Learning Aims – lecture 2

- To know the terms polar and non-polar and how it relates to membranes and ions. To appreciate ions are hydrated in solution.
- To relate the basic components of an electrical circuit to a potential difference across the cell membrane
- To state factors which influence the passive flux of molecules across a cell membrane, as expressed by Ficks' Law
- To distinguish simple and facilitated diffusion, and facilitated diffusion via channels and carriers
- To briefly describe an example of a facilitated diffusion transport process including physiological relevance and protein involved
- To be able to define ion channels and their basic properties of gating and selectivity
- To recognise osmosis as water diffusion and relate tonicity and water flux across cell membranes. To know what aquaporins are.

Diffusion

Fick's law

$$\text{net flux} = PA(\Delta C)$$

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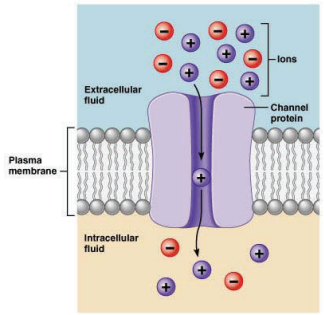
Facilitated Diffusion – 2. Ion Channel Mediated

Transmembrane proteins with aqueous pores that allow diffusion of ions



Germann & Stanfield Fig. 4.13

- Ion Flux is downhill (passive)
- Selective (e.g, a cation channel)
- *The cation influx shown in the schematic diagram makes the voltage inside the cell more positive.*

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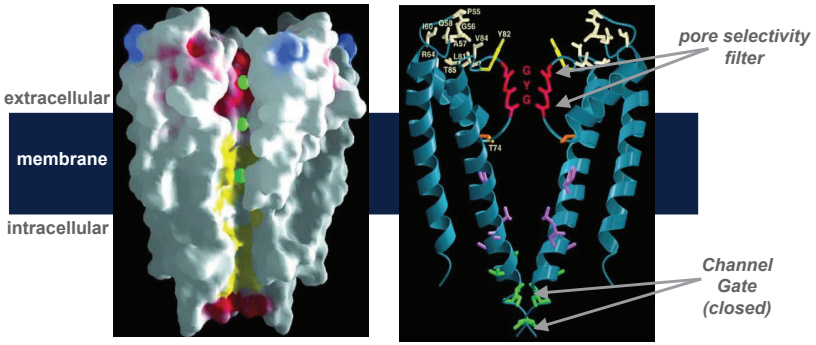
Facilitated Diffusion – Ion Channel Mediated

What it really looks like …… (Science, 1998)

The Structure of the Potassium Channel: Molecular Basis of K^+ Conduction and Selectivity

Declan A. Doyle, João Morais Cabral, Richard A. Pfuetzner, Anling Kuo, Jacqueline M. Gulbis, Steven L. Cohen, Brian T. Chait, Roderick MacKinnon*



Doyle et al., Science, 1998

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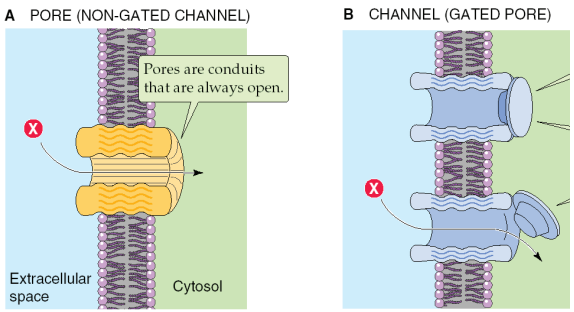
Facilitated Diffusion – Ion Channel Mediated

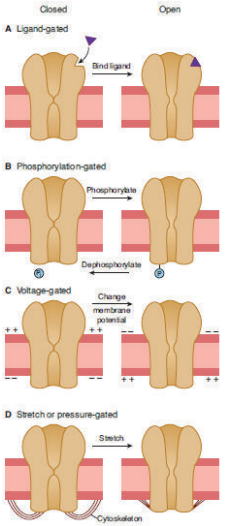
Can be always open, or can be "gated"……



from *Medical Physiology* by Boron and Boulpaep, Saunders, Philadelphia, 2003

Gating of Ion Channels

-changing between non-conducting to conducting conformations



Main modes of ion channel gating (& examples):

extracellular ligand (Panel A in Figure)
e.g. a neurotransmitter chemical released at a synapses)

intracellular phosphorylation (panel B) or binding of an intracellular ligand
e.g. change in phosphorylation due to metabotropic receptors can open r close different K^+ channels; an intracellular 2nd messenger like cyclic MP can open channels in sensory cells.)

A Change in the membrane potential (panel C)
e.g., the voltage-gated Na+ and K+ channels involved in action otentials)

Stretch of the membrane (panel D)
e.g., large mechanosensitive channels that allow protozoa to adjust to ifferent tonicity environments, channels in sensory nerve endings that etect touch)

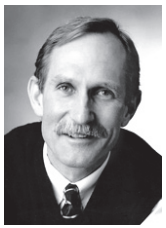
"background", "leak" channels or "Pores"
e.g., non-gated or weakly gated K^+ channels that help to set the resting membrane potential)

Ganong, Review of Medical Physiology, 23rd Ed, 2010

A Nobel Prize for Ion Channels, 2003

"for discoveries concerning channels in cell membranes"

Peter Agre
"for the discovery of water channels"

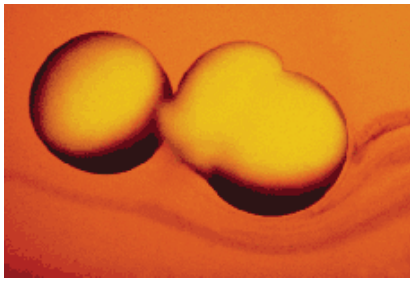


Rod MacKinnon
"for structural and mechanistic studies of ion channels"

http://nobelprize.org/nobel_prizes/chemistry/laureates/2003/index.html, accessed 16/04/2010

Osmosis - water diffusion

Serendipitous discovery of the water channels or "Aquaporins"



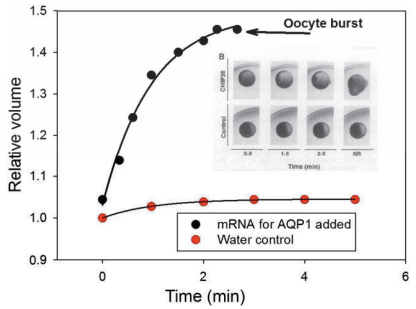
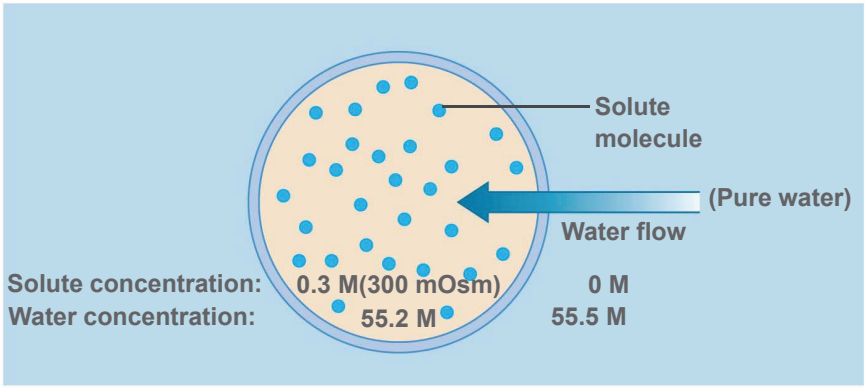


Figure 2.18 Increased osmotic water permeability of CHIP28 RNA-injected Xenopus oocytes. After 72 h, control-injected and CHIP28/AQP1 RNA-injected (10 ng) oocytes were transferred from 200 to 70 mosM modified Barth's buffer, and changes in size were observed by videomicroscopy. Osmotic swelling of representative control-injected (red circles) and CHIP28/AQP1 RNA-injected (black circles) oocytes. Time of rupture is denoted by the arrow. (Inset) Photos of injected oocytes at indicated times.
Redrawn from Preston GM, Carroll TP, Guggino WB, Agre P. Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein. Science. 1992;256:385_7.

Osmosis - water diffusion

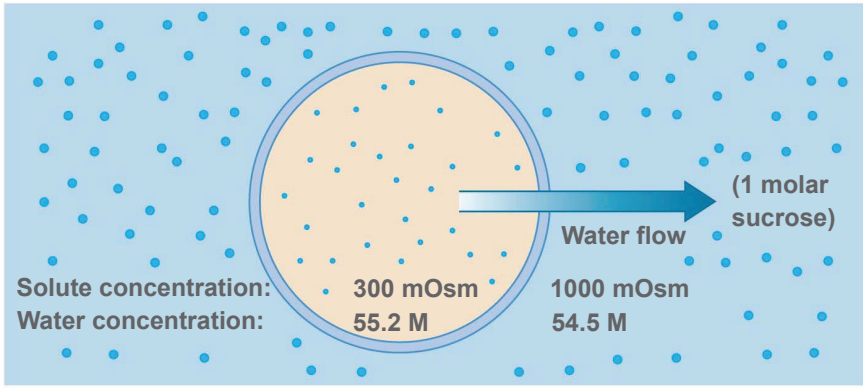
e.g., a red blood cell placed in water



(a)

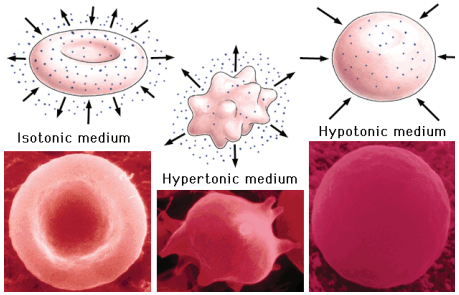
Osmosis - water diffusion

e.g., a red blood cell placed in high salt or sucrose solution



(b)

Osmosis - water diffusion – to be further addressed in Cell Homeostasis Prac



- If cell is placed in EXT solution that is:

isotonic (same tonicity): *- no change in cell volume as no force to move water in or out*
hypertonic: *- cell will shrink as water leaves the cell*
hypotonic: *- cell will expand as water enters the cell*

- A physiological solution contains solute (water) and osmolytes (particles) that exert an osmotic pressure. Typical values of osmolarity in physiological solutions is ~300 mOsm. Typically the osmolarity of the intracellular (INT) and Extracellular (EXT) solutions are equal, and the cell is at equilibrium as their is no water concentration gradient across the membrane and no water will flow. However there can be transient changes to this equilibrium as a result of cell activity or changes in water consumption or loss. When there is a different osmolarity across the membrane, water will diffuse down its concentration gradient. If a swelling cell doesn't release osmolytes (e.g. via stretch-activated channels or via the Na^+ pump) it may burst.

How can substances cross the cell membrane?

1. Passive diffusion
   1. *Simple Diffusion*
   2. *Facilitated Diffusion (carriers & channels)*

2. Active Transport

3. Via incorporation into lipid vesicle ("sac")
[eg, exo- & endo-cytosis]

Multiple Choice

Cells constantly need to metabolize glucose for energy. Glucose levels inside and outside cells during rest/fasting is about 5 mM. After a meal (when blood glucose levels can be > 10 mM), which of the following may be true?

- glucose can move into cells via simple diffusion
- glucose can move into cells via facilitated diffusion
- glucose will come out of cells as it gets drawn away buy the blood flow
- influx of glucose across the membrane can saturate
- one way to increase the rate of storage of glucose inside cells is to increase the number of transporters

How can substances cross the cell membrane?

1. Passive diffusion
2. Active Transport
3. Via incorporation in sis]

http://www.abc.net.au/news/2014-09-29/volcanic-ash-rises-from-mount-ontake/5776418, accessed 4/10/2014

How can substances cross the cell membrane?

1. Passive diffusion
   1. Simple Diffusion
   2. Facilitated Diffusion (carriers & channels)
2. Active Transport — **Requires ENERGY, uphill movement of solutes**
3. Via incorporation into lipid vesicle ("sac") [exo- & endo-cytosis]

How can substances cross the cell membrane?

1. Passive diffusion
   1. Simple Diffusion
   2. Facilitated Diffusion (carriers & channels)
2. Active Transport
   1. Primary Active Transport
   2. Secondary Active Transport
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How do things move across the cell membrane?

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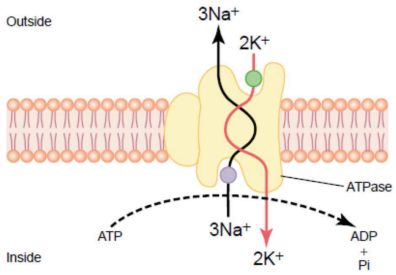


Guyton & Hall, Textbook of Medical Physiology, 2006

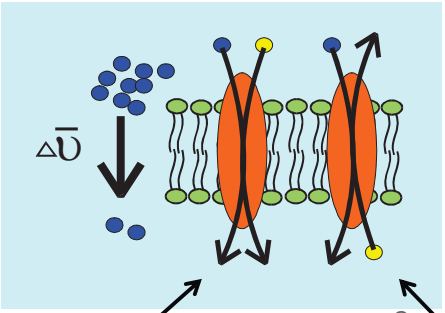
The "Na⁺-pump"

- Essential for life!, ubiquitous
- a primary active transporter
- direct coupling between ATP & transport
- establishes Na+ and K+ gradients
- energy for secondary transport
- energy for electrical signalling

Component:	Intracellular concentration	Extracellular concentration
Na^+	5 - 15 mM	145 mM
K^+	140 mM	5 mM
Mg^{2+}	0.5 mM	1 - 2 mM
Ca^{2+}	0.1 µM	1 - 2 mM
Cl^-	5 - 15 mM	110 mM
pH (= -log $[H^+]$)	7.1	7.4

Secondary Active Transporters

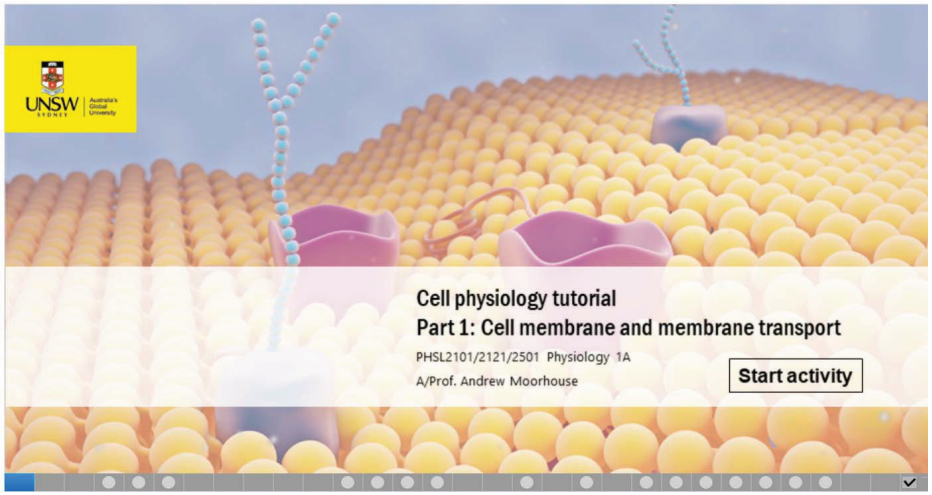
These transporters are a broad and diverse group of membrane proteins that do not directly couple energy utilization to transport but rather ***use an electrochemical gradient*** established by primary transporters.



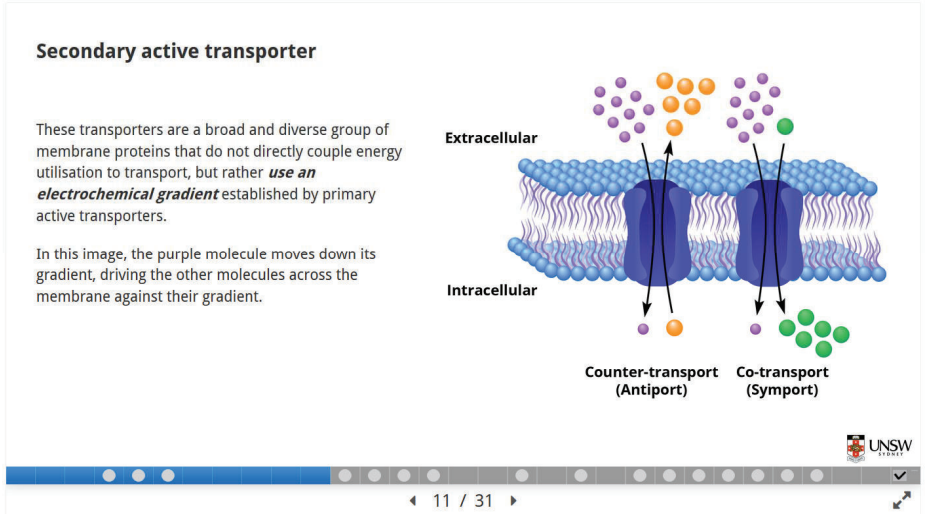
Two broad types of secondary active transporters, depending on directions of substrate transport:

* co-transporters (symports)
* exchangers (antiports; counter-transport)

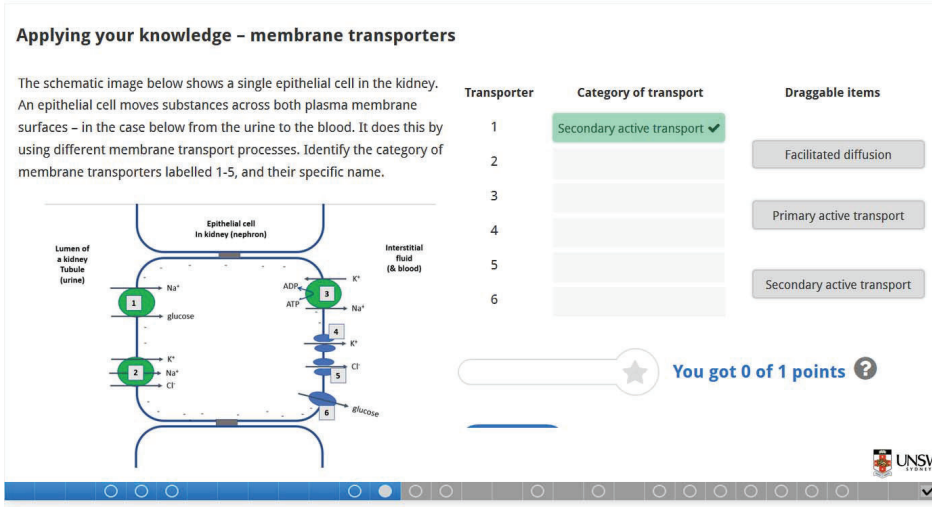
You're ready for the 1st tutorial exercise on Moodle



Review and test your knowledge of membrane transport



Apply the knowledge to physiological conditions



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3. Via incorporation into lipid vesicle (“sac”) [exo- & endo-cytosis

Exocytosis and endocytosis: Definitions

Movie: http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120068/bio02.swf::Endocytosis%20and%20Exocytosis

Endocytosis:
- from outside to in
- phagocytosis (particle / bacteria / food, e.g. microglia in brain)
- pinocytosis (water / liquid)
- receptor mediated endocytosis (specific molecules)

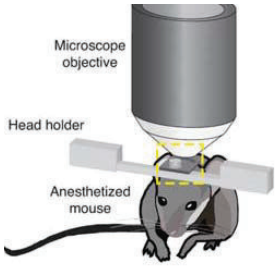
Exocytosis:
- from inside to outside,
- vesicle containing substance fuses with plasma membrane

Features:
- larger peptides, proteins, immune cells,
- complexity, involving different signaling pathways
- transport of proteins within the cell

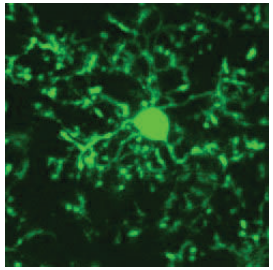
Exocytosis and endocytosis: Example: microglial phagocytosis

1. Microglia phagocytose debris from infections or dying nerve cells in disease, and apoptotic cells during development or brain regeneration

e.g. Davalos & Nimmerjahn, Nature, 2005, amazing movie of microglia migration is here
e.g., Koizumi et al (Nature,) image of microglia in a dish phagocytosing a fluorescent bead is here



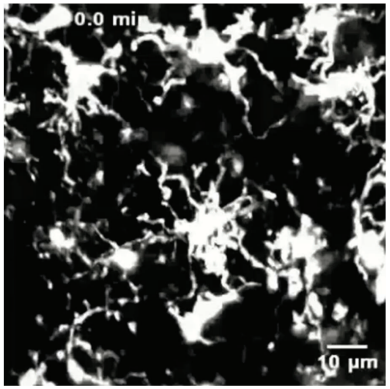
In vivo imaging in transgenic mice



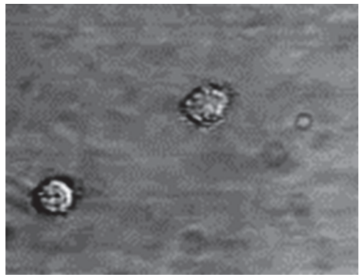
A microglial cell in the brain of living mouse expressing green fluorescent protein

1. Left: Nimmerjahn, 2012, Frontiers.; 2. ©MPI for Medical research, Max Planck Inst, Nimmerjahn

Exocytosis and endocytosis: Example: microglial phagocytosis In vivo

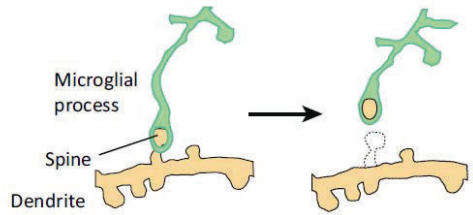


Exocytosis and endocytosis: Example: microglial phagocytosis In vitro



Exocytosis and endocytosis: Example: microglial phagocytosis

1. Microglia phagocytose debris from infections or dying nerve cells in disease, and apoptotic cells during development or brain regeneration
e.g. Davalos & Nimmerjahn, Nature, 2005, movie

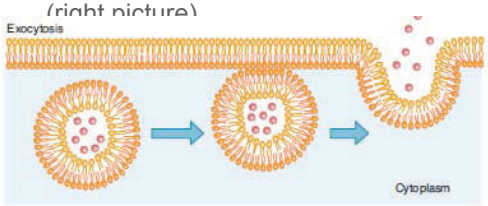


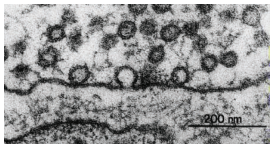
Microglia also regulate brain homeostasis by phagocytosis of surplus or injured synapses

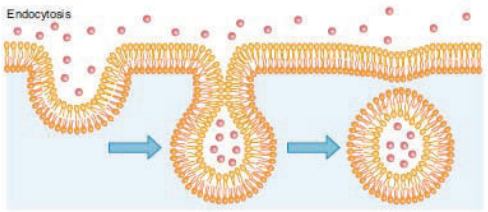
Wake, Moorhouse, Miyamoto, Nabekura 2013. Trends in Neuroscience

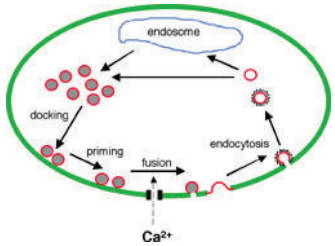
Exocytosis and endocytosis: Learning by examples

2. At nerve synapses, neurotransmitters vesicles are released by exocytosis (left picture) and then recycled by endocytosis (right picture)









Ganong's Review of Medical Physiology, Barret et al., 23rd Edition Fig 2.12

How can substances cross the cell membrane?

1. Passive diffusion
   1. *Simple Diffusion*
   2. *Facilitated Diffusion (carriers & channels)*
2. Active Transport
   1. *Primary Active Transport*
   2. *Secondary Active Transport*
3. Via incorporation into lipid vesicle ("sac")
   1. *Exocytosis*
   2. *Endocytosis*

Next important objectives

Concept of electrochemical gradients and electrochemical equilibrium

Why is the resting membrane potential about -70 mV??

What ions and channels are involved in the nerve Action Potential?

Learning Aims – lecture 3

- To be able to define ion channels and their basic properties of gating and selectivity
- To recognise osmosis as water diffusion and relate tonicity and water flux across cell membranes. (also in Prac 1)
- To be able to define primary and secondary active transport, and co-transport and counter-transport, and give examples of each
- To know the basic cellular function of the Na^+ pump and the ions that it transports, including the directions and quantities
- To be able to briefly describe exocytosis and endocytosis using physiological examples