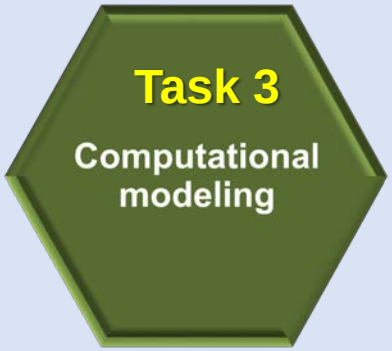
Synergy for Task 3: Computational modeling



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MIT

Science generally involves experiments & theory/models.

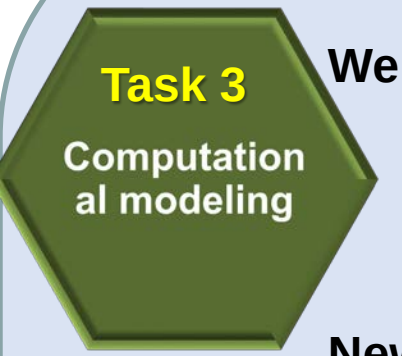
Biophysics is central to understanding how electric fields alter structure or modulate processes, and includes cell membrane electroporation (EP). Maybe straightforward to explain bipolar cancellation (BPC). **Not so!**

TP = Transient pore (lipidic); CP = Complex pore (hybrid)

Standard Model failed. A new Cell EP model is able to account for key responses of the experimental groups (Pakhomov, Pakhomova, Craviso, Vernier).

Our models use Xiao waveforms and will benefit from Yakovlev electromechanics tools.

Task 3 – Why is our modeling essential?



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We show biophysical mechanism that can account for BPC.

- **Based on cytoplasmic molecules**
- **Decreased tracer uptake by a 2nd pulse of opposite polarity**
- **Partial recovery of uptake with a delay between pulses**

New updated extended cell model:

- **Field-driven insertion of cytoplasmic molecules into TPs**
- **Describes both membrane- and cell-level interactions**
  - short-lived transient pores (lifetime 100 ns)
  - long-lived complex pores (lifetime 100 s)
- **Quantifies electrodiffusion of tracer molecules post-pulse**
  - dominant in uptake experiments that last several orders of magnitude longer than the pulse duration

Occluding molecules yield complex pores (CPs)

- **lifetimes up to ~1,000 s**

Partial occlusion hinders tracer molecule transport into cell (measured)

Synergy

40 year old "standard model" of electroporation is inadequate

Continuum & MD multi-scale

Engineering Core

Novel bio-electro-mechanical tools

AFRL

Waveforms are model inputs

Post-pulse Um: ms and s after pulse

Um during pulse

Computational modeling

Molecular modeling and ultra-short pulses

Vernier group experimental results:

- CPs (post-pulse tracer) drift
- calcein efflux data validates post-pulse transport

New EP model under continual development. Capable of bipolar cancellation. Results in new biophysical phenomena.

Biophysical mechanisms and biomembranes

Neurosecretion

Bipolar cancellation and CANCAN stimulation

- Experimental Data: Tracer uptake from BPC from experimental groups tests the theoretical/computational model
- Modeling suggests experiments

Bipolar cancellation poses a challenge to biophysics. New discoveries of biophysical mechanisms were needed. BPC in turn can be applied to new and exciting applications.

Biophysics: effects mechanisms limitations

Traditional/Transient Pores (TPs)

(as revealed by molecular dynamics – MD)

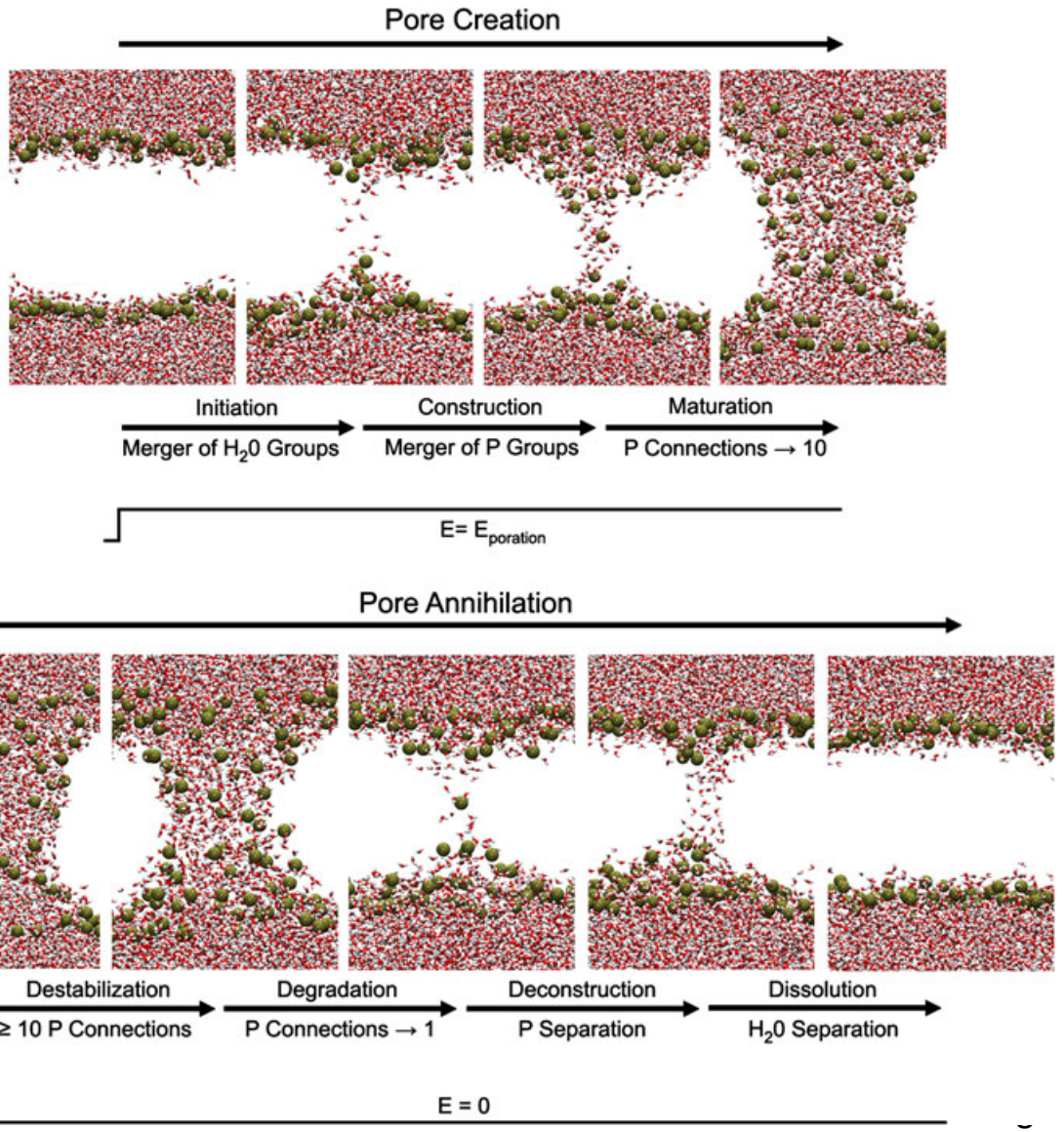
Pore birth

Pore lifetime is more important

100 ns lifetime pores vanish before observations

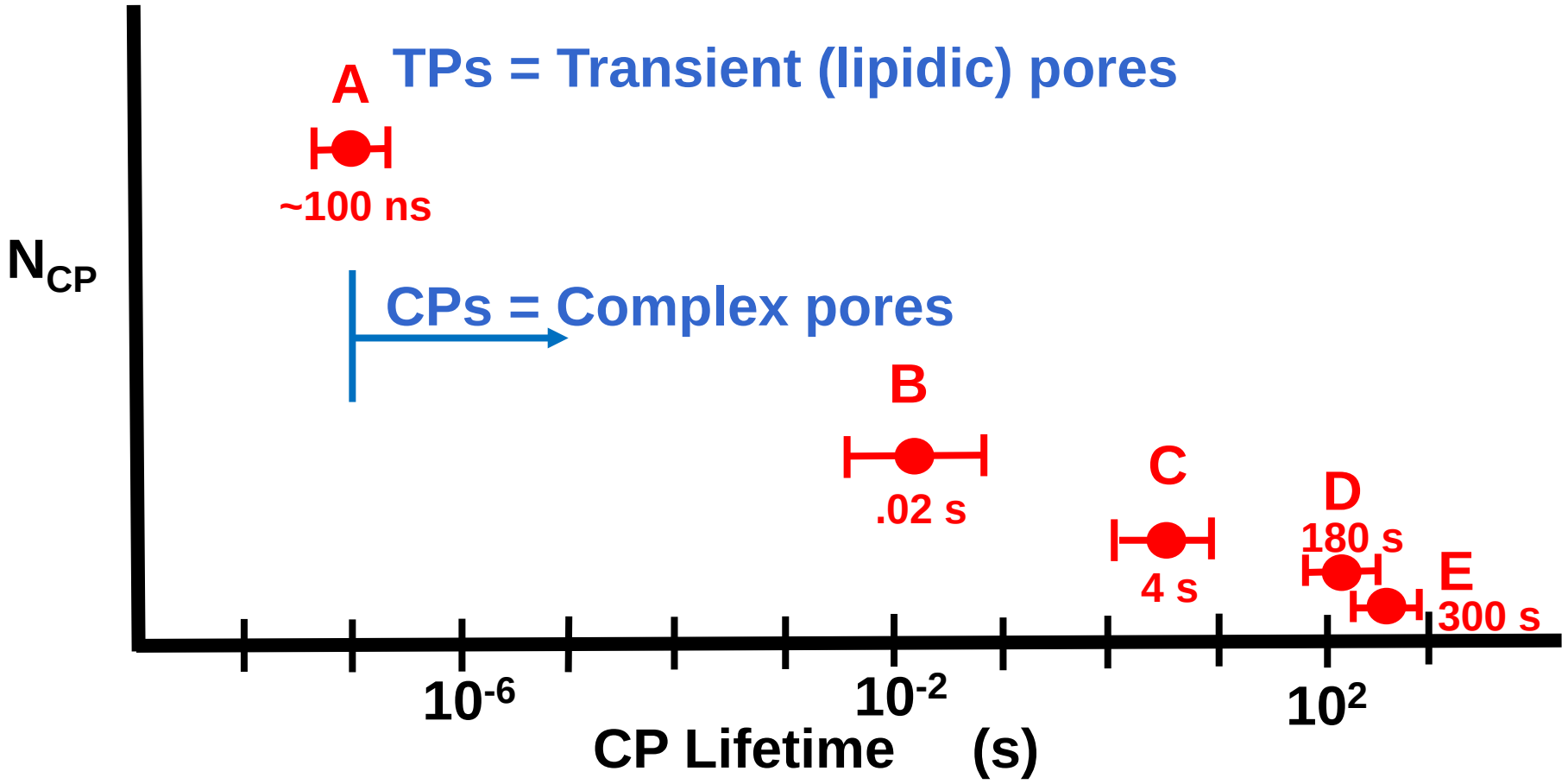
Pore death

~100 ns lifetimes



(Levine and Vernier JMB 2010)

Broad Lifetime Distribution Concept



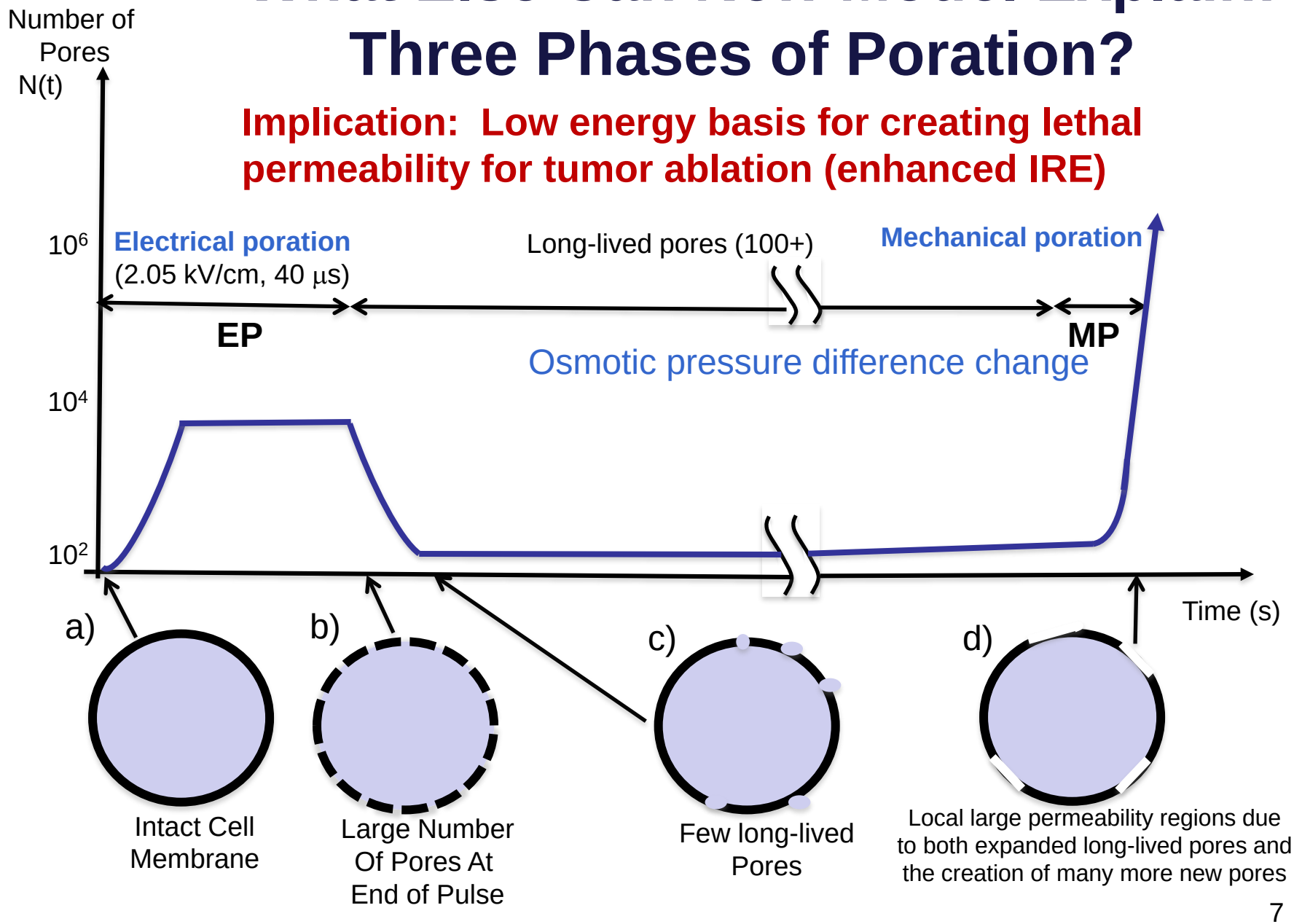
Guess-estimates are based on order of magnitude TP numbers, and experiments with ~1 to 300 s observation times

Further experiments are essential.

(Stern et al., arXiv 25 AUG 2017)

What Else Can New Model Explain: Three Phases of Poration?

Implication: Low energy basis for creating lethal permeability for tumor ablation (enhanced IRE)



Crowded cytoplasm: Simulation demonstration

(McGuffee & Elcock, PLoS Comp. Biol. 2010)

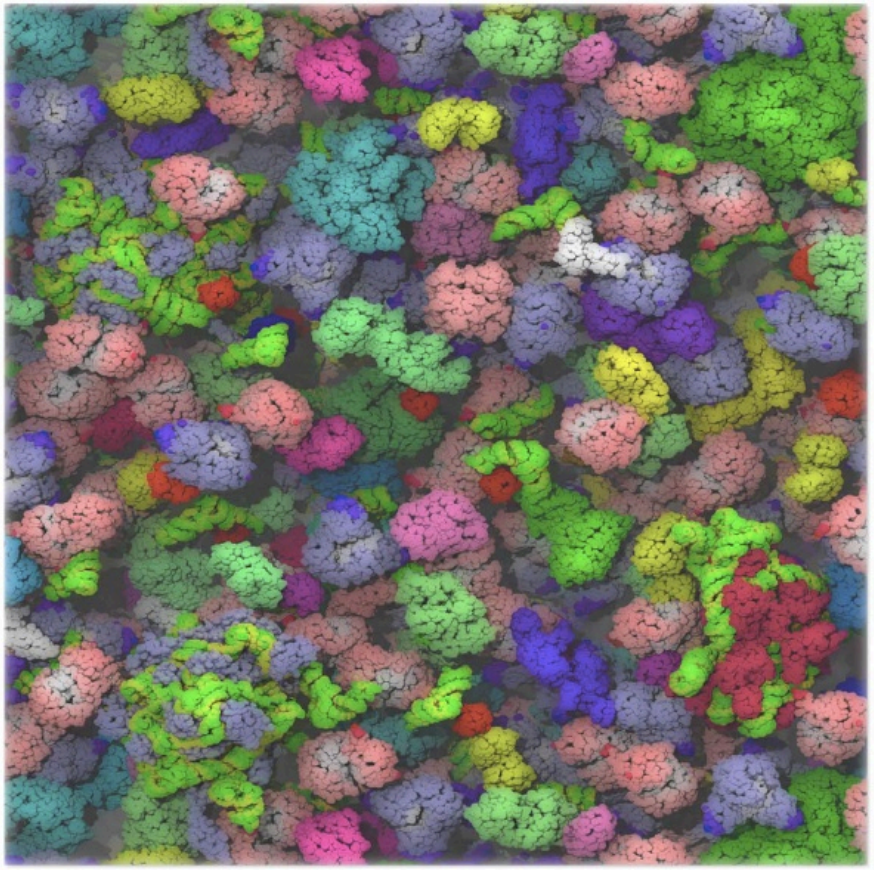
Prior cell EP models treat cytoplasm as simple aqueous electrolyte

Name	Mw	#
Adk	24	14
AhpC	187	7
Asd	80	4
Bcp	11	8
CspC	7	72
CysK	64	13
DapA	125	2
DnaK	41	11
Efp	20	14
Eno	91	18
Fba	78	6
Frr	21	7
FusA	69	22

Name	Mw	#
GapA	142	10
GlnA	621	1
GltD	94	3
GlyA	91	15
GpmA	55	4
Hns	5	7
Hup	15	12
IcdA	92	43
IlvC	54	18
Mdh	65	13
MetE	84	213
Mop	845	2

Name	Mw	#
PanB	140	2
Pgk	41	26
Pnp	190	3
Ppa	116	9
PpiB	18	7
PurA	94	4
PurC	42	7
Pyr	308	3
RpiA	46	3
Rpo	260	4
SerC	79	11
SodA	46	13
SodB	42	9

Name	Mw	#
Suc	142	4
Tig	48	9
TpiA	54	5
Tsf	61	12
TufA	84	181
Upp	45	11
UspA	31	7
50S	1,355	10
30S	788	10
tRNA-C	24	37
tRNA-Q	24	37
tRNA-F	25	37
GFP	26	8



In new, extended model we recognize the complex cytoplasm.

We hypothesize complex pores (CPs), and estimate some CP functions.

Typical cytoplasmic proteins + GFP shown (color for distinction). These contact or are close to the PM, so interactions are expected.