Focus

How do cells work?

from the ground up

(water and the cell)

cell biological concepts today (Alberts *et al.* text)

...

...

mechanisms based on channels, pumps

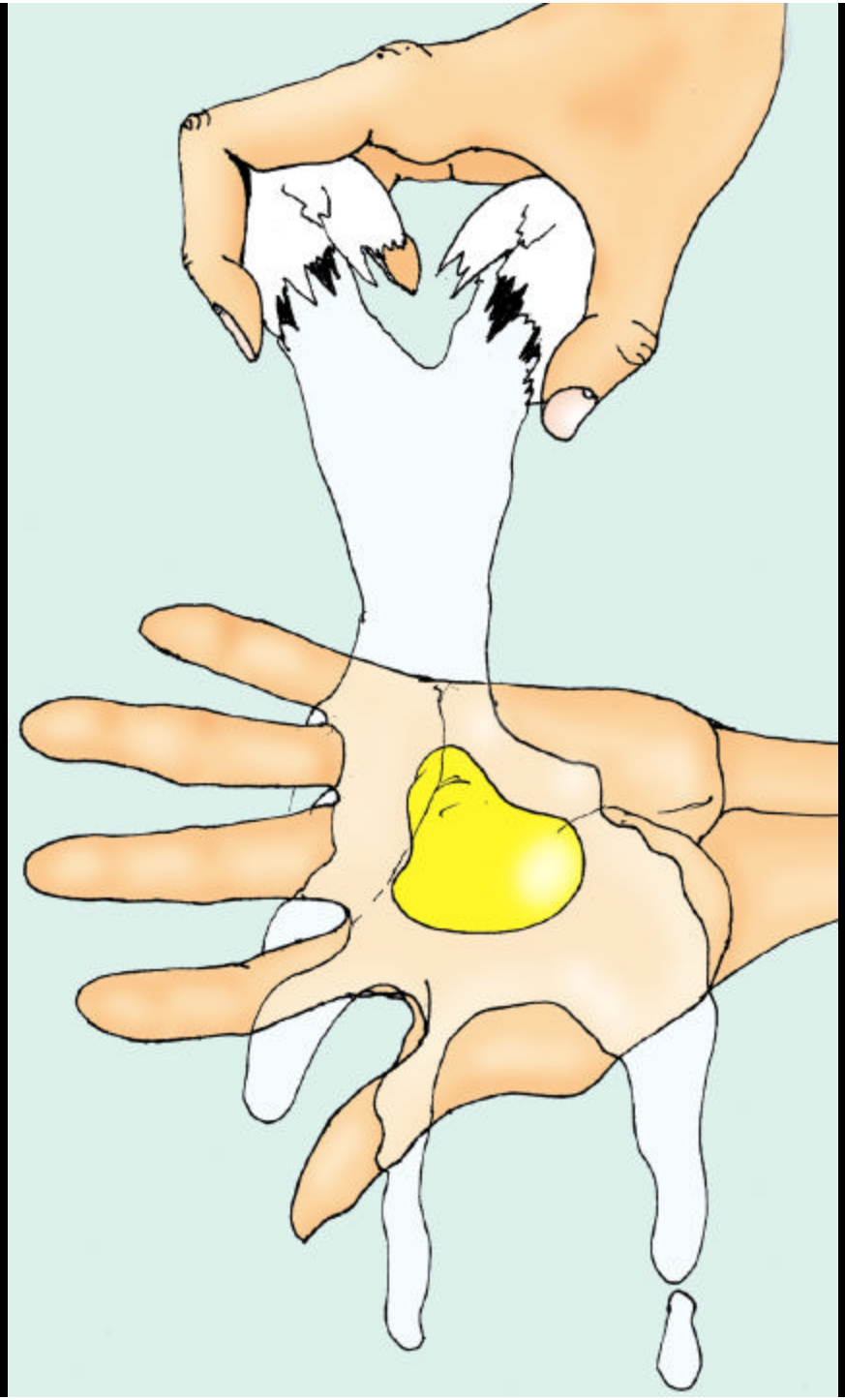
channels, pumps for exchange

continuous barrier req'd

dissolved solutes

aqueous sol'n

cytoplasm



cell biological concepts today (Alberts *et al.* text)

...

...

mechanisms based on channels, pumps

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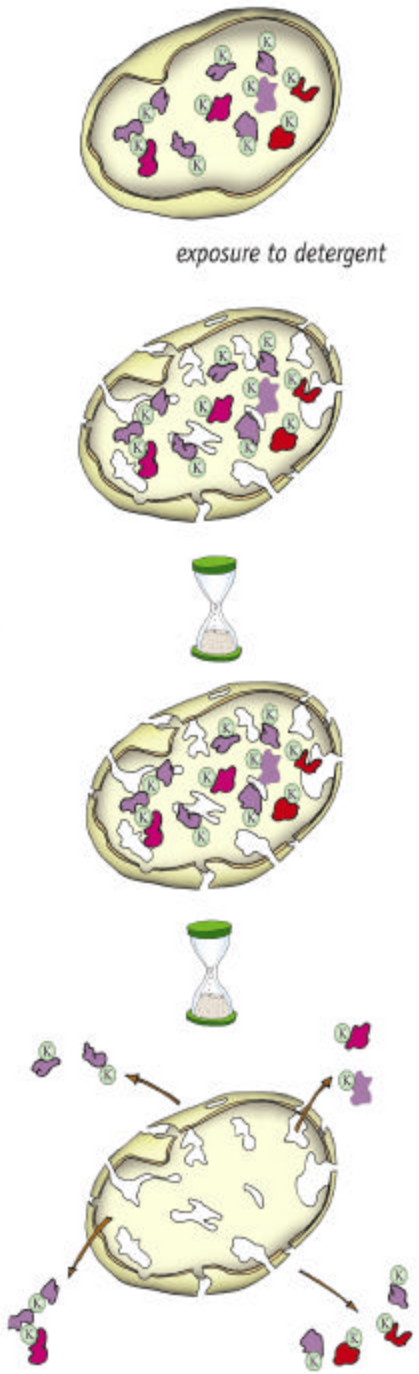
continuous barrier req'd

dissolved solutes

aqueous sol'n ?

cytoplasm

Kellermayer et al., 1986
Cameron et al., 1996
etc.



Conclusion: K^+ not freely diffusible

cell biological concepts today (Alberts *et al.* text)

...

...

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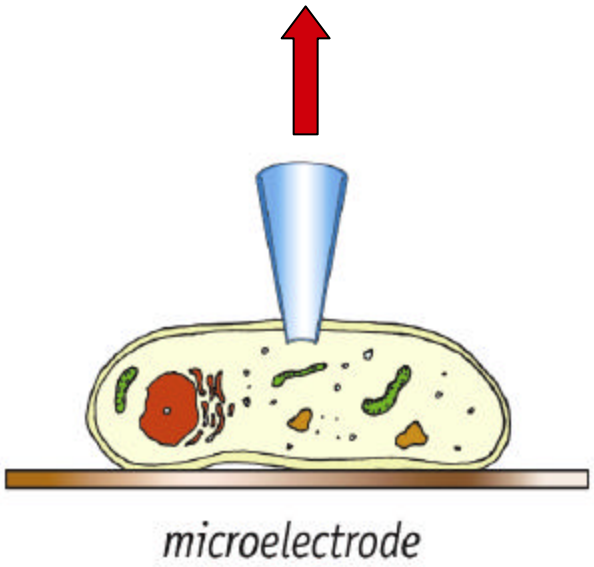
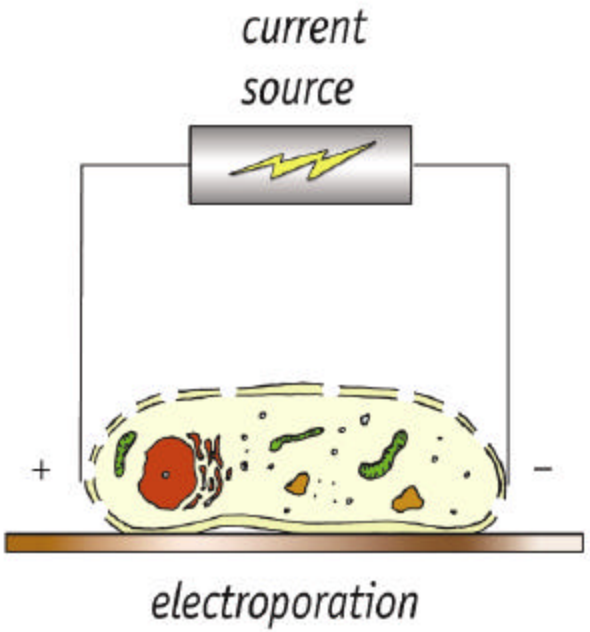
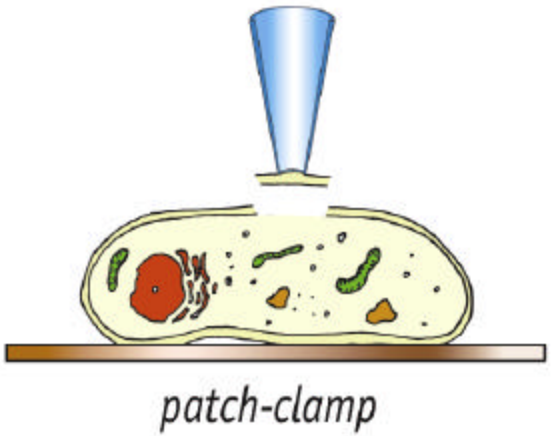
aqueous sol'n ?

cytoplasm


microelectrode


current
source
+
–
electroporation


patch-clamp

cell biological concepts today (Alberts *et al.* text)

...

...

mechanisms based on channels, pumps

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dissolved solutes ?

aqueous sol'n ?

cytoplasm

Pumps: require energy

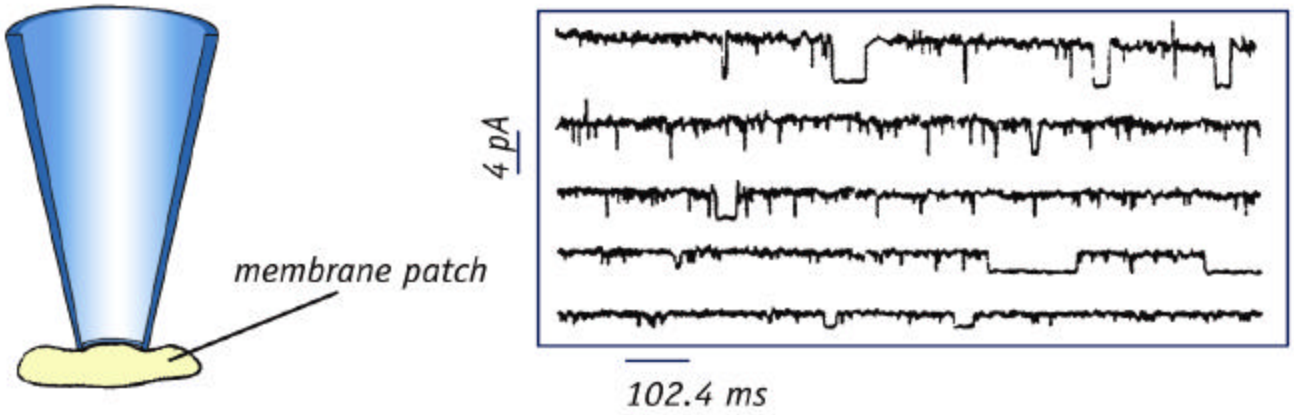
- Na-pump consumes 30 - 35% of cell's energy supply

- >100 additional cell-membrane pumps
- mitochondrial membrane pumps
- endoplasmic reticular membrane pumps
- *etc.*

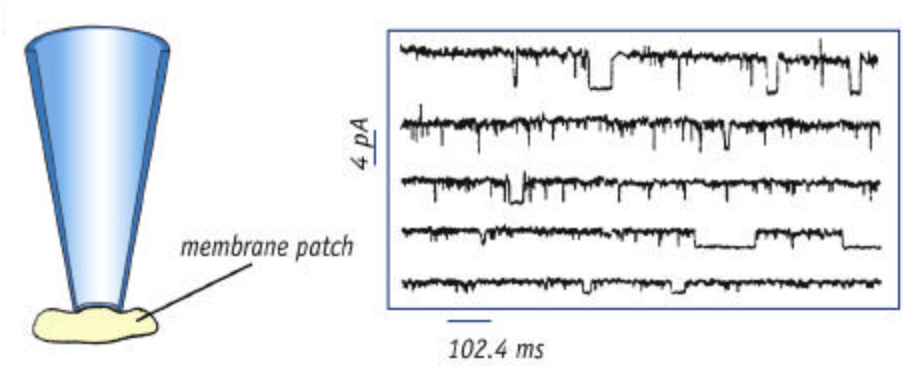
Is there enough energy?

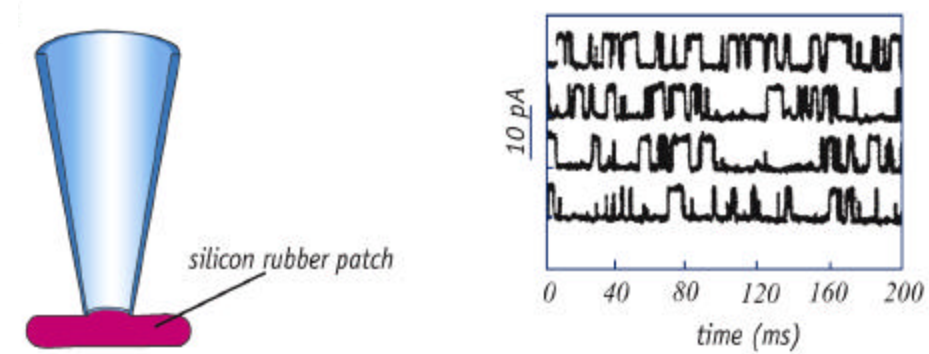
Evidence for channels ….

Patch-clamp experiment (Neher and Sakmann)



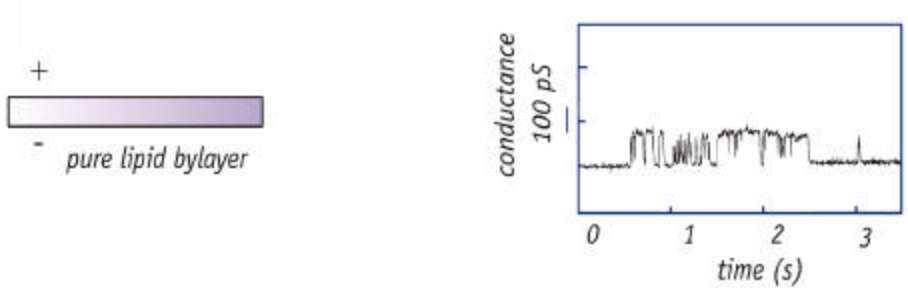
Sachs and Qin, 1993



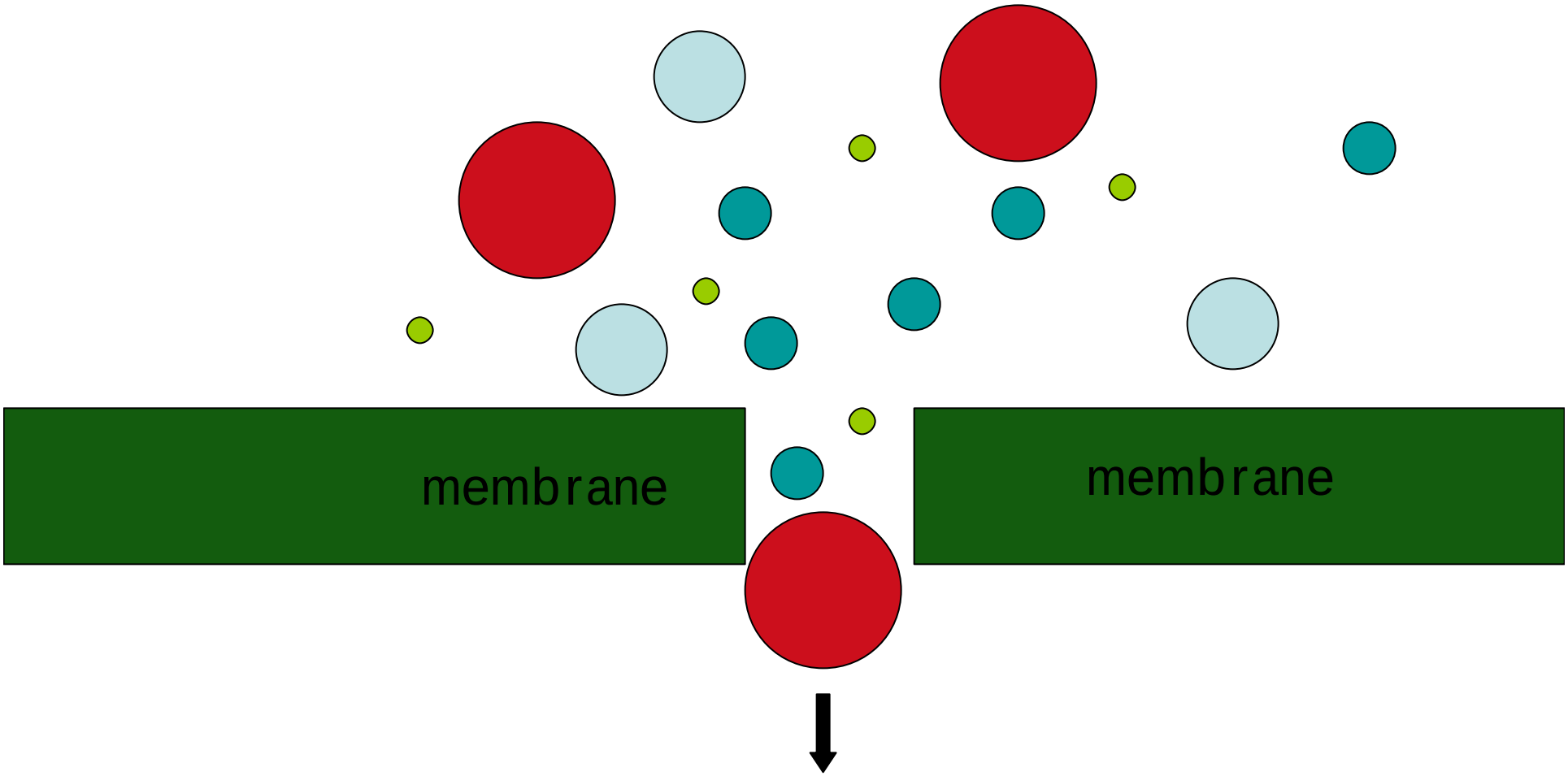


Lev et al., 1993

Woodbury, 1989



Largest channels and small solutes




DOGS ONLY

cell biological concepts today (Alberts *et al.* text)

...

...

mechanisms based on channels, pumps

channels, pumps for exchange ?

continuous barrier req'd ?

dissolved solutes ?

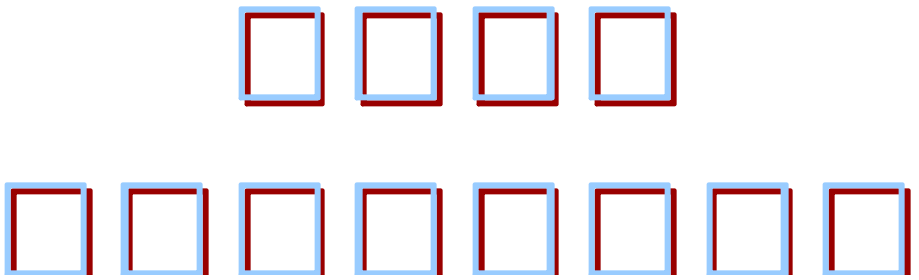
aqueous sol'n ?

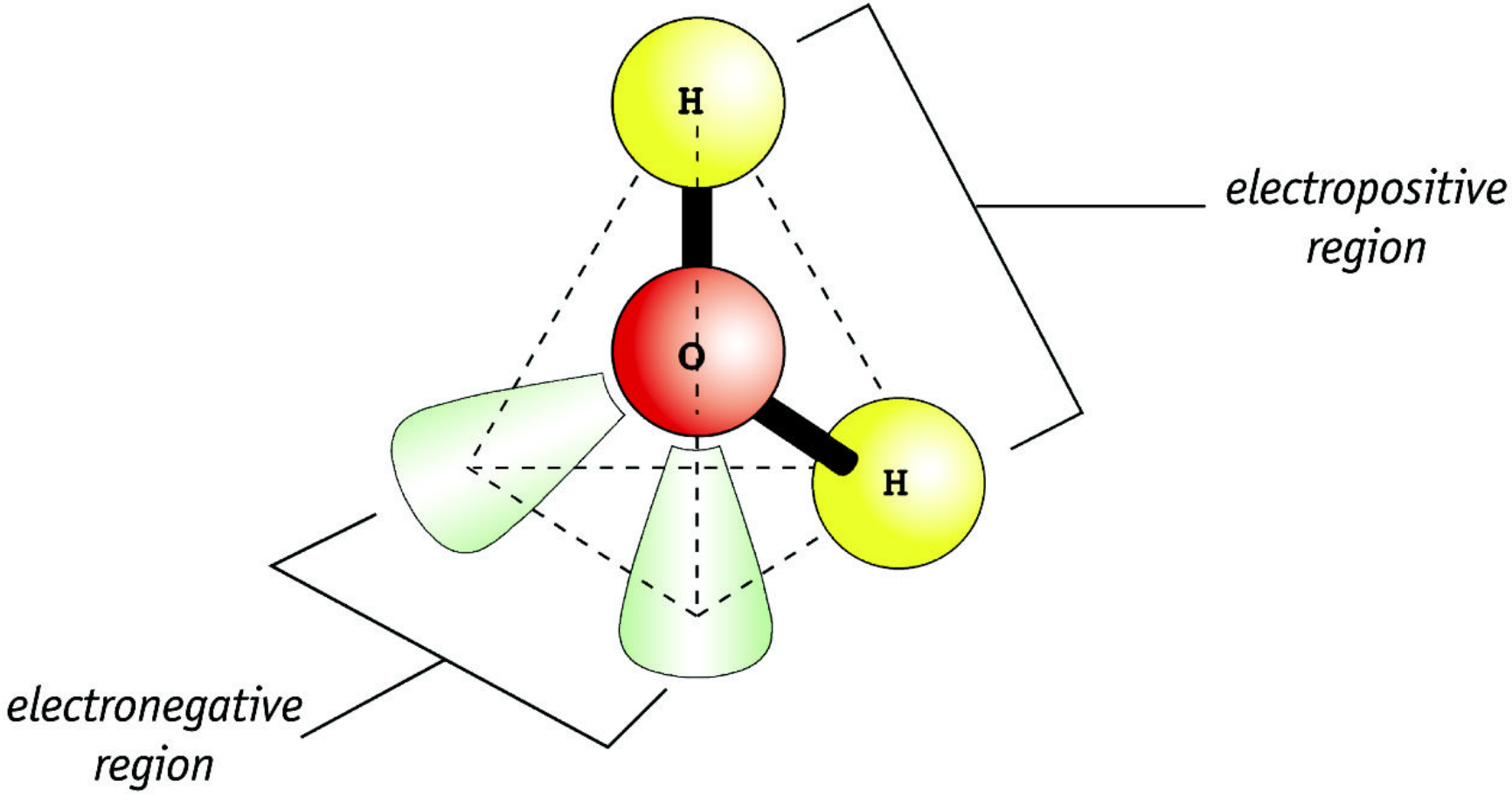
cytoplasm

Begin at bottom: *gel*

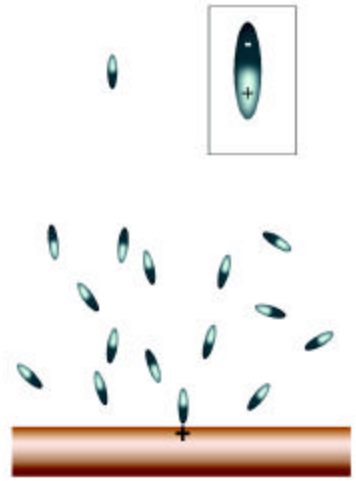
Most abundant component of gel -

Water

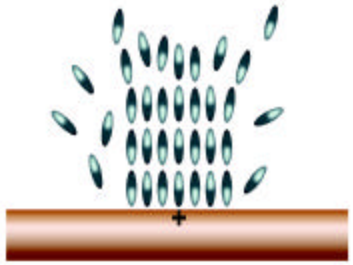




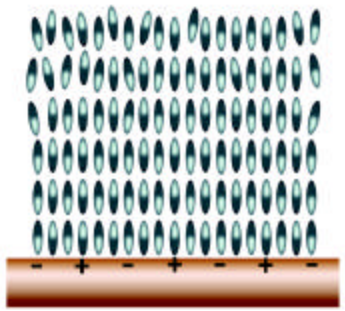
Water is a *dipole*



A.

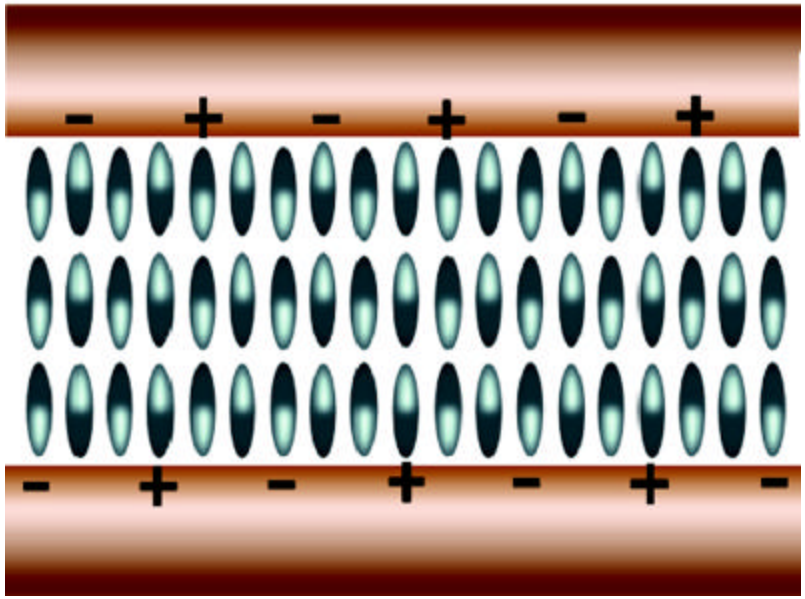


B.



C.

Bonding effect

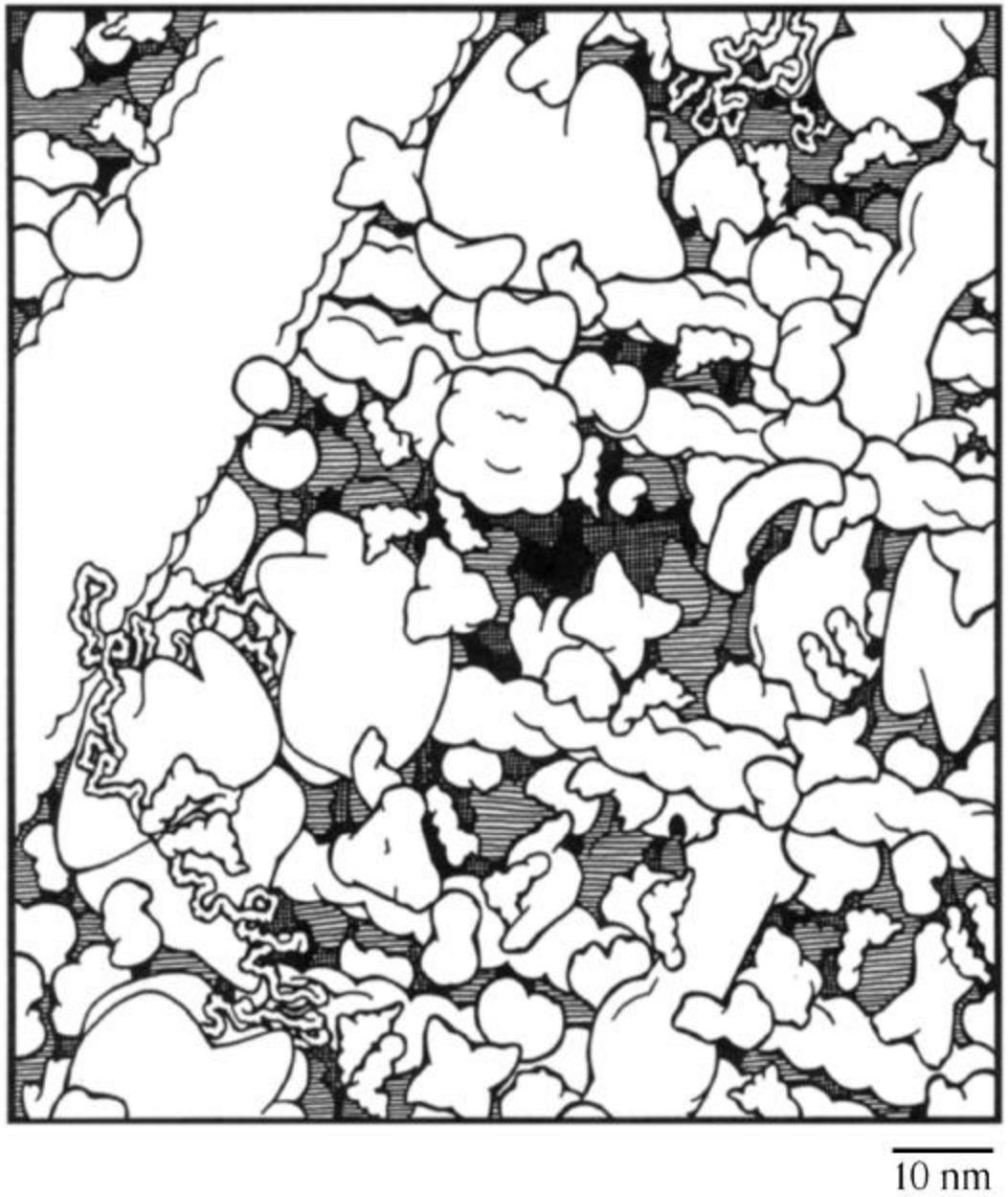


glue-like behavior

Is water *in the cell* structured?

Requirements:

- charged surfaces
- surfaces closely packed



(Goodsell)

Average surface-to-surface distance
< 5 nm

Literature estimates:
1.5 - 2.5 nm

Therefore, conditions ideal for structuring

Evidence that cytoplasmic water is structured:

- Nuclear magnetic resonance
- Ultrahigh frequency dielectric dispersion
- Quasi-electric neutron scattering

Details in recent review articles (e.g., Mentre, 1995; Vogler, 1998)

Structured water differs from bulk water

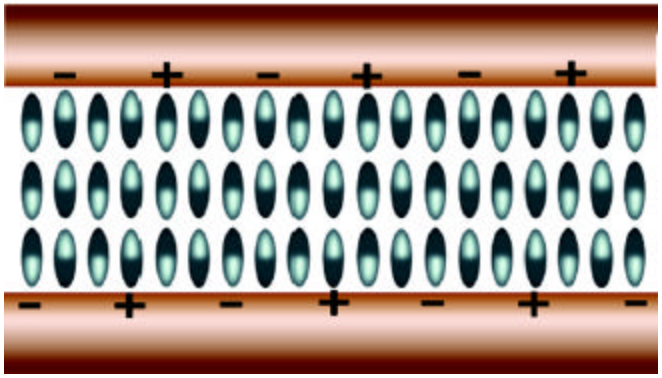
How does this impact function?

Static properties of cell

- Ion distribution (in *vs.* out)
  - *partitioning:* $\text{inside}_{\text{structured}}$ *vs.* $\text{outside}_{\text{bulk}}$
  - *association:* with cell $\text{proteins}_{\text{in}}$
- Cell potential

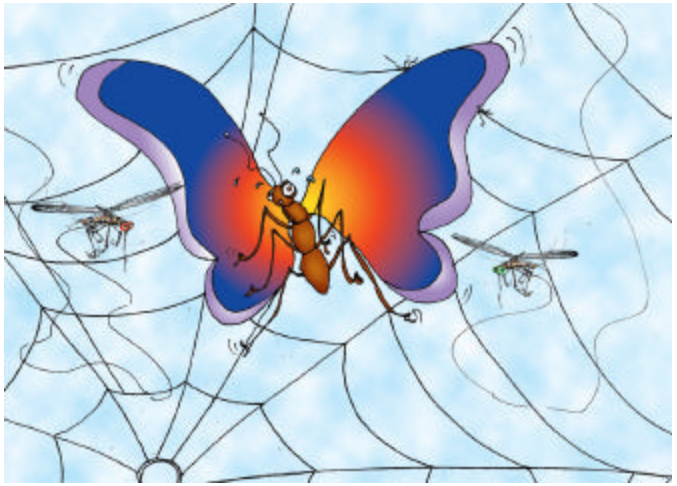
Partitioning between structured and bulk water

Energy term:



Larger solutes dig hole with more difficulty than smaller ones

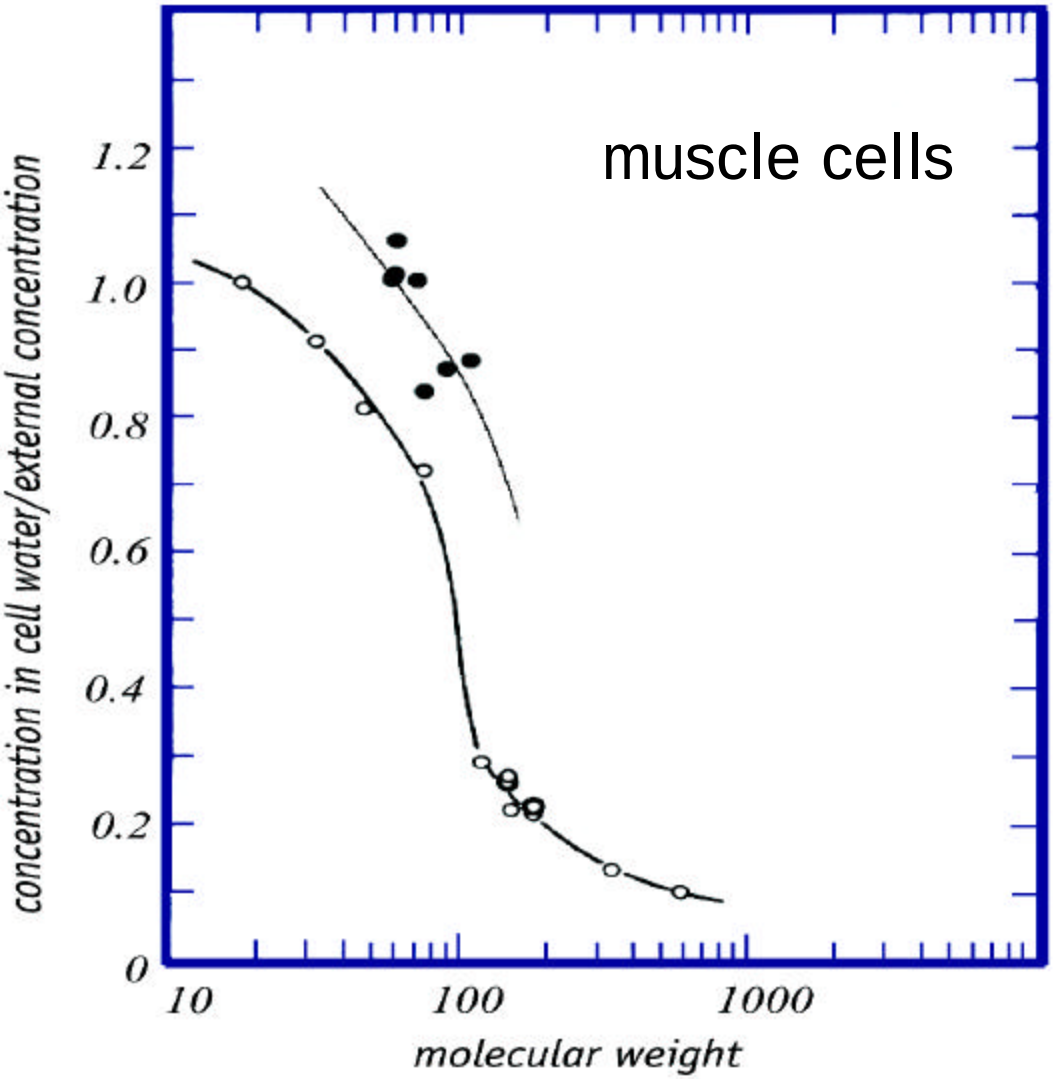
Entropy term:



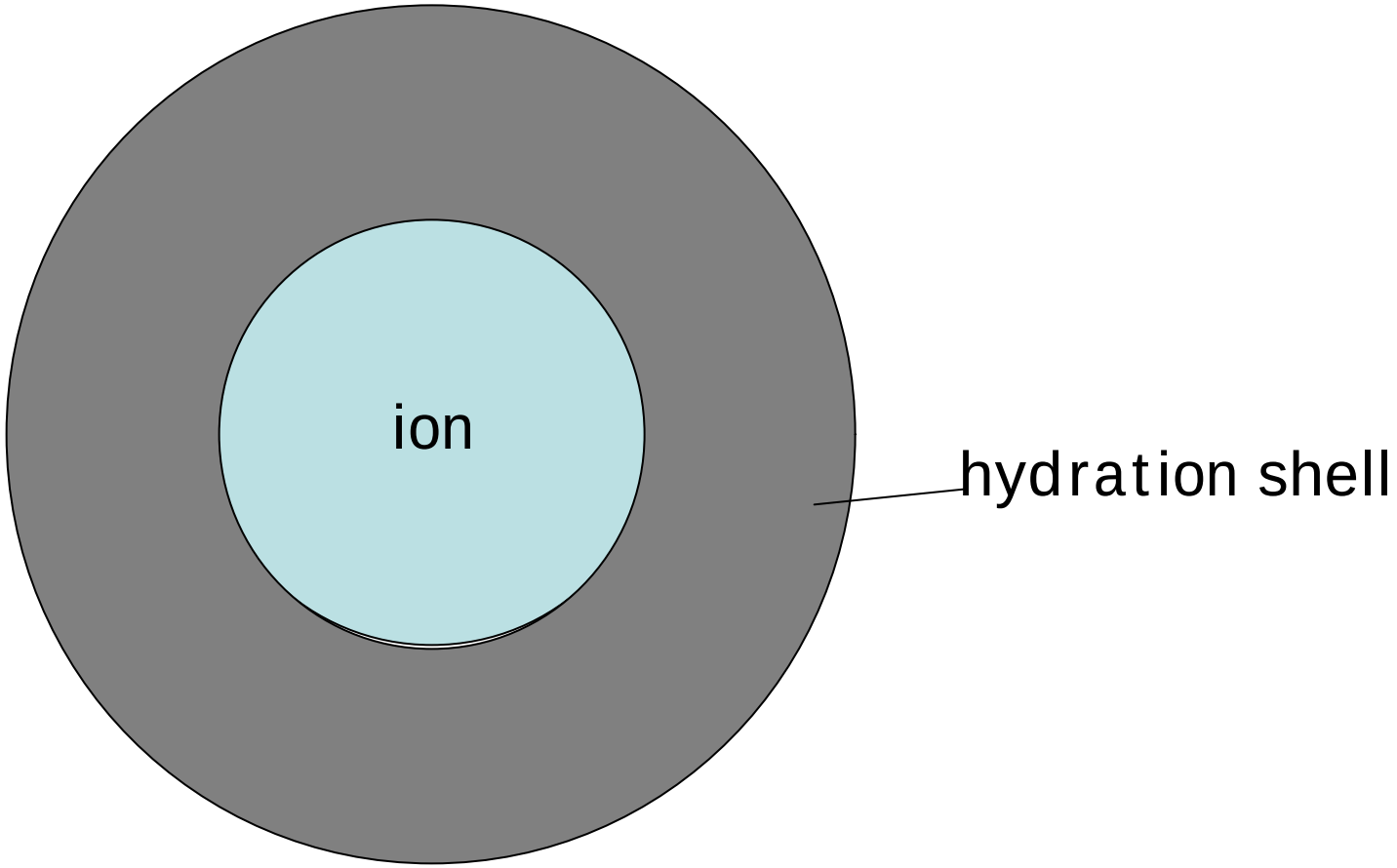
Larger solutes more constrained than smaller ones

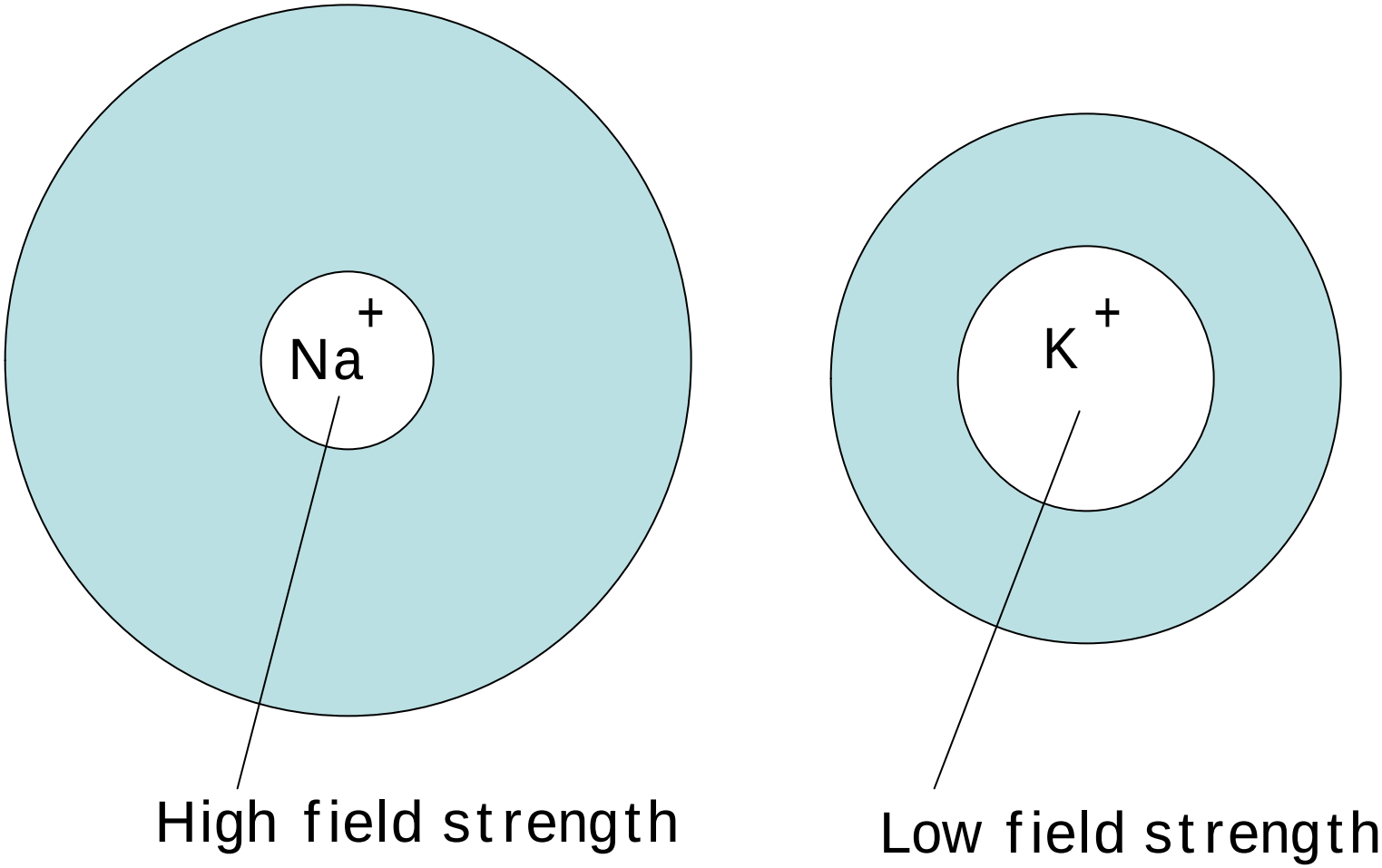
Conclusion: lgr. solutes partition more profoundly toward bulk

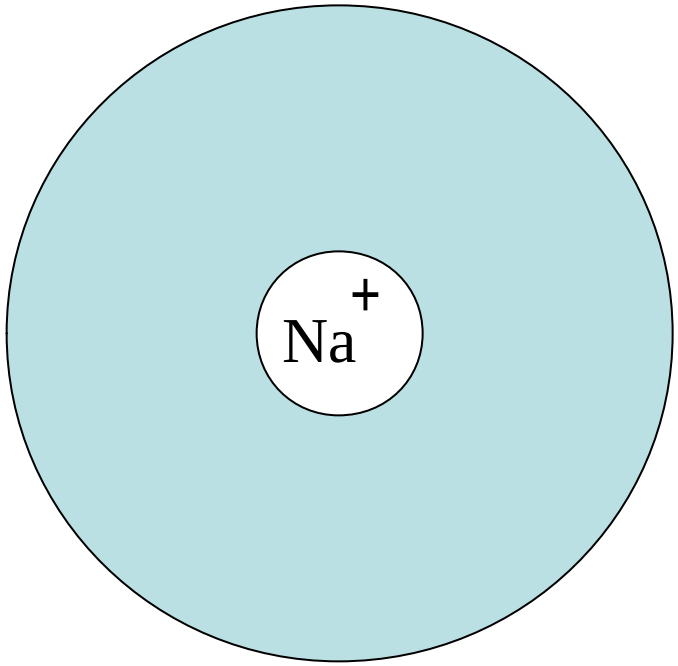
Testing size-based solute exclusion…

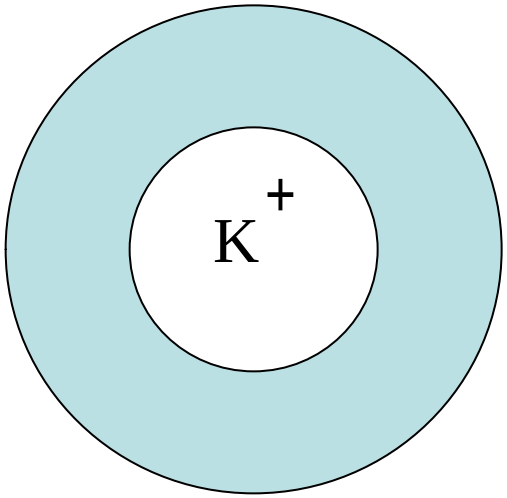


Ions...




Na+
K+
High field strength
Low field strength





Lower solubility in structured water

Higher solubility in structured water

Lower affinity for charged surfaces

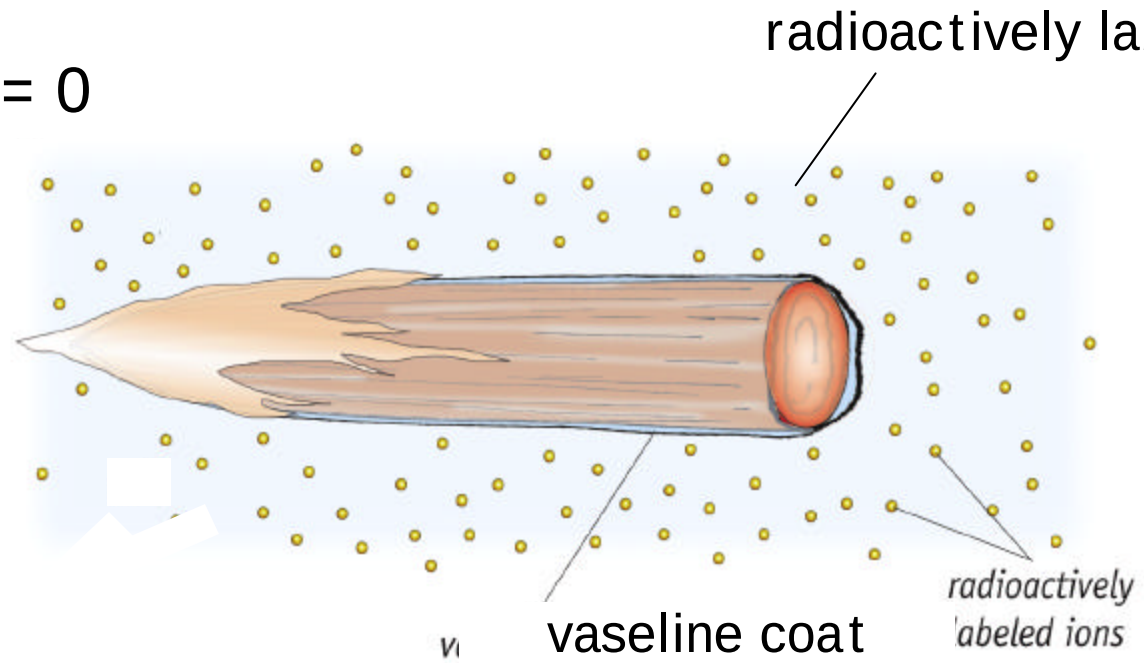
Higher affinity for charged surfaces

Less in cell

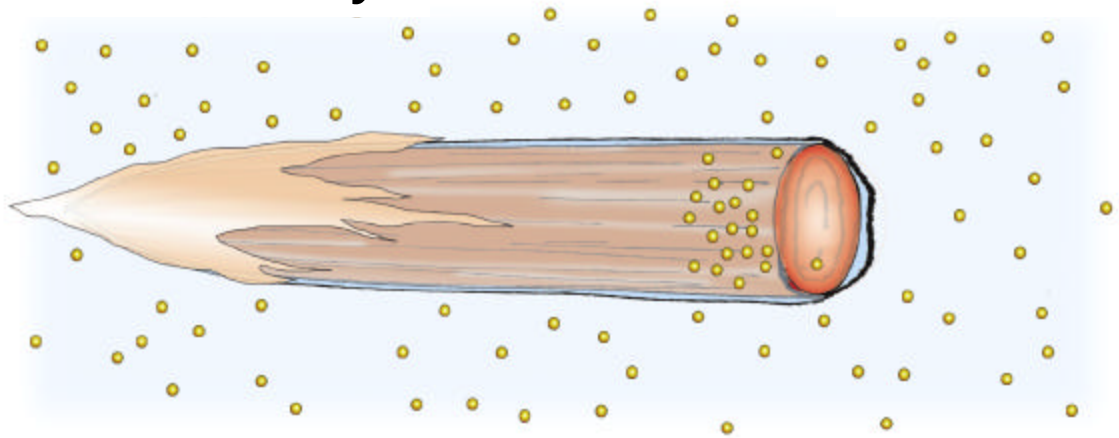
More in cell

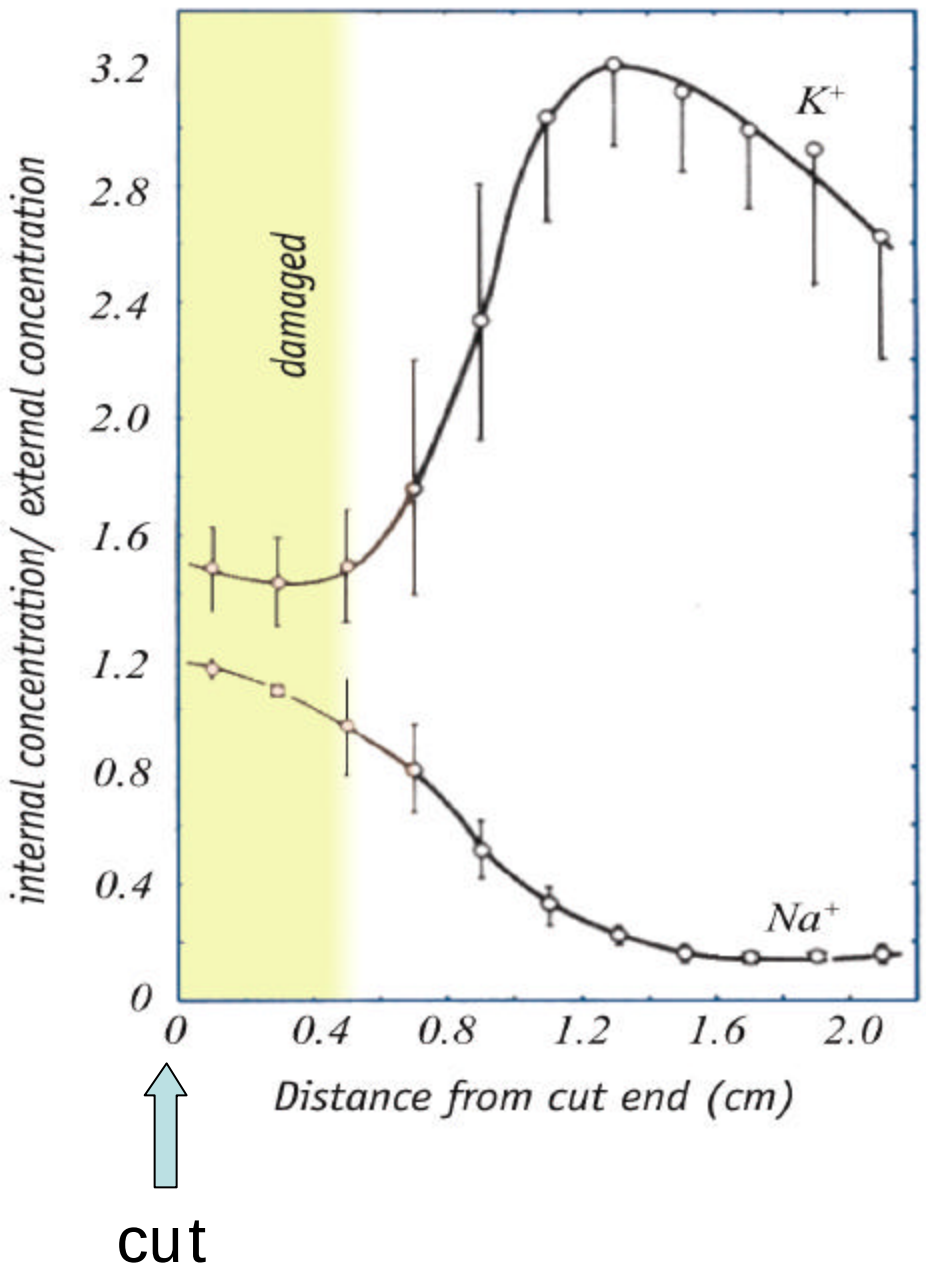
Testing this prediction in the cell…

t = 0

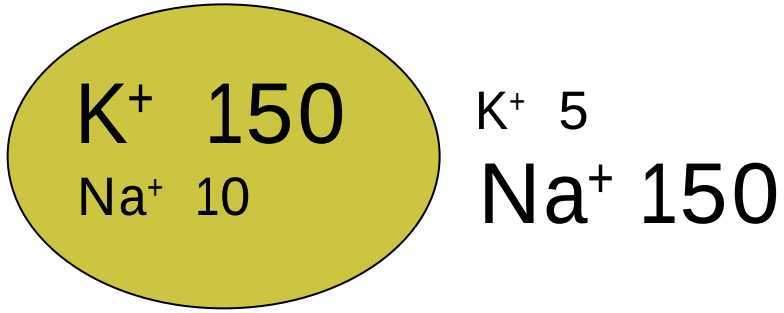


t = several days




internal concentration/ external concentration
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
damaged
K+
Na+
0
0.4
0.8
1.2
1.6
2.0
Distance from cut end (cm)
cut

Conclusion: origin of ion gradients

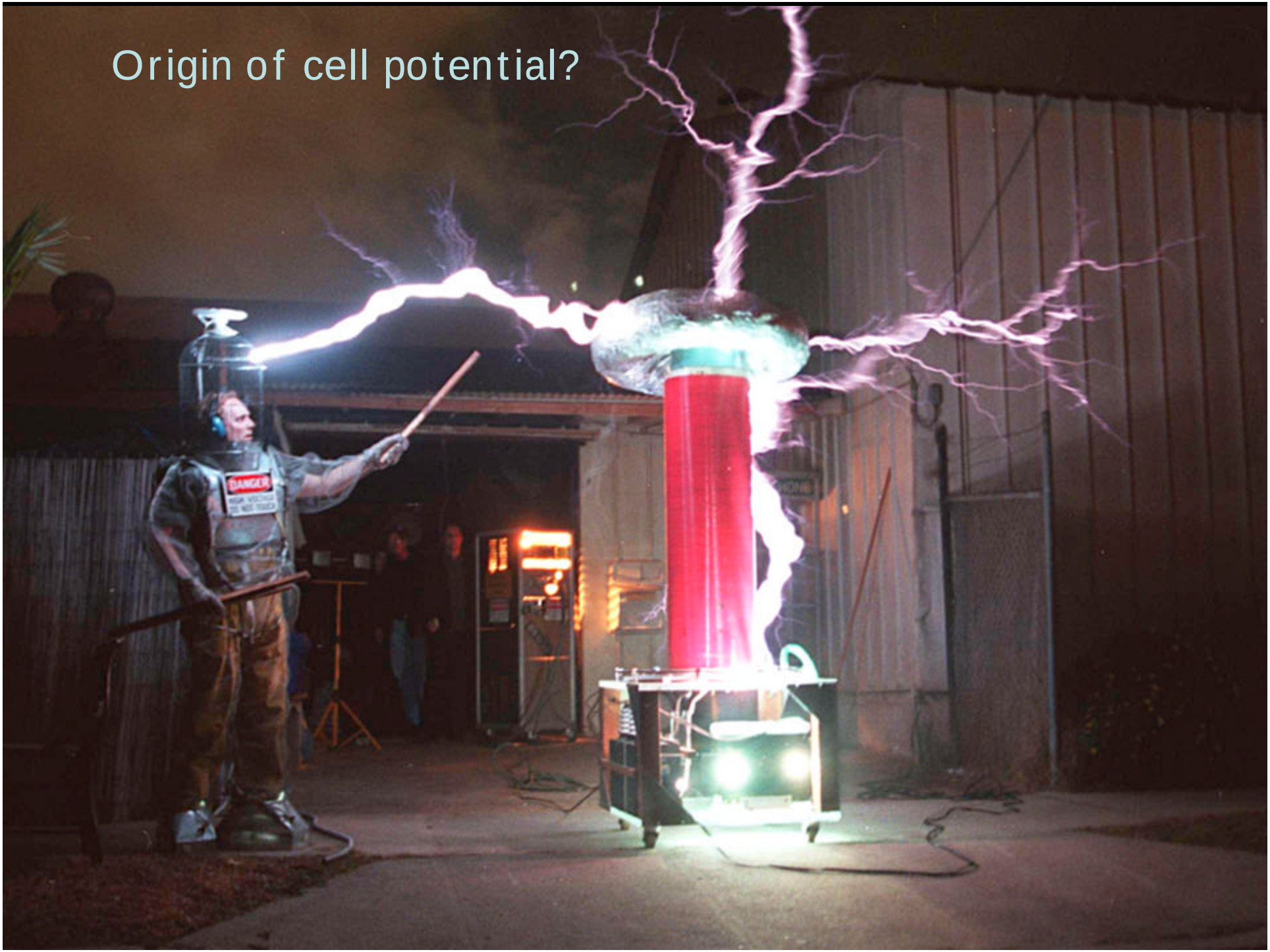


Na: solute exclusion*

K: affinity to protein charges*

*similar behavior in gels

Origin of cell potential?



How much net charge in cell?

Protein

- Negative charge: 1.6 mol/kg
- Positive charge: 1.01 mol/kg
- Net protein charge: 0.6 mol/kg (-) (Wiggins, 1990)

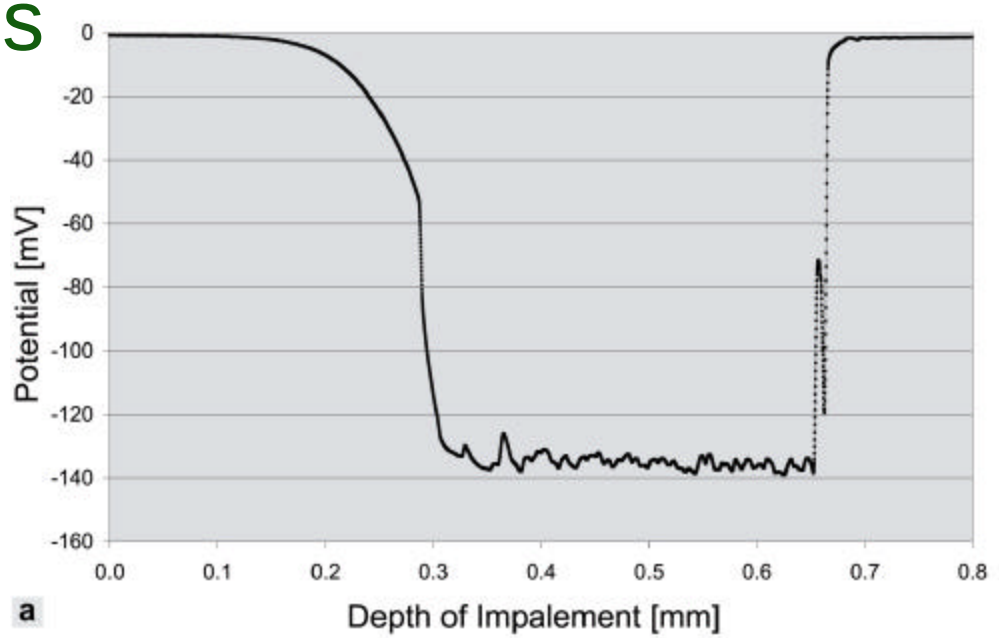
Ions

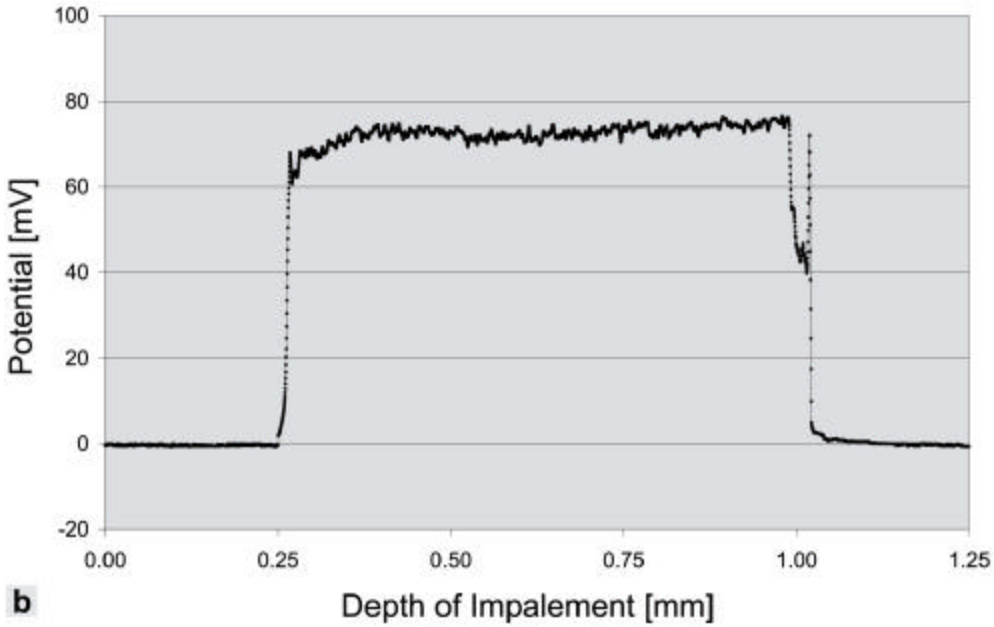
- Potassium ion: 0.5 mol/kg (+)
- Chloride ion: 0.2 mol/kg (-)
- Net ion charge: 0.3 mol/kg (+)

- NET CYTOPLASMIC CHARGE: 0.3 mol/kg (-)

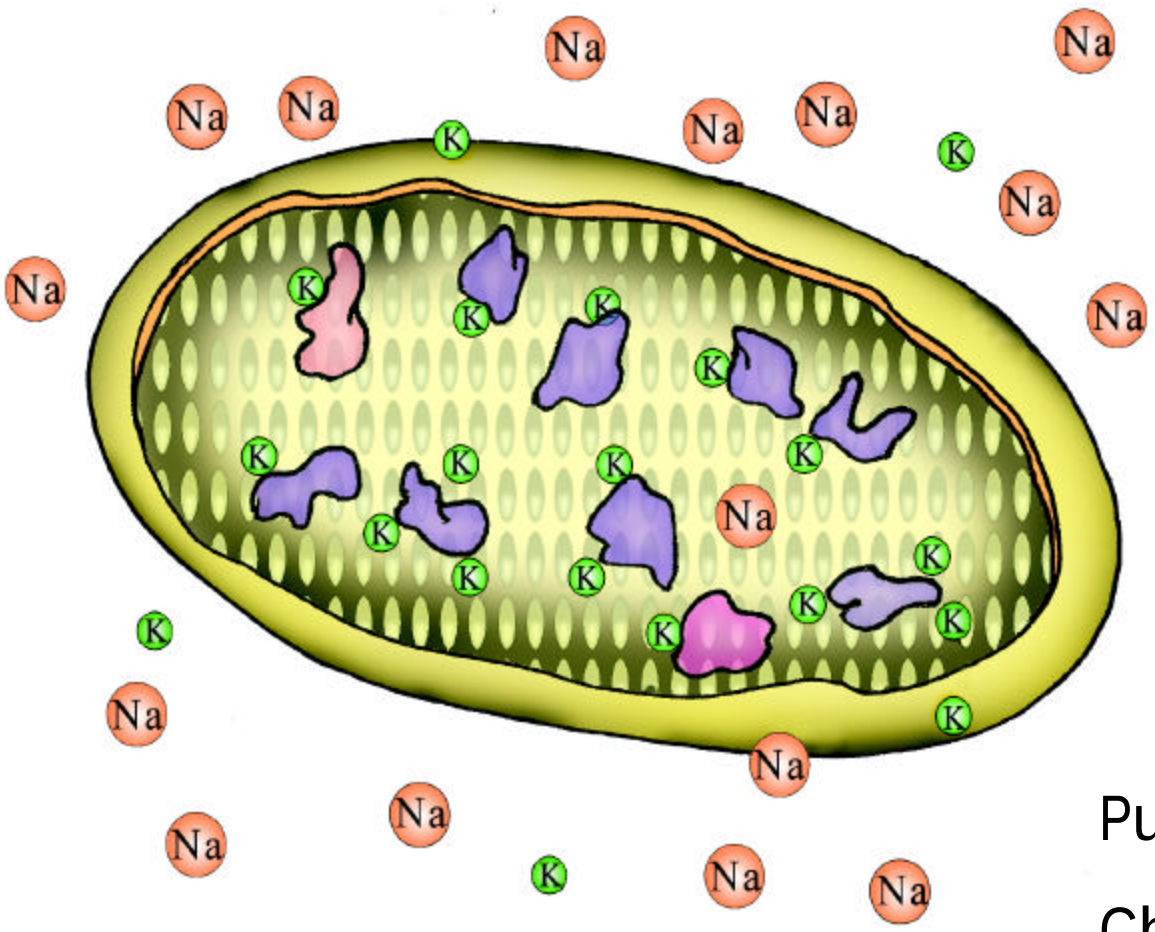
potentials in gels

(Courtesy, R. Gülch)





Summary of static features…



Pumps not required

Channels not required

Energy not required

…cell biological concepts today…

…

…

mechanisms based on channels, pumps

channels, pumps for exchange

continuous barrier req'd

dissolved solutes

aqueous sol'n

cytoplasm

standard paradigm

real cell biological mechanisms?

…

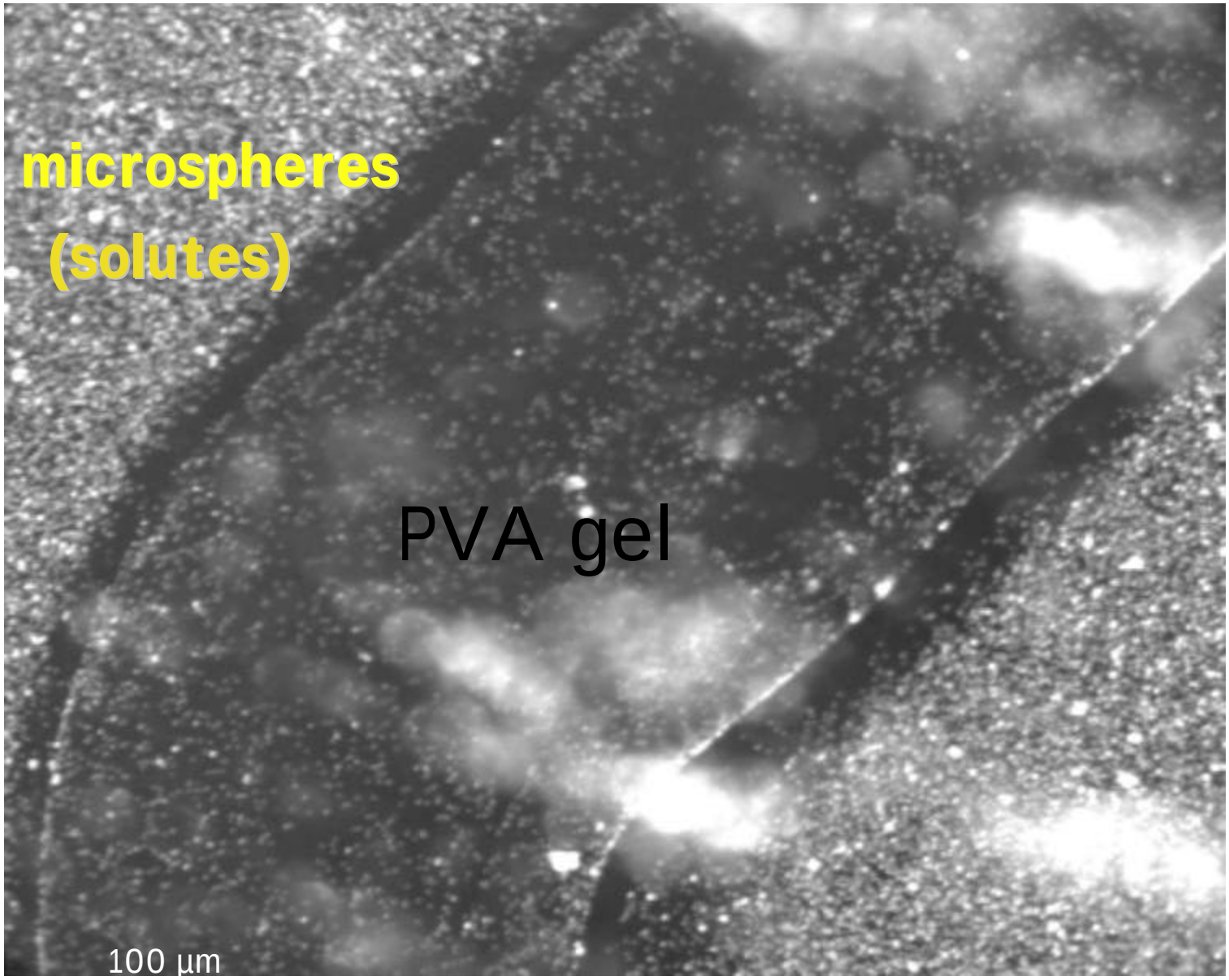
matrix adsorbs water, ions

gel matrix

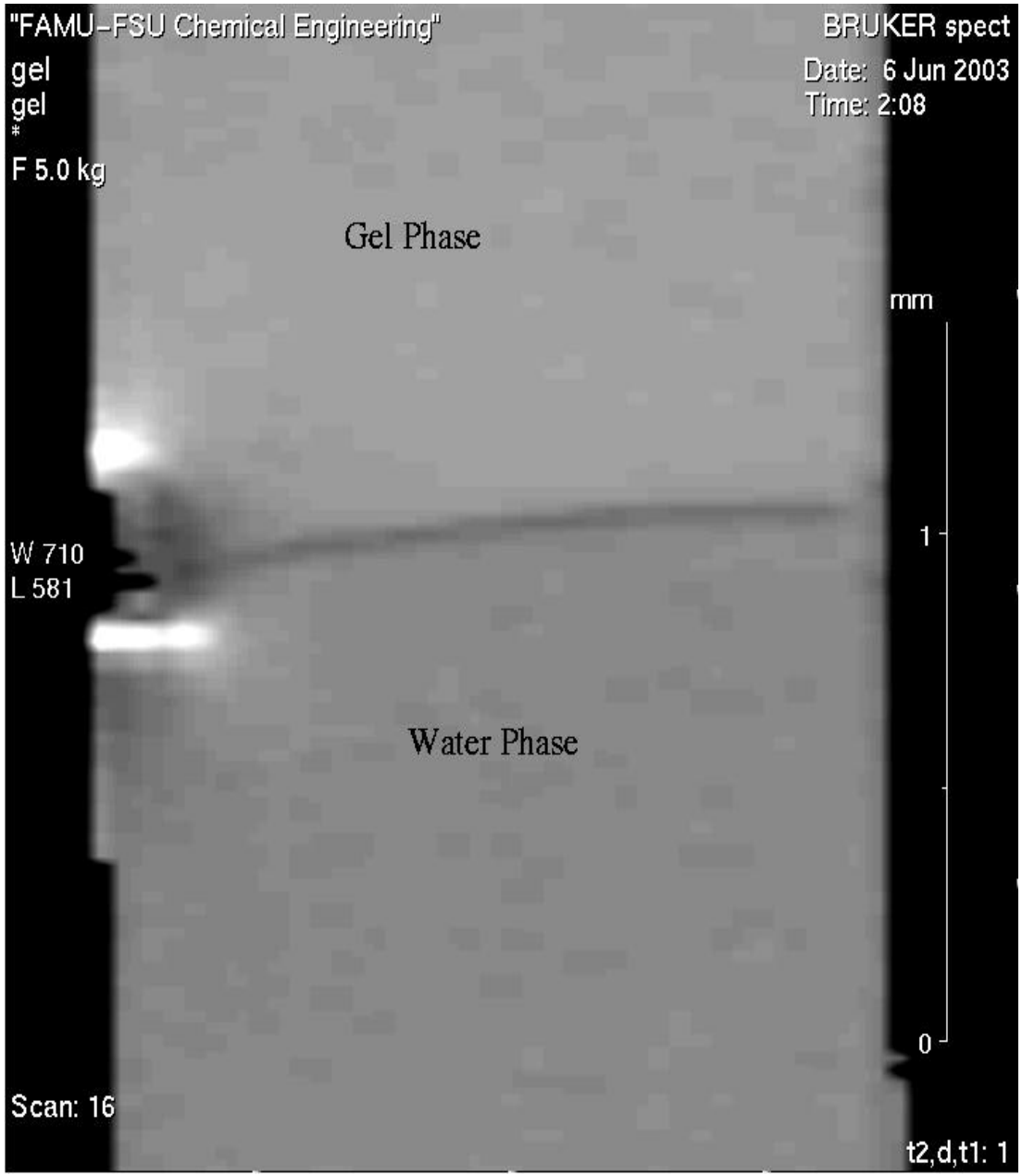
cytoplasm

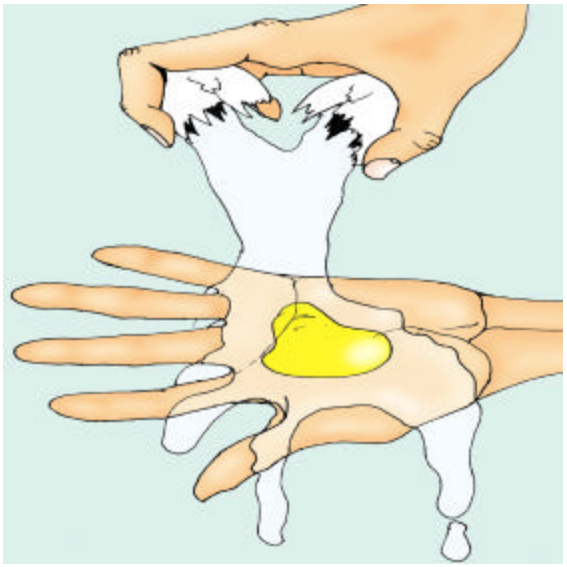
new paradigm

Structured water beyond gel surface?



Zheng and Pollack, PRE, 2003


"FAMU-FSU Chemical Engineering"
BRUKER spect
gel
gel
*
Date: 6 Jun 2003
Time: 2:08
F 5.0 kg
Gel Phase
mm
1
W 710
L 581
Water Phase
0
Scan: 16
t2,d,t1: 1

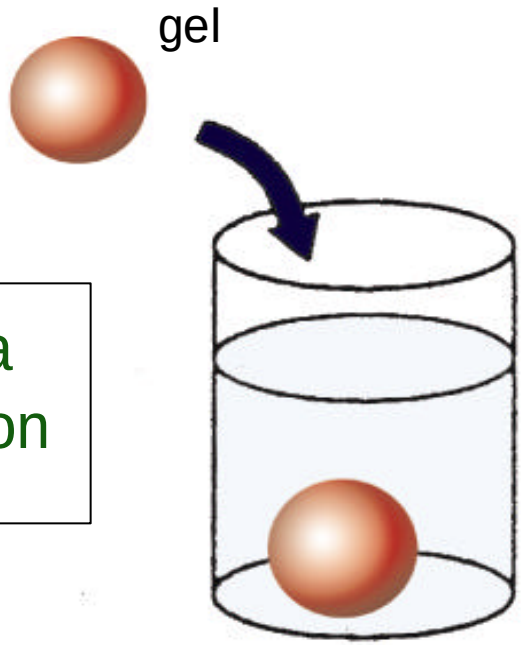


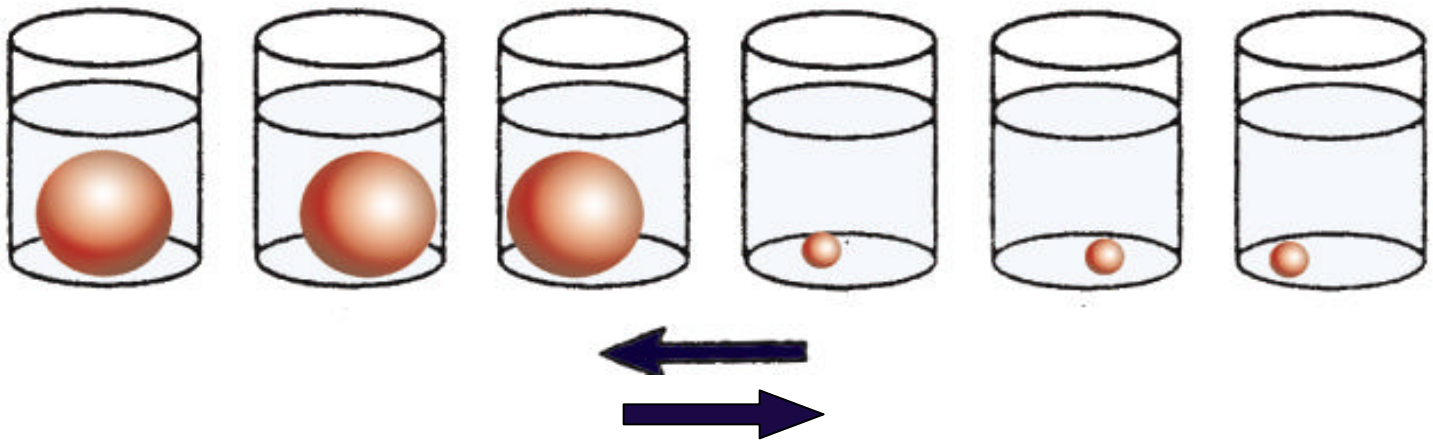
Within this “new” framework, how to approach cell function?

Do gels “act”?

phase-transition

phase-transition is a
“critical” phenomenon

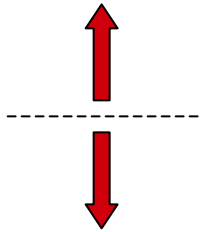




temperature, solvent composition, pH, ions, electric field, UV light, specific molecules or chemicals

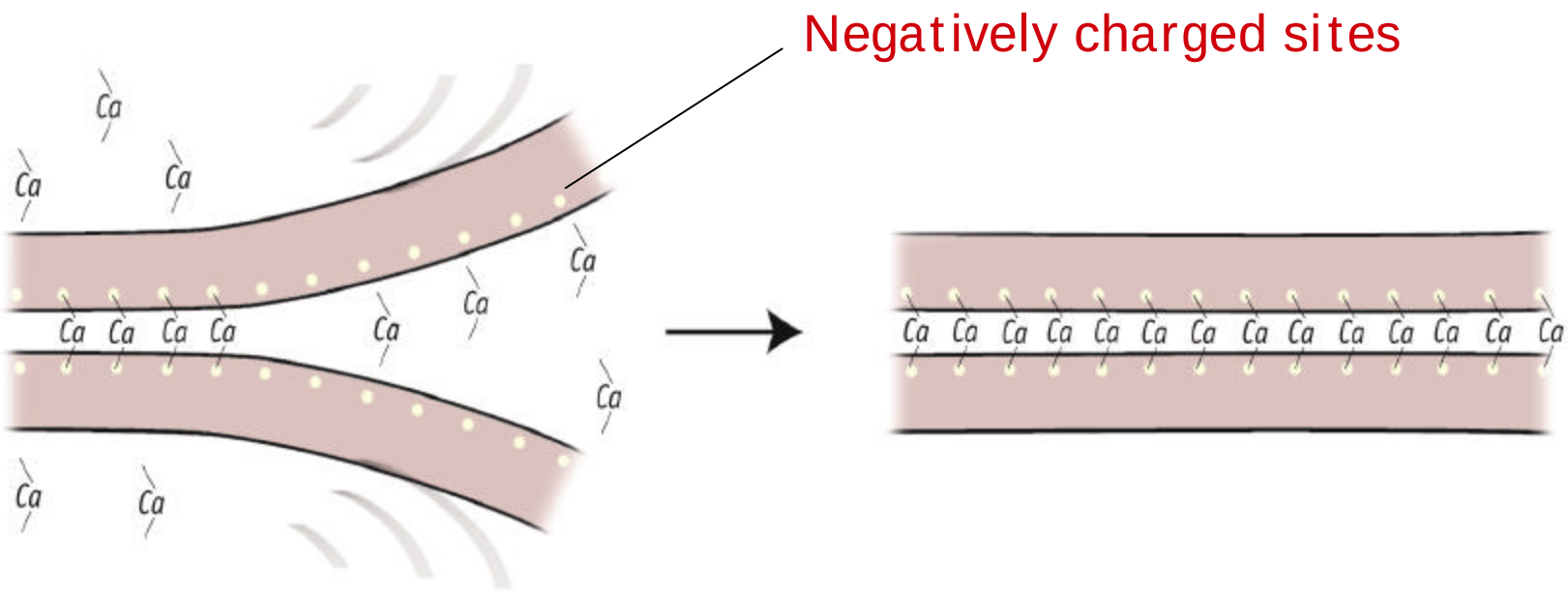
How does the phase-transition work?

Polymer-polymer affinity dominates: Condensed

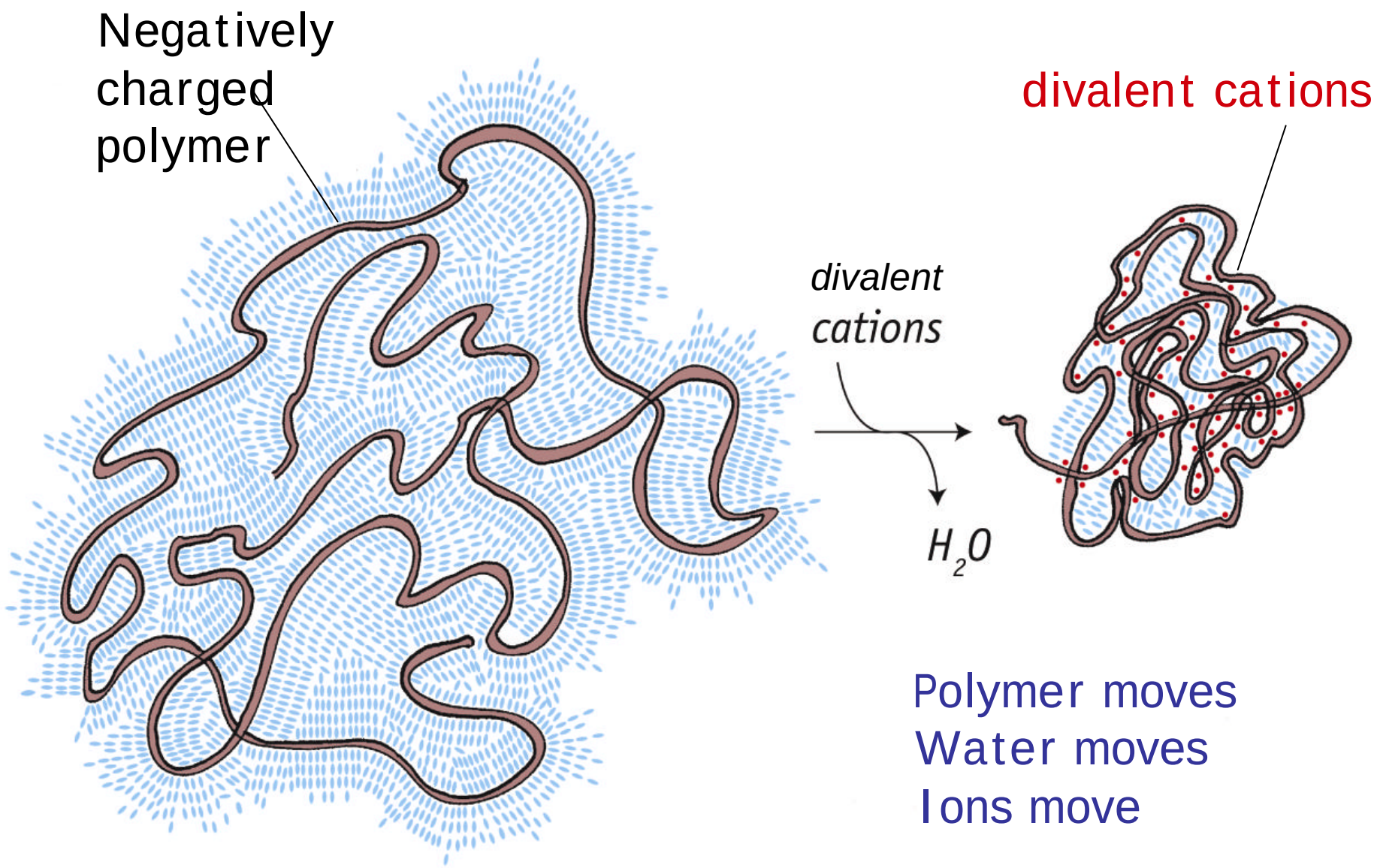


Polymer-water affinity dominates: EXPANDED

Divalent cations induce condensation

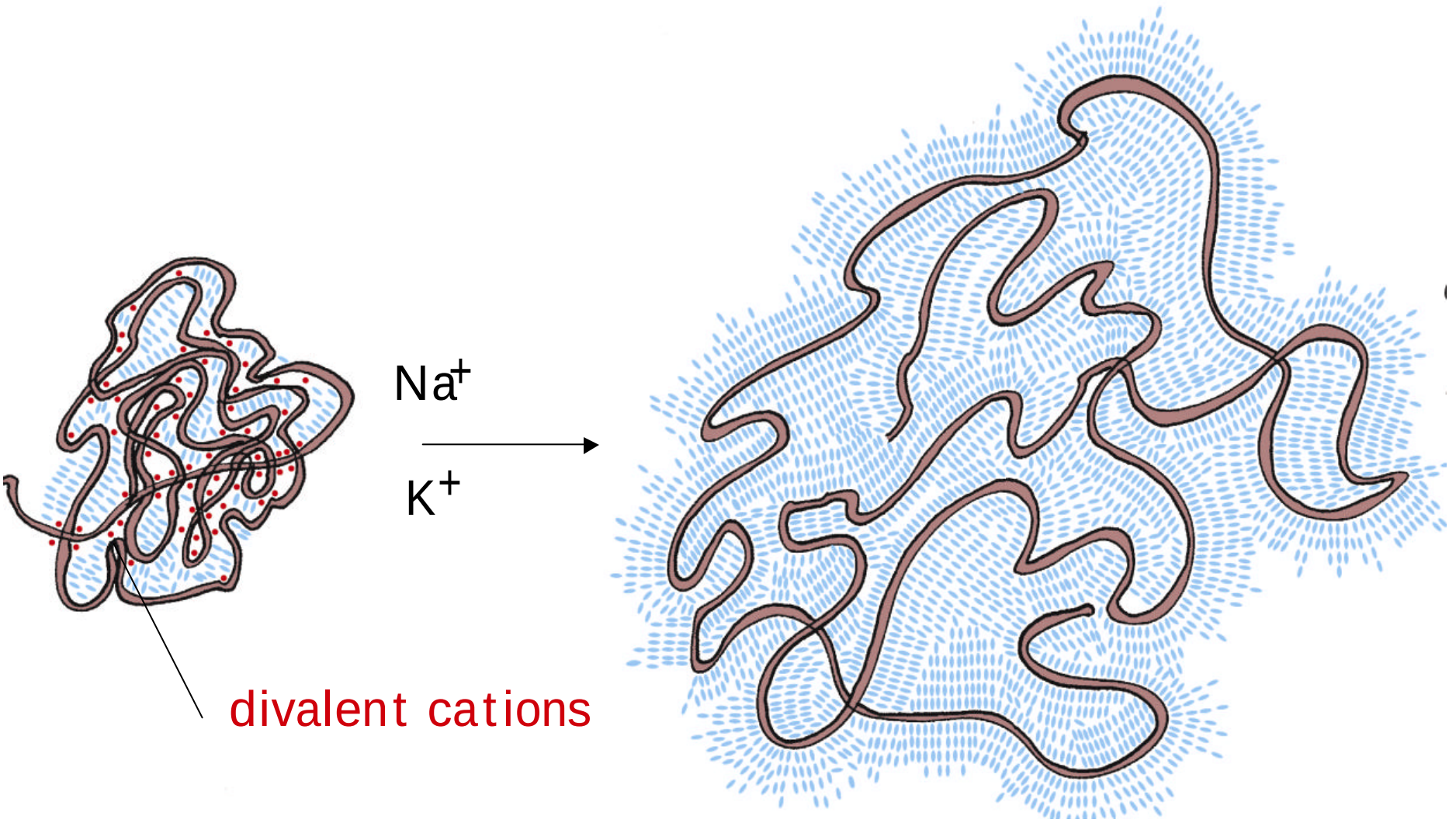


(reversed by monovalent cations)



Polymer moves
Water moves
Ions move

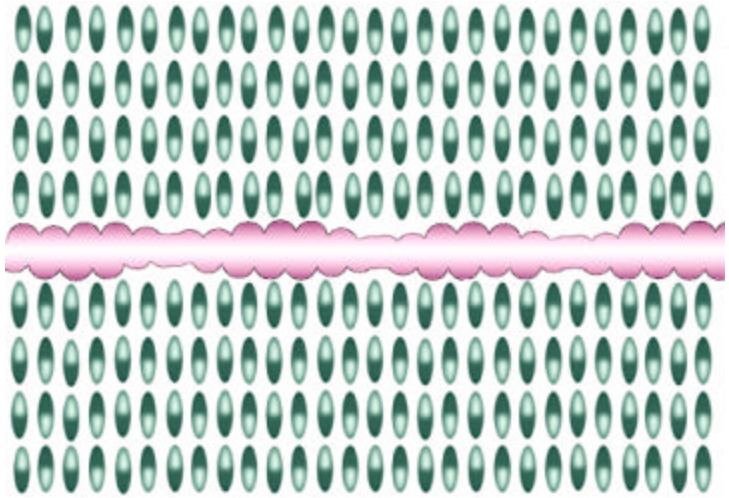
Reverse:



(Divalent cations released)

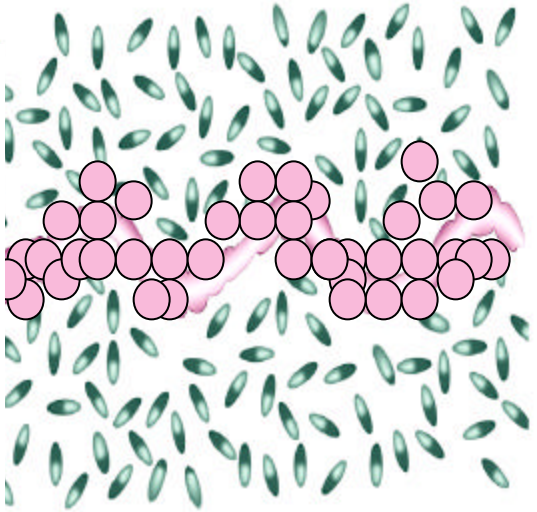
Isotropic

Linear



structured

Divalent cations cross-link polymer



melted

Hypothesis: cell action occurs by phase-transitions

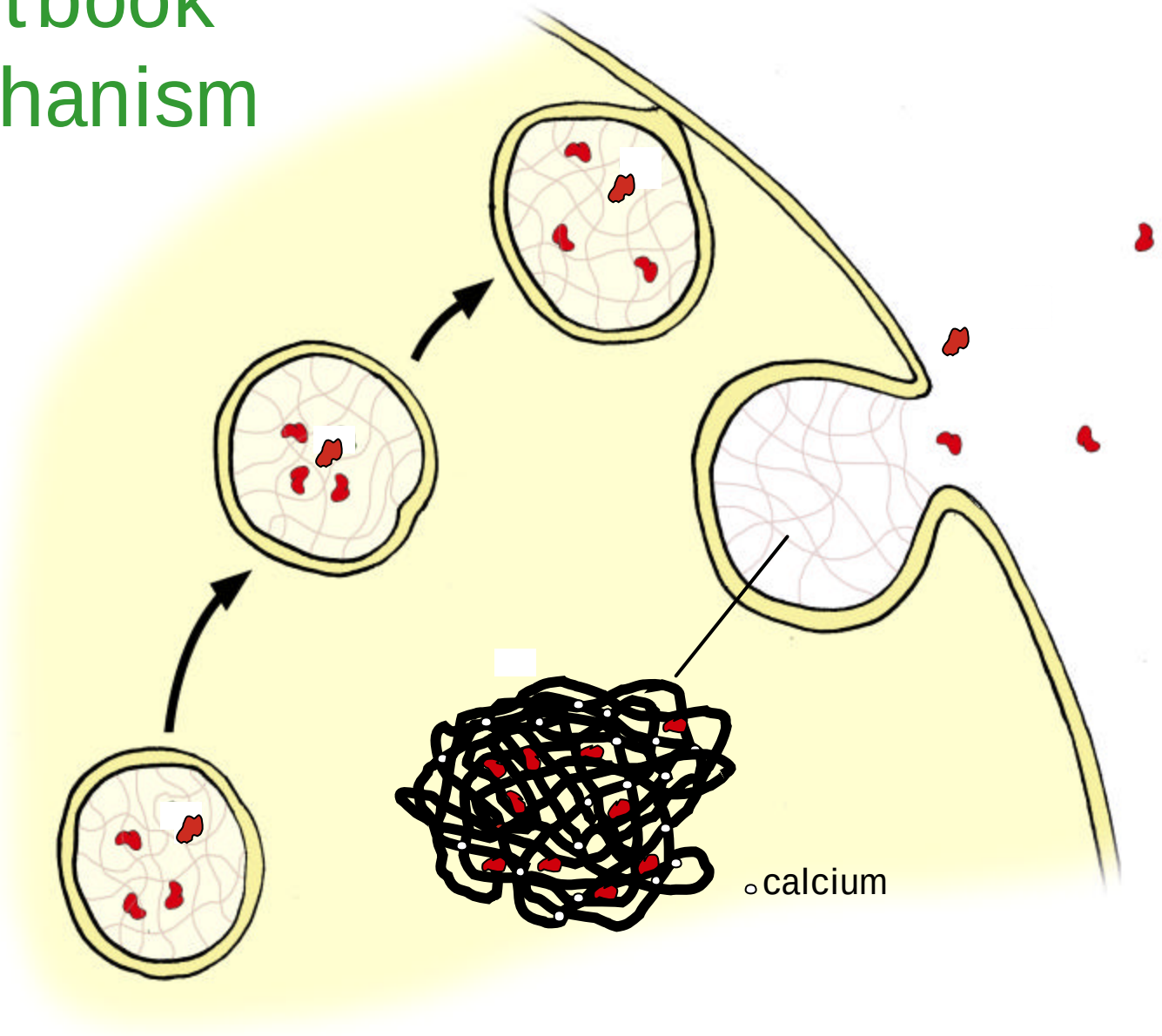
(i.e., phase-transition within each organelle)

Processes considered today

- Secretion
- action potential
- intracellular transport
- contraction
- cell motility
- ciliary and flagellar action
- mitosis
- transmembrane transport

Problem: Molecules trapped in network

Textbook mechanism

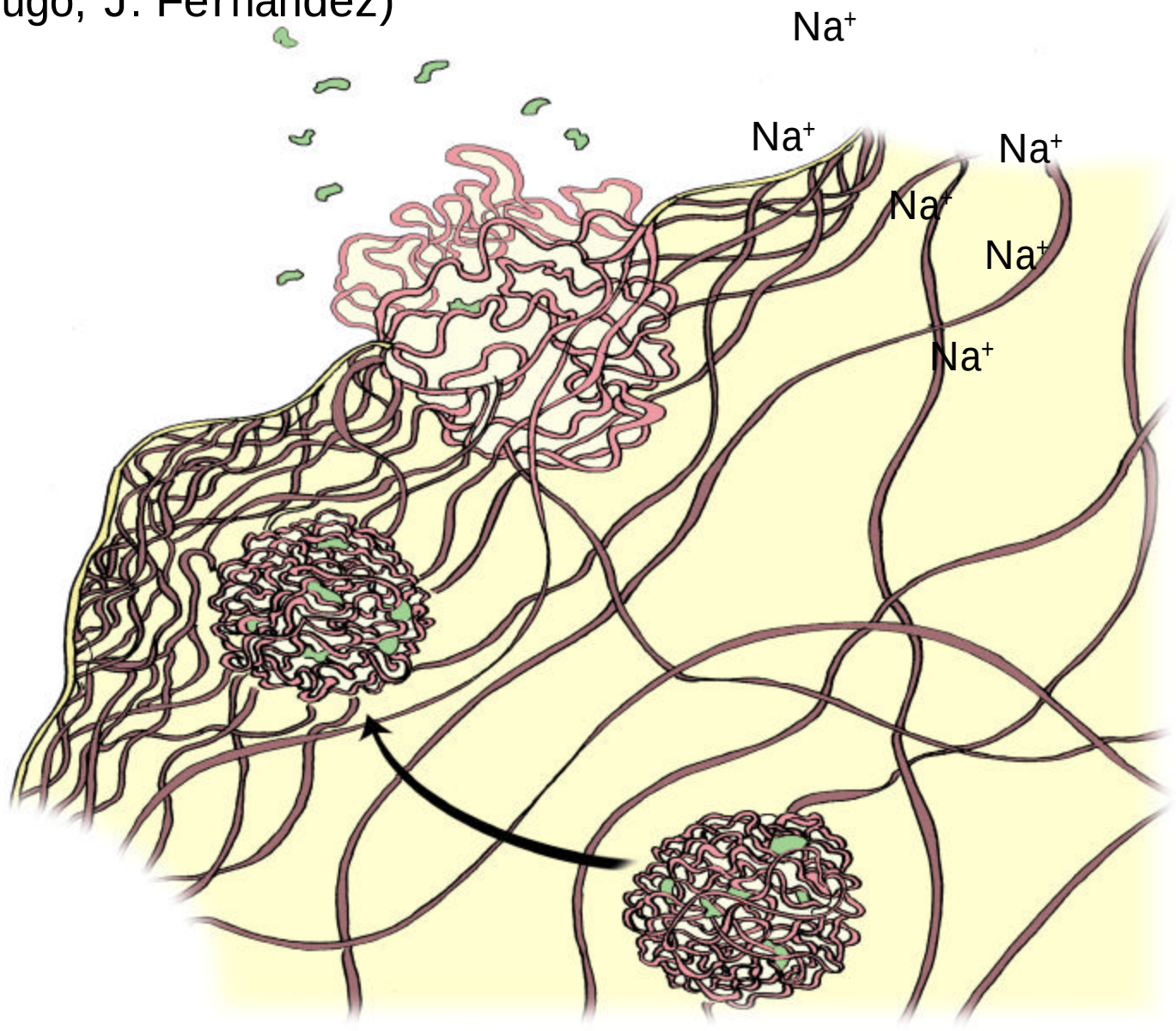


How can molecules exit?

phase transition

Proposed mechanism:

(After: P. Verdugo, J. Fernandez)



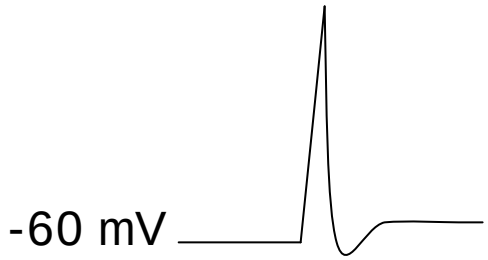
Goblet Cells Before and After UTP Stimulation

10 μm

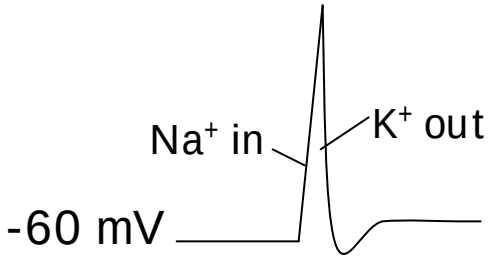
Before

After

Action potential

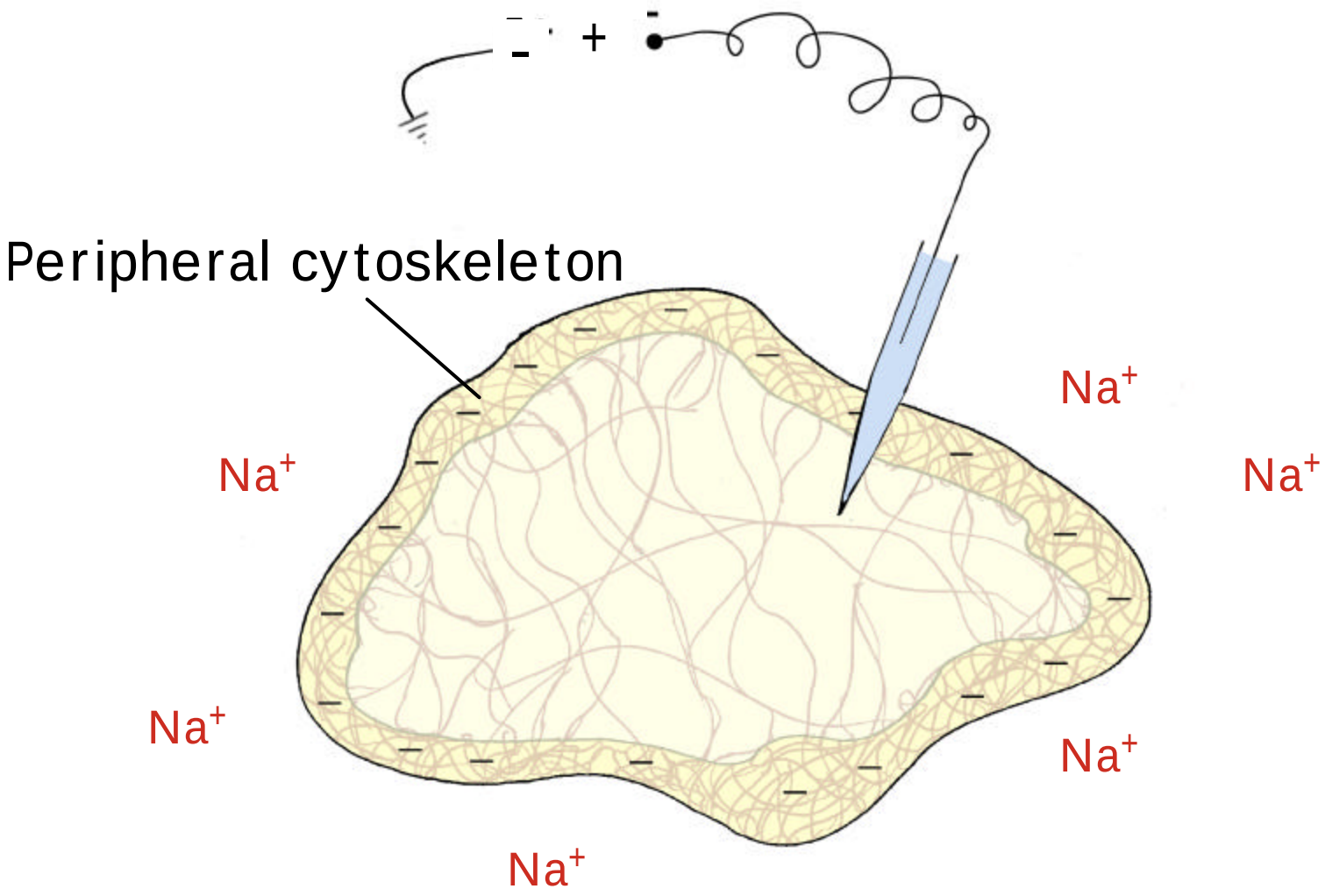


Conventional view
(Na channels; K channels)



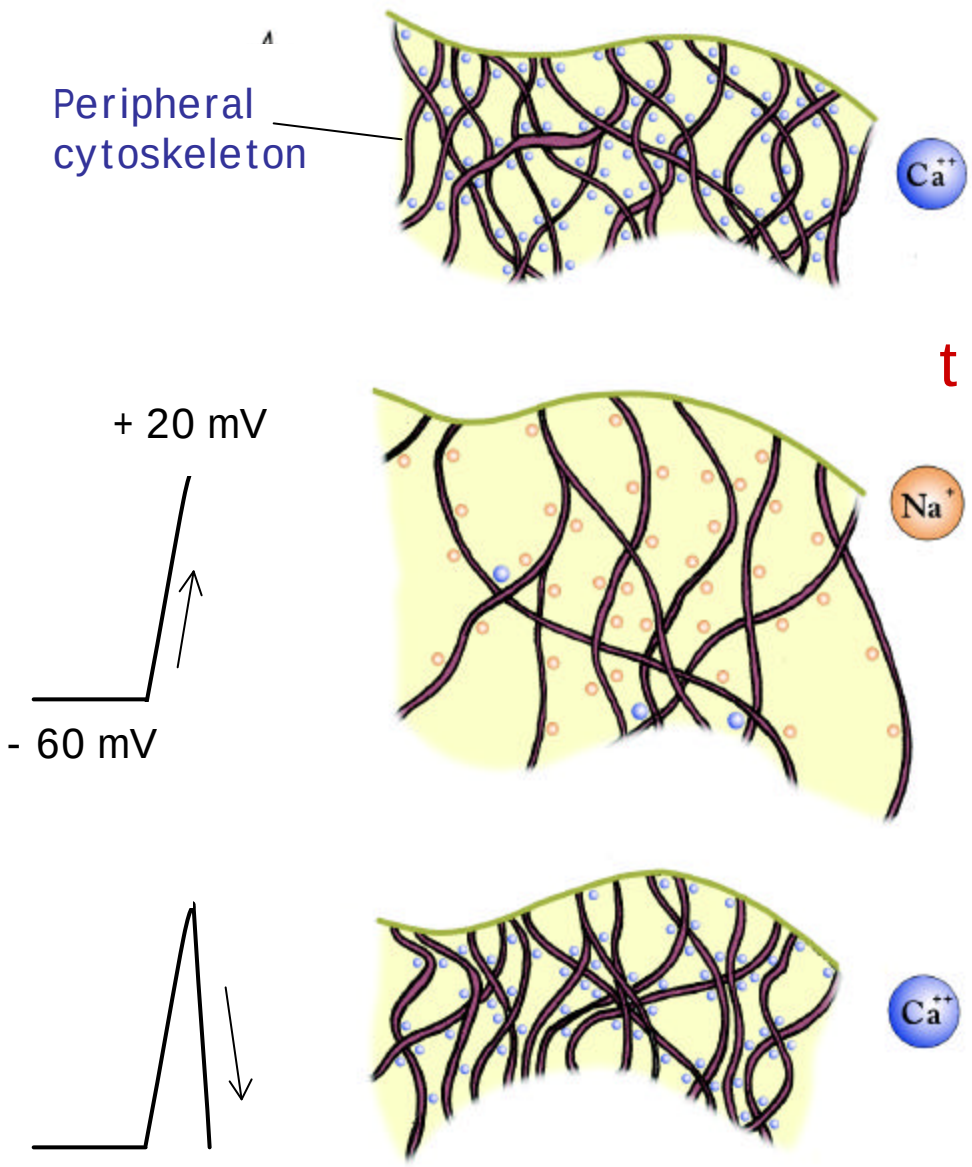
- Remove outside sodium: action potentials remain
- Remove inside potassium: action potentials remain

(numerous papers)



Na^+ penetrates into condensed gel: Another phase transition?

Tasaki [NIH], Matsumoto [Japan]:



condensed state

trigger

unzip
water entry
increased permeability
more sodium entry
positive swing of potential

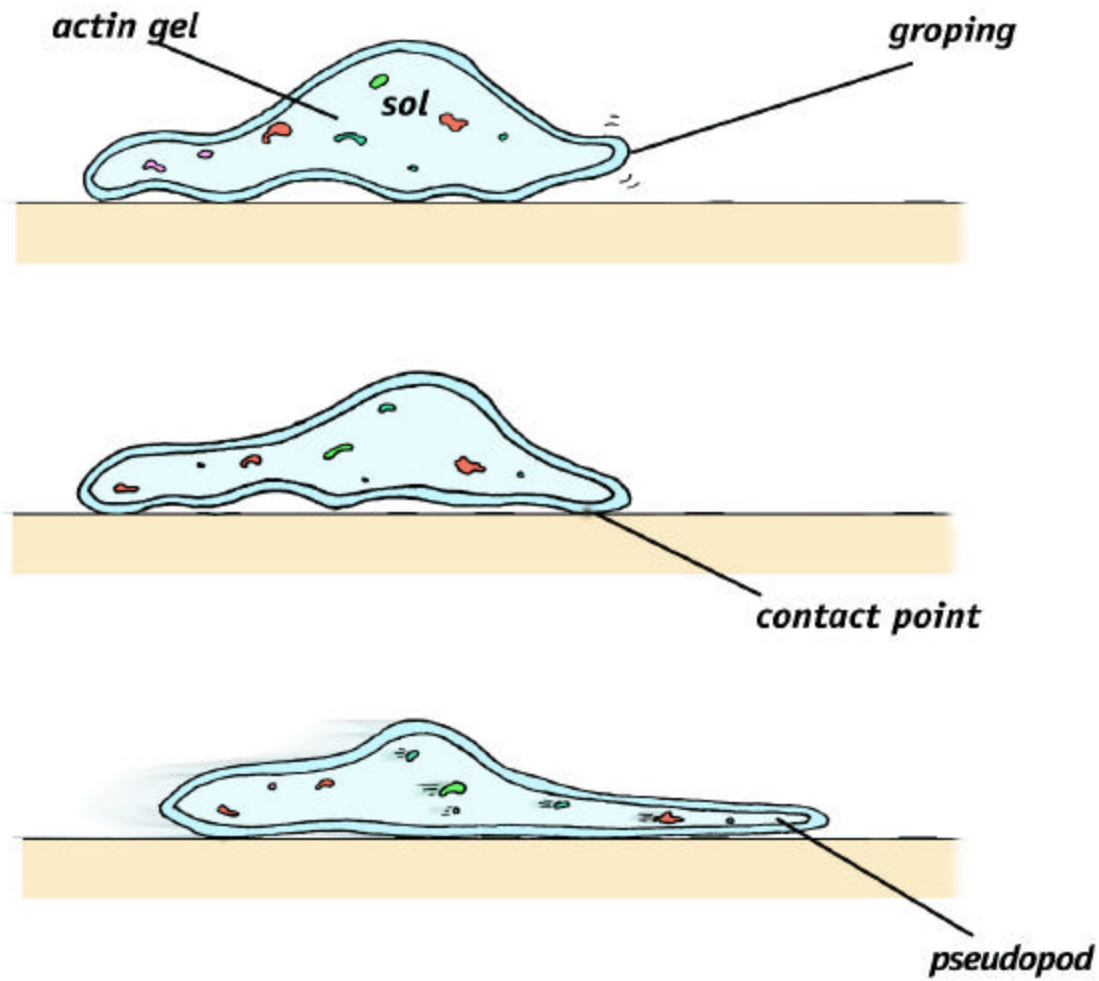
network collapses
initial condition reset

Finally…

Linearly oriented polymers

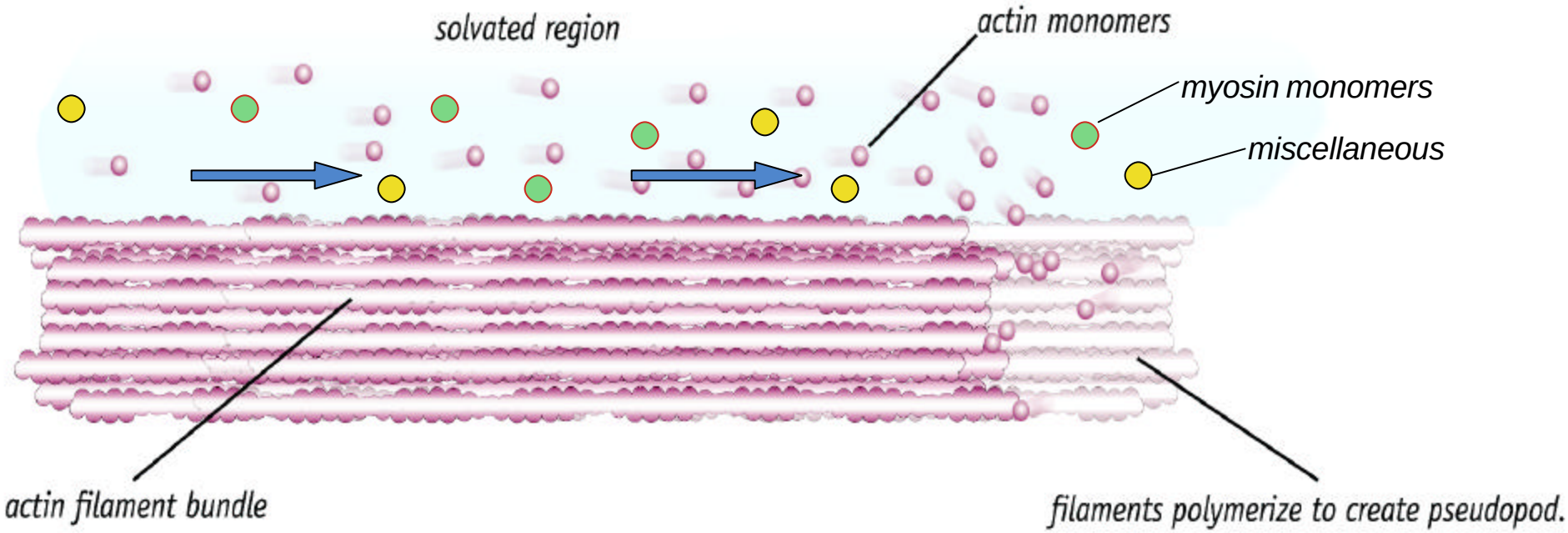
- Actin filaments (streaming)
- Muscle contraction

Cell movement



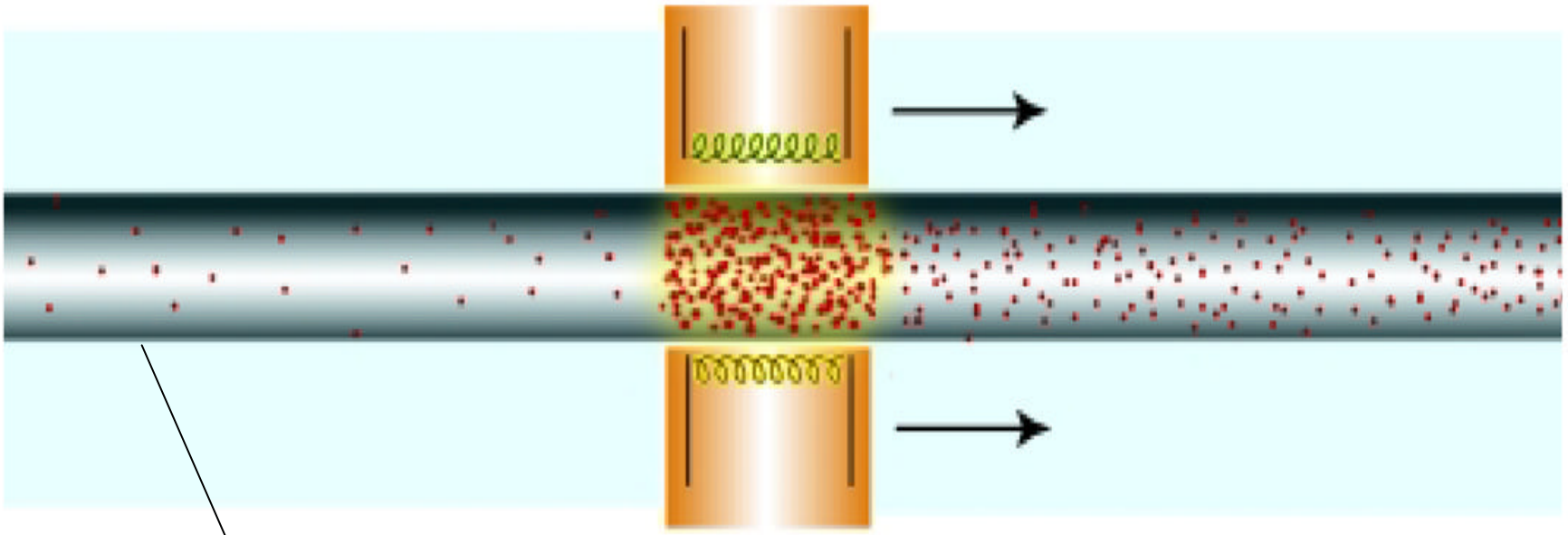
Internal mass movement

Basis…



Phase-transition mechanism?

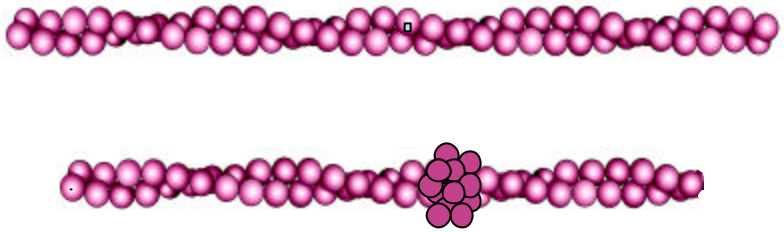
Phase transition transports solutes: *Zone refining*



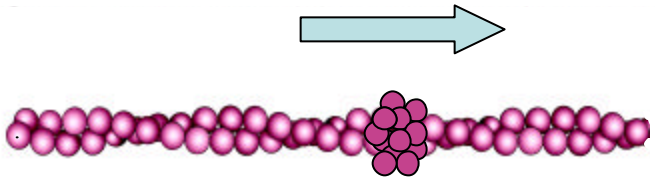
silicon, or germanium

Similar principle along actin bundle?

Requirement 1: local phase change



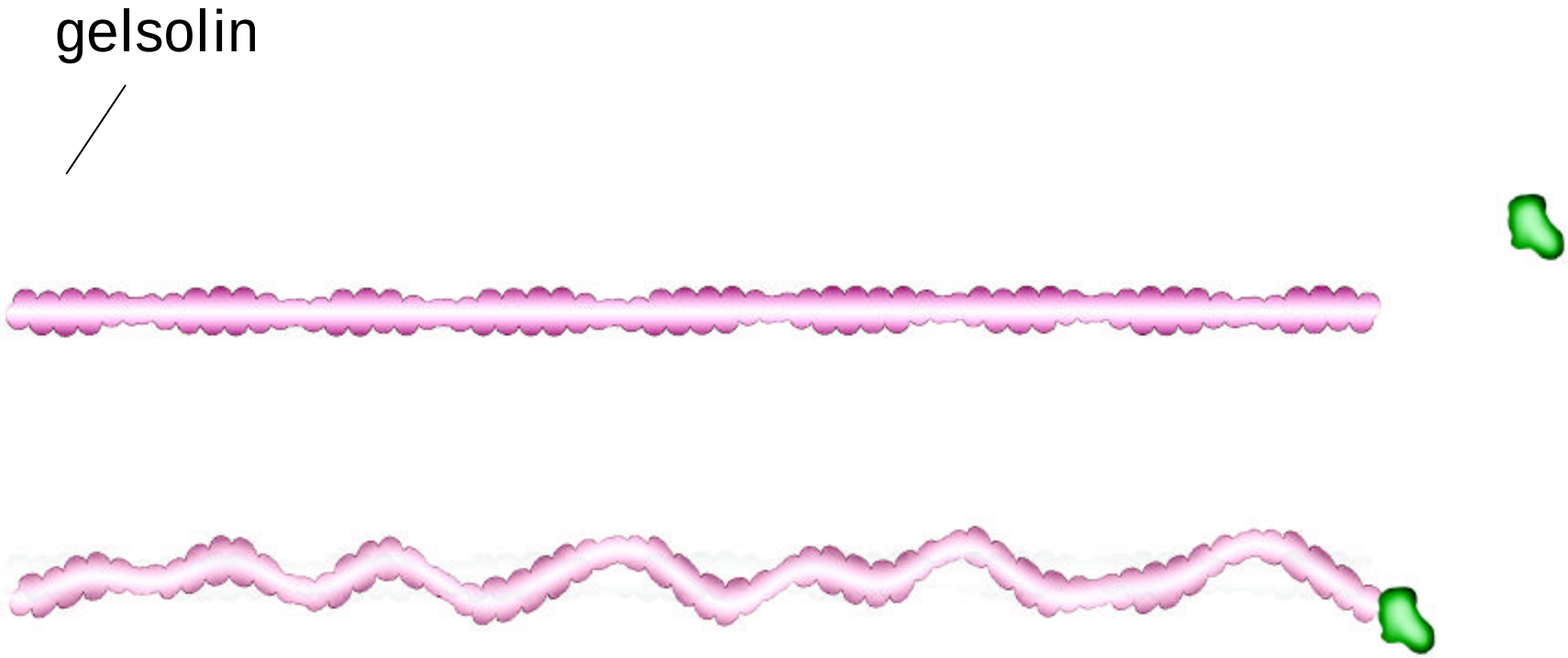
Requirement 2: propagation



Evidence for structural change in actin

- Length changes in streaming bundles, ~15% (Kamiya)
- Actin intra-monomer spacing changes 17% - fluorescence energy transfer (Miki & Koyama)
- Actin repeat changes by ~15% - X-ray diffraction of actin-profilin crystals (Schutt and Lindberg)
- Monomers rotate on myosin exposure - fluorescence polarization (Yanagida & Oosawa)

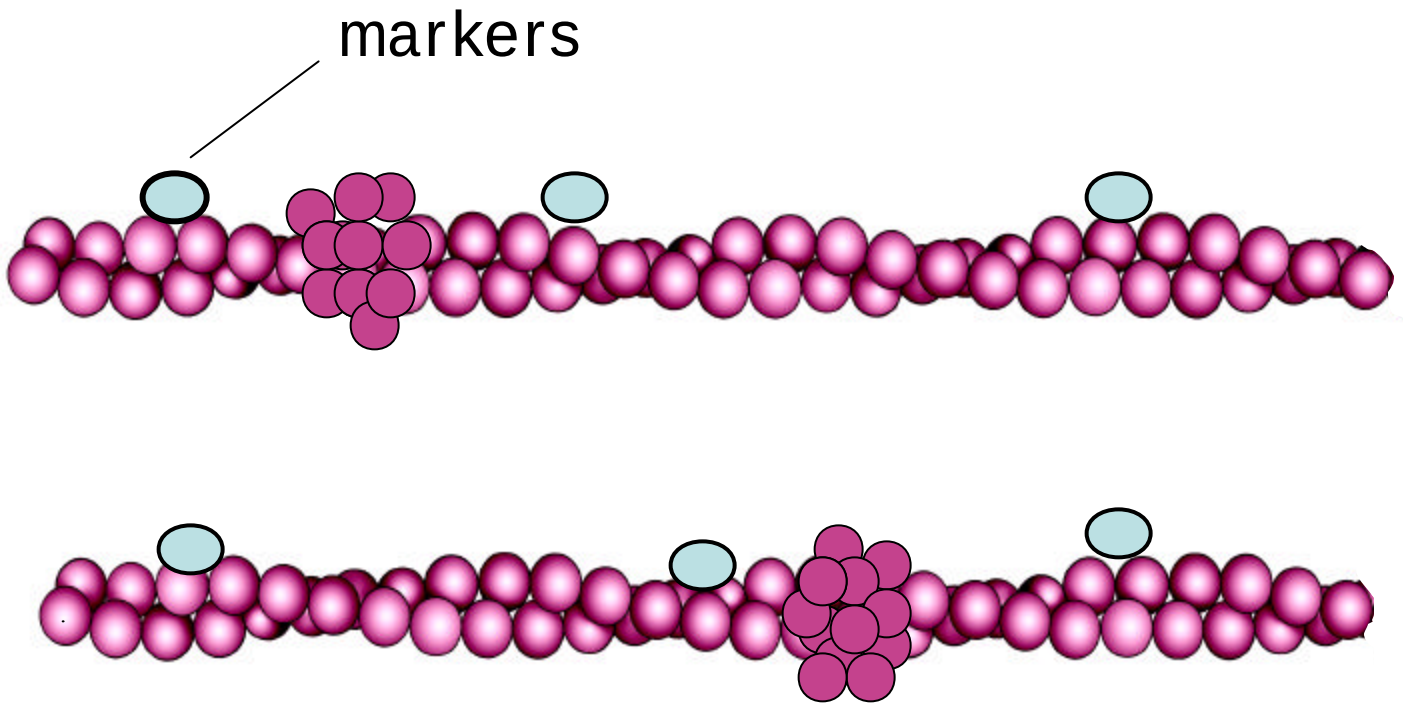
Evidence for propagation (#1)



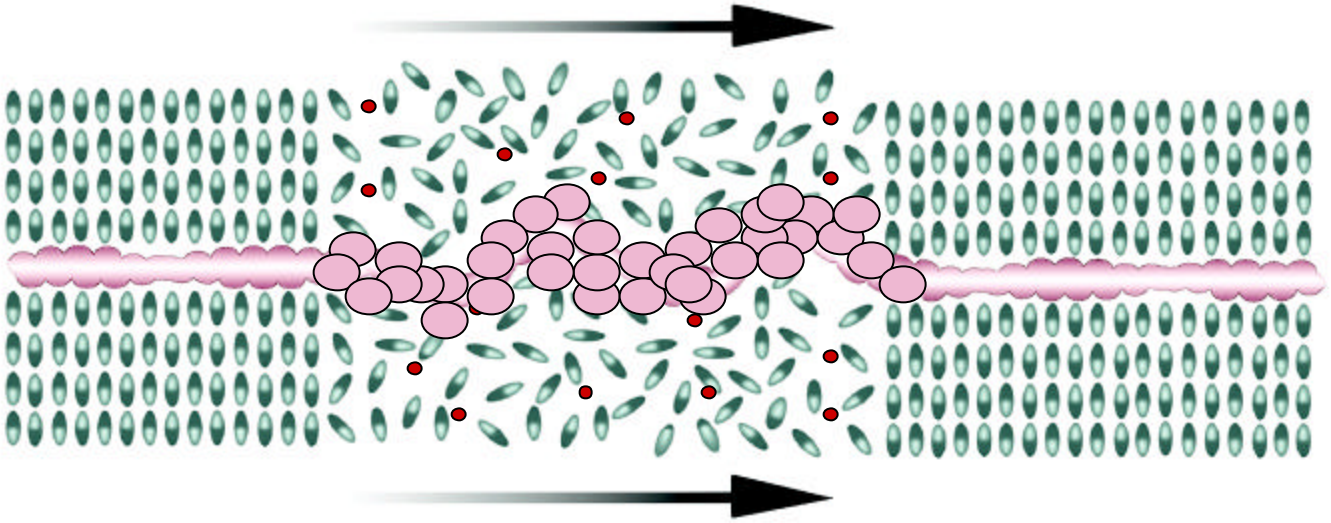
(Prochniewicz et al., 1996)

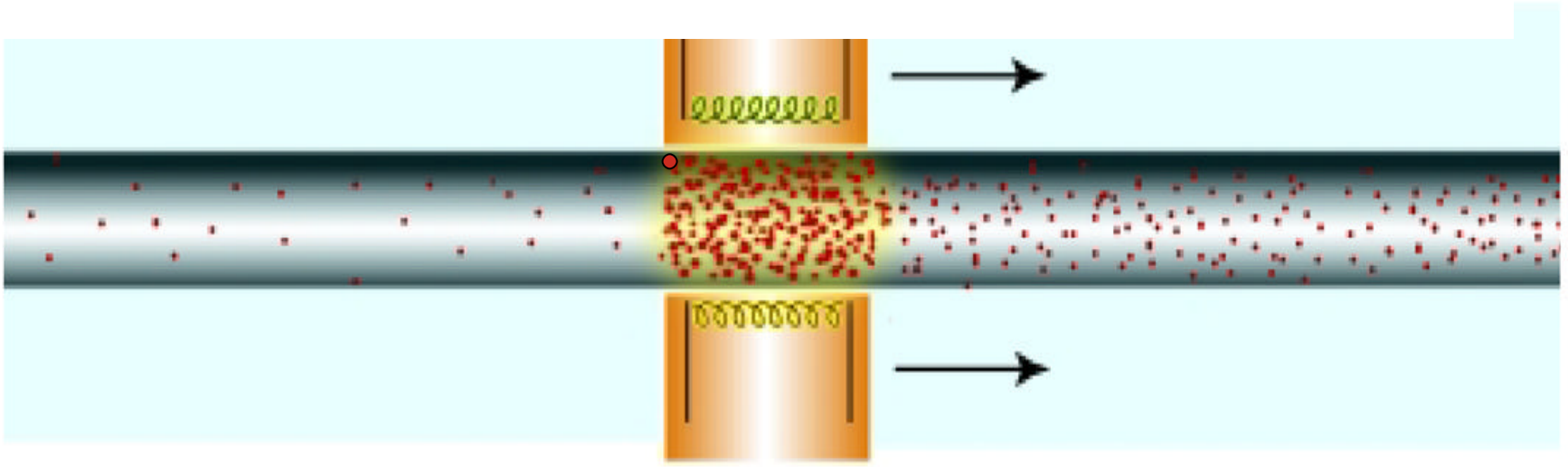
Evidence for propagation (#2)

Actin filament translating over a bed of myosin



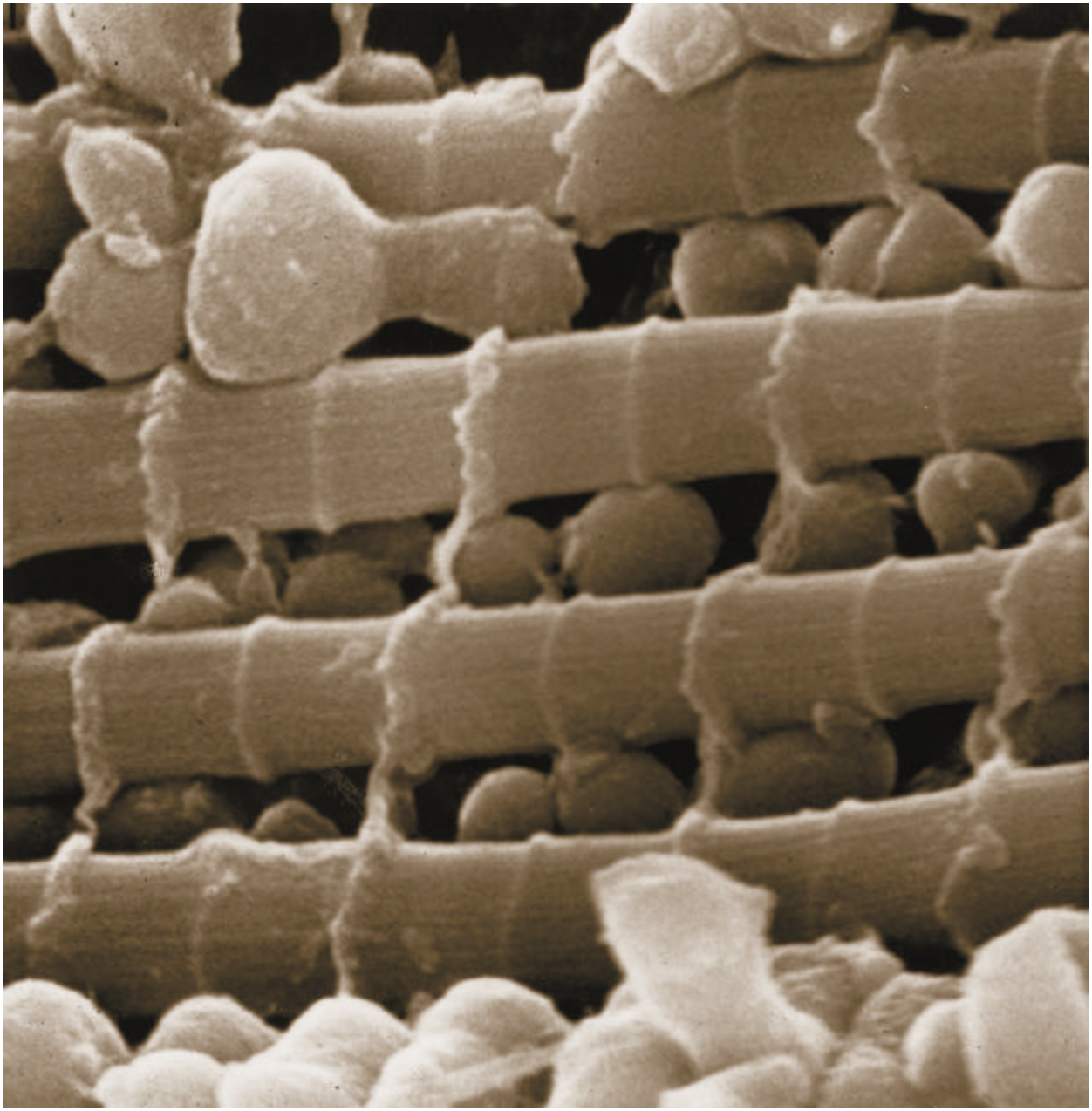
Hatori et al. (1996, 1998)



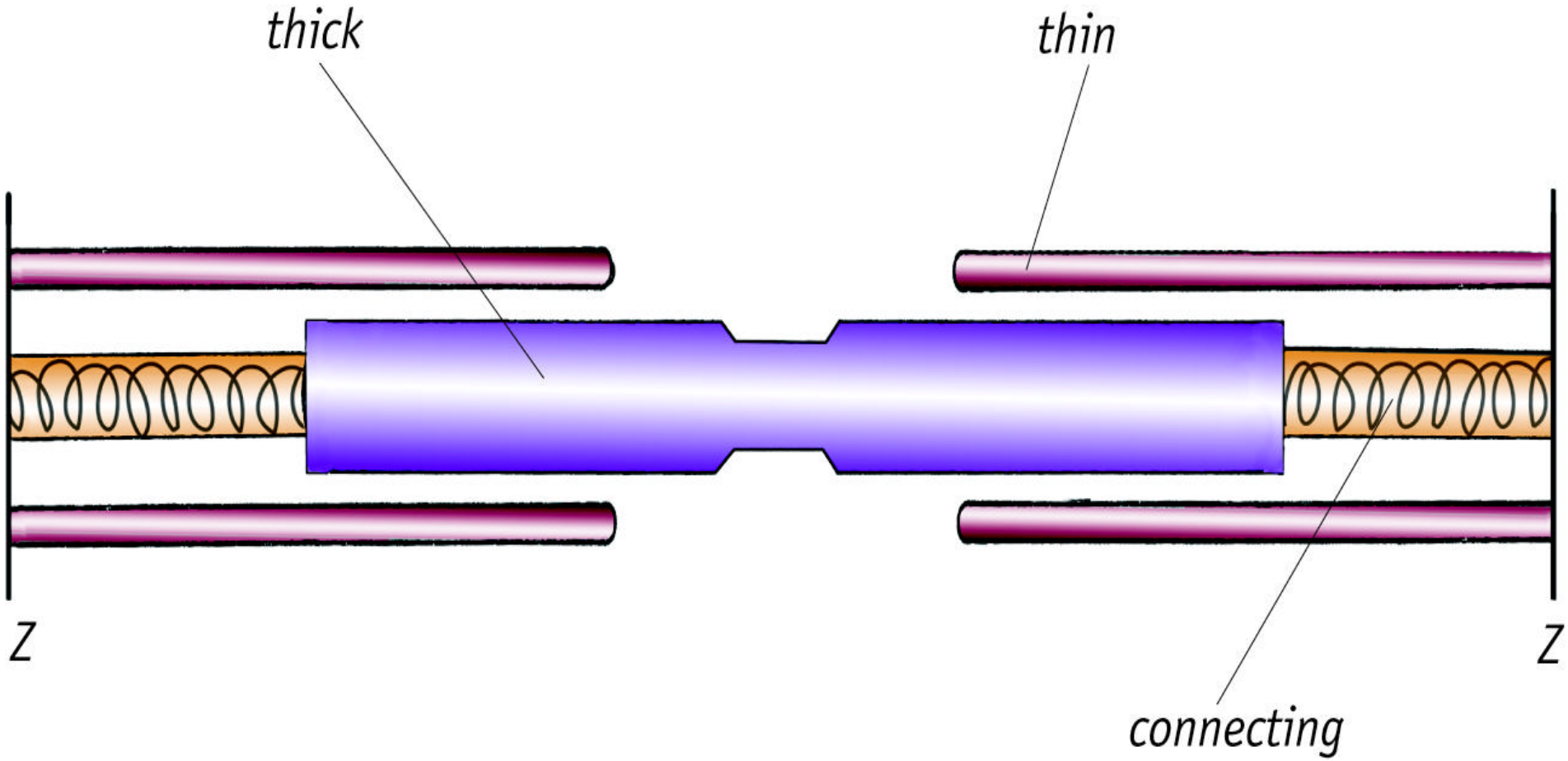


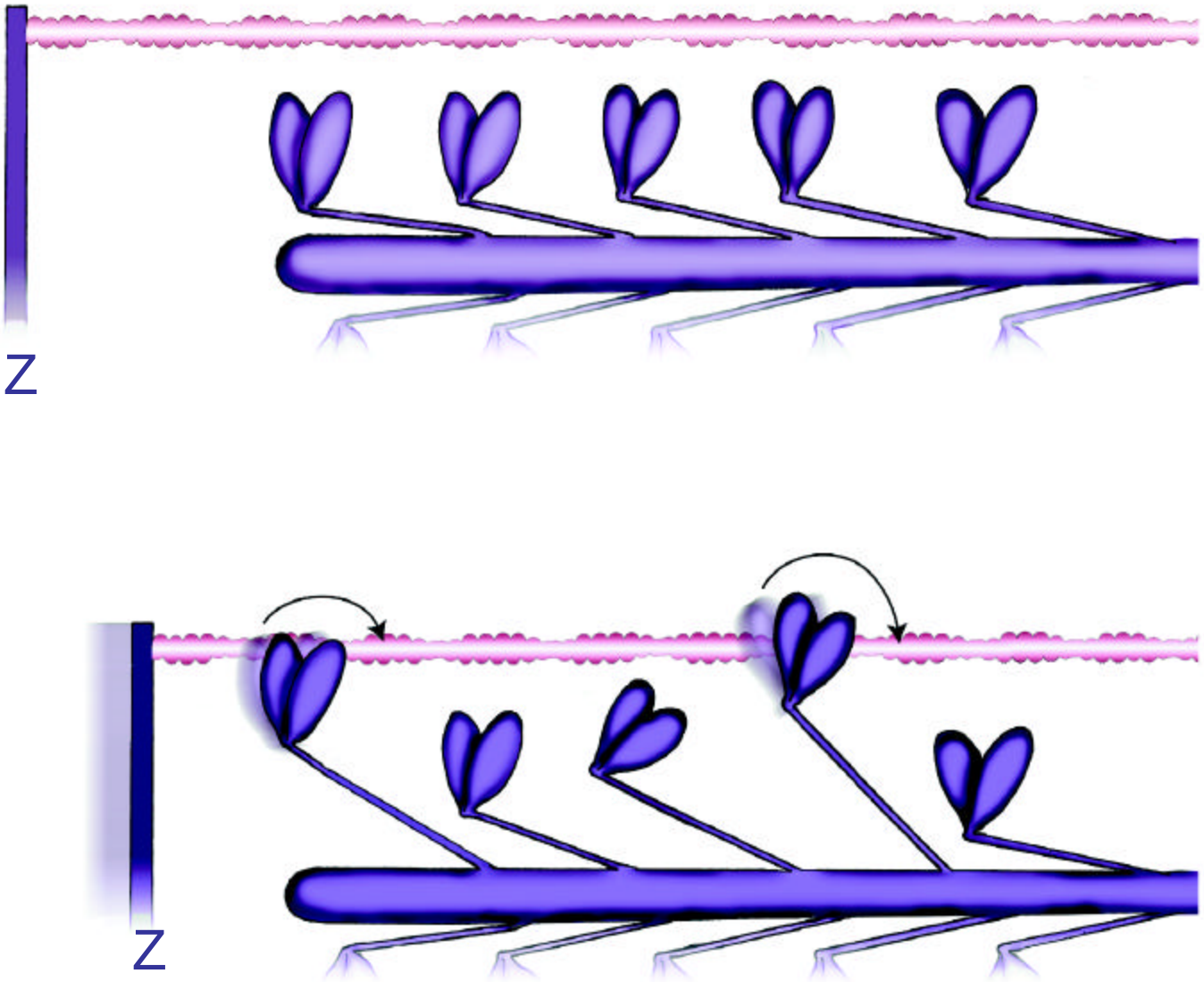
melt zone

Muscle Contraction

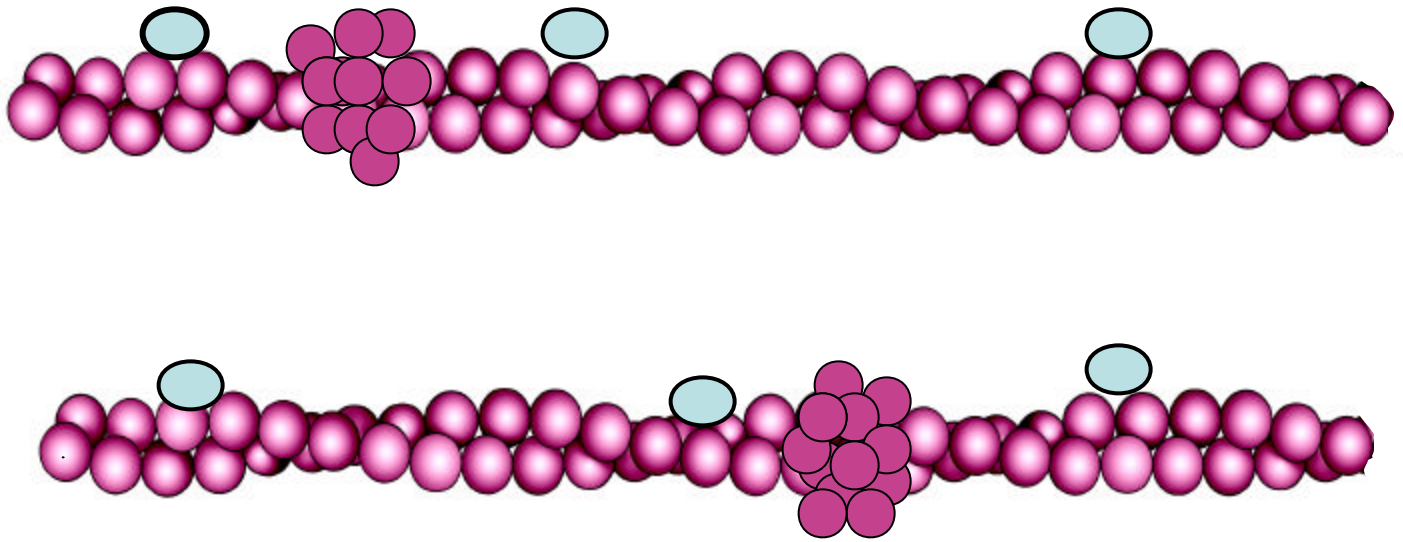


Contractile filaments

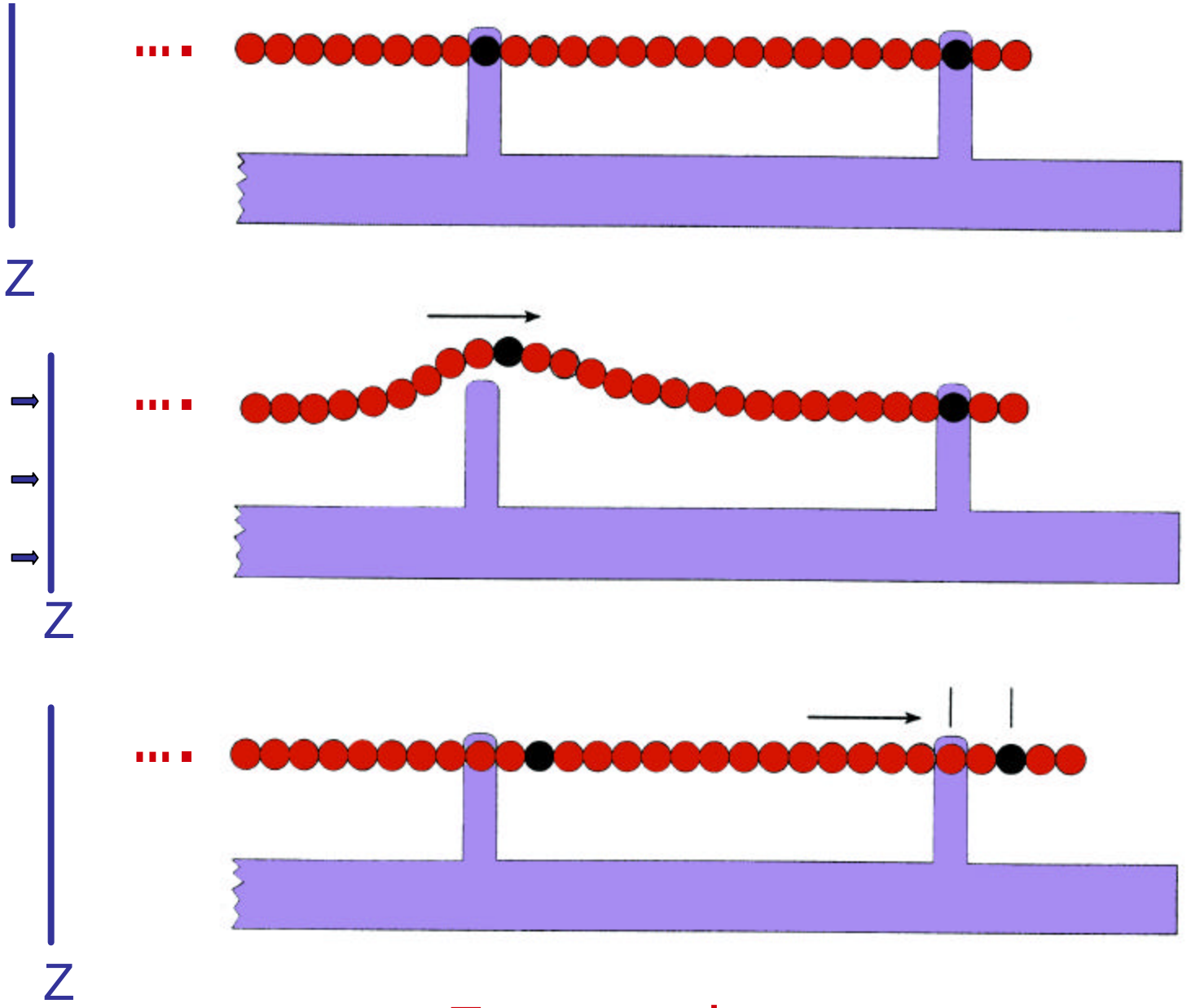




cf. Iwazumi, Schutt and Lindberg, Oplatka, Pollack

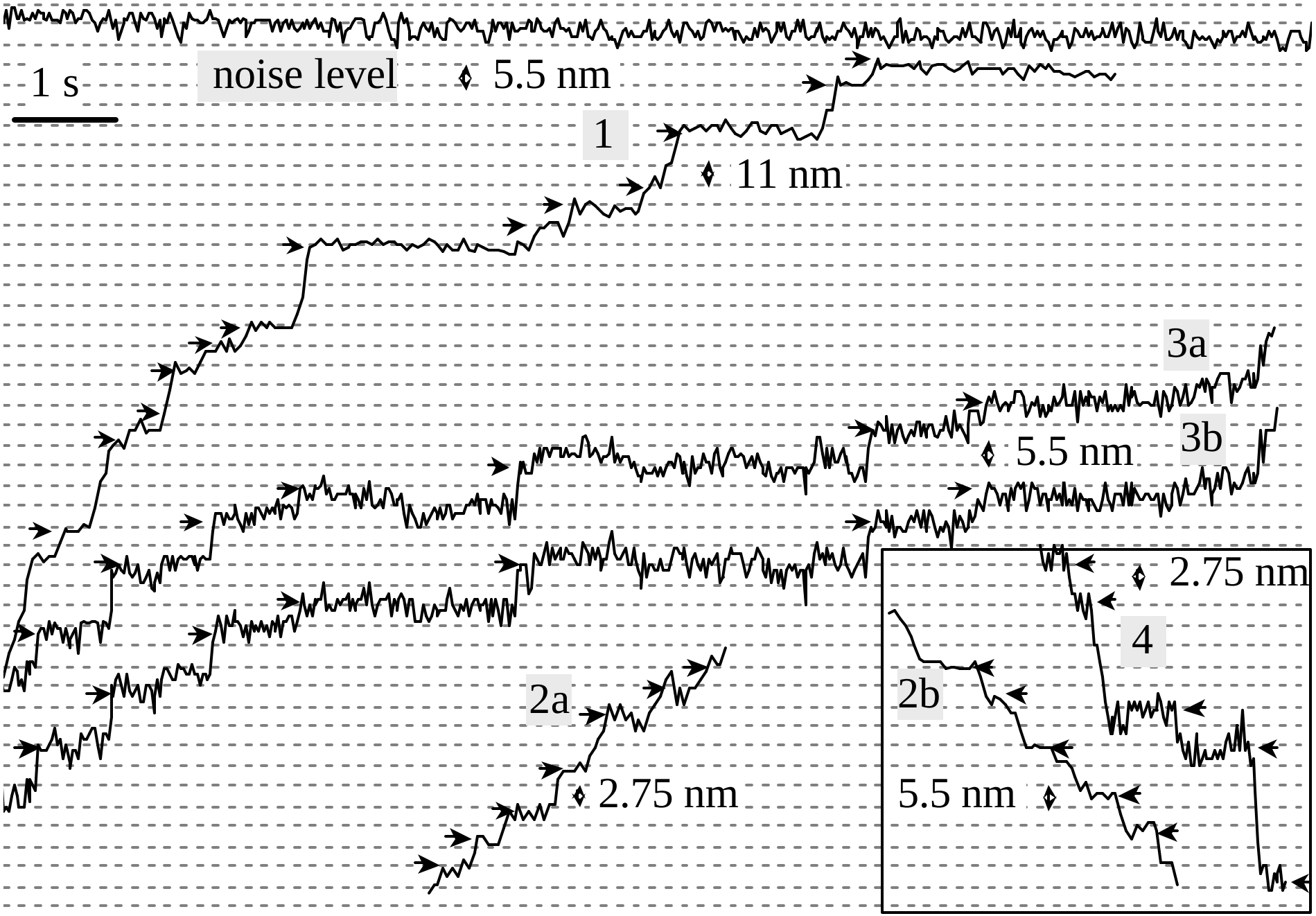


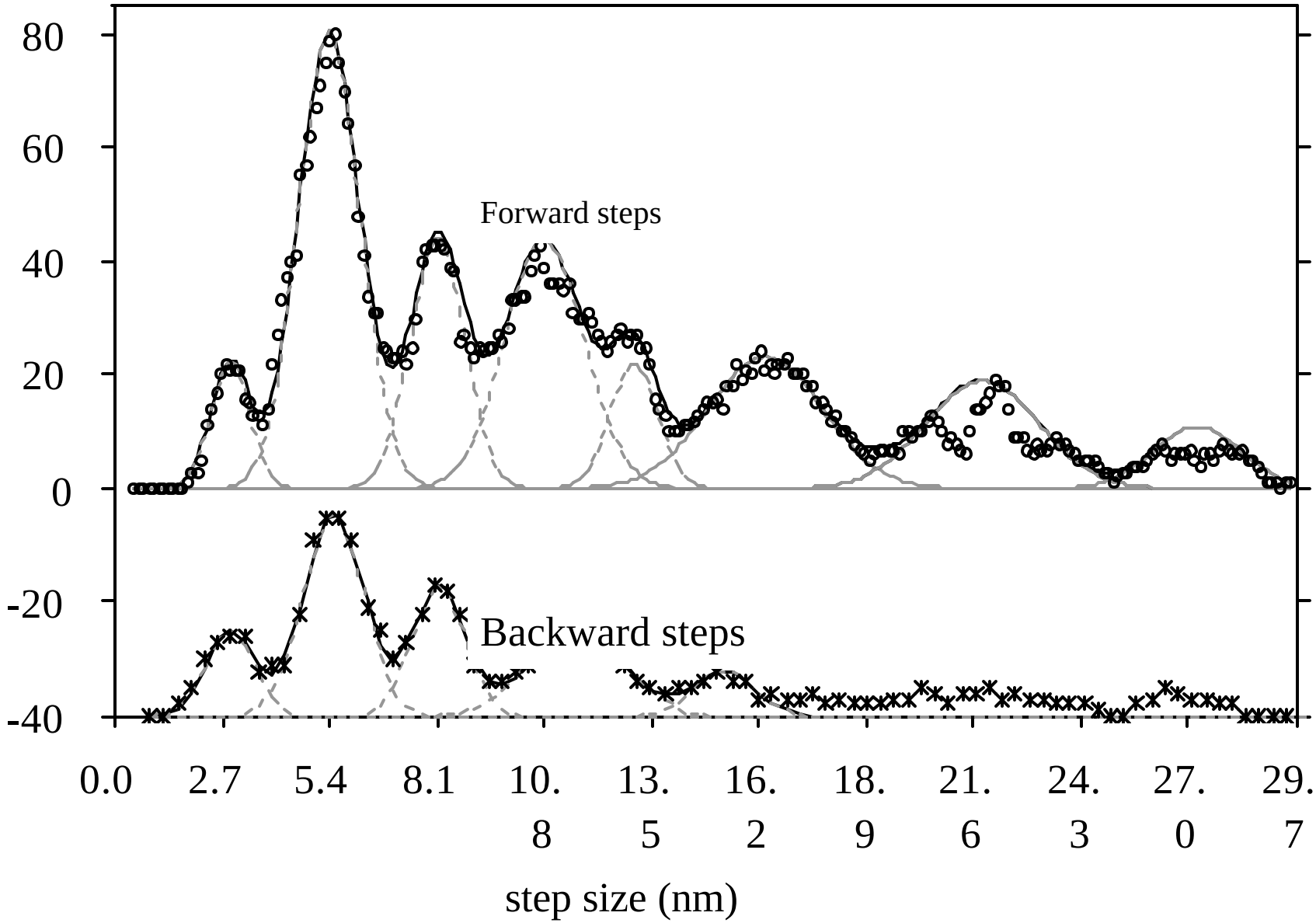
Propagating phase-change mechanism?



Expectations: one step per wave

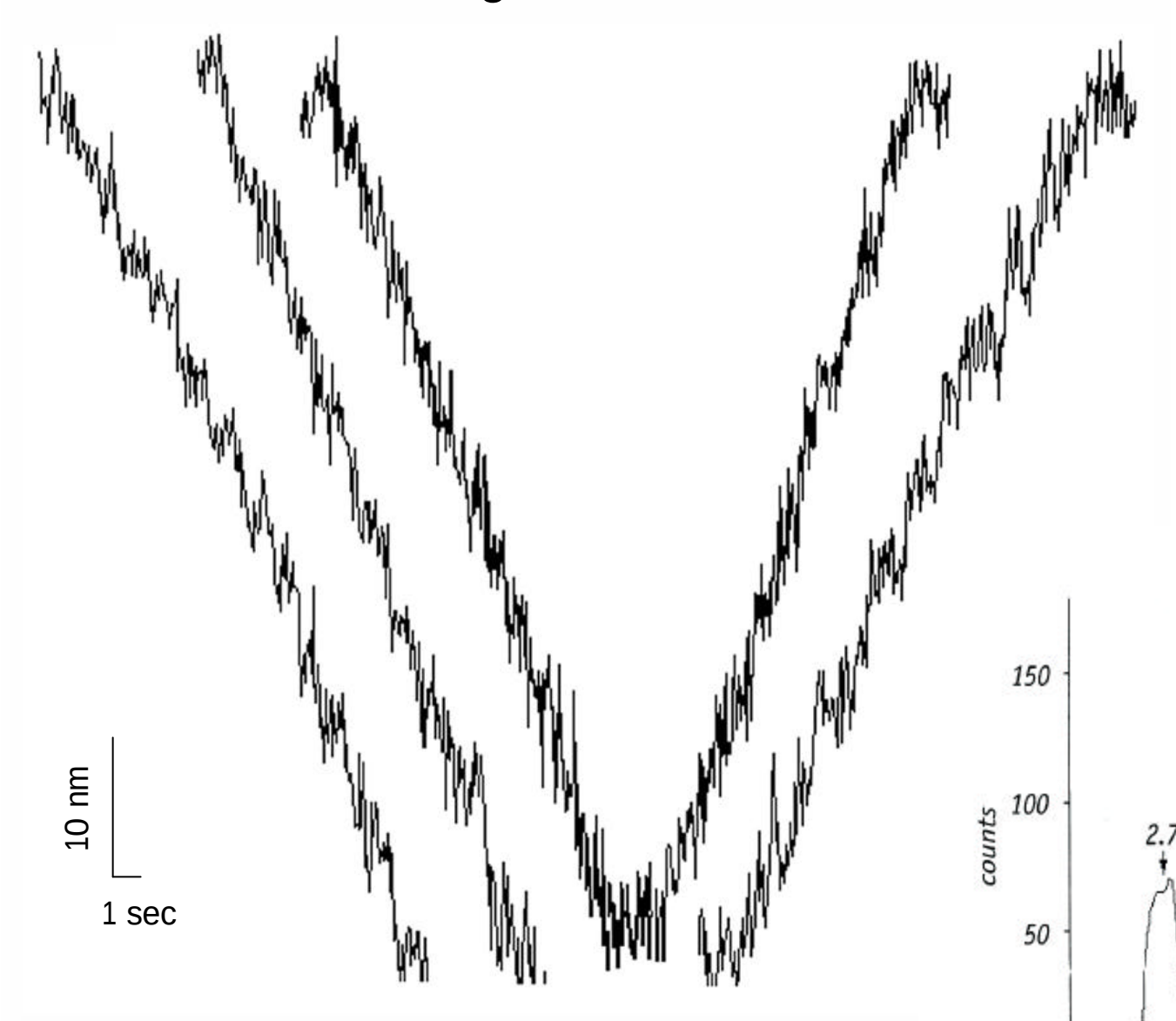
step size *n* x 2.7 nm

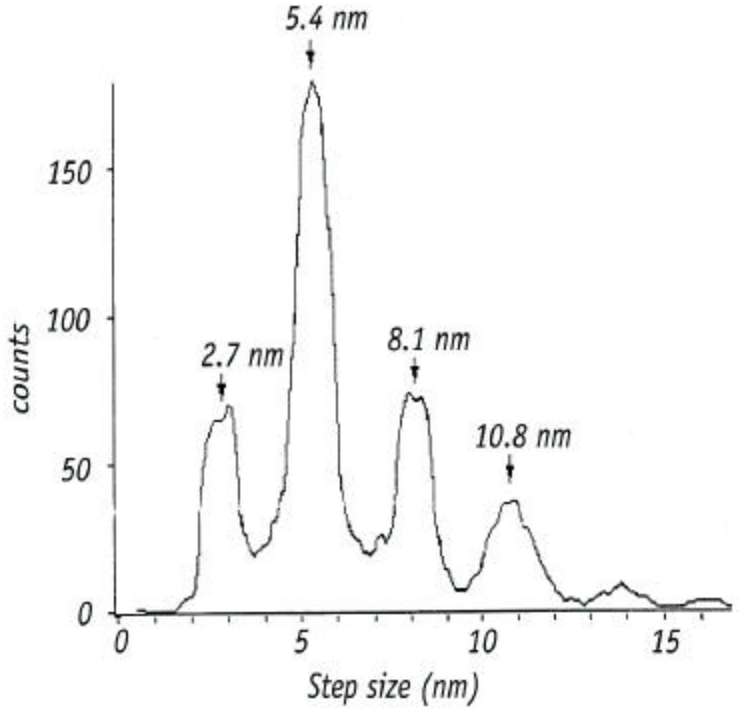

1 s
noise level
5.5 nm
1
11 nm
3a
3b
5.5 nm
2a
2.75 nm
2.75 nm
4
2b
5.5 nm



(Liu and Pollack, Biophys J. 2003)

Single sarcomere





(Yakovenko et al., 2002)

Conclusion

Muscle contraction could well involve a phase-transition propagating along the actin filament

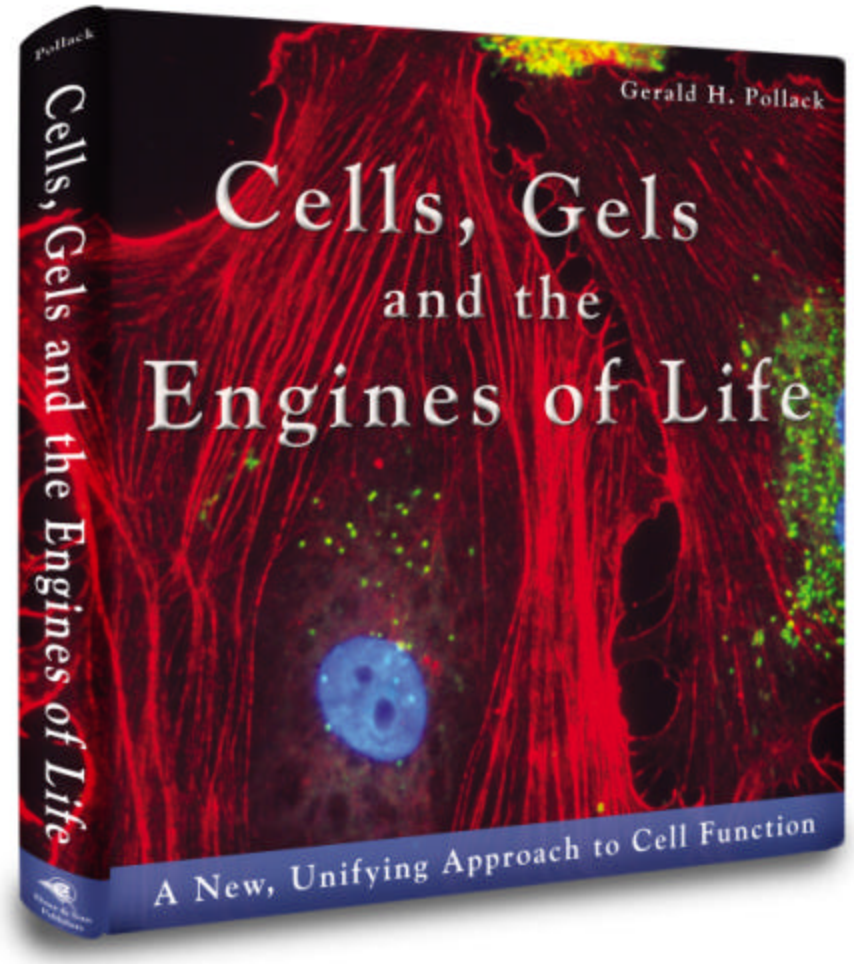
(the same propagating transition that drives streaming)

Phase-transitions implicated in:

- secretion
- action potential
- intracellular transport
- contraction
- cell motility
- ciliary and flagellar action
- mitosis
- transmembrane transport

How do cells work?

Phase-transition



"A 305 page preface to the future of cell biology" *Cell*, July 2002

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