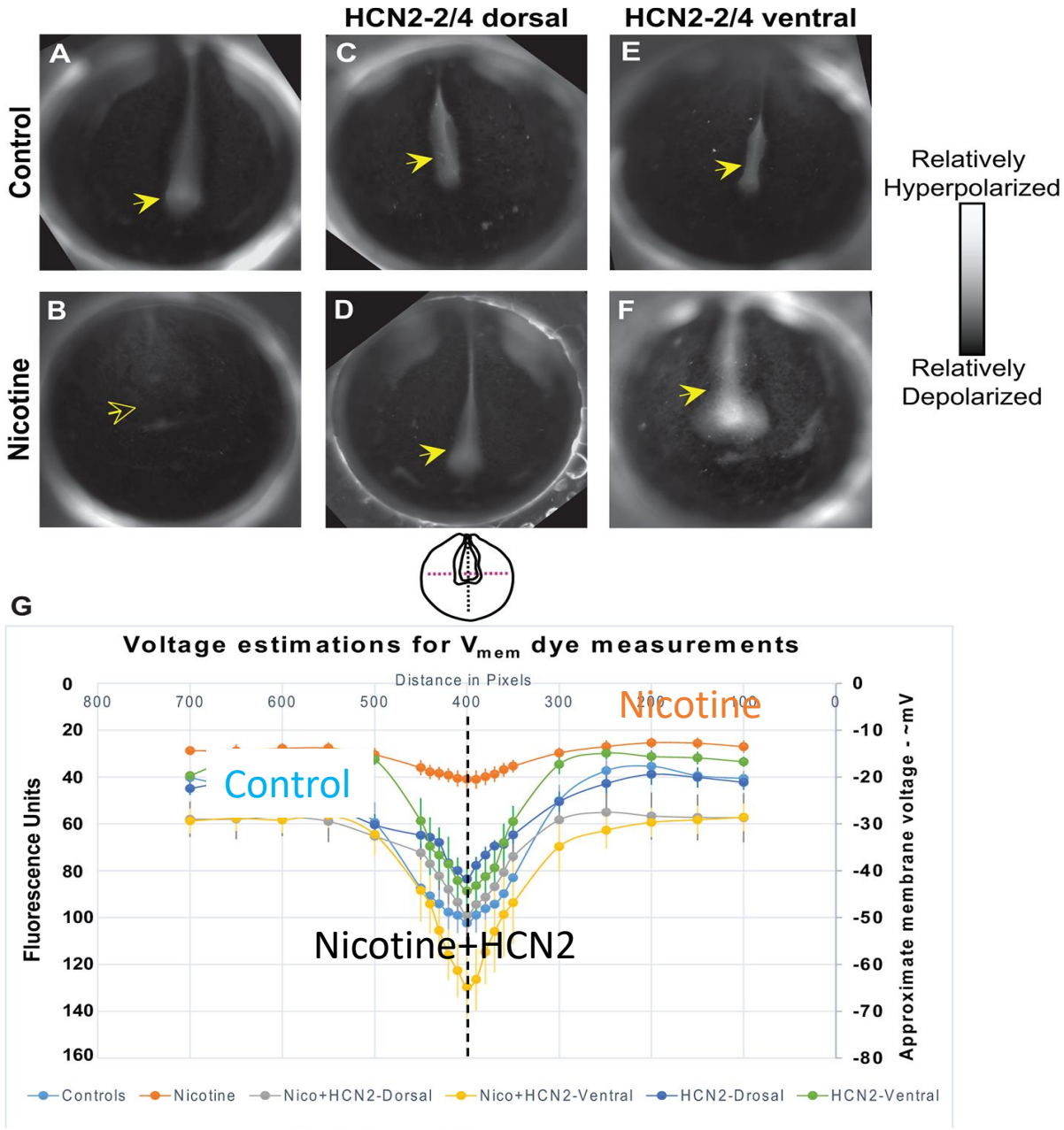
Planaria can regenerate their body upon amputation.

By controlling the *multicellular potential map*, normal, headless, and double-head planaria can be obtained.



Embryonic exposure to teratogen *nicotine* results in *brain defects,* apparently by *disrupting* the *multicellular potential* endogenous pre-pattern.

Upregulation of an *ion channel* (*HCN2*) can recover the potential pre-pattern *at distance*, acting to reverse profound defects and *rescue* brain anatomy and learning.



Nat. Commun. 2018 10.1038/s41467-018-03334-5
Front. in Cell. Neurosci. 2020 10.3389/fncel.2020.00136
iScience 2023 10.1016/j.isci.2023.108398

4. Bioelectrical model assumptions and equations

J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641
Cell 2021
10.1016/j.cell.2021.02.03

General ideas to be incorporated in a *biophysical* model:

- *complex patterns* can result from relatively *simple local rules* of interaction between individual units with *no central coordination* due to *multiscale competencies* provided by *multicellular fields*;

- *a cell* has *limited local information*; however, the whole *multicellular system* of locally interacting cells may have a *complex representation* of *itself* and the *external environment*; and

- aggregates of neurons, non-excitable cells, and bacteria, may show spatio-temporal patterns characterized by *polarized/depolarized multicellular regions*.

The above facts suggest that *slow bioelectrical mechanisms* in ensembles of non-excitable cells, reminiscent of the relatively fast processes found in neural networks, should be explored.

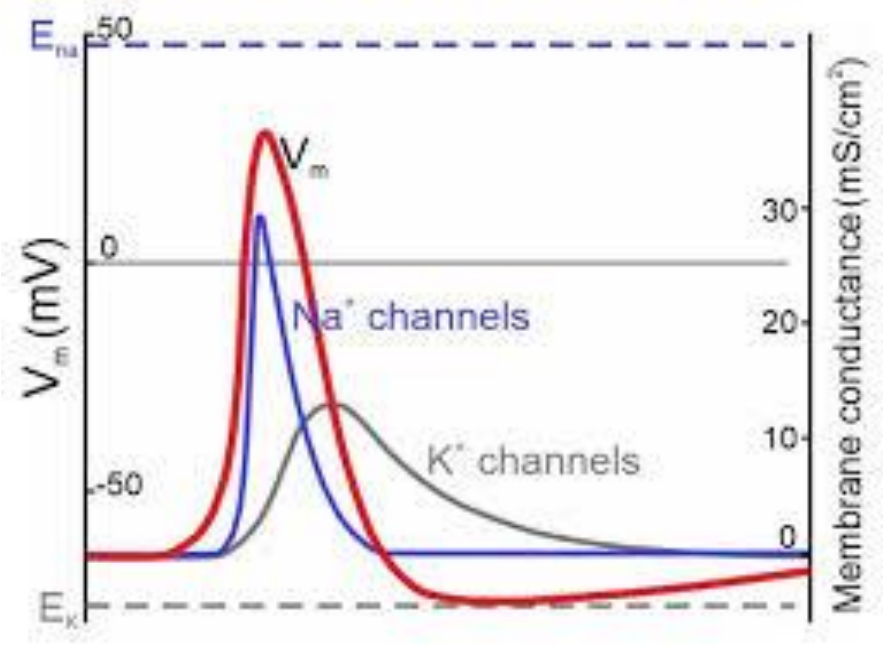
Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

Crucial facts to develop for a simple *bioelectrical* model:

1) many *ion channels* are *voltage-sensitive* and operate over a *wide voltage window*: channels can *polarize* or *depolarize* the cell depending on the particular cell state and the environmental conditions;

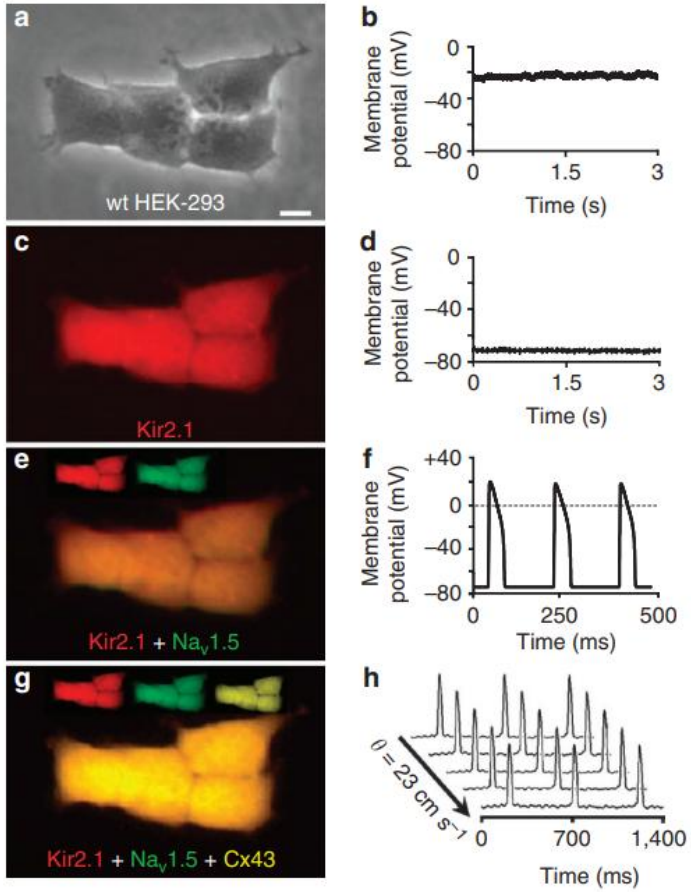
2) the voltage-gated conductances of a *small number* of *counteracting channels* can regulate the polarized (*pol*) and depolarized (*dep*) cell states.

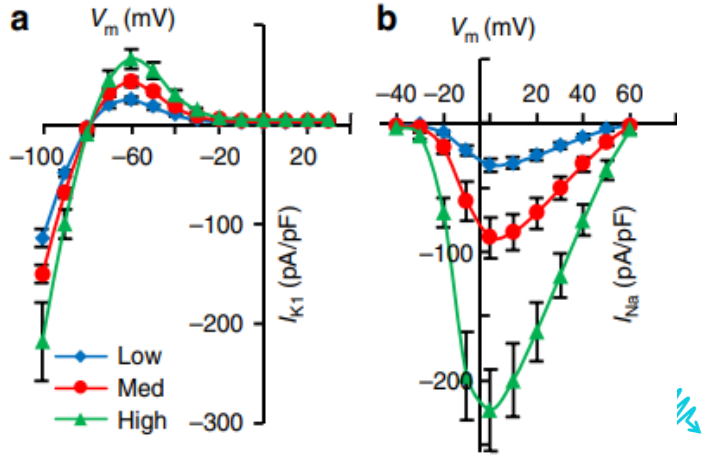
In a different but related problem, the action potential concerns the combined action of *sodium* and *potassium* channels in *depolarization* and *polarization* phenomena;

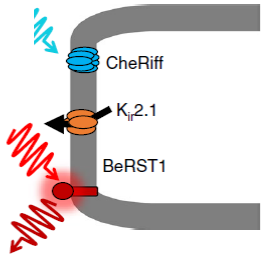


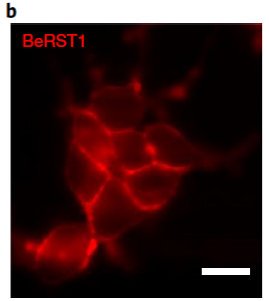
3) biosynthetic *excitable tissues* can be designed from non-excitable cells using a *small number* of channels and intercellular junctions, with the formation of *domains* of *bi-stable cell potentials*;

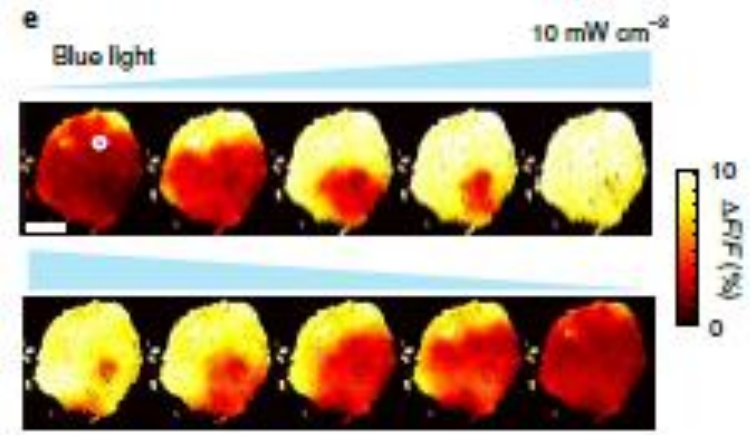
Nat. Commun. 2011, 2016
10.1038/ncomms1302
10.1038/ncomms13132
Nat. Phys. 2020
10.1038/s41567-019-0765-4

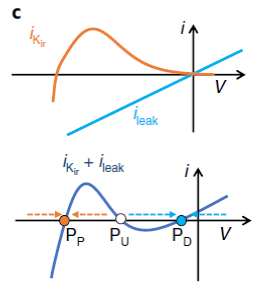


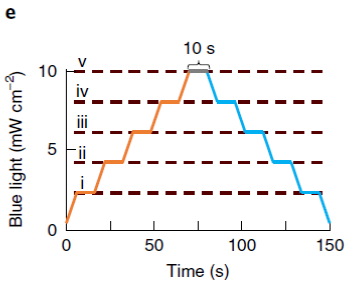


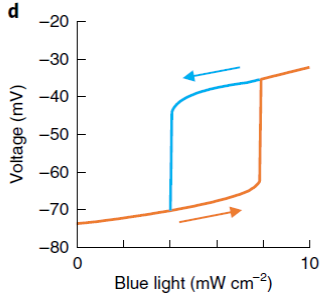


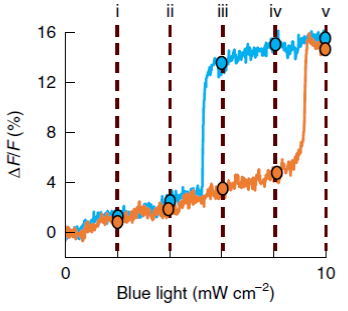












J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641
Cell 2021
10.1016/j.cell.2021.02.03
Commun. Biol. 2024
10.1038/s42003-024-06037-4

4) channel proteins and cell *membrane potential* influence *transcription via second-messengers* such as calcium, serotonin or butyrate that can regulate downstream processes. The *coupling* between *bioelectricity* and *transcription* can encode *spatio-temporal information*; and

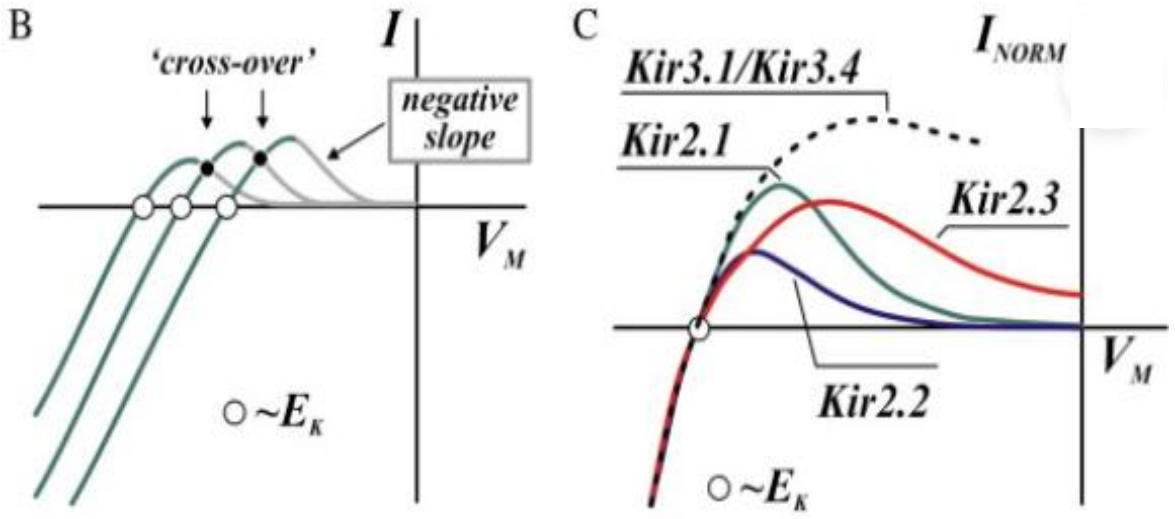
5) the cell state is *updated* in response to *environmental changes*. Because of the intercellular connectivity, average *multicellular potentials* can *suppress perturbations* in local cell polarization but may also *permit morphologically instructive* transitions induced by environmental changes.

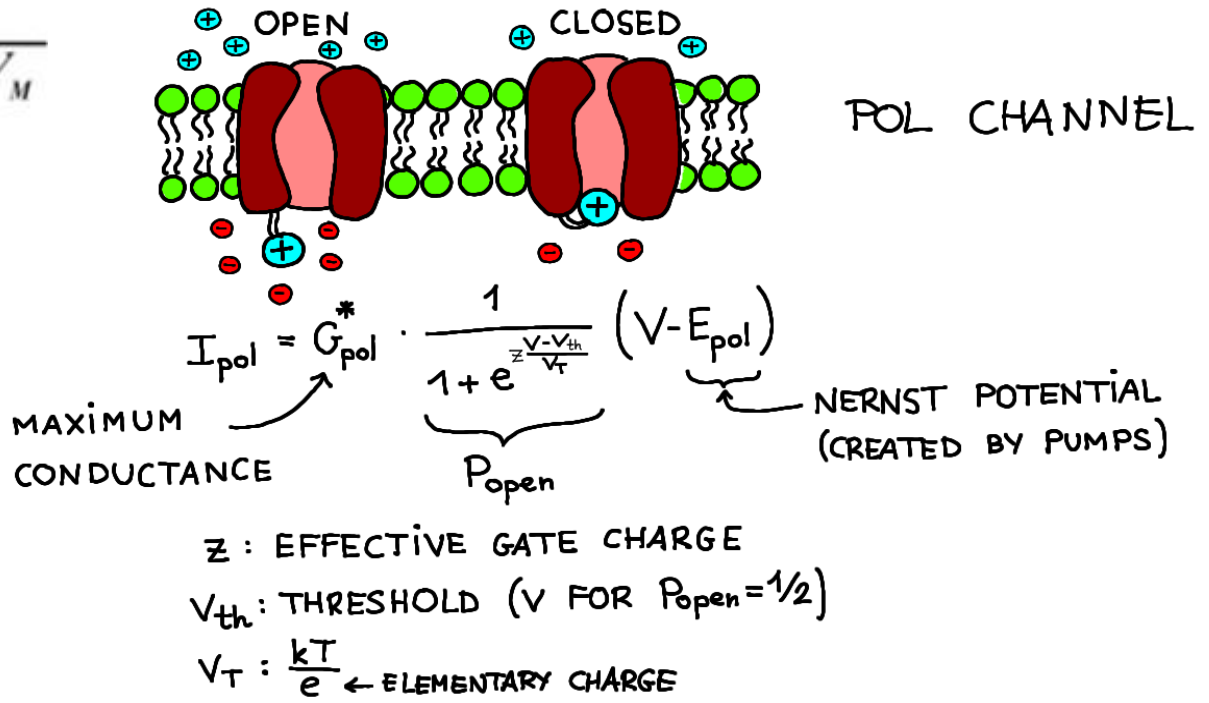
Bioelectricity can contribute to the *adaptive* phenomena observed in complex *cognitive systems* that show abilities to *solve problems* and deal with *novel challenges*.

4a Single-cell bioelectrical model

J. Mol. Cell. Cardiol. 2009
10.1016/j.yjmcc.2009.08.013
J. Phys. Chem. B 2014
10.1021/jp508304h

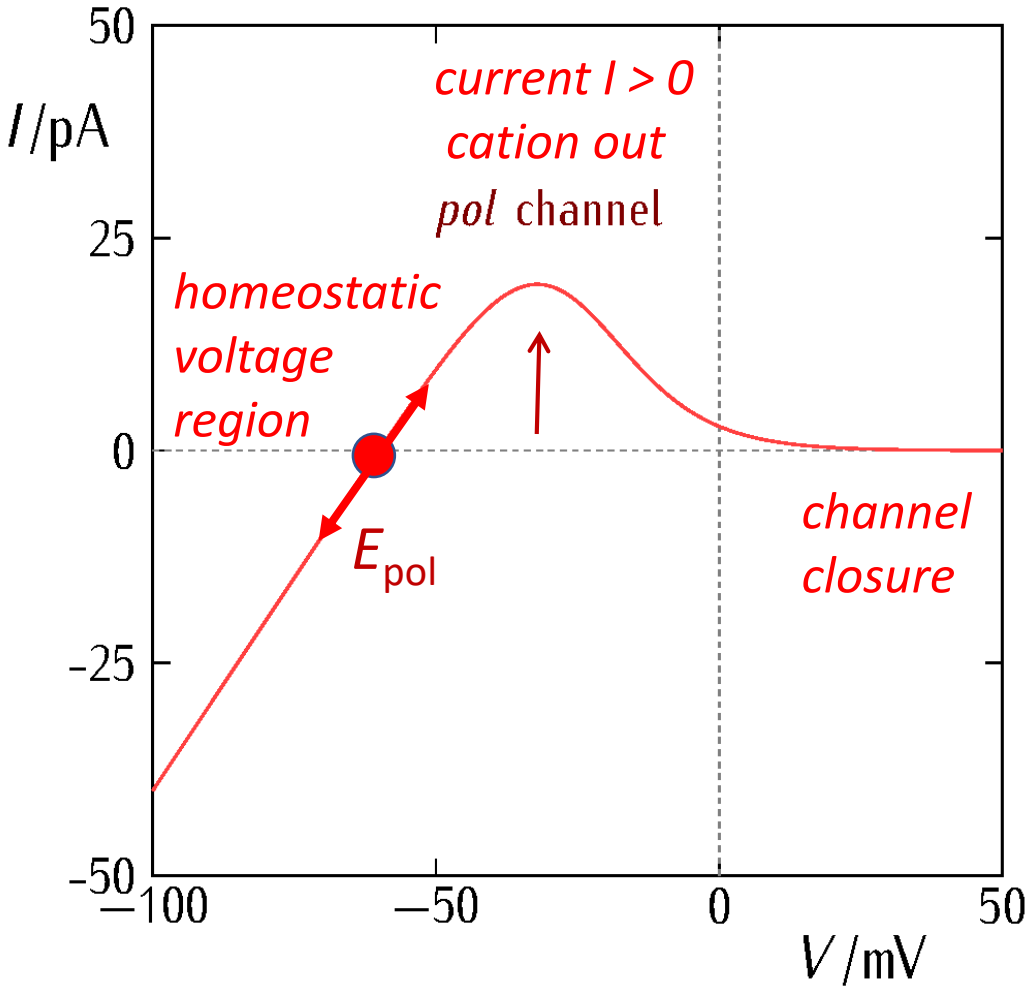
K^+ channel
I-V curves





In the model, a *generic pol* conductance simulates a *diverse family* of channels acting to *polarize* the cell.

J. Phys. Chem. B 2014
10.1021/jp508304h



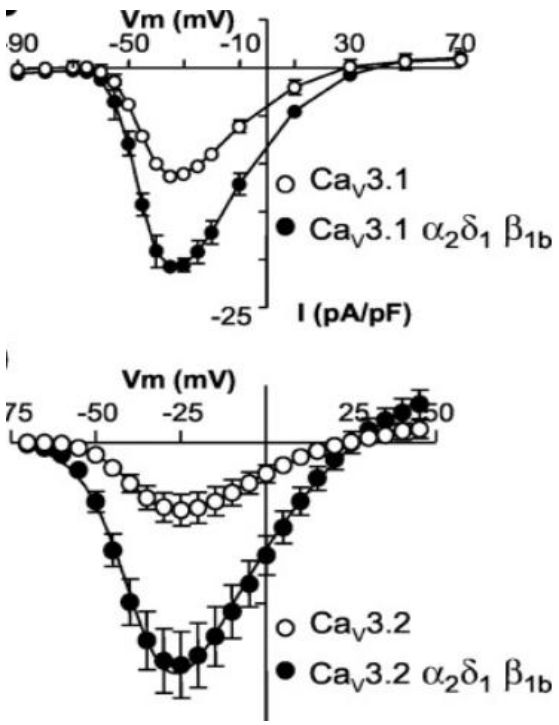
context-sensitive:

channel can repolarize the cell by correcting a *small* depolarization (*homeostatic* region, disruptive event *suppressed*)

but

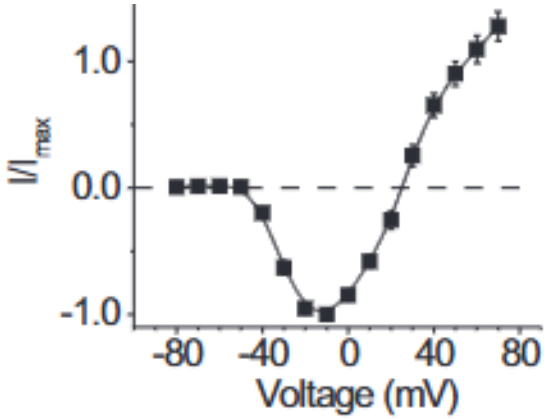
can permit a *large* depolarization (channel *closure*), thus allowing a

bioelectrical regionalization

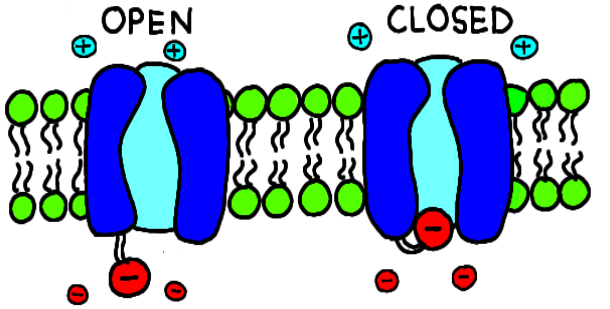


Ca^{2+} channel
I-V curves

Na^+ channel
I-V curve



PNAS 2012
10.1073/pnas.1115575109
J. Biol. Chem. 2004
10.1074/jbc.M313450200
J. Phys. Chem. B 2014
10.1021/jp508304h



DEP CHANNEL

$$I_{dep} = G^*_{dep} \cdot \frac{1}{1+e^{z\frac{V-V_{th}}{V_T}}} (V-E_{dep})$$

MAXIMUM CONDUCTANCE

P_{open}

NERNST POTENTIAL (CREATED BY PUMPS)

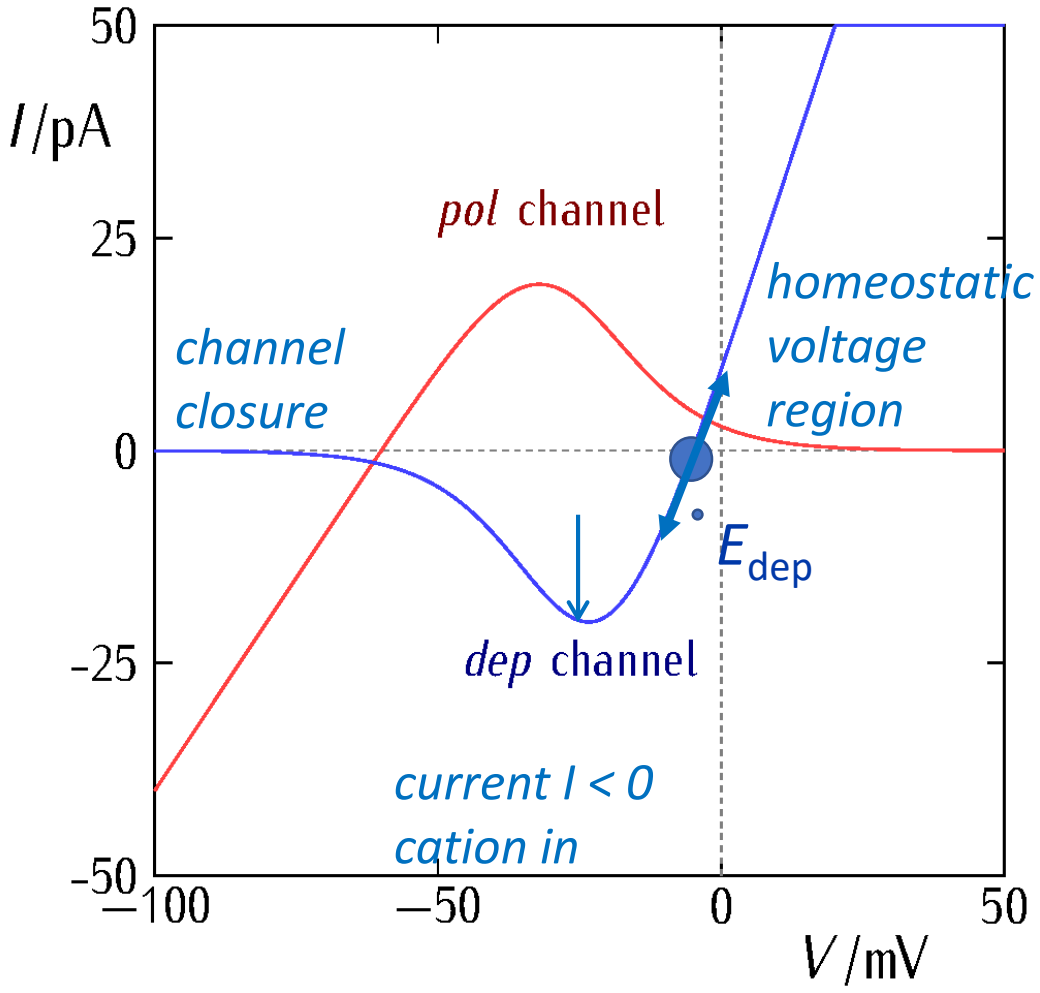
z : EFFECTIVE GATE CHARGE ($z<0$)

V_{th} : THRESHOLD (V FOR $P_{open}=1/2$)

V_T : $\frac{kT}{e}$ ← ELEMENTARY CHARGE

In the model, a *generic dep* conductance simulates a *diverse family* of channels acting to *depolarize* the cell.

J. Phys. Chem. B 2014
10.1021/jp508304h
Sci. Rep. 2016
10.1038/srep20403



context-sensitive:

channel can depolarize by correcting a *small* repolarization (*homeostatic* region, disruptive event *suppressed*)

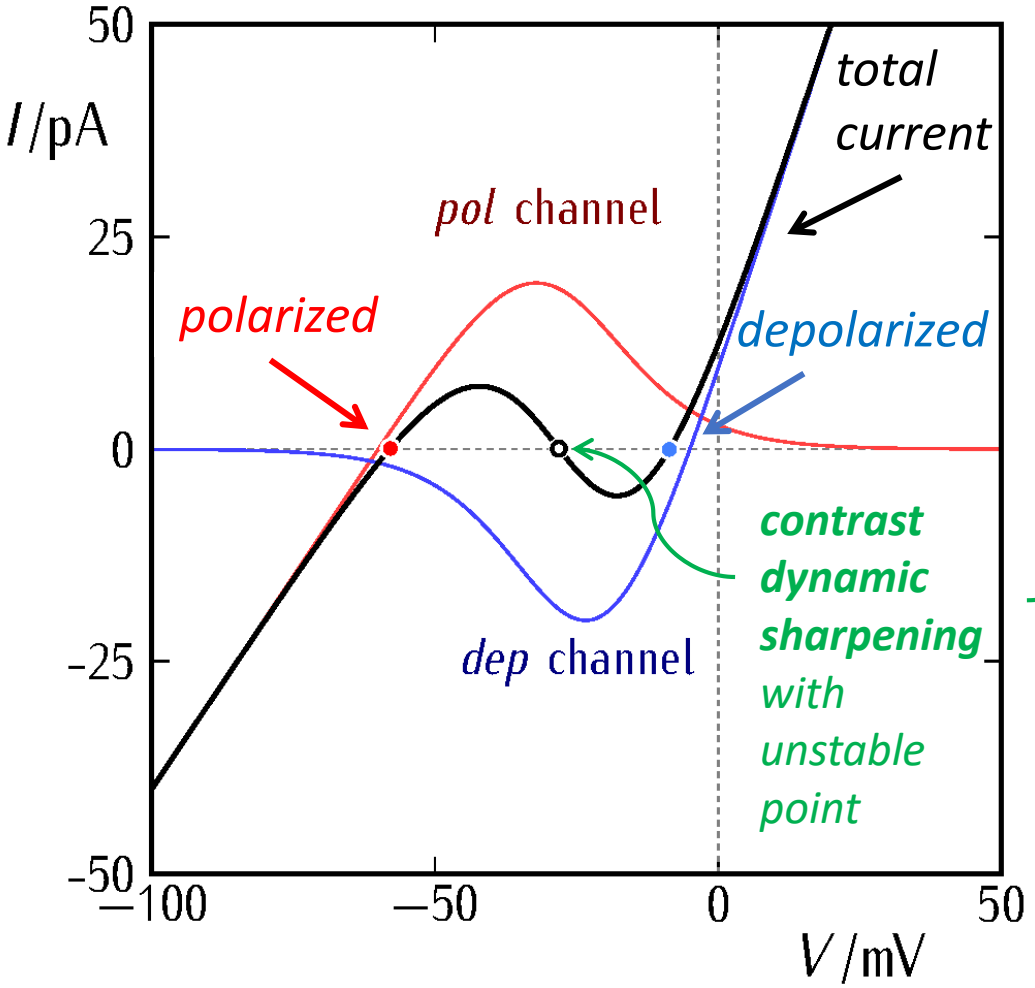
but

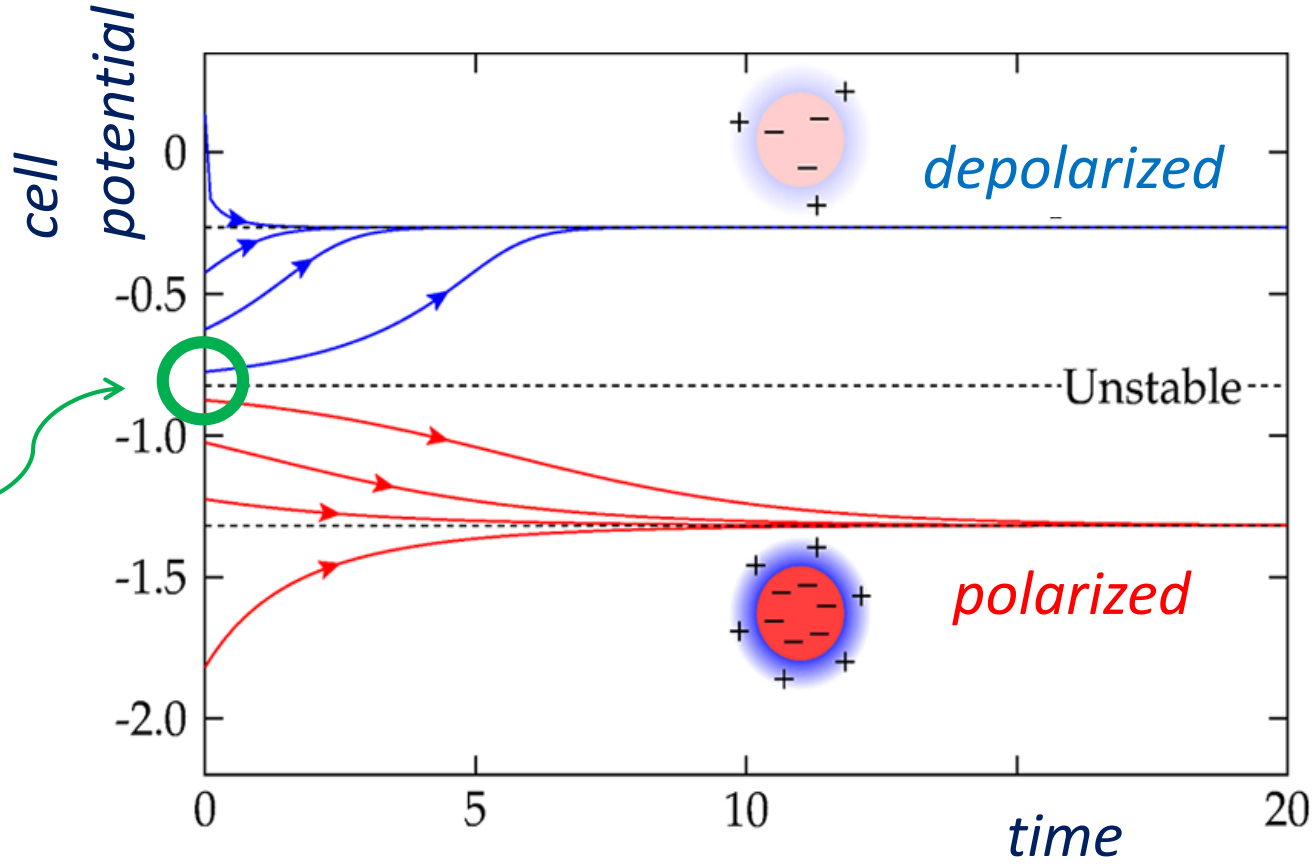
can permit a *large* repolarization (channel *closure*), thus allowing a

bioelectrical regionalization

J. Phys. Chem. B 2014
10.1021/jp508304h

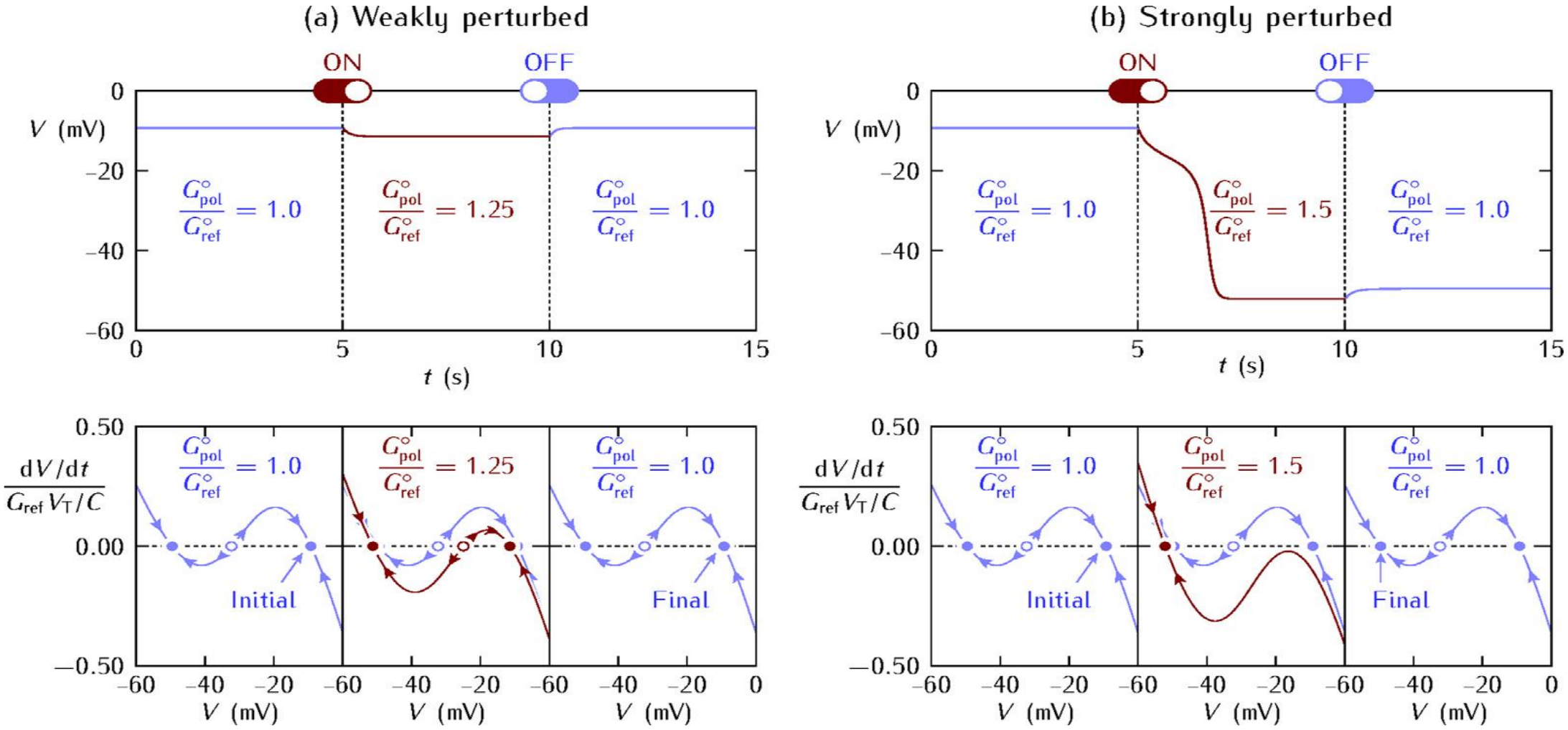
Contrast dynamic sharpening: the *combination* of the two channels over a *wide voltage window* may *polarize* or *depolarize* the cell depending on the particular cell state and the environmental conditions. The *combined action* of the two generic channels *pol/dep* gives a cell potential *bi-stability* that allows *contrast sharpening* in *bioelectrical regionalization*.





Channels may support *stable single-cell memories* based on membrane potentials. A *perturbation* or *disruptive* event (e.g. the over-expression or blocking of a specific channel) changes the state of a cell of capacitance *C only* if sufficiently *strong*. Instructive changes could be established by *temporarily limited actions*.

Prog. Biophys. Mol. Biol. 2019 10.1016/j.pbiolmolbio.2019.06.004



$C(dV/dt) = -I_{pol} - I_{dep}$

4b The coupling between bioelectricity and transcription

Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717

Having described

how ion channel proteins can contribute to the cell membrane potential,

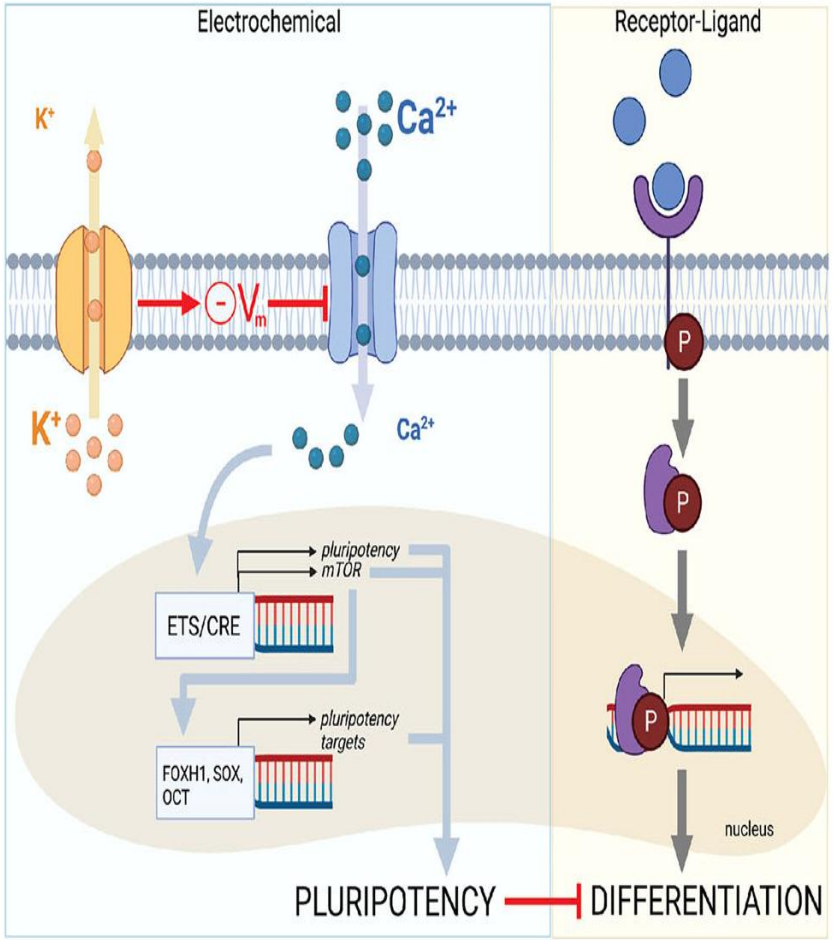
we should address now

how the feedback bioelectrical mechanisms can regulate protein expression (*left*)

as a complementary view to receptor-ligand mechanisms (*right*).

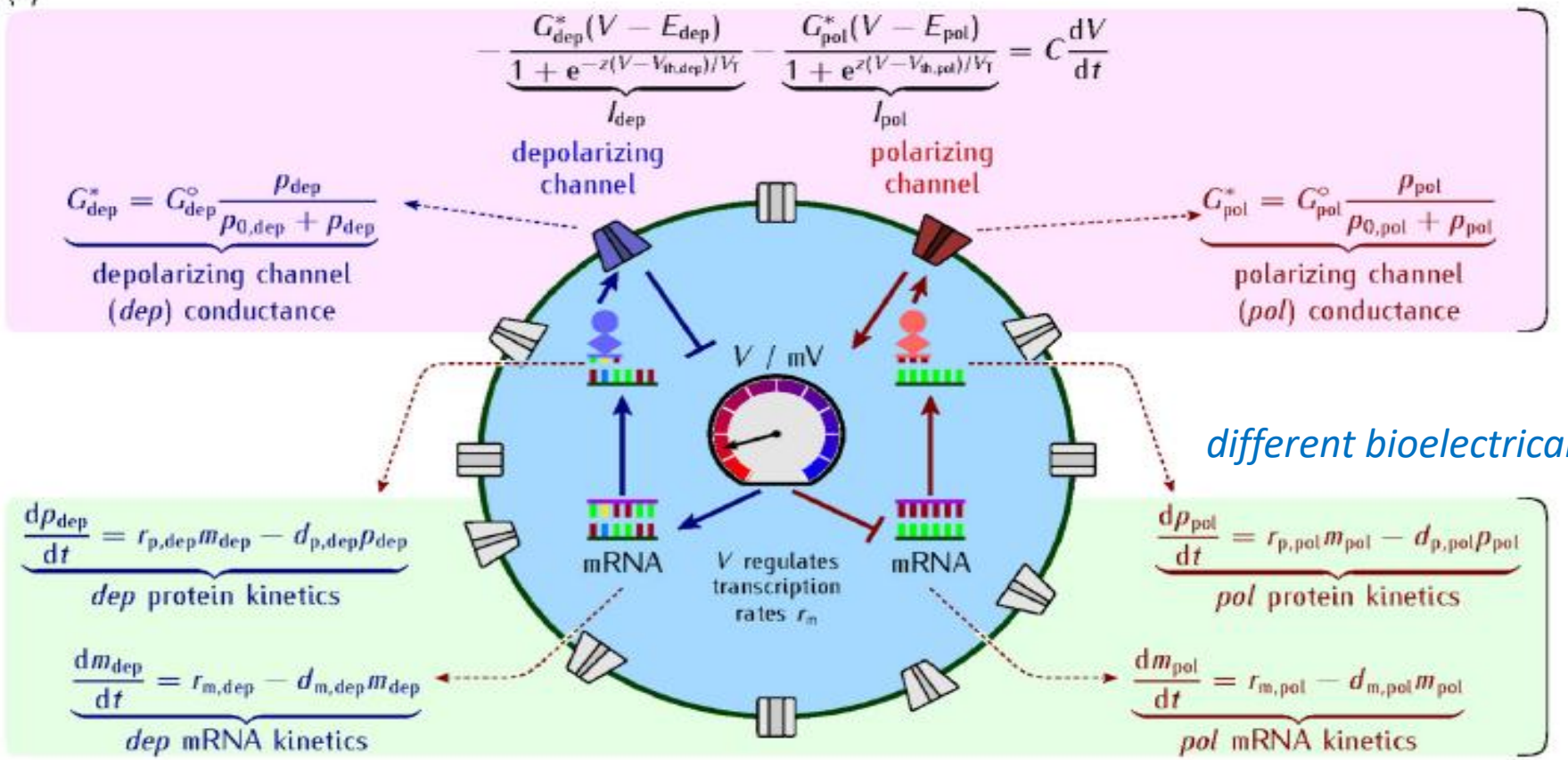
The *spatio-temporal coupling* between the *cell potentials* and *signaling ions and molecules*:

the local concentration *S* of a *regulatory agent* that acts as a *transcription factor* can be influenced by the cell potential, which is in turn modulated by the relevant channels. For instance, the opening of a *voltage-gated calcium channel* caused by the cell depolarization provokes the calcium entry and trigger downstream transcriptional processes.



Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717

(a)

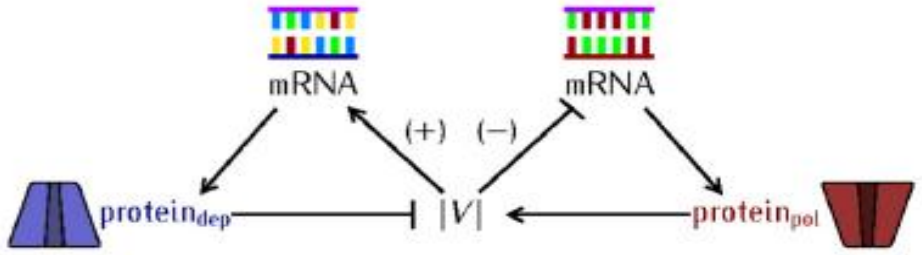


Bioelectrical network
(ion channel dynamics: *seconds to minutes*)

different bioelectrical and *transcriptional time scales*

Transcriptional network
(ion channels expression: *hours to days*)

(b)



The *cell membrane potential V* can influence *transcription via second-messengers*, e.g. calcium: its cytoplasm *concentration S* may show a *positive* (*dep* channel here) or *negative* (*pol* channel here) regulation on *V*.

Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

In this bioelectrical model:

- the transcription and translation of the *dep* and *pol* channel proteins is described in terms of the *mRNA* (m_{dep} and m_{pol}) and *protein* (p_{dep} and p_{pol}) *concentrations*;

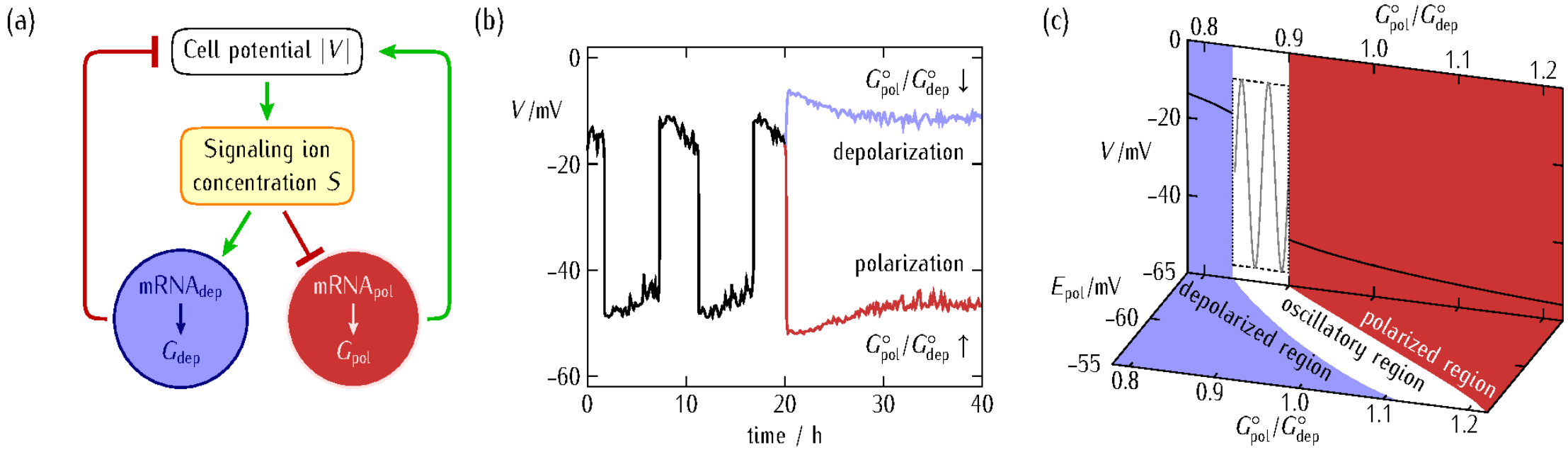
- the *rate constants* $r_{m,k}$ and $r_{p,k}$ (k = *dep*, *pol*) regulate the mRNA transcription and protein translation processes, respectively, which occur with the respective *degradations rates* $d_{m,k}$ and $d_{p,k}$ (k = *dep*, *pol*);

- the *conductances* $G^*_k = G^o_k\ p_k/(p_{0,k} + p_k)$ (k = *dep*, *pol*) follow a *Hill kinetics* with the respective protein concentrations p_k, where G^o_k is the *maximum conductance* and $p_{o,k}$ is the *reference protein concentration* which gives the half-maximum conductance. This kinetic saturation of the *dep* and *pol* channel conductances reflects the finite values of the transcription and translation rates and may also incorporate the limits imposed by the protein trafficking to and insertion in the cell membrane. The action of specific blockers can also be introduced by decreasing the effective values of the maximum conductance G^o_k; and

- the *coupling* between transcription and bioelectricity results in *cell potential V-dependent mRNA transcription rates* $r_{m,k}(V)$ where $r^o_{m,k}$ are the reference values (k = *dep*, *pol*). Thus, *V* modulates the protein concentrations and the resulting channel conductances through the transcription while the channel conductances modulate *V* through the post-translational bioelectricity. Note that this coupling should be *system specific*.

J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

the model allows bi-stable and oscillatory single-cell cell potentials



(a) The *cell potential V* characterizing the *pol* and *dep* states, regulated by the *bioelectrical* and *transcriptional feedback*, for the case of a *signaling ion* of *concentration S*. (b) The coupling between bioelectricity and transcription leads to *pol* and *dep stable* and *oscillatory* cell potentials, modulated by the maximum conductances ratio and the equilibrium potentials. Here, the channel protein regulation is *negative* for the *pol* channel and *positive* for the *dep* channel. (c) The conductance ratio and equilibrium potential define a *configuration space* for the bioelectrical transitions between the cell states.

limitations of the single-cell model

The *bioelectrical-transcriptional coupling* is *system-dependent:* theoretical models may not be universal and depend on the *different channels and mechanisms* relevant to each *experimental case*.

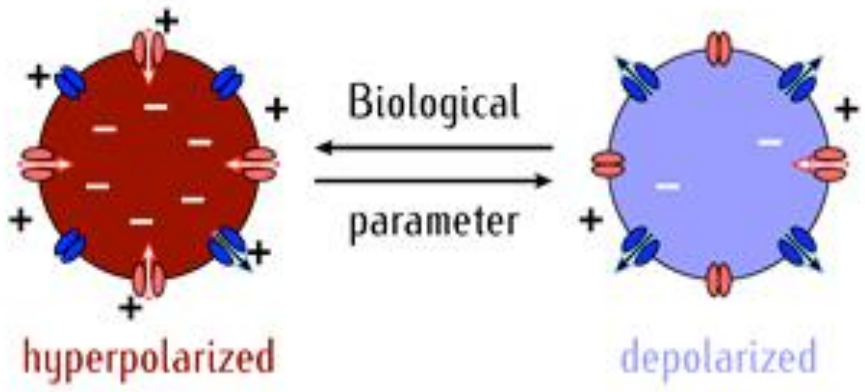
Because channels *polarize* or *depolarize* the cell depending on the particular *cell state* and the *environmental conditions*, externally-induced bioelectrical actions are not only *system-dependent* but also *context-dependent*.

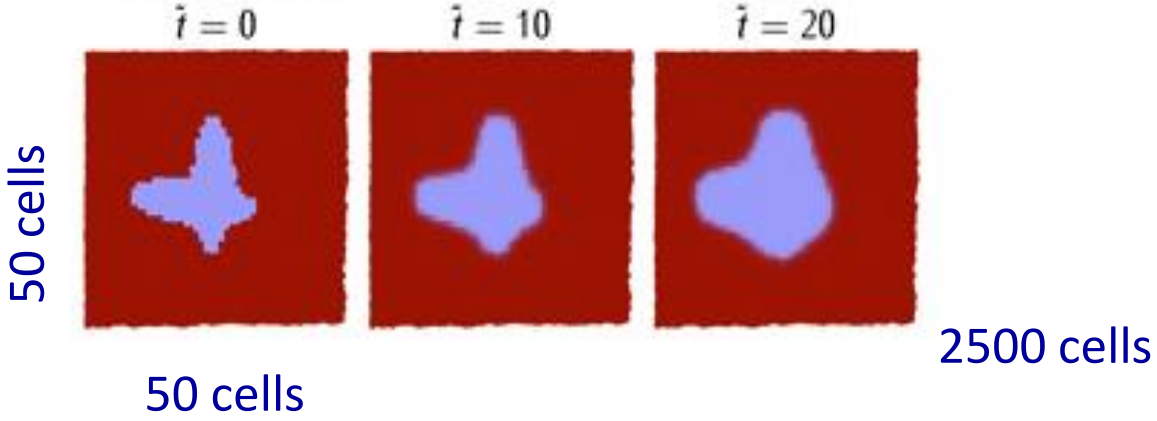
These *serious limitations* should be taken into account when attempting to describe *cell potentials* in particular experimental cases.

the cell as a bioelectric dynamical system

J. Phys. Chem. B 2014, 2015, 2017
10.1021/jp508304h
10.1021/jp512900x
10.1021/acs.jpcb.7b04774
Sci. Rep. 2016, 2016
10.1038/srep20403
10.1038/srep35201

Low (*depolarized*) and high (*polarized*) cell potential *states* and *transitions* between them.





Transitions are induced by changes in:

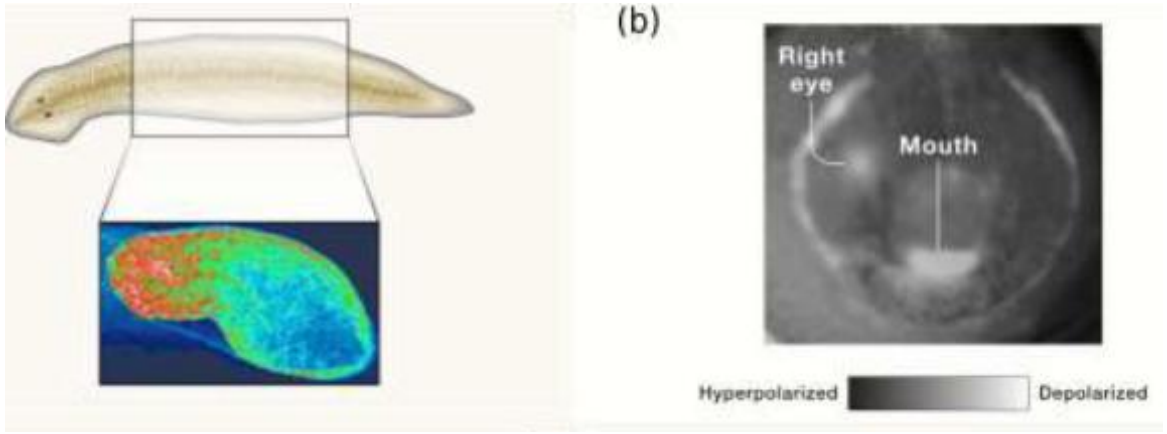
1) *ionic concentrations* (equilibrium potentials) and

2) relative values of the *polarizing* and *depolarizing* channels *conductances*.

However, *single-cell* states are coupled at the *multicellular* level. Thus, an *extended* model that includes the *intercellular gap junction* conductance is needed.

4c Extension to the multicelular case: intercellular gap junctions (with José A. Manzanares)

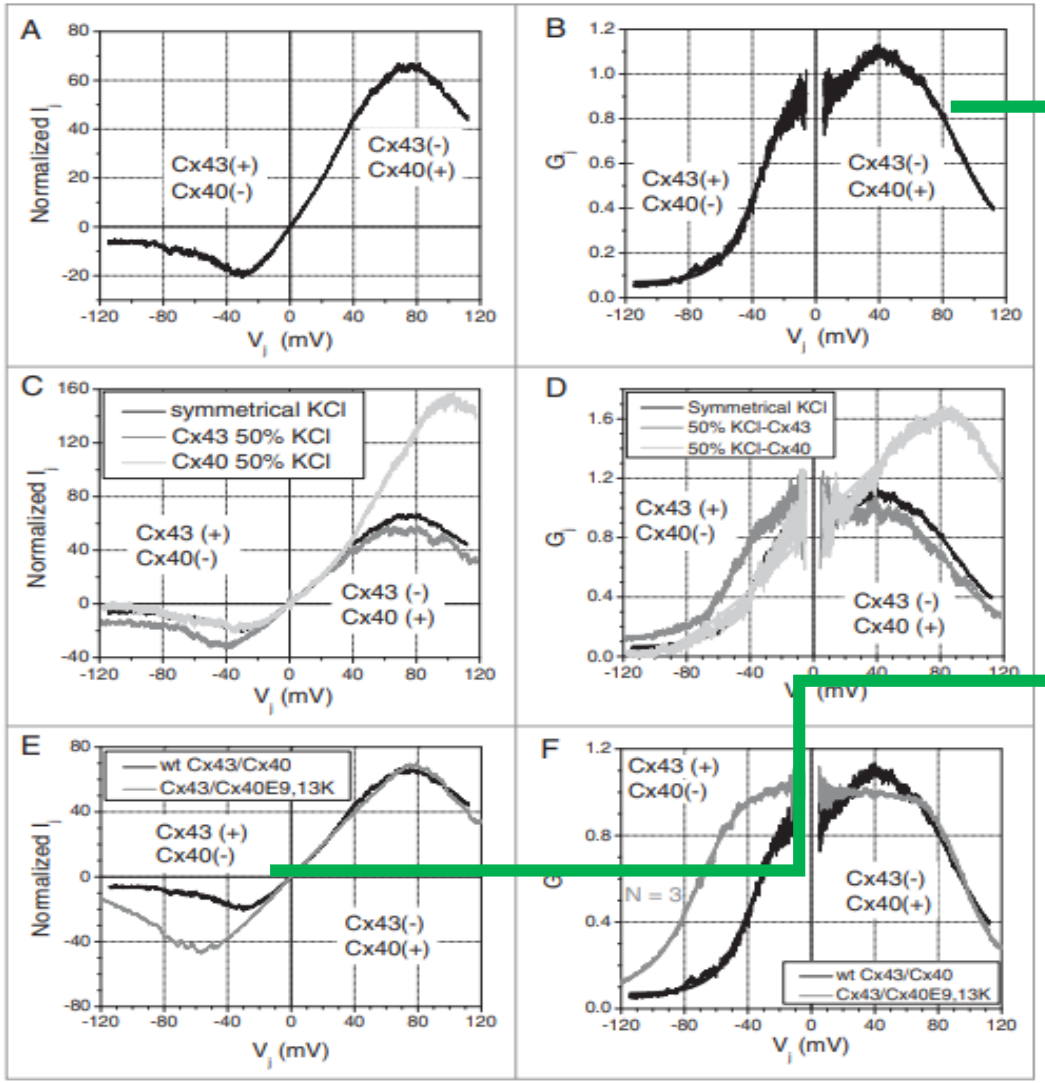
Because of intercellular connectivity, the *single-cell* coupling between bioelectricity and transcription can be extended to the *multicellular level*. In this way, *average potentials* could either *collectively counteract* disruptive local perturbations in cell polarization or *facilitate* morphologically instructive global transitions, as needed in the *different biological stages* shown.



The *bioelectrical regionalization* of the multicellular ensemble in *polarized/depolarized* regions converts *single-cell* states into *multicellular* states. Because cells are sensitive to spatio-temporal bioelectrical patterns, the *different multicellular potential maps* give *distinct downstream gene expression patterns*.

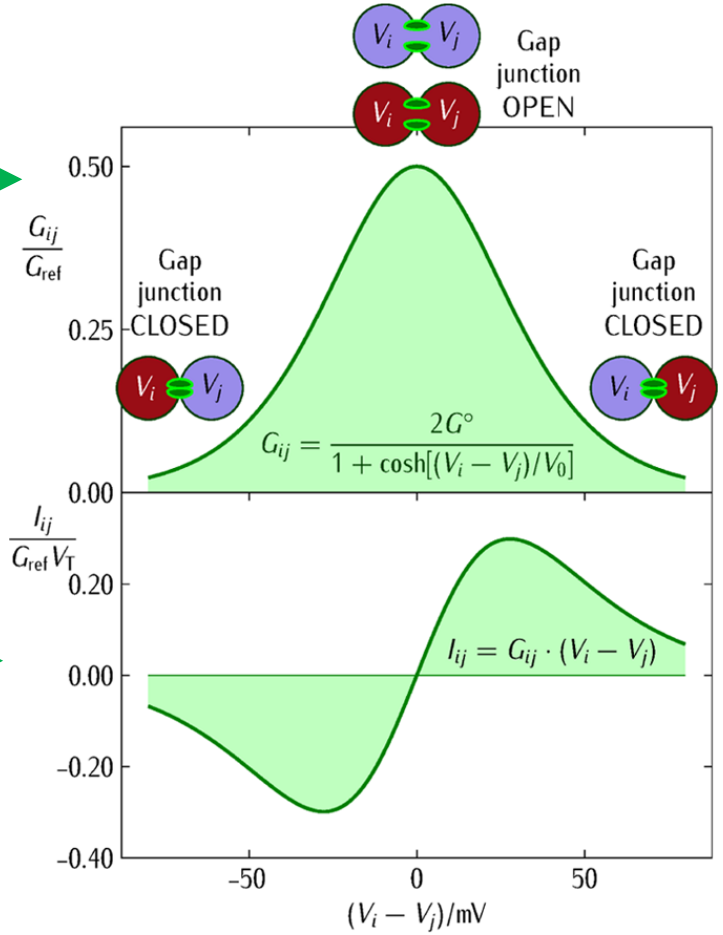
Channels 2014 10.4161/19336950.2014.949188
J. Phys. Chem. B 2019 10.1021/acs.jpcb.9b01717

Experimentally, the *junction current* and *conductance* G_{ij} between two neighboring cells i and j depends on the *intercellular potential difference* $V_i - V_j$

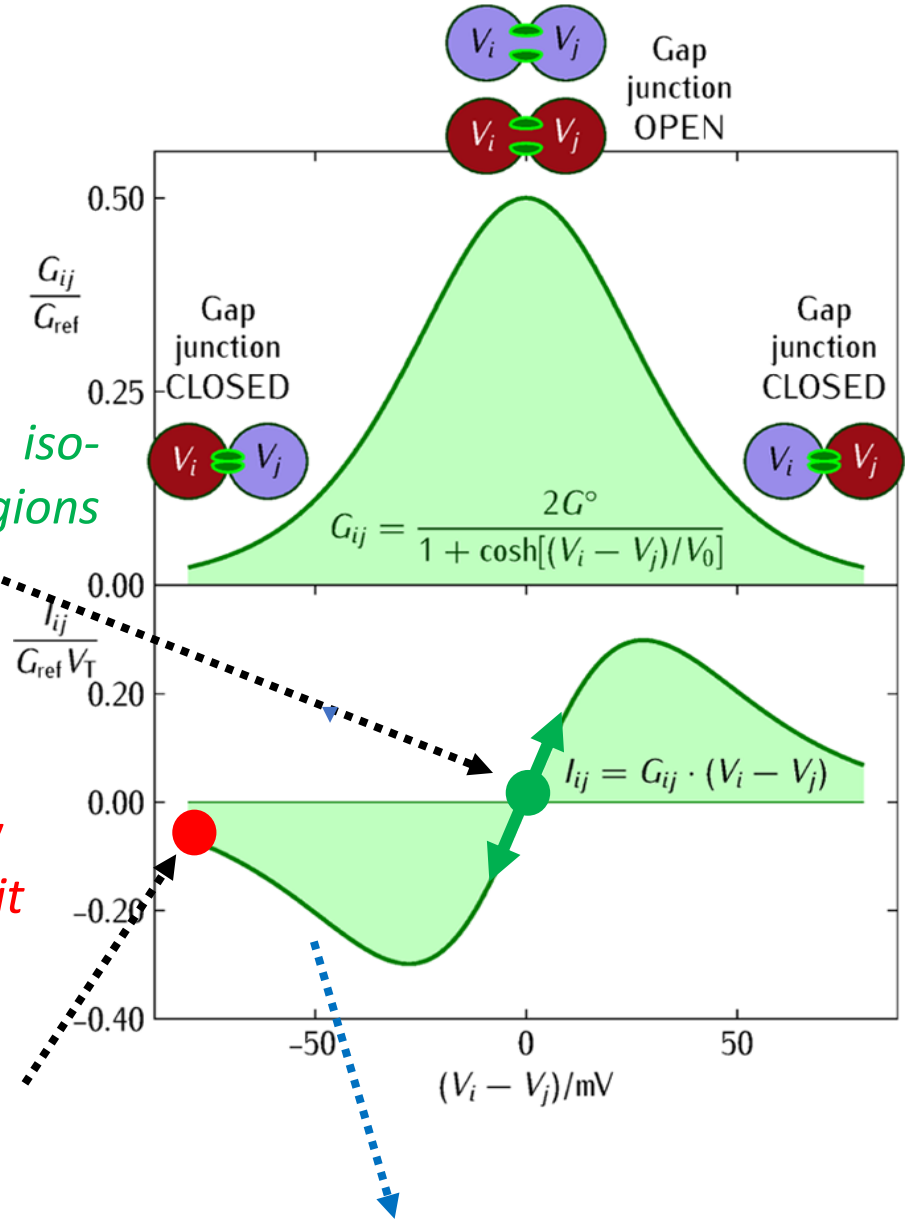


model conductance

model current



J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004



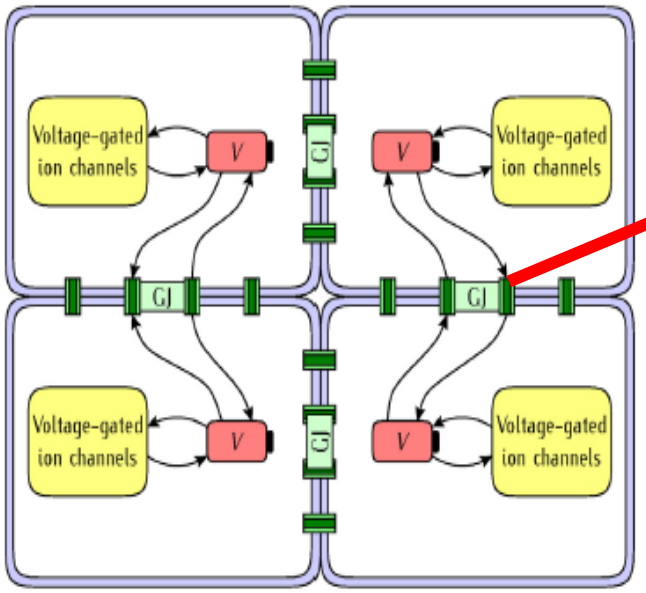
***open**: keeps iso-potential regions (**homeostasis**)*

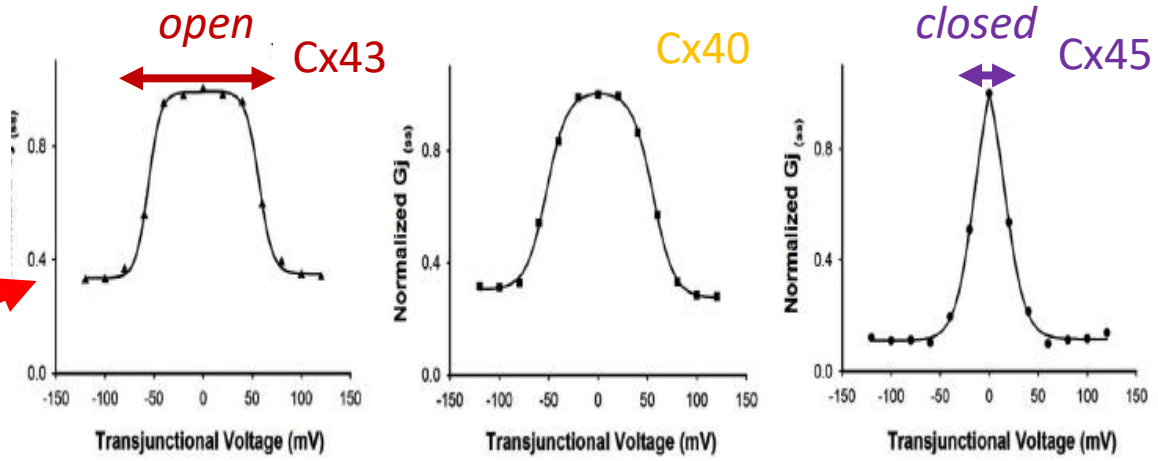
***closed**: inhibitory connections permit bioelectrical regionalization (**development**)*

Negative Differential Resistance (NDR) region

Contrast dynamic sharpening: *gap junctions* operate over a wide voltage window: they may *polarize* or *depolarize* the cell depending on the bioelectrical neighborhood and environmental conditions, which contributes to the spatio-temporal contrast needed for *bioelectrical regionalization*.

PLoS ONE 2013
10.1371/journal.pone.0060506
Cell Rep. 2015
10.1016/j.celrep.2015.04.021
Phys. Rep. 2023
0.1016/j.physrep.2022.12.003
Cancers 2021
10.3390/cancers13215300

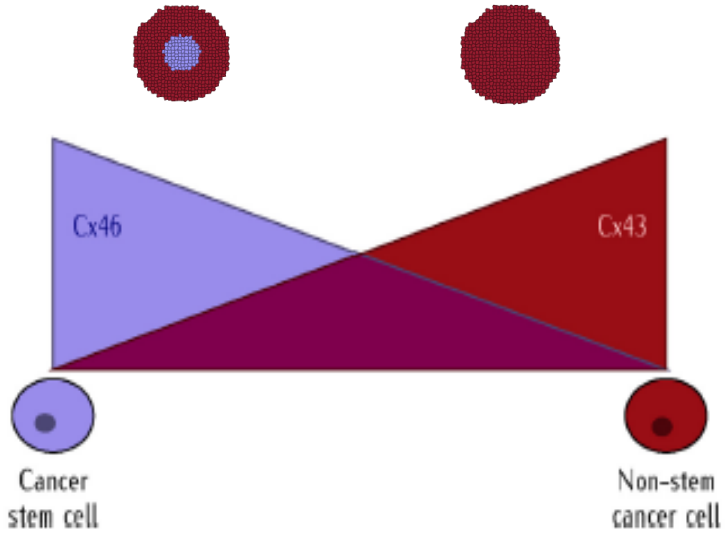




Sensitivity to transjunctional voltage: closing promotes *bioelectrical regionalization*

Single-cell potentials (*V*) *influence* and *are influenced* by voltage-gated ion channels. In turn, *this feedback* is *coupled by* the *intercellular gap junctions* at the multicellular level.

closed: *regionalization?* *open*: *no regionalization?*



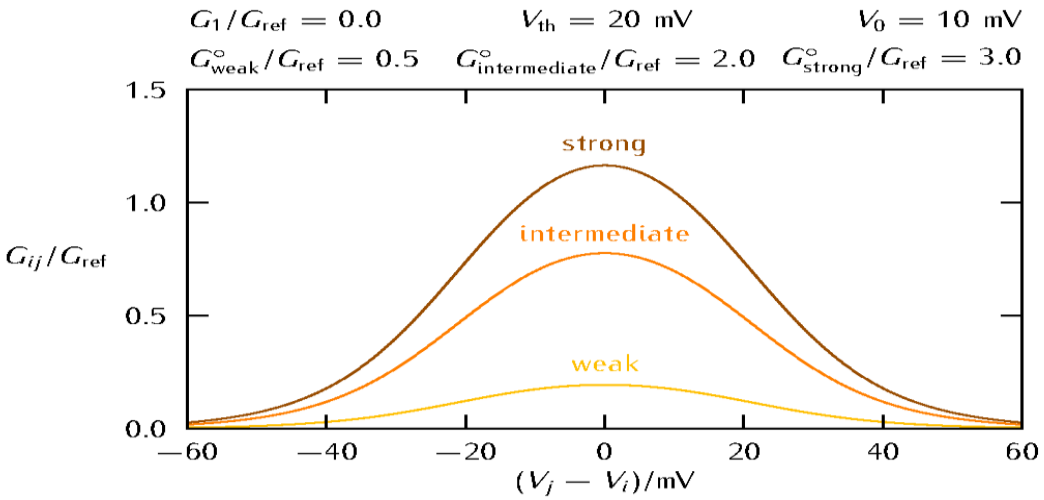
Glioblastoma cancer stem cells show particular junction proteins.
Different junctions with *distinct voltage-sensitivities* can perturb multicellular domains.
But other *biochemical & biomechanical* must also be present!

Cancers 2021
10.3390/cancers13215300
Phys. Rev. E 2020
10.1103/PhysRevE.102.052412

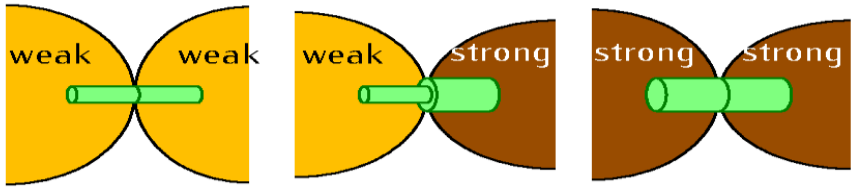
(a)

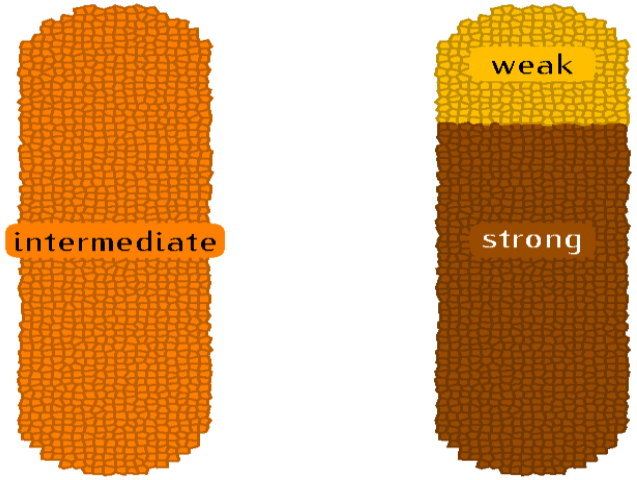
$$G_{ij} = \frac{G_i^\circ G_j^\circ/(G_i^\circ + G_j^\circ)}{\{1+\exp[-(V_j - V_i - V_{\text{th}})/V_0]\}\{1+\exp[(V_j - V_i - V_{\text{th}})/V_0]\}}$$

(b)



(c)





(a) Junction conductances of different connexin proteins obtained with distinct parameters.

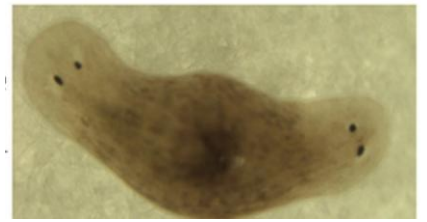
(b) The cells *i* and *j* have maximum conductances that depend on the *individual polarization state* of the cell, which influences the expression of the junction protein.

(c) Homogeneous spatial distribution of junction conductances (*left*) or *regionalizations* into *different modules* (*right*) are possible.

Together, these facts show a *rich network connectivity dynamics*, as suggested by the variety of bioelectric patterns found experimentally.

bioelectrical configuration space for flatworm morphology depends on junction connectivity

Bioelectrochem. 2020
10.1016/j.bioelechem.2019.107410

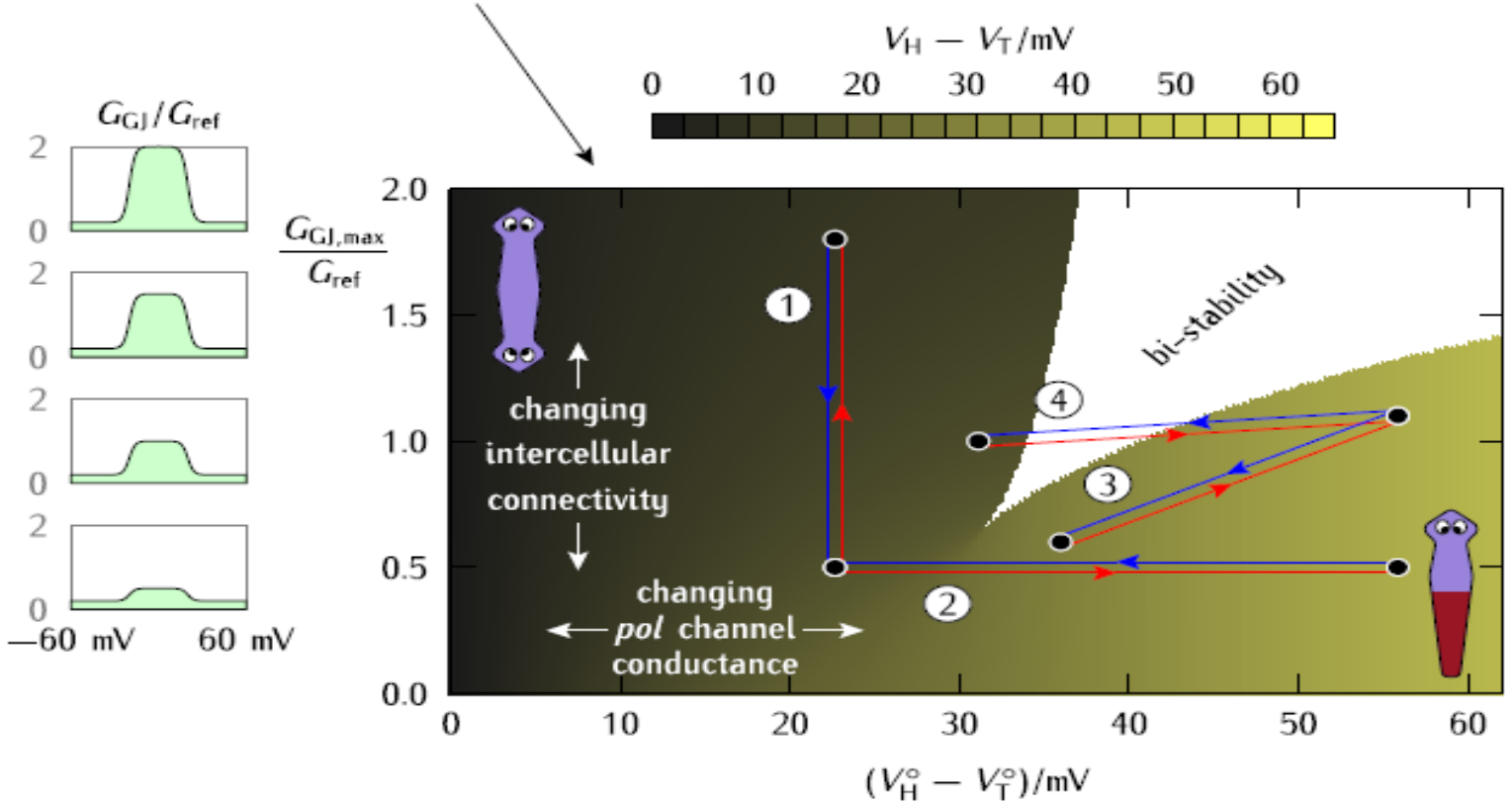


Steady-state solution for isolated cells:

$$V_H^\circ \equiv \frac{G^\circ_{pol,H}E_{pol,H} + G^\circ_{dep,H}E_{dep,H} - I_{pump,H}}{G^\circ_{pol,H} + G^\circ_{dep,H}}; \quad V_T^\circ \equiv \frac{G^\circ_{pol,T}E_{pol,T} + G^\circ_{dep,T}E_{dep,T} - I_{pump,T}}{G^\circ_{pol,T} + G^\circ_{dep,T}}$$

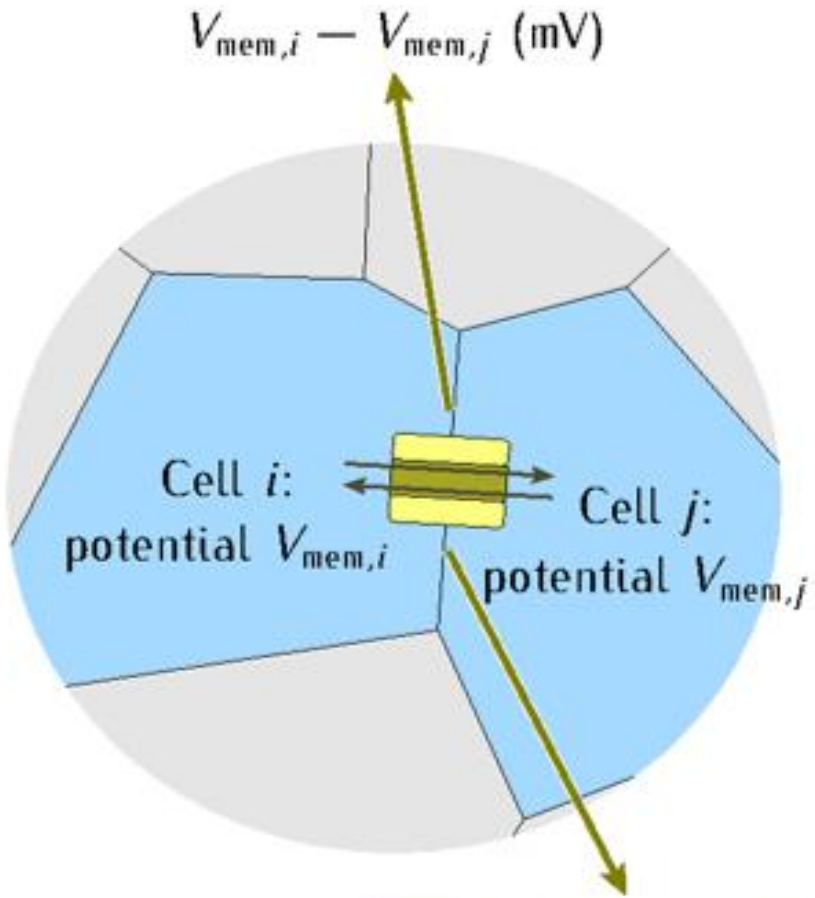
Steady-state solution for coupled cells:

$$\left[1 + \frac{G_{GJ}(V_H - V_T)}{G^\circ_{pol,H} + G^\circ_{dep,H}} + \frac{G_{GJ}(V_H - V_T)}{G^\circ_{pol,T} + G^\circ_{dep,T}}\right](V_H - V_T) - (V_H^\circ - V_T^\circ) = 0$$



Model equations and resulting *configuration space* for the *head-tail* potential difference $V_H - V_T$ as a function of the *isolated cell* potential difference (*superindex* o) and the *maximum gap junction conductance* $G_{GJ;max}/G_{ref}$. The color gradient indicates the different *monostable solutions;* the central white zone is the *bi-stability region*. The arrows show the trajectories of different processes.

J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004



$$C_i \frac{dV_{mem,i}}{dt} = \underbrace{-I_{pol} - I_{dep}}_{\text{single cell currents to extracellular environment}} + \underbrace{\sum_{j \in nn} G_{ij} \cdot (V_{mem,j} - V_{mem,i})}_{\text{gap junction coupling current between neighboring cells}}$$

The membrane potential V_{mem} of cell i changes because of the channel currents I_{pol} and I_{dep} and the *intercellular coupling current* I_{ij} between cell i and all neighboring cells j.

Crucially, the junction allows the *cytoplasm-coupled cells* of a spatial module to form an *isopotential region* that can be *regulated as a whole*, as suggested by the experimental patterns of electrical potentials.

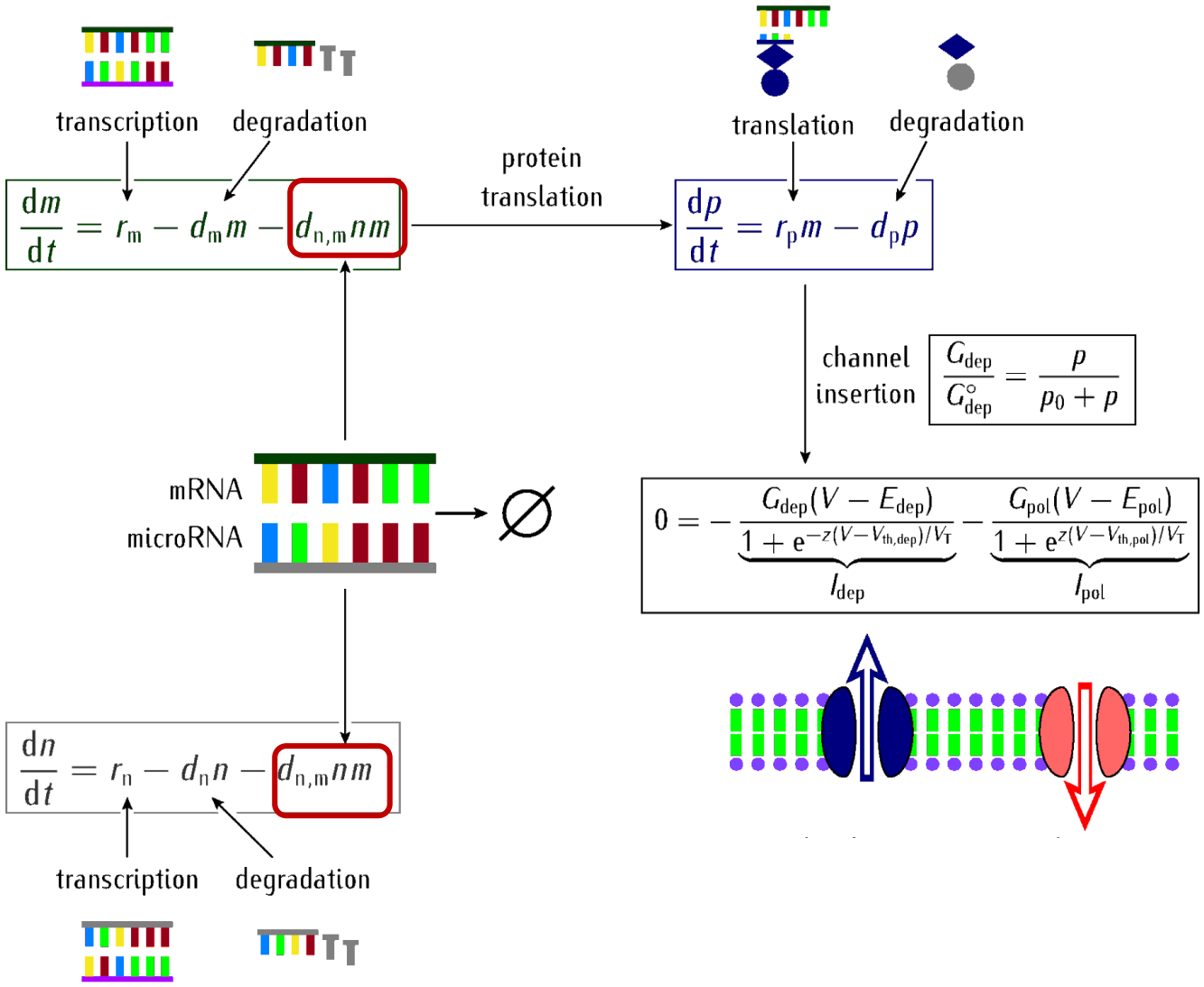
not only intercellular currents : gap junction-mediated transfer of signaling ions/molecules, e.g. microRNA

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774

miRNA-mediated intercellular communication supported by gap junctions might regulate *gene expression in neighboring cells* during development.

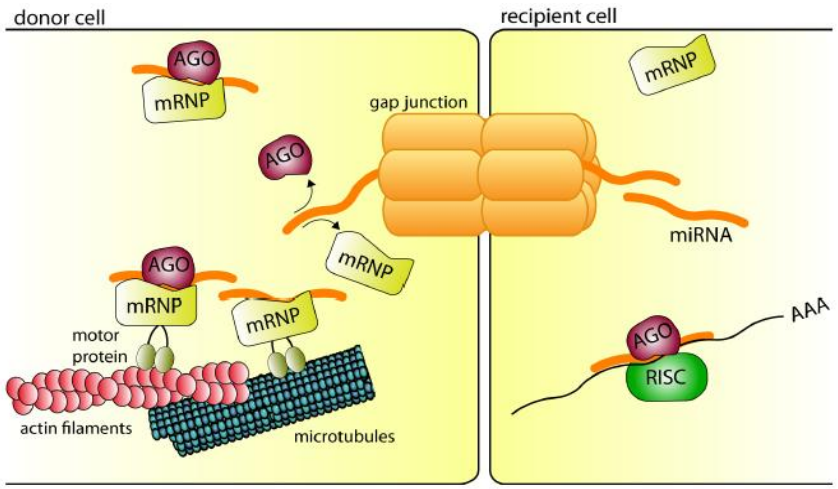
Cell-specific delivery by tuning the *junction protein* and *miRNA characteristics* establish *spatio-temporal regions* with *different bioelectrical states*.

A biophysical model where the miRNA acts on the mRNA of a specific channel protein thus modulating the *expression of* the *ion channel* (*right*).



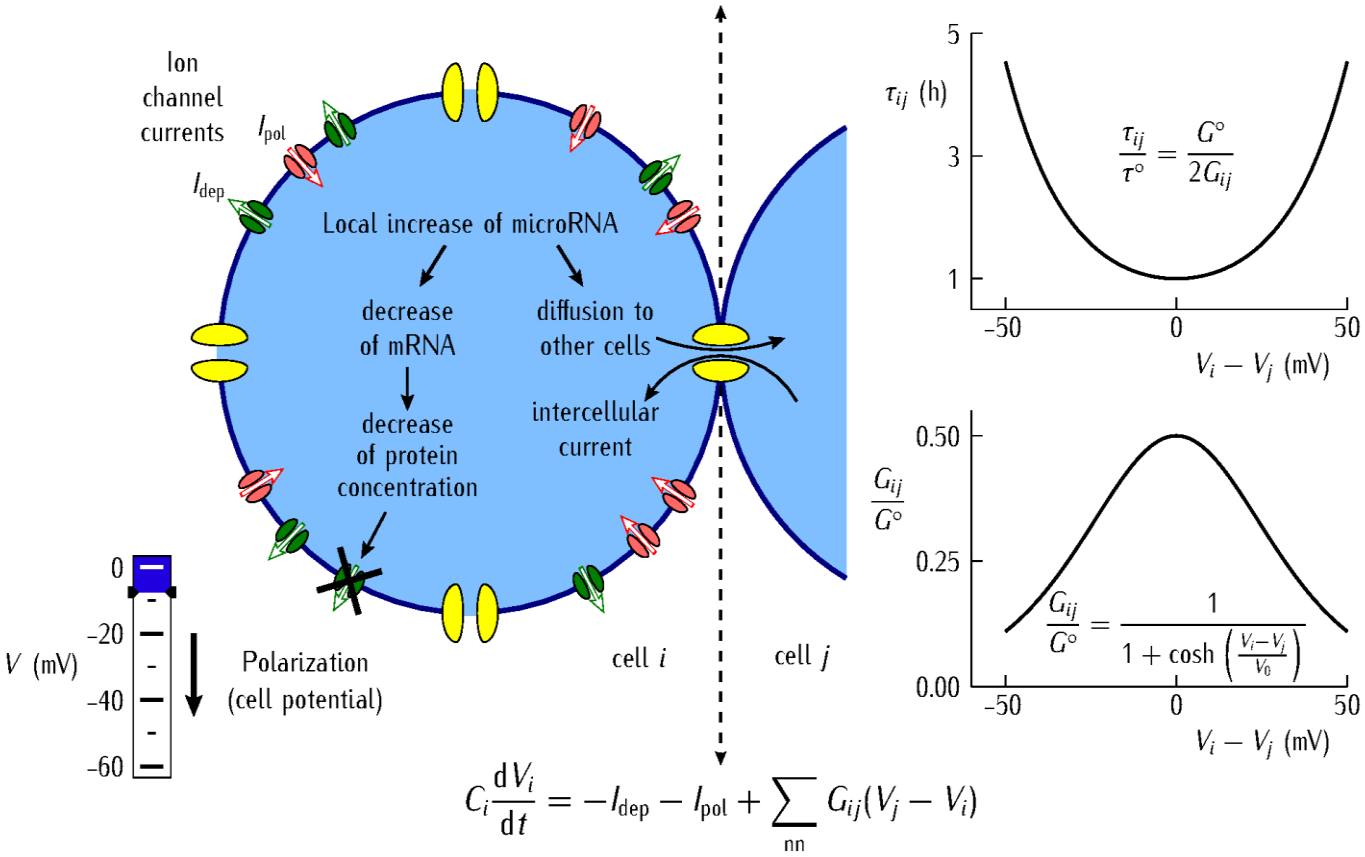
extended model for microRNA intercellular transference

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774
Traffic. 2018
10.1111/tra.12606



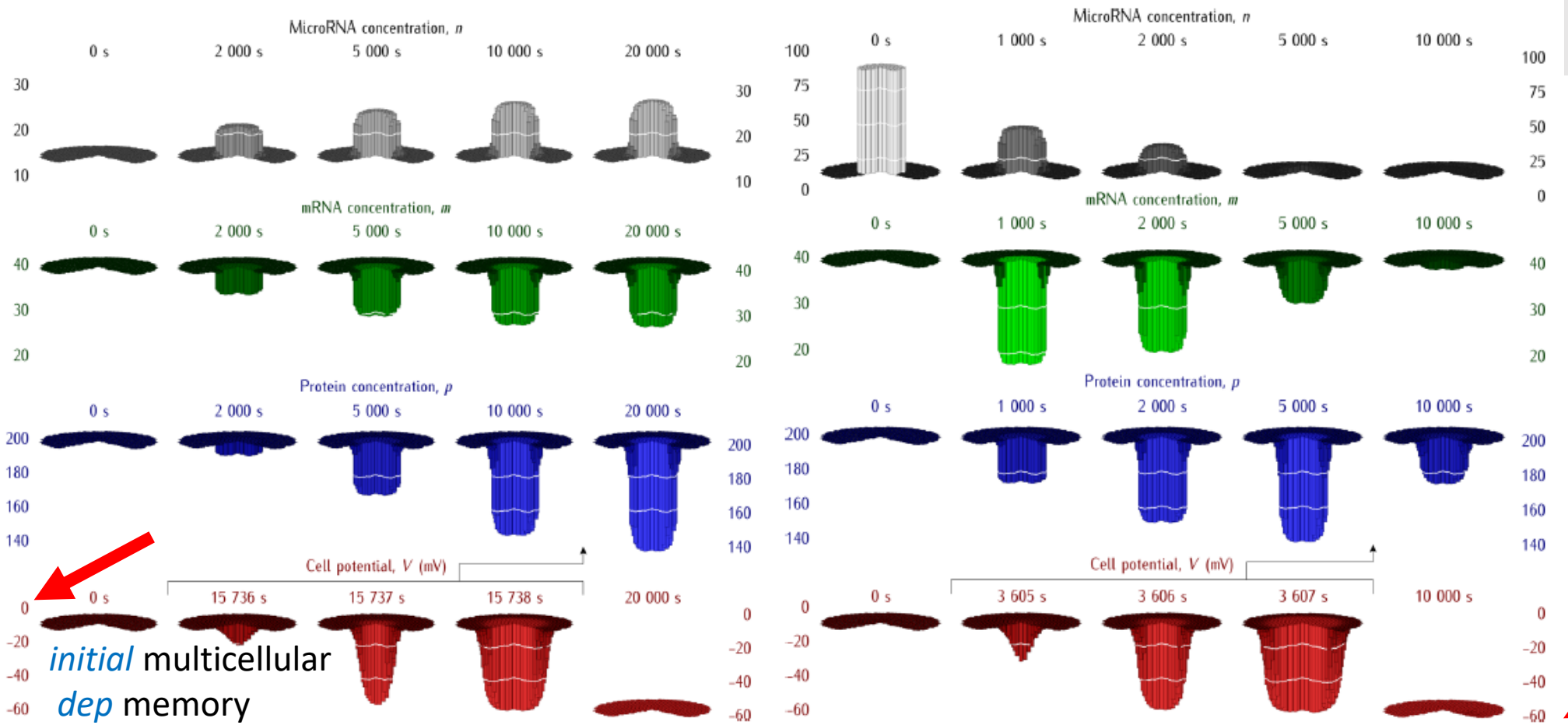
Translocation of miRNA to *gap junctional* cell-cell contacts driven by passive diffusion of miRNA molecules, e.g. bound to AGO proteins or other non-AGO proteins (mRNPs) in the cell.

$$\frac{dn_i}{dt} = r_n - d_n n_i - d_{nm} m_i n_i + \sum_{nn} \frac{1}{\tau_{ij}}(n_j - n_i)$$



$$C_i \frac{dV_i}{dt} = -I_{dep} - I_{pol} + \sum_{nn} G_{ij}(V_j - V_i)$$

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774



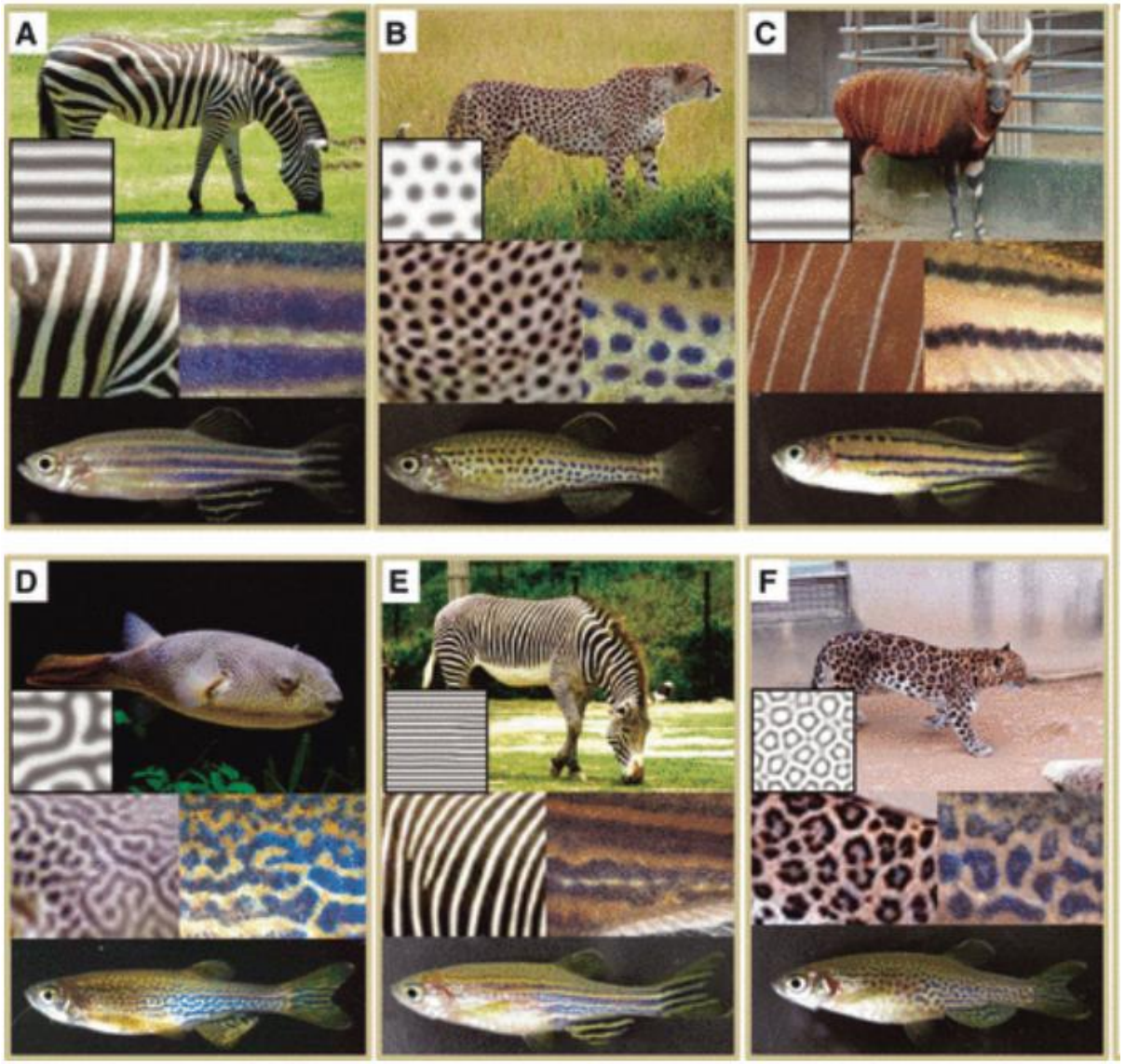
miRNA could change a multicellular bioelectrical memory

Time changes of *dep* channel *miRNA*, *mRNA*, and *protein* concentrations and the *cell potential* after the local increase of the miRNA transcription rate from 1.5 min^{-1} (*external* multicellular domain) to 2.0 min^{-1} (*central* domain). The miRNA transference across the intercellular junctions changes the d*ep* channel protein concentration, forcing the *repolarization* from the *initially depolarized state* (*left*). Time changes following a *local transfer of miRNA* over the central domain. Now, the initial *mRNA* and *protein concentrations* are *recovered at long times* because of the finite amount of miRNA transferred but *repolarization is maintained* (*right*).

other experimental systems: gap junction-assisted changing of skin patterns

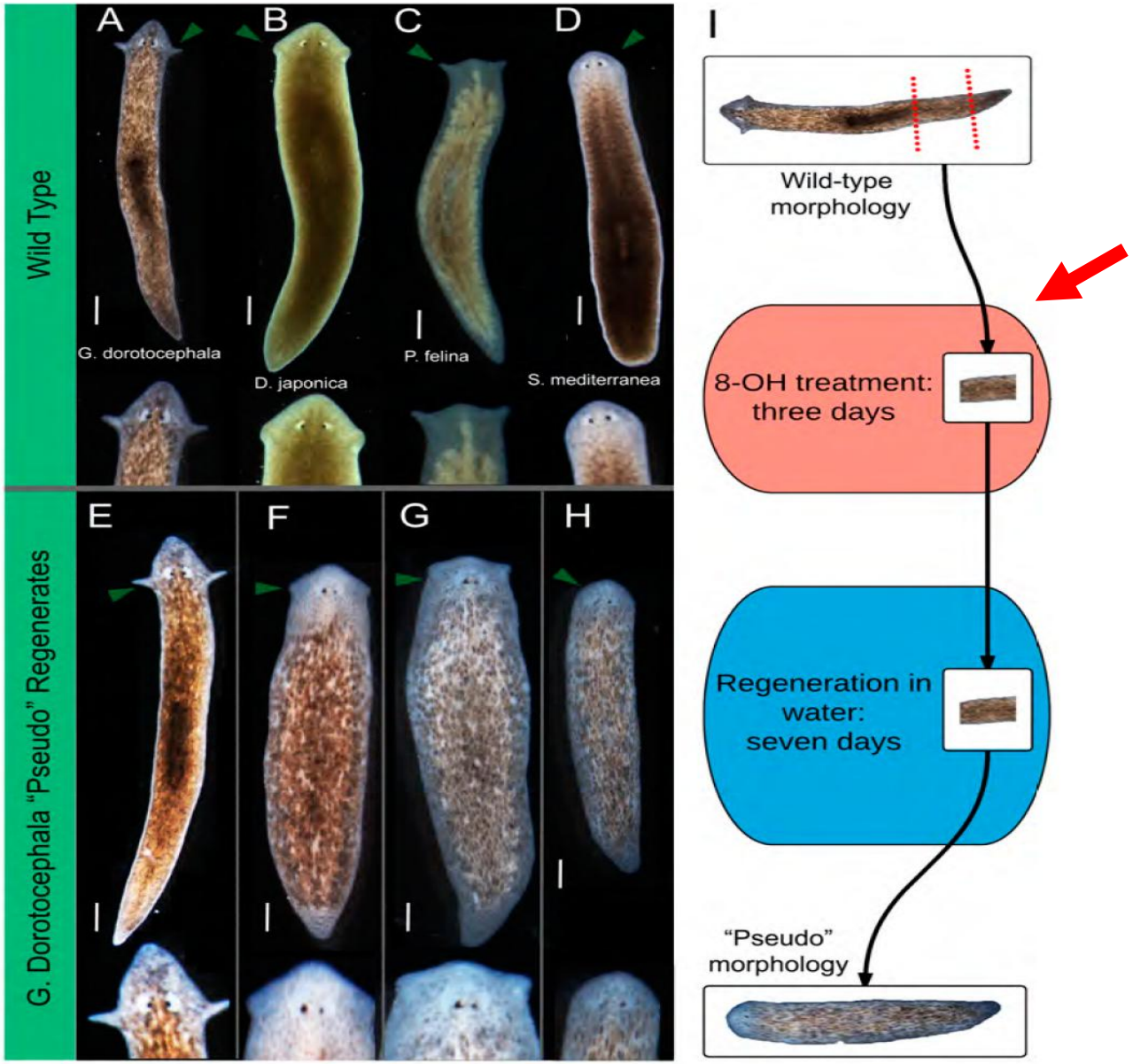
In a *biochemically-related context*, it has been suggested that gap junctions tune the *reaction-diffusion* (Turing type) processes responsible for the *skin patterns* observed in zebrafish, where slight junction changes lead to pattern diversity.

However, it is difficult to identify the molecules specific to particular cellular processes because of the limited selectivity of the gap-junctions.



Pigment Cell Melanoma Res. 2012
10.1111/j.1755-148X.2012.00984.x
J. Biol. Chem. 2016
10.1074/jbc.M115.673129

Int. J. Mol. Sci. 2015
10.3390/ijms161126065

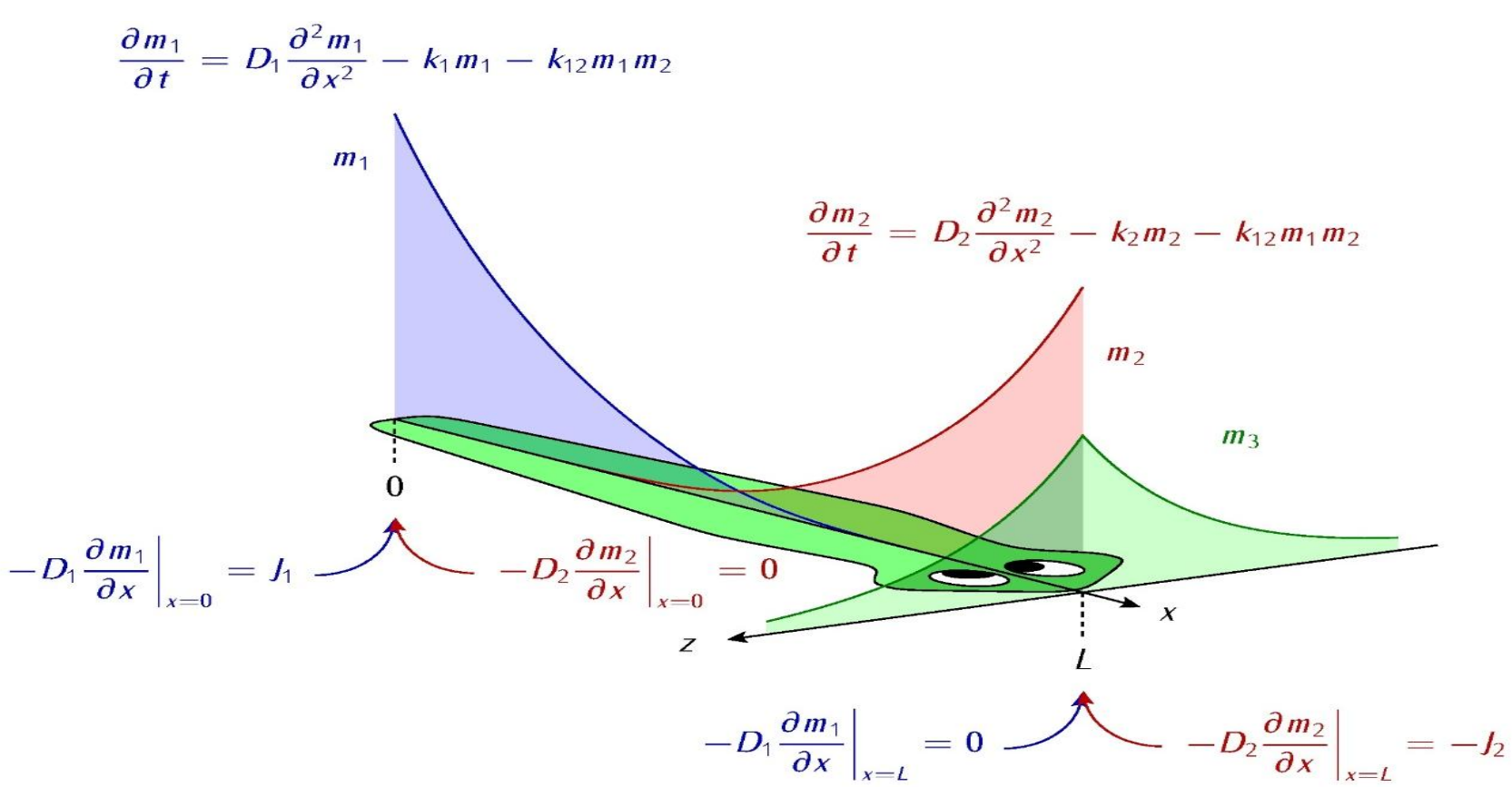


more actions on gap junctions: octanol blocking may change planaria head morphology

Different *head morphologies* observed after *octanol* treatment of *four species* of *planaria* flatworm (A–D).

The green arrows indicate the distinct head shapes (E–H) obtained when the *pre-tail fragments* are *amputated* from G. dorotocephala *worms* and treated according to the experimental procedure. The scale bar corresponds to 0.5 mm.

Biosystems 2021
10.1016/j.biosystems.2021.104511

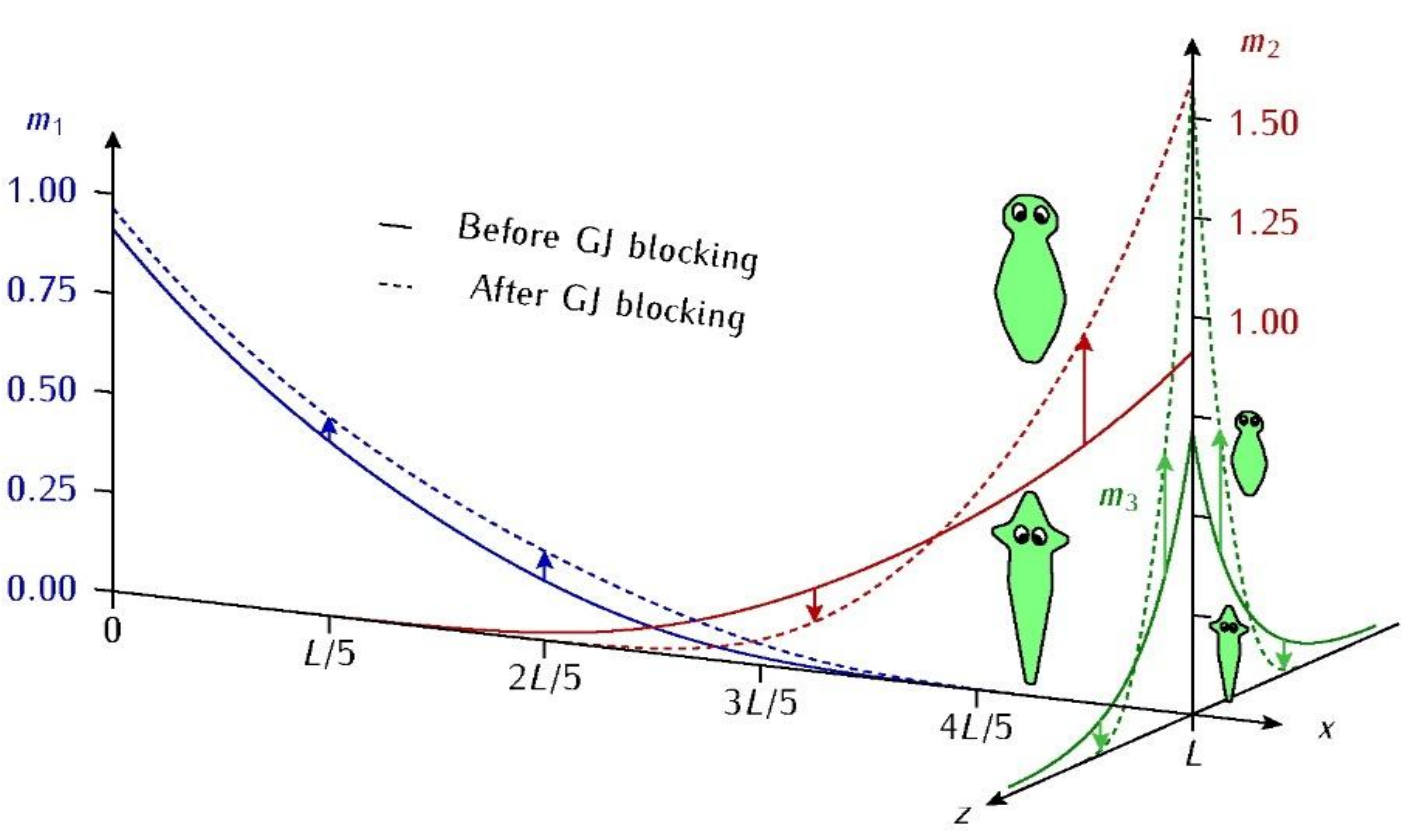


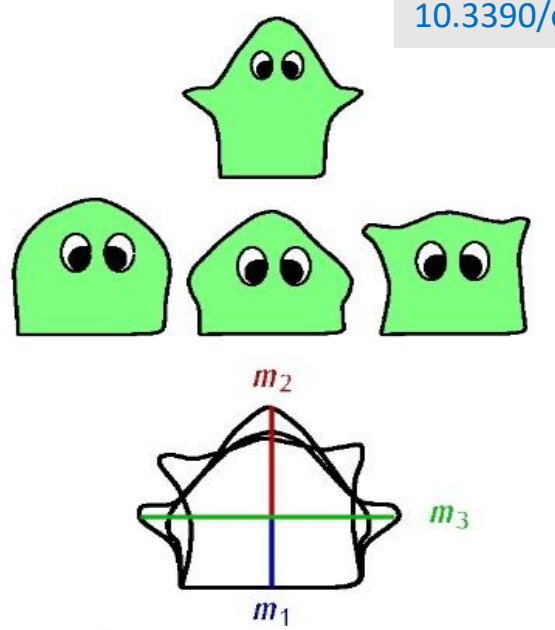
model equations for planaria head morphology

Reaction-diffusion equations with boundary conditions at x = 0 (*tail*) and L (*head*). The *spatial regionalization* of two *morphogen concentrations* m_1 and m_2 along the x axis establishes the *antero-posterior morphology* of the multicellular system. Because the position of the *central wavefront* between the two concentrations is regulated by the diffusion coefficients D_1 and D_2, the *gap junction blocking* eventually impacts on the *spatial patterns*. A third morphogen of concentration m_3 that diffuses independently along the orthogonal direction (z axis) may also influence lateral morphology.

Biosystems 2021
10.1016/j.biosystems.2021.104511
Entropy 2022
10.3390/e24060819

model qualitative results





Anteroposterior and *auricular* morphogen concentrations modulate *shape morphology*

Simulations suggest changes in the *instructive patterns* of the antero-posterior morphogens 1 and 2 and the auricular morphogen 3 resulting from the *gap junction blocking*. Do they lead to different *expression patterns* and *head shapes*? This result emphasizes the importance of identifying *biological morphospaces* defined by *experimentally accessible* magnitudes.

5. Theoretical results of qualitative relevance

The progress from an *individual cell* to *multicellularity* leads to *average biophysical fields* that influence *development* and *regeneration* (a *high level of abstraction*).

In *Bioelectricity,* an initial

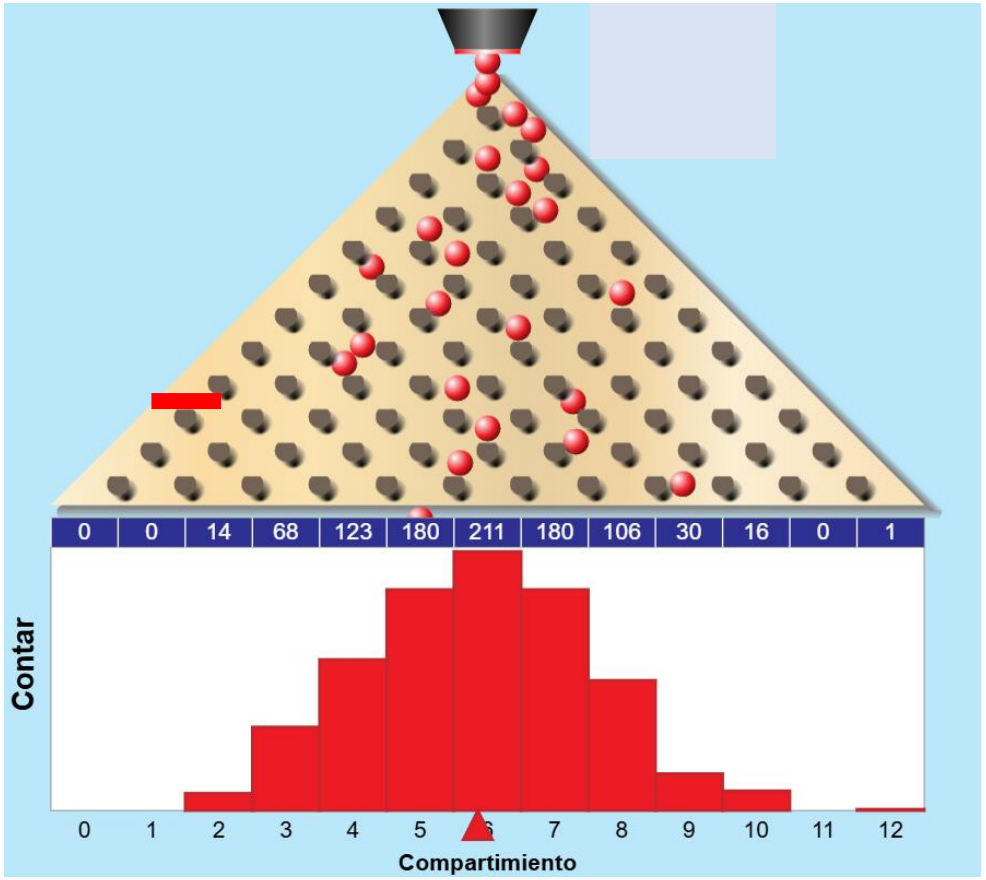
small asymmetry, *local disruptive event*, *change in environmental conditions*, etc.

triggers subsequent processes where the coupling between *bioelectricity* and *transcription* may *oppose to* or *amplify* the initial perturbation to *reestablish previous* or *establish new* multicellular programs.

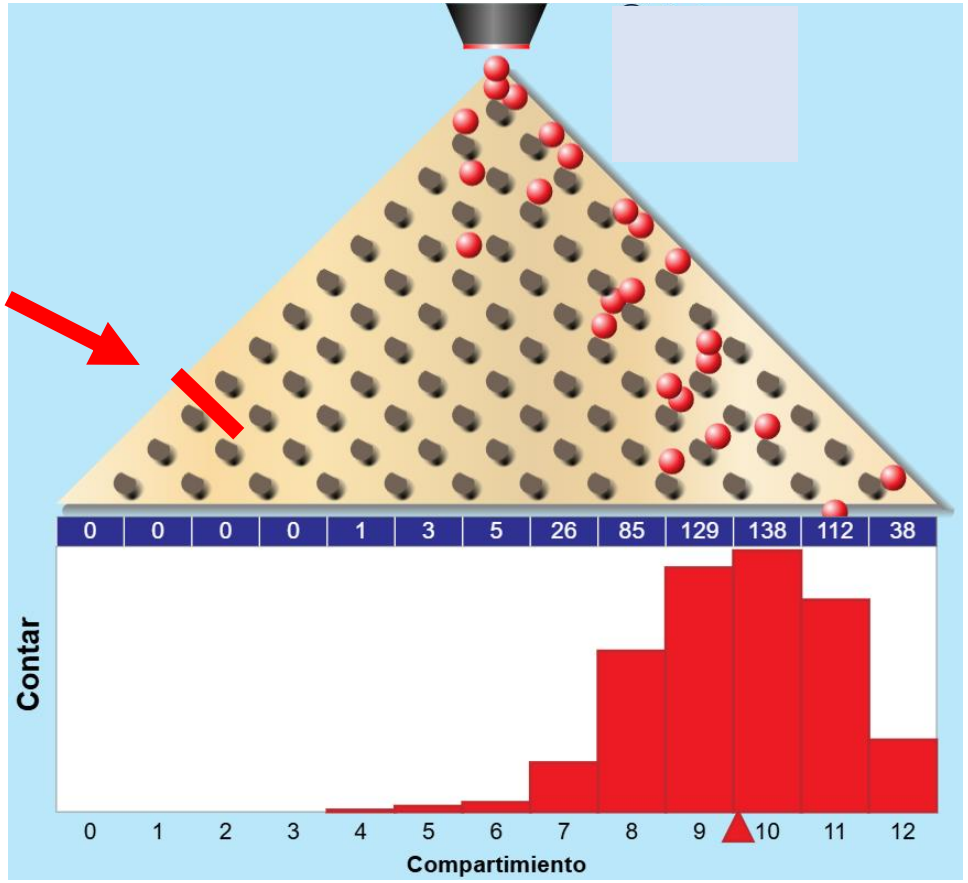
The following *model simulations* explore the *qualitative consequences* of different *symmetry-breaking* and *disruptive* phenomena, with an emphasis on *bioelectricity*. Other *biochemical* and *biomechanical* processes not included here may also be relevant in real systems.

PhET Interactive Sims. Univ. Colorado at Boulder 2024
https://phet.colorado.edu/es/simulations/plinko-probability

A previous observation: *complex phenomena* such as *symmetry-breaking* and *shape regulation* can arise in relatively *simple systems* composed by multiple units.



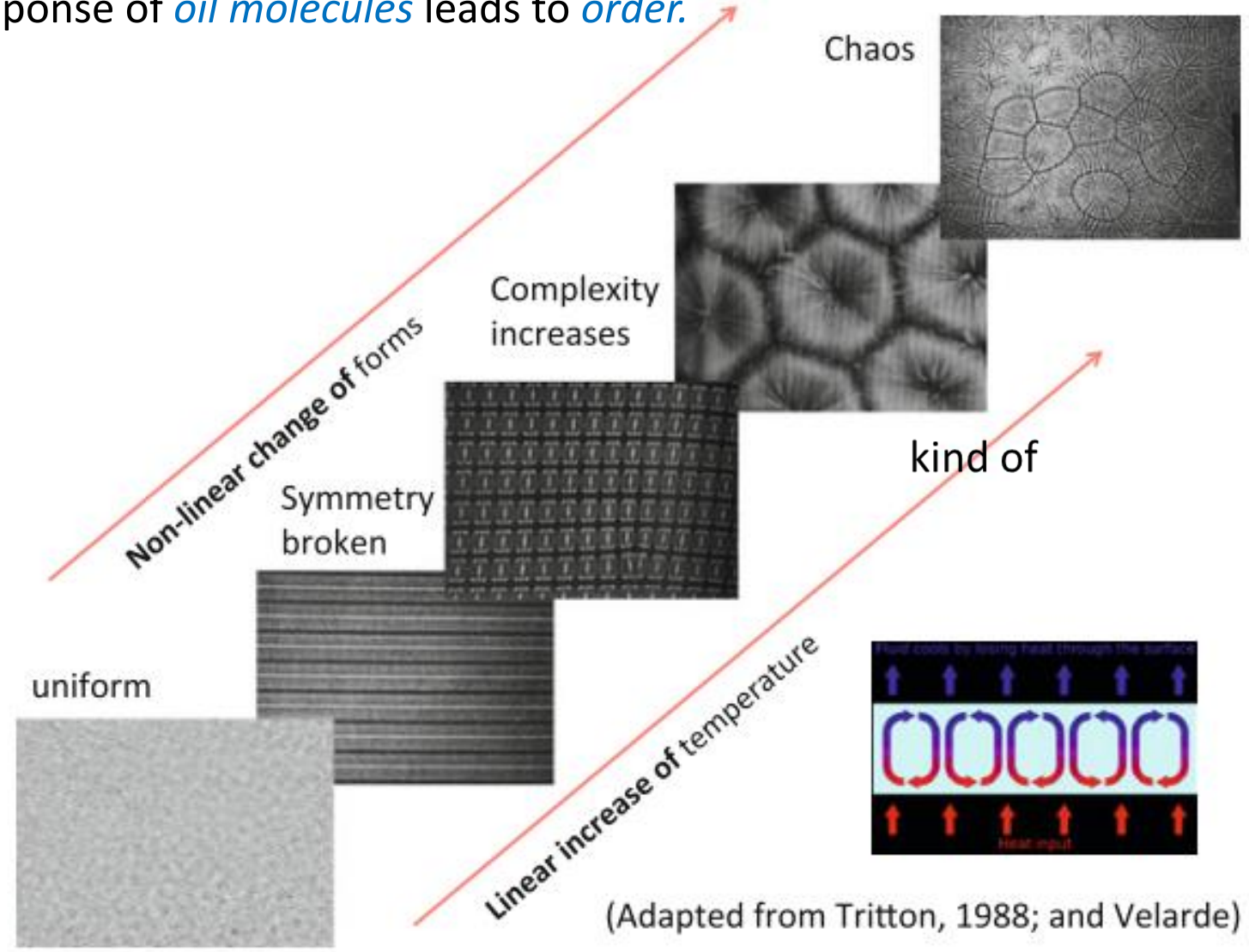
central



left-right

The Frontiers Collection (Springer Verlag) 2013
10.1007/978-3-642-36086-2_9

Rayleigh-Bénard cells: the *collective* response of *oil molecules* leads to *order.*



When heating from below a thin layer of oil spread out on a plane surface, *convection currents* with peculiar geometric shapes (lines or polygons) *self-organize*.

The shapes change abruptly when the temperature gradient goes over particular *thresholds*.

This non-linearity may *mimic* self-organization in *living systems*

[Photos adapted from Tritton (1988); and Manuel Velarde, Universidad Complutense, Madrid].

Sci. Rep. 2016
10.1038/srep204031
Ageing Res. Rev. 2024
10.1016/j.arr.2024.102310

In contrast to the *universality* of equilibrium phase transitions, *no general theory* predicts what organization arises far from equilibrium in *biological systems*: systems have *specific details* that matter.

However, two *bioelectrical features* are central to our model:

Contrast dynamic sharpening: *channels* and *gap junctions* operate over *wide voltage windows*; they may *polarize* or *depolarize* depending on the cell state and the neighboring conditions, providing the spatio-temporal contrast needed for *bioelectrical regionalization*.

Degraded pattern recovering: "corrupted images" due to a *local cell depolarization/polarization* (single-cell failure) or a *decrease of connectivity* (intercellular failure) can still be recovered by *single-cell* (e.g. channel redundancy re-establishes the normal cell state) or *multicellular* (e.g. community effects re-establish intercellular communication) mechanisms.

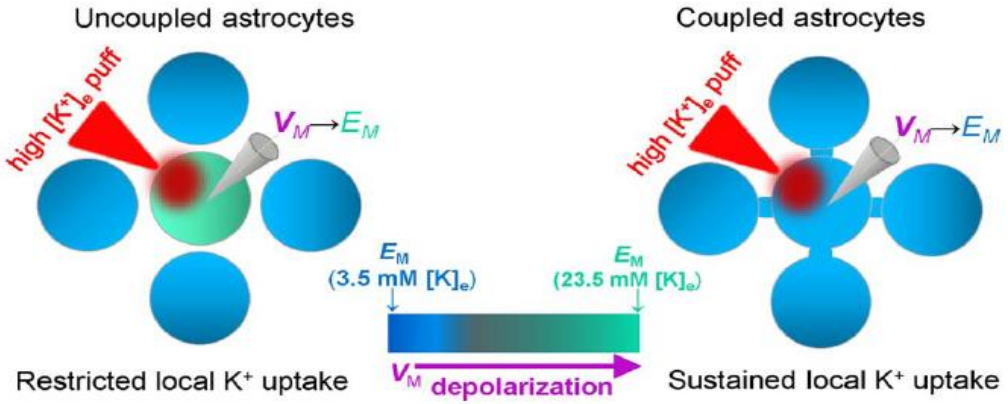
the bioelectrical community effect

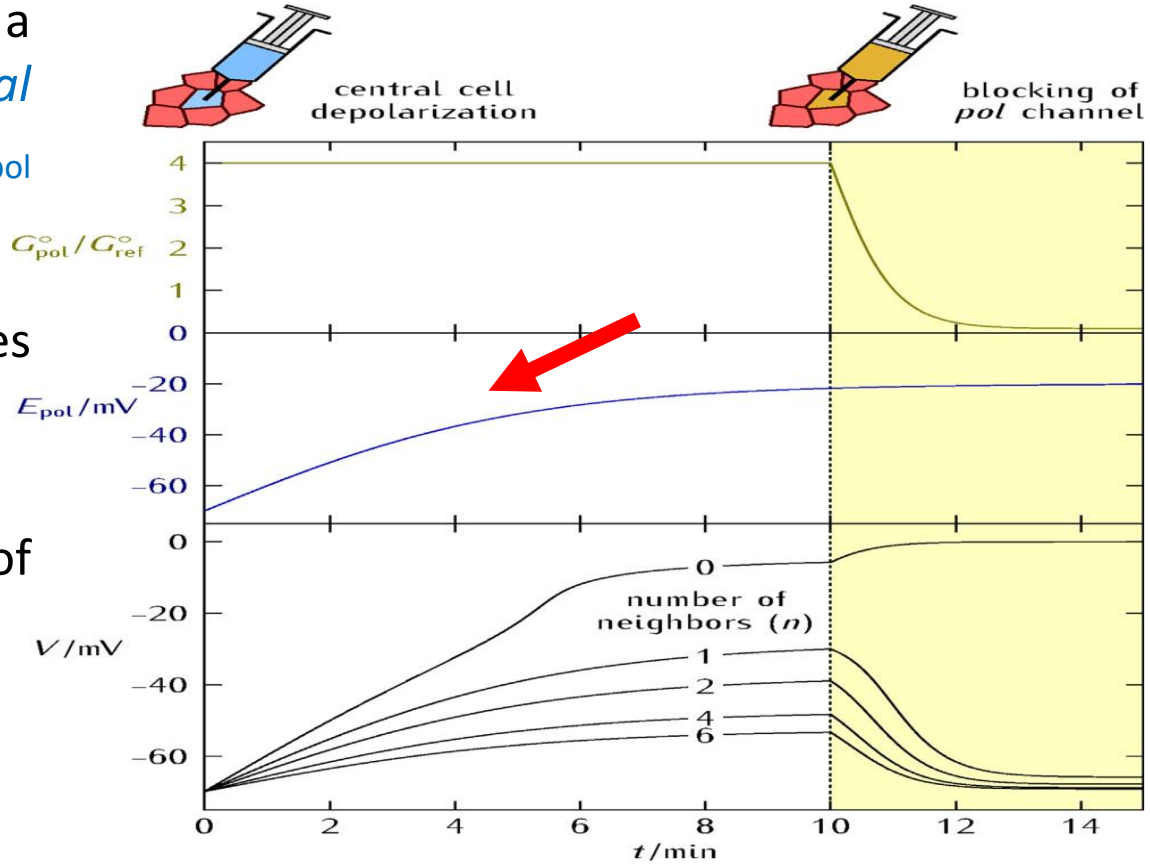
The *gap junction-mediated* transfer of *electrical* and *chemical* signals in a *multicellular community* provides a *feeling of belonging* to a cluster.

The *buffer effect* due to the *neighboring cells* can *correct* a perturbation, which is caused here by the *abnormal depolarization* of the *central* cell due to a local change in E_{pol} (it could also be caused by the *pol* channel *blocking*).

The community effect *opposing* the *disruptive* event increases with the *number of neighbors* and the *junction conductance*.

Each central cell *bioelectrically "measures"* the number of neighbors!

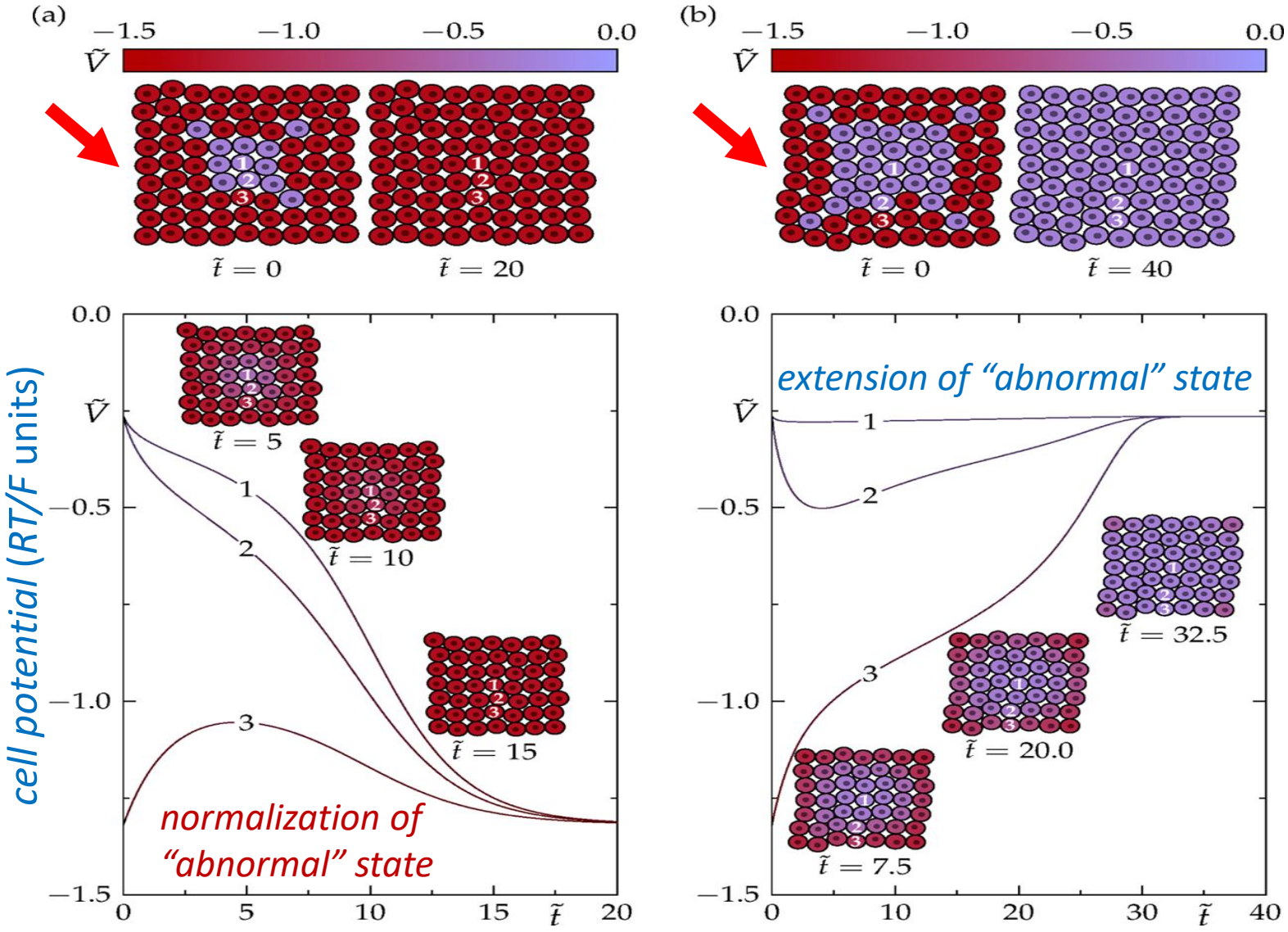




Glia 2015
10.1002/glia.22924
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004

J. Phys. Chem. B 2015
10.1021/jp512900x
Cancers 2021
10.3390/cancers13215300

a bioelectrical community effect



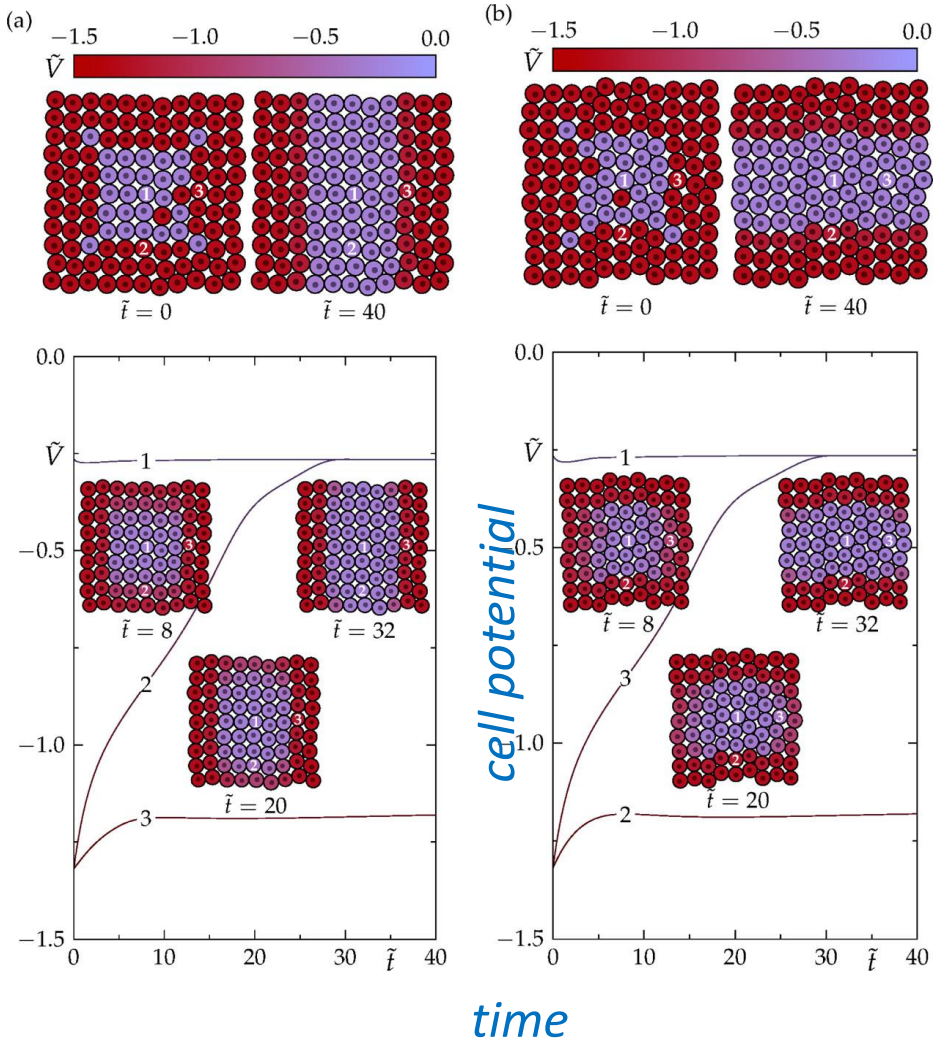
Bioelectrically *suppressing* (*left*) or *enhancing* (*right*) a disruptive local *depolarization* event:

for *small regions* of "*abnormal*" cells, the community effect allows a *normalization* of the bioelectrical cell state (*left*).

This normalization is *not possible* for *large "abnormal" regions* (*right*).

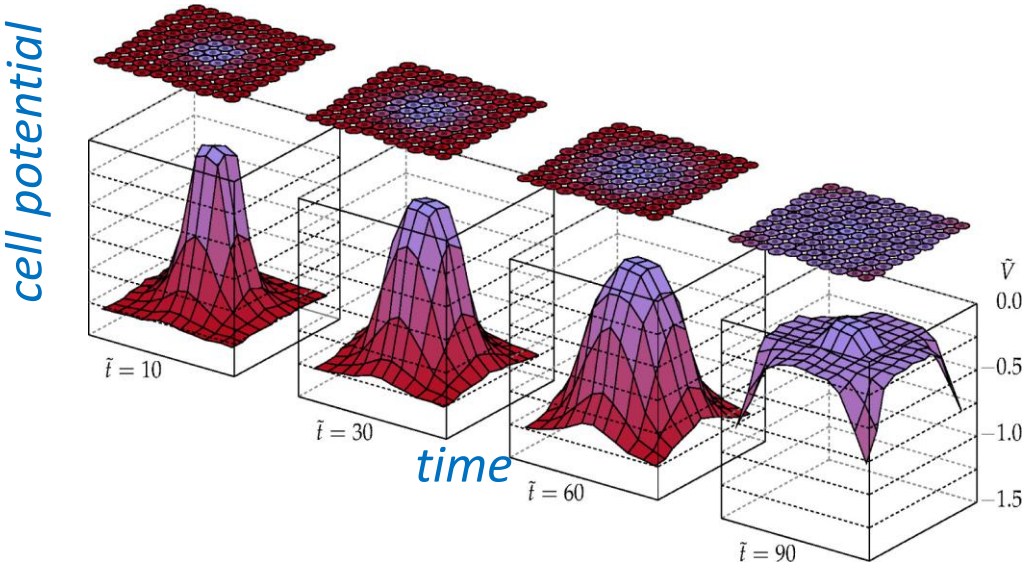
Thus, the *intercellular connectivity* could *normalize small regions* but also *spread the abnormal cell state* in the case of large enough regions.

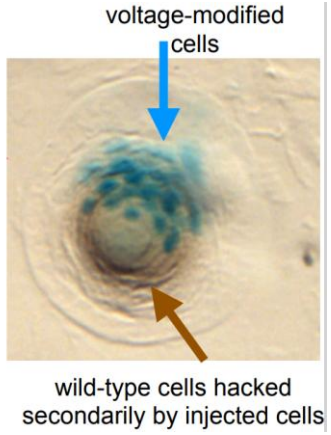
A *pre-pattern* of intercellular *gap junctions* allows imprinting a *directionality* to the tissue *re-writing*.



J. Phys. Chem. B 2015
10.1021/jp512900x
Commun. Biol. 2024
10.1038/s42003-024-06037-4

bioelectrical recruiting by *depolarizing wave?*

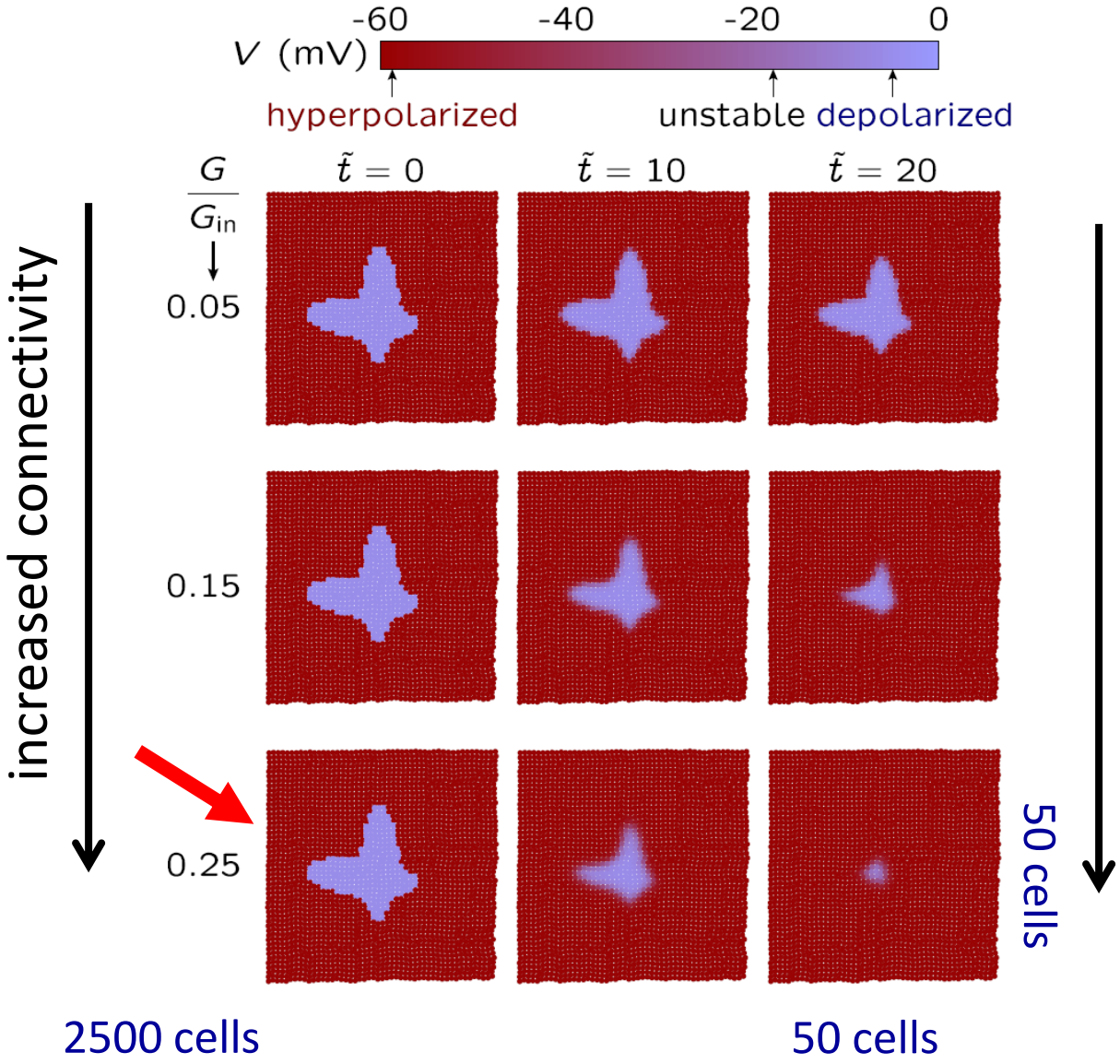




A *patch of cells* in a depolarized (*blue*) state, established by a pulse current, a mis-expression, or a blocking of the *pol* potassium channel, recruits the initially polarized (*red*) neighbors to *re-write* a *bioelectric pattern.*

Operational limitation: this recruiting is *not* always possible because it depends critically on the *relative stability* of the model *pol* and *dep* cell states.

Sci. Rep. 2016
10.1038/srep204031
Cancers 2021
10.3390/cancers13215300



stage-dependent behavior of intercellular junctions:

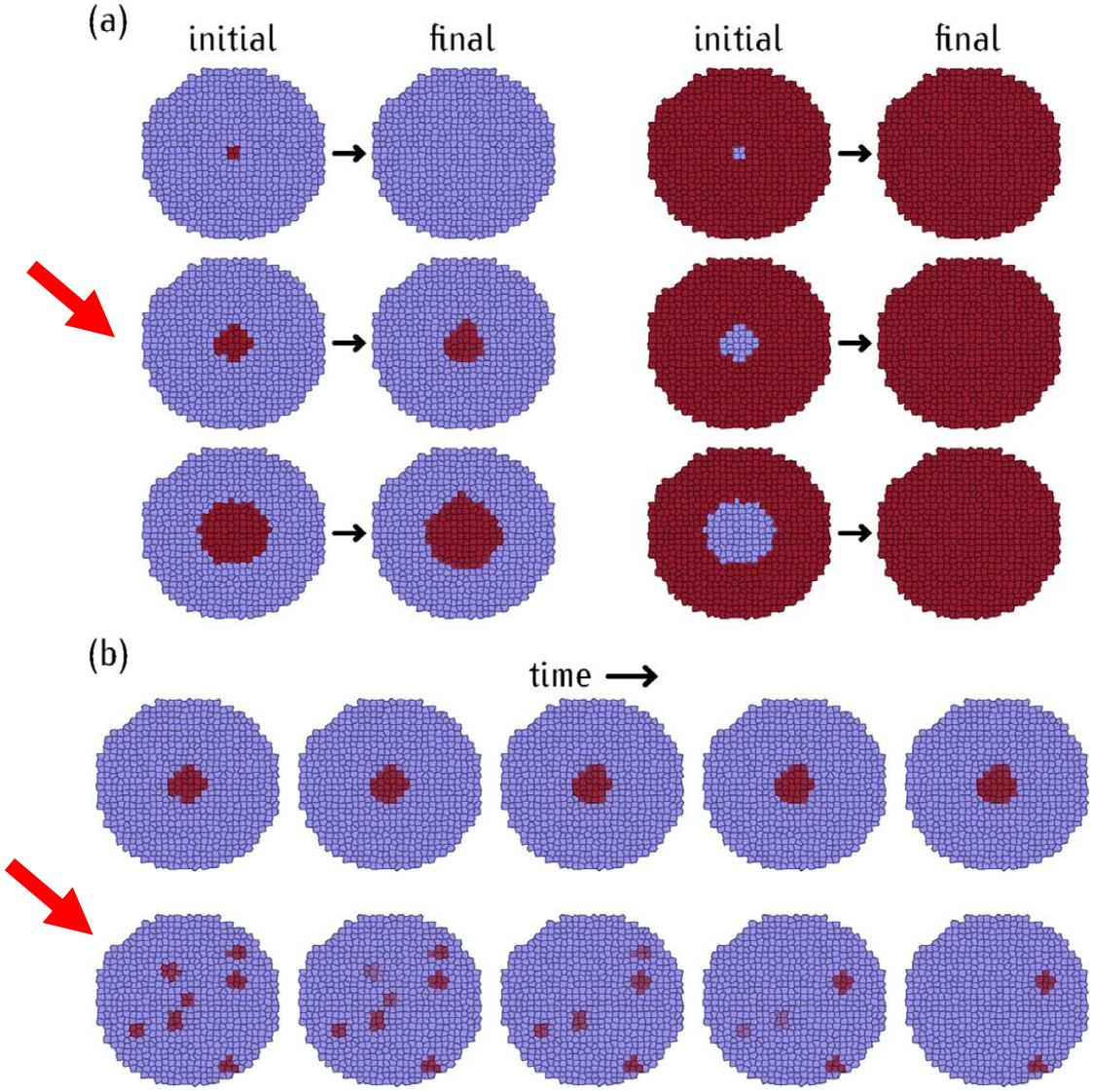
- at *early* stages, a disruptive event could be *bioelectrically suppressed* by *increasing* the connectivity of the *small abnormal* region with the normal neighborhood.

However,

- at *final* rather than initial stages, the disruptive event should be *suppressed* by *decreasing* rather than increasing the *internal* connectivity (*cohesion*) of the now *large abnormal* region, as shown in 10.1103/PhysRevE.102.052412).

Experimental limitations: 1) not only *electrical* but also *chemical* signals are transferred through gap junctions; and 2) bioelectrical *suppression* or *enhancement* of a disruptive event depends on the *relative* stability of the *pol* and *dep* cell states.

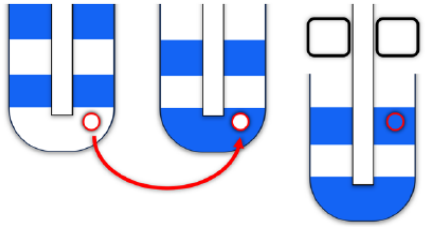
Phys. Rev. E 2020
10.1103/PhysRevE.102.052412
Commun. Biol. 2024
10.1038/s42003-024-06037-4



(a) The *community* effect is *size-sensitive* and depends also on which *isolated cell state* is dominant at the *single-cell* level: an "abnormal" *initially depolarized central region* can be *normalized* (*right*) but a polarized central region could resist the community normalization effect (*left*) because the *pol* state is dominant over *dep* state in this simulation.

(b) A *big patch* of cells shows a *stronger community* effect than many *small patches*: small bioelectrically "abnormal" regions hardly progress within a *highly-connected* multicellular ensemble.

A *biochemical* reminiscence (cell grafting in *segmentation*)?

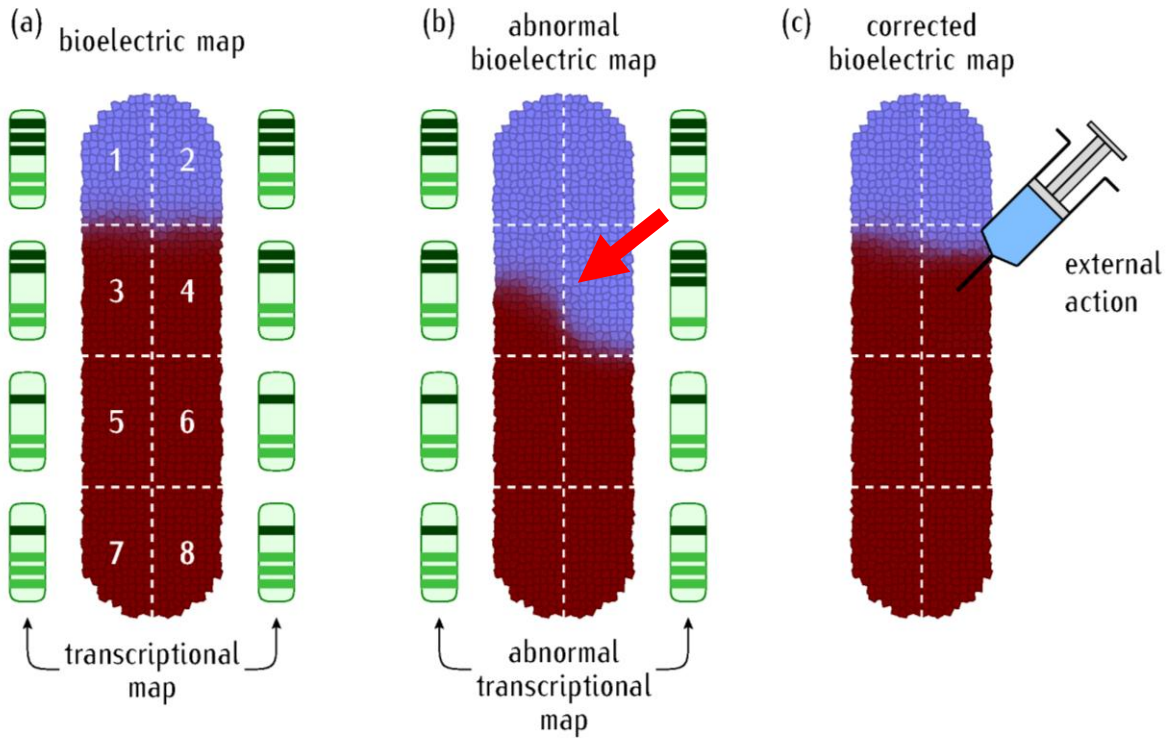


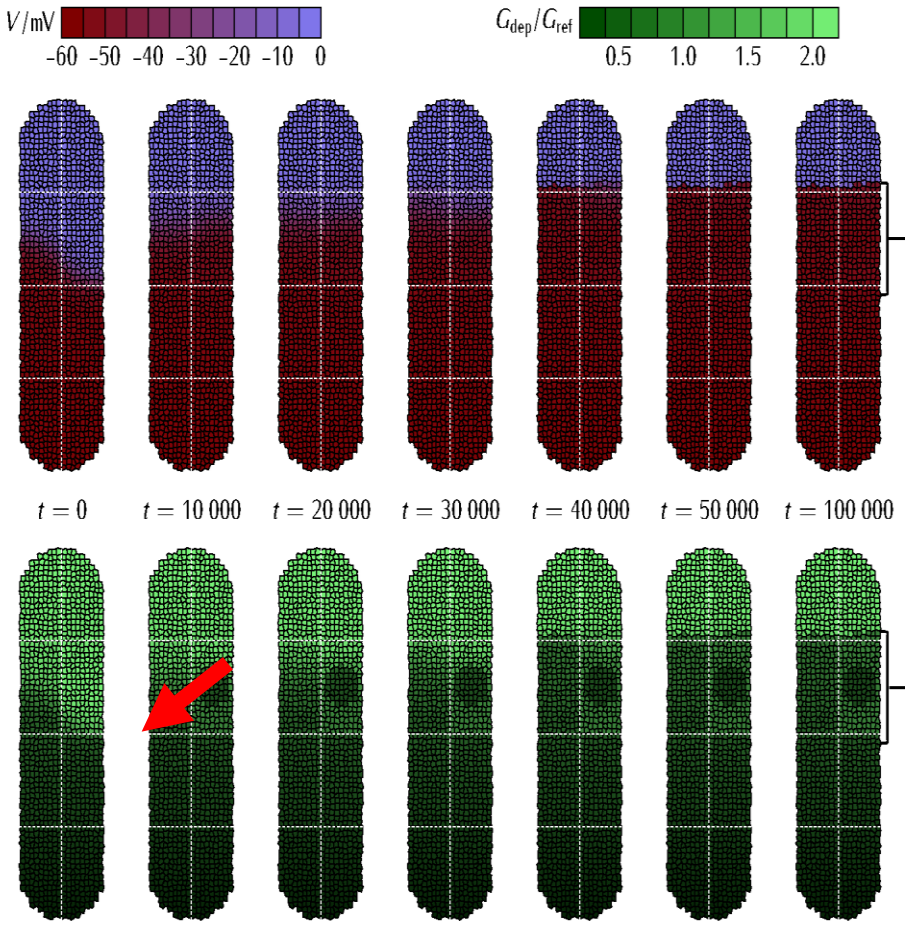
correction and completion: can locally degraded patterns be restored?

Biochim. Biophys. Acta 2023
10.1016/j.bbagen.2023.130440

Because of the *coupling* between *bioelectricity* and *transcription*, the simulations suggest that *corrupted spatio-temporal maps* could be *corrected* by *external actions:*

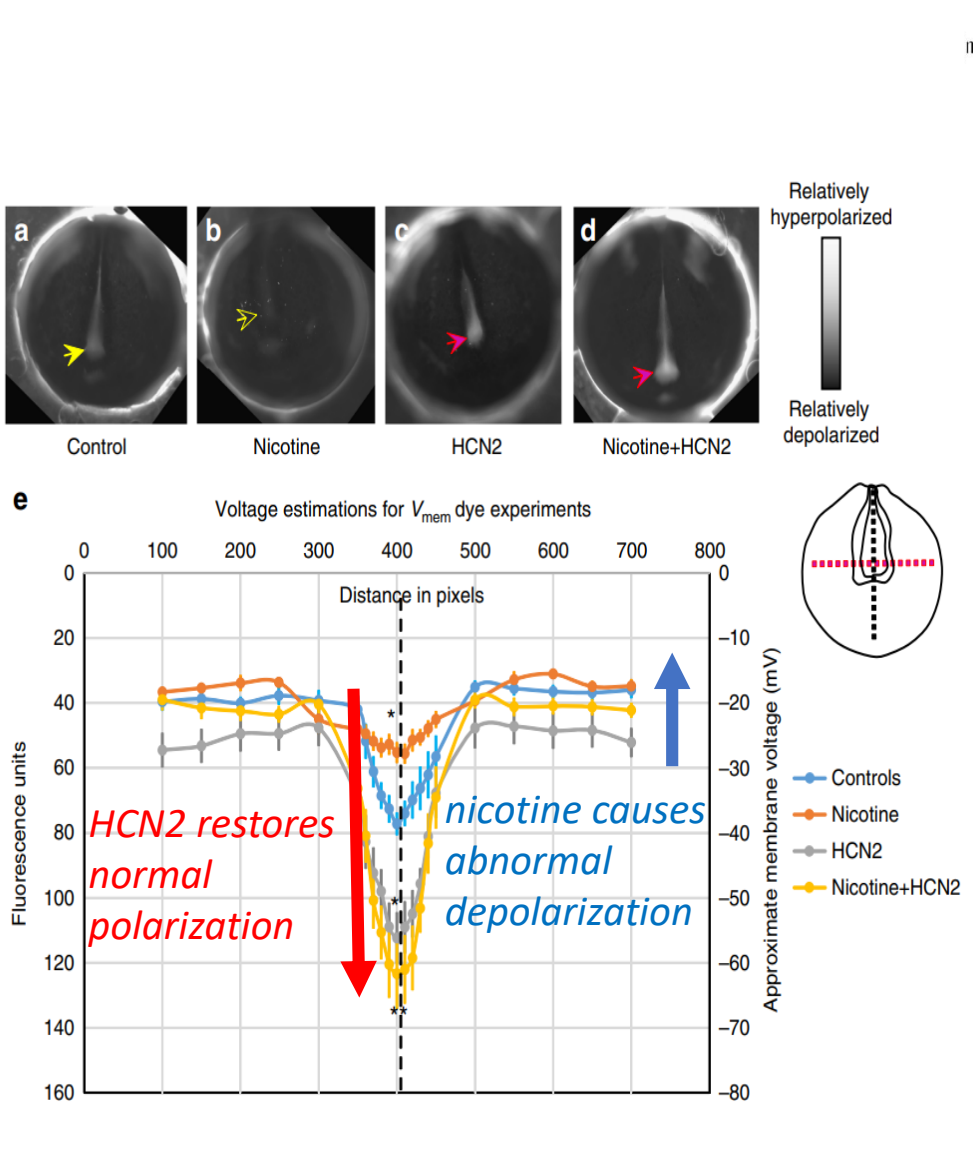
the *blocking* of an *incorrect* (*dep*) channel or the *upregulation* of a *rescue* (*pol*) channel *restores* the *correct pattern*.

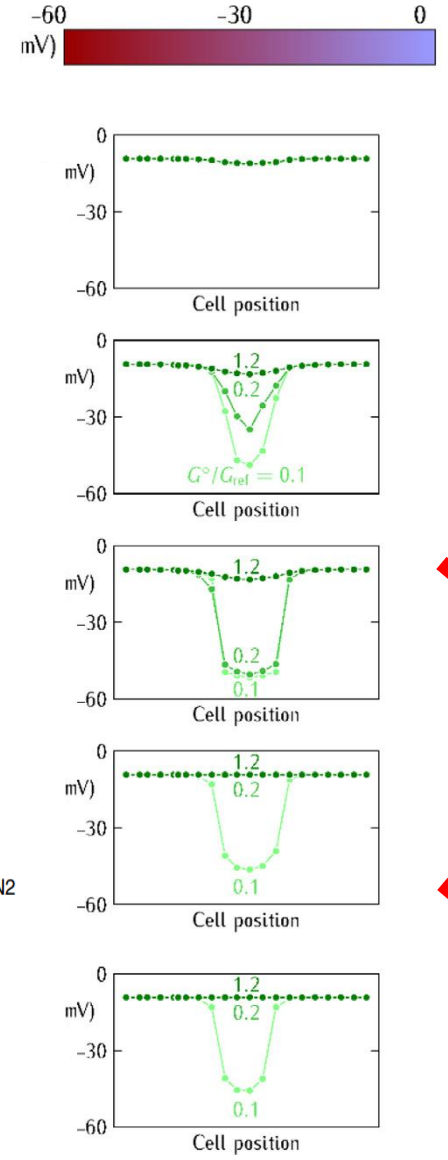




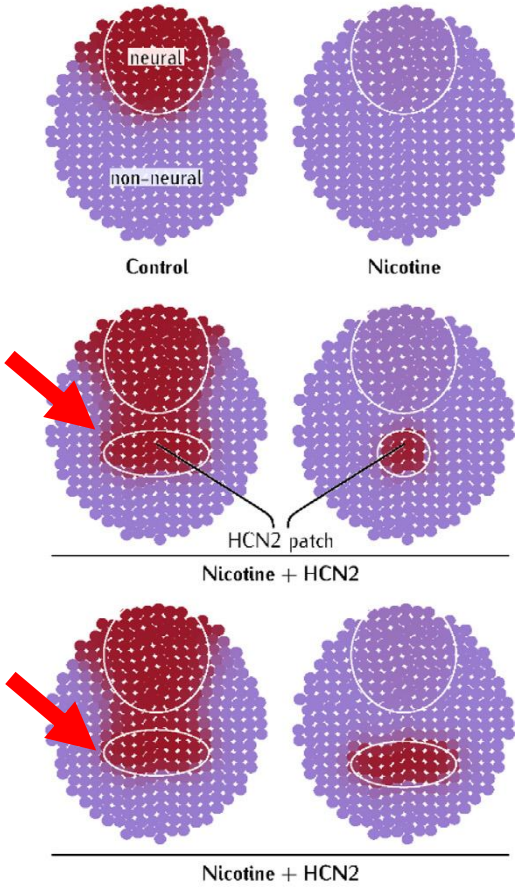
relative potentials between separated cell groups influence transcriptional patterns: are distant actions (polarization waves) possible?

Nat. Commun. 2018
10.1038/s41467-018-03334-5
Front. Cell. Neurosci. 2020
10.3389/fncel.2020.00136





distant cell bioelectrical recruiting

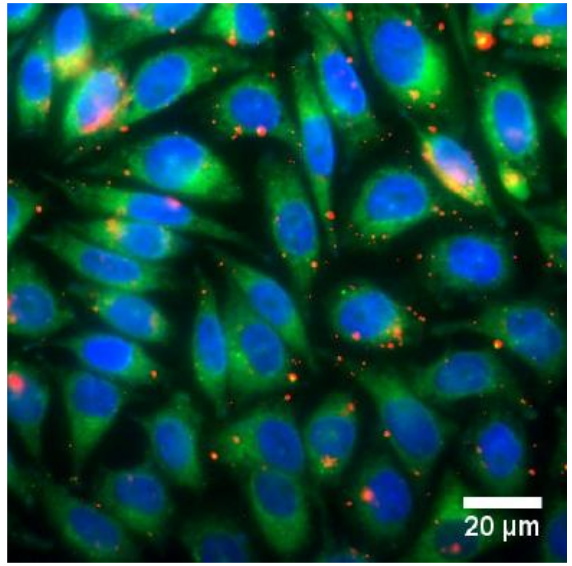


Incorporation of a *polarizing channel* (*HCN2*) partially recovers, *at distance*, the *potential pre-pattern* partly distorted by previous nicotine exposition.

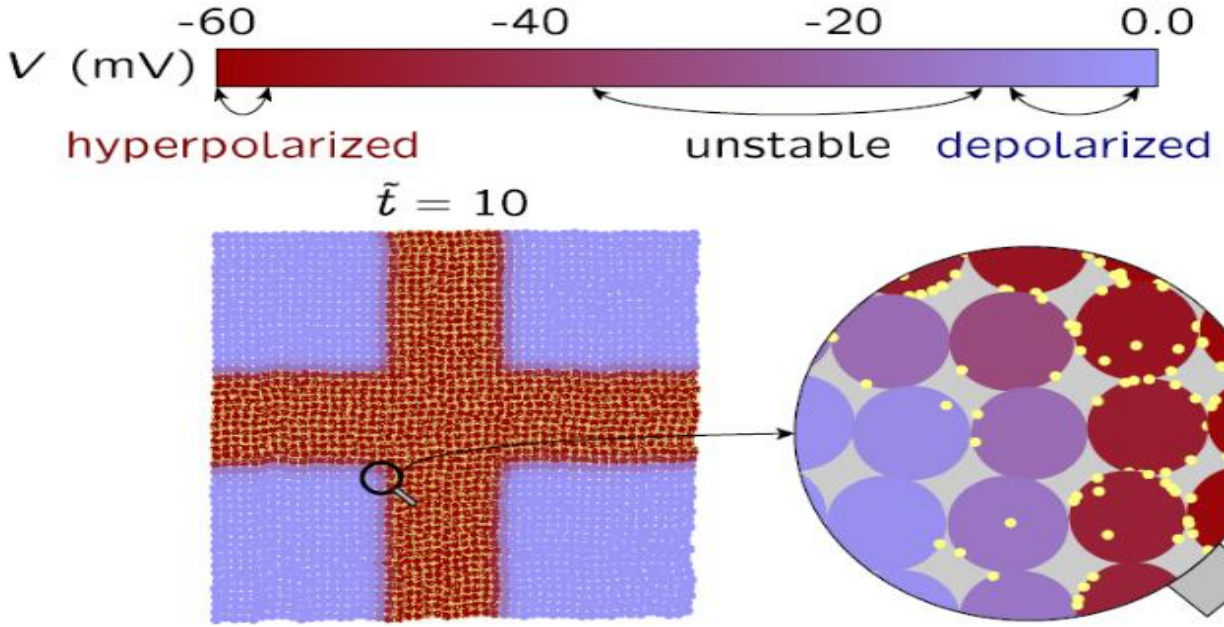
Size and *distance* effects influence the *repolarization*.

are other correcting actions possible (nanoparticles seeding)?

Nanoscale 2013
10.1039/C3NR01667F
RSC Adv. 2015
10.1039/C4RA15727C
Small 2024
10.1002/smll.202404152
Sci. Rep. 2016
10.1038/srep20403
Phys. Rep. 2023
0.1016/j.physrep.2022.12.003



The 60 nm *amine-modified polystyrene nanoparticles* (*small red circles*) bound to the *Chinese Hamster Ovary* (CHO) *cell membranes* can be observed by flux cytometry, dyes, and fluorescence microscopy techniques (*up*).



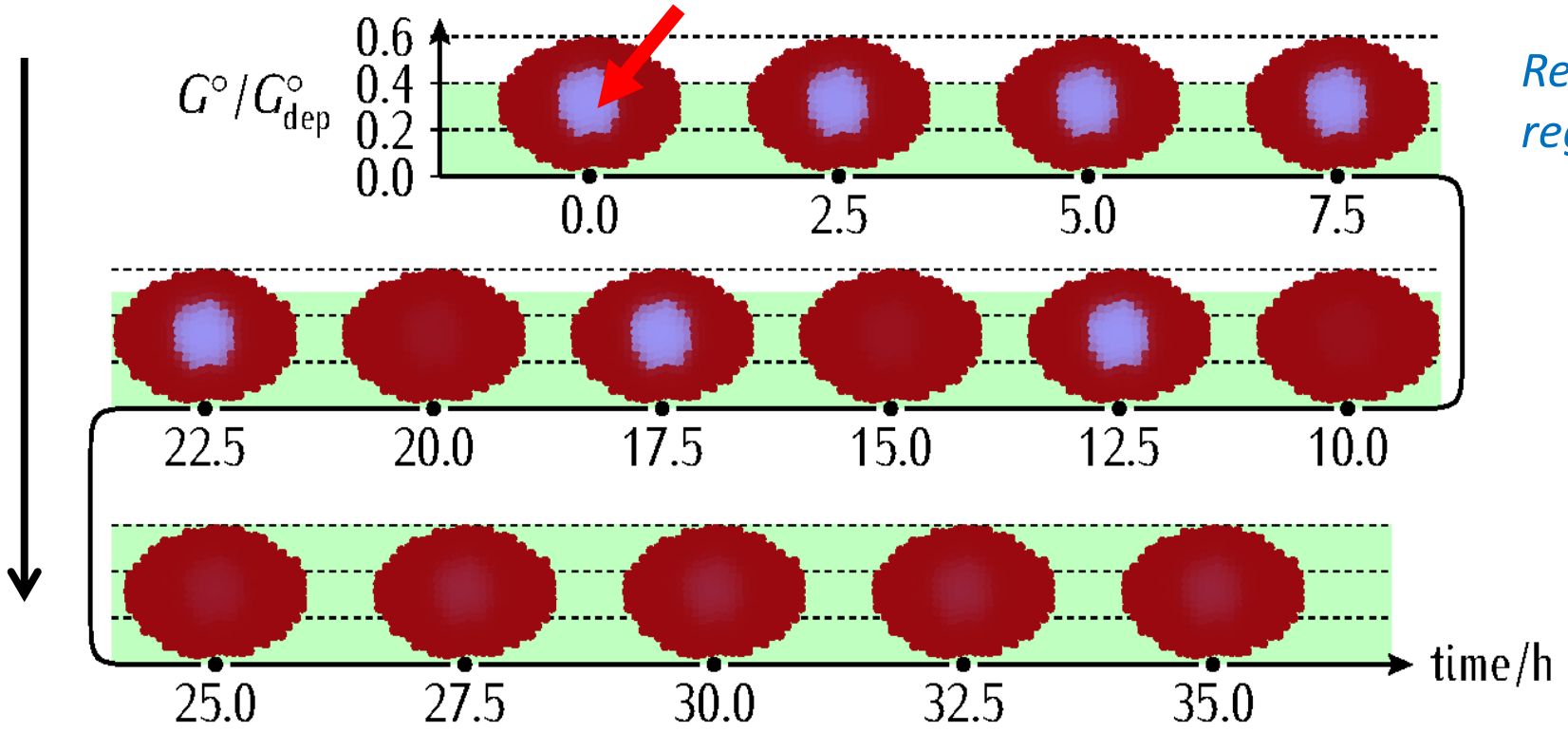
The bioelectrical simulation suggests that the *spatial distribution* of *positively charged nanoparticles* (*small yellow circles*) follows the *cell potential pattern*: particle accumulation is more significant around polarized (*more negative*) cells than around depolarized cells. The inset zooms a small region of the multicellular system (*down*).

Limitation: not only electrical but also *hydrophobic/hydrophilic* contributions to *nanoparticle binding.*

Phys. Rep. 2023
0.1016/j.physrep.2022.12.003

oscillatory multicellular potentials: acting on the intercellular connectivity

Increasing connectivity



Regionalization by exogenous grafted region or endogenous disruptive event

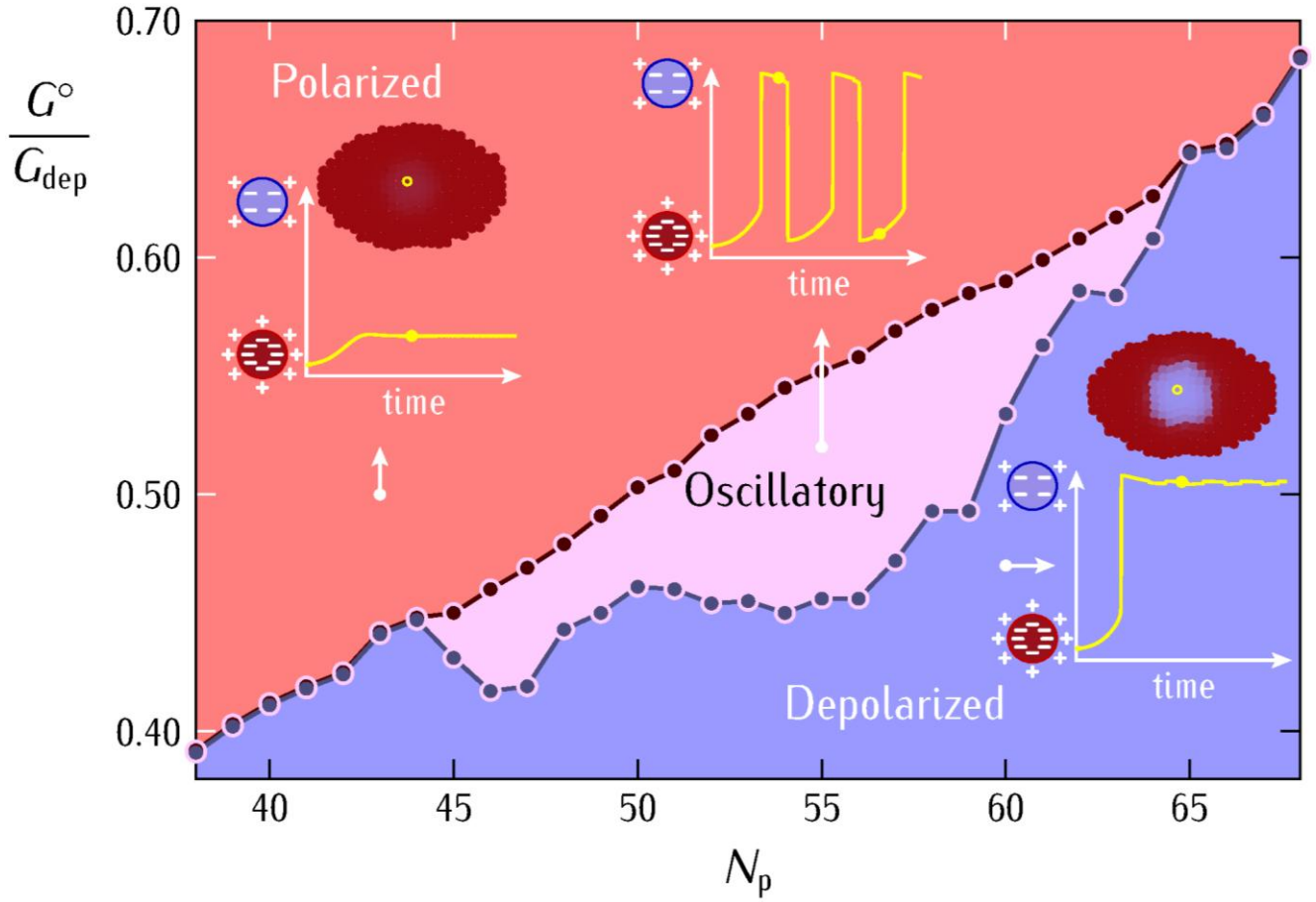
Collective oscillation

Normalization: isopotential system

Transitions between *depolarized* and *polarized* multicellular states, including *collective oscillations*, can be modulated by the *intercellular connectivity*. Note that *three* possible bioelectrical states can be realized in the central region, which suggests that external *instructive actions* on the *gap junctions* should be possible.

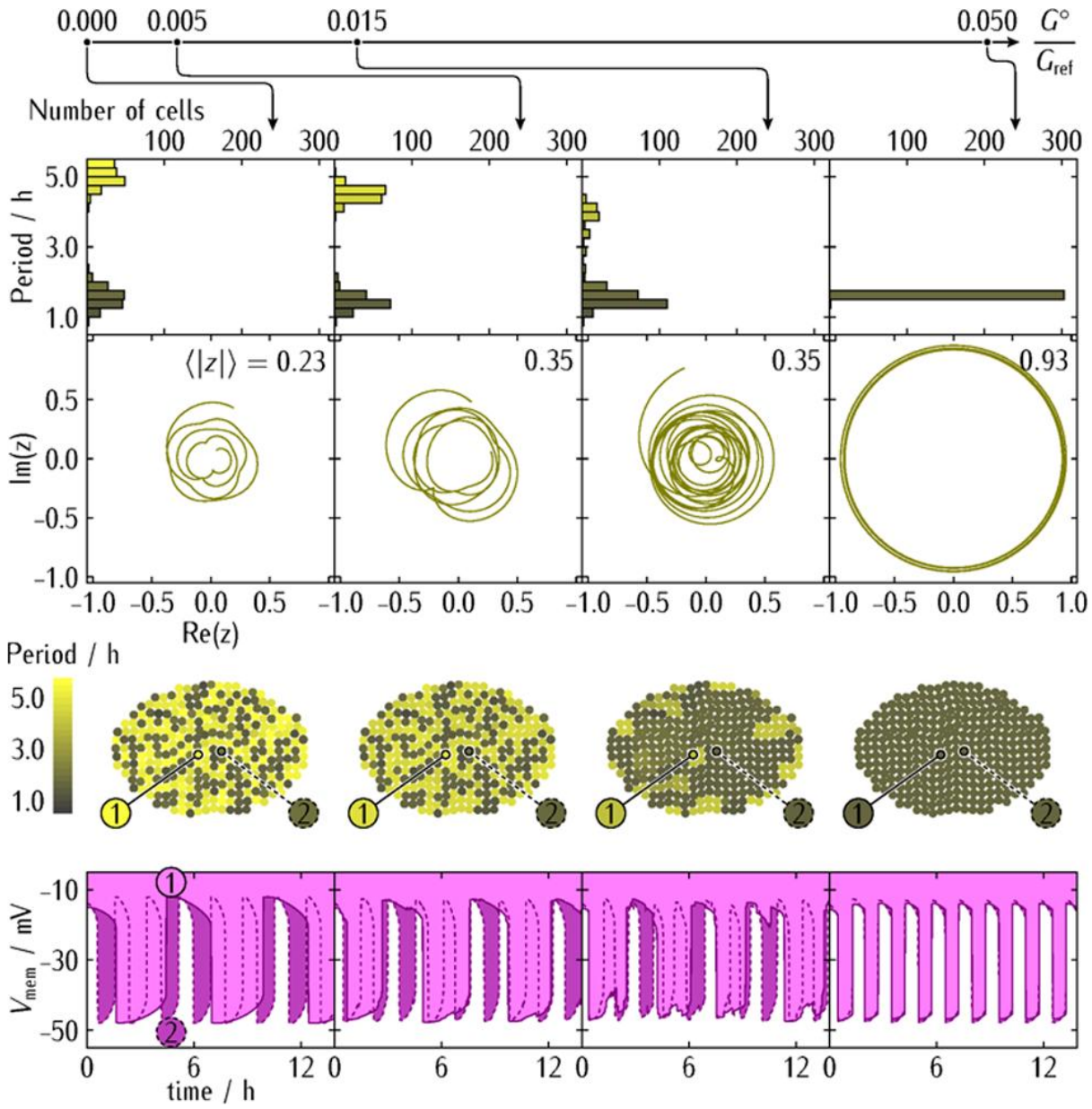
configuration phase space for central region: different bioelectrical states can be established by changing intercellular connectivity

Phys. Rep. 2023
0.1016/j.physrep.2022.12.003



Polarized, depolarized and oscillatory *multicellular states* as a function of the intercellular *junction conductance* and the *number of cells* in the central region*:* exploring the *bioelectrical* rather than *genetic* phase space.

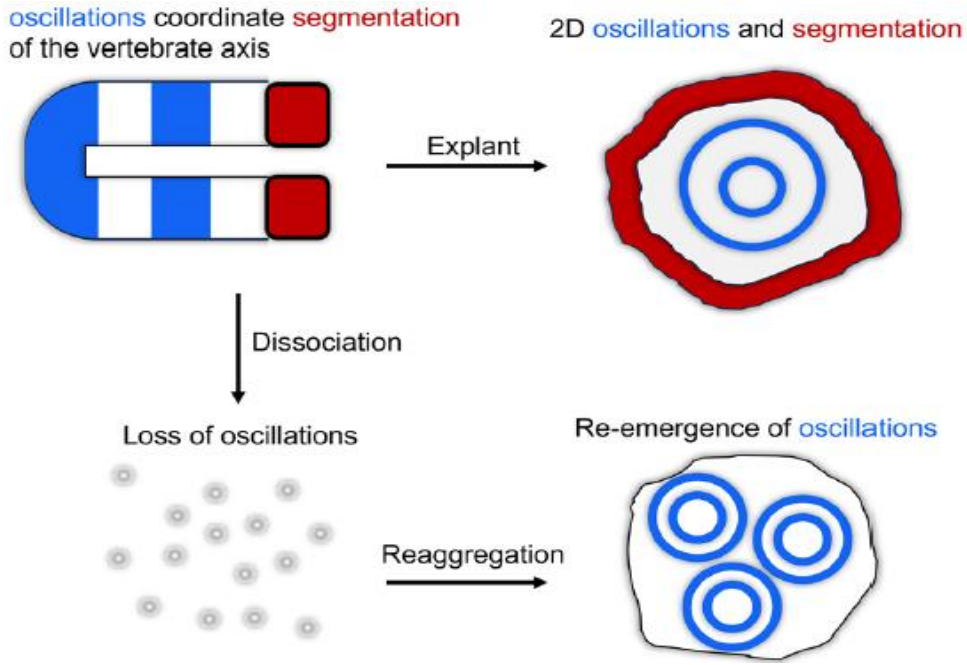
increasing intercellular connectivity →



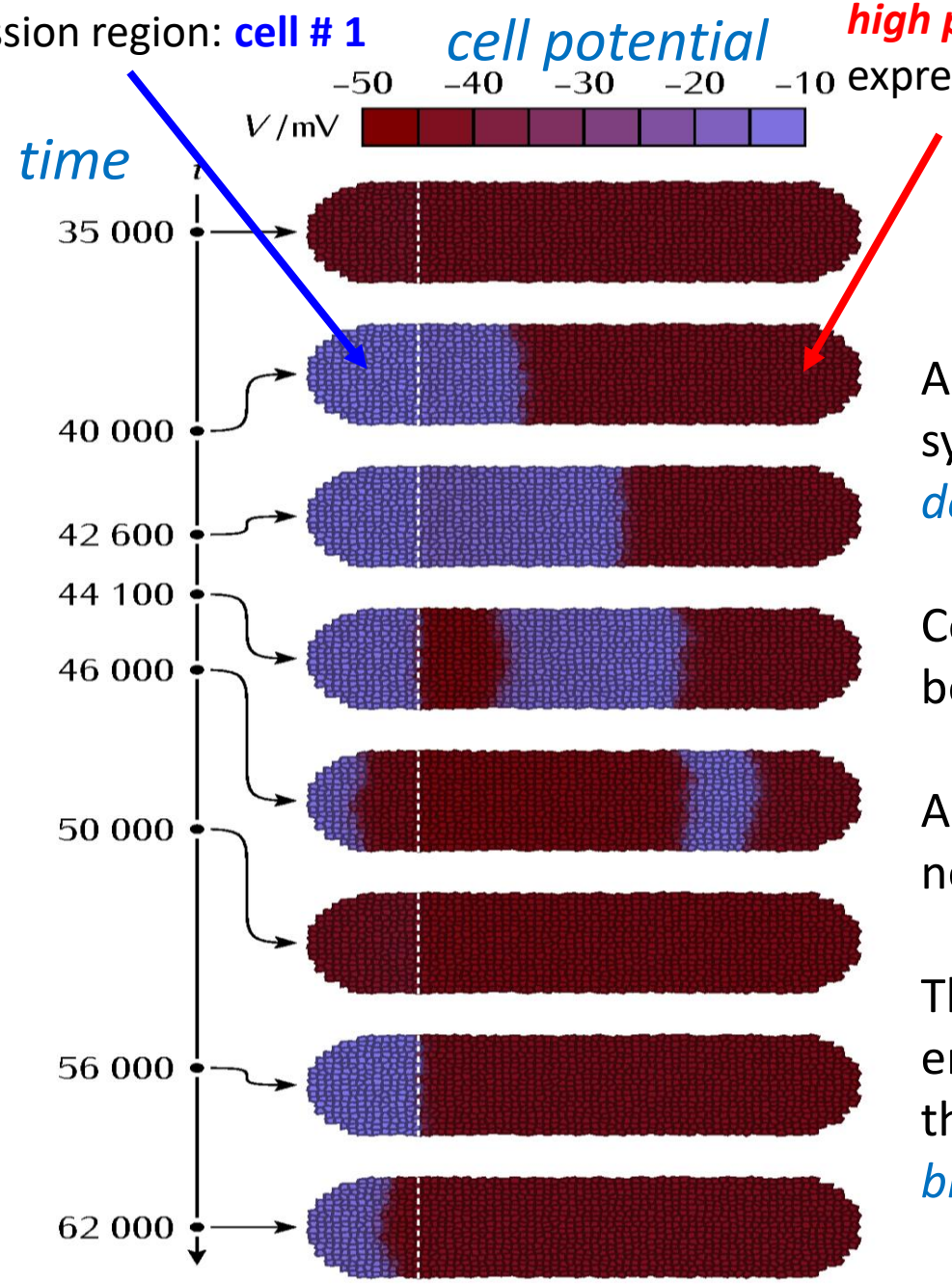
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Commun. Biol. 2024
10.1038/s42003-024-06037-4

Synchronization phenomena: the modulation of the *junction conductance* can establish *regions* with the same *oscillatory phase* of *bioelectricity* and *transcription* (*left*).

A *biochemical* reminiscence?: when the tissue is dissociated, cells stop oscillating, but coordinated oscillations arise by re-aggregation (*bottom*).



Comp. Biol. Med. 2024
10.1016/j.compbiomed.2024.108964



symmetry breaking amplified: bioelectrical-transcriptional waves

A *depolarization wave* propagates across the multicellular system evidenced by the *spatio-temporal bands* of alternate *dep* and *pol* regions.

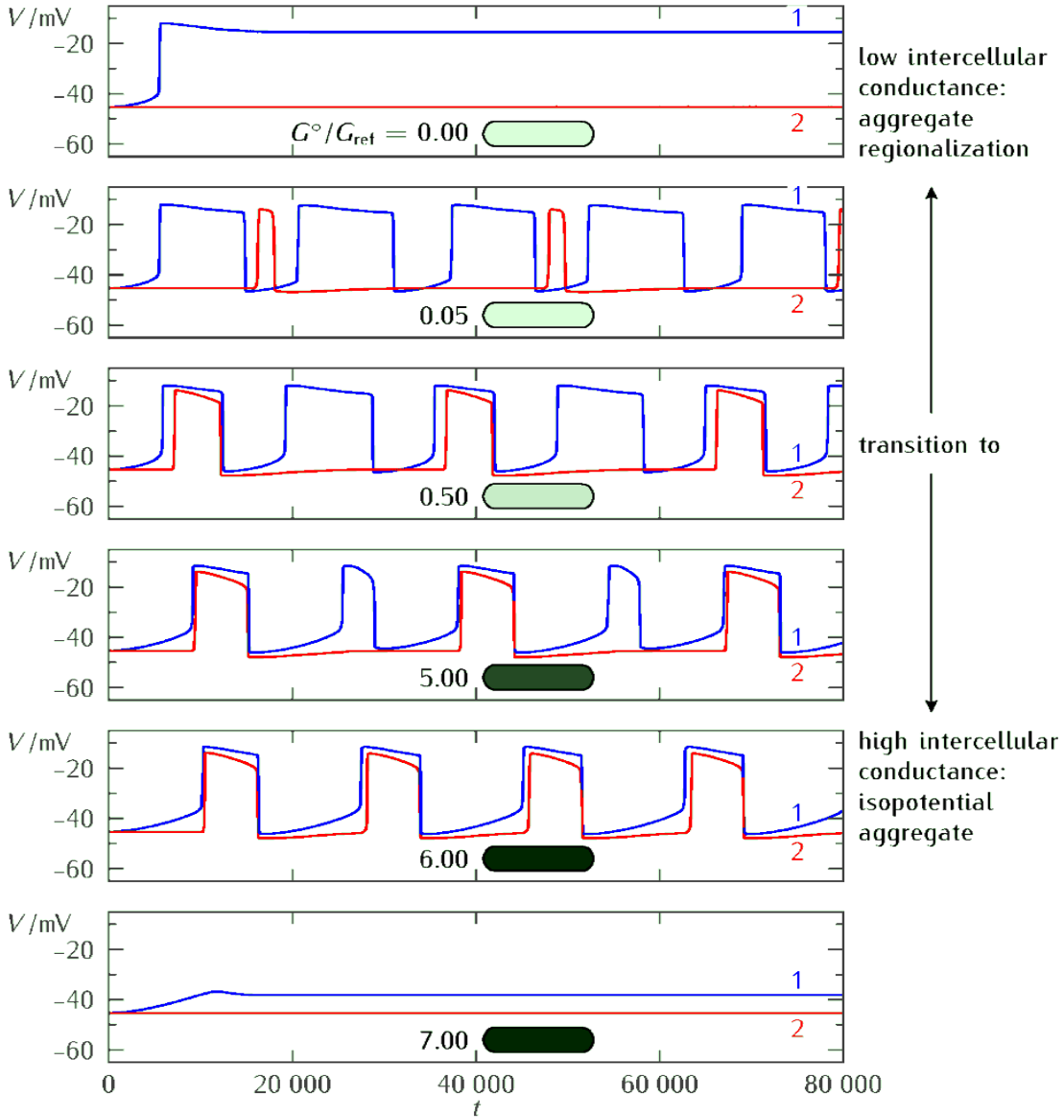
Could the coexistence of *phase* and *antiphase regions* regions be *developmentally* relevant?

A *polarized central region* between two depolarized neighboring regions can *arise* and *expand* at long times.

The *coupling* between *bioelectricity* and *transcription* can encode *spatio-temporal information* (e.g. segmentation) through the *response* of *multicellular potentials* to a *symmetry-breaking* (the gradient of *pol* channel expression here).

transitions between bioelectrical states regulated by intercellular gap junctions

Comp. Biol. Med. 2024
10.1016/j.compbiomed.2024.108964



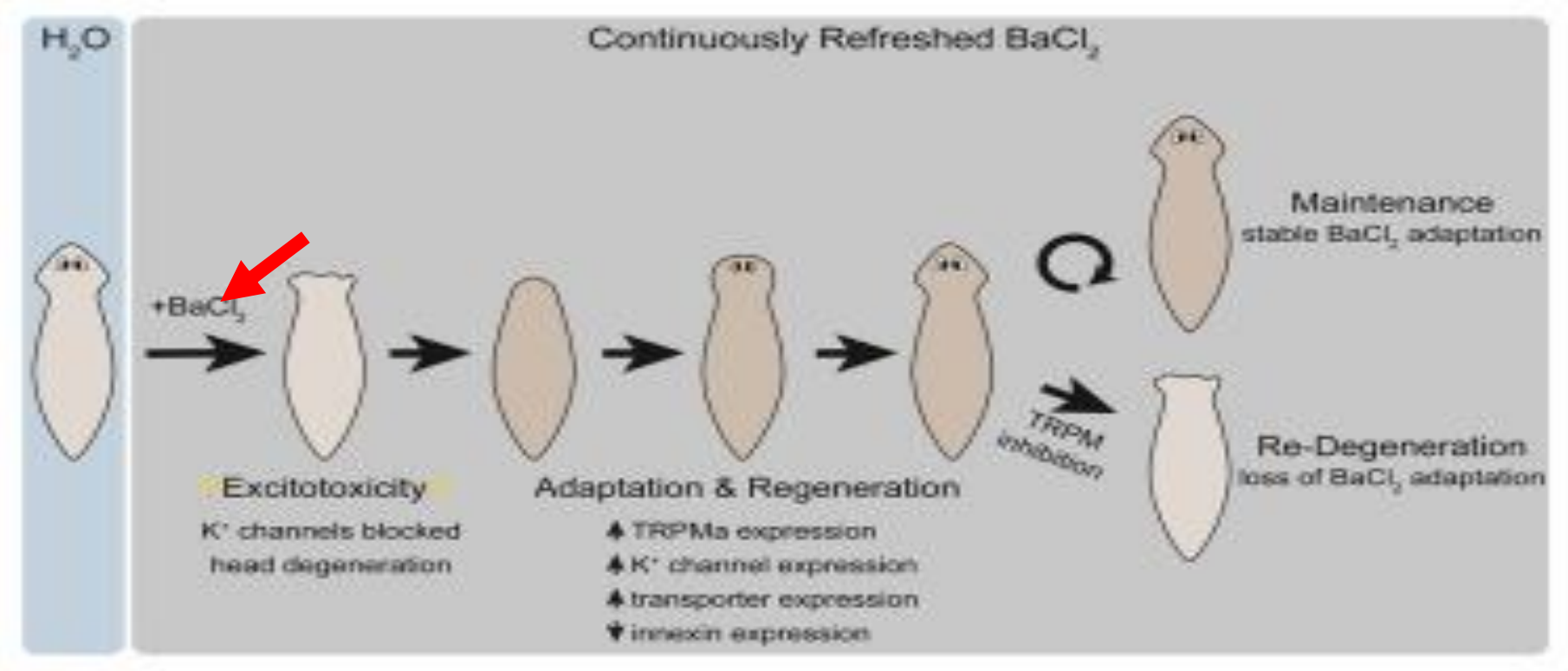
At *zero connectivity* (*top*), the *depolarized* (cell # 1, *left* region) and *polarized* (cell # 2, *right* region) are *independent* and show different potentials. *Trivial two-region encoding*.

Intermediate connectivity couples the *left* and *right* regions in *phase* and *antiphase* oscillations, leading to a rich dynamics and the eventual *synchronization* of *distant* cell potentials: a *developmentally-relevant* case?

At *high connectivity* (*bottom*), an homogenous *iso-potential* system is obtained: *nothing can be encoded*.

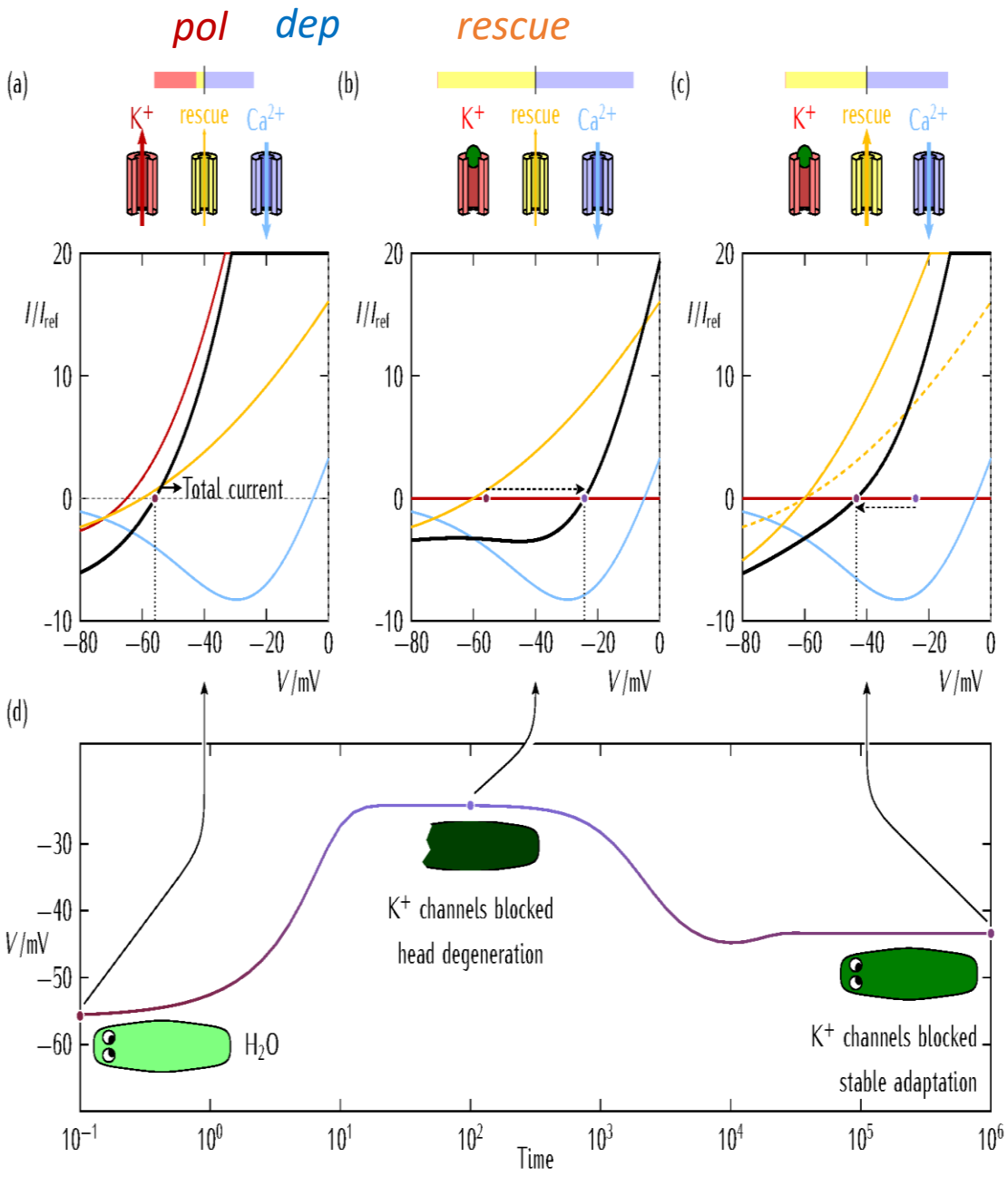
iScience 2019
10.1016/j.isci.2019.11.014

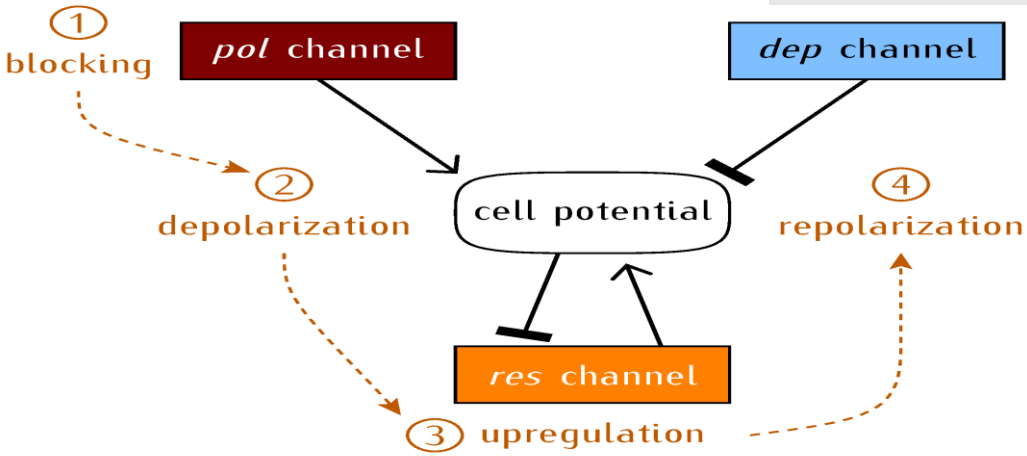
planarian regeneration as a model for acquired tolerance to toxic environments



Exposure to barium chloride ($BaCl_2$) provokes a rapid degeneration of anterior tissue in *Dugesia japonica*. However, continued exposure to fresh solution of $BaCl_2$ results in regeneration of heads that are insensitive to $BaCl_2$. A model of *adaptation to toxicity* shows the central role of *ion channels* in *adaptive plasticity*, which may provide a target for *biomedical* strategies.

Sci. Rep. 2024
10.1038/s41598-024-79087-7





Bioelectrical model: ion channels and *cell polarization*

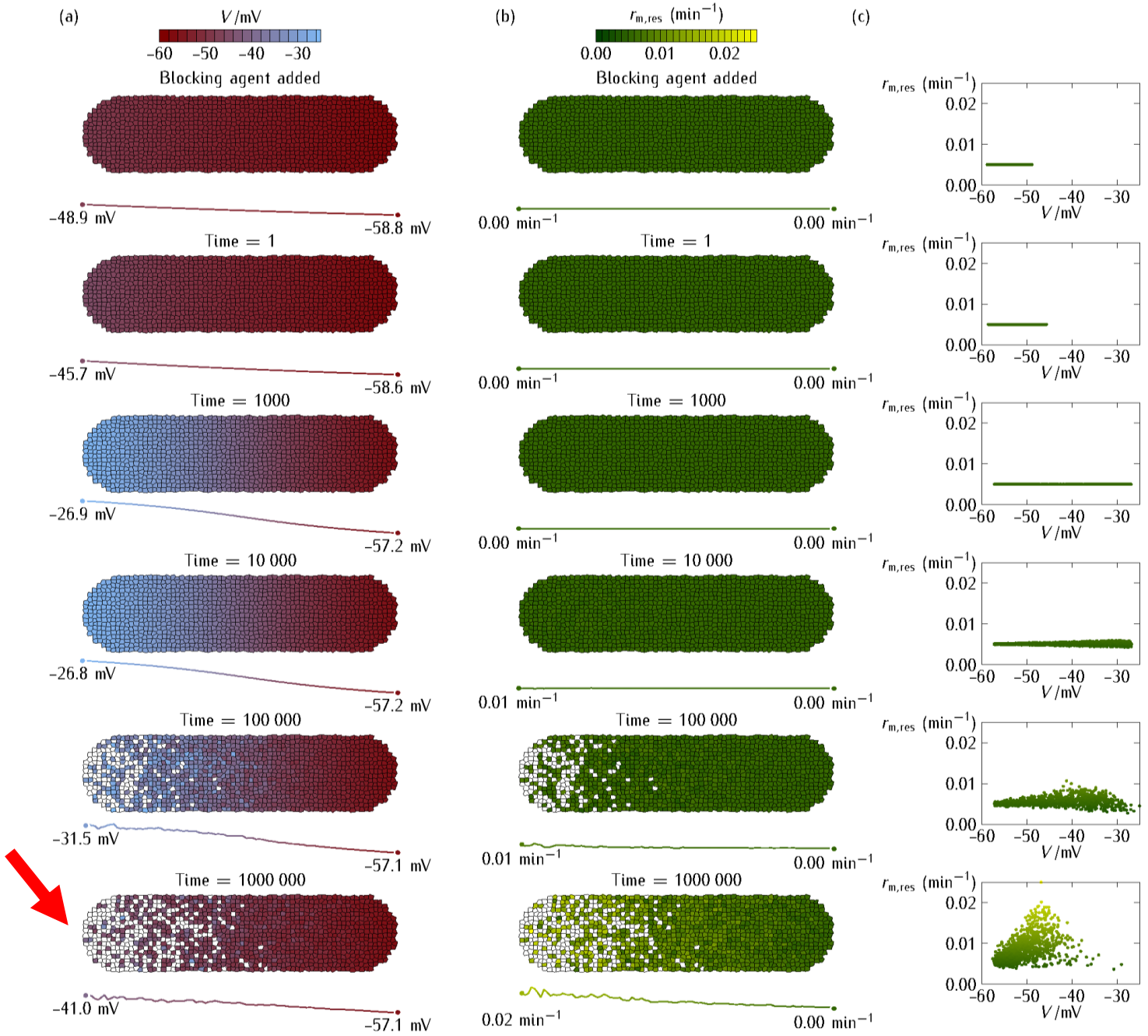
(a) The *I-V* curves of two opposing channels show the outward (*red*) and inward (*blue*) currents by the potassium and calcium channels before blocking.

(b) When the potassium channel is blocked, the depolarized cell potential marks the onset of the outward *rescue* channel.

(c) Cell physiological *repolarization* can eventually be achieved by the *increased expression* of the *compensatory rescue* channel.

(d) Time trace of the head cell potential.

Sci. Rep. 2024
10.1038/s41598-024-79087-7

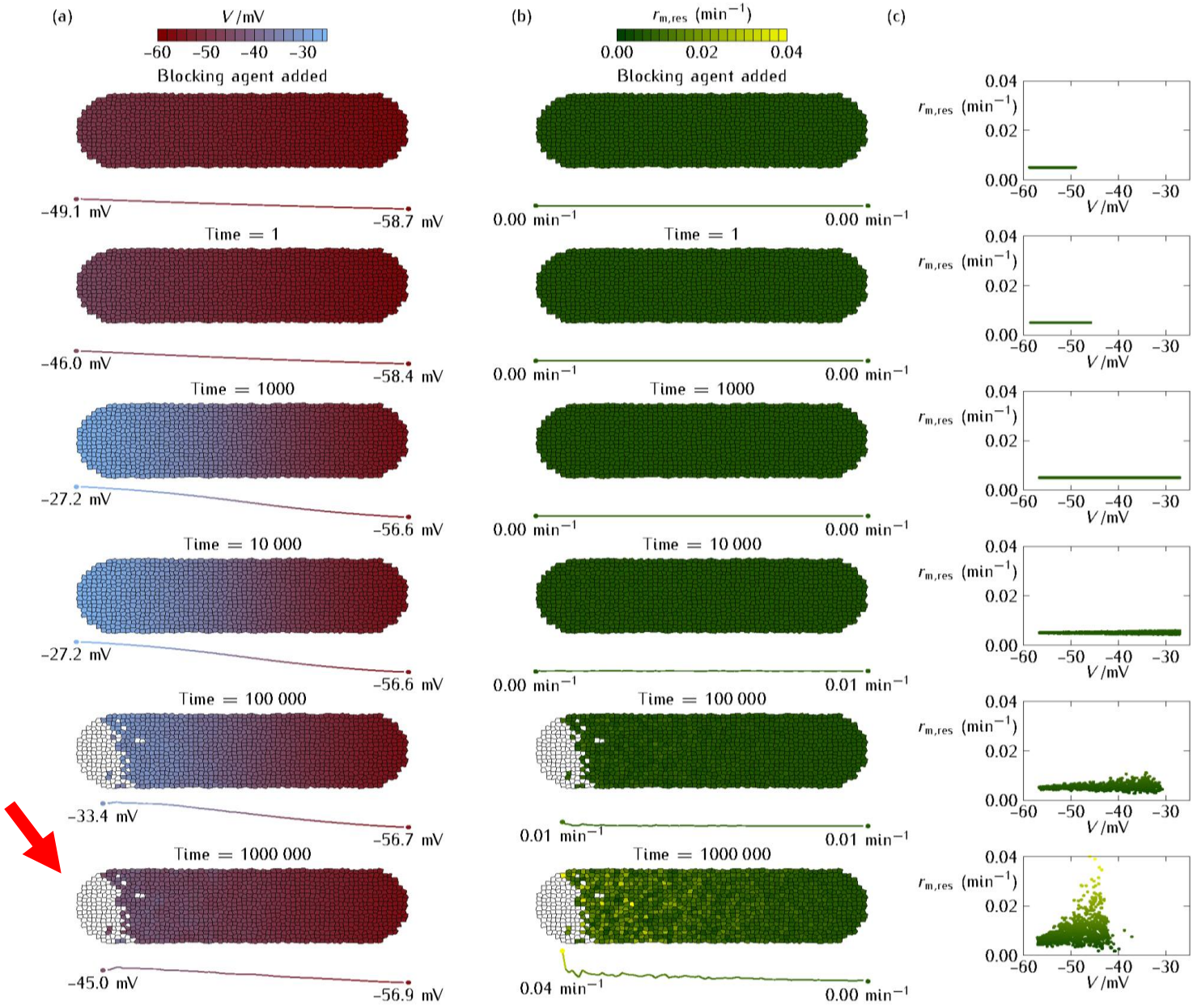


The single-cell state noisy updating can be *slightly biased* to upregulate the *res* channel that acts to reestablish cell polarization.

However, model simulations of the aggregate at *zero* junction conductance (isolated cells, *no* intercellular connectivity) show the *limited compensatory effect* of the *res* channel: note the significant *extension* of *dead* (white) *cells* in the head.

(a) Cell potentials *V*.
(b) Cell rate constants of the upregulated *res* channel.
(c) Rate vs. *V* plot for the living cells (points).

Sci. Rep. 2024
10.1038/s41598-024-79087-7



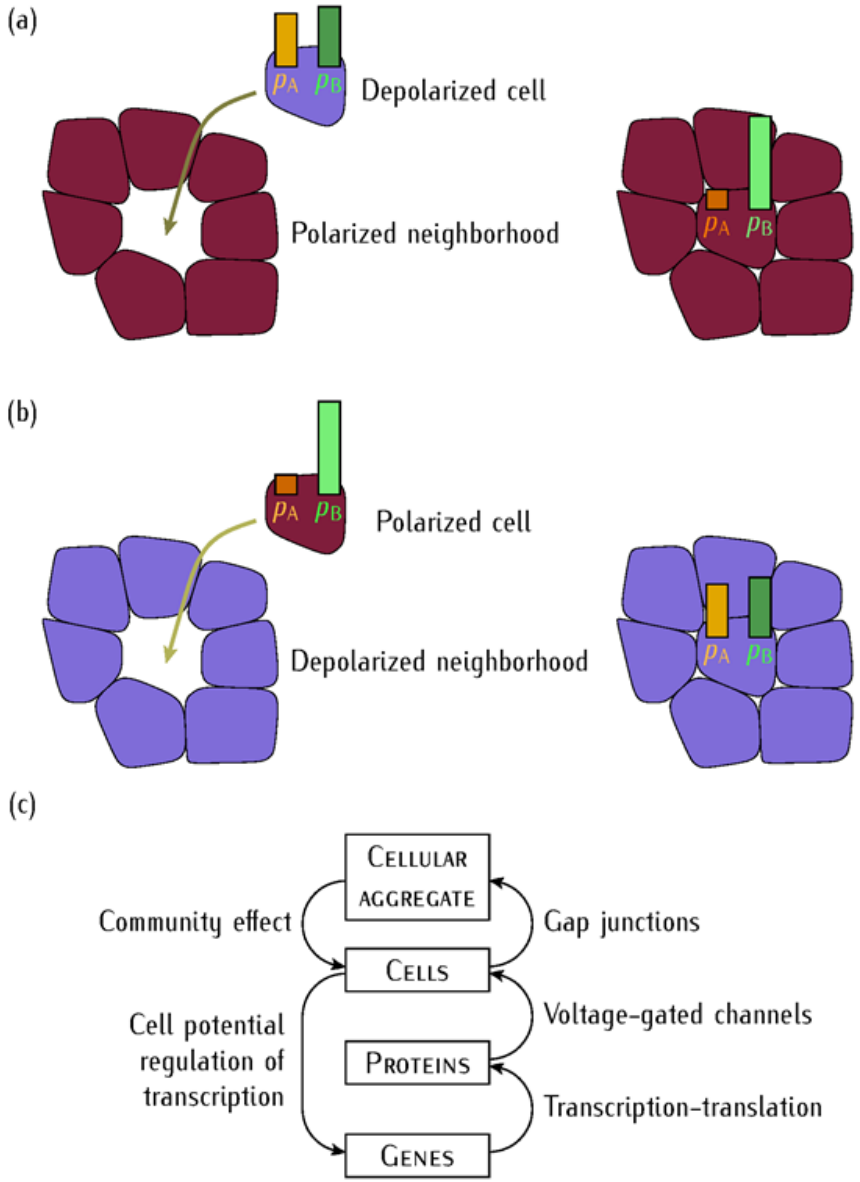
The model simulations of the multicellular aggregate at *non-zero* junction conductance (intercellular connectivity) show the significant *compensatory effect* of the *res* channel: note the *limited extension* of *dead* (white) *cells* in the head.

The result suggests a *collective* contribution to the *adaptive response* that may contribute to the bioelectrical *pattern completion* (head re-establishment here).

(a) Cell potentials *V*.
(b) Cell rate constants of the upregulated *res* channel.
(c) Rate vs. *V* plot for the living cells (points).

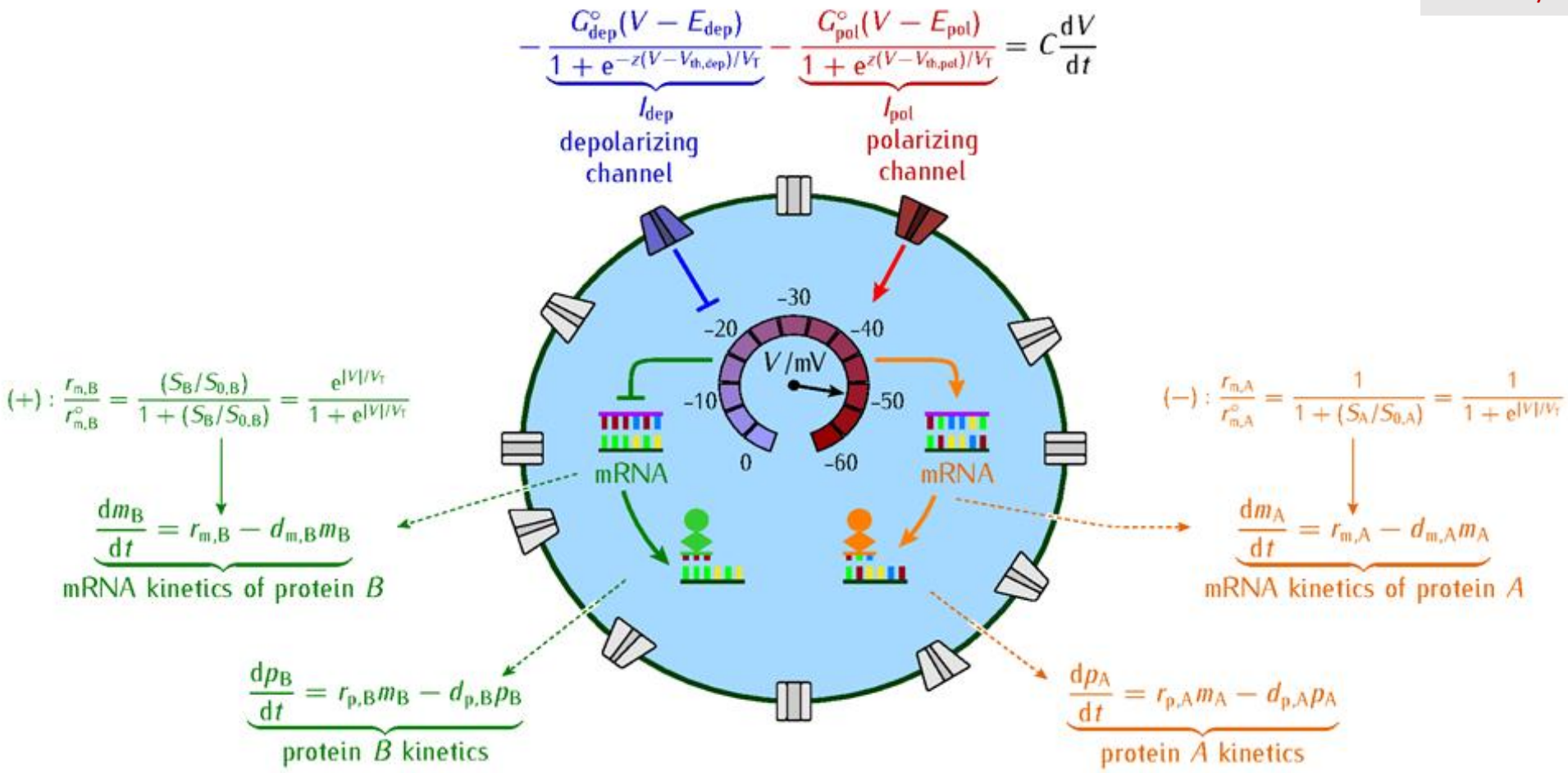
top-down effects on cell membrane potential and protein transcription

Sci. Rep. 2025
10.1038/s41598-025-31696-6



(a) A multicellular aggregate with a *central* and *n neighboring cells*. Intially, t = 0 s, the central cell is in a *depolarized* (*blue*) state characterized by a low absolute value of the (*negative*) membrane potential V while the neighboring cells are in *polarized* (*red*) states. At $t > 0$, the *intercellular connectivity* is established (*arrow*), allowing the polarized neighboring cells to repolarize the central cell. The expressions of *two proteins* A and B are coupled through the cell potential V. Because of the different regulations assumed for the *V*-dependent transcriptional rates of proteins A and B, the initially similar expression levels and concentrations p_A and p_B can become distinct after the central cell repolarization. (b) In the *opposite case*, the central cell would be polarized while the neighboring cells are depolarized at time t = 0 s. Now, the initially distinct concentrations of proteins A and B can become similar after the central cell depolarization. (c) The *interplay between the bioelectrical and biochemical networks* is described using the *top-down* and *bottom-up* mechanisms shown in the scheme.

Sci. Rep. 2025
10.1038/s41598-025-31696-6

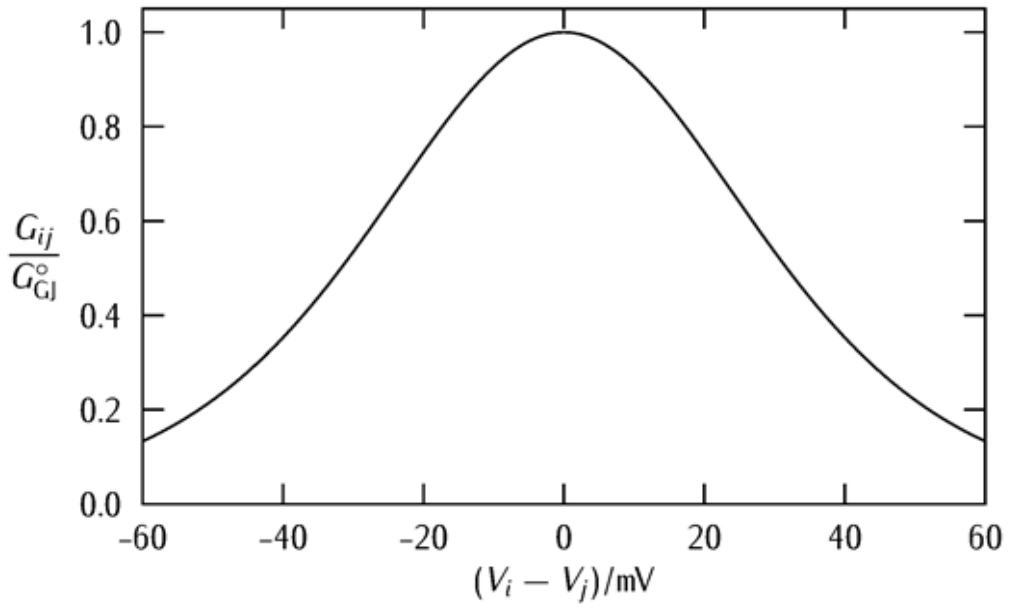


Bioelectrical and transcriptional feedback for *two proteins A and B* whose transcriptions are modulated by the cell potential *V*, regulated in turn by two *dep* and *pol* channel conductances. Proteins A (negative regulation, –) and B (positive regulation, +) have *opposite* dependencies of their *transcription rates on the absolute value of V*.

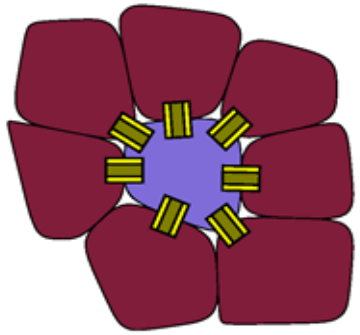
Sci. Rep. 2025
10.1038/s41598-025-31696-6

(a)

$$\frac{G_{ij}}{G_{GJ}^{\circ}} = \frac{2}{1+\cosh[(V_i - V_j)/V_0]}$$



(b)

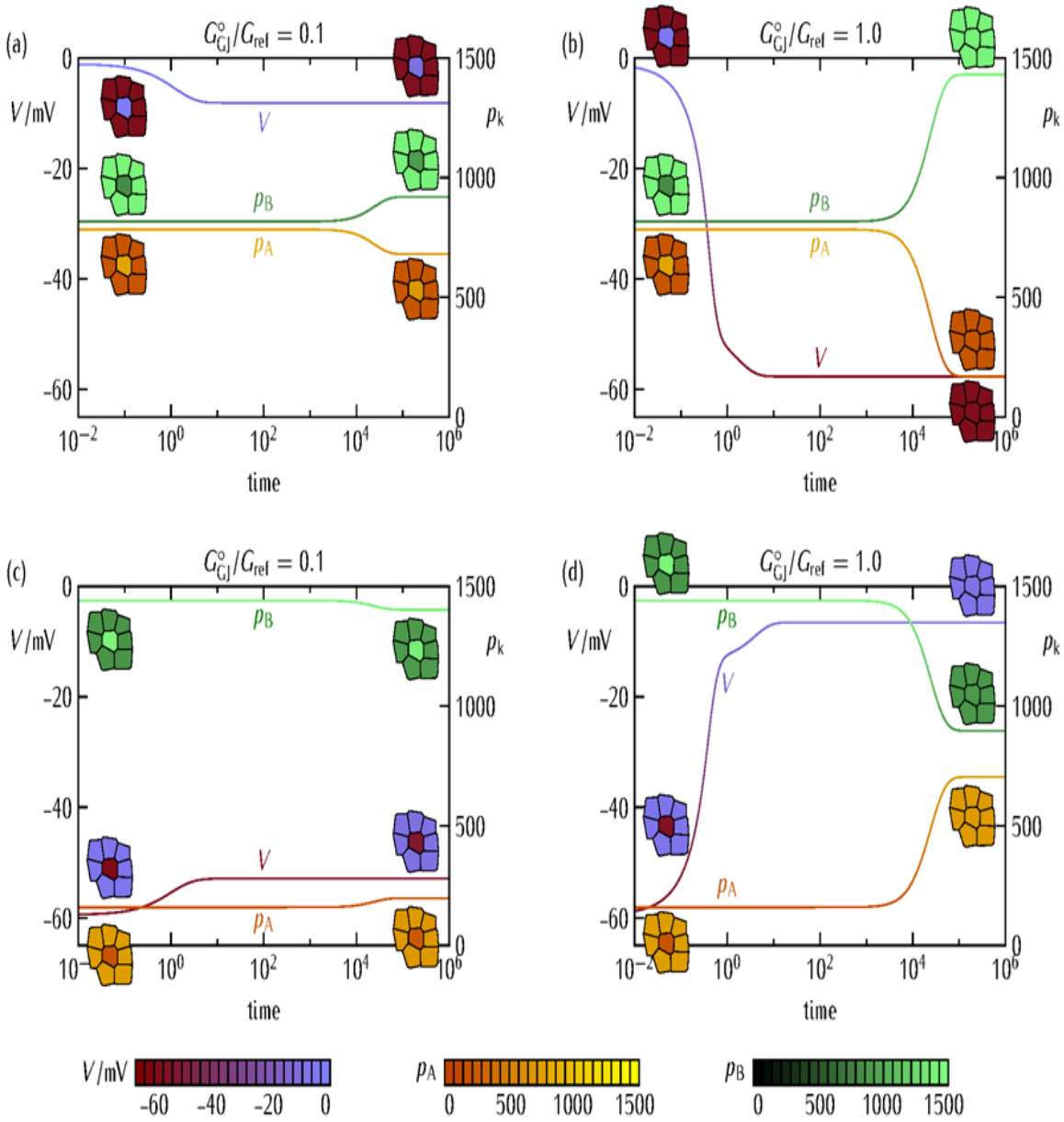


$$C\frac{dV_i}{dt} = -I_{pol} - I_{dep} + \sum_{j \in nn} G_{ij}(V_j - V_i)$$

(a) The conductance G_{ij} of the *intercellular junction between two neighboring cells i and j* as a function of the cell potentials difference $V_i - V_j$. The reference potential is V_0 and G_{GJ}^{o} is the maximum conductance.

(b) The potential V_i of the central cell changes because of the channel currents I_{dep} and I_{pol} and the sum of the intercellular currents between cell *i* and its nearest neighboring (nn) cells *j* *bioelectrically connected* to cell i through *gap junctions.*

Sci. Rep. 2025
10.1038/s41598-025-31696-6



(a) Time-dependent central cell potential V and protein concentrations p_k (k = A, B) in the initially *depolarized central cell* for $n = 7$ *polarized neighboring cells* and *low* intercellular conductance ratio (*weak* connectivity, *top*). (b) The case of (a) but now with *strong* connectivity. (c) Time-dependent cell potential and concentrations of proteins A and B in the initially *polarized central* cell for $n = 7$ *depolarized neighboring cells* and *weak* connectivity. (d) The case of (c) but now with *strong* connectivity. The scales (*bottom*) correspond to the central and neighboring cells potentials and the protein concentrations, which are relative numbers that depend on the system considered, in the central cell. The *insets* show the *cell states initially* and at *steady state*. The time units are those of the cell potential relaxation time (0.1 – 1 s), so that the protein concentration relaxation times would be in the delayed range 1 h – 1 day.

Sci. Rep. 2025
10.1038/s41598-025-31696-6

The above *top-down perspective* for the *multicellular interplay* between *bioelectricity* and *protein transcription* is *complementary* to the usual *bottom-up description*. The non-excitable cell bioelectrical representation of the external environment, including the neighboring cells, is based on voltage-gated ion channels and intercellular junctions and makes *three predictions*:

(i) *shifts in membrane potential V* allow *transitions between gene expression states;*
(ii) *depolarized cells* cannot *control gene expression* as effectively as polarized cells; and
(iii) *community effects* permit to extend the *single-cell control* to the *multicellular level*.

Because the spatio-temporal distributions of instructive signaling ions and molecules depend on the local electric potentials, *different multicellular potentials* correlate with *distinct downstream gene expression patterns*.

Because a central cell is able measure the number of neighboring cells and learn their bioelectrical state from the downward-induced membrane potential changes, *multiscale bioelectricity* can operate as a *top-down mechanism* in *development and regeneration*.

other bioelectrical models: BETSE simulator (Alexis Pietak)

Front. Bioeng. Biotechnol. 2016
10.3389/fbioe.2016.00055
J. R. Soc. Interface 2017
10.1098/rsif.2017.0425

frontiers in Bioengineering and Biotechnology

ORIGINAL RESEARCH
published: 06 July 2016
doi: 10.3389/fbioe.2016.00055

Exploring Instructive Physiological Signaling with the Bioelectric Tissue Simulation Engine

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Bioelectric cell properties have been revealed as powerful targets for modulating stem cell function, regenerative response, developmental patterning, and tumor reprograming. Spatio-temporal distributions of endogenous resting potential, ion flows, and electric fields are influenced not only by the genome and external signals but also by their own intrinsic dynamics. Ion channels and electrical synapses (gap junctions) both determine, and are themselves gated by, cellular resting potential. Thus, the origin and progression of bioelectric patterns in multicellular tissues is complex, which hampers the rational control of voltage distributions for biomedical interventions. To improve understanding of these dynamics and facilitate the development of bioelectric pattern control strategies, we developed the BioElectric Tissue Simulation Engine (BETSE), a finite volume method multiphysics simulator, which predicts bioelectric patterns and their spatio-temporal dynamics by modeling ion channel and gap junction activity and tracking changes to the fundamental property of ion concentration. We validate performance of the simulator by matching experimentally obtained data on membrane permeability, ion concentration and resting potential to simulated values, and by demonstrating the expected outcomes for a range of well-known cases, such as predicting the correct transmembrane voltage changes for perturbation of single cell membrane states and environmental ion concentrations, in addition to the development of realistic transepithelial potentials and bioelectric wounding signals. *In silico* experiments reveal factors influencing transmembrane potential are significantly different in gap junction-networked cell clusters with tight junctions, and identify non-linear feedback mechanisms capable of generating strong, emergent, cluster-wide resting potential gradients. The BETSE platform will enable a deep understanding of local and long-range bioelectrical dynamics in tissues, and assist the development of specific interventions to achieve greater control of pattern during morphogenesis and remodeling.

Keywords: bioelectric simulation, pattern formation, resting potential, transmembrane voltage

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1. INTRODUCTION

1.1. Bioelectricity: Why Model Electrical Activity in Non-Neural Cells?

Explaining and learning to control large-scale pattern is a central unsolved problem, with implications for mitigation of birth defects, and the advancement of regenerative medicine and synthetic bioengineering. The dynamics of signals orchestrating large-scale order *in vivo* are a key area of

INTERFACE

rsif.royalsocietypublishing.org

Research

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Subject Areas:
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THE ROYAL SOCIETY PUBLISHING

Bioelectric gene and reaction networks: computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation

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ML, 0000-0001-7292-8084

Gene regulatory networks (GRNs) describe interactions between gene products and transcription factors that control gene expression. In combination with reaction–diffusion models, GRNs have enhanced comprehension of biological pattern formation. However, although it is well known that biological systems exploit an interplay of genetic and physical mechanisms, instructive factors such as transmembrane potential (V_{mem}) have not been integrated into full GRN models. Here we extend regulatory networks to include bioelectric signalling, developing a novel synthesis: the bioelectricity-integrated gene and reaction (BIGR) network. Using *in silico* simulations, we highlight the capacity for V_{mem} to alter steady-state concentrations of key signalling molecules inside and out of cells. We characterize fundamental feedbacks where V_{mem} both controls, and is in turn regulated by, biochemical signals and thereby demonstrate V_{mem} homeostatic control, V_{mem} memory and V_{mem} controlled state switching. BIGR networks demonstrating hysteresis are identified as a mechanisms through which more complex patterns of stable V_{mem} spots and stripes, along with correlated concentration patterns, can spontaneously emerge. As further proof of principle, we present and analyse a BIGR network model that mechanistically explains key aspects of the remarkable regenerative powers of creatures such as planarian flatworms. The functional properties of BIGR networks generate the first testable, quantitative hypotheses for biophysical mechanisms underlying the stability and adaptive regulation of anatomical bioelectric pattern.

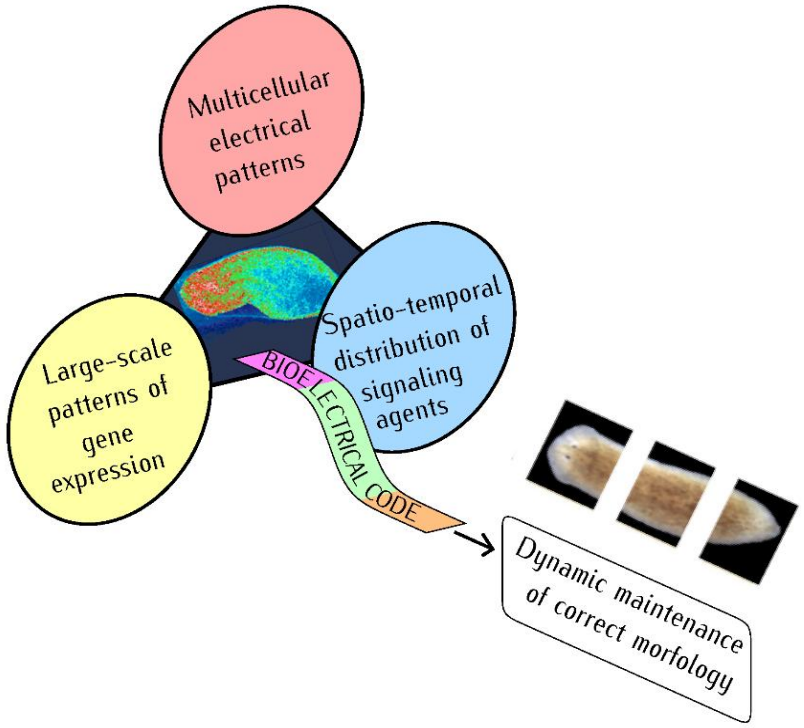
1. Introduction

Large-scale biological patterning in development, regeneration and disease remains among the most fundamental and important questions facing modern biology. Metazoan organisms reliably self-assemble a complex body plan from a single fertilized egg cell; furthermore, many animals, such as salamanders and planaria, are able to repair or remodel their bodies back to the correct shape despite injury and other types of drastic perturbation such as limb amputation [1,2]. Understanding the mechanisms that control the formation and regulation of organism-scale biological patterns may allow us to mitigate birth defects, implement organ regeneration strategies and to prevent, heal or even reprogramme the cancer state [3]. It is crucial to begin to understand and exploit the multicellular algorithms and dynamics that control anatomy and its remodelling, in addition to the details of subcellular signalling pathways.

Biological pattern formation is highly complex, involving numerous biomolecular mechanisms that lead to formation of instructive chemical patterns in a tissue collective, as well as mechanical considerations concerning shape changes and movements of individual cells and the tissue substratum as a whole. From the chemical patterning perspective pioneered by Turing [4] and Wolpert [5] (among others [6–8]), individual cells produce a variety of substances which may: (i) have the capacity to influence the production of other substances via genetic expression

6. Where we go: identifying key bioelectrical steps in biological complexity

6.a Bioelectrical limitations



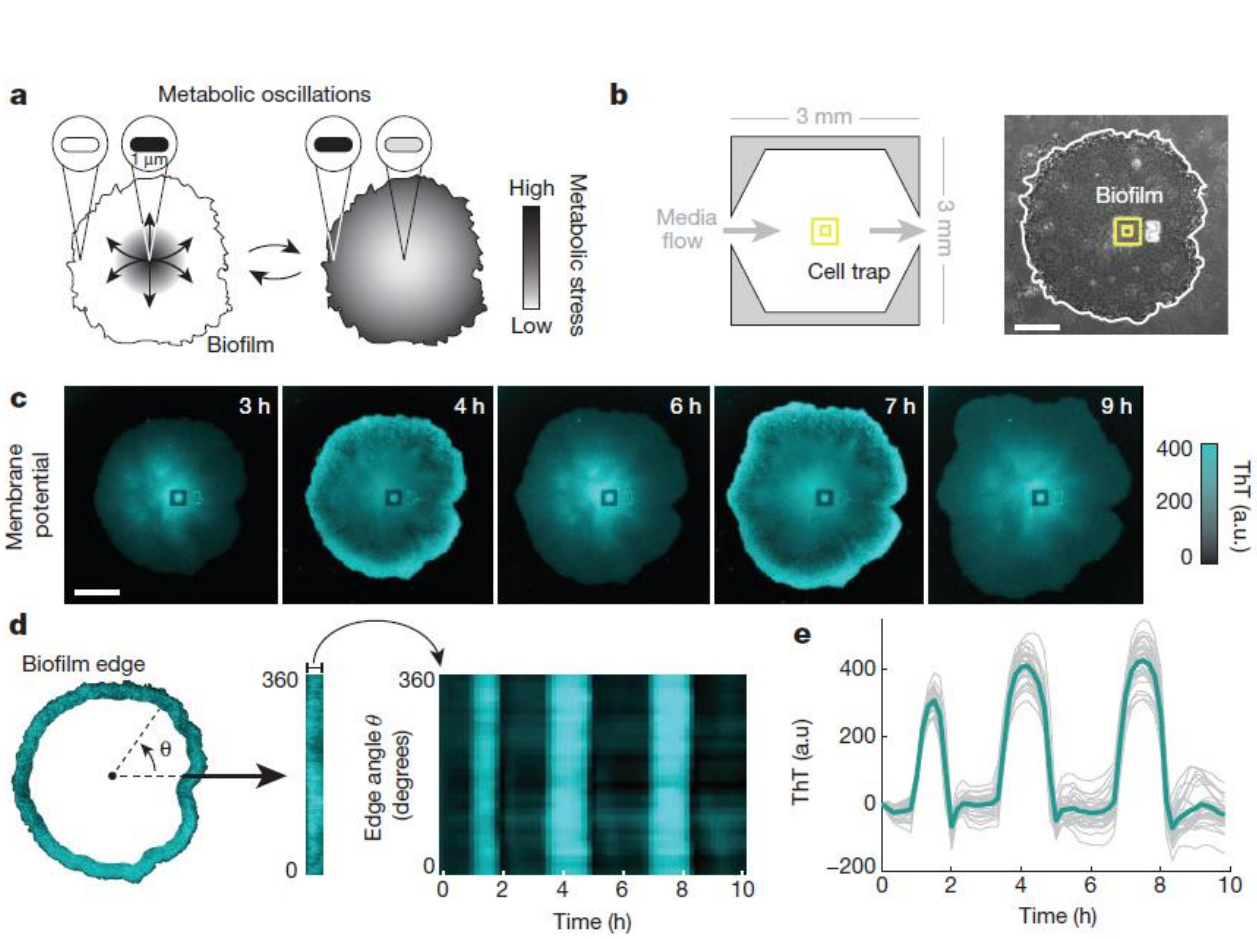
The model simulations explore the *interplay* between *transcription* and *bioelectricity*, modulated by the *single-cell states* and the *intercellular coupling*, in the establishment of *instructive* spatio-temporal patterns at the *multicellular level*. Could *perturbative* changes be *suppressed* or *enhanced* by interrelated *bioelectrical* and *biochemical* processes?

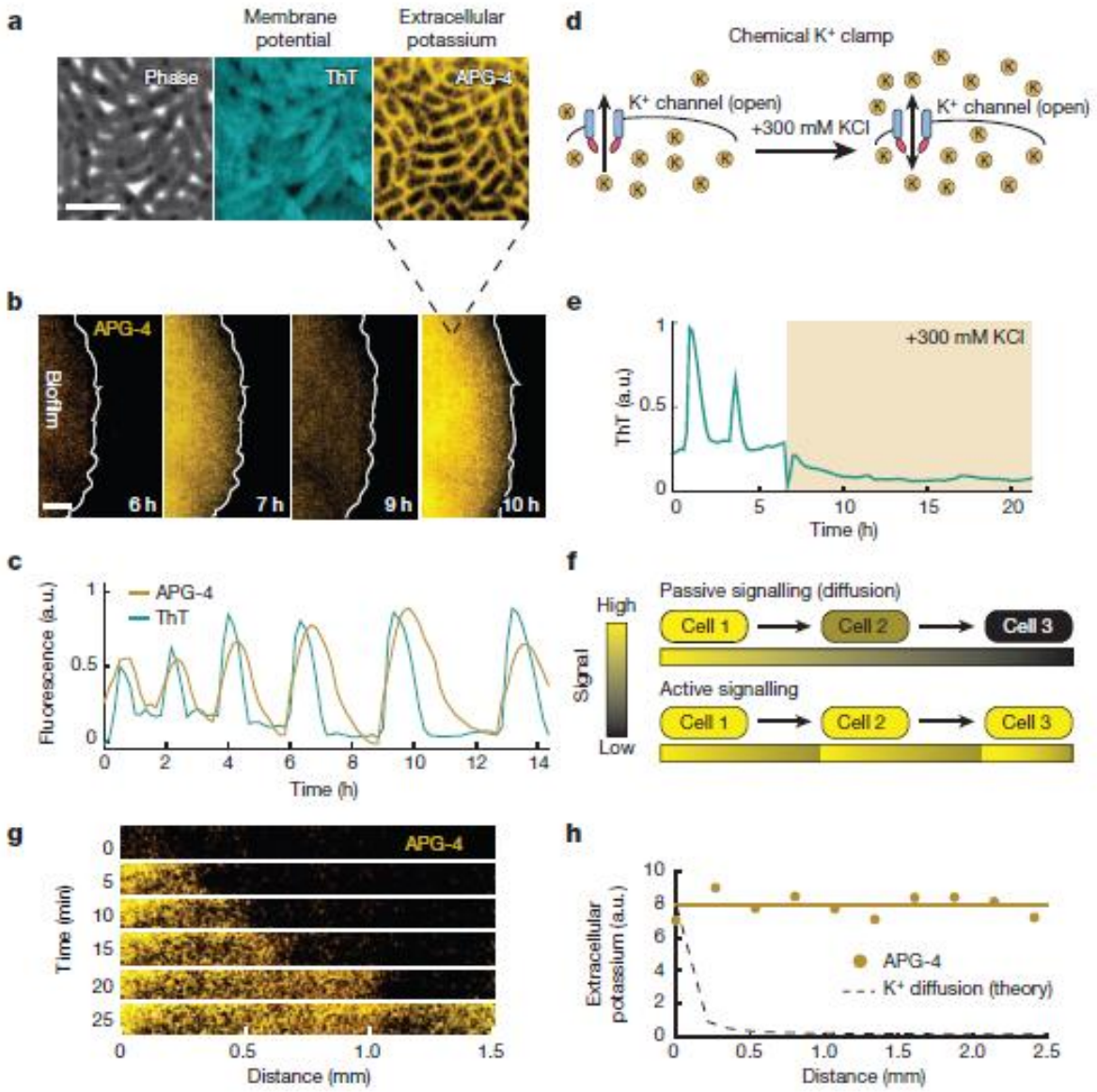
Although the physical model is exceedingly *simple* for quantitative descriptions, it suggests a *rich* phenomenology, based on the *multicellular coupling* between *bioelectricity* and *transcription,* that can be explored further. Also, the core concepts are general enough to permit future *extensions*.

In moving forward, however more *realistic* models must incorporate *additional contributions* to morphogenesis/regeneration, together with the *coordinated integration* of controlling signals. In particular, concerted *bioelectrical-biomechanical-biochemical* actions should act together to modulate expression patterns, as shown in the following cases.

bioelectrical and biochemical coupling in bacterial communities

Nature 2015
10.1038/nature15709

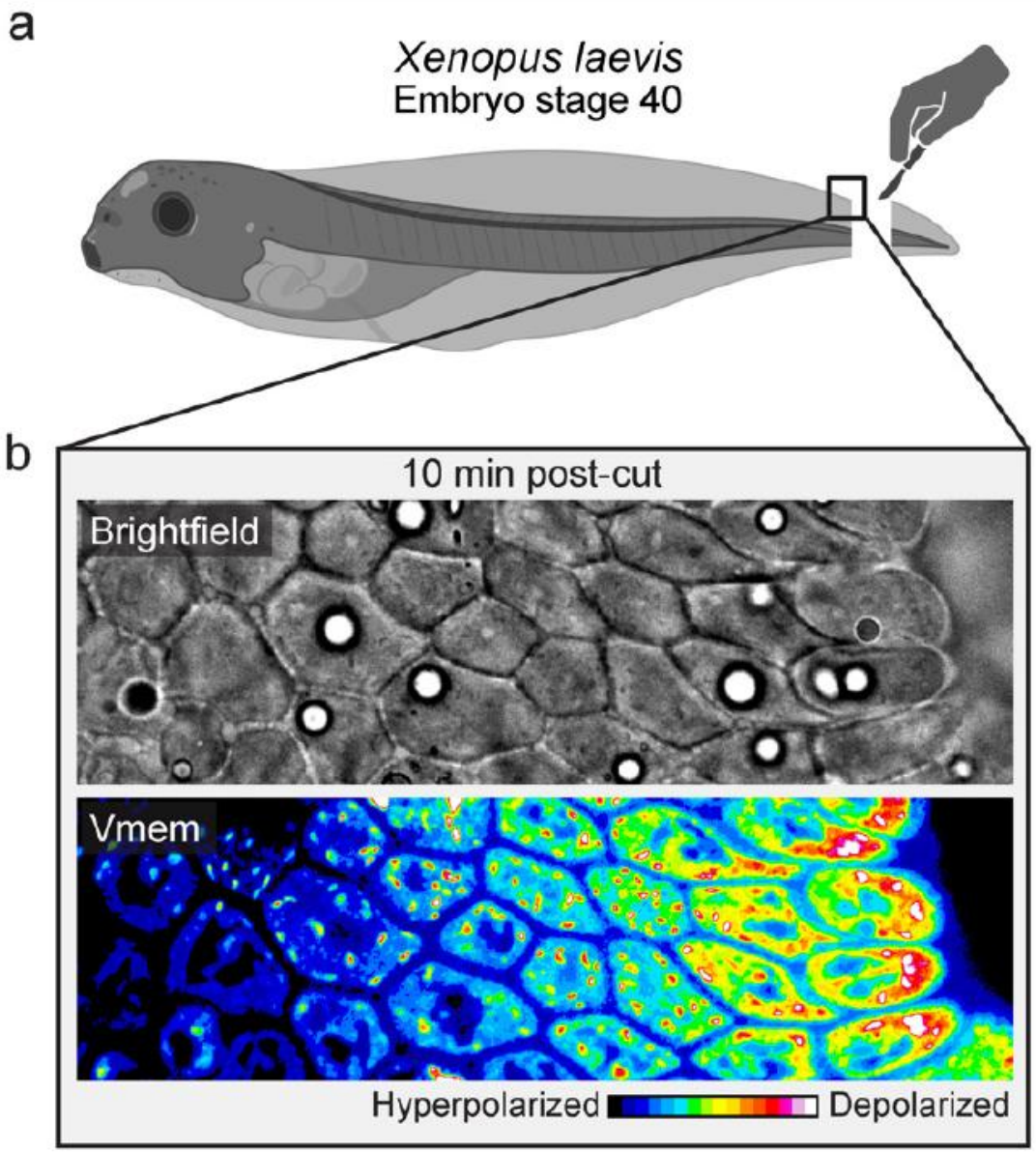




Biofilms produce *synchronized* oscillations in membrane potential. *Ion channels* and *potassium* release are involved in active signal propagation within the biofilm.

membrane potential coupled with cellular mechano-transduction

bioRxiv 2024
10.1101/2023.11.02.565386



The *interplay of membrane potential and cytoplasmic dilution* at the initiation of *Xenopus* tail regeneration after amputation (a) and the membrane potential (DiSBAC2(3) dye) images of the wound edge (b) suggest that cell layers are progressively depolarized, from the deep tissue towards the wound edge.

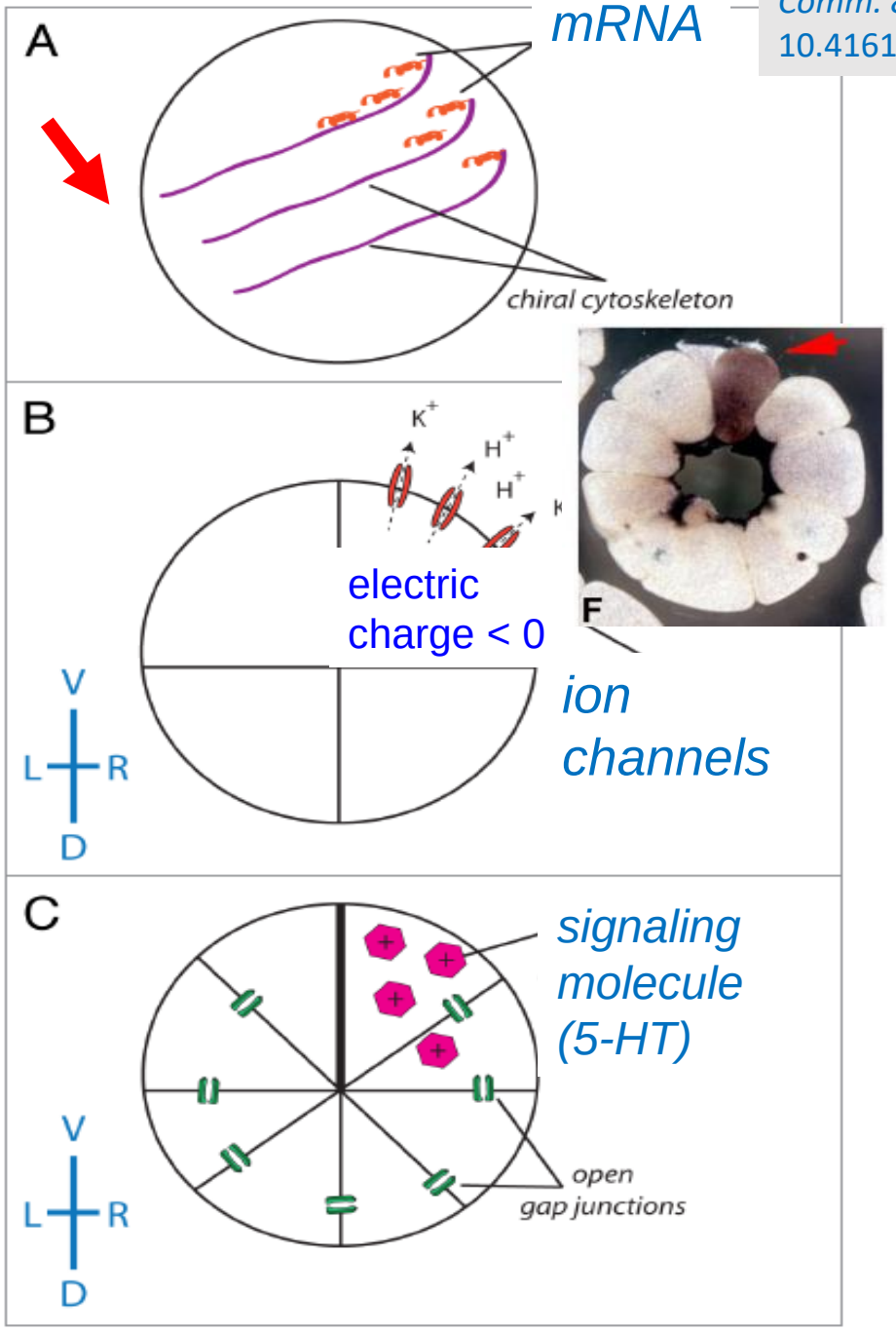
mechano-transduction, bioelectricity and biochemical networks acting together in Xenopus frog model

(A) Cytoskeleton *asymmetrically* distributes *mRNAs* encoding *ion transporters.*

(B) The biased expression gives an *electric potential* difference.

(C) Negative potential in the ventral right blastomere yields a *net flow* of positively charged *signaling molecules* (serotonin).

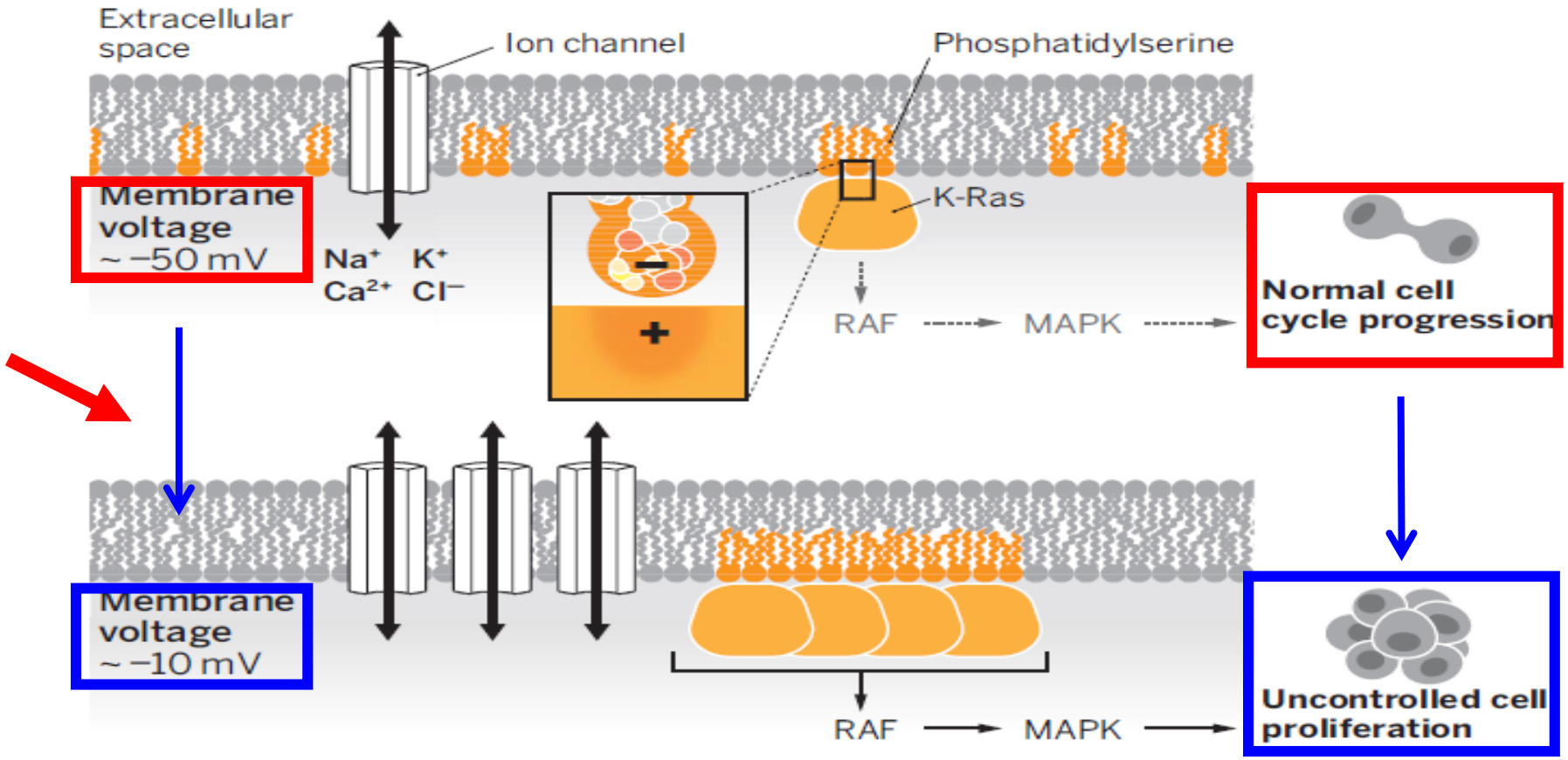
The *electric potential* regulates the spatial distribution of *signaling molecules* and *ions* over multicellular domains but *biomechanical* effects are also present.



Comm. & Integr. Biol. 2016
10.4161/cib.27155

mechano-transduction, bioelectricity, and biochemistry coupled in tumorigenesis

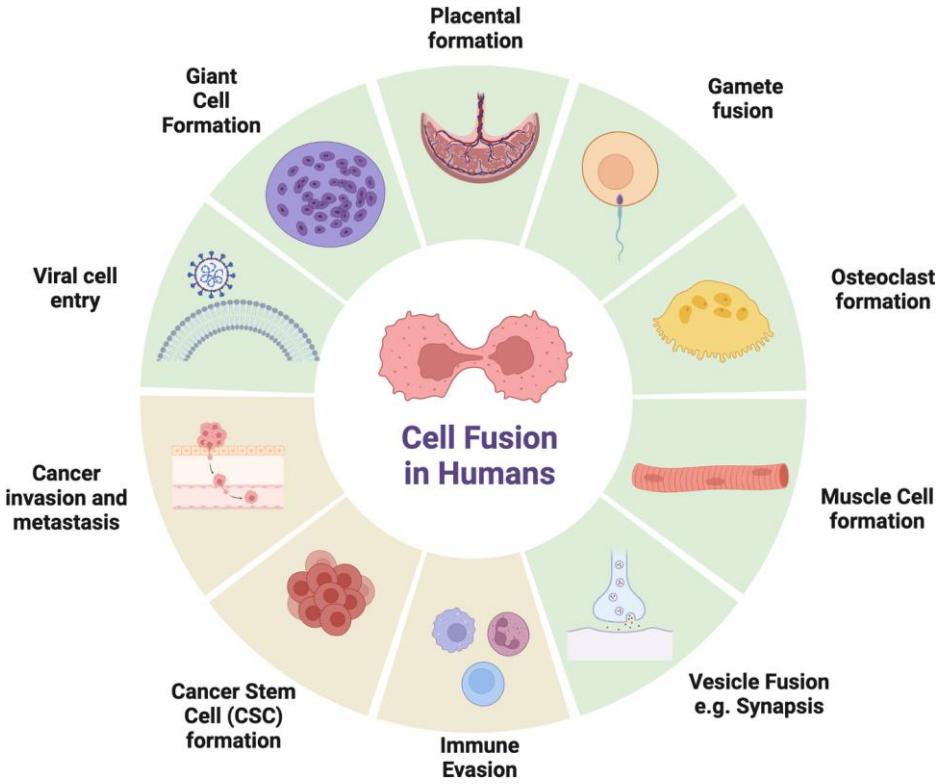
Science 2015
10.1126/science.aaa5619
10.1126/science.aad0874



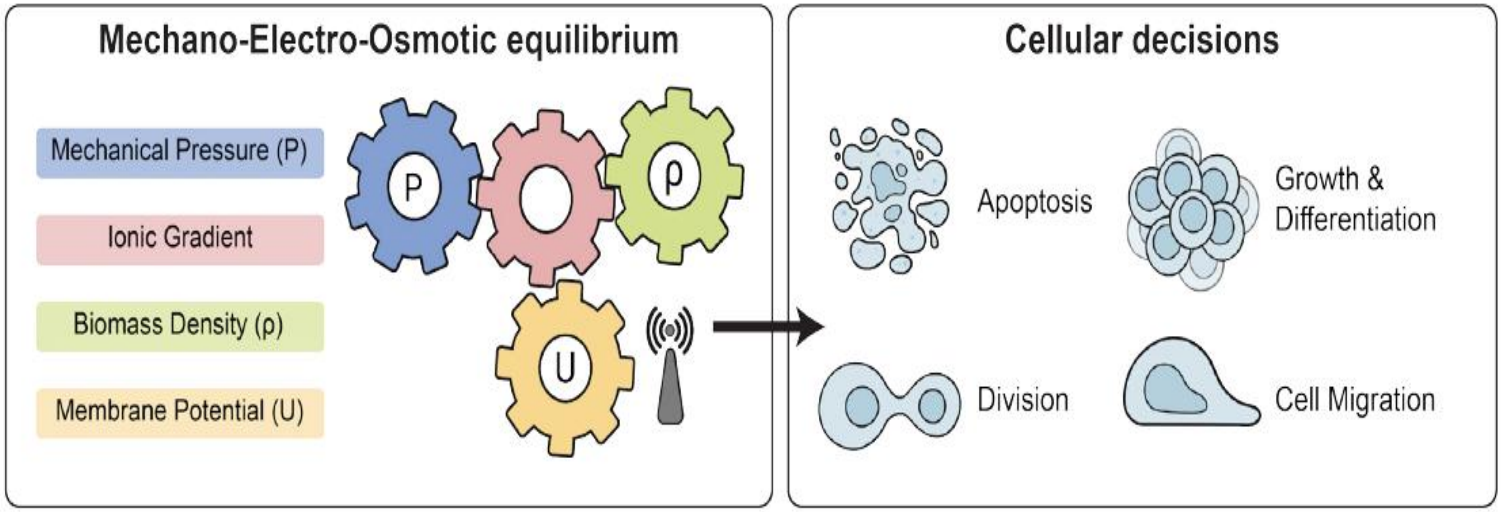
Cell *depolarization*: the membrane as a *biomechanical field-effect transistor.* The control of signaling via specific lipids and proteins suggests *bioelectrical actions*.

However, significant *clinical risks* exist: channels regulate *multiple functions* (e.g. cardiac rhythm), being involved in other cell properties (adhesion, volume regulation, apoptosis).

Int. J. Biochem. Cell Biol. 2024
10.1016/j.biocel.2024.106649
bioRxiv 2024
10.1101/2023.11.02.565386



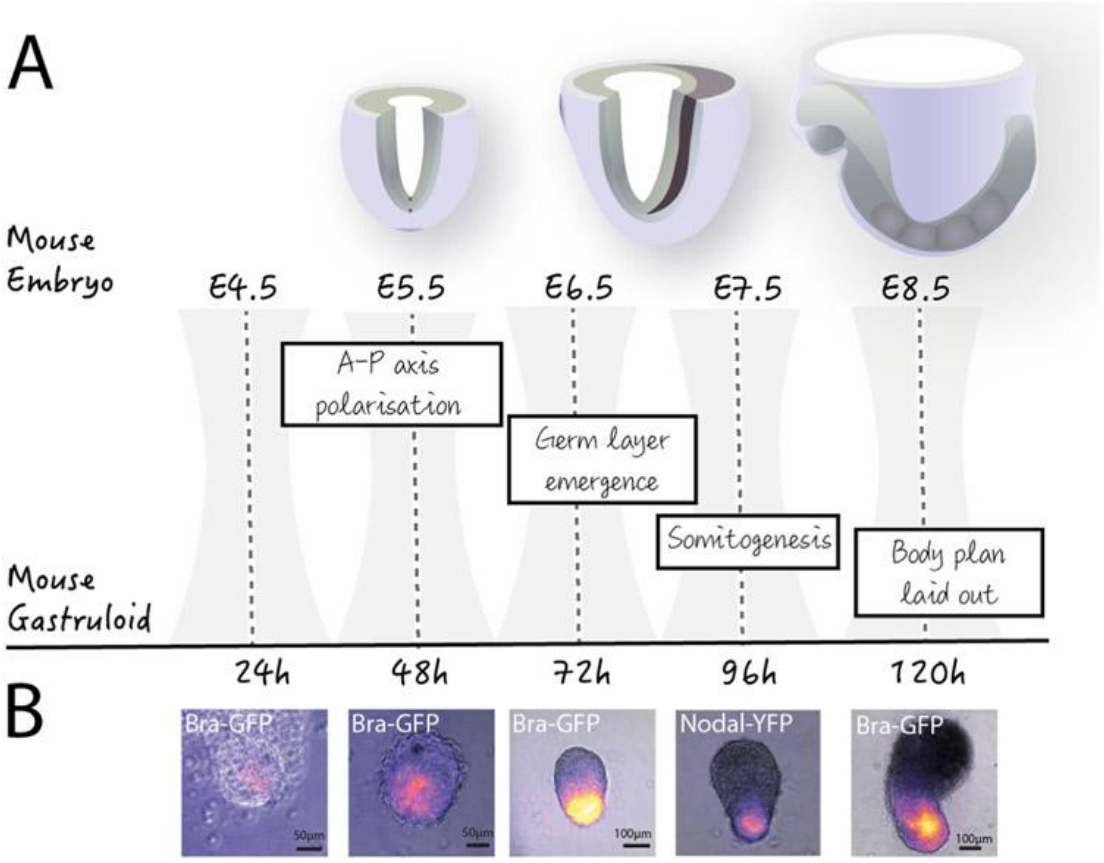
biomechanical pressure, cytoplasmic biomass density and membrane potential



cell-cell fusion: the next cancer hallmark? Healthy tissues (green) and cancerous contexts (yellow)

gastruloid biomechanics: pluripotent stem cell models of mammalian gastrulation and embryo engineering

Dev Biol. 2022
10.1016/j.ydbio.2022.05.002



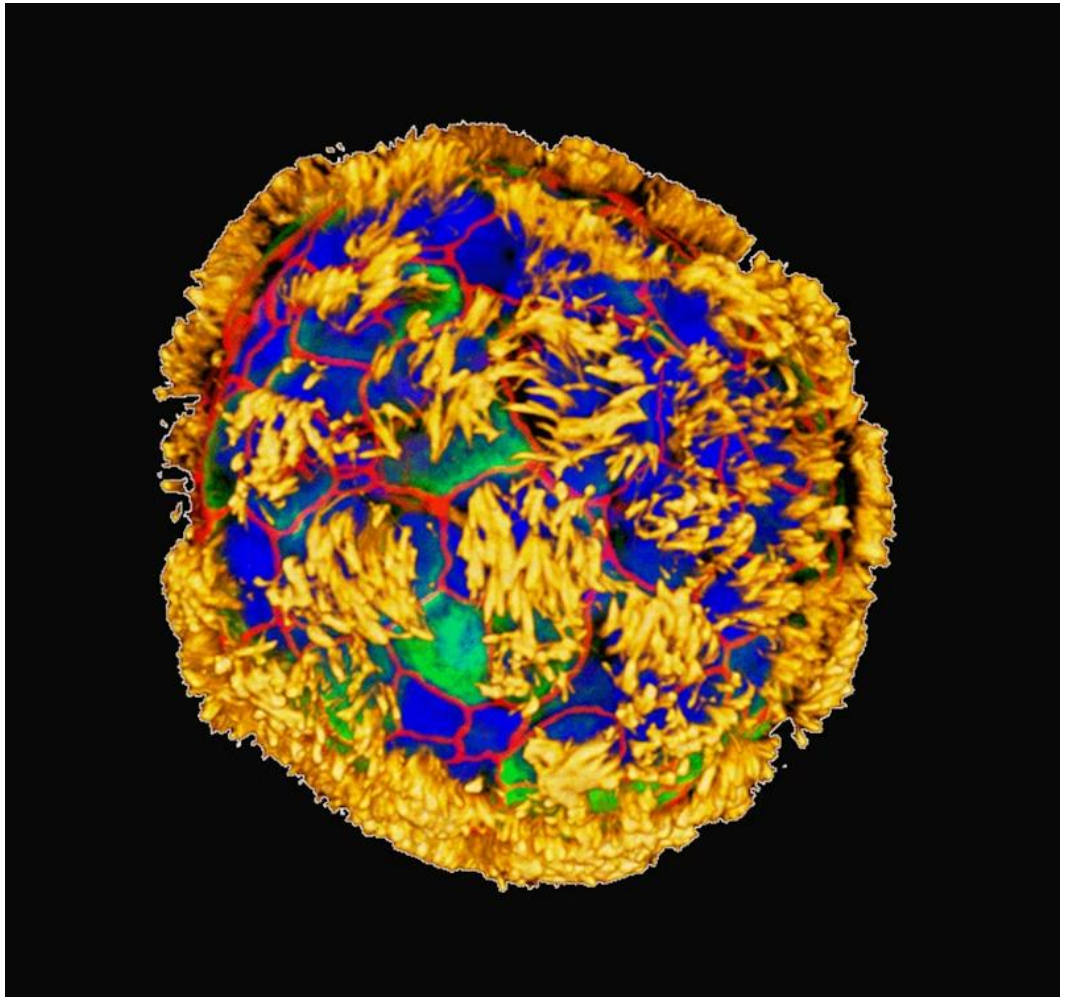
Relationship between mouse embryo (A) and gastruloid (B) development. Gastruloids and polarization of *Bra* and *Nodal* gene expression. The regulation of the fate of *stem cells* and *biomechanical field* effects influence the target shape.

Adv. Sci. 2024
10.1002/advs.20230

anthrobots: what are the multicellular fields that govern what cells can do besides create default features in the body?

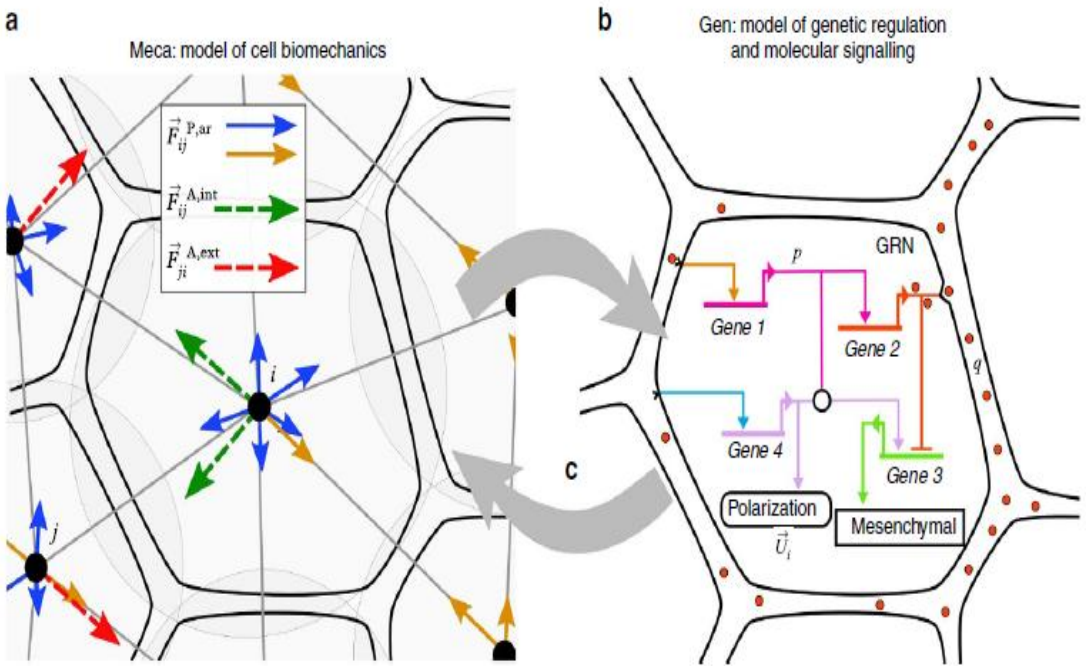
Human tracheal skin cells self-assemble into *multi-cellular moving* Anthrobots with cilia on their surface (*yellow*) distributed in different patterns.

Surface patterns of cilia are correlated with different movement patterns.

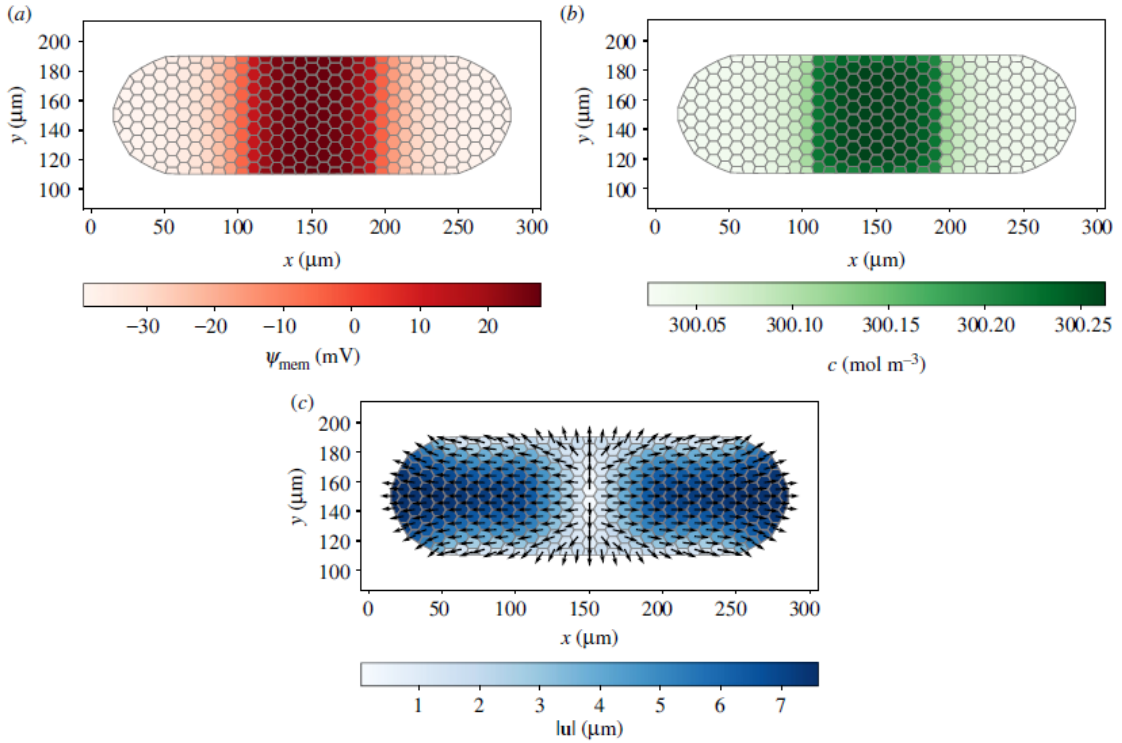


coupling biomechanical, bioelectrical, and biochemical models

Nat. Commun. 2017 10.1038/ncomms13929
J. R. Soc. Interface 2020 10.1098/rsif.2020.0177

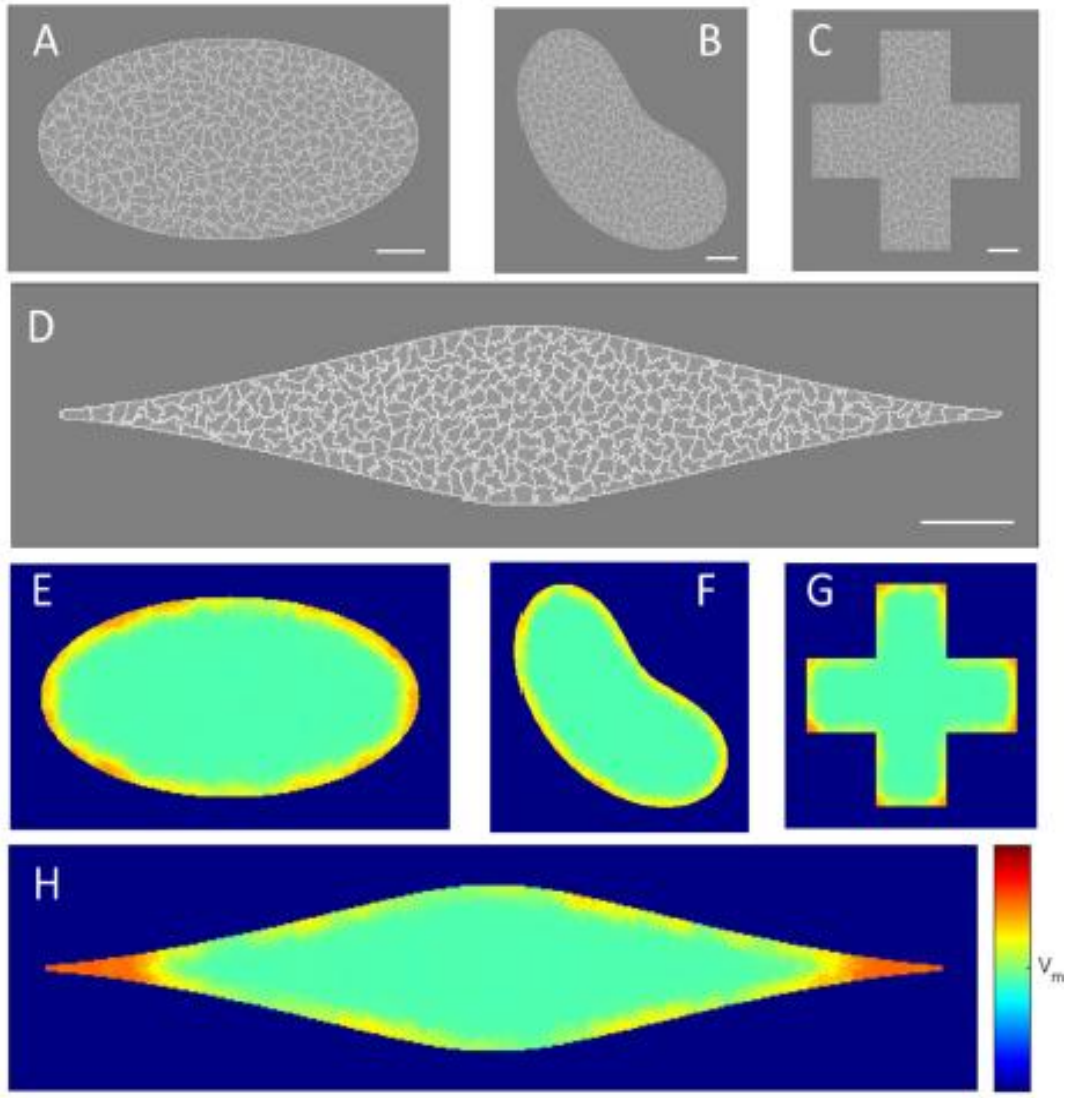


Schematic overview of the *MecaGen* model *coupling* the cell *biomechanical* properties to its *biochemical* activity. Mechanical parameters are specified by the gene expression dynamics and molecular state. Conversely, spatial rearrangements among cells impact protein synthesis via signaling and mechanical stress.

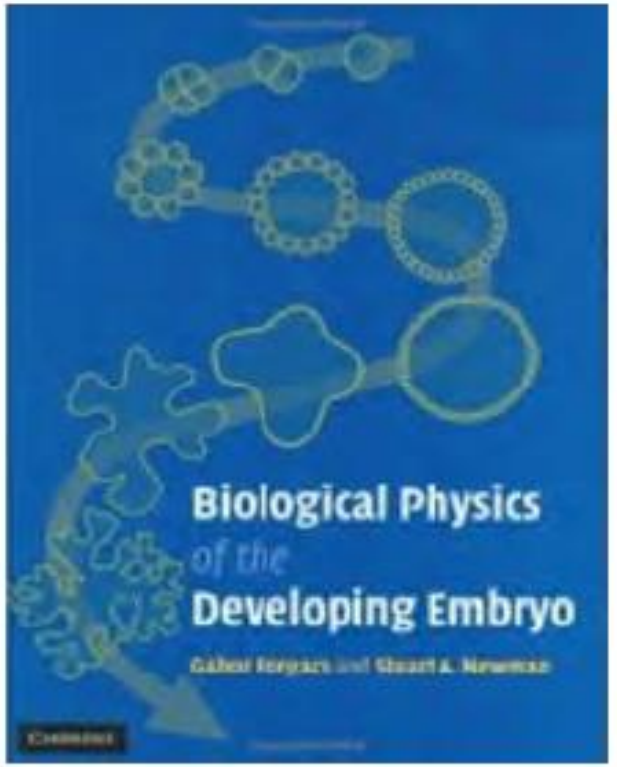


Modeling the *coupling* of *mechanics* with *bioelectricity* and its role in *morphogenesis*: cell membrane potential (a), osmotic concentration (b) and displacement vector (c) at a fixed time. The central depolarized region in which ions accumulate determines a symmetric horizontal elongation of the cluster.

J. Theoret. Biol. 2023
10.1016/j.jtbi.2022.111338
Biological Physics of the Developing Embryo 2005



Shape effects matter: simulated average *multicellular potentials* V_m in *different geometries*: elliptical, bean-like, cross, and eye-like (A to H).



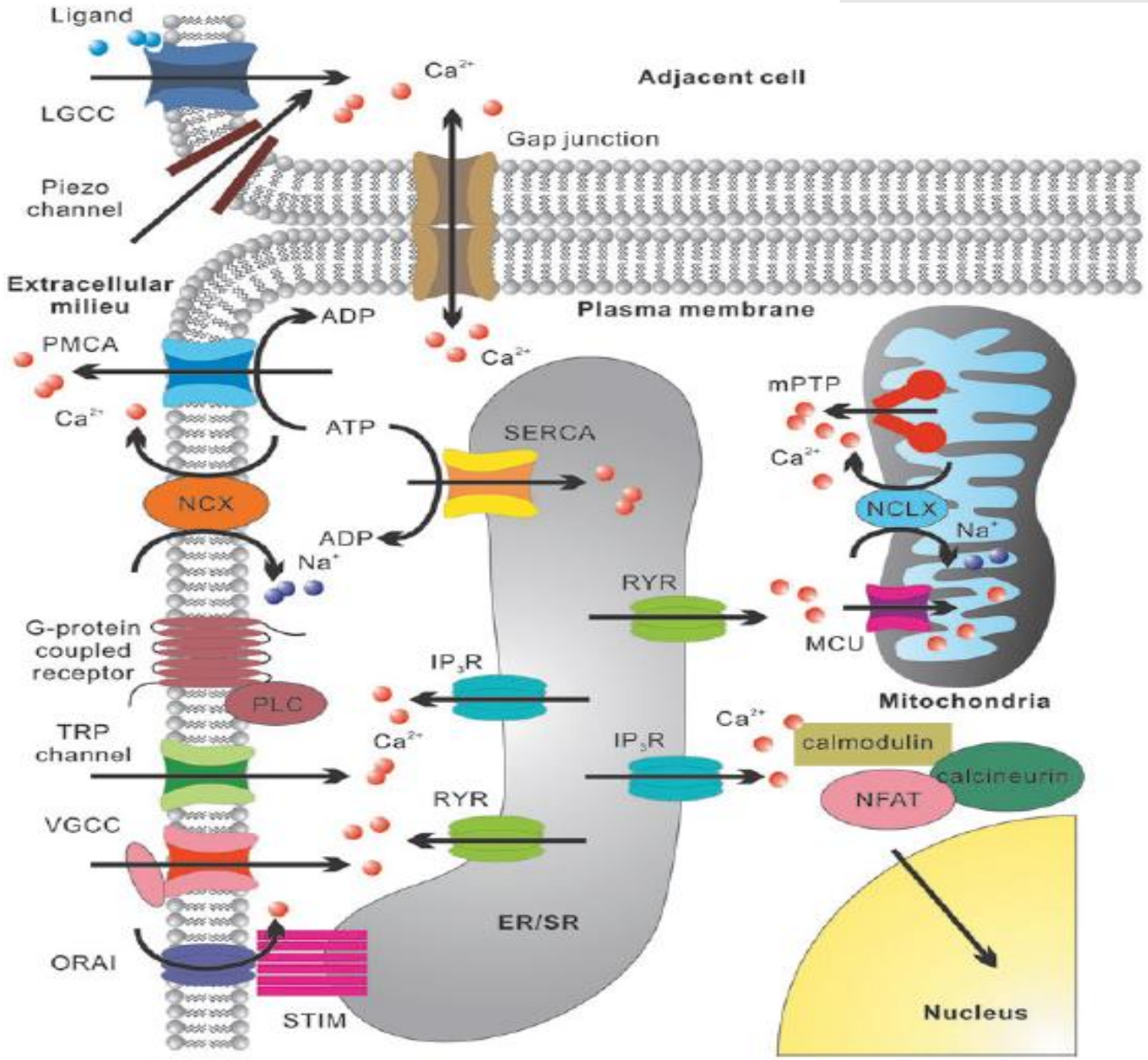
Biological Physics of the Developing Embryo
Gabor Forgacs and Stuart A. Newman. Cambridge University Press, 2005. *Biomechanical* compartment formation and gastrulation are central in *morphogenetic* processes.

Bioelectricity 2020
10.1089/bioe.2020.0001

multitude of ion transporters, not only voltage-gated channels, can establish bioelectrical and biochemical feedbacks

Biological complexity: schematic representation of key regulators of cytosolic Ca^{2+} levels.

Clearly, *extensions* of the bioelectrical model must be considered in *real cases*.



6.b Bioelectrical opportunities

1) The progress from an *individual cell* to *multicellularity* leads to *average* biophysical magnitudes. They constitute *higher levels of abstraction* that provide an actuation layer *complementary* to cell-level management.

2) Differences between *multicellular* regions influence *gene expression*: cells are sensitive to *bioelectrical spatio-temporal patterns* to develop specific programs by *collective* decision. Both *spatial regions* and *time windows* characterize bioelectricity.

3) A *data-driven bioelectrical view*, complementary to biochemical and biomechanical models, can provide *qualitative insights* into bioelectricity and transcription: reduce complexity by identifying those magnitudes relevant to *average multicellular potentials*.

4) *Main features of multicellular fields –a Statistical Physics view*:

Phys. Rep. 2023
10.1016/j.physrep.2022.12.003

- basic *units* need *not* be *equal* for a *robust collective response*: individual heterogeneity, which allows the *diversity* needed to respond to environmental changes, can be compensated by intercellular *connectivity* and *redundancy* mechanisms;

- *multicellular fields* can manage individual *variability* and *environmental noise*, avoiding tight regulation at the single-cell level. Also, *low-dimension average responses* tend to demand *small system resources* compared with an exhaustive control of every unit;

- *transitions* between *instructive multicellular patterns* can be obtained by *local remodeling* of the *intercellular connectivity*; while this fact may increase system *vulnerability*, it can also allow external *correcting actions*; and

- if *morphogenetic processes* are exceedingly *complex to micromanage* (“programming at the bottom layer with *uncertain consequences* on upper layers”), identifying and manipulating *average multicellular fields* can offer new opportunities.

5) The *bioelectric state* of non-neural tissues may be modified by molecular-genetics, pharmacological, and opto-genetical techniques to *open* and *close* ion channels and gap junctions, thus modulating *instructive patterns*. An *analogy*: different *bioelectrical* patterns *downstream* produce distinct *biochemical* patterns whose outcomes act as *morphological attractors.* Collective decision-making ensures robustness and error correction of these outcomes. However, when this self-stabilizing dynamics fails, a *bioelectrical drug* may still correct the resulting *wrong attractor* by re-establishing the genuine pattern. In this context, the *bioelectric concepts* introduced here can suggest particular hypotheses that could be tested by *reductionist procedures* in *model systems*.

There is a *significant limitation* inherent to biosystems: *specific actions* are *system-dependent* because multiple single-cell and multicellular feedbacks between channels and junctions may polarize or depolarize a cell in a *context-sensitive* way. Ion channels and intercellular junctions control cardiac rhythm, muscle contraction, and neurological functions at *different levels* of biological organization. Thus, a detailed knowledge of the *target* and *anti-target* channels, together with the possible feedback mechanisms, is required to implement any bioelectrical action; with the potential *risk* of *unknown* non-canonical channel functions that can lead to *unexpected* and *adverse events*.

Cell 2021
10.1016/j.cell.2021.02.034
Biochim. Biophys. Acta 2023
10.1016/j.bbagen.2023.130440
Bioelectricity 2022
10.1089/bioe.2022.0014
Annu. Rev. Cell Dev. Biol. 2015
10.1146/annurev-cellbio-100814-125338
Development 2021
10.1242/dev.180794

https://www.uv.es/membrane/index.html

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