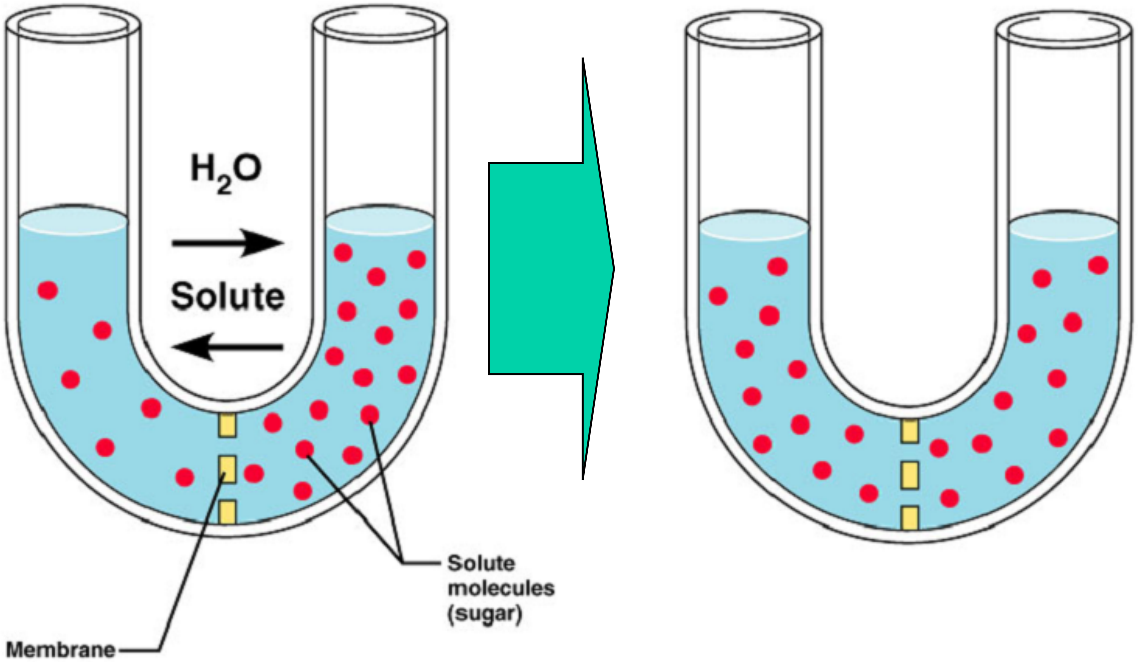
Osmosis


Osmosis
+PÜR MEDICAL SKINCARE
hydralift
firming gel mask
30 mL

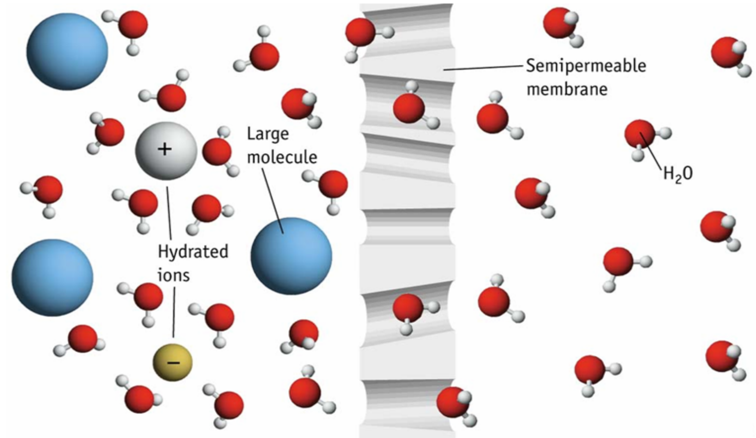
Osmosis can be thought of as the driving force for particle motions along a gradient.

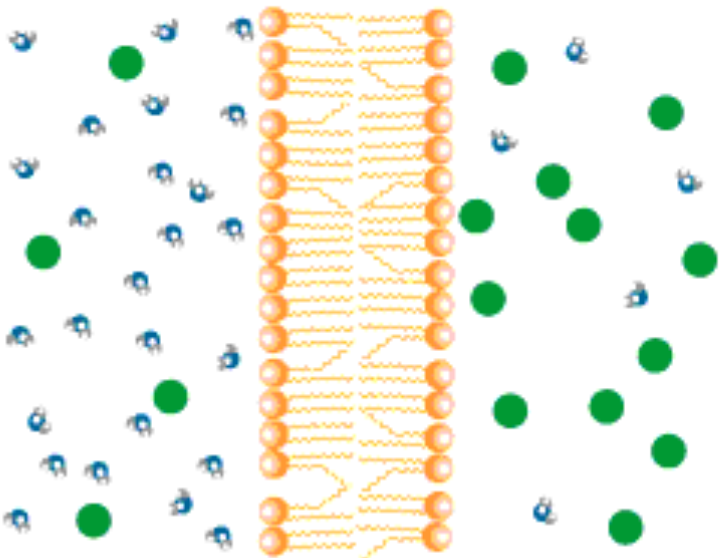
This is an entropic „force" that tends to make the concentration uniform in any region of space.

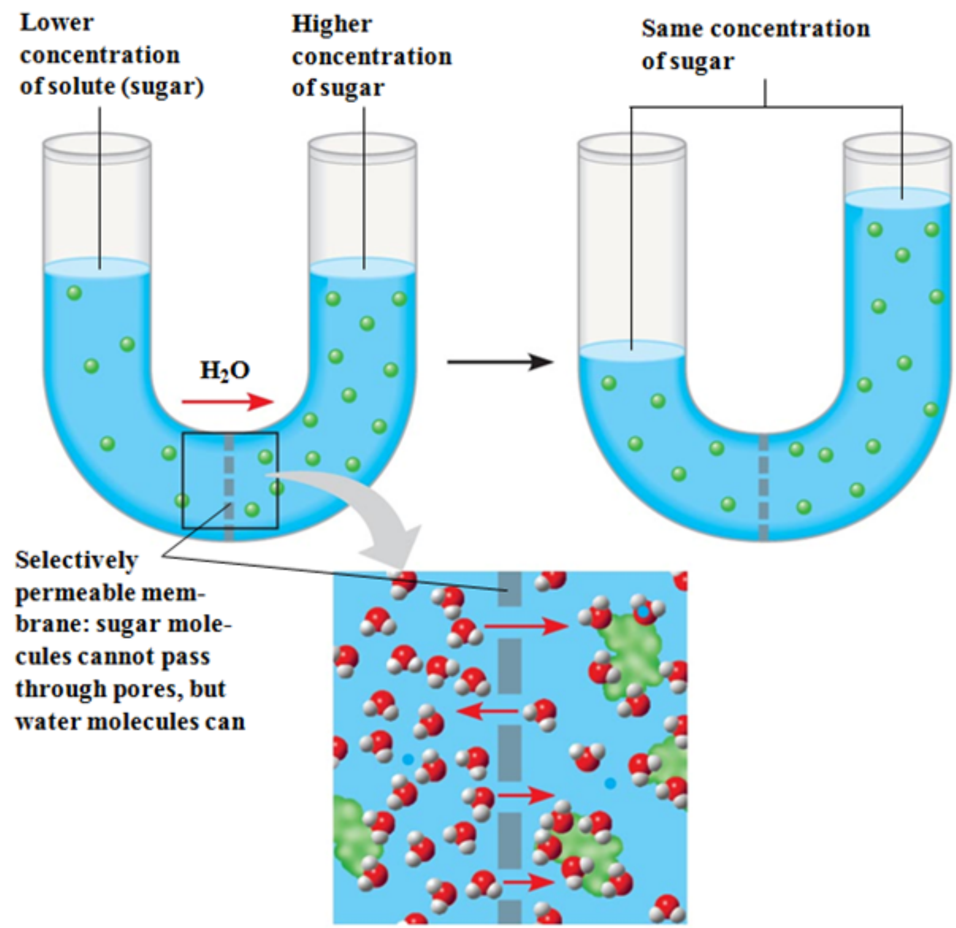


Membrane permeable to both solute molecules and water

A semi-permeable membrane.







Osmotic pressure: force required to <u>prevent</u> osmosis.

Osmotically active = solutes which can't diffuse through the semipermeable membrane.

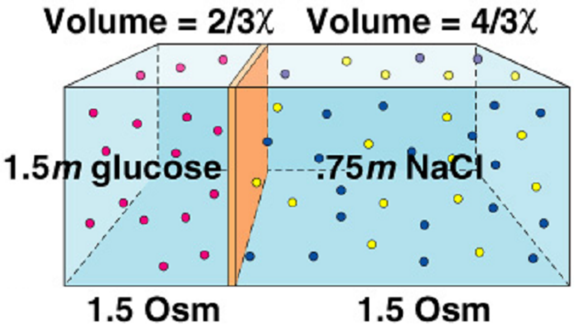
Way to measure osmolality:

Each Osm (of any solute) lowers the freezing point of water by ~ 2º C

The osmolarity of a solution is equal to the molarity of the particles dissolved in it.

1. 10 mmoles/liter of glucose = 10 mosmoles/liter.
2. 10 mmoles/liter of NaCl = 20 mosmoles/liter.
3. 10 mmoles/liter of $CaCl_2$ = ???

In a simple solutions the effect is additive.

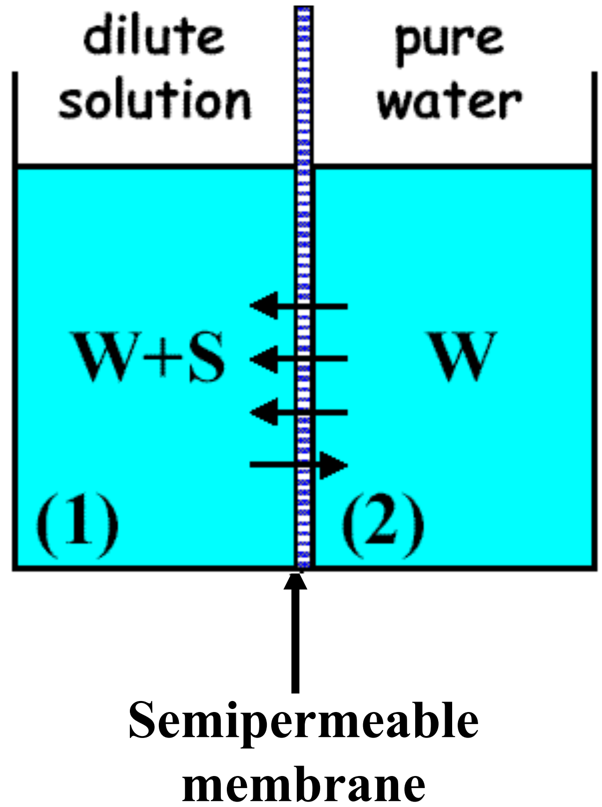


Chemical Potential of Water

$$X_w(1) < 1 \Rightarrow X_w(2) = 1$$

$$\mu_w = \mu_w^0 + RT \ln X_w + PV_w$$

μ_w^0 – standard chemical potential of water
X_w – molar fraction of water
P – pressure
V_w – molar volume of water



Solutes Decrease the Chemical Potential of Water

Addition of an impermeable solute to one compartment drives the system out of equilibrium.

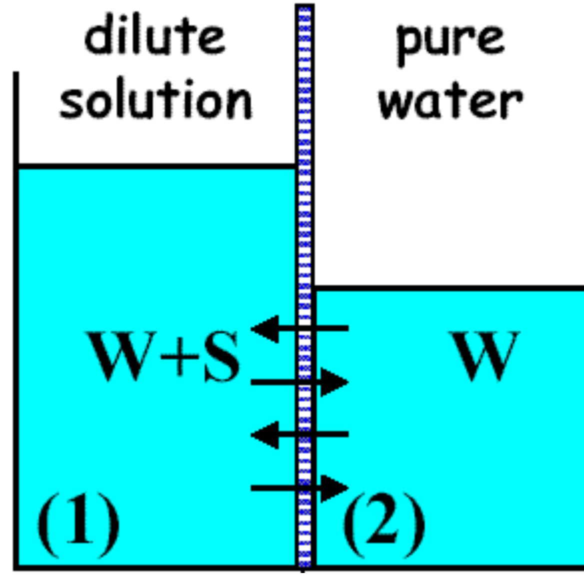
$$RT \ln X_w(1) < RT \ln X_w(2) \Rightarrow \mu_w(1) < \mu_w(2)$$

There is a net water flow from compartment (2) to compartment (1).

Osmotic Equilibrium

$$X_w(1) < 1 \Rightarrow X_w(2) = 1$$

At the equilibrium the chemical potential of any species is the same at every point in the system.



$$\mu_w(1) = \mu_w(2)$$

$$\mu_w^0(1) + RT\ln X_w(1) + P(1)V_w = \mu_w^0(2) + RT\ln X_w(2) + P(2)V_w$$

$$RT\ln X_w(1) + P(1)V_w = P(2)V_w$$

$$V_w\Delta P = -RT\ln X_w(1)$$

$$X_w + X_s = 1$$

$$\ln X_w = \ln(1 - X_s) \cong -X_s$$

$$V_w\Delta P = RTX_s$$

Solute molar fraction in physiological (dilute) solutions is much smaller than water molar fraction.

$$X_s << 1$$

$$\Pi = \Delta P = \frac{RT}{V_w}X_s$$

Osmotic pressure

$$\Pi = \Delta P = \frac{RT}{V_w} X_s$$

Solute concentration (~0.1M) in physiological (dilute) solutions is much smaller than water concentration (55M).

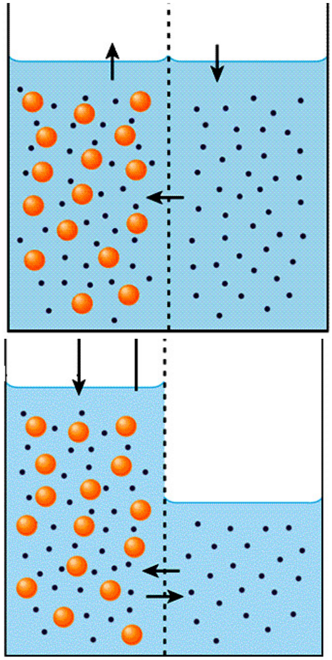
$$n_s << n_w$$

$$X_s = \frac{n_s}{n_s + n_w} \approx \frac{n_s}{n_w} = \frac{n_s}{n_w}\frac{V_w}{V_w} = \frac{n_s}{V_{tot}} V_w = C_s V_w$$

vant'Hoff's law
(the osmotic pressure)

$$\Pi = \Delta P = \frac{RT}{V_w} C_s V_w = RTC_s$$

Osmotic Flow

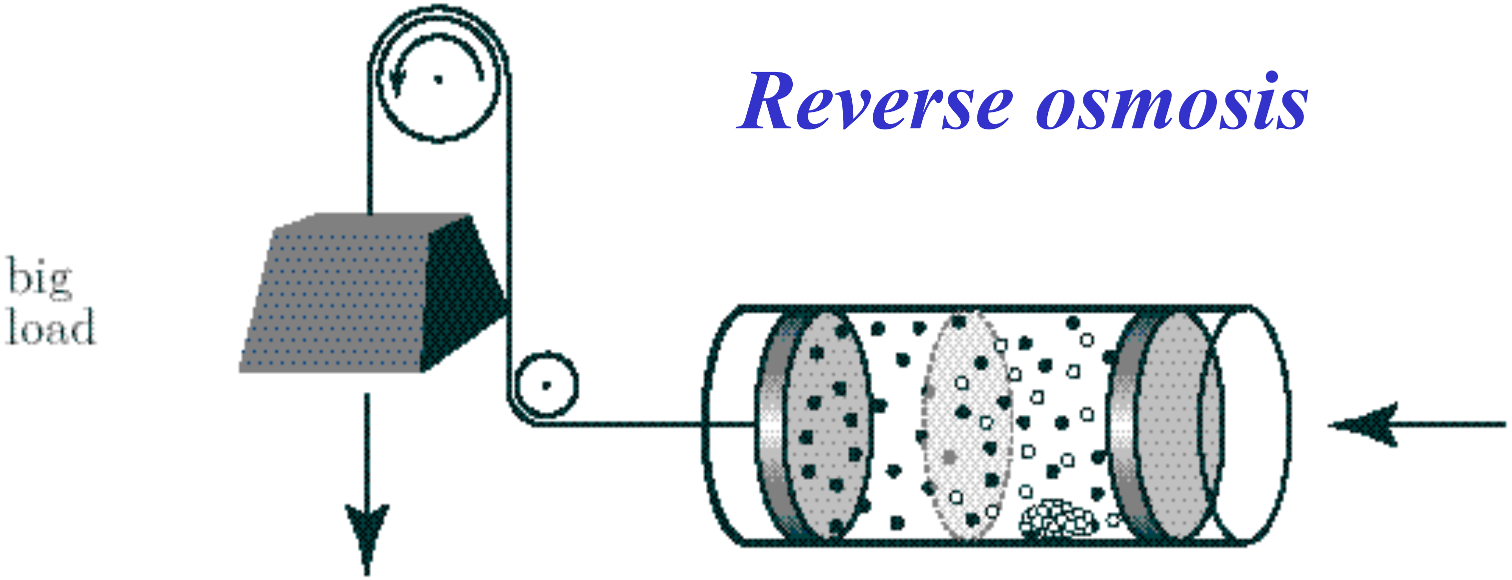


Water flows from the solution with a low osmotic pressure to the solution with a high osmotic pressure.

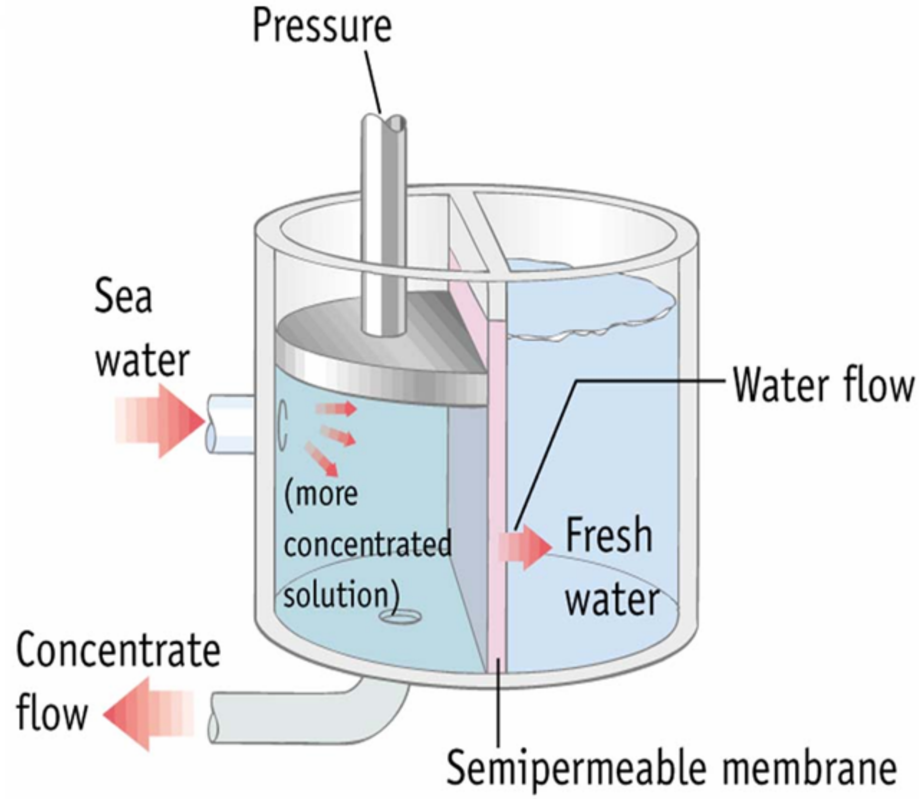
At equilibrium $\Delta P - \Delta\Pi = 0$

$$\Delta\Pi = \Delta P = RT\Delta C_s$$

Reverse osmosis

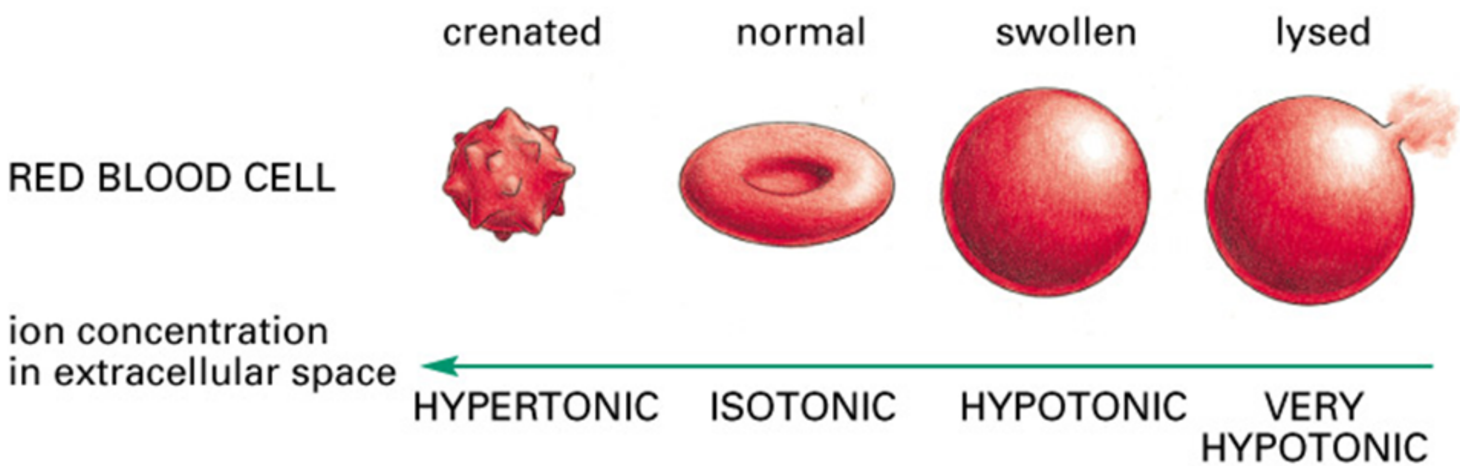


Reverse Osmosis is Used for Water Purification

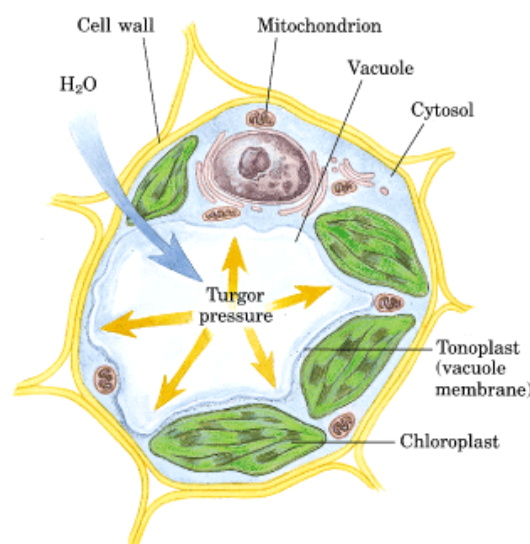


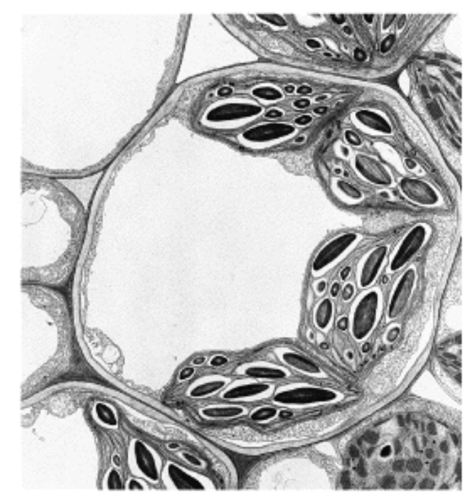
What osmotic pressure difference can do to cells?

Plasma: 0.3 Osm (or 300 mOsm)



Turgor

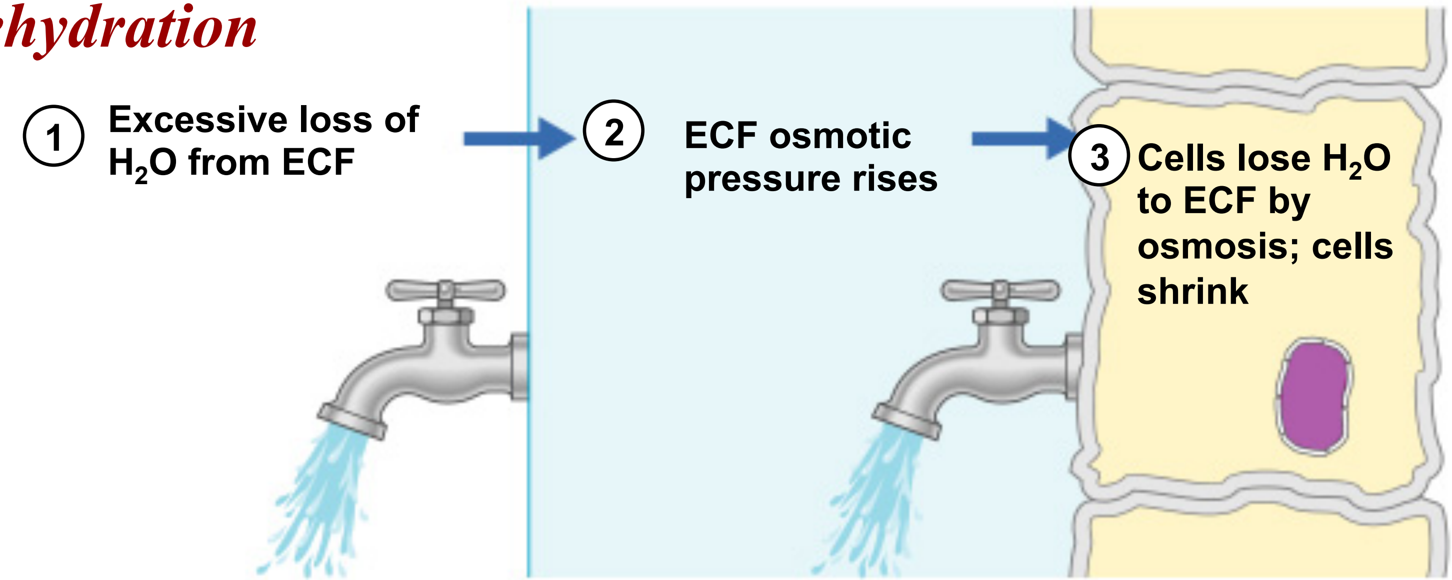




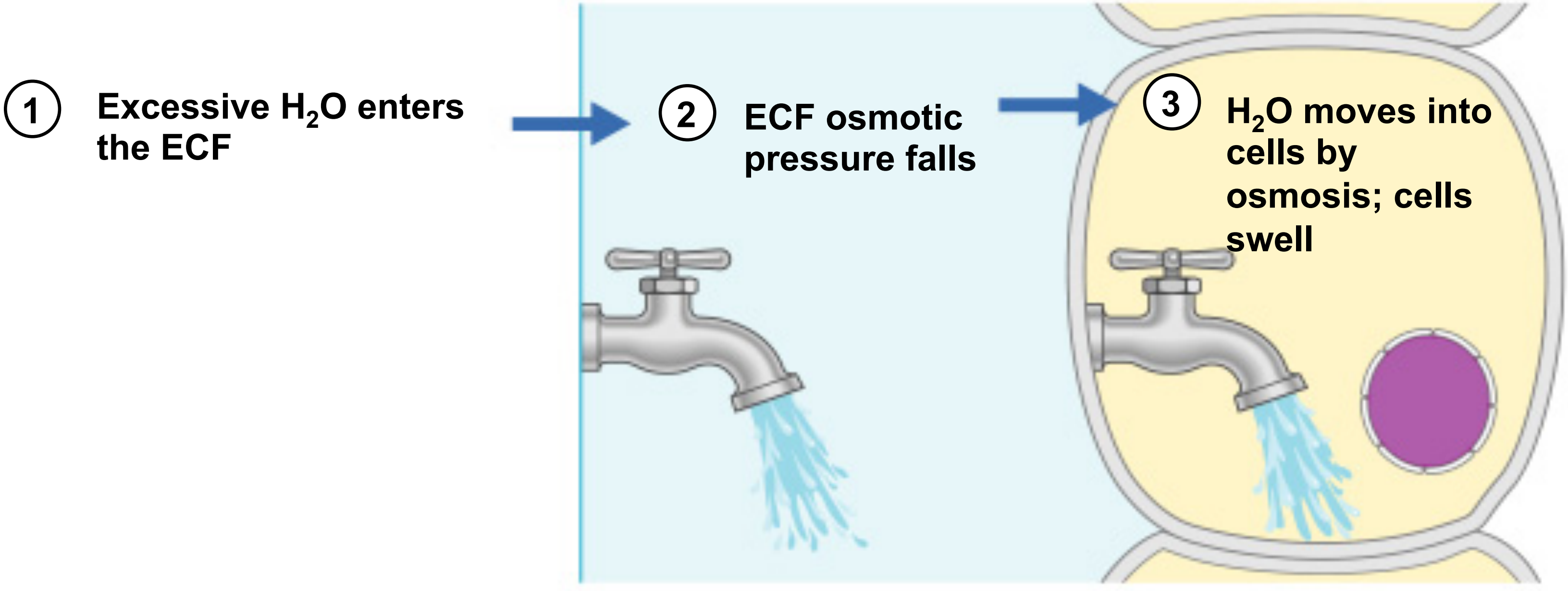
Osmotic regulation is an advantage of multicellularity

- *individual cells in solution are vulnerable to changes in their environment*
- *multicellular organisms control the environment in which their cells live*
- *cells in multicellular organisms flourish in a constant environment of body fluids, which invariably differ from the environment in which the organism lives*

Hypertonic Dehydration

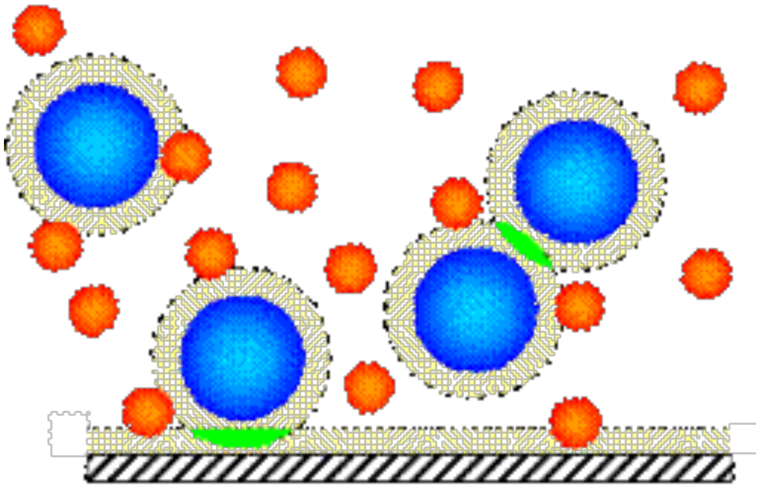


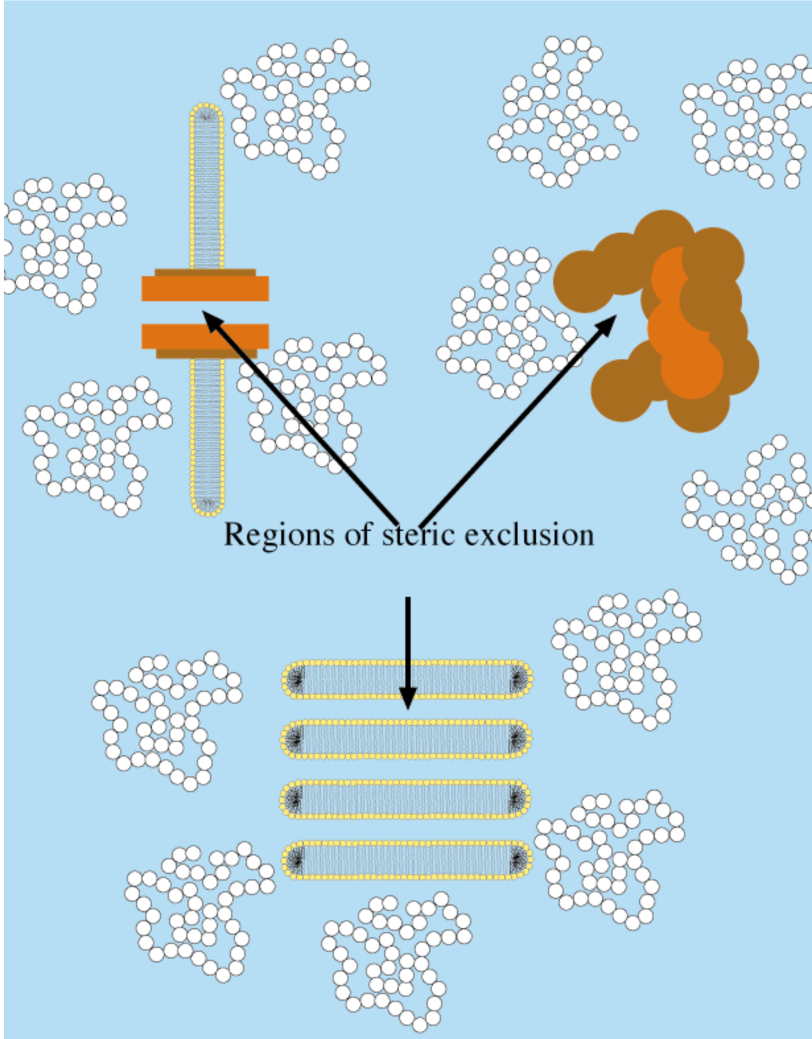
Hypotonic Hydration



Entropy driven aggregation

➢ Each of the large objects is surrounded by a depletion zone of thickness equal to the radius a of the small particles.



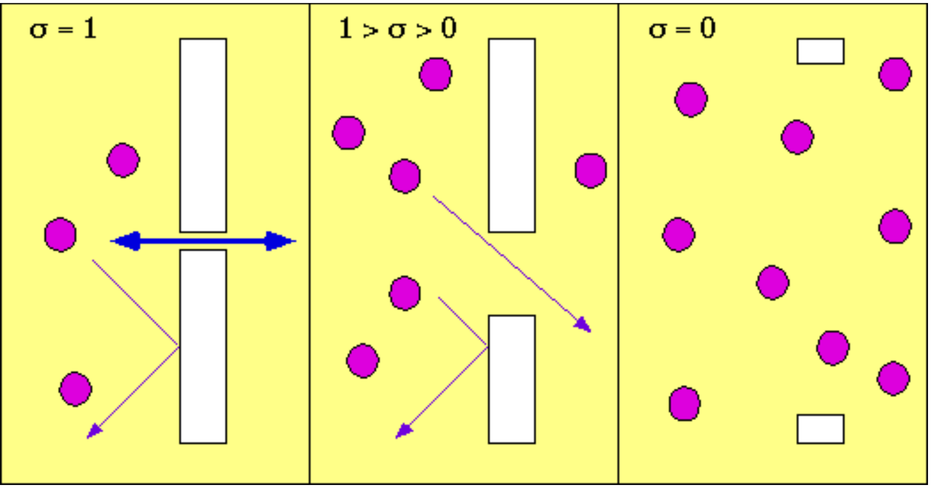


➢ The depletion zone reduces the volume available to the small particles – ***eliminating it would increase their entropy and hence lower their free energy***.

The osmotic pressure $\Pi = gRTC$

σ – selectivity/reflection coefficient

It is a measure of ***the probability of the molecule crossing the membrane***.



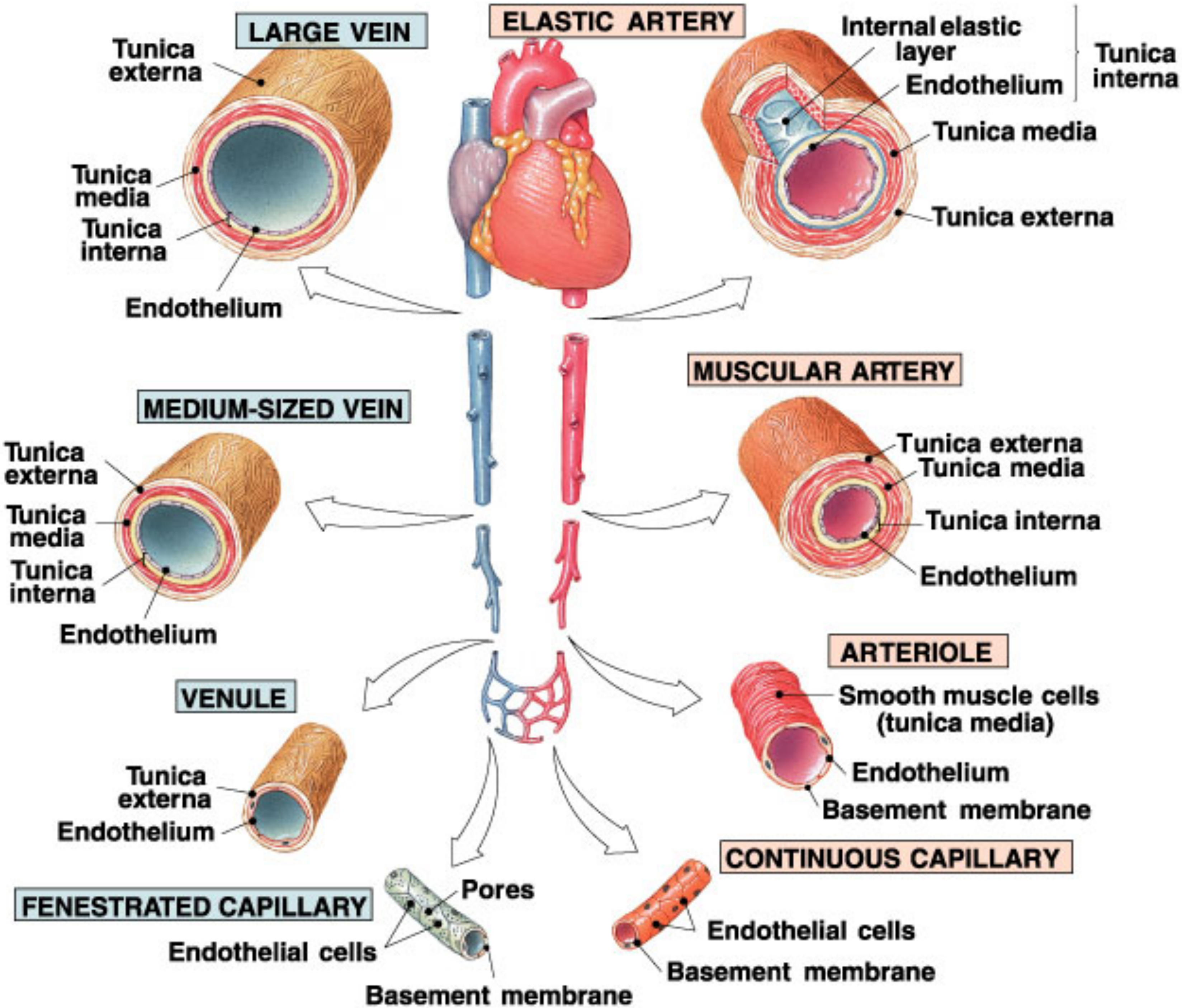
semipermeable membrane ***non-selective membrane***

The effective osmotic pressure depends on the reflection coefficient:

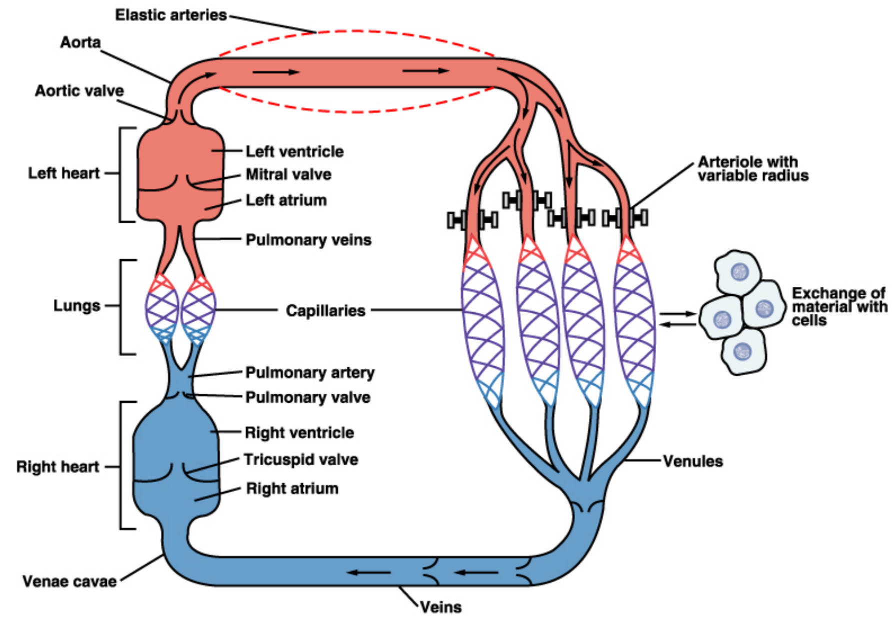
$$\Pi_{eff} = \sigma\Pi = \sigma gRTC$$

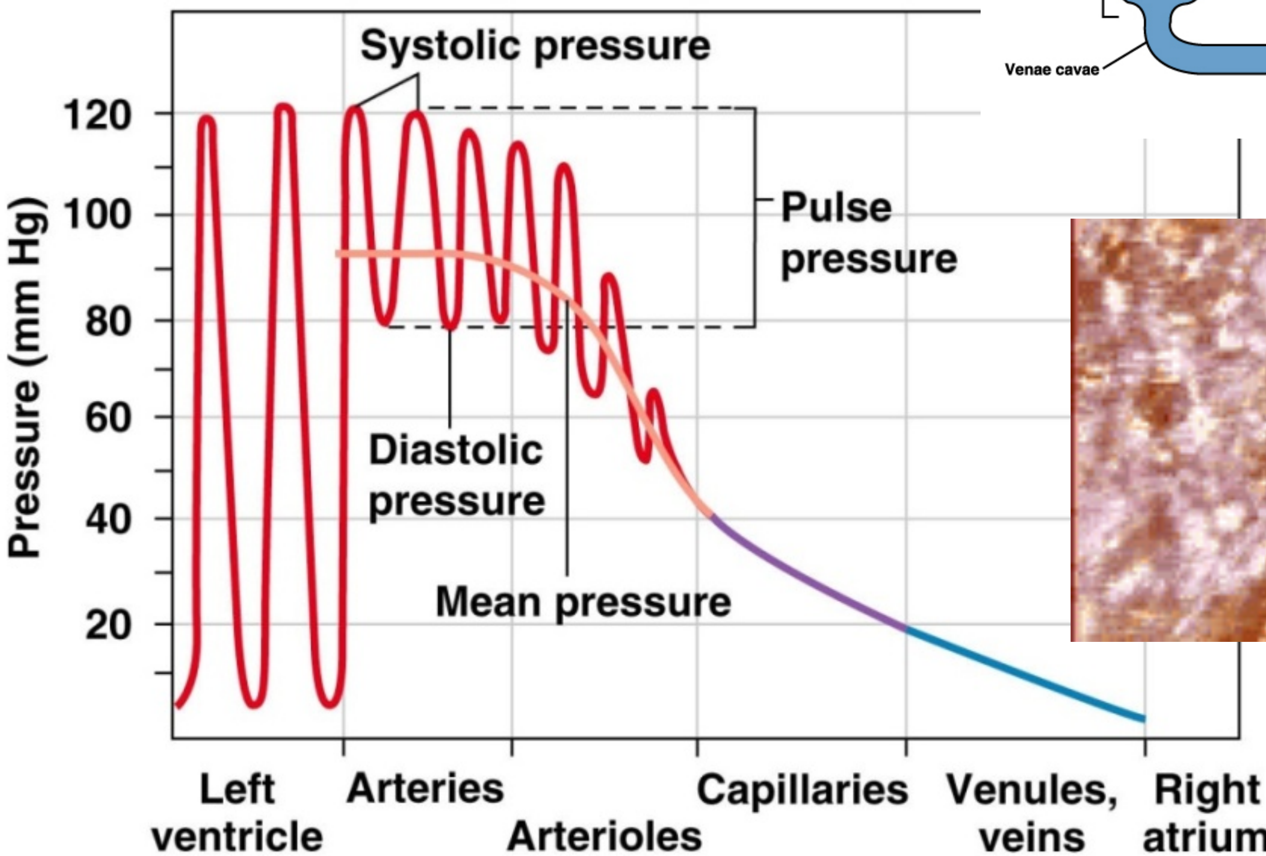
Bulk flow of water through barrier

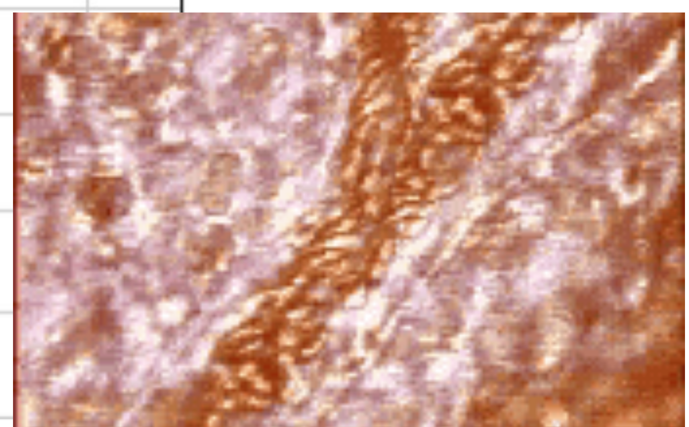
$$J_V = L_P\left(\Delta P - \sigma\Delta\Pi\right)$$


LARGE VEIN
Tunica externa
Tunica media
Tunica interna
Endothelium
ELASTIC ARTERY
Internal elastic layer
Endothelium
Tunica interna
Tunica media
Tunica externa
MEDIUM-SIZED VEIN
Tunica externa
Tunica media
Tunica interna
Endothelium
MUSCULAR ARTERY
Tunica externa
Tunica media
Tunica interna
Endothelium
VENULE
Tunica externa
Endothelium
ARTERIOLE
Smooth muscle cells (tunica media)
Endothelium
Basement membrane
FENESTRATED CAPILLARY
Pores
Endothelial cells
Basement membrane
CONTINUOUS CAPILLARY
Endothelial cells
Basement membrane

Blood pressure along the circulation

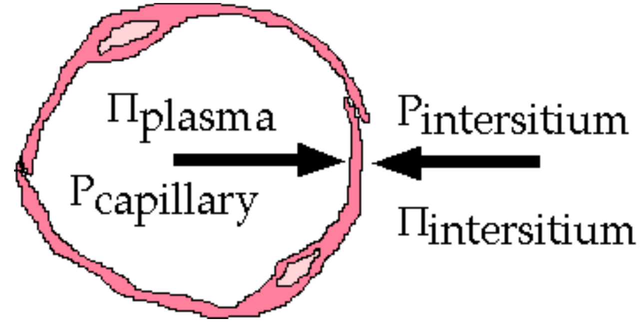






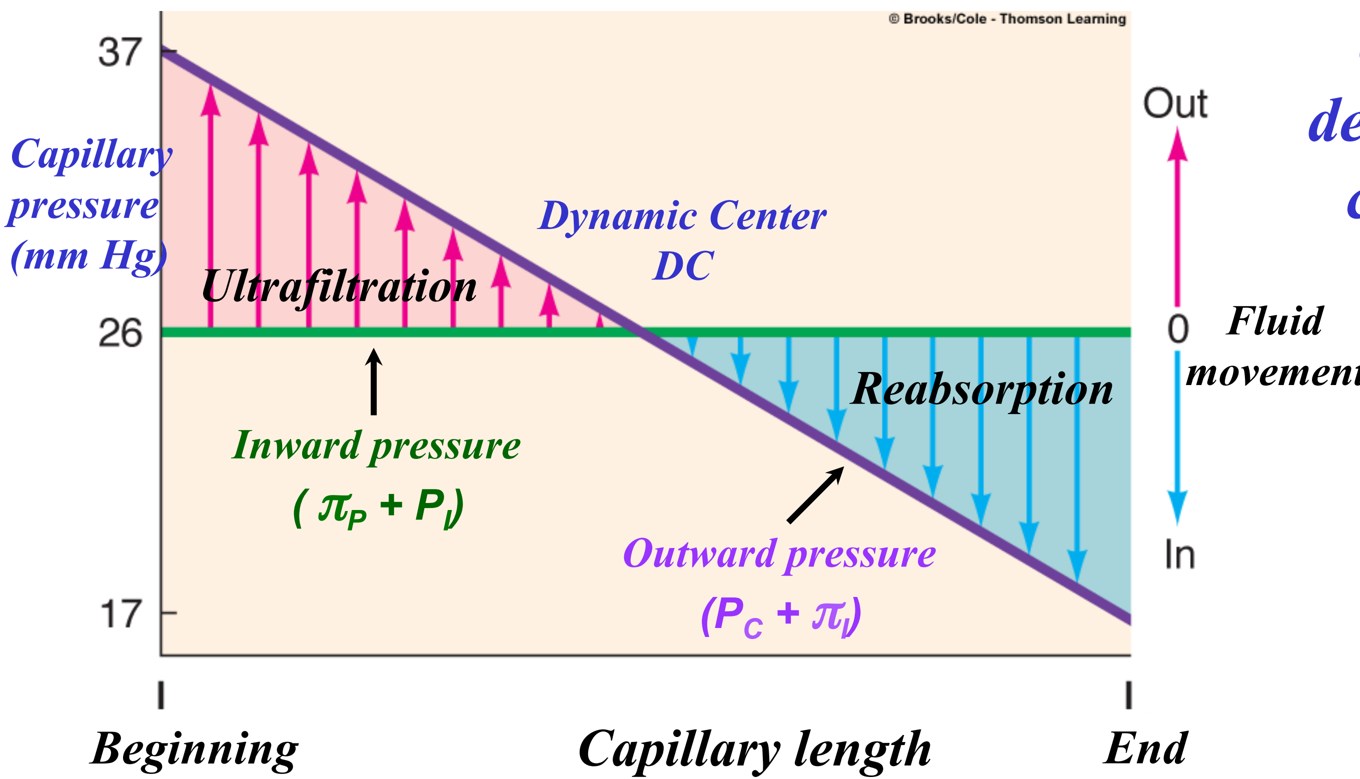
$$J_V = L_P A\left[\left(P_p - P_i\right) - \sigma\left(\Pi_p - \Pi_i\right)\right]$$

$$K_f = L_P A$$



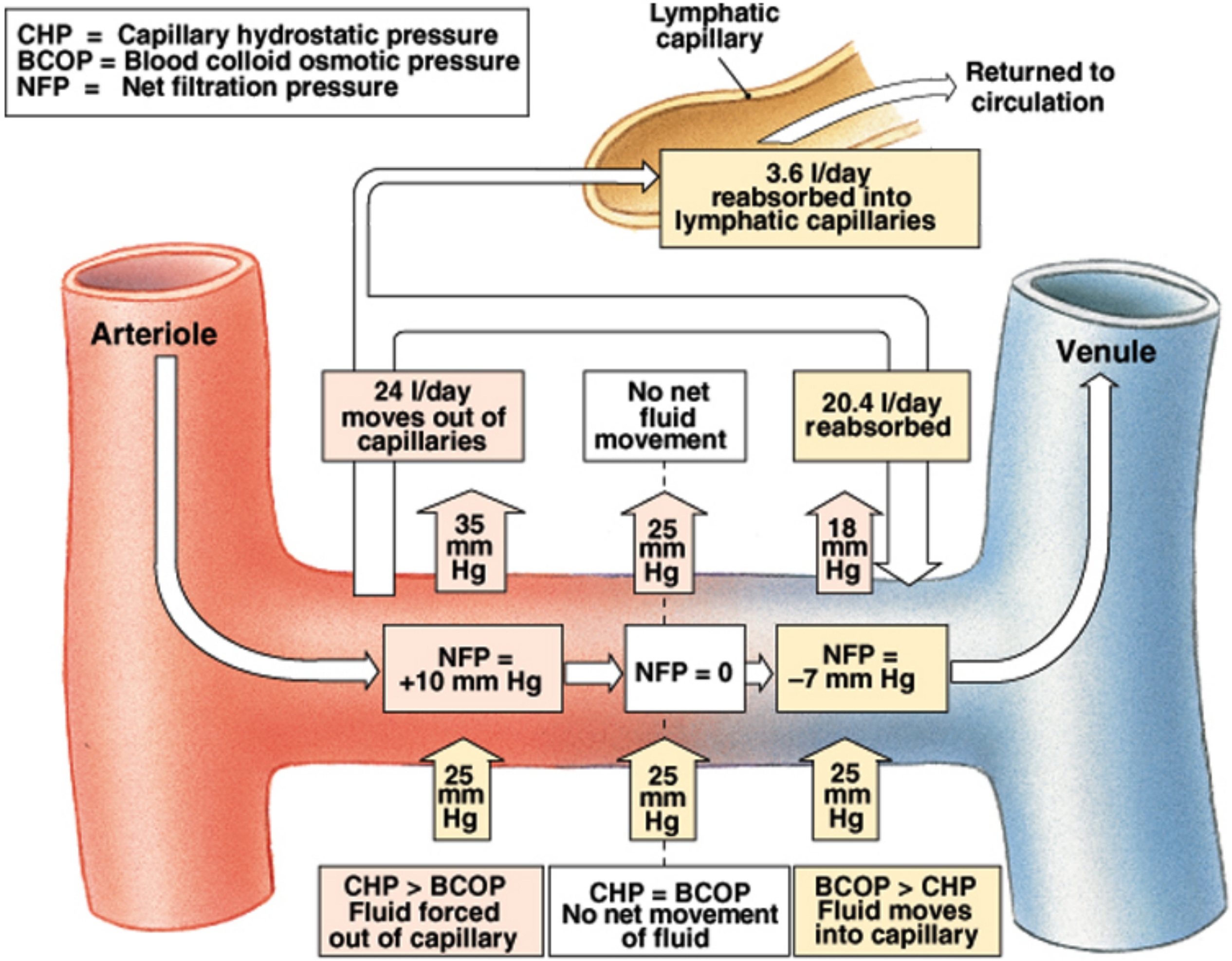
L_p – hydraulic Conductivity, indicates the leakiness of the capillary wall.

K_f – Capillary Filtration Coefficient, the hydraulic conductance per unit surface area for exchange.



The number of open capillaries determines the total capillary surface area (A).

Capillary Exchange



Blood velocity ~ 100 to 1000 μm/s

The interstitial fluid velocity ~ 0.1 μm/s

Fluid velocity in the lymphatic capillaries ~ 1 to 10 μm/s

Edema

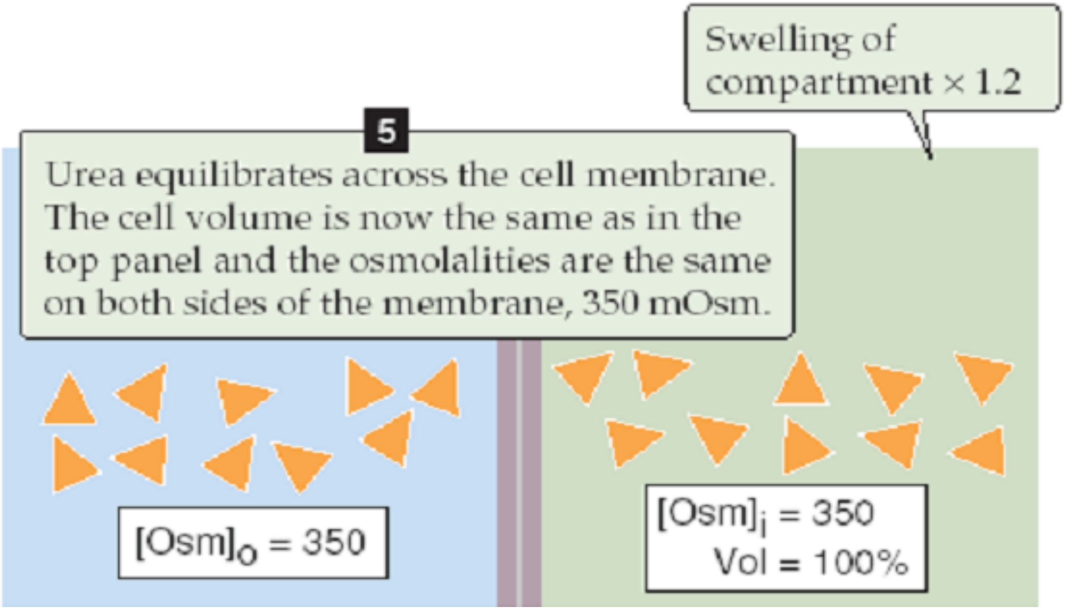
Due to disruption of capillary exchange

2 major causes:

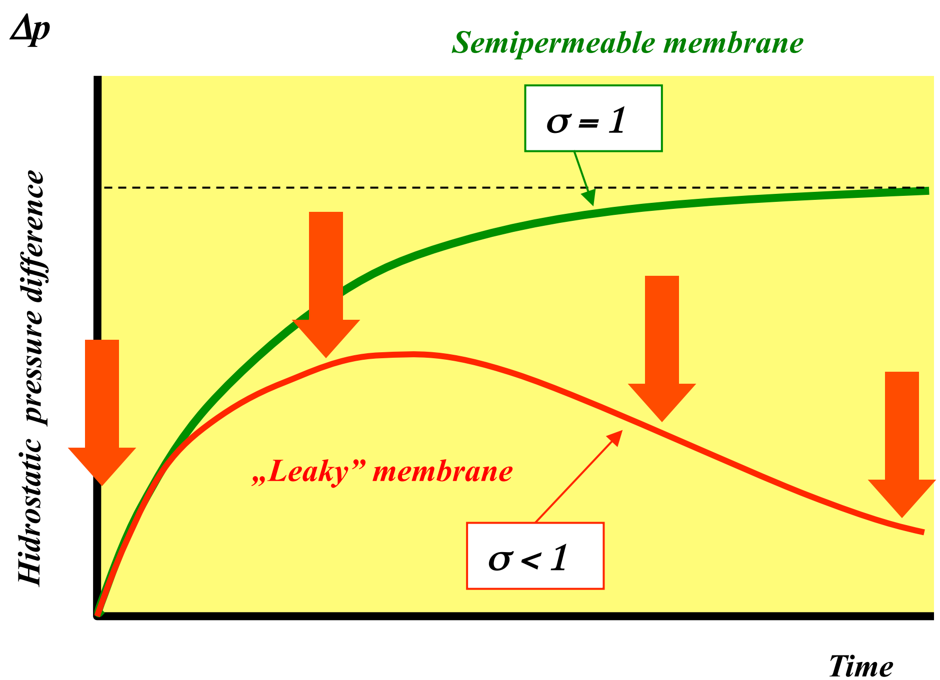
1. **Blockage of lymph drainage**
   - **Cancer & fibrotic growth**
   - **Pathogens**
   - **Pregnancy**

2. **Capillary filtration > absorption**
   - **Venous pressure ↑ due to right / left heart failure, backs up in to capillaries**
   - **Plasma protein concentration ↓ due to liver failure or severe malnutrition (Kwashiorkor) reduces colloid osmotic pressure**
   - **↑ in interstitial protein**

Effect of urea on cell volume (σ <1).



The cell membrane is far more permeable to water than to urea.

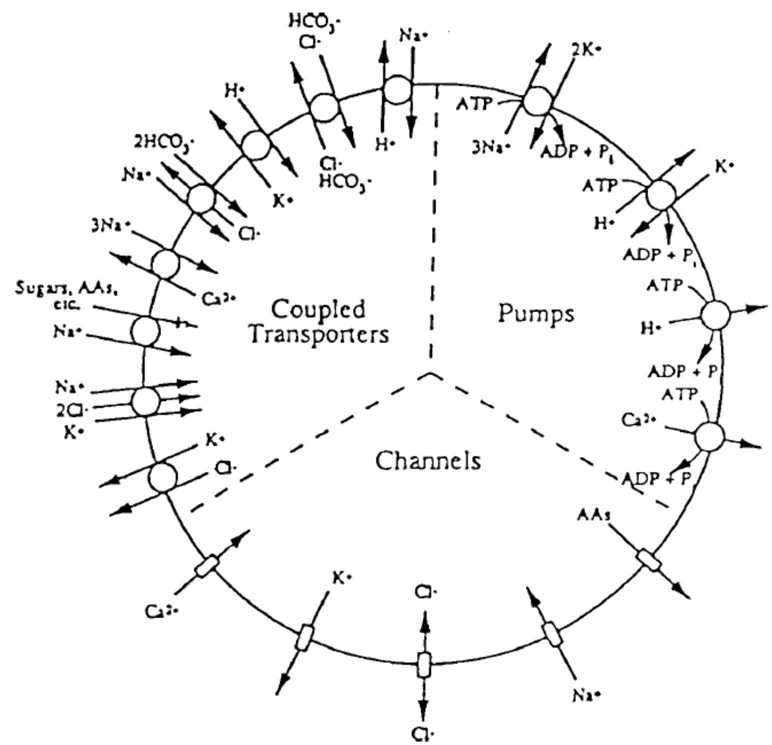


Important summary points about osmosis

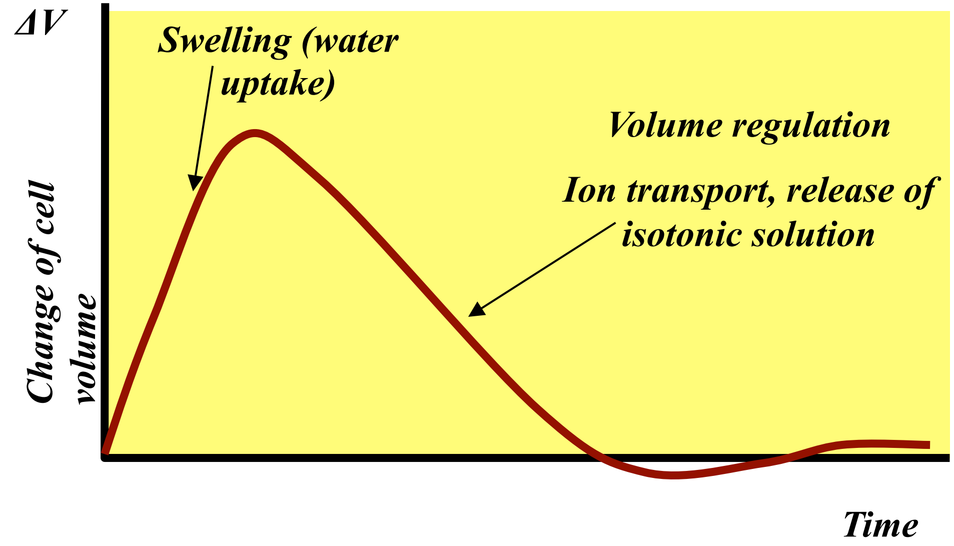
1. The steady-state volume of the cell is determined by the concentrations of impermeant ions.

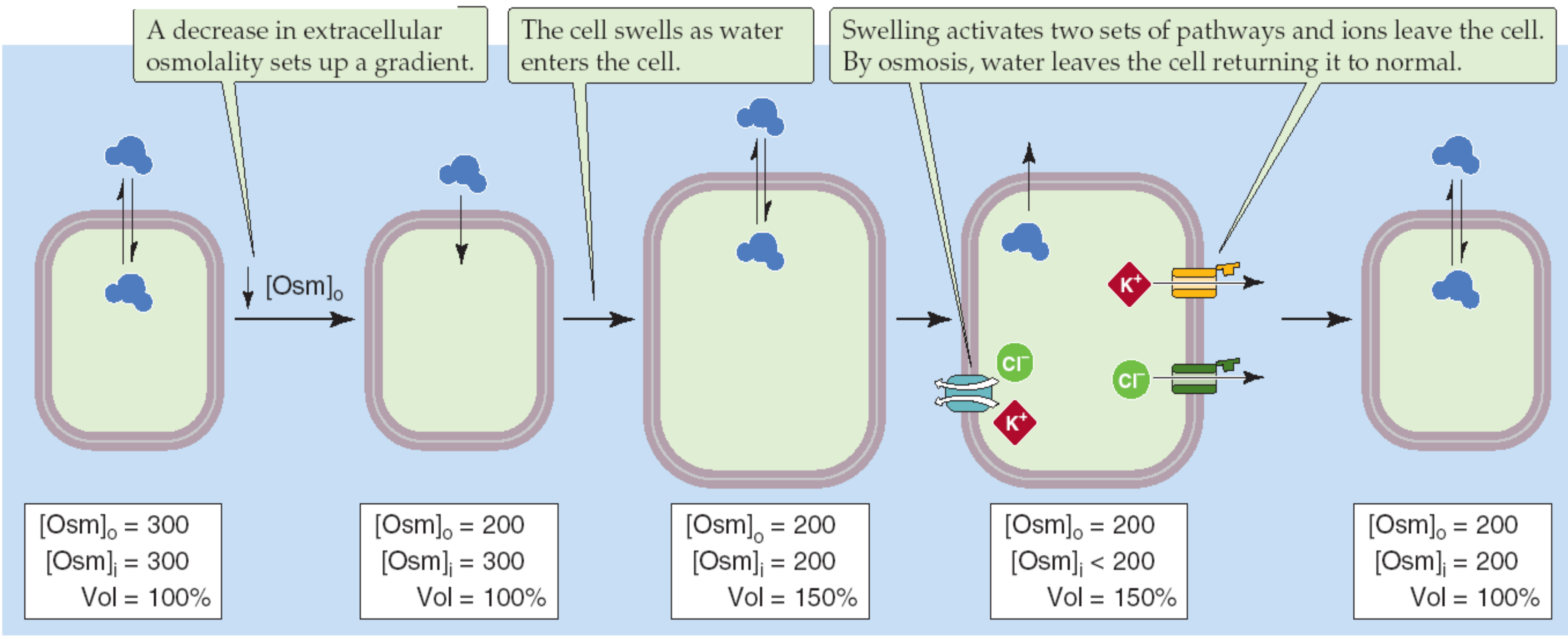
2. Permeant solutes redistribute according to the rules of electrodiffusion, and hence affect only the transient volume of the cell.

3. The more permeant the solute, the more transient its effects on volume.



Volume regulation of living animal cells

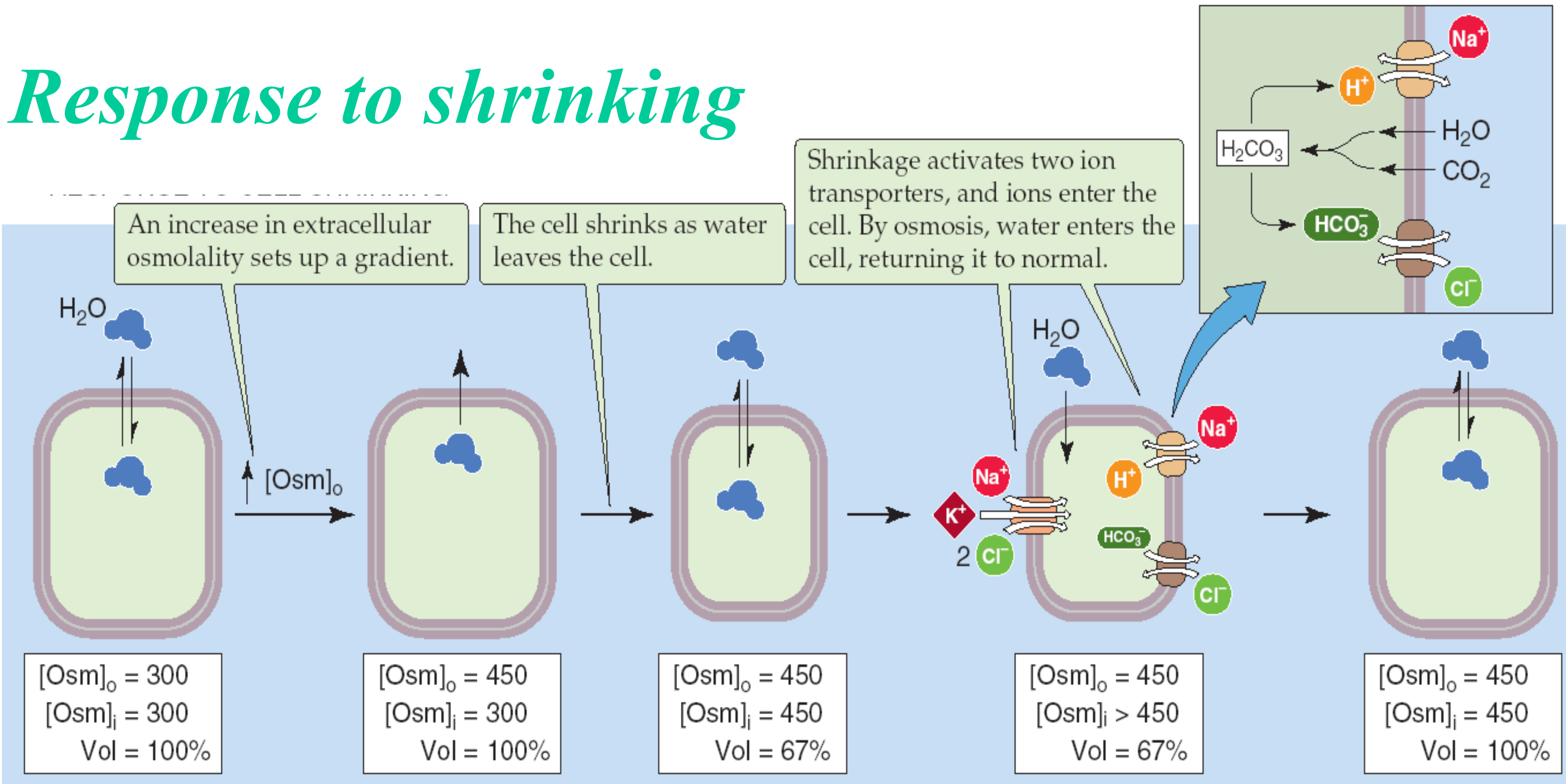




Osmoconformers – animals, like sea slugs, that allow the osmolarity of their internal environment to change with that of the external environment.

Osmoregulators – animals that do not allow the osmolarity of their internal environment to change.

Response to shrinking



The activation energy (E_a) required for water diffusion in an entirely aqueous environment – ***5 kcal/mol***.

The activation energy (E_a) required for water diffusion through the lipid bilayer – ***10-20 kcal/mol.***

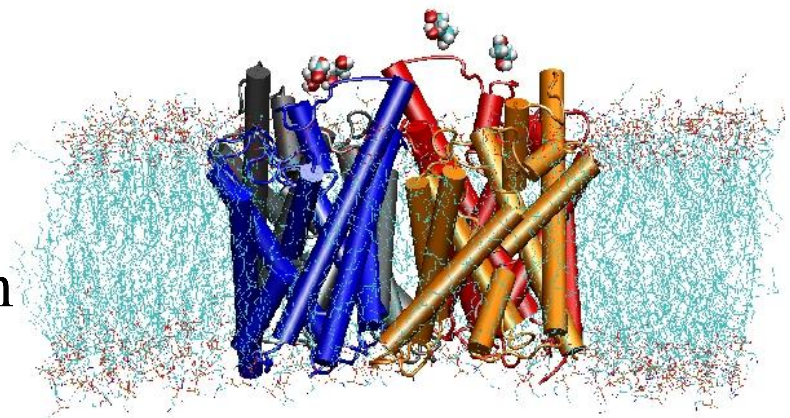
Water Transport Across Cell Membrane

always passive; bidirectional; osmosis-driven

Diffusion through lipid bilayers

slow, but enough for many purposes

Channel-mediated

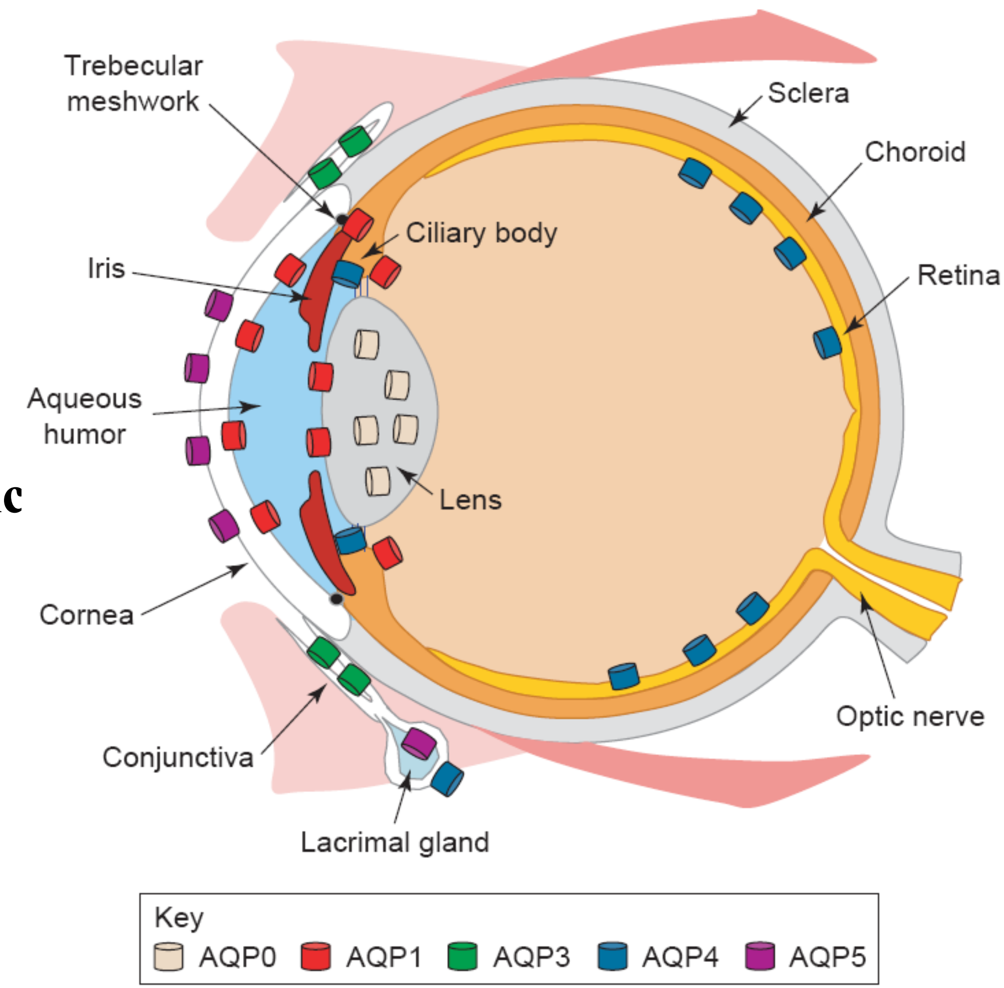


- Fast adjustment of water concentration is necessary (RBC, brain, lung).
- Large volumes of water needed to be transported (kidneys).

Aquaporins perform a variety of physiological functions.

- **concentrate urine in the kidney,**
- **are integral to fat metabolism and obesity,**
- **maintain lens transparency in the eye,**
- **maintain water homeostasis within the brain,**
- **extrude sweat from the skin,**
- **are implicated in cell migration during tumor growth,**
- **help suspend fish eggs in seawater,**
- **facilitate a rapid response to osmotic shock in yeast,**
- **tightly regulate cell osmolarity within plants.**

Distribution of aquaporins in human eye.



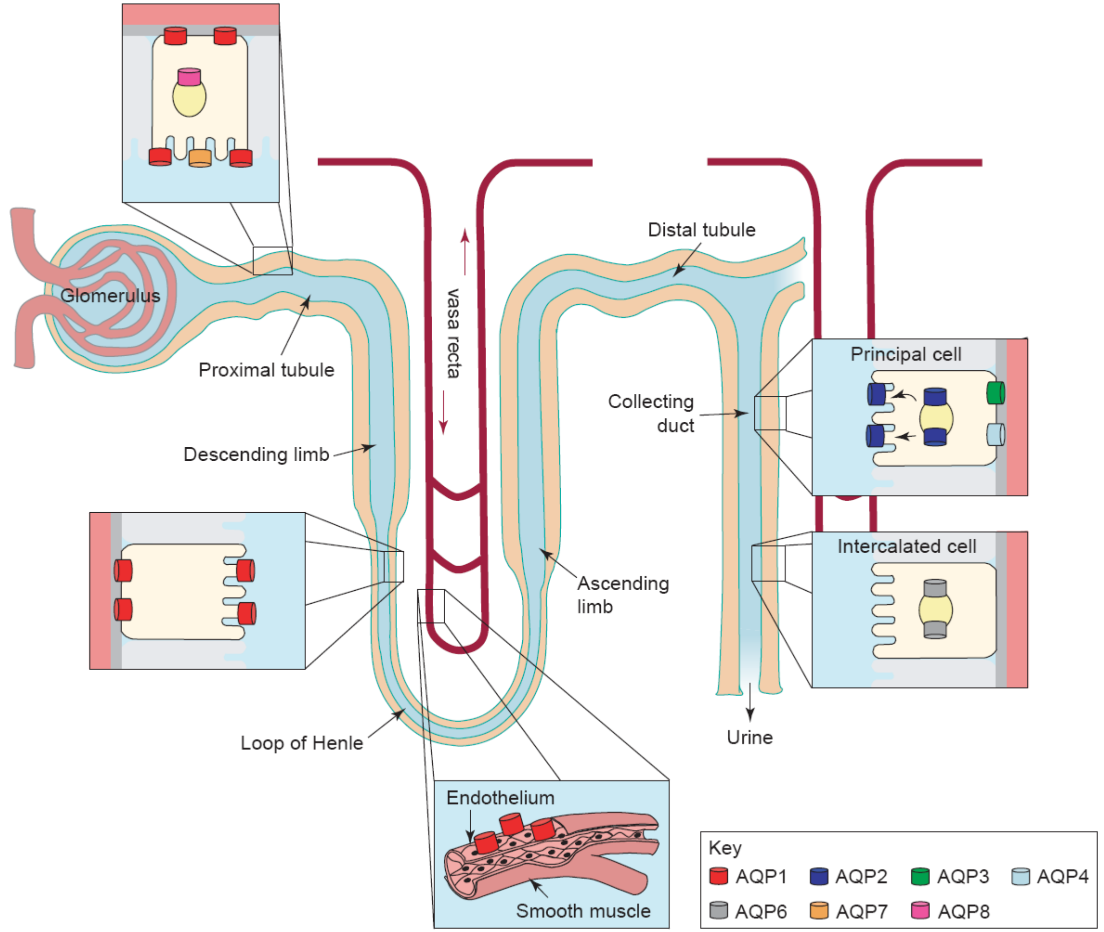
Aquaporins in the Kidney

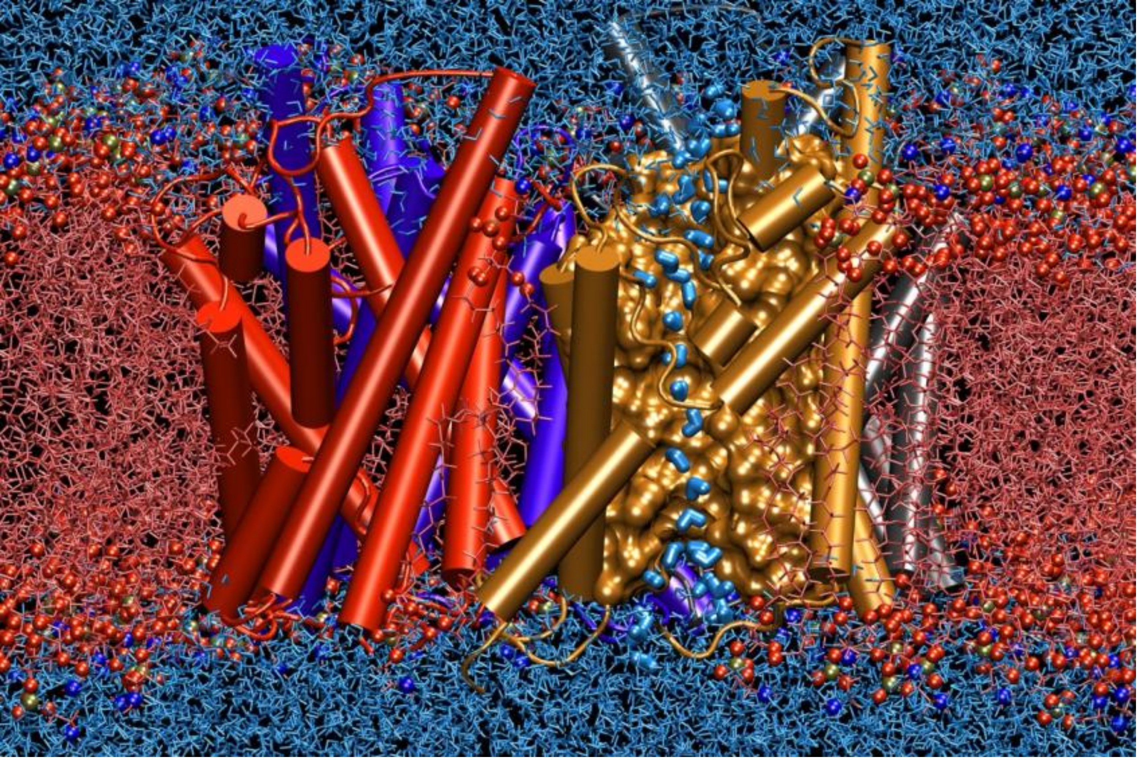
It filters and eliminates toxic substances from the blood.

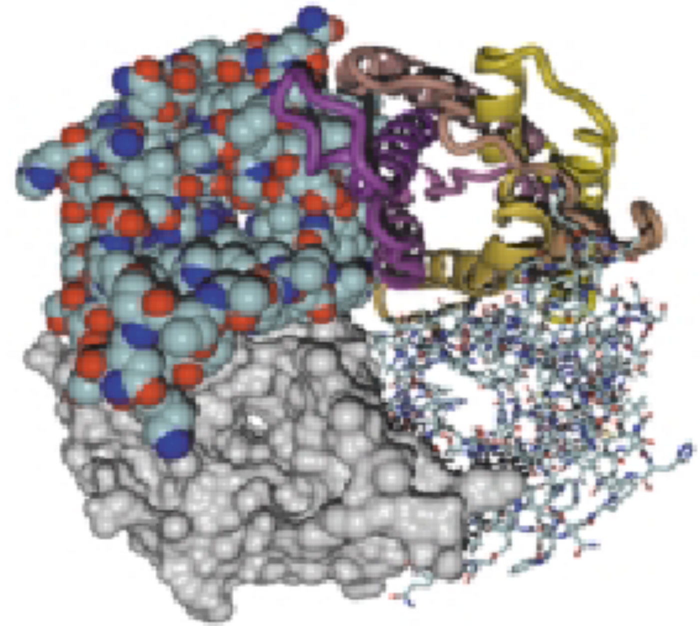
This is achieved by the filtration of blood in nephrons, which have important functions in the reabsorption of water, active solute transport and acid–base balance.

- Adult human kidneys filter >150 l of blood each day.

- To maintain water balance, > 99% of water is reabsorbed before it leaves the kidney as urine.

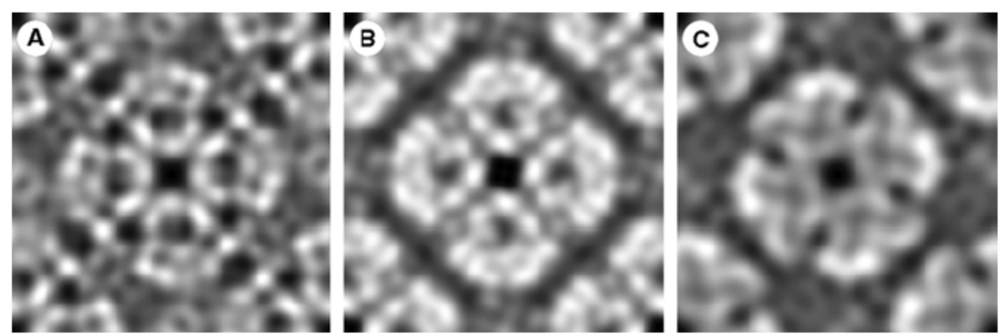






The AQP1 tetramer

Cryo-electron microscopy maps of water channel proteins (viewed from cytoplasmic side).



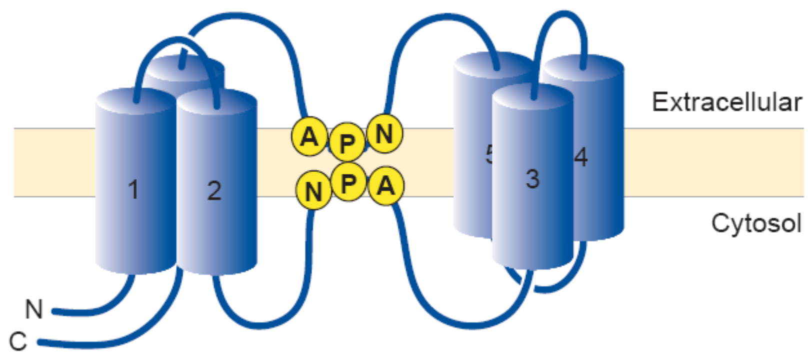
Red blood cell water channel AQP1

The lens fiber water channel MIP or AQP0

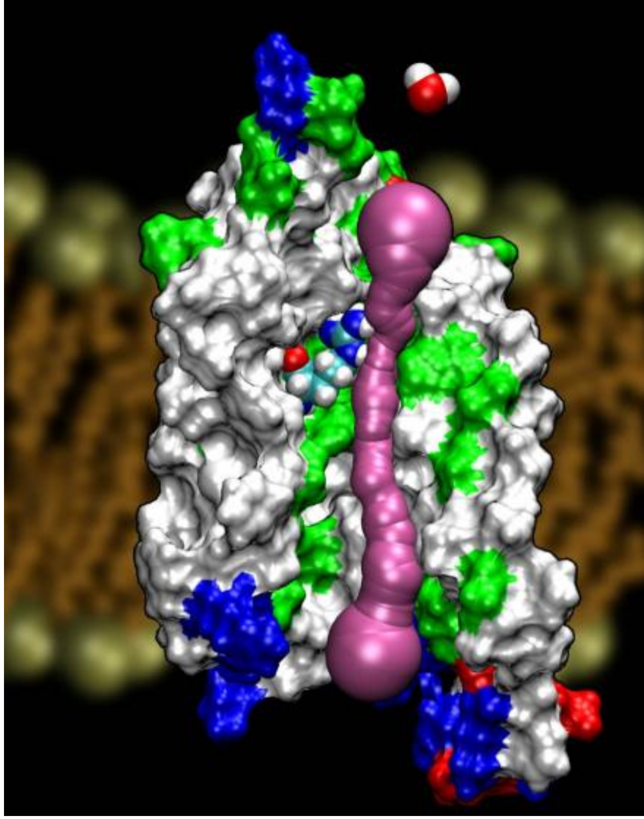
The bacterial water channel AqpZ

Protein	**Cell Type**	**Function?**
AQP-0	Eye lens cells	Water balance in lens
AQP-1	Red blood cells	Osmotic protection
AQP-1	Kidney tubules	Concentration of urine
AQP-1	Brain	Production of CSF
AQP-2	Kidney collecting ducts	Mediates action of ADH
AQP-4	Lungs	Bronchial fluid secretion
AQP-5	Salivary glands	Production of saliva
AQP-5	Lacrimal glands	Production of tears

Topology of aquaporins

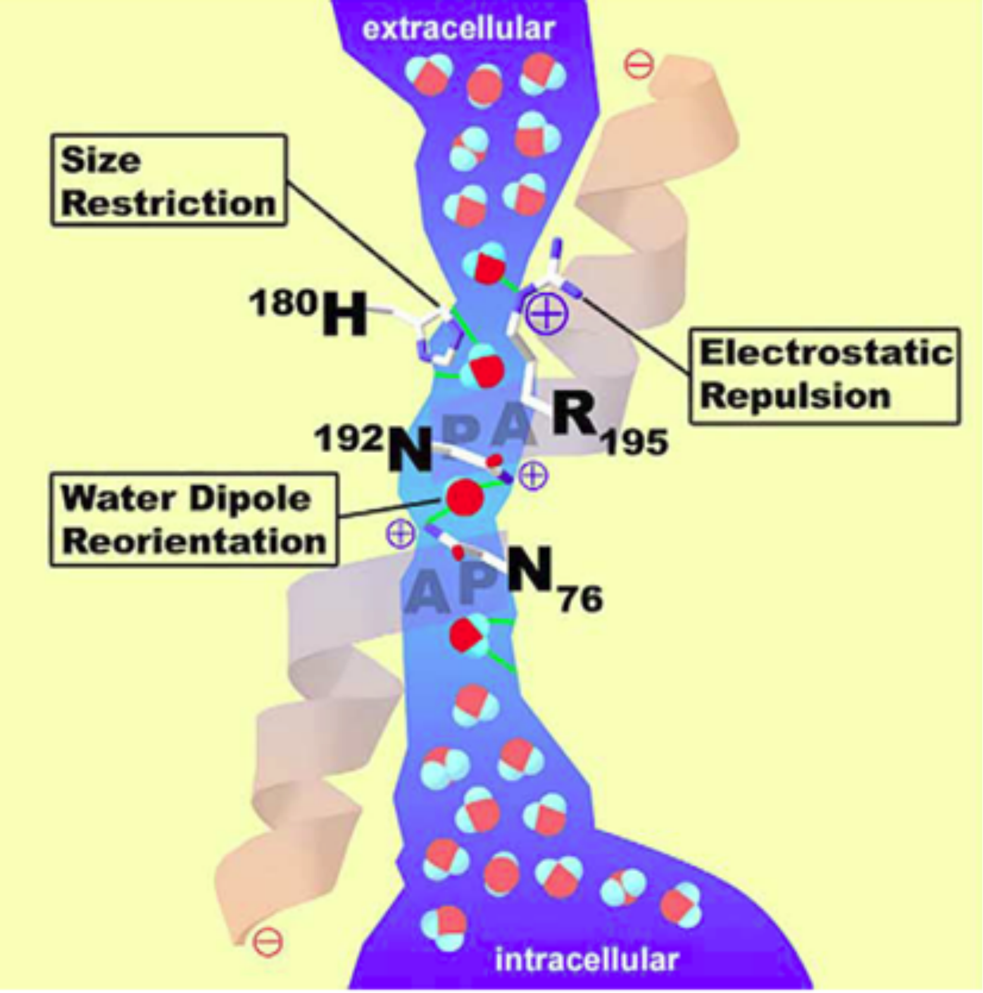


Six transmembrane domains and the conserved NPA-containing loops that form the selectivity filter of the water-conducting pore.

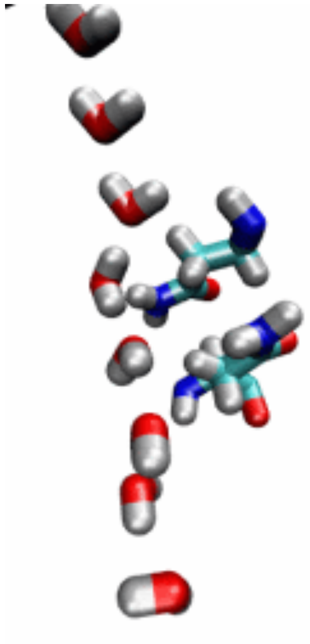


AQP1 comprises cone-shaped water-filled extracellular and intracellular vestibules that are separated by a 20 Å long channel ~2.8 Å at its narrowest point.

Hydrogen bonding between water molecules occurs within the AQP pore, except at its narrowest point.



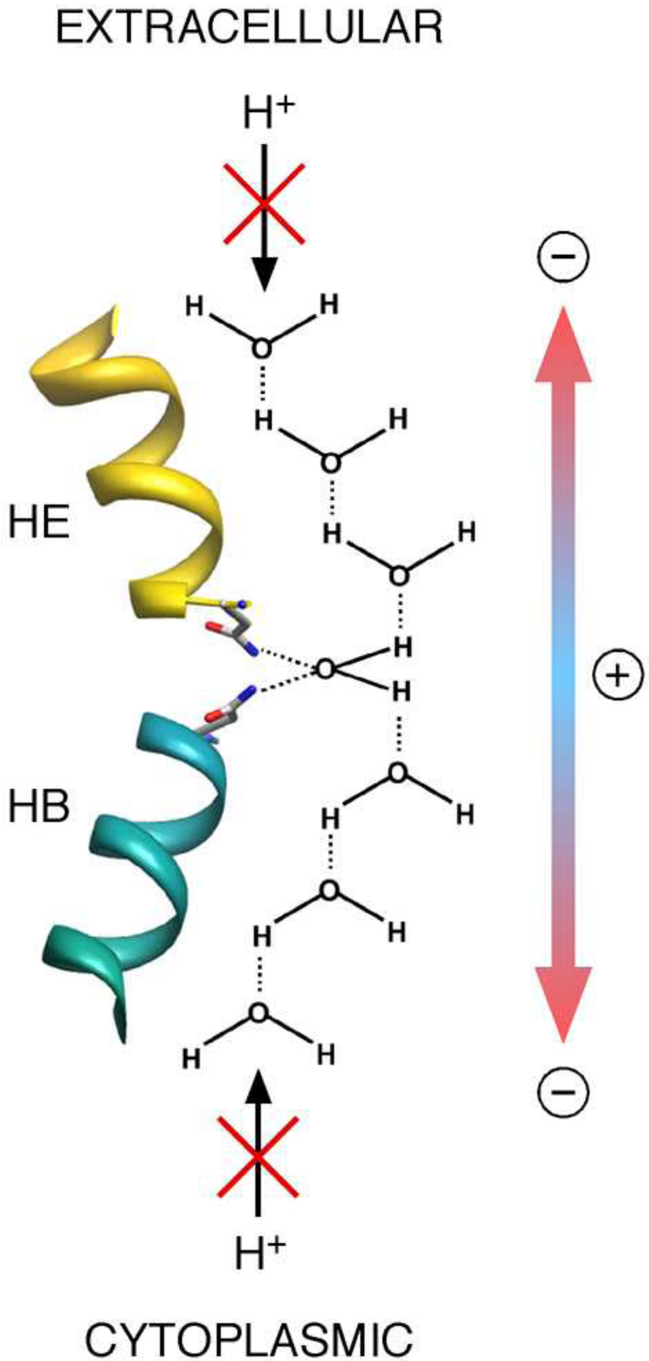
Water molecules rotate by about 180° during passage.



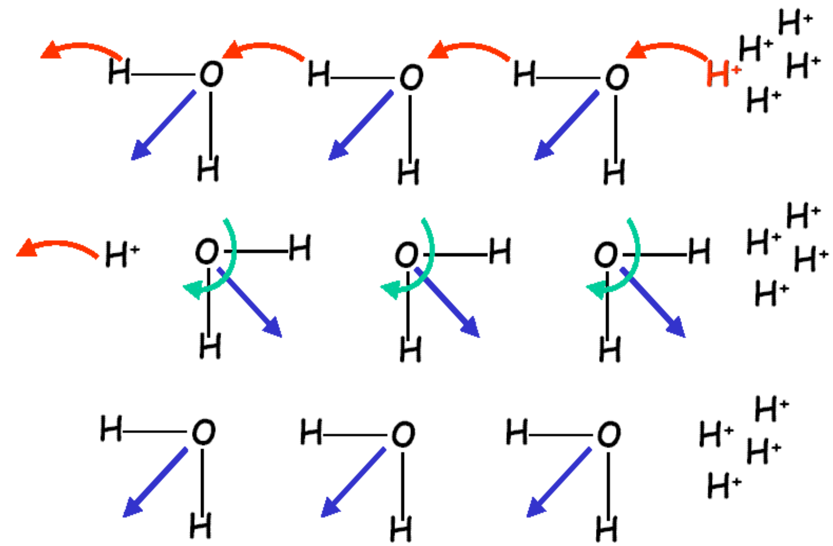
Water–water interactions are distorted with respect to bulk .

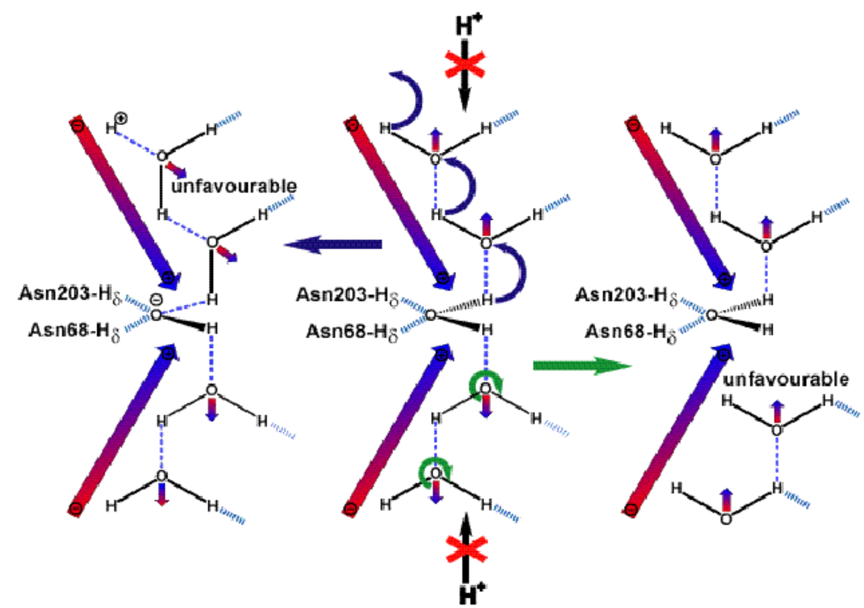
Peter Agre , David Kozono, FEBS Letters 555 (2003) 72-78

Impermeability for protons

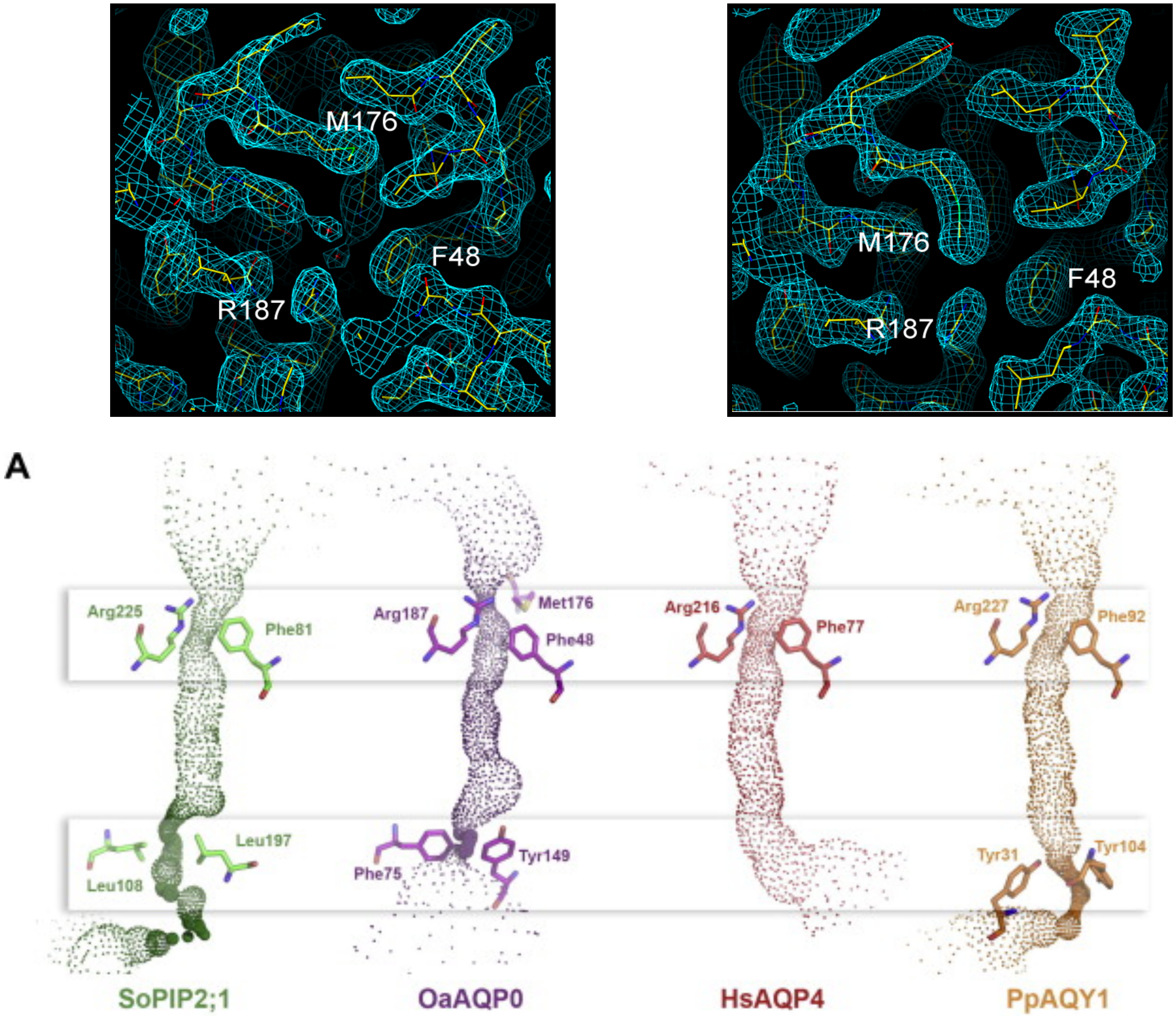


Proton transfer in bulk water





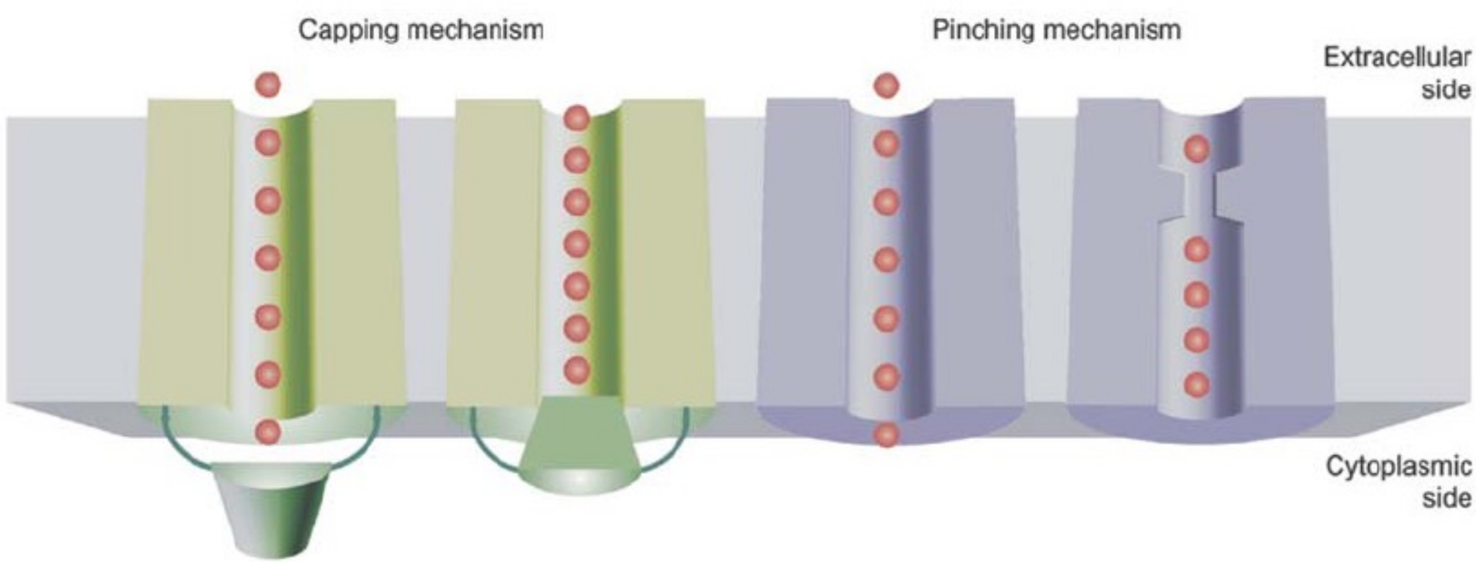
An aquaporins gate



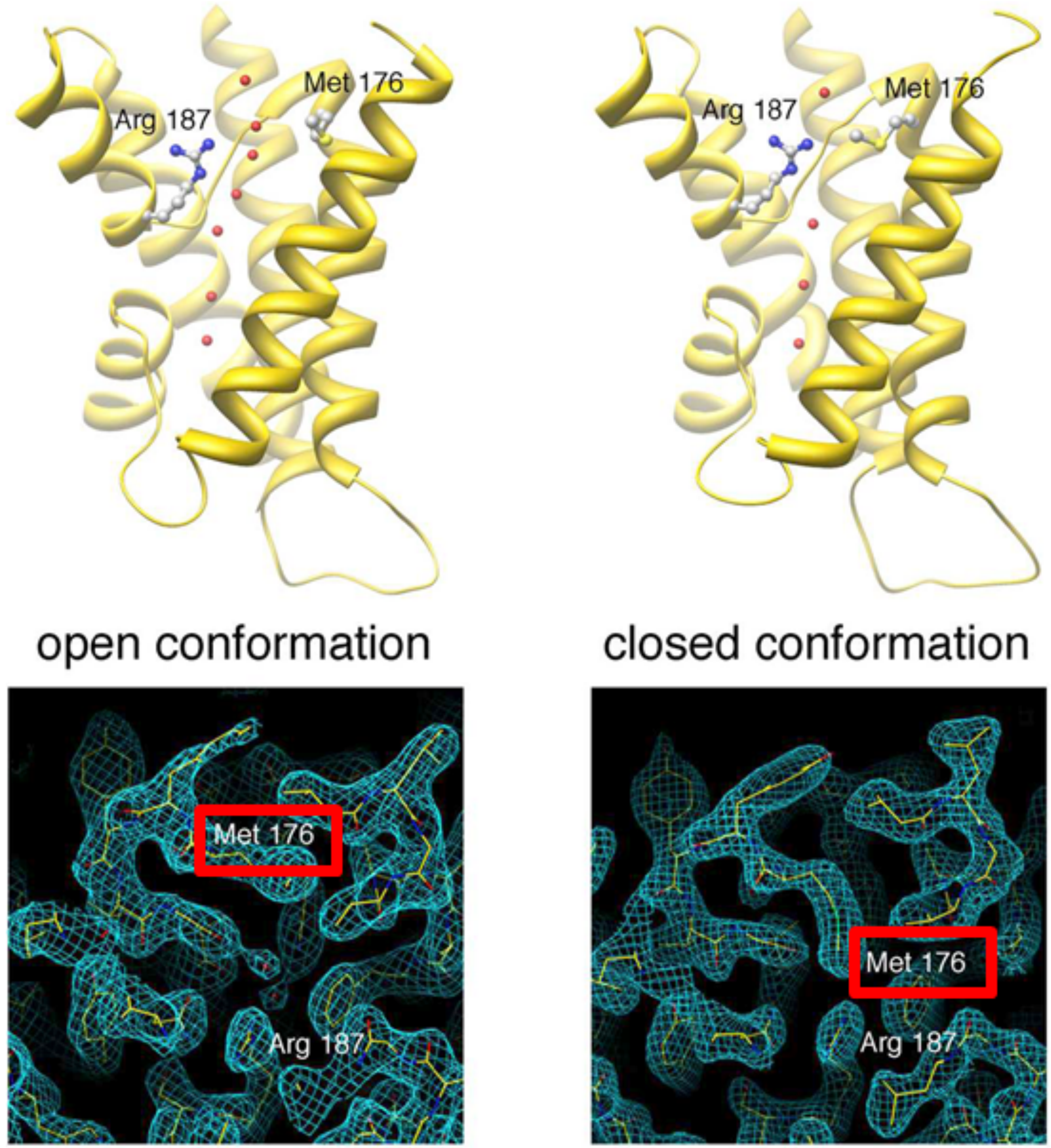
Eukaryotic aquaporins are frequently regulated by phosphorylation, pH, divalent cations, interactions with other proteins and osmolarity.

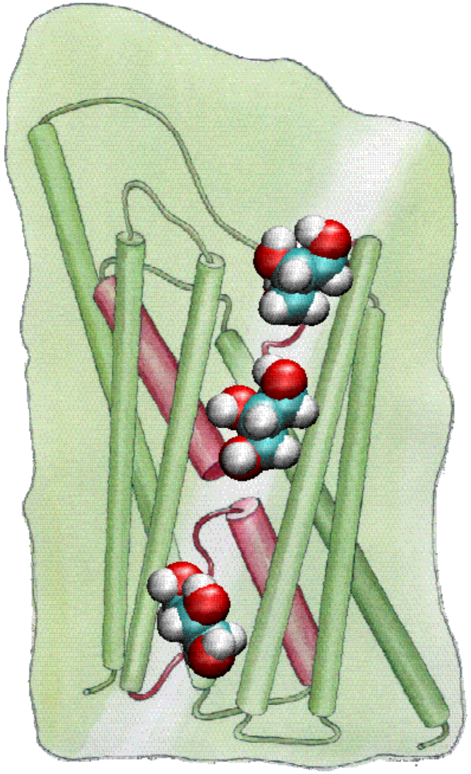
Aquaporin regulation at the protein level includes induced targeting of aquaporins to different membranes as well as direct gating of the aquaporins in situ in the membrane.

The capping (SoPIP2;1) and pinching (AQP0 and AQPZ) mechanisms of aquaporin gating.



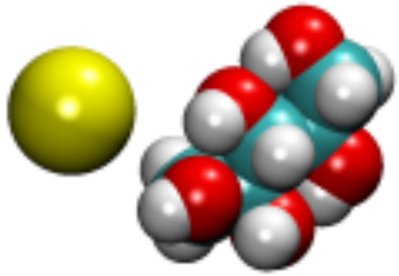
Open and closed conformations of the AQP0 water pore





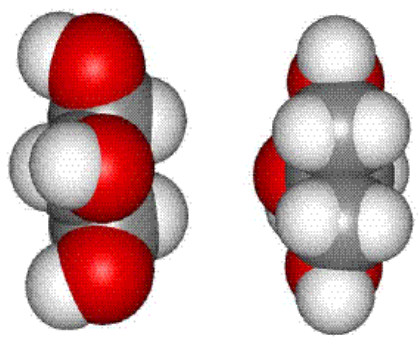
The E. coli Glycerol Facilitator (GlpF)

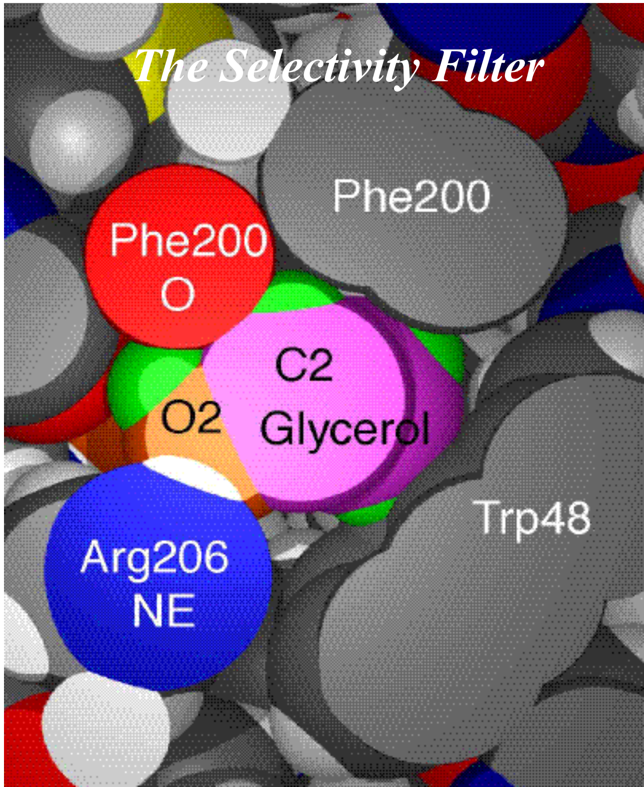
It is an aquaporin with an extra feature – ***it allows small, linear sugars such as glycerol and ribitol, but not ions, to pass.***



A sodium ion and ribitol molecule.

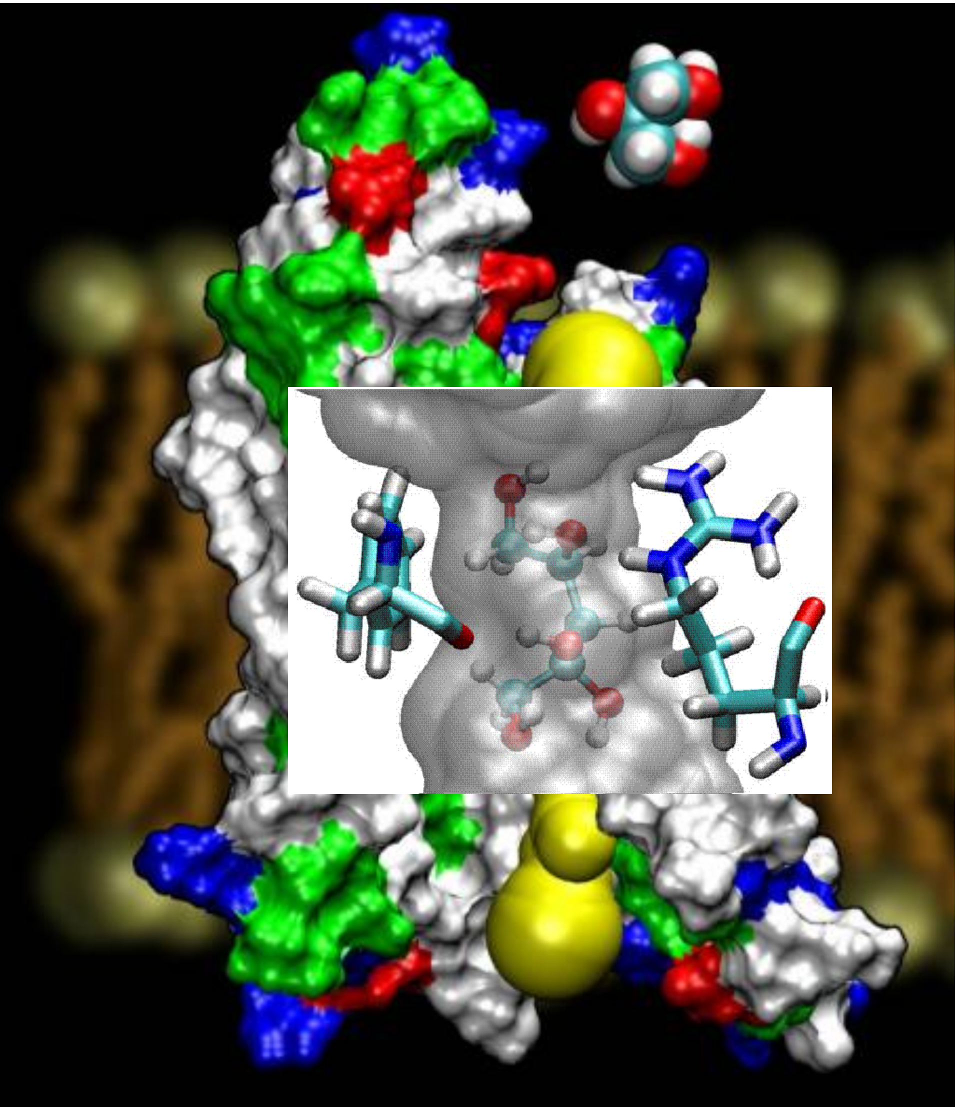
Complementarity

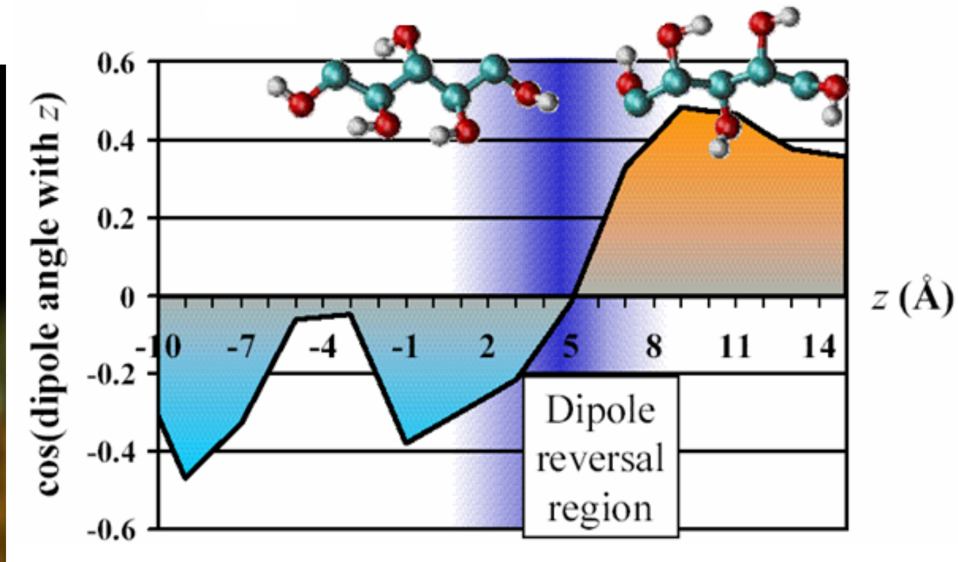


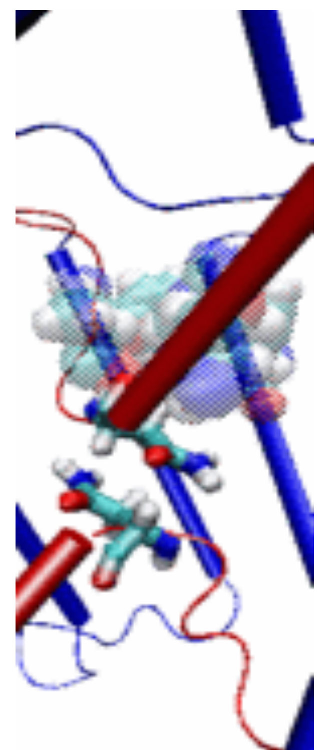


glycerol molecule ⇔ channel

The role of the selectivity filter







Cellular Volume Homeostasis

Physiology and pathophysiology of cell volume change

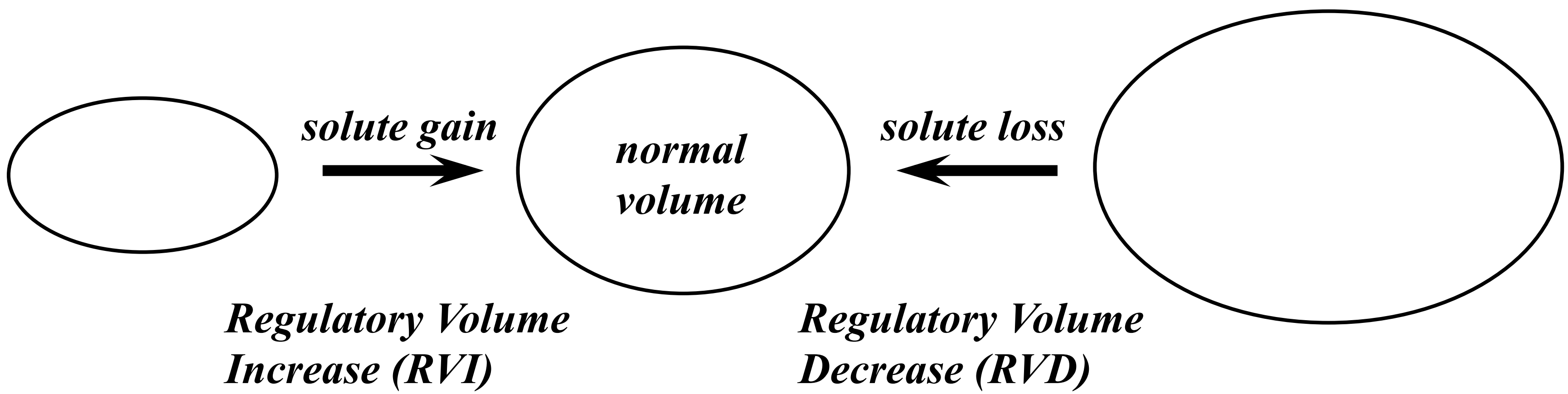
- ***Physiology: all cells are exposed to isosmotic volume perturbations***

- ***Physiology: organisms and cells that live in osmotically unstable environments***

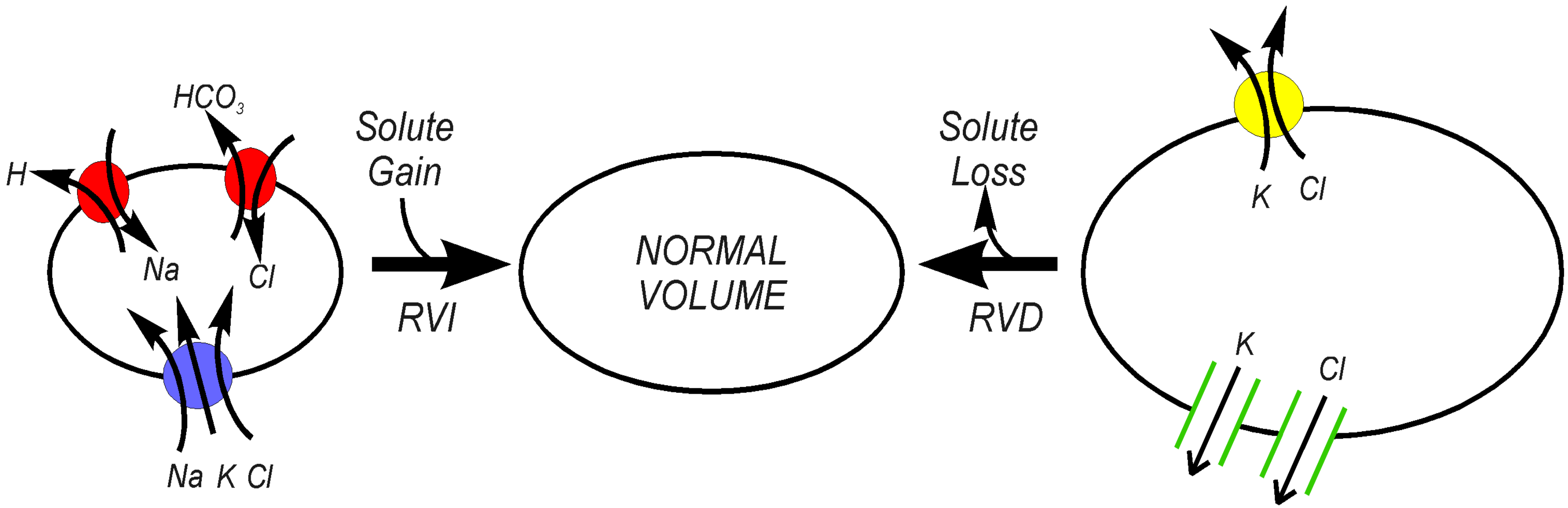
  >intertidal zone
  >gut
  >kidney

- ***Pathophysiology: e.g., systemic osmolality disturbances, anoxia and ischemia, reperfusion injury, diabetes, sickle cell crisis***

Cell volume is regulated by the gain and loss of osmotically active solutes



Volume regulatory electrolyte gain and loss are mediated by rapid changes in membrane transport



- ***Advantages:** allows cell to rapidly correct their volume by activating pre-existing transport pathways*

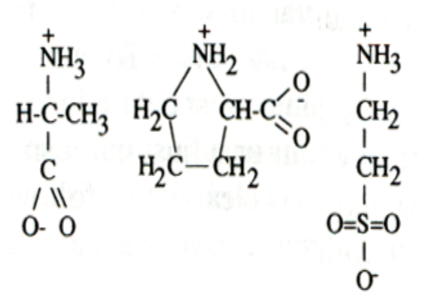
- ***Disadvantages:** disruption of intracellular ion concentrations and cytoplasmic ionic strength*

Organic osmolytes allow cells to maintain long-term stability of cytoplasmic ionic strength

Three major classes of organic osmolytes:

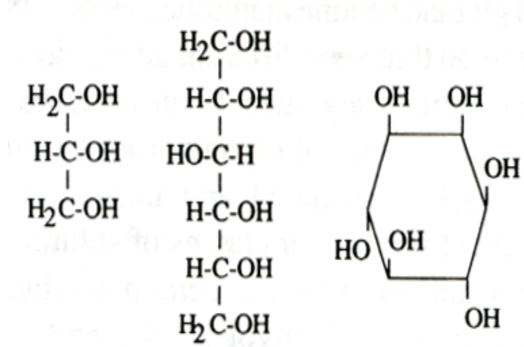
Amino acids

Alanine *Proline* *Taurine*



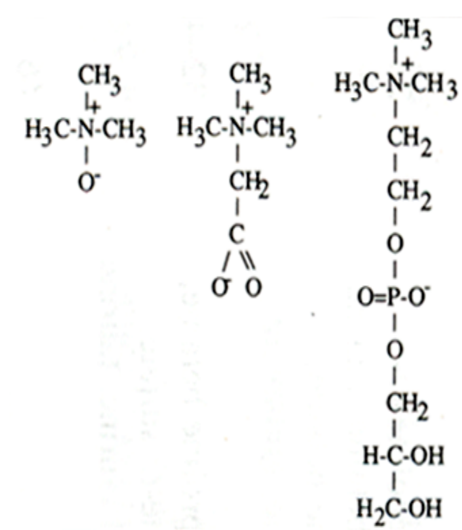
Polyols

Glycerol *Sorbitol* *myo-Inositol*



Methylamines

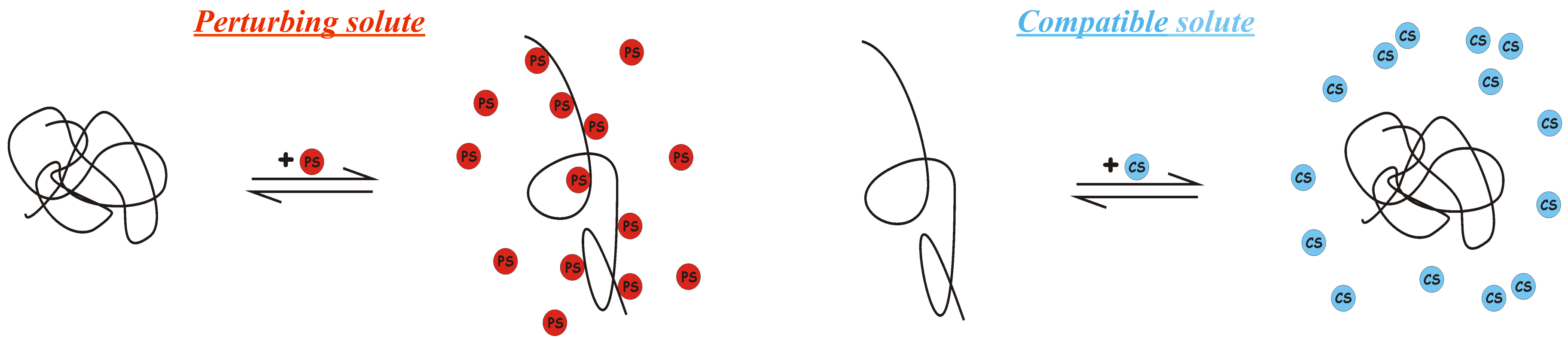
TMAO *Betaine* *GPC*



Organic osmolytes are "compatible" or "non-perturbing" solutes

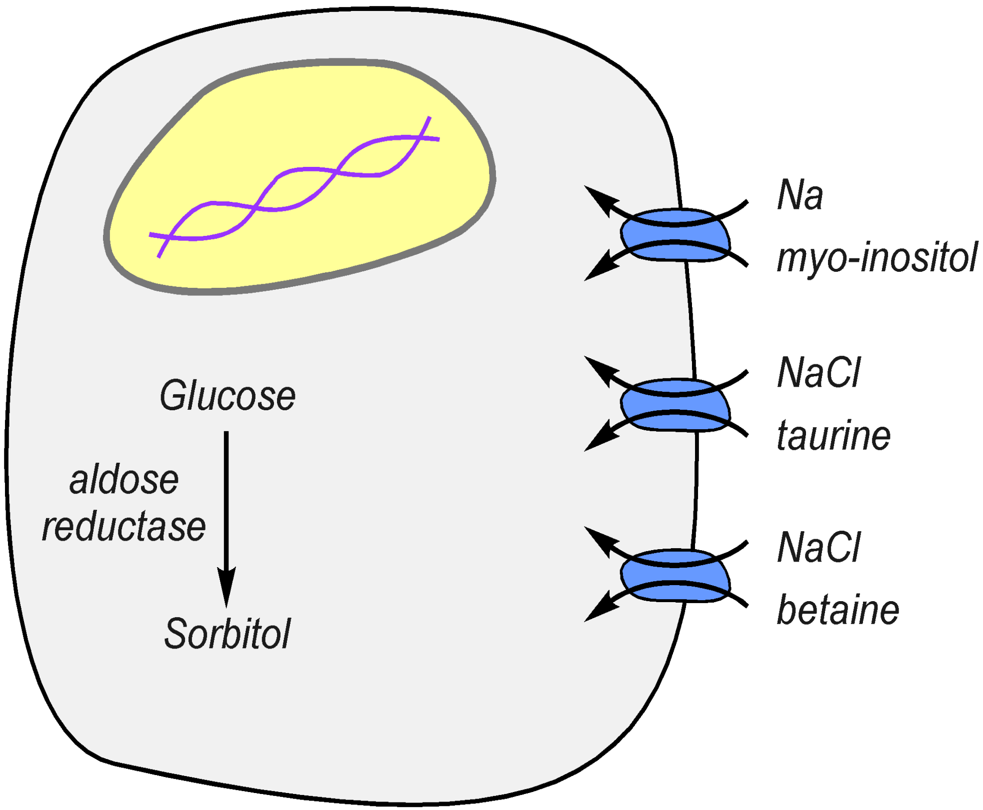
- *Compatible solutes are an ubiquitous solution to osmotic stress; used by all organisms for cellular osmoregulation*
- *High water solubility: accumulated to cytoplasmic concentrations of 10s to 100s of millimolar*
- *Compatible solutes do not perturb macromolecular structure or function when present at high concentrations*

Compatible solutes are excluded from the surface of macromolecules



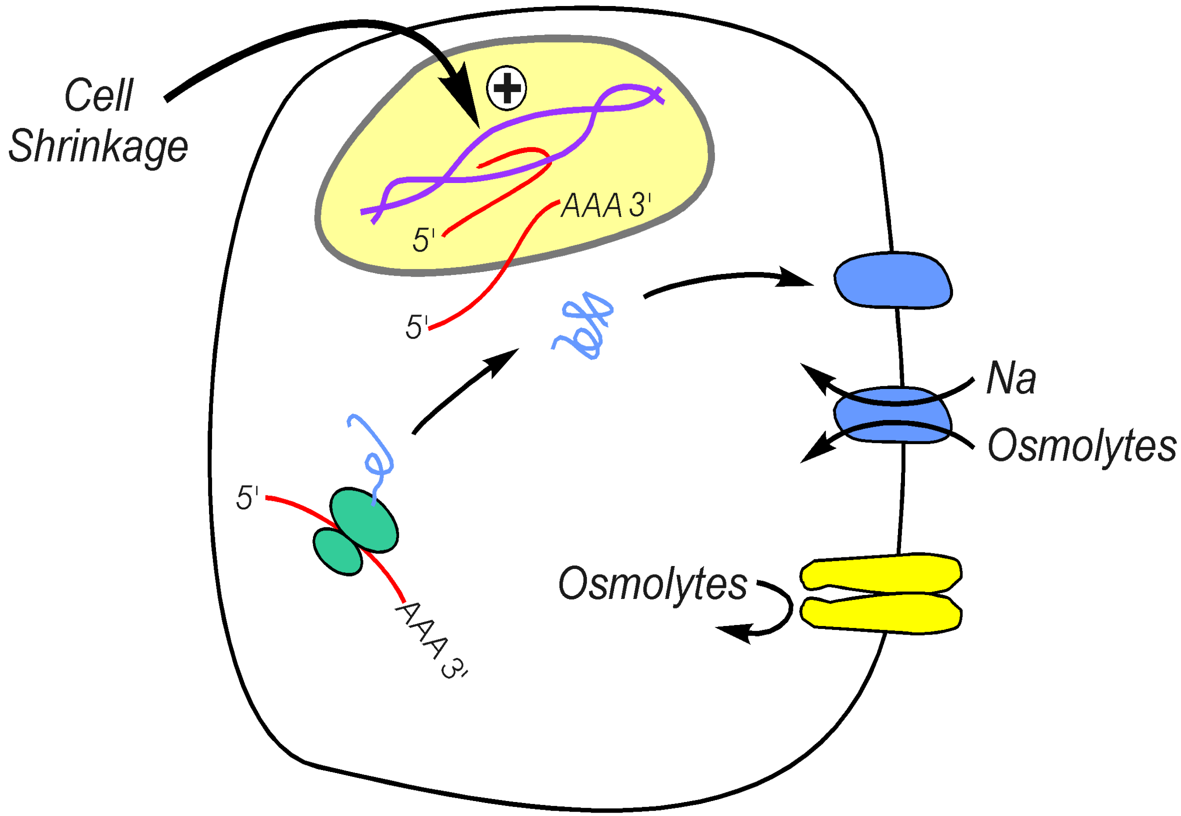
- ***No net charge at physiological pH***
- ***Lack strongly hydrophobic regions***
- ***Steric properties***

Organic osmolyte accumulation occurs by changes in synthesis or membrane transport



- ***Metabolically expensive: organic osmolytes are accumulated against concentration gradients of up to 10^7-fold***

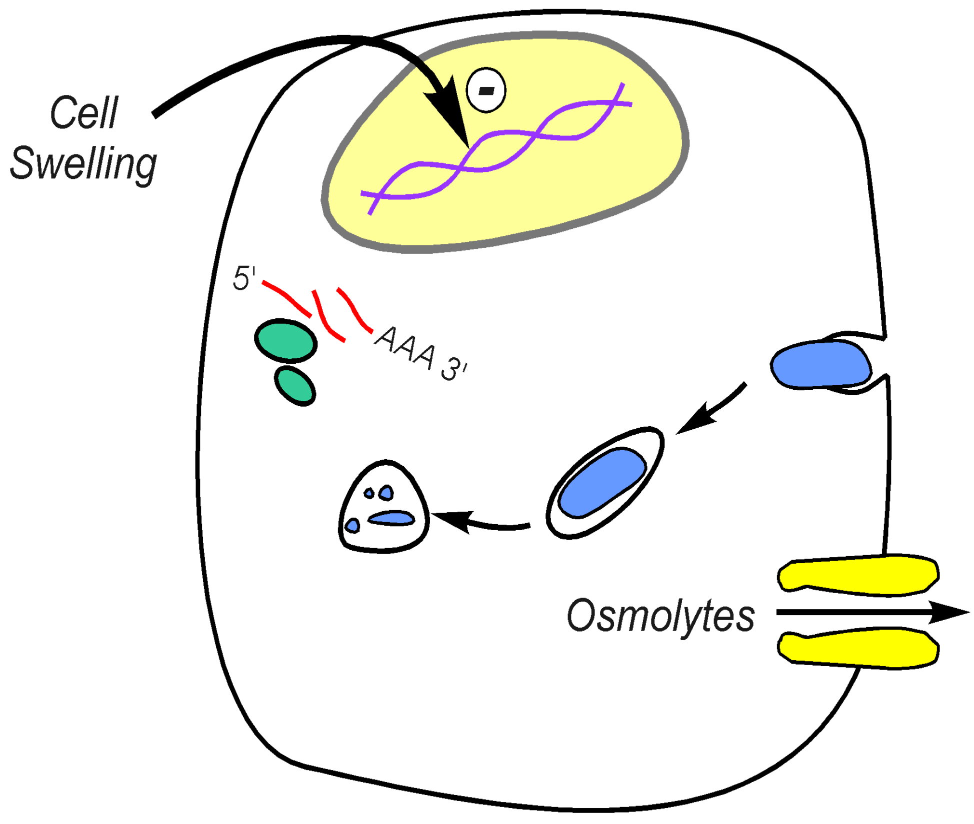
Organic osmolyte accumulation requires increased gene expression



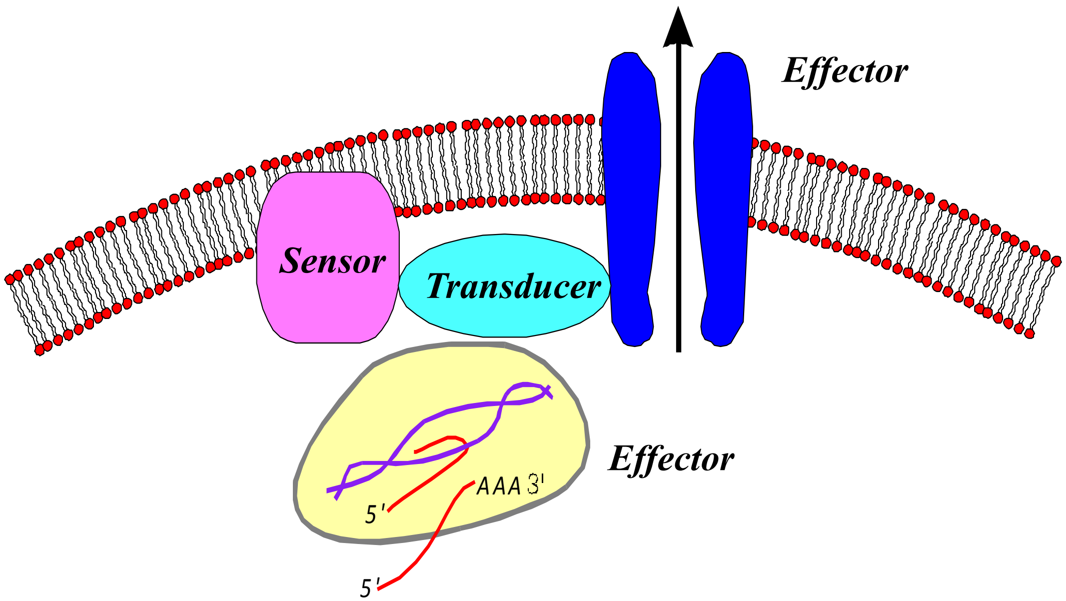
- ***Slow: requires many hours of exposure to osmotic stress***

Organic osmolyte loss is mediated by:

1. ***Decreases in gene expression: rapid and slow components***
2. ***Increase in passive efflux: rapid***



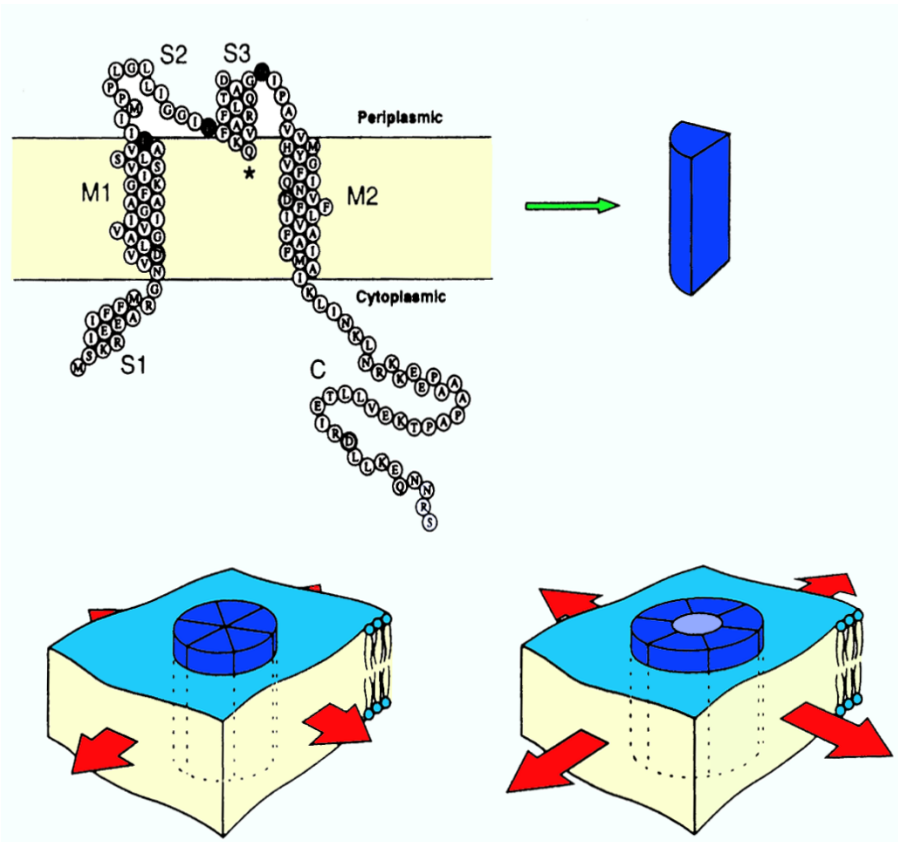
How do cells detect volume changes?



- *Signals: mechanical stress; dilution and concentration of cytoplasmic constituents*
- *Signal transduction: kinases and phosphatases*

Mechanical stress (bilayer model): force transduction via the lipid bilayer

E. coli MscL channel



From Sukharev et al. Ann. Rev. Physiol. 59:633-657, 1997

MscL channel

- *swelling/stretch-activated*
- *cloned protein activated by bilayer stretch*

ProP transporter

- *shrinkage-activated*
- *cloned protein activated by liposome shrinkage*

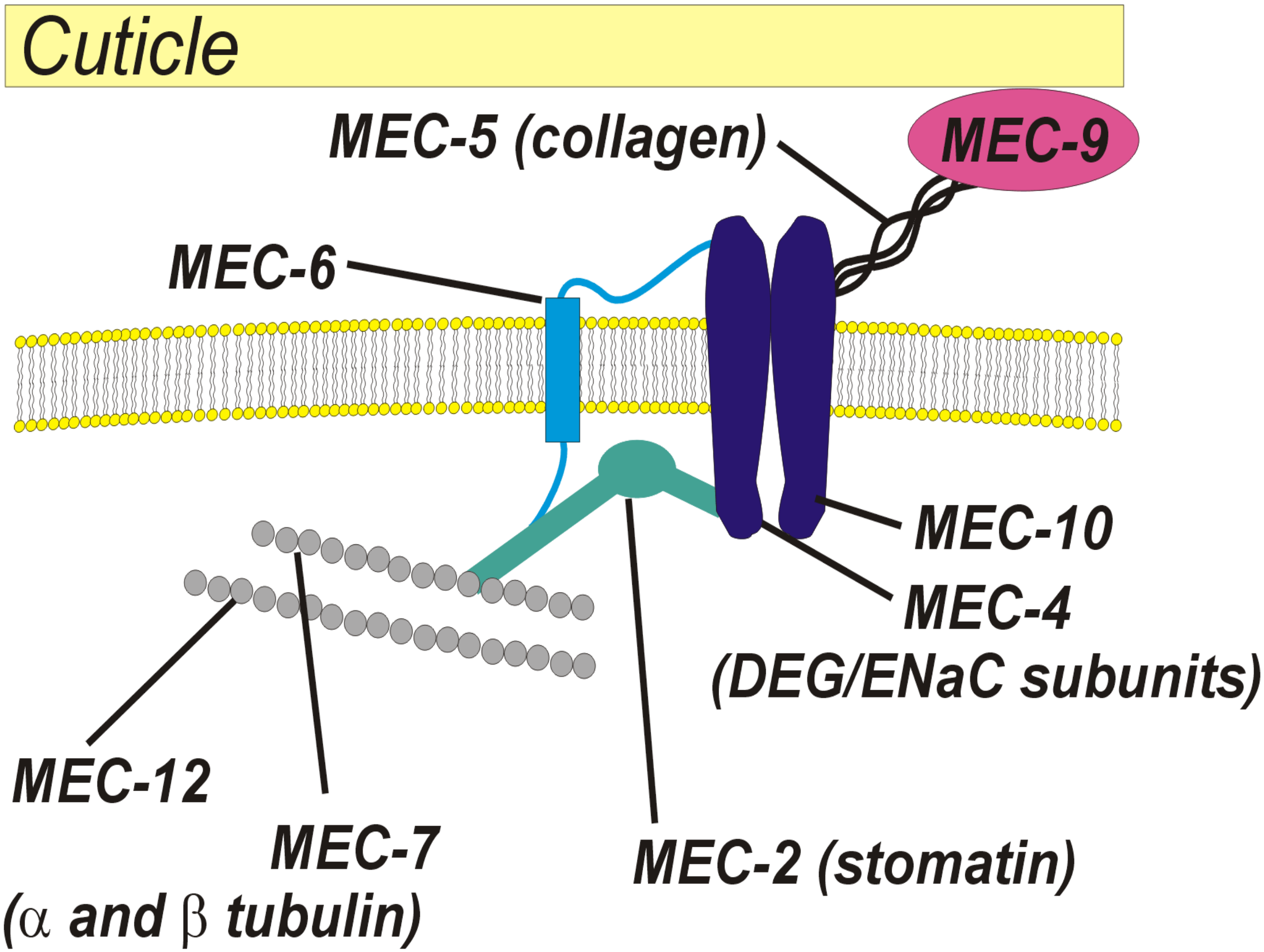
Membrane-bound enzymes

- *PLA_2*
- *GTPases*

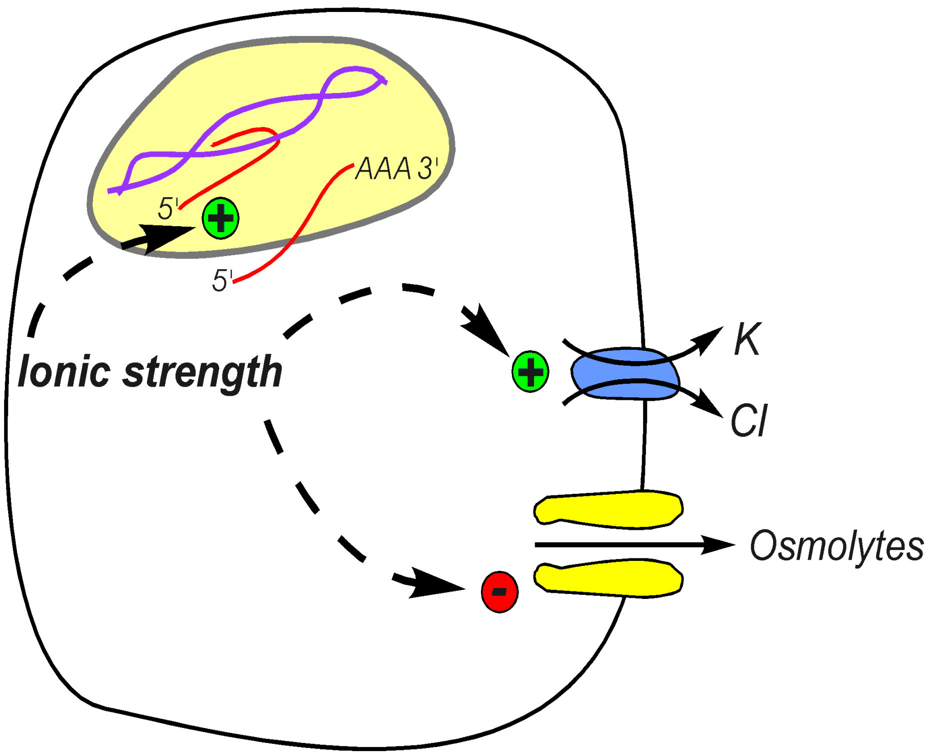
Model channels

- *alamethicin*
- *gramicidin*

Mechanical stress (tethered model): force transduction via cytoskeletal/extracellular proteins

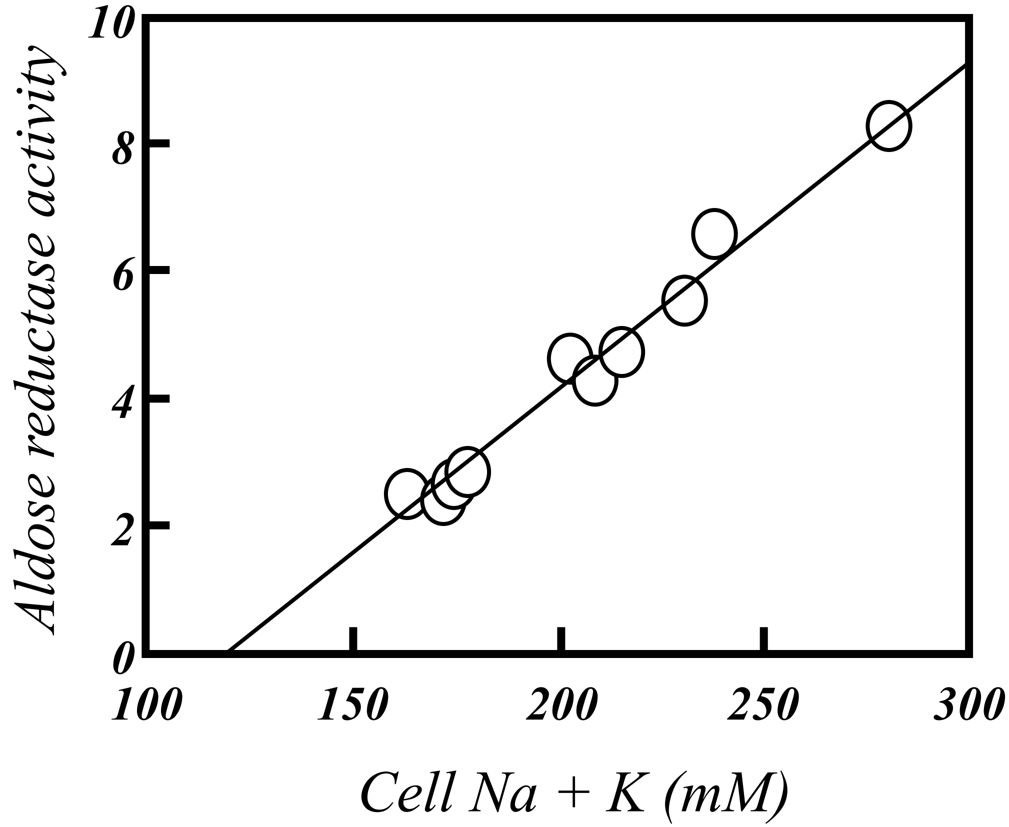


Cytoplasmic dilution/concentration of small solutes



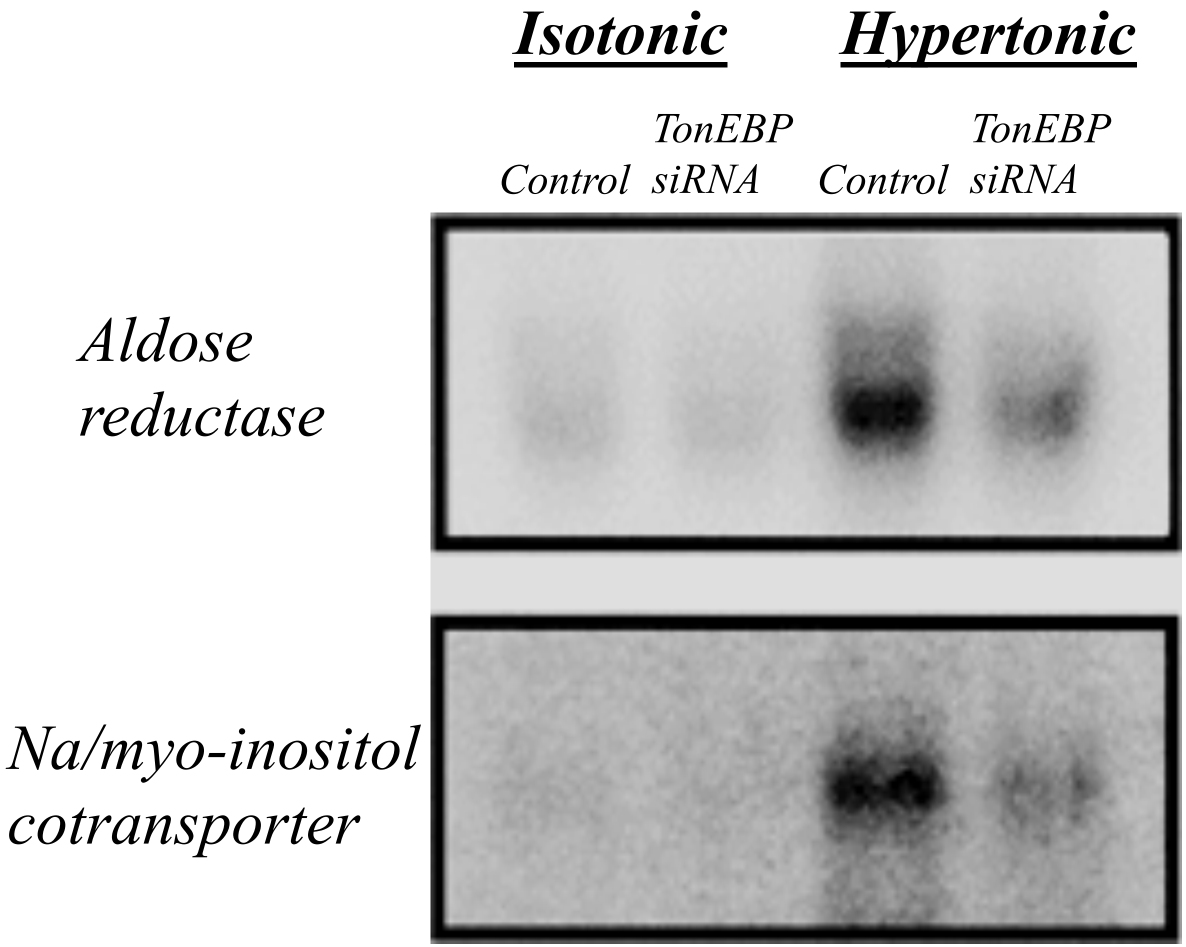
- ***Intracellular ionic strength***
- ***Specific ions (e.g., K^+)***
- ***Other solutes??***

Increased cell ionic strength increases expression of organic osmolyte transporters and synthesis enzymes



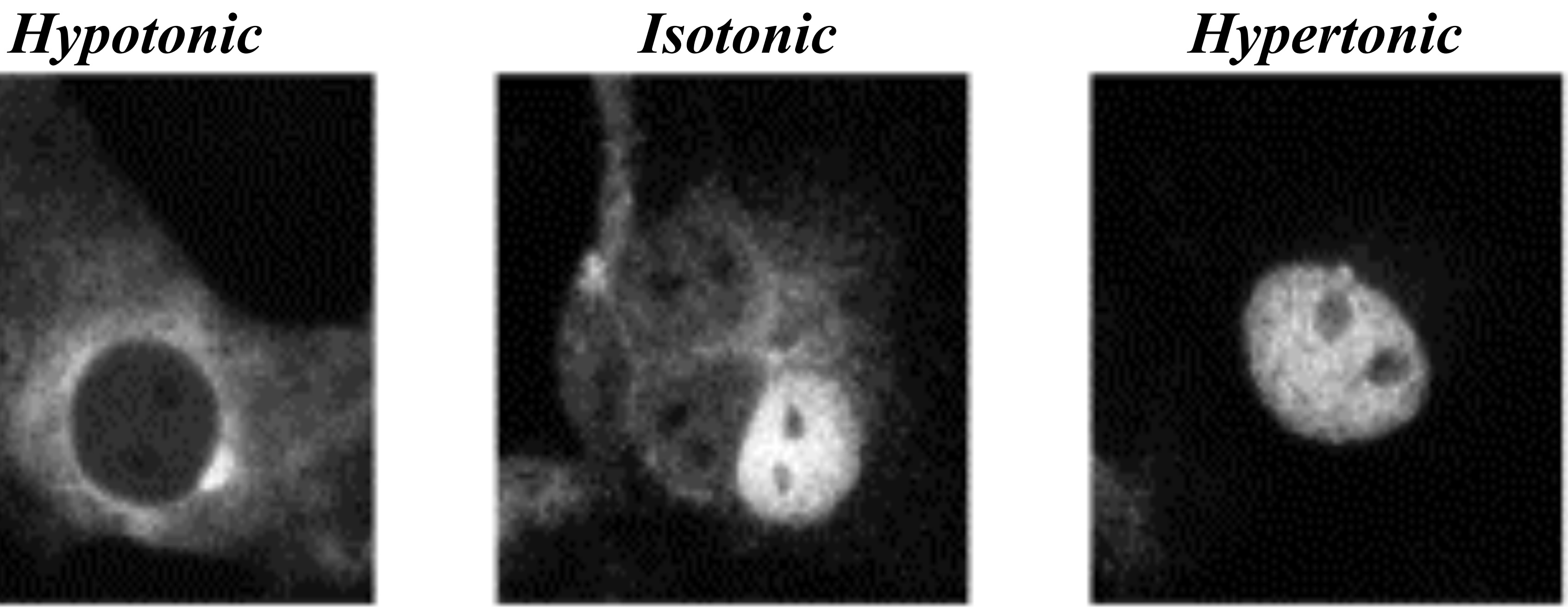
Uchida et al., Am. J. Physiol. 256:C614-C620, 1989

The transcriptional activator TonEBP regulates hypertonicity-induced gene expression



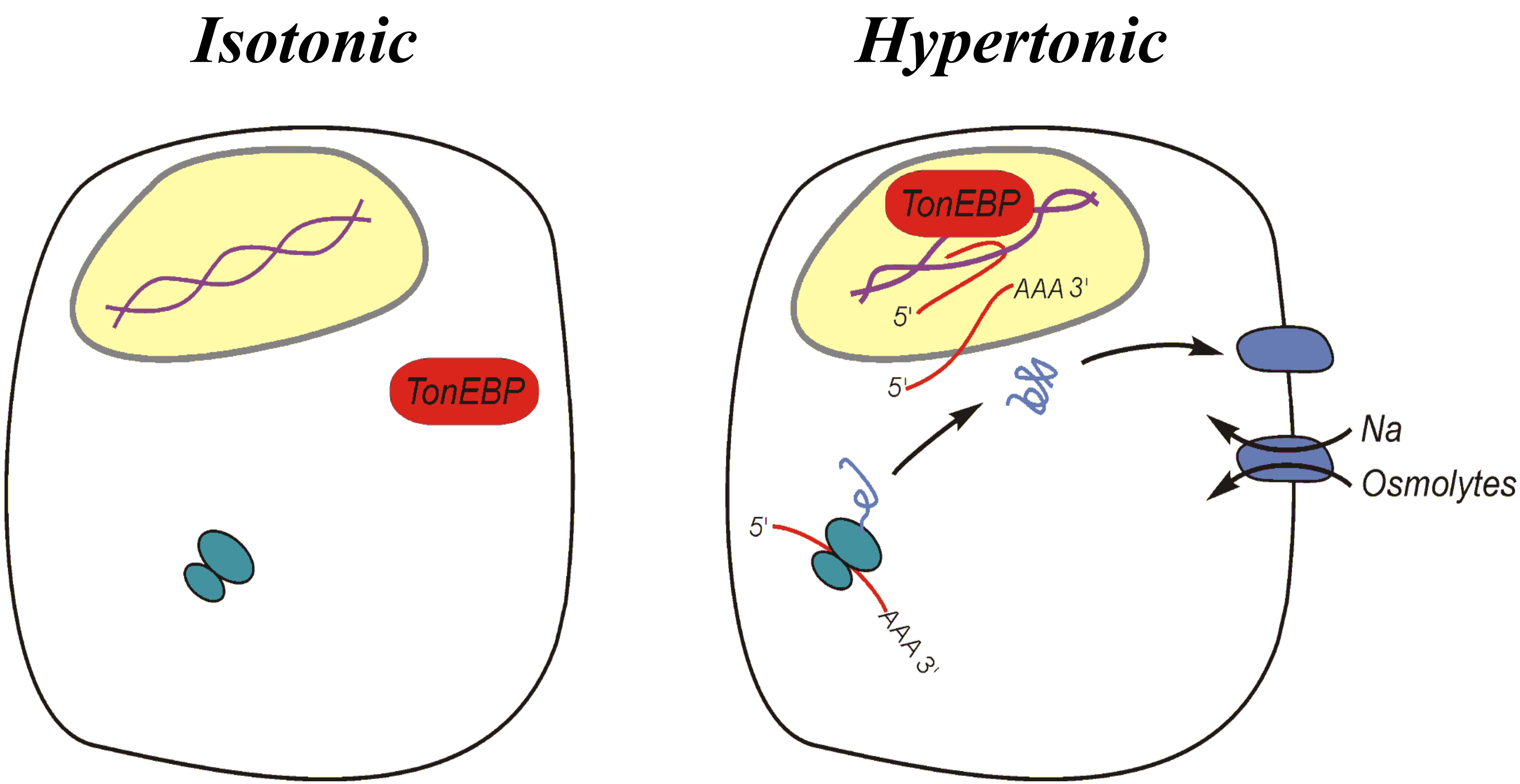
Young et al., J. Am. Soc. Nephrol. 14:283-288, 2003

TonEBP translocates into the cell nucleus in response to hypertonic stress

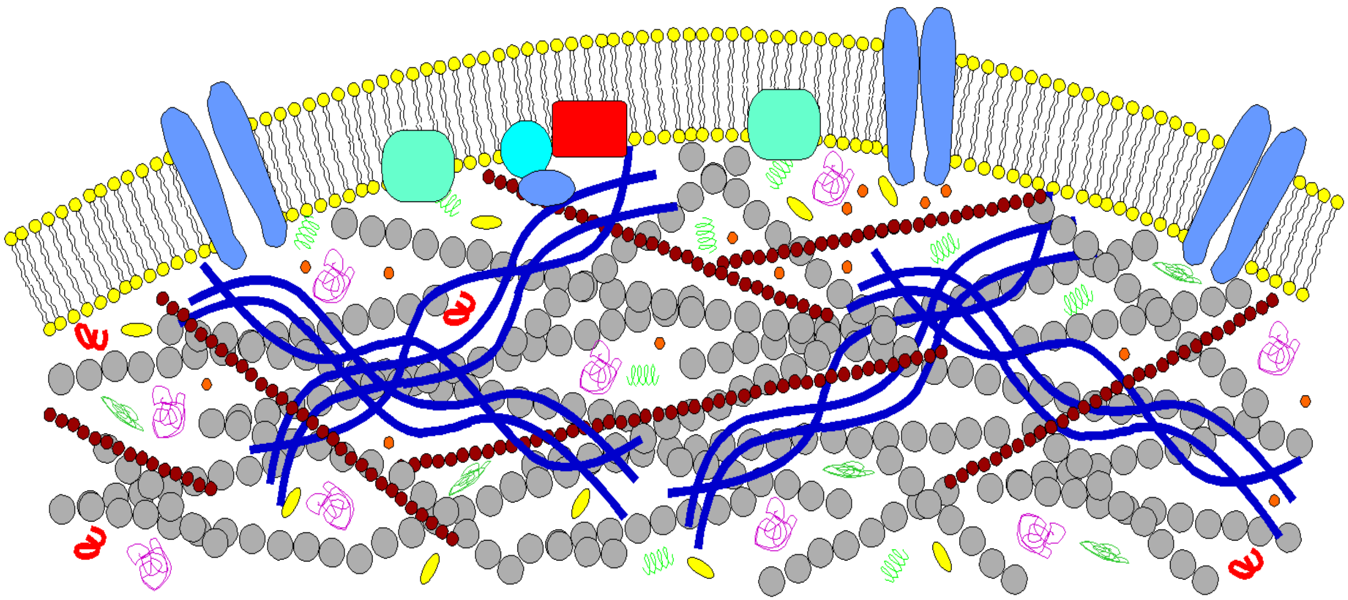


Lee et al., Biochem. Biophys. Res. Comm. 294:968-975, 2002

Regulation of gene expression by TonEBP

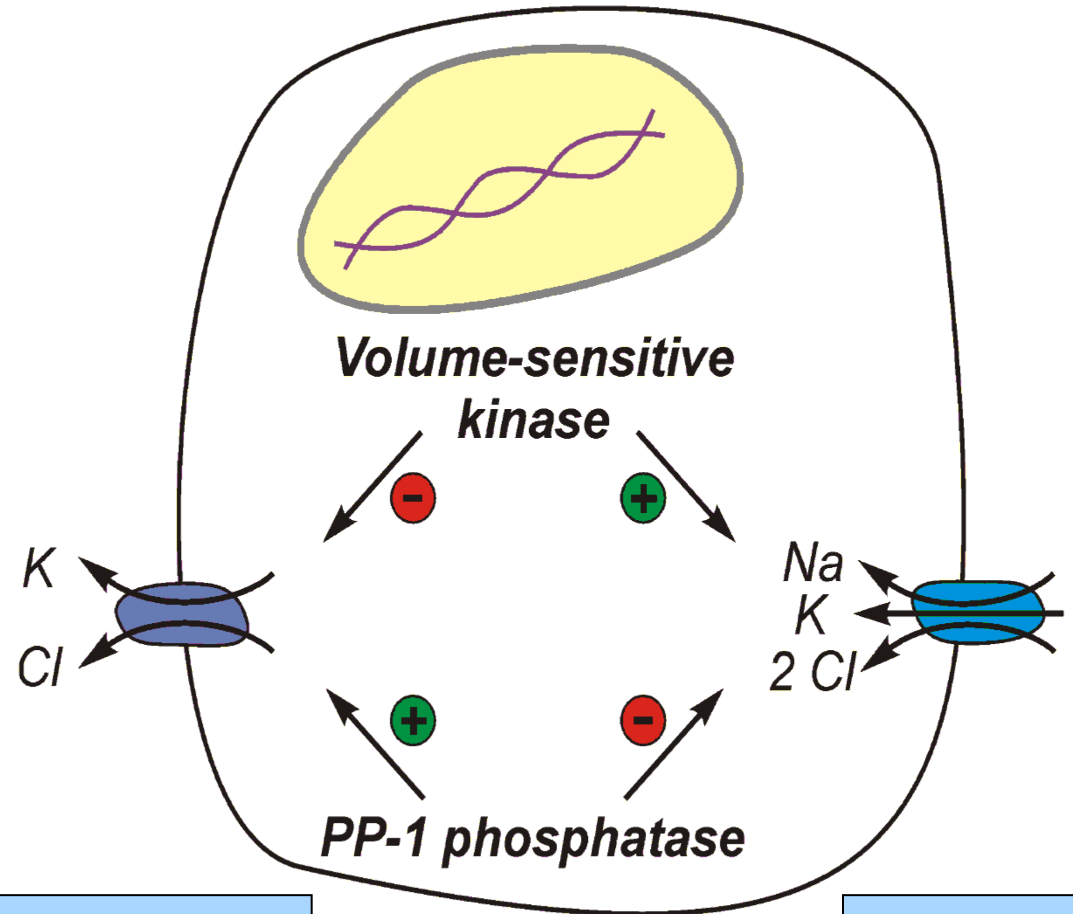


Cytoplasmic dilution/concentration: macromolecular crowding and confinement



- ***Crowding and confinement alter macromolecule thermodynamic activity, structure and interactions***
- ***Small changes in crowding and confinement can lead to large changes in the activity of signaling pathways, gene transcription, channel/transporter activity, etc.***

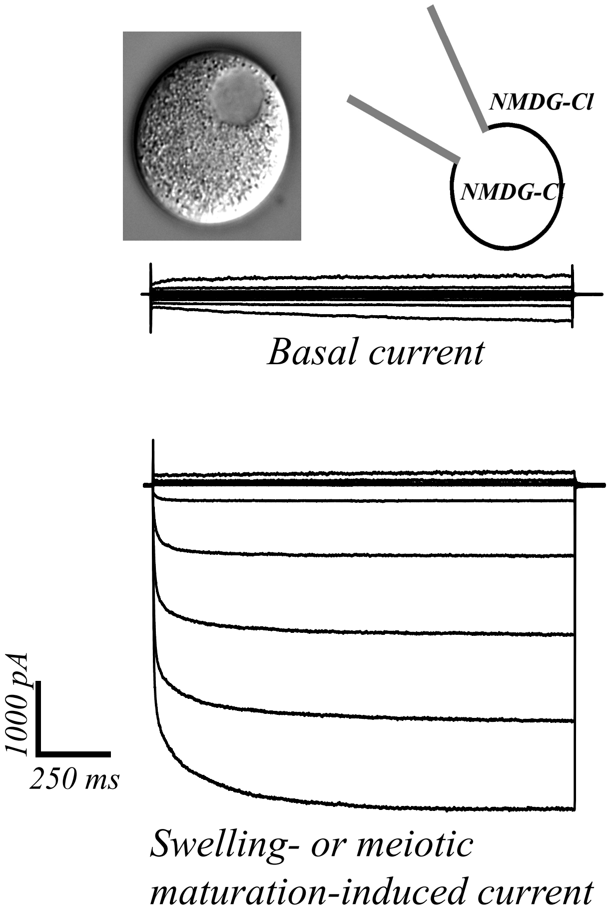
Signal transduction: the case for kinases and phosphatases

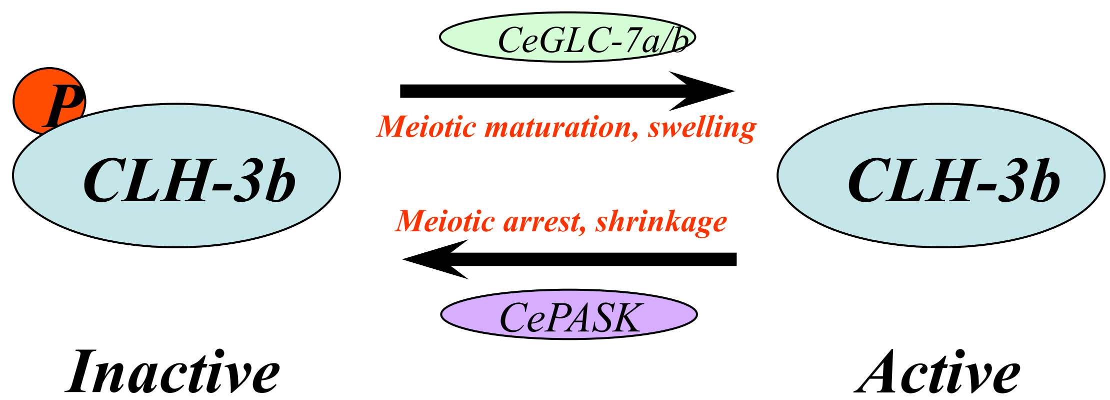


Swelling-activated RVD

Shrinkage-activated RVI

CePASK regulates the C. elegans volume-sensitive ClC channel CLH-3b





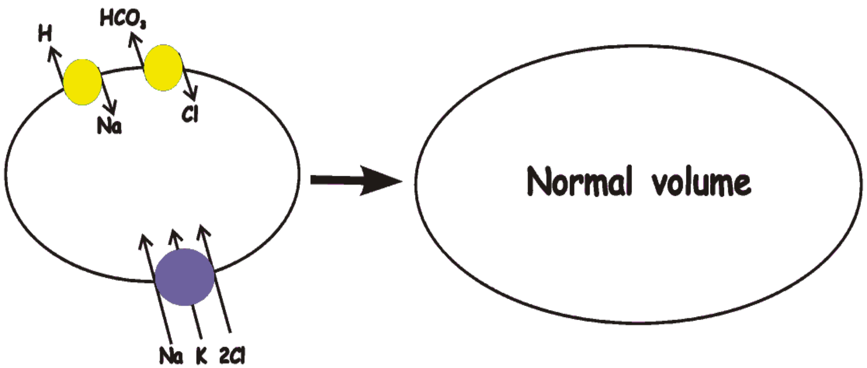
Denton, Rutledge, Nehrke, Strange

How cell volume is perturbed matters

Cells sense:

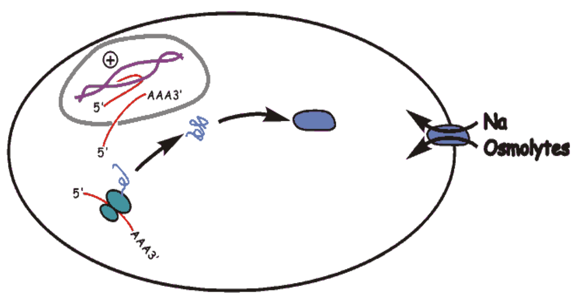
- ***Extent of volume change***
- ***Rate of volume change***
- ***Mechanism of volume change (anisosmotic vs. isosmotic)***

The cellular osmotic stress response



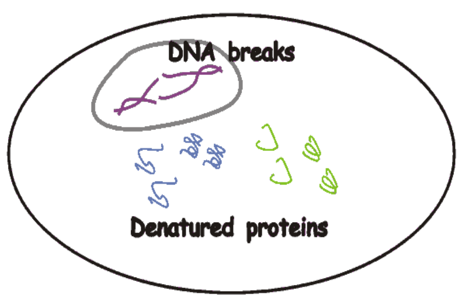
Volume recovery

- *Rapid electrolyte accumulation/loss*



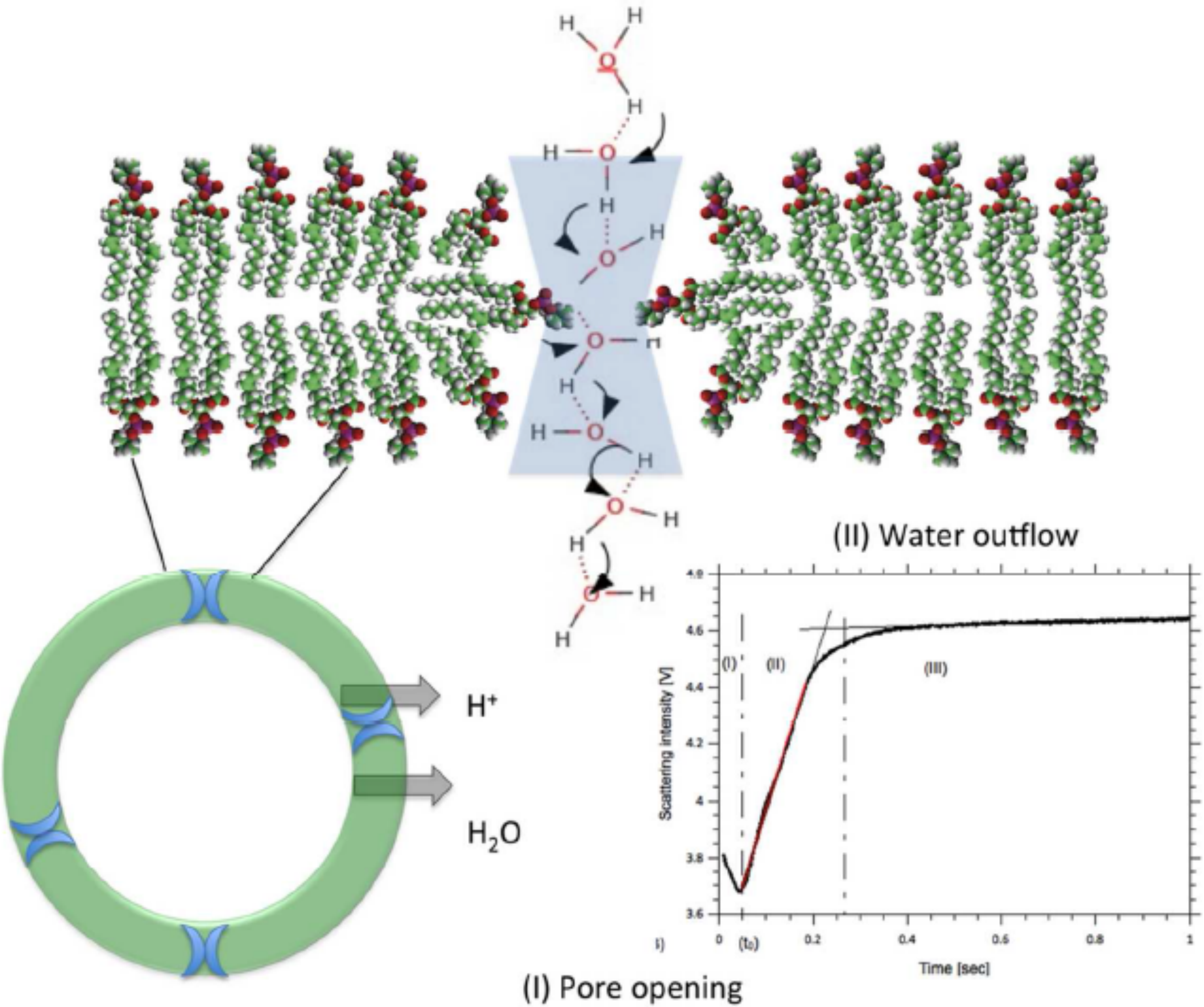
Organic osmolyte homeostasis

- *Slow accumulation*
- *Rapid efflux/loss*



Damage detection/repair

- *Detection*
- *Cell cycle arrest*
- *Repair or apoptosis*

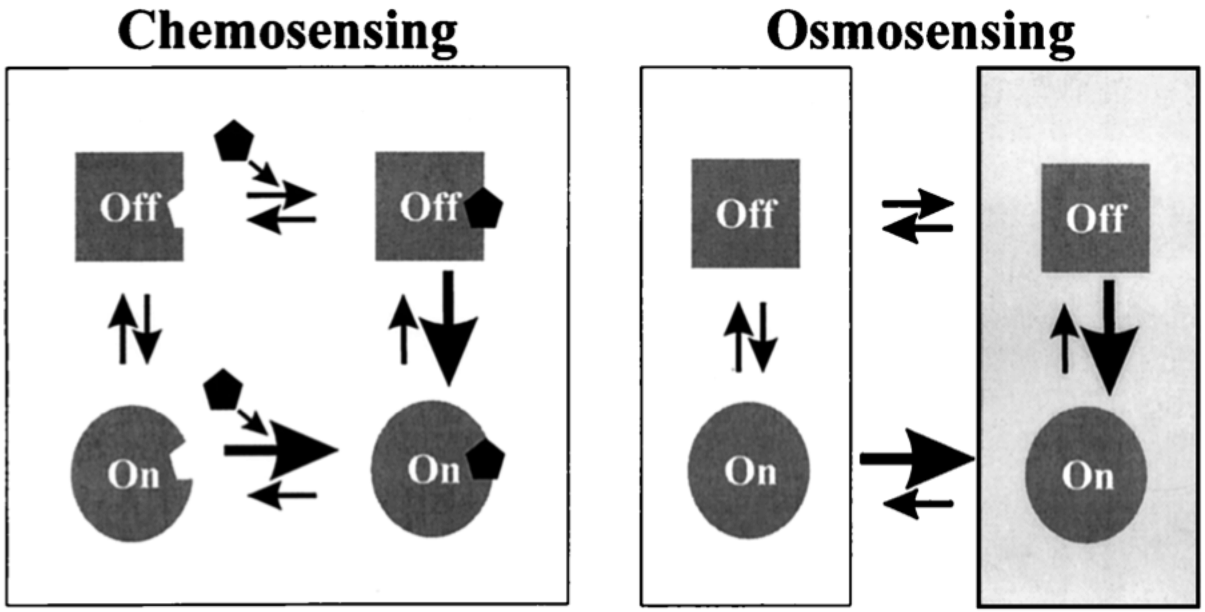

H⁺
H_2O
(II) Water outflow
Scattering intensity [V]
4.8
4.6
4.4
4.2
4
3.8
3.6
(I)
(II)
(III)
0
(t₀)
0.2
0.4
0.6
0.8
1
Time [sec]
(I) Pore opening

Phase	Structural Change	Approximate Duration	Physiological Change
III	Cell Wall and Nucleoid Remodeled DNA/Protein Synthesis Resume Cell Growth and Division Resume Co-solvent Composition Adjusted	**1 or more hours**	Osmoresponsive Genes Expressed (e.g. *proP*, *proU*, *kdpFABC*, *betT*) Compatible Solute Uptake/Efflux Cycle Established
II	Nucleic Acid Counterions Replaced Rehydration Begins	**20 to 60 minutes**	Putrescine Extruded K^+ Glutamate and Compatible Solutes Accumulate Respiration Resumed (Reduced Rate) $\Delta\tilde{\mu}_{H^+}$ Restored ATP Level Restored
I	Cell Dehydrates, Shrinks Cytoplasmic a_w Decreased Cytoplasmic Crowding Increased Wall/Membrane Strain Altered	**1 to 2 minutes**	Respiration and Most Transport Cease; Trk/ProP Activate ΔpH Increased Transiently ATP Level Increased Transiently
Shift	**Upshift: $\Delta\Pi$ decreased, $\Delta\Pi < \Delta P$, $\Delta\mu_w < 0$** ↑ **Time 0: $\Delta\Pi = \Delta P$, $\Delta\mu_w = 0$** ↓ **Downshift: $\Delta\Pi$ increased, $\Delta\Pi > \Delta P$, $\Delta\mu_w > 0$**		
I	Cell Hydrates, Swells Cytoplasmic a_w Elevated Cytoplasmic Crowding Decreased Wall/Membrane Strain Is Altered	**< 1 minute**	Channels Open
II	Cell Shrinks Cytoplasmic Crowding Increased	**1 to 2 minutes**	Co-solvents and Water Extruded $\Delta\tilde{\mu}_{H^+}$ Collapsed?
III	?	**10 to 20 minutes**	Channels Close $\Delta\tilde{\mu}_{H^+}$ Restored Co-solvents Re-accumulate

Phases of the osmotic stress response for E. coli K-12.

Structural and physiological responses triggered by osmotic shifts (up or down) imposed at time zero proceed in parallel along the indicated, approximate timescales.

Chemosensing versus osmosensing.

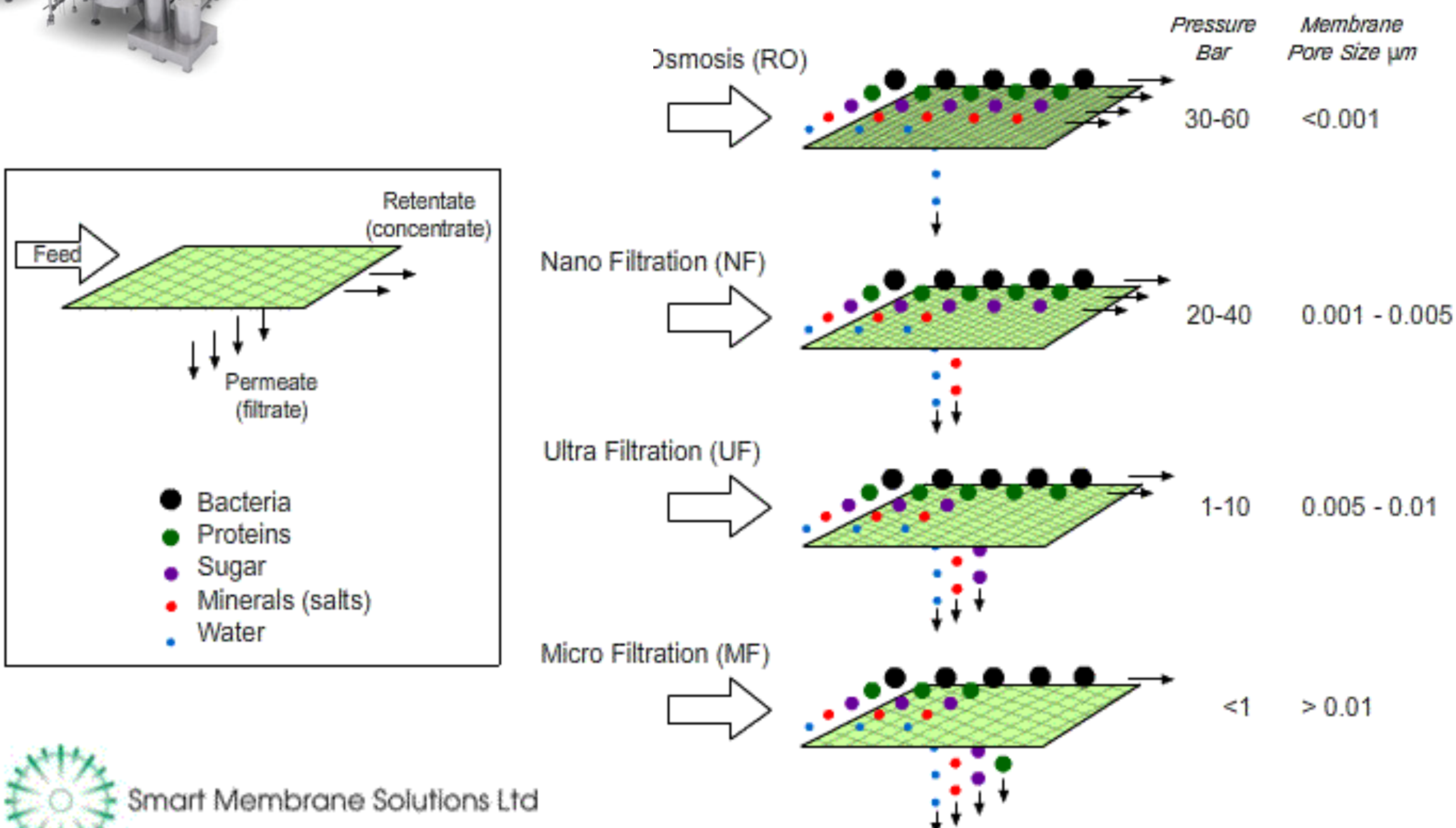


***Chemosensors** detect the biochemistry of cellular environments, including changes in nutrient supplies and signals with biological origins.*

***Osmosensors** detect changes in extracellular water activity (direct osmosensing) or resulting changes in cell composition or structure (indirect osmosensing).*

Potential stimuli for membrane- or nucleoid-based osmosensors

Compartment sampled	Stimulus detected, change in[b]:
Periplasm	Thickness
	Turgor pressure
	Concn of a specific cosolvent (e.g., glucan)
	Macromolecular crowding
	Osmolality
	Ionic strength
Cytoplasmic membrane	**Osmolality gradient**
	Lateral pressure
	Bilayer curvature
	Head group charge density
	Head group hydrogen bonding
	Head group hydration
	Thickness
	Lateral phospholipid distribution
	Intermonolayer phospholipid distribution
Cytoplasm	**Osmolality**
	Ionic strength
	Concn of kosmotropes
	Concn of a specific cosolvent (e.g., K glutamate)
	Macromolecular crowding or confinement
Nucleoid	Turgor pressure
	Counterion composition
	Protein composition
	Macromolecular crowding
	DNA topology



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