認知單元

Cell membrane
Ion channel and diseases
細胞膜
離子通道與疾病

陳建璋
中央研究院生物醫學科學研究所
ccchen@ibms.sinica.edu.tw
2652-3522

11/30/2024

大綱

- 細胞，細胞膜，細胞膜蛋白質
- 研究離子通道的方法
- 離子通道的種類、功能和相關疾病
- 疼痛的研究

Size of Cells
細胞的大小

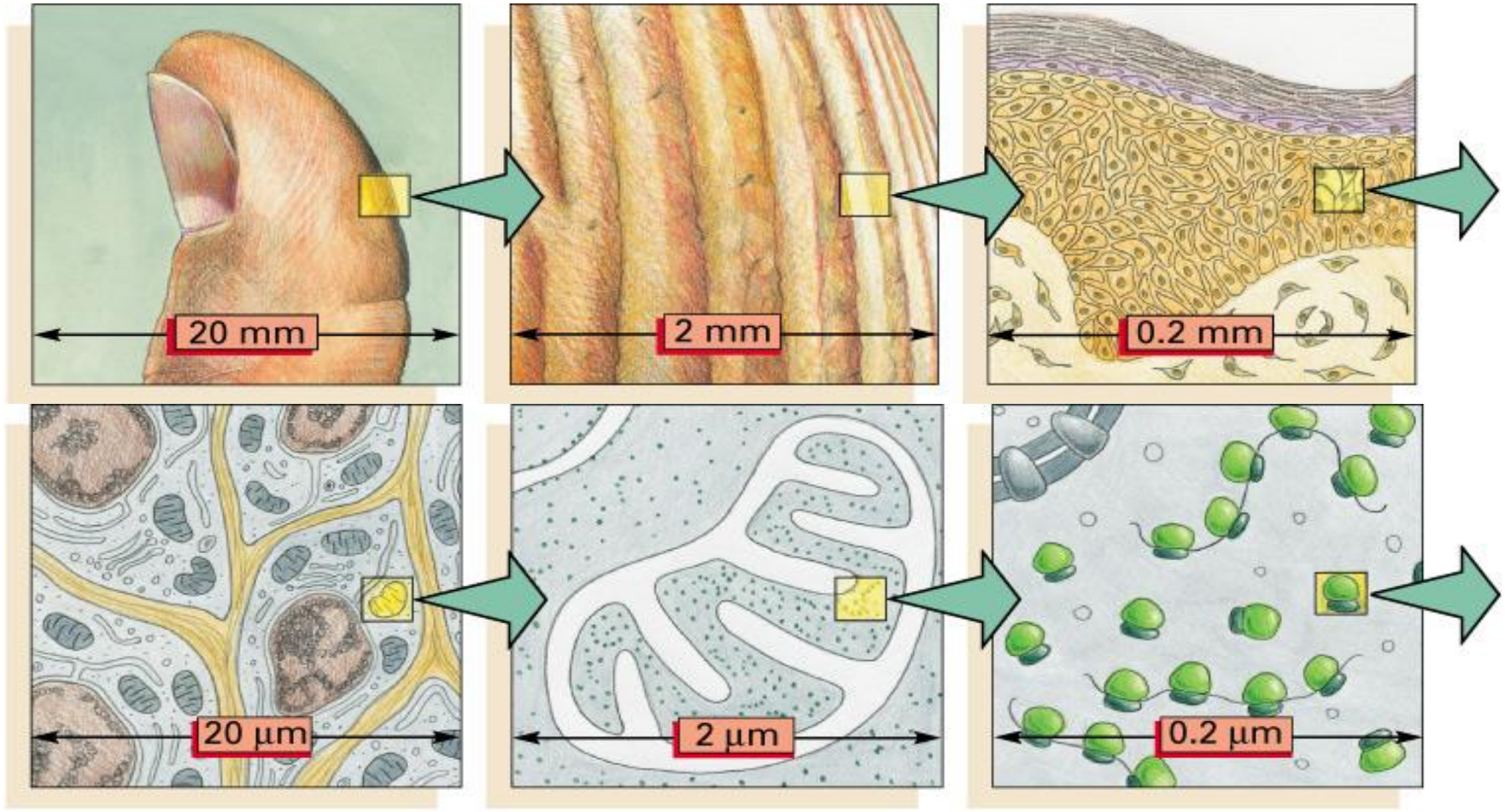
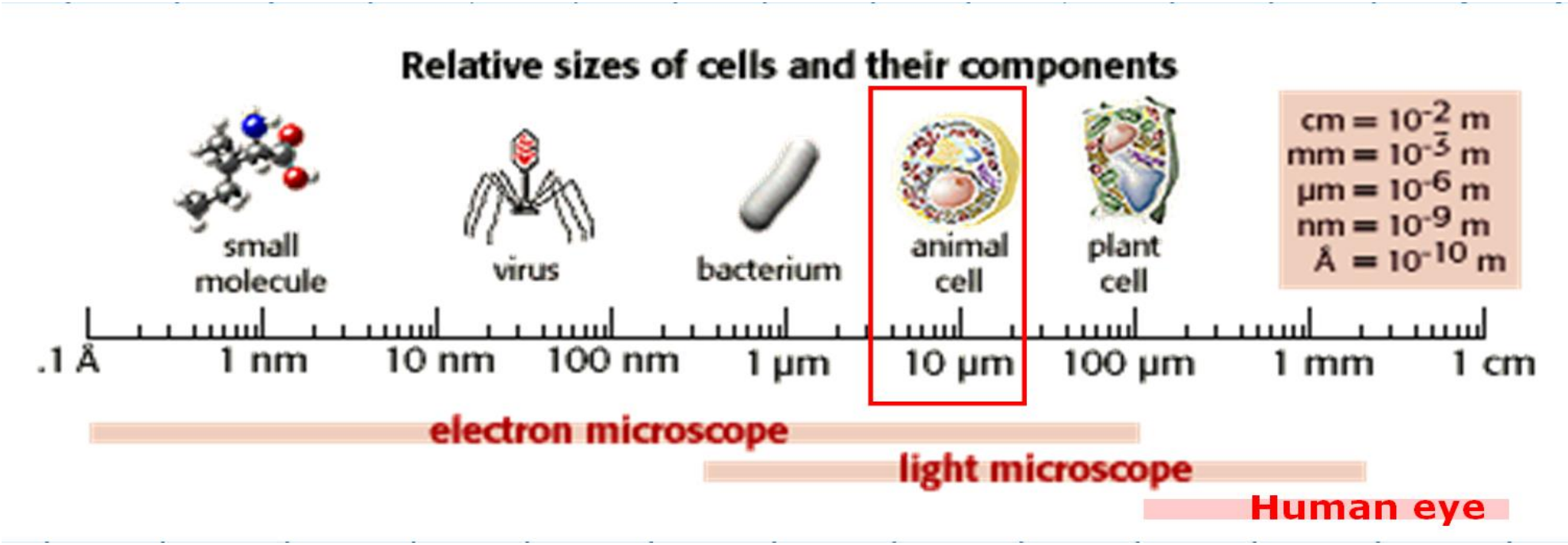


Figure 9–1 part 2 of 3. Molecular Biology of the Cell, 4th Edition.



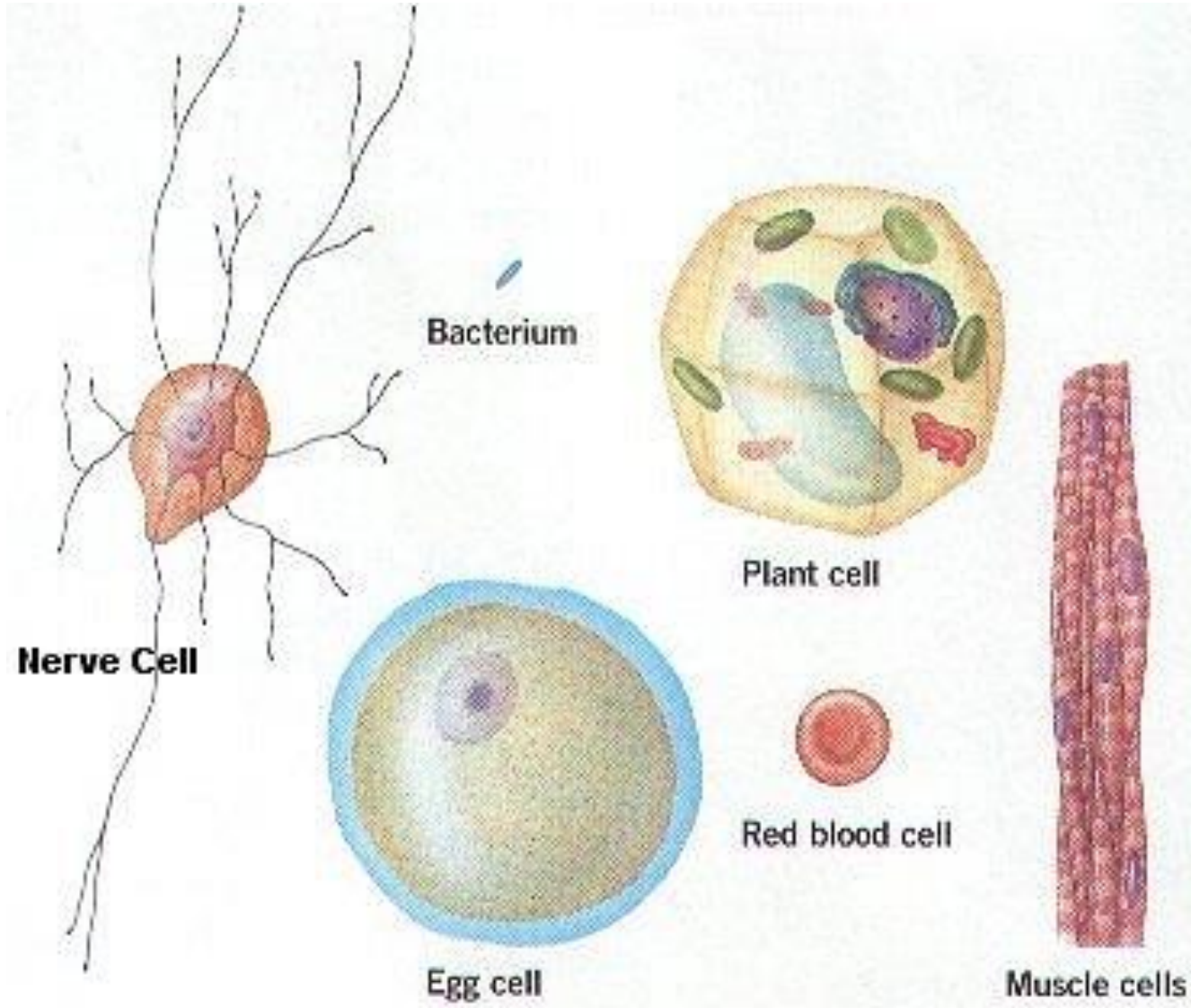
Diameter of a typical animal cell - 10 to 20 microns

micron = micrometer = um = 0.001 mm

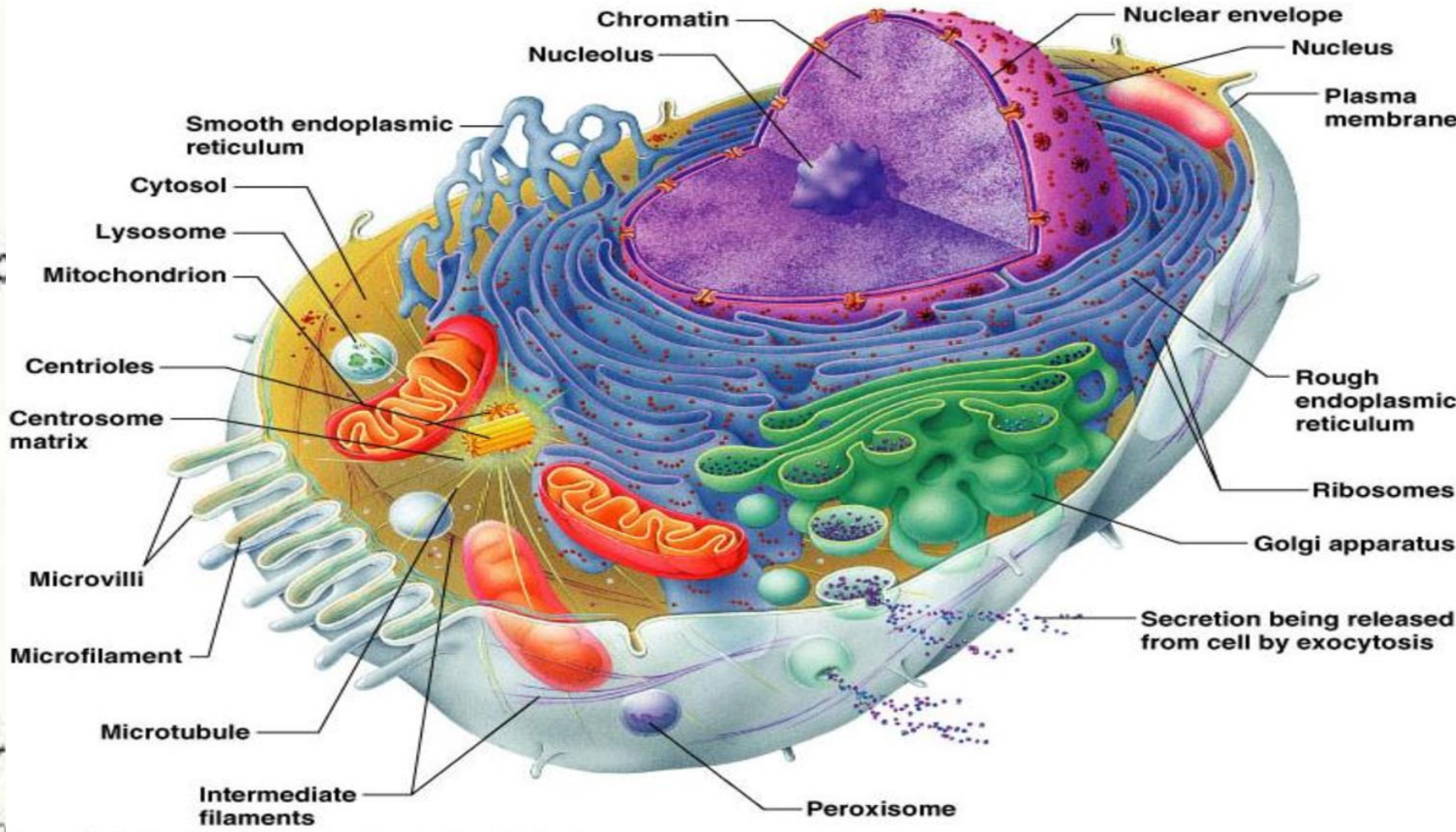
針頭的直徑大約是1 毫米, 因此細胞是大約1/100 針頭的直徑

http://sps.k12.ar.us/massengale/cell%20%20notes%20bi.htm

Cells come with different shape and size
不同形狀及大小的細胞

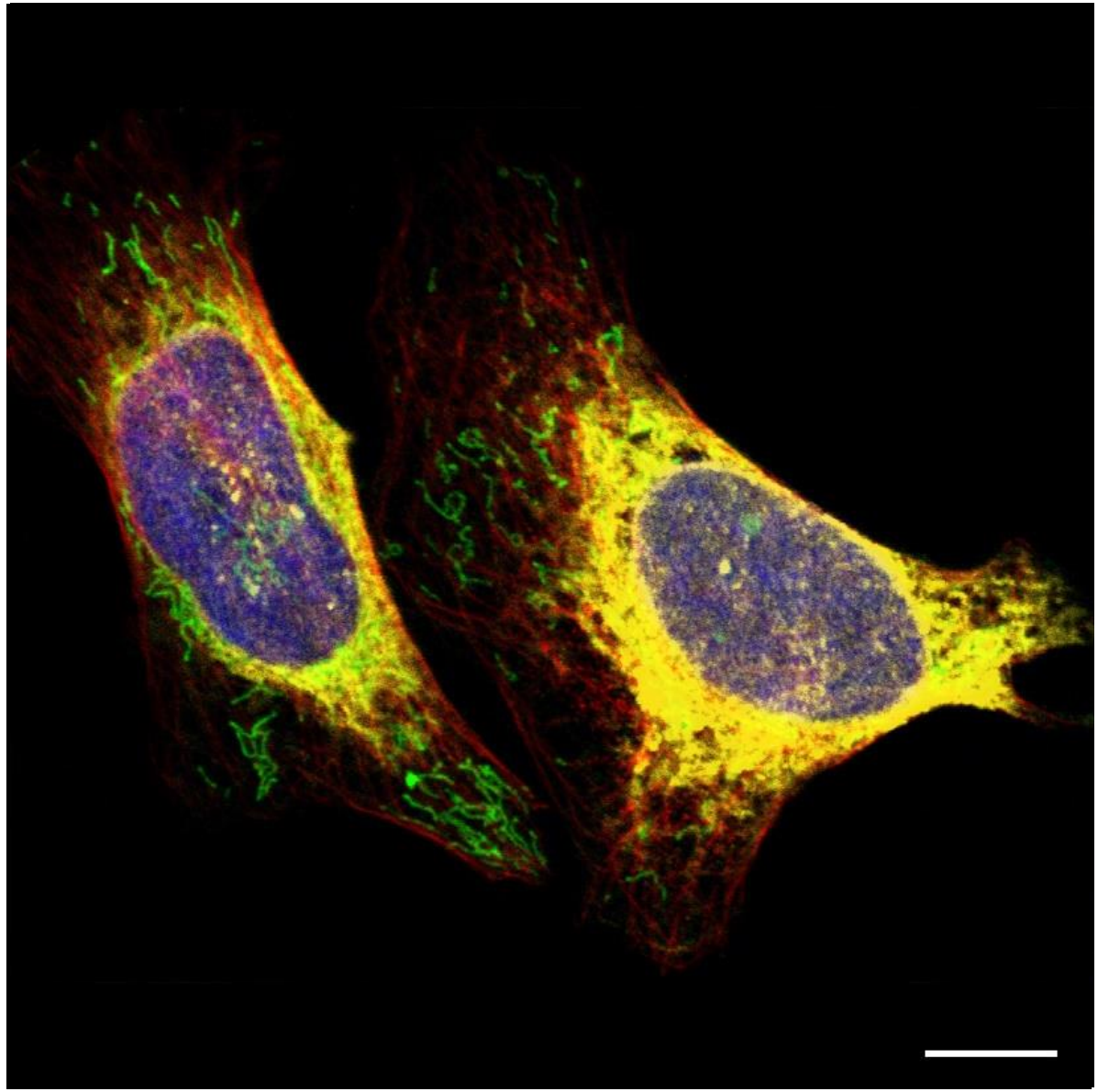


Structure of a Generalized Cell



http://www.infovisual.info/03/001_en.html

http://www.transcendancing.net/tag/cell-structure/



http://www.proteinatlas.org/learn/dictionary/cell/mitochondria

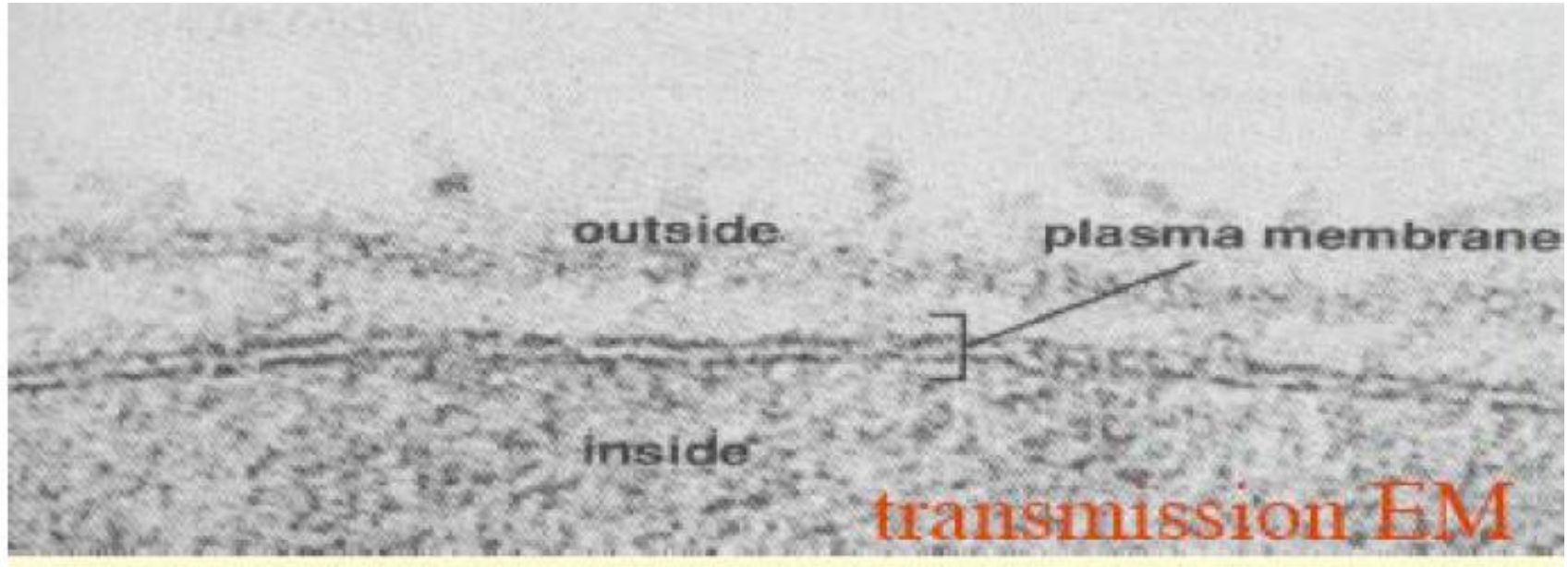
細胞就像一座城堡

http://www.kottke.org/plus/photos/200105europe/castle.jpg

Membrane Functions/細胞膜功能

Form compartments/ 隔間

7-8 nm



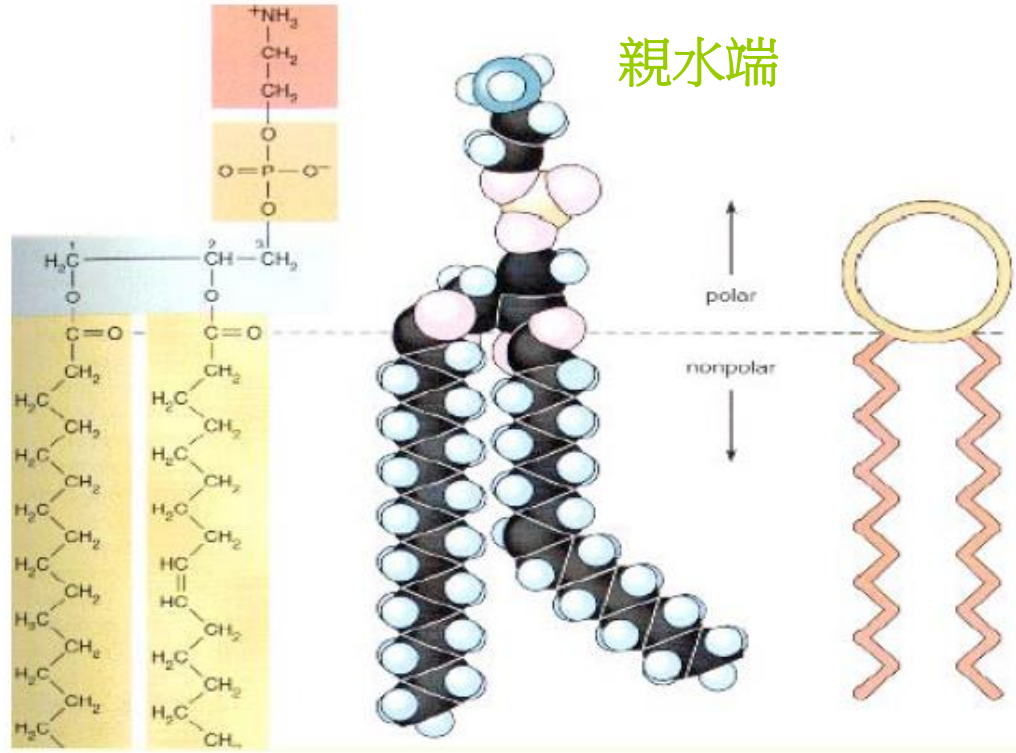
Cell Membrane

Phospholipids and proteins
First compartment formed

Prokaryotes (bacteria)/原核生物
Just one compartment

Eukaryotic cells)/真核生物
Many different compartments

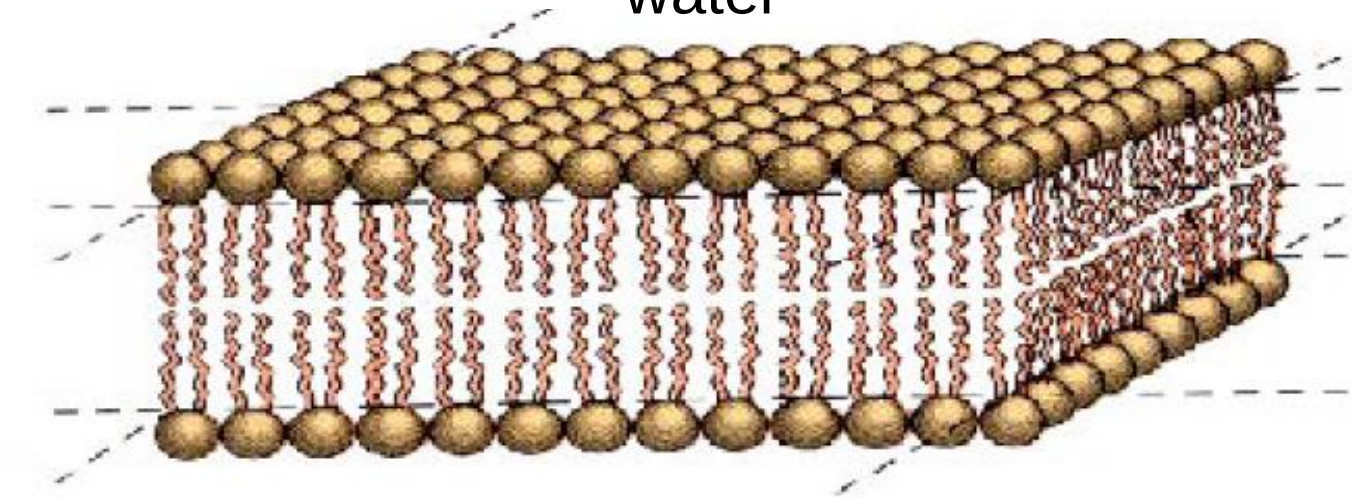
磷脂質



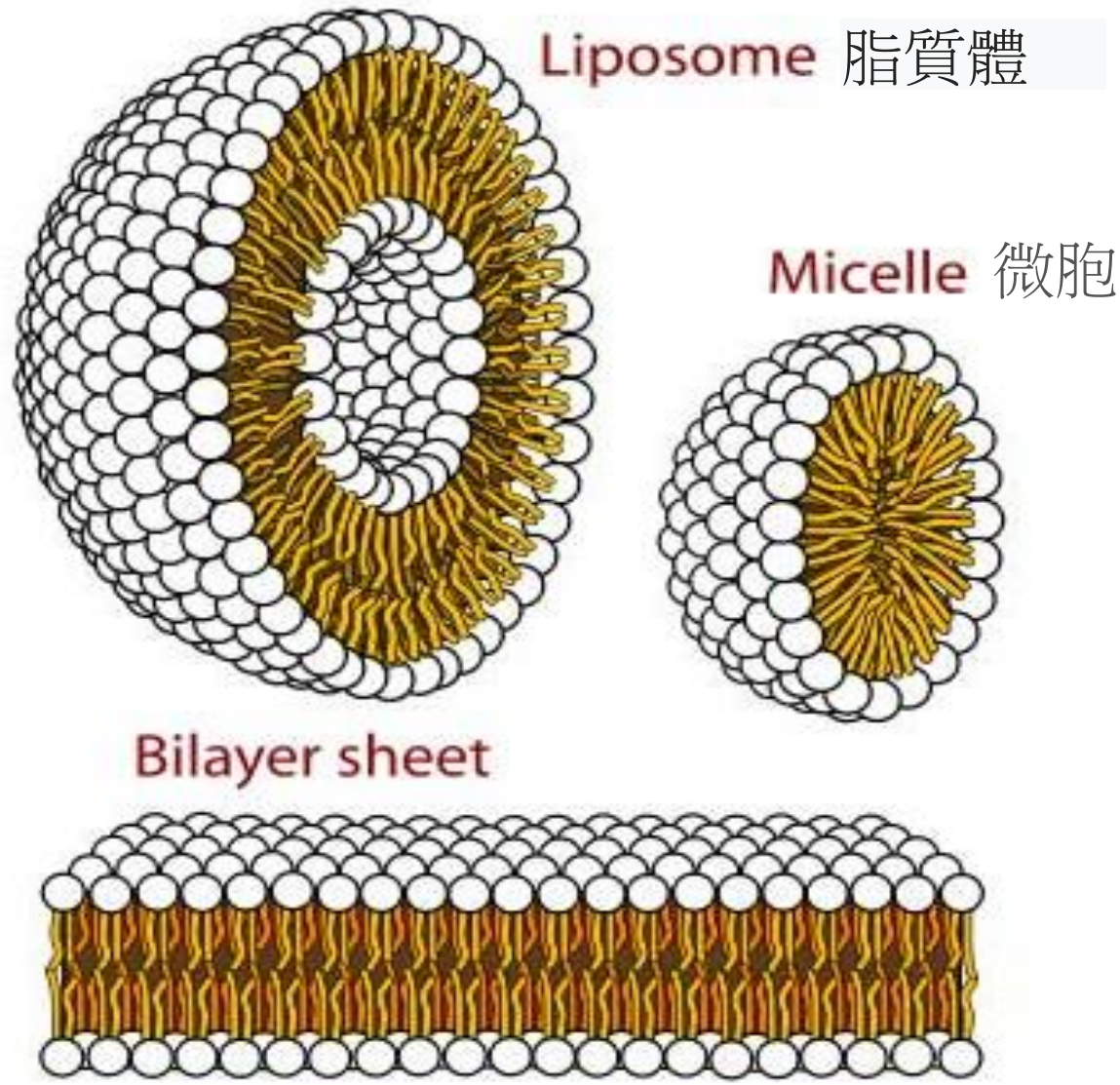
air

water

water

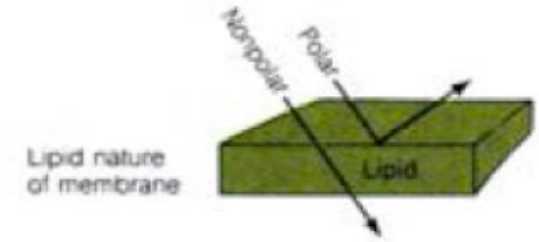


water

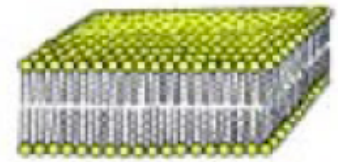

Liposome 脂質體
Micelle 微胞
Bilayer sheet

Membranes- History 1

- 1890 Charles Overton
  - selective permeation of membranes
    - non-polar pass through (lipid soluble)
    - polar refractory
  - lipids present as a coat

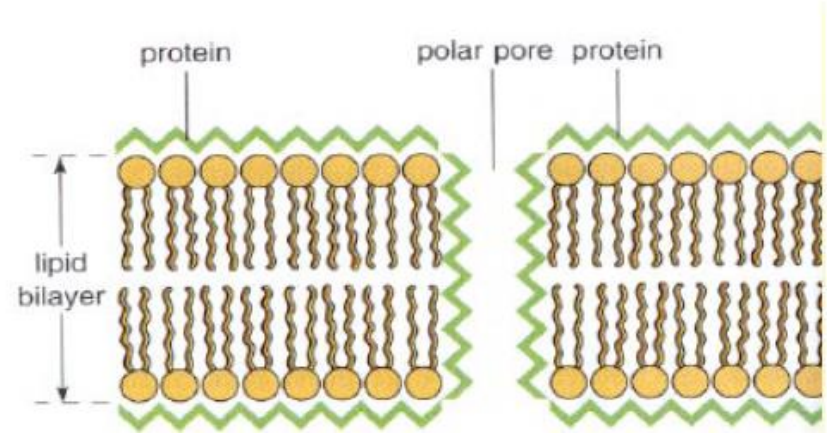


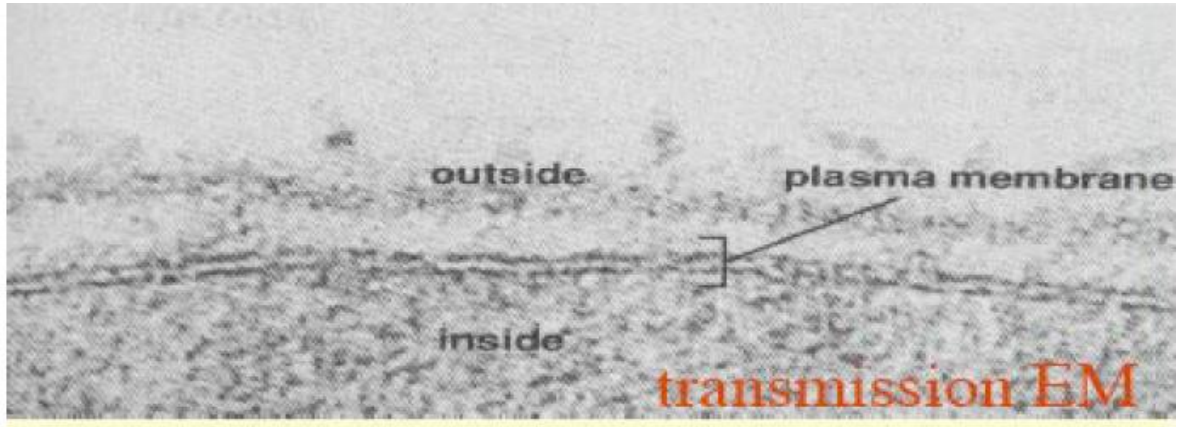
- 1905 Irving Langmuir
  - lipids faced with heads towards water away from organic solvents
- 1925 Gorter & Grendel
  - monolayer of lipid isolated from rbc's
  - 2x surface area of cell (bilayer)



Membranes- History 2

- 1930-40 Danielle-Davson Model
  - Proteins coat a bilayer with polar “pores”

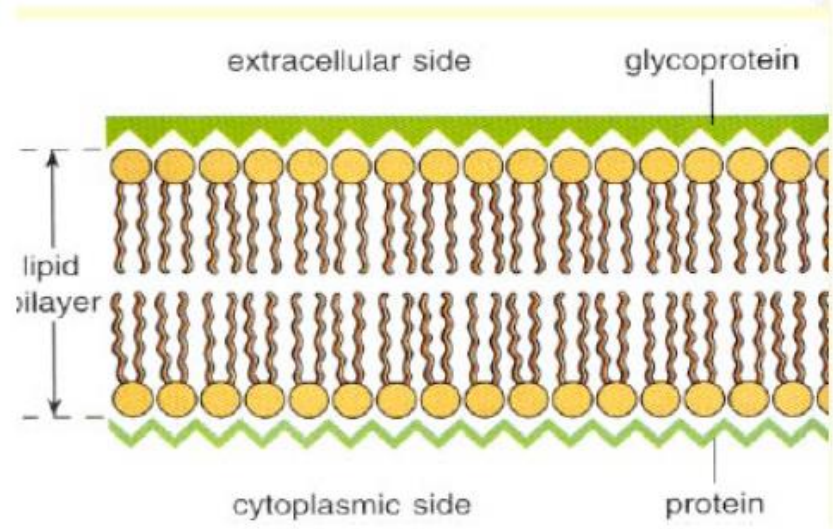




加了蛋白質

Sandwich model

- 1960s Robertson Modification
  - Glycoprotein on one side, therefore asymmetric



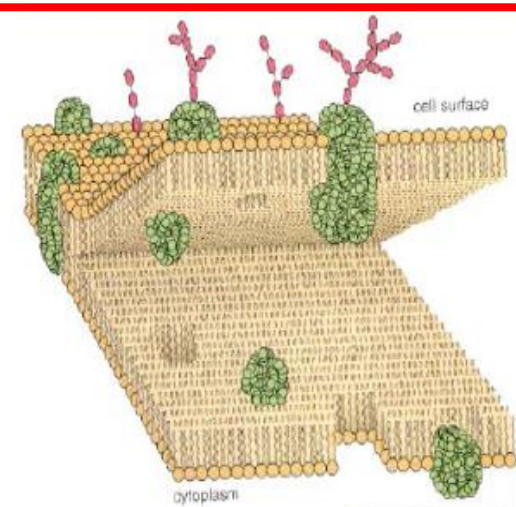
加了醣蛋白

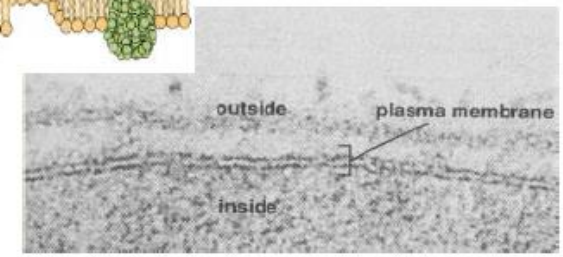
Q1: not all the membrane are the same, different lipid and protein compositions

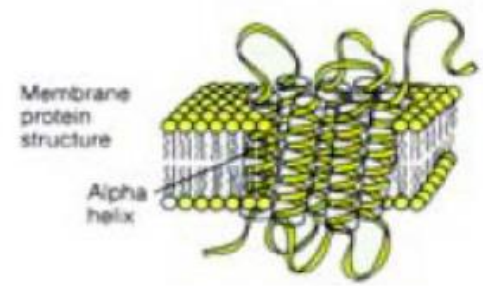
Q2: location of membrane proteins

Membranes- History 3

- 1972 Singer & Nicholson Model
  - proteins “floating” within lipid bilayer like a “liquid” surface
- 1975 Unwin & Henderson
  - integral membrane proteins
  - both hydrophobic and hydrophilic
    - alternating -phobic and -philic represent trans-membrane loops.
  - Glycoproteins
    - carbohydrate groups on outer surface







Fluid mosaic model 流動鑲嵌模型

Freeze-fracture method 冷凍碎裂法

Fluid Mosaic Model

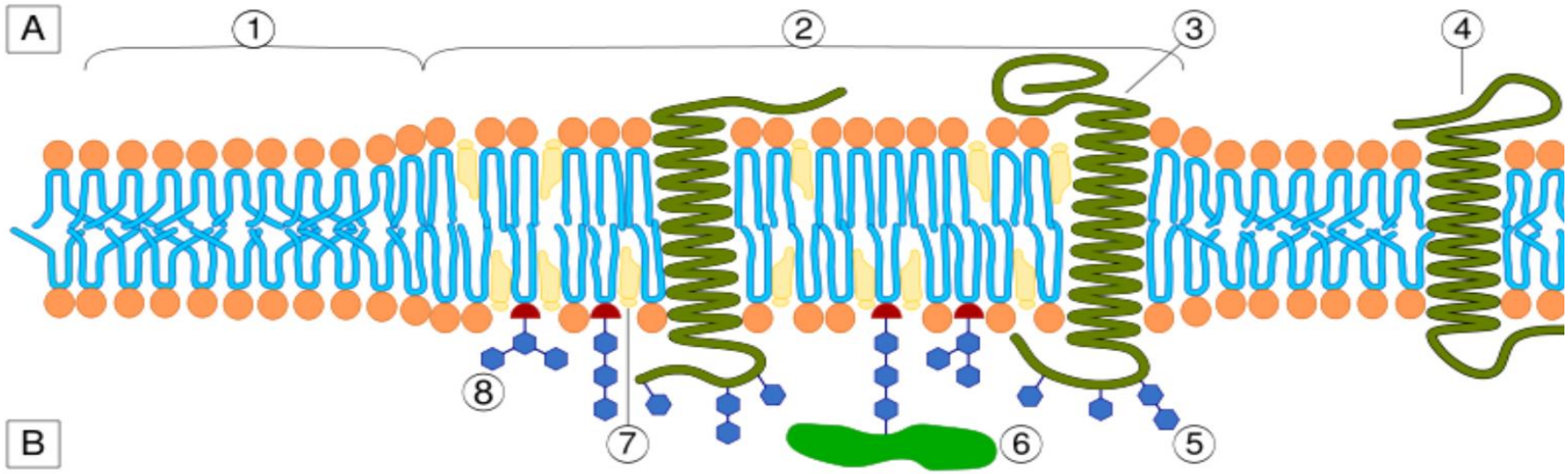
Membranes- History 4

Lipid raft/脂筏

- 1997 “Membrane Rafts”
  - “A new aspect of cell membrane structure is presented, based on the dynamic clustering of sphingolipids and cholesterol to form rafts that move within the fluid bilayer. It is proposed that these rafts function as platforms for the attachment of proteins when membranes are moved around inside the cell and during signal transduction.”
    - Simons K, Ikonen E. Nature 1997 Jun 5;387(6633):569-72

鞘脂

Lipid raft (脂筏)



A Intracellular space or cytosol
B Extracellular space or vesicle/Golgi apparatus lumen
1. Non-raft membrane
2. Lipid raft
3. Lipid raft associated transmembrane protein
4. Non-raft membrane protein
5. Glycosylation modifications (on glycoproteins and glycolipids)
6. GPI-anchored protein
7. Cholesterol 膽固醇
8. Glycolipid

Caveolae (胞膜窖)

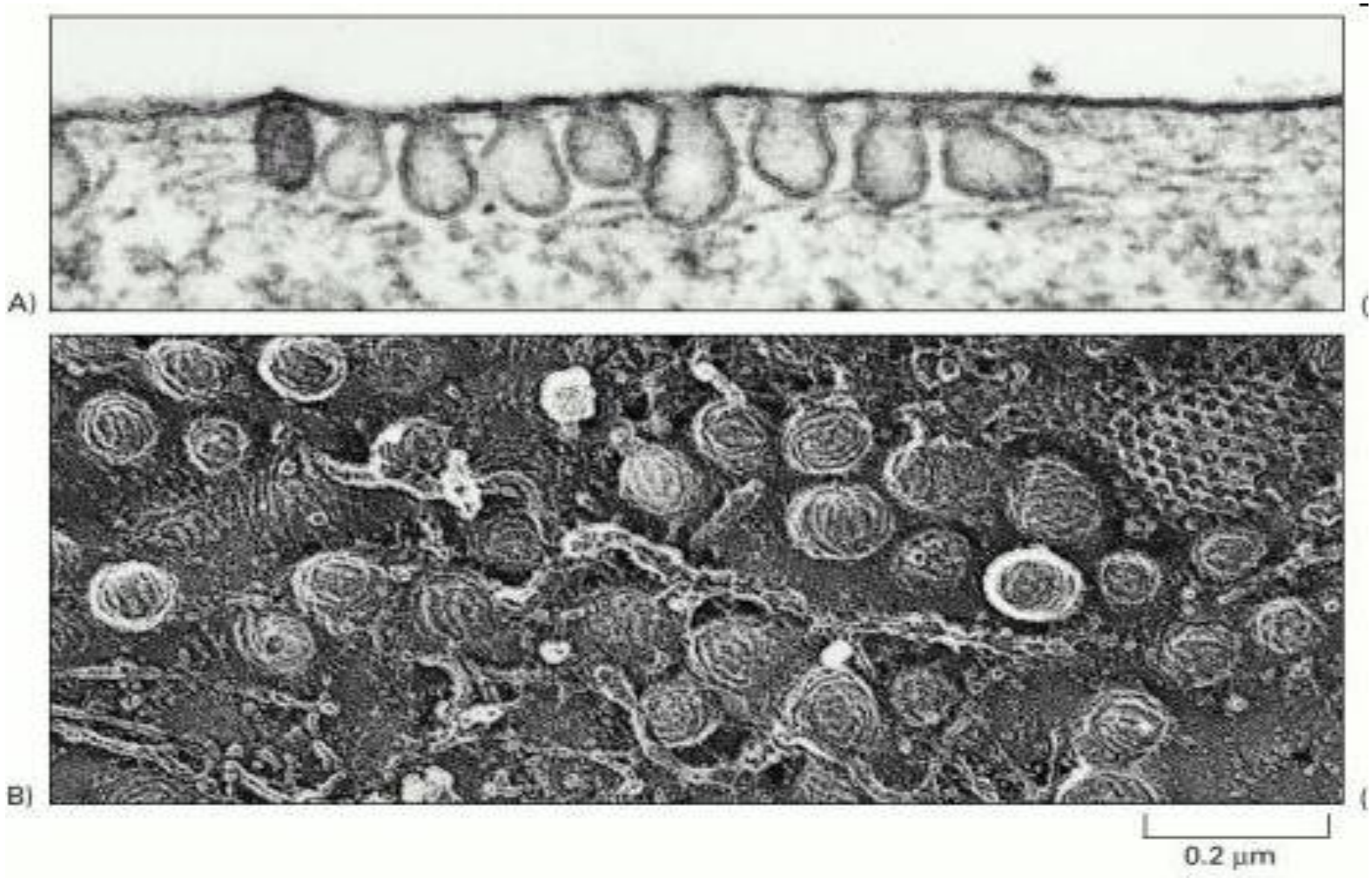


Figure 13-42. Caveolae in the plasma membrane of a fibroblast.

Discover in 1953

細胞膜的流體性質

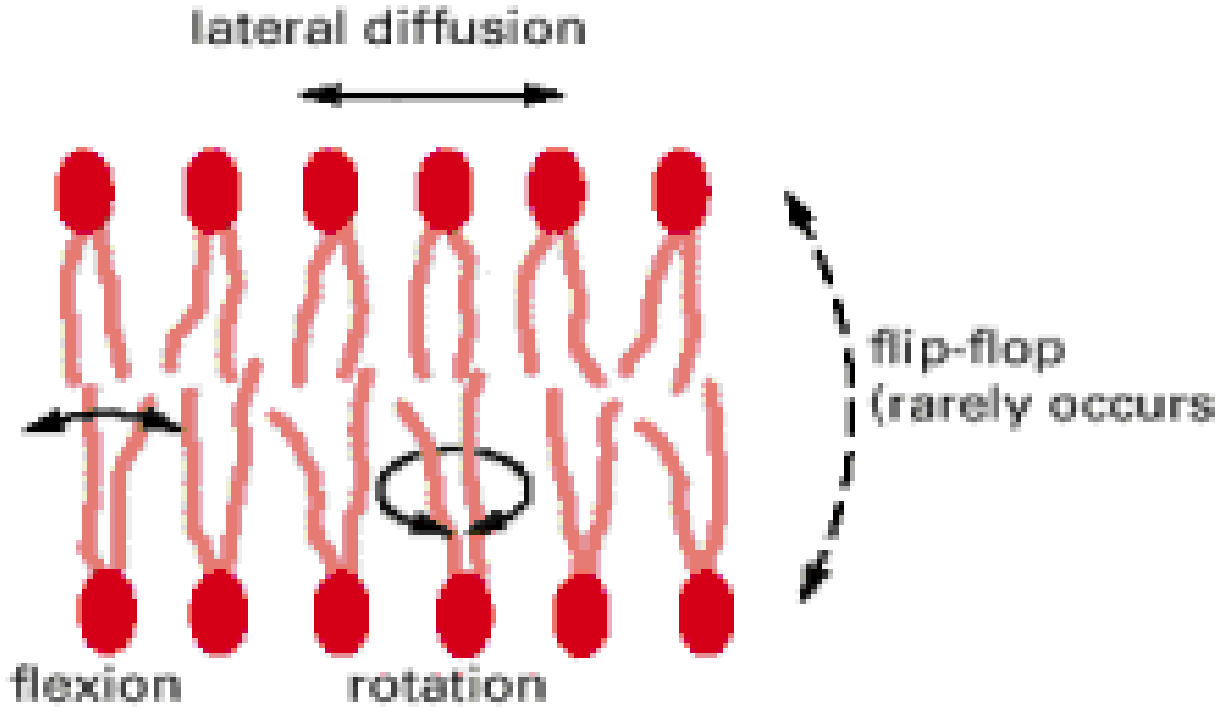
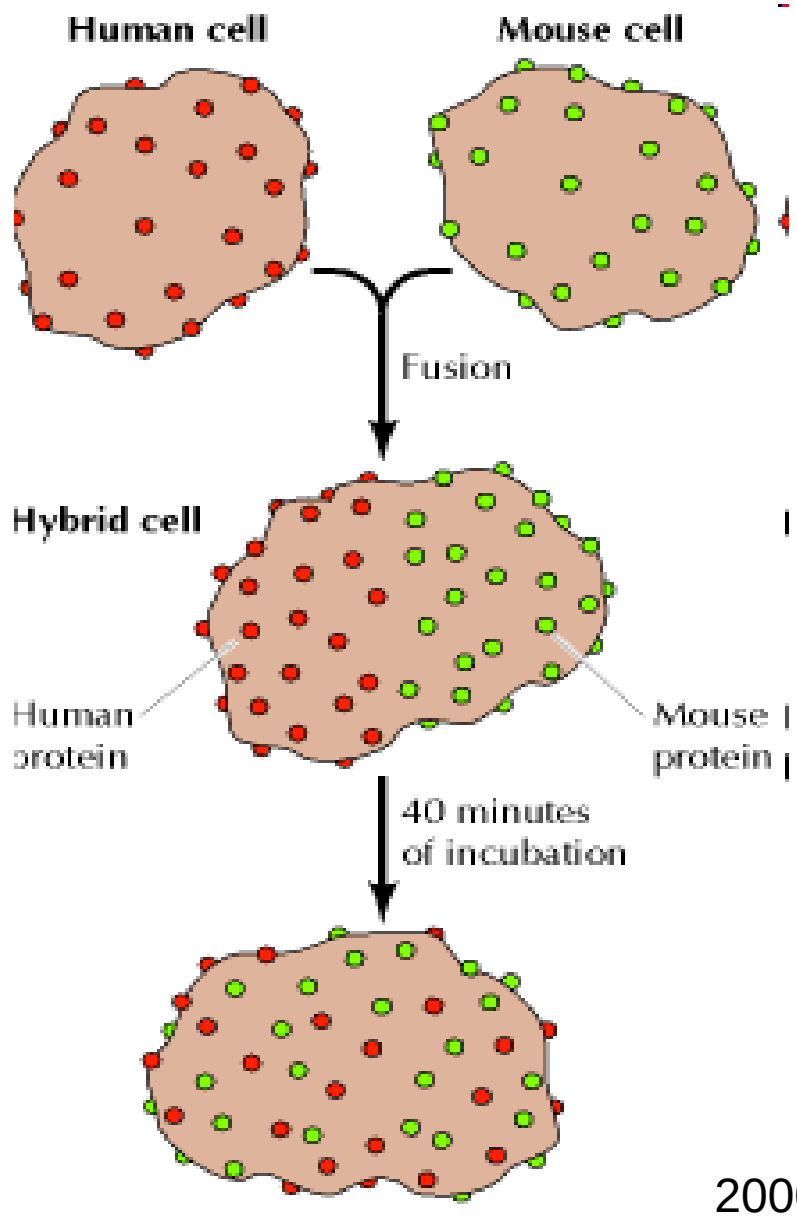


Figure 10-8. Phospholipid mobility.

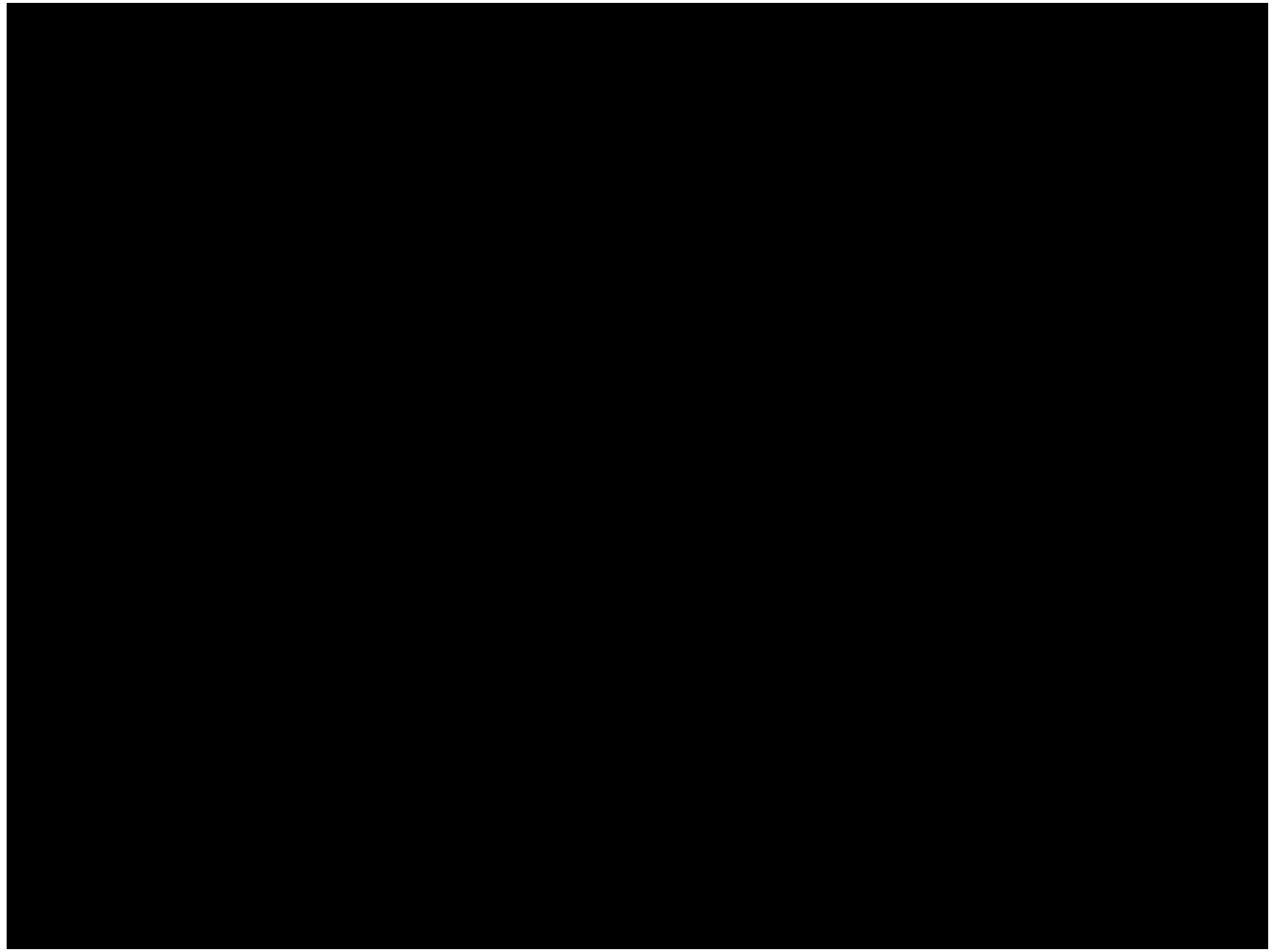
Membrane Fluidity



螢光標示的膜蛋白

細胞融合

殺手 T 細胞

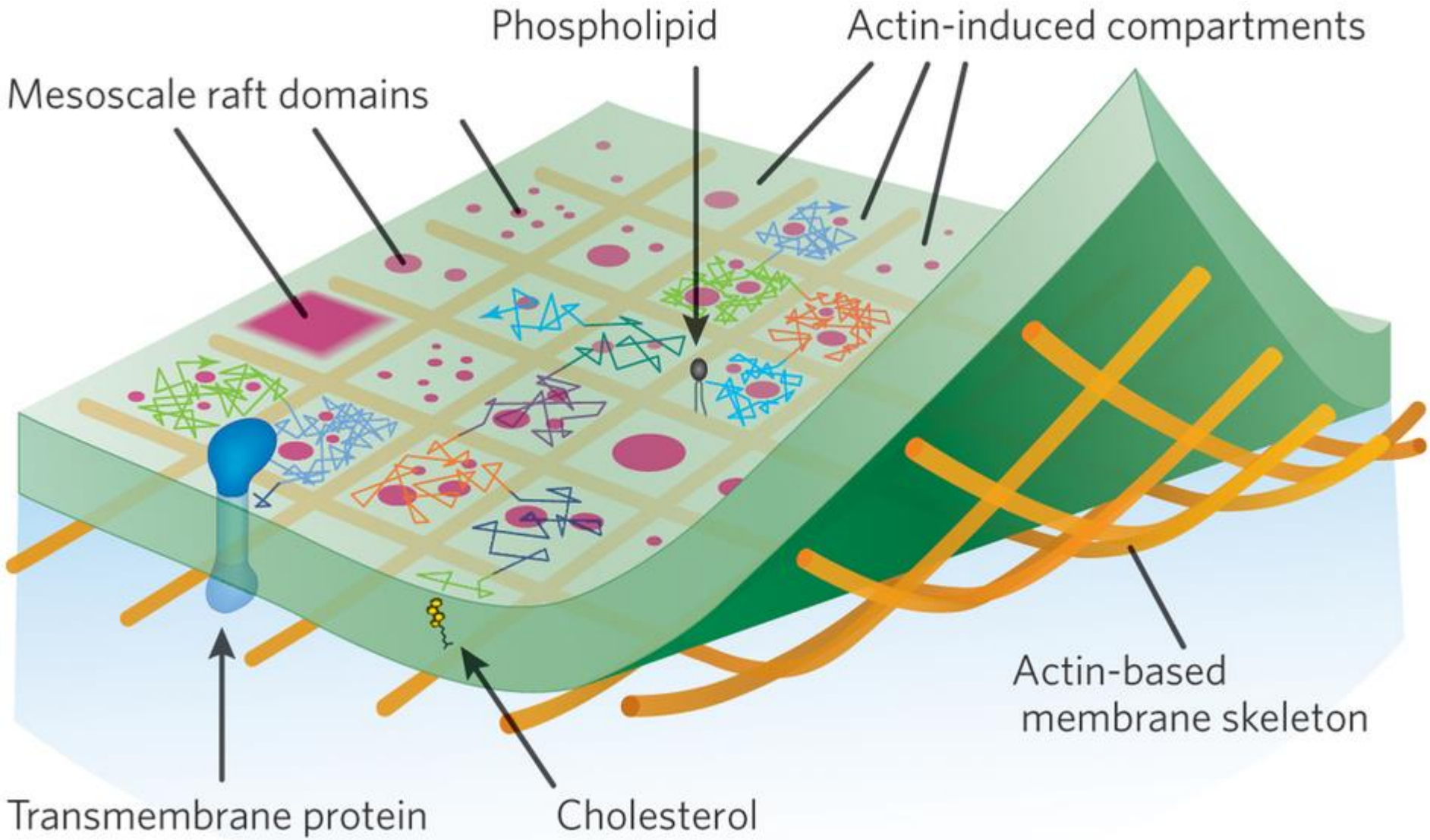


https://www.youtube.com/watch?v=ntk8XsxVDi0

Professor Akihiro Kusumi
Institute for Integrated Cell-Material Sciences, Kyoto University
Okinawa Institute of Science and Technology

Membrane mechanisms: Concerted action of membrane domains for signal transduction in the plasma membrane revealed by single-molecule tracking

Watch video 1-1:50



Nature Chemical Biology **10**, 524–532 (2014)

Animal cell membrane
細胞膜

雙層磷脂質

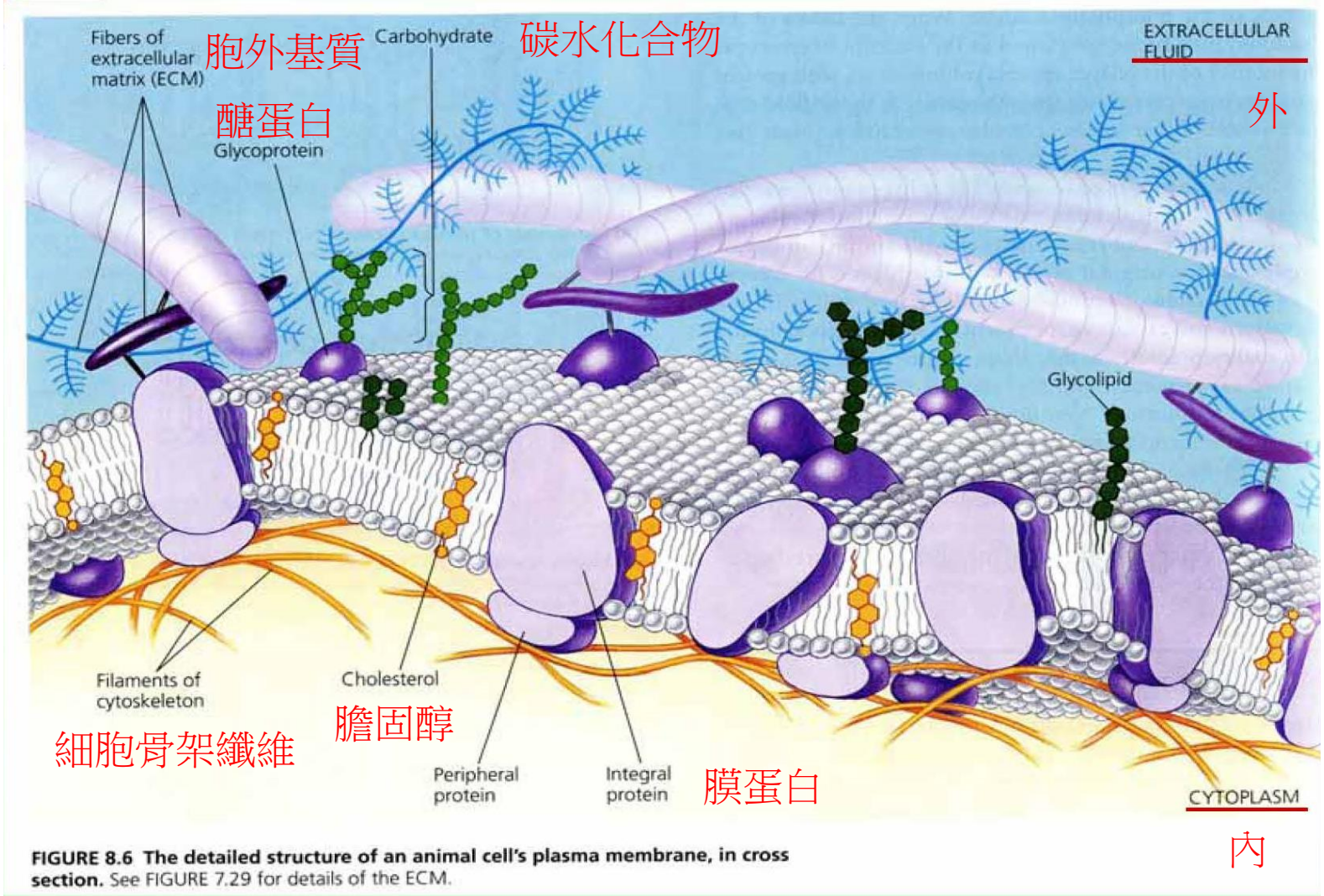


FIGURE 8.6 The detailed structure of an animal cell's plasma membrane, in cross section. See FIGURE 7.29 for details of the ECM.

Campbell/Biology

Functions of membrane proteins
細胞膜蛋白質的功能

1. 作為接收外界訊號的偵測器，準確地接收外界聲光的刺激，或是來自身體其他細胞的激素或神經傳導物等訊息。
2. 作為與外界交換物質的通道。
3. 作為細胞與細胞間的連接。
4. 作為細胞與細胞間的辨認。
5. 作為細胞內與細胞外的連接。

細胞膜的通透性

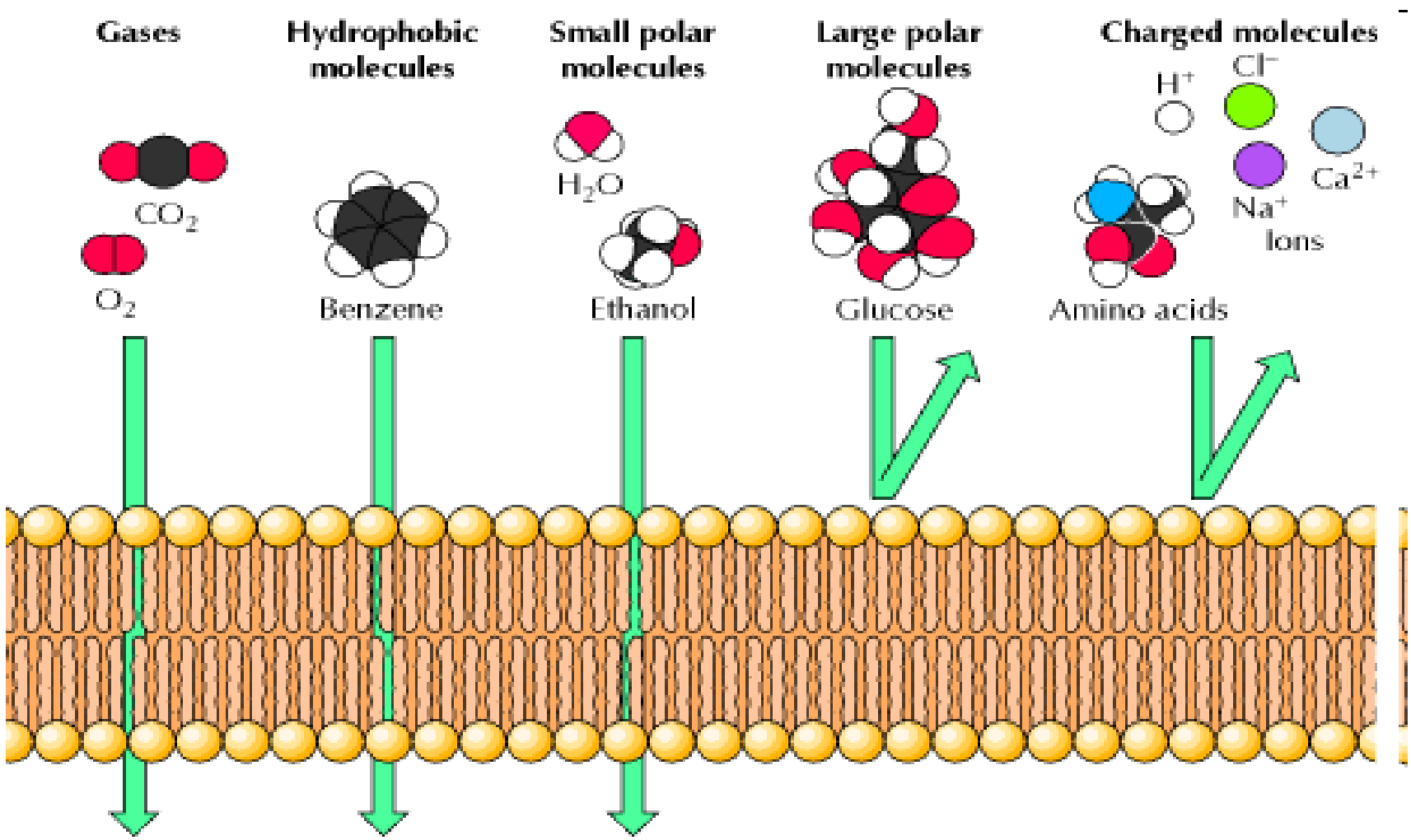


Figure 12.15. Permeability of phospholipid bilayers

Passive transport

Active transport

吞噬作用

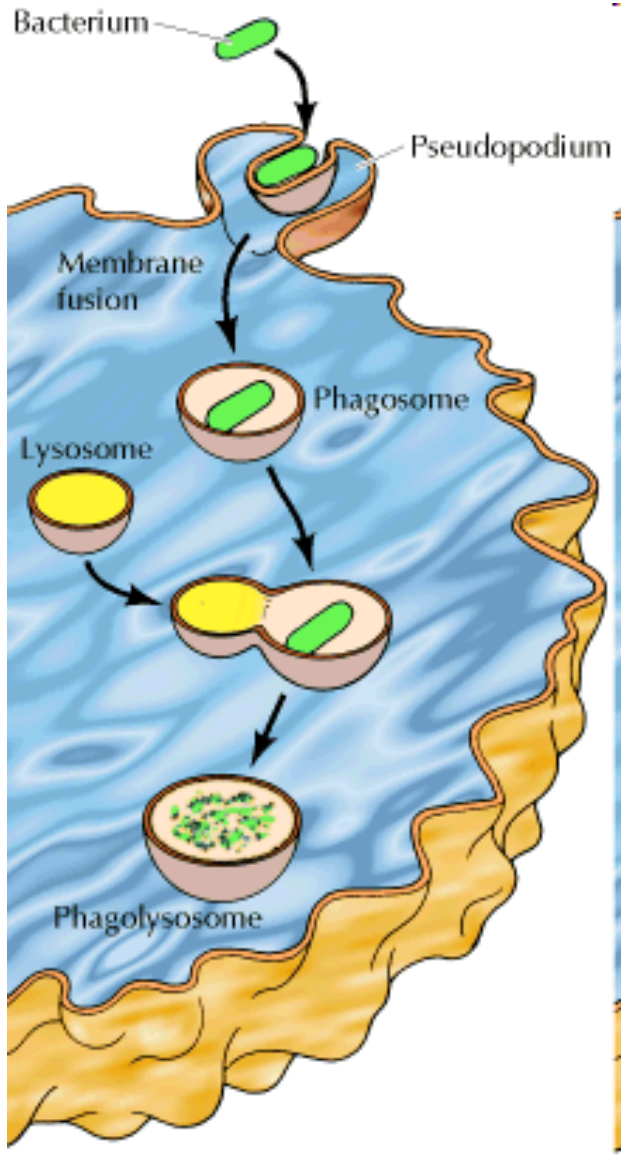
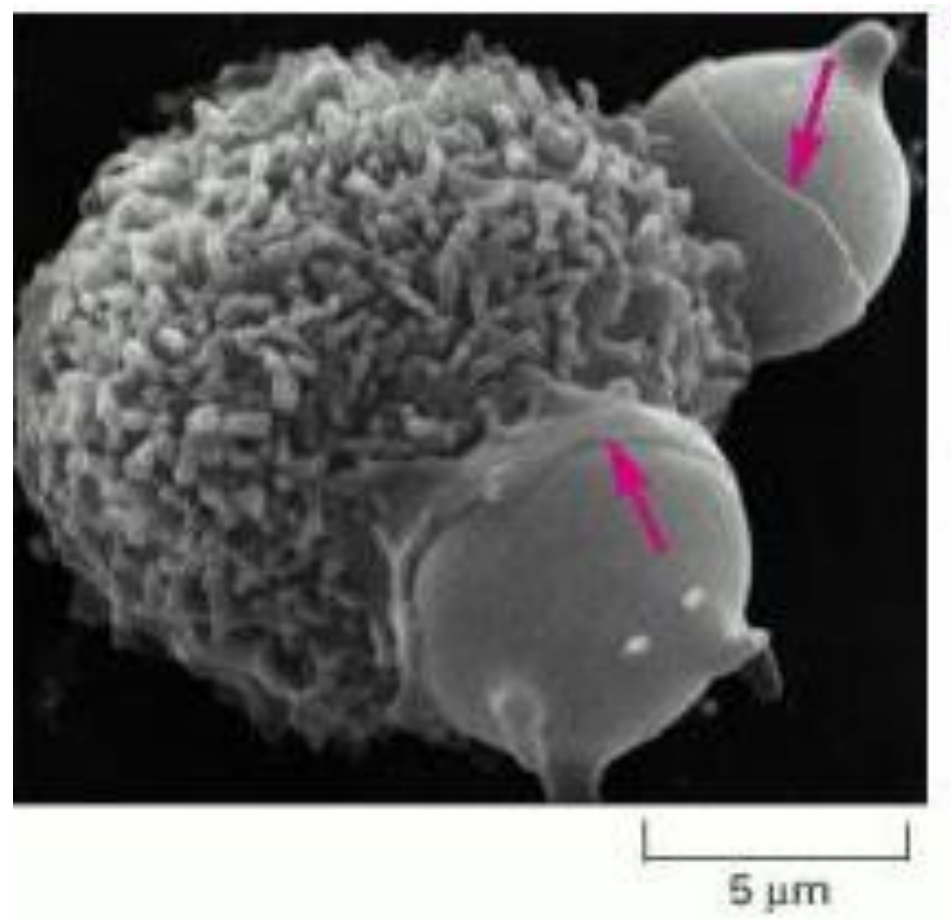
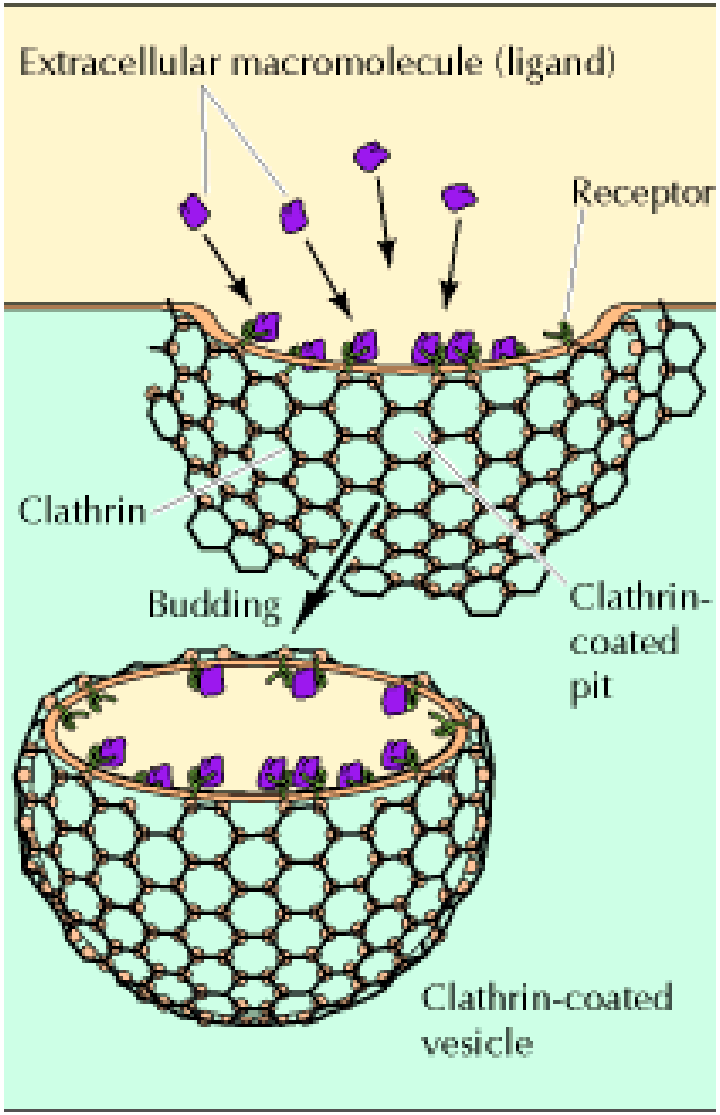


Figure 12.34. Phagocytosis



Phagocytosis by a macrophage.

胞吞作用



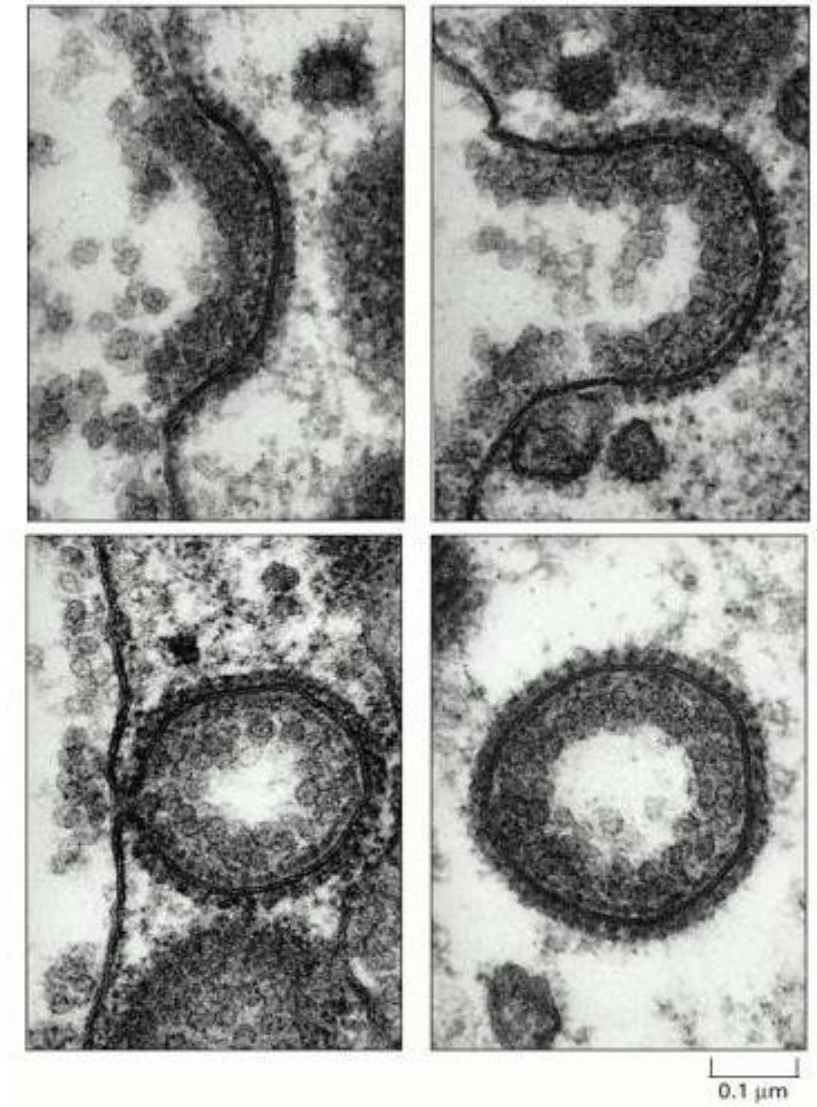


Figure 12.36. Clathrin-coated vesicle formation

Exocytosis & Endocytosis

https://www.youtube.com/watch?v=r2PiumV8KEY&index=13&list=PLXwnjgs_UWpLcVHARCbbglQJPwFl-kD_v

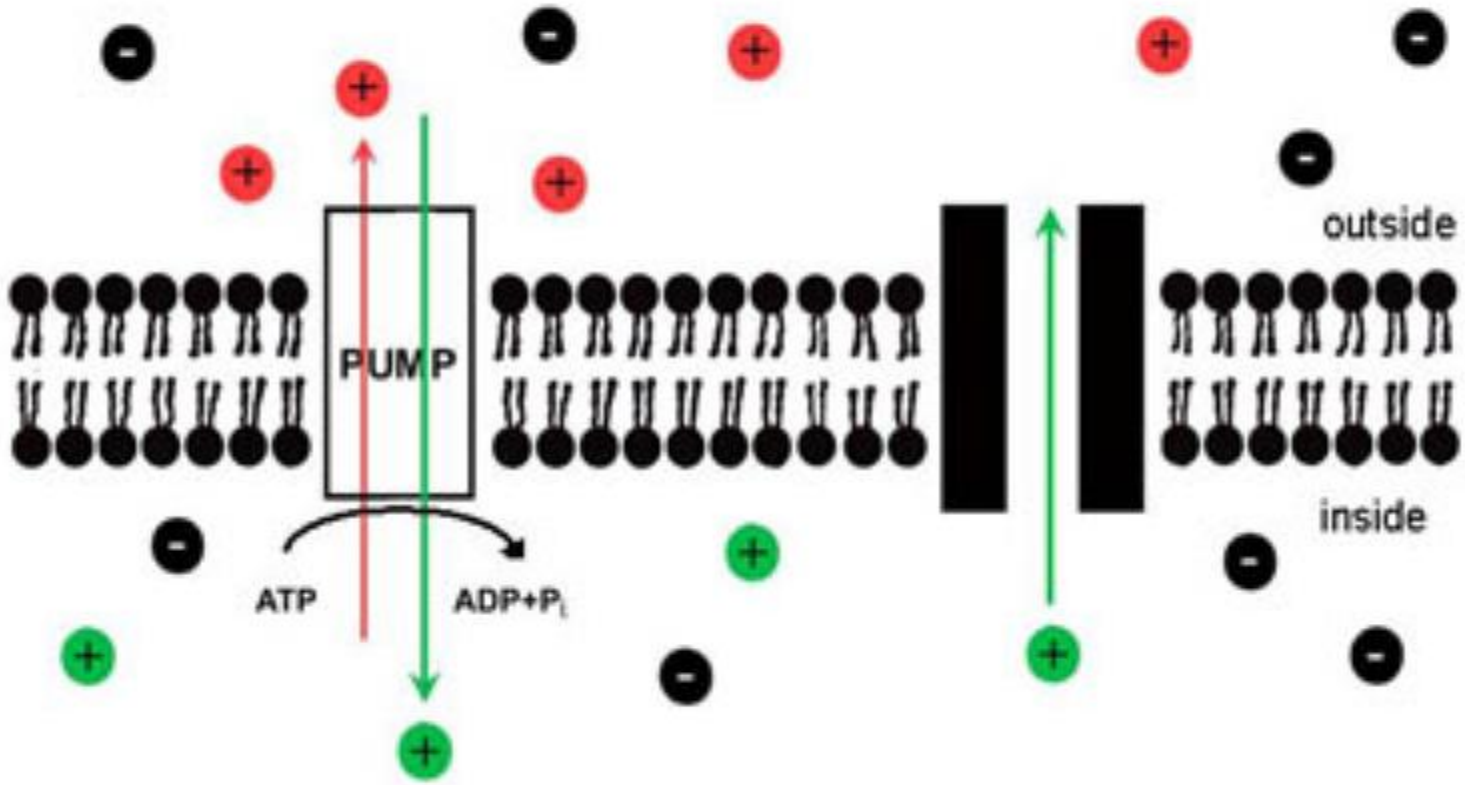
- 細胞，細胞膜，細胞膜蛋白質
- 研究離子通道的方法
- 離子通道的種類、功能和相關疾病
- 疼痛的研究

TABLE 11-1 A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell

COMPONENT	INTRACELLULAR CONCENTRATION (mM) 内	EXTRACELLULAR CONCENTRATION (mM) 外
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions*		
Cl^-	5–15	110

*The cell must contain equal quantities of positive and negative charges (that is, be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cellular constituents are negatively charged (HCO_3^-, PO_4^{3-}, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions. There is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, but this is mostly bound to proteins and other substances and, for Ca^{2+}, stored within various organelles.

Pumps build ion gradients, ion channels dissipate gradients



Active transport

Passive transport

Na^+/K^+ Pump
鈉鉀幫浦

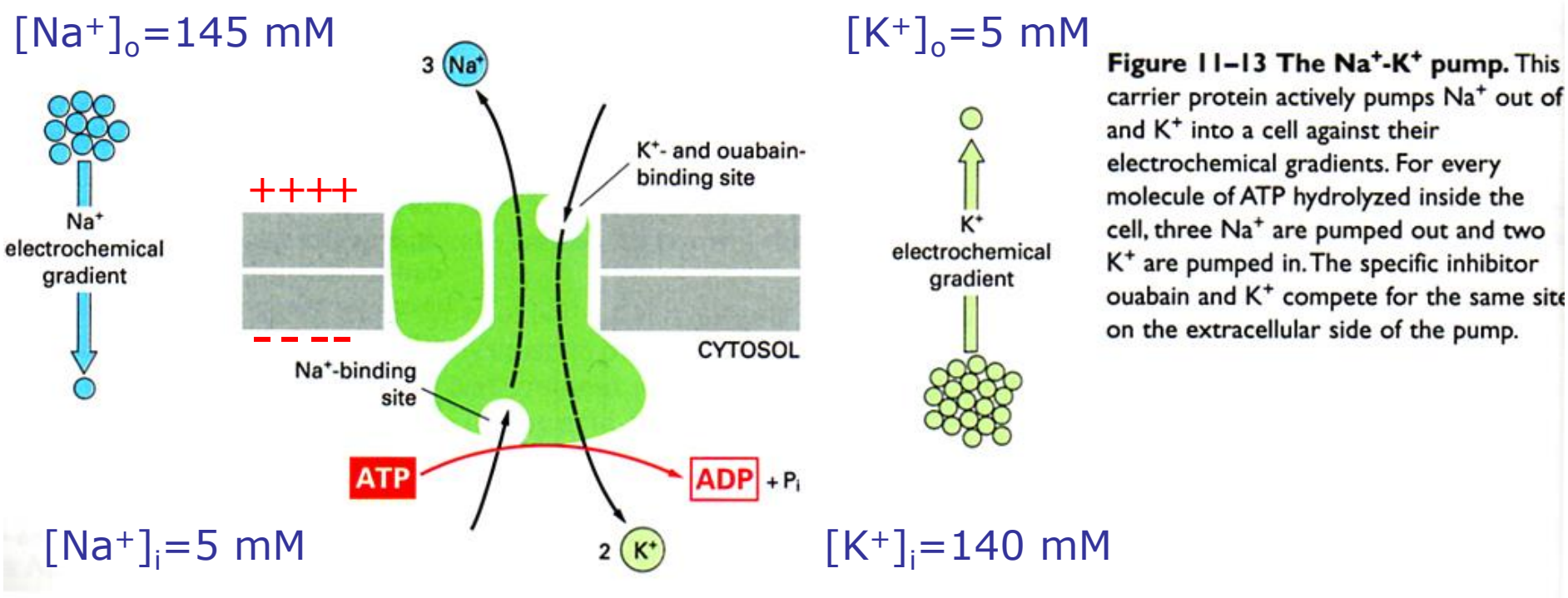


Figure 11–13 The Na^+-K^+ pump. This carrier protein actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, three Na^+ are pumped out and two K^+ are pumped in. The specific inhibitor ouabain and K^+ compete for the same site on the extracellular side of the pump.

Molecular Biology of the Cell

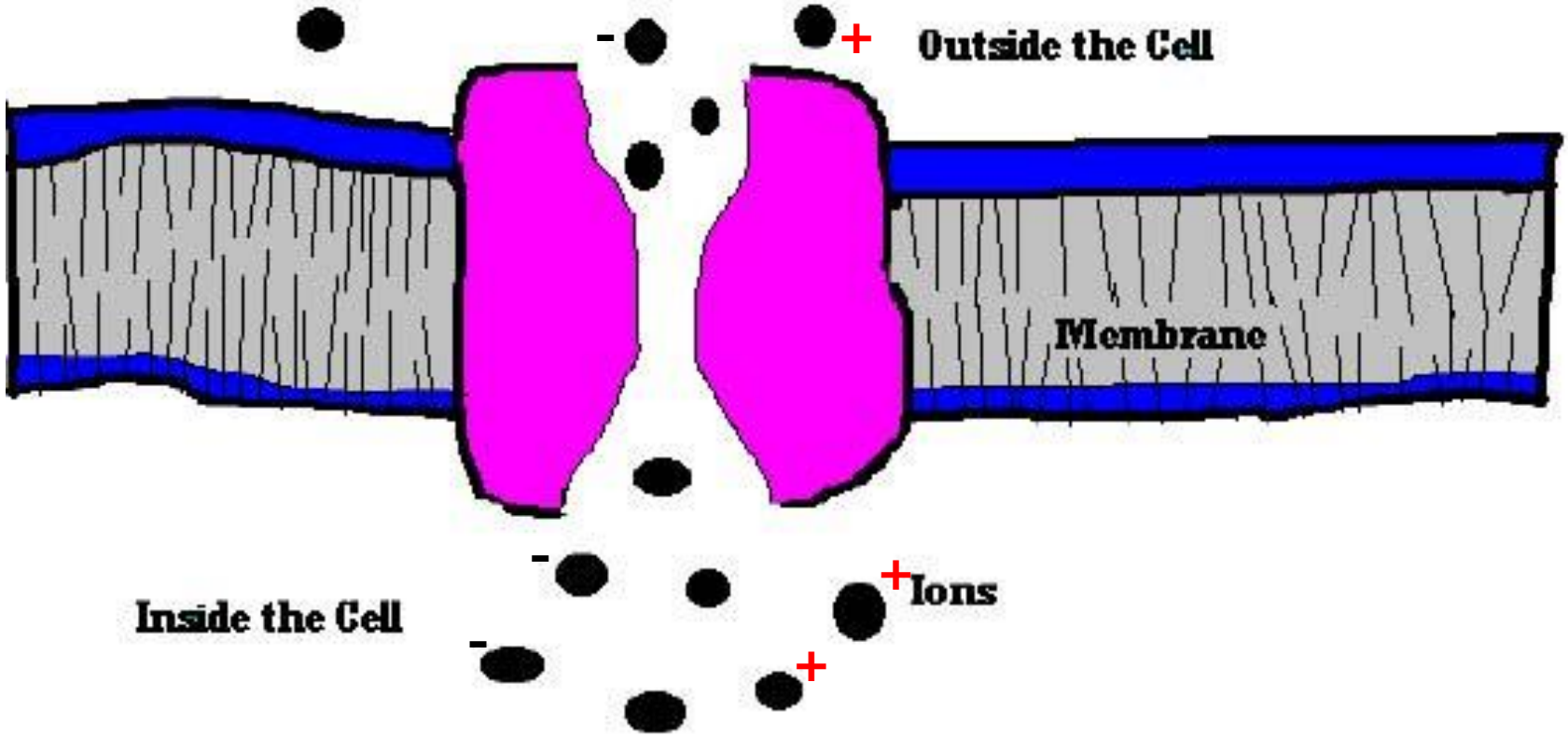
polarization
細胞膜極化

https://www.youtube.com/watch?v=QD0pOVbVUQQ&index=7&list=PLXwnjgs_UWpLcVHARCbbglQJPwFl-kD_v

Nerve transduction
神經傳導

可興奮性細胞
- 神經細胞
- 骨骼肌細胞
- 心肌細胞
- 平滑肌細胞

How to study ion channel?
如何研究離子通道?



Ohm's Law: V =IR 電壓＝電流 X 電阻

http://opal.msu.montana.edu/cftr/IonChannelPrimers/beginners.htm

Voltage-clamp technique 電壓箝制技術

二十世紀中葉（1950年代）：

霍去金 (**Hodgkin**) &赫胥黎 (**Huxley**)研究烏賊的巨大神經元軸突

→發現神經細胞內外具有電位差（神經細胞內的電位較低≒－65mV）

(1)無脊椎動物（烏賊、龍蝦、蚯蚓）具有巨大的神經元

(2)烏賊的神經元軸突直徑＞1mm

可用來測量靜止膜**電位**的伏特數（烏賊的靜止膜電位≒－65mV）

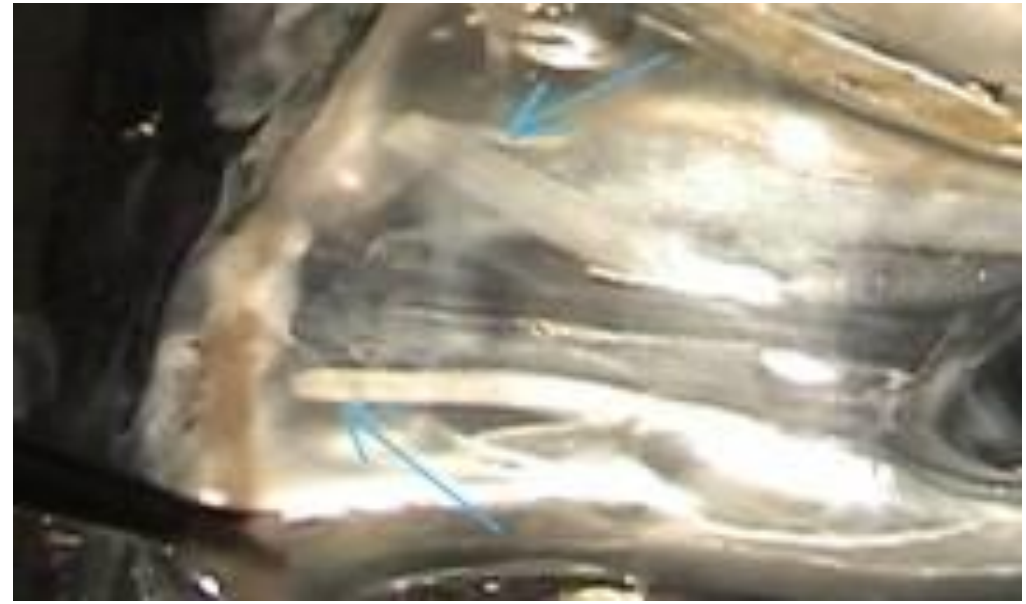
可用來記錄在神經衝動傳遞時，離子流動所造成的電位變化

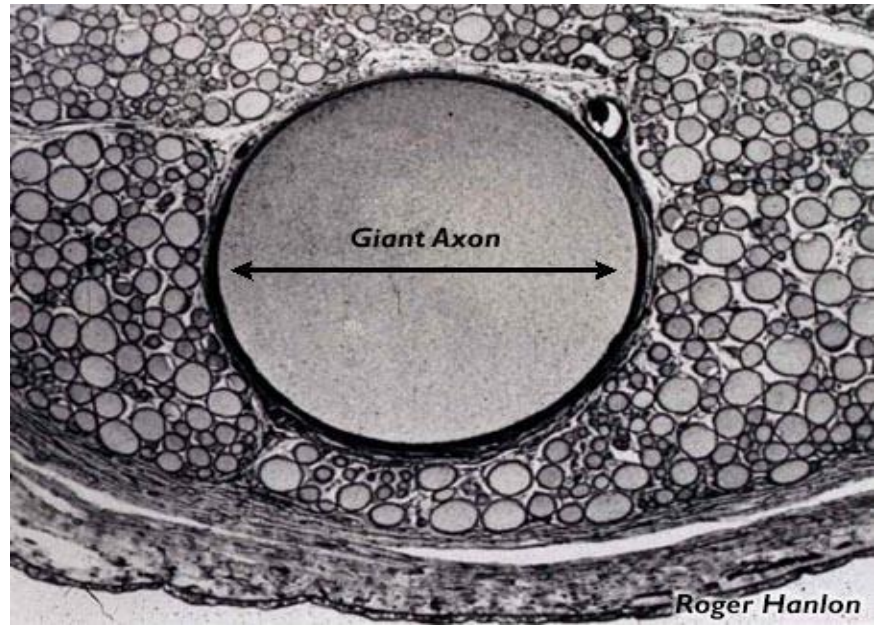
(烏賊的動作電位≒＋40mV）

Hodgkin

Huxley

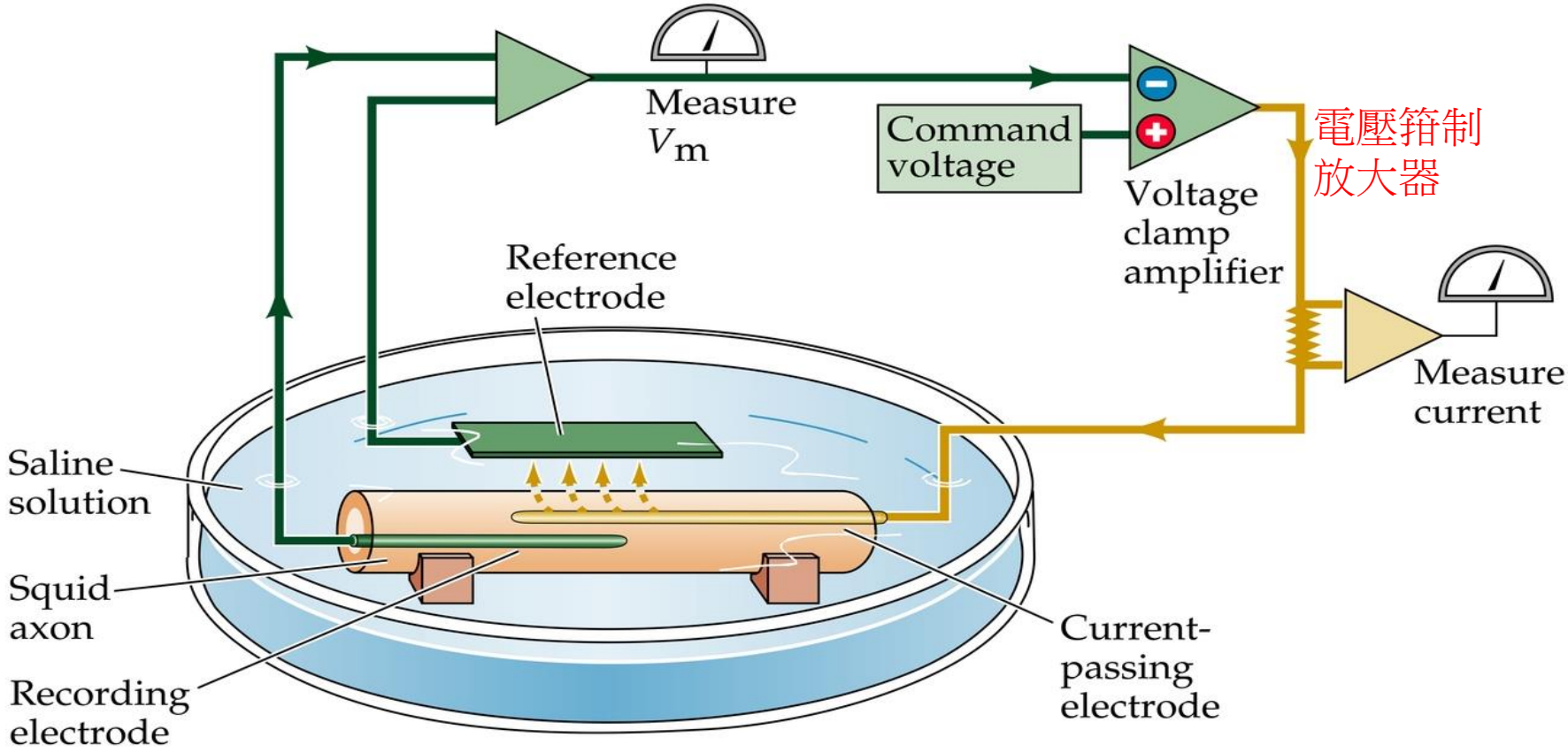
Nobel Prize in 1963





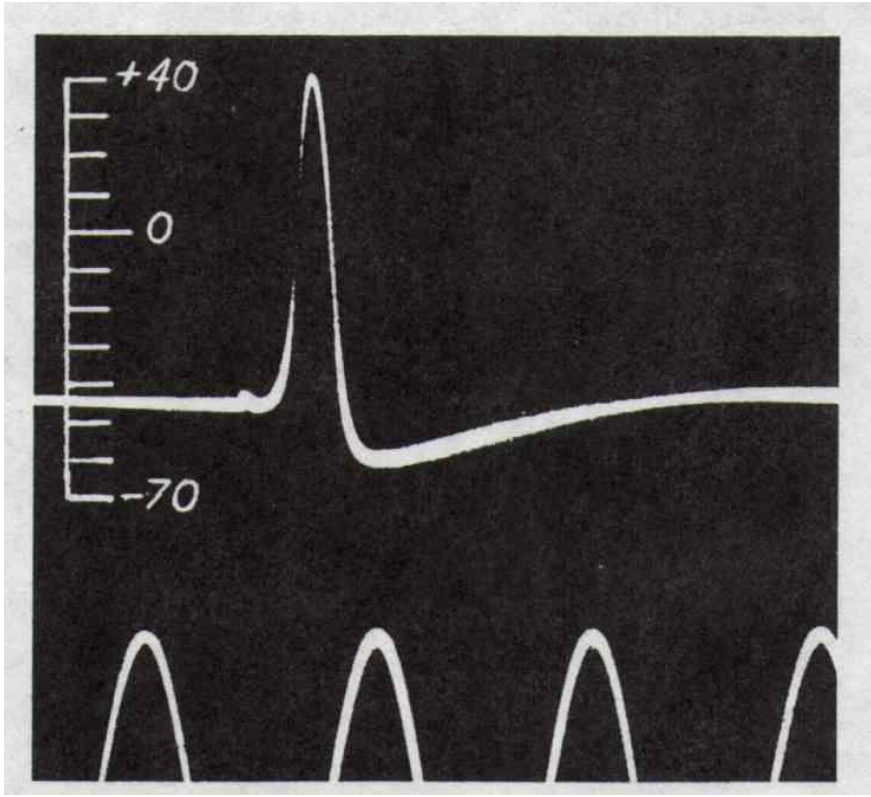
1 mm dia

Voltage-clamp methodology of Hodgkin and Huxley

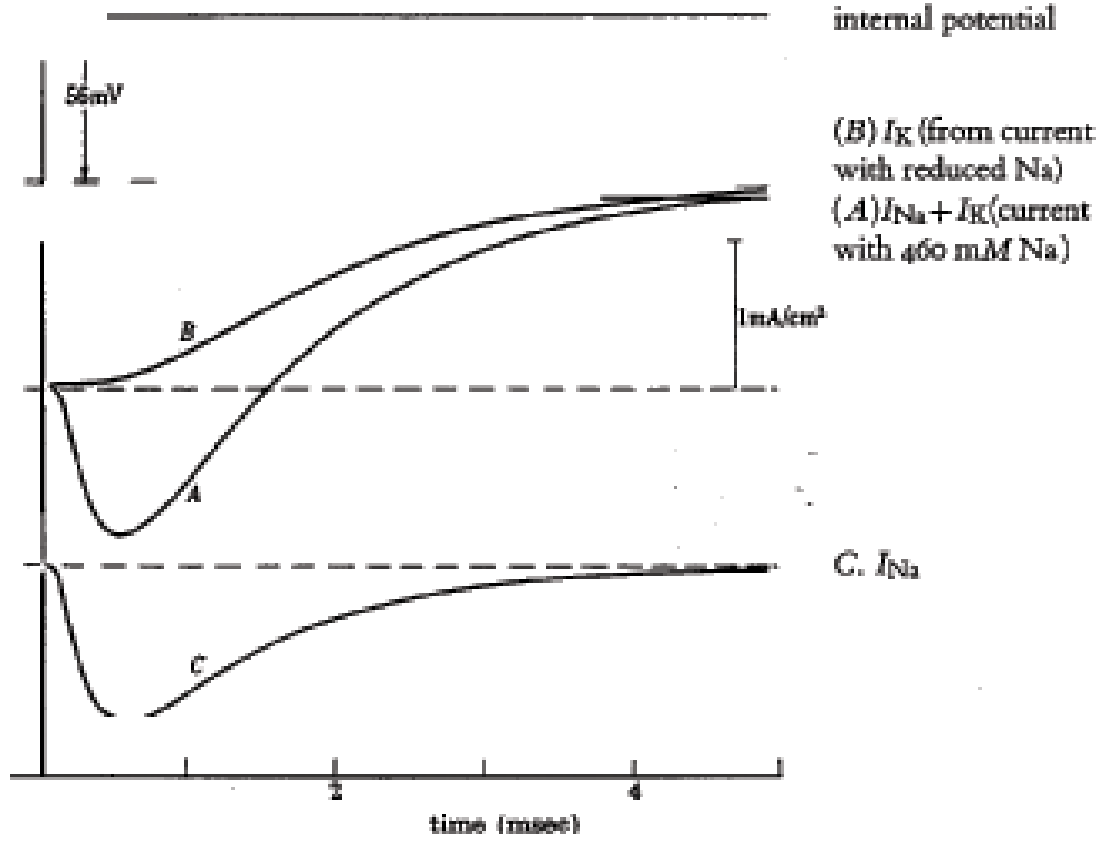


Neuroscience by Purves et al.

Action potential and Na^+ and K^+ currents recorded from squid axon



最早的細胞內紀錄-動作電位
1939

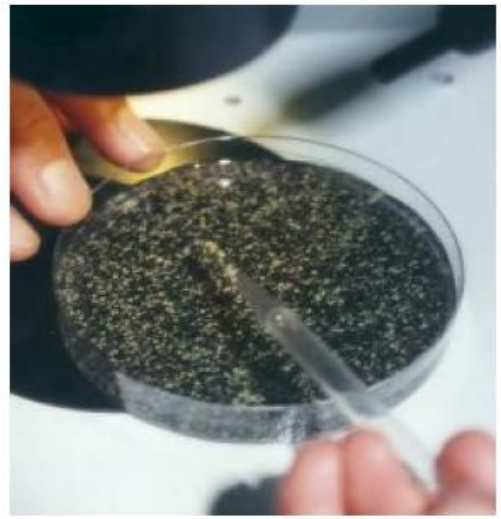


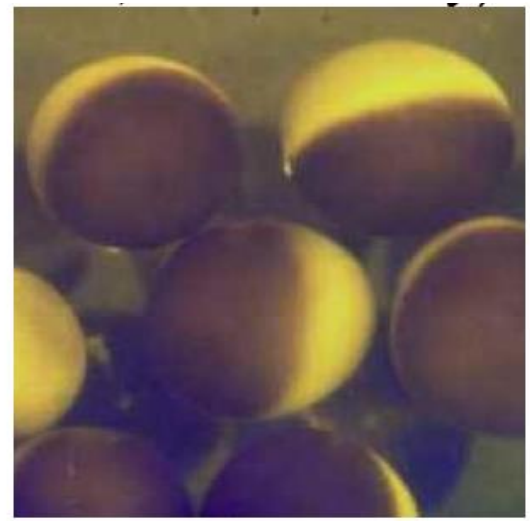
Hodgkin and Huxley

Gurdon J. B., Lane D. C., Woodland H. R., and Marbaix G. (1971) Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. *Nature (Lond.)* **233**, 177–182.

Gundersen Miledi R., and Parker I. (1984) Messenger RNA from human brain induces drug-and voltage-operated channels in *Xenopus* oocytes. *Nature (Lond.)* **308**, 421–424.

Inject mRNA in oocytes; wait 2-5 days; protein in membrane.

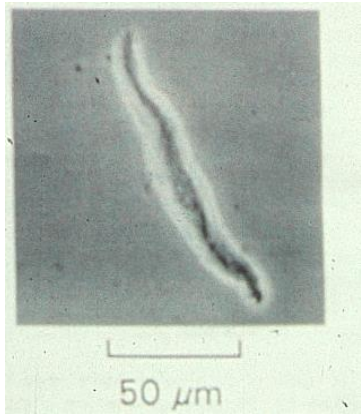


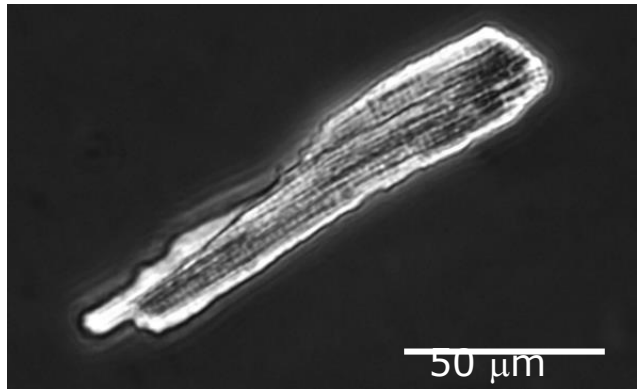


www.mpibp-frankfurt.mpg.de/schwarz/oocytes.html

How to record a single cell?
如何記錄單一細胞？

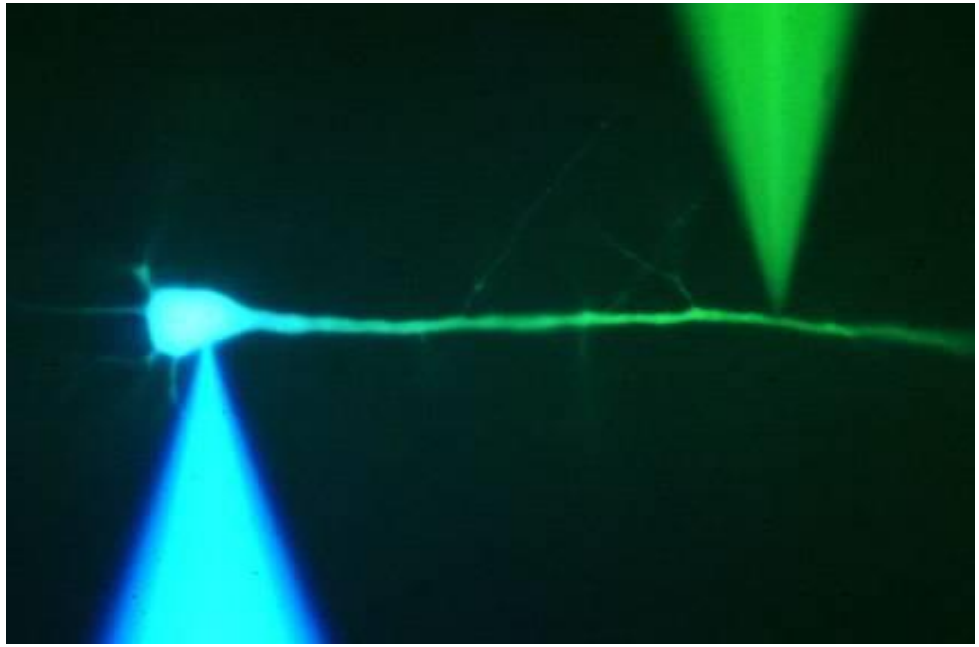
Smooth muscle cells
(diameter <10 μm)
平滑肌細胞





Mouse
ventricular myocytes
小鼠心室細胞

Cortical
pyramidal neuron



Stuart and Sakmann, 1994

Patch-Clamp Technique 「膜片箝制」

Erwin Neher

Bert Sakmann

Nobel Prize in 1991

Patch-Clamp Setup

Optical parts

Microscope
CCD camera

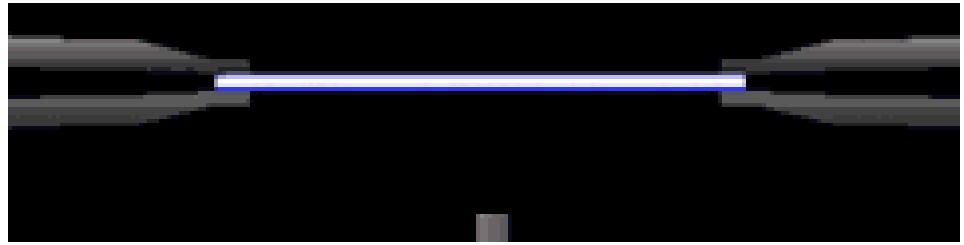
Mechanical parts

Vibration-free table
Micromanipulators

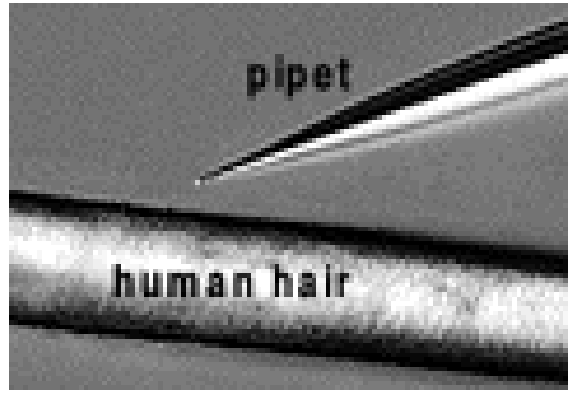
Electrical parts

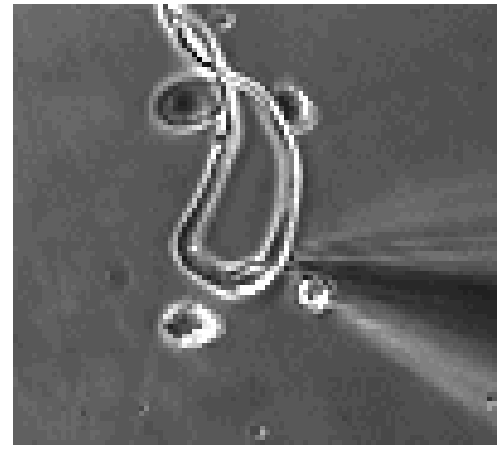
Amplifiers
Oscilloscope
AD/DA Converter
Computer

Patch-Clamp Technique「膜片箝制」的技術

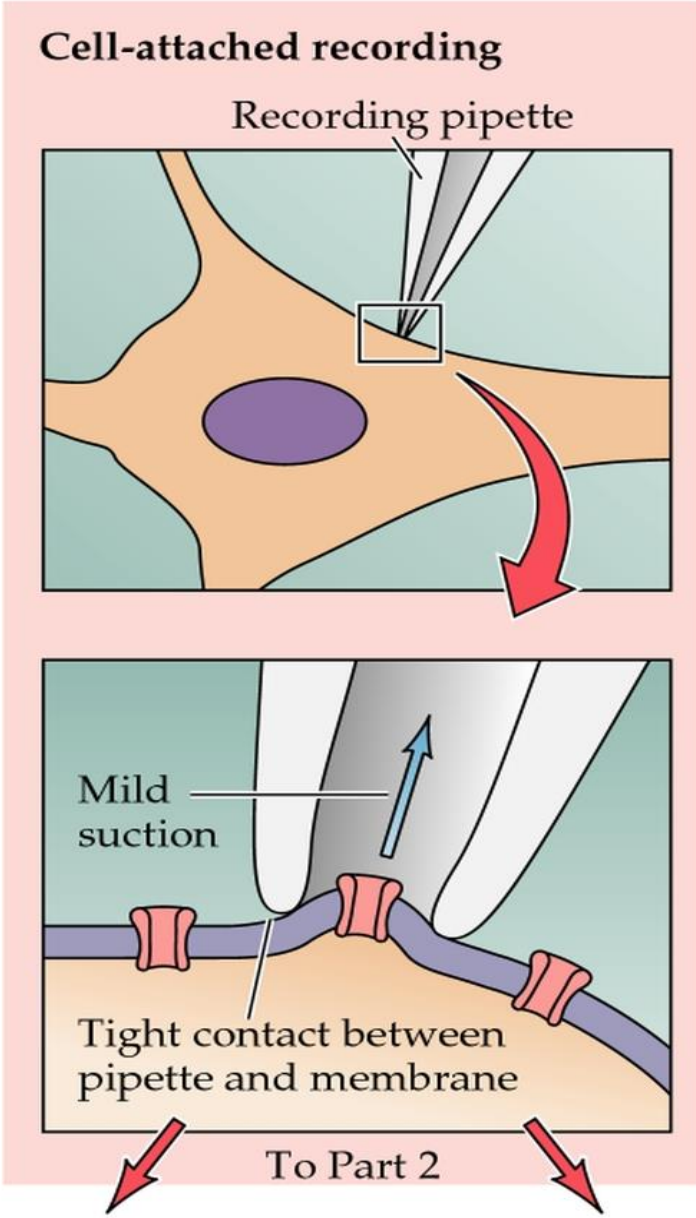


電極尖端
1-2 μM



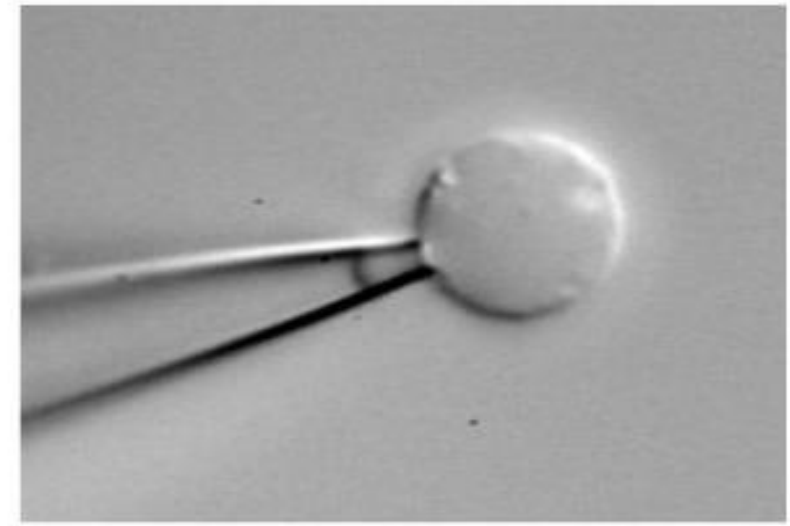


http://www.cellsalive.com/patch.htm



電壓＝電流 X 電阻

- Tight seal onto the membrane – GΩ seal
- Ion channel is trapped under the pipette.
-
-



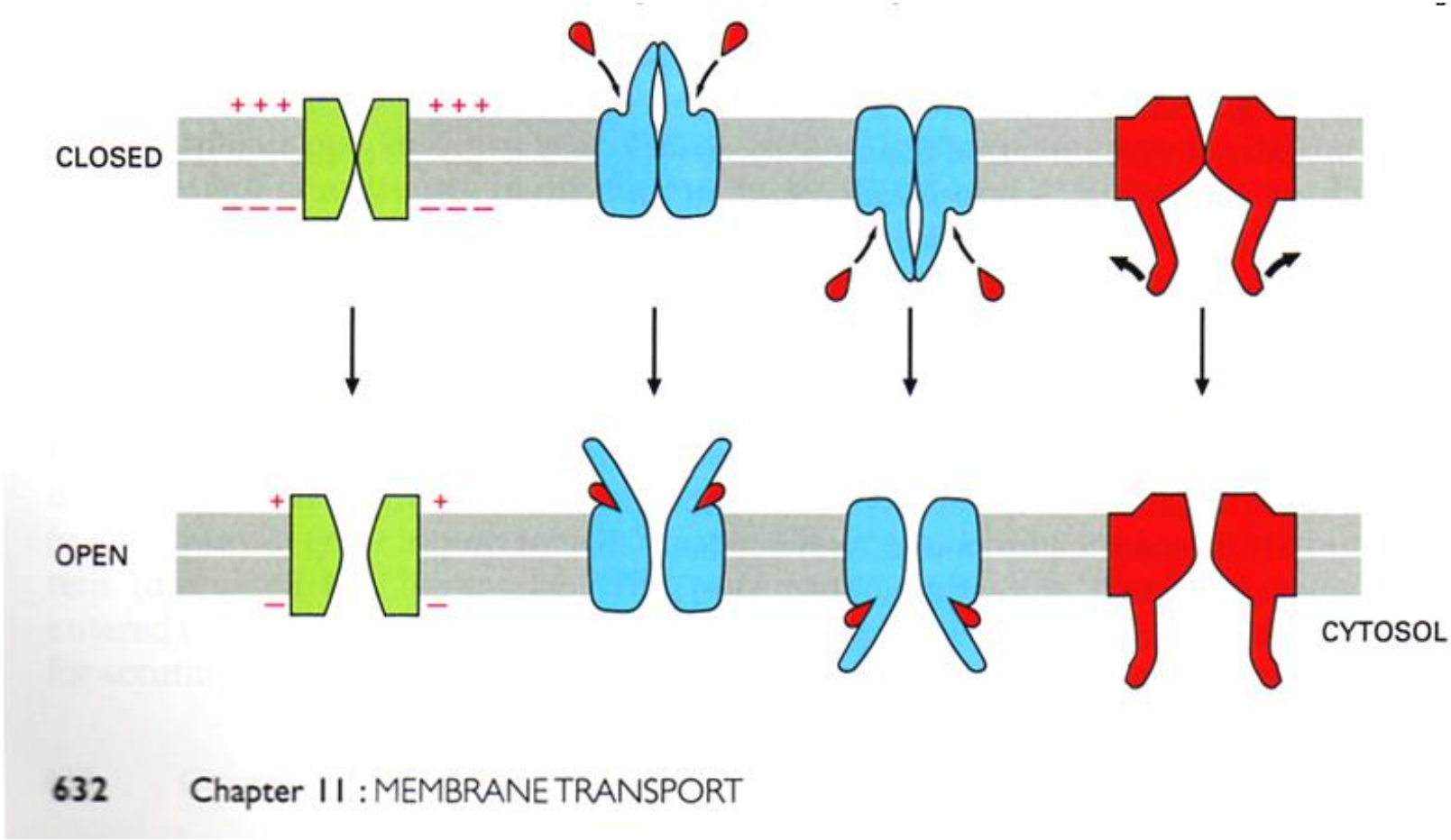
y

Neuroscience by Purves et al.

- 細胞，細胞膜，細胞膜蛋白質
- 研究離子通道的方法
- 離子通道的種類、功能和相關疾病種類
- 疼痛的研究

Types of ion channels
離子通道的種類

Voltage-gated 電壓驅動式　　Ligand-gated 配體驅動式　　Mechanical-gated 機械驅動式



Molecular Biology of the Cell

Types of ion channels
離子通道的種類

電壓驅動式離子通道

Na^+ 鈉離子　K^+ 鉀離子　Ca^{2+} 鈣離子

可興奮性細胞
- 神經細胞
- 骨骼肌細胞
- 心肌細胞
- 平滑肌細胞

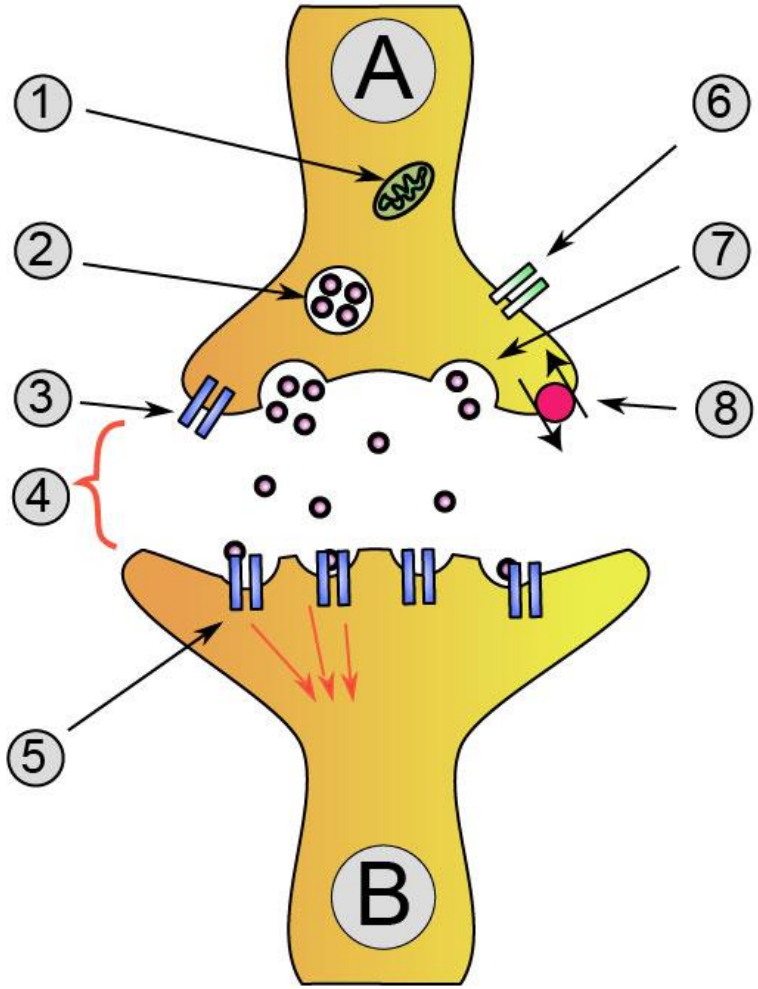
配體驅動式離子通道

acetylcholine 乙烯膽鹼　glutamate 谷氨酸鹽　glycine 甘氨酸

Excitable neuron
興奮性神經細胞

Inhibitory neuron
抑制性神經細胞

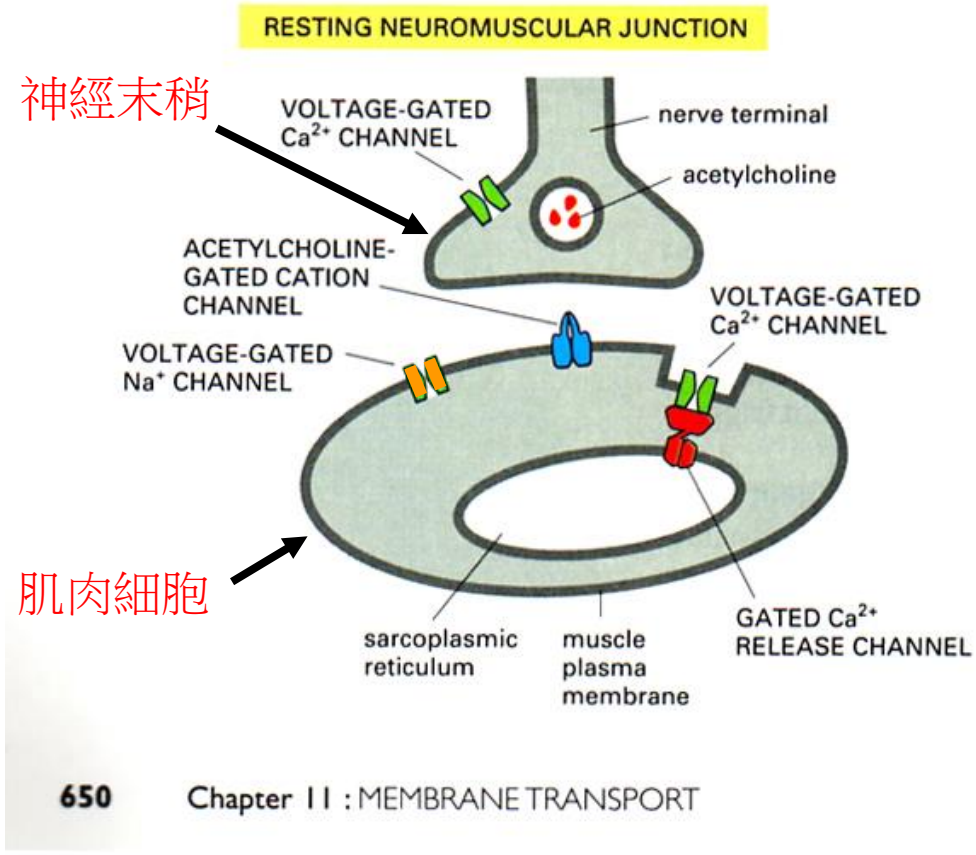
典型的突觸結構

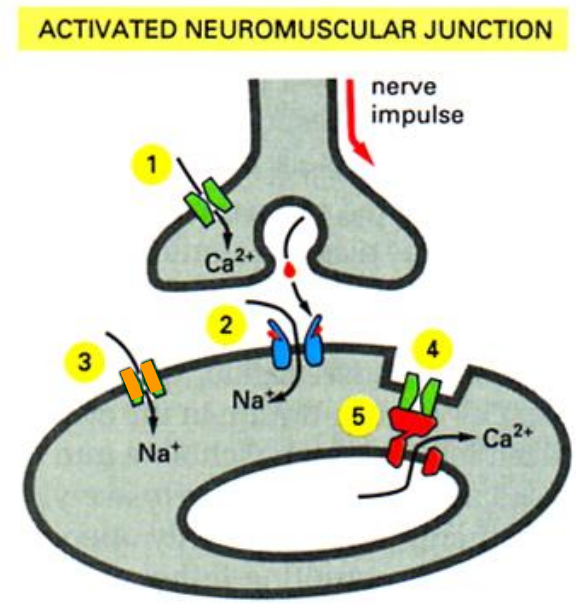


A. 突觸前膜。
B. 突觸後膜。
1. **線粒體**：突觸的能量供應者。
2. **突觸小泡**：內含待釋放的神經遞質。
3. 突觸前膜上的神經遞質**受體**。
4. 突觸間隙。
5. 突觸後膜上的神經遞質**受體**。
6. 突觸前膜上的**鈣通道**。
7. 突觸前膜。
8. 離子幫浦。

維基百科

Neuromuscular junction
神經肌肉接點

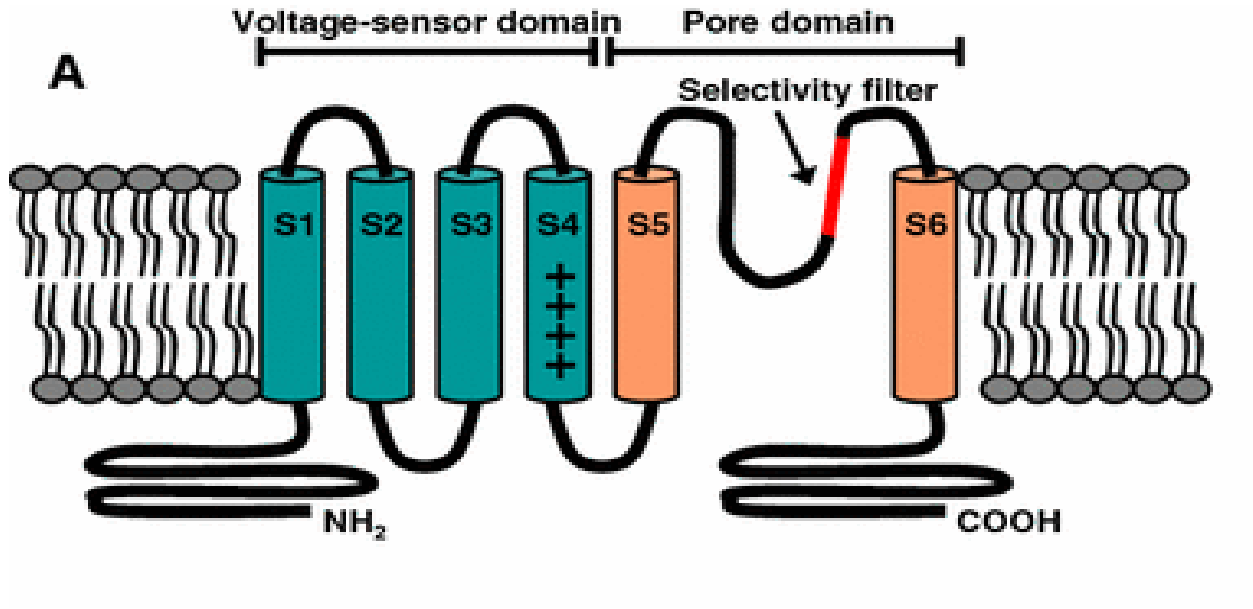


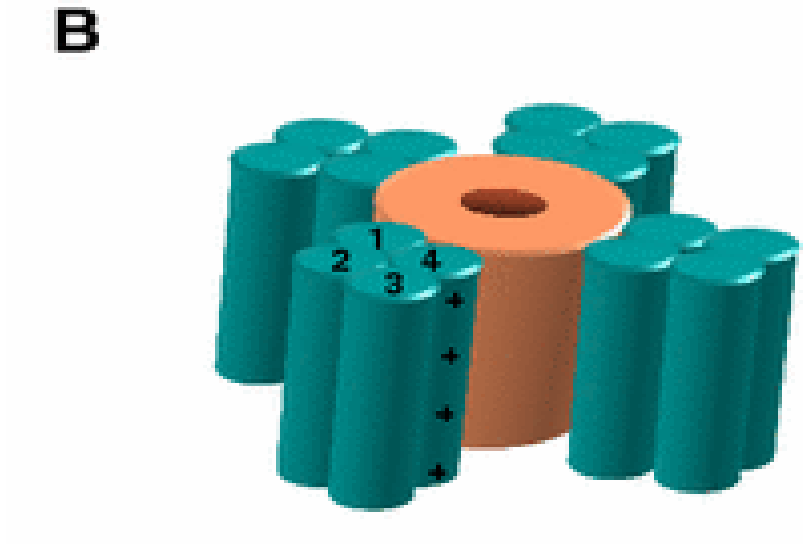



乙烯膽鹼接受器


電壓驅動式鈣離子通道


電壓驅動式鈉離子通道

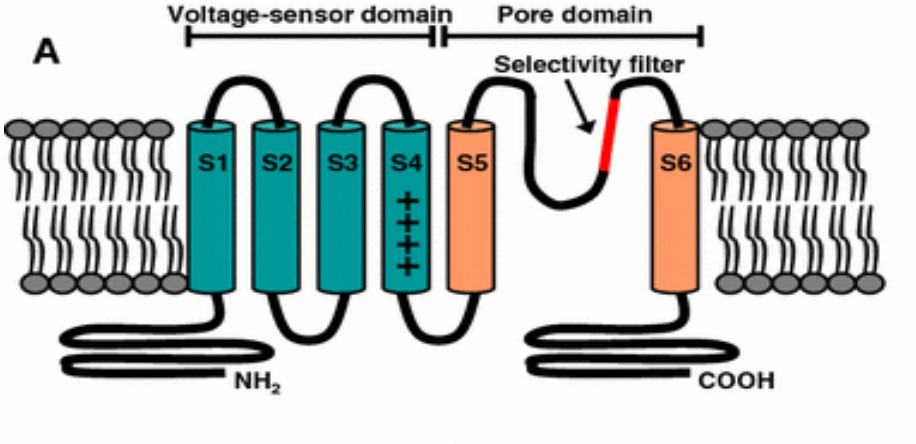


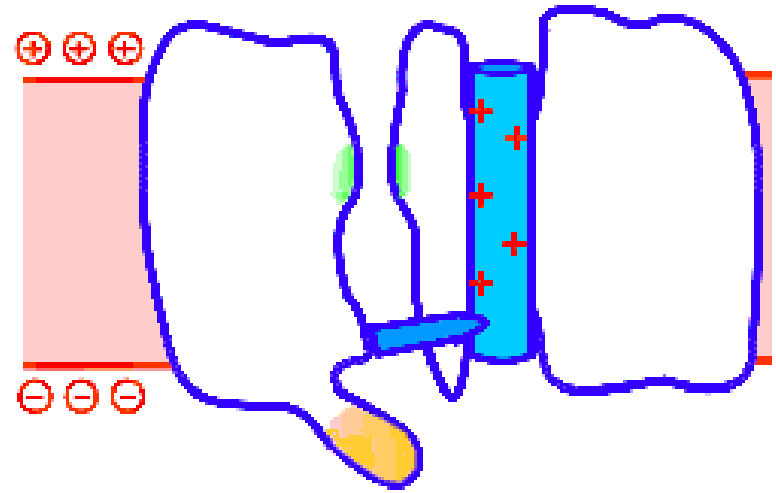


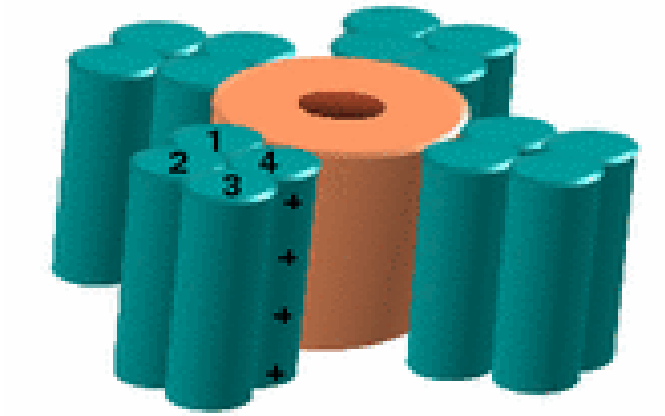
Cell Biochemistry and Biophysics 2008

How does the channel sense change in voltage?
離子通道如何感應膜電位的變化？

- Positively charged residues within the S4 TM segment.
- Evidence that the segment rotates out of the membrane in response to change in voltage: gating charges



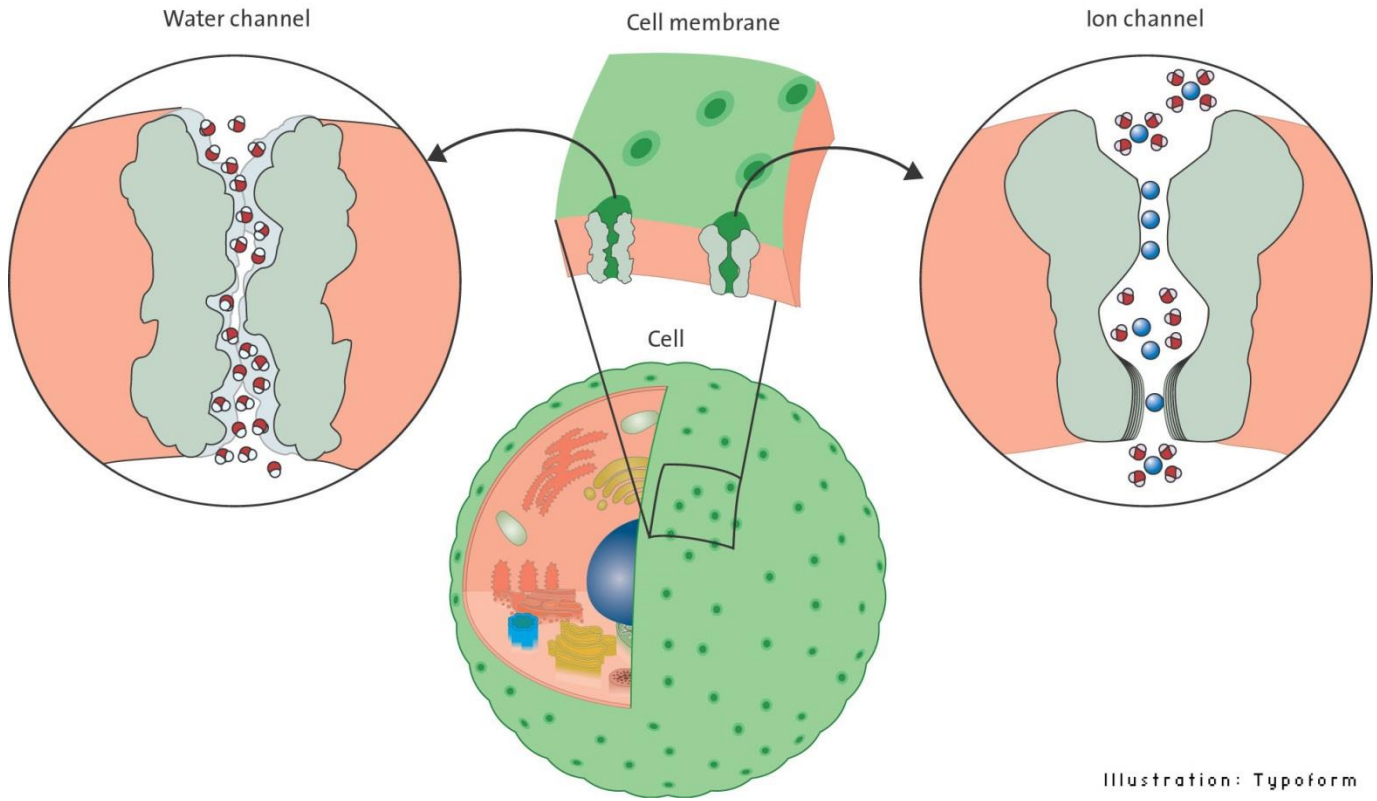




The third Nobel Prize for ion channel research

Agre

阿格雷



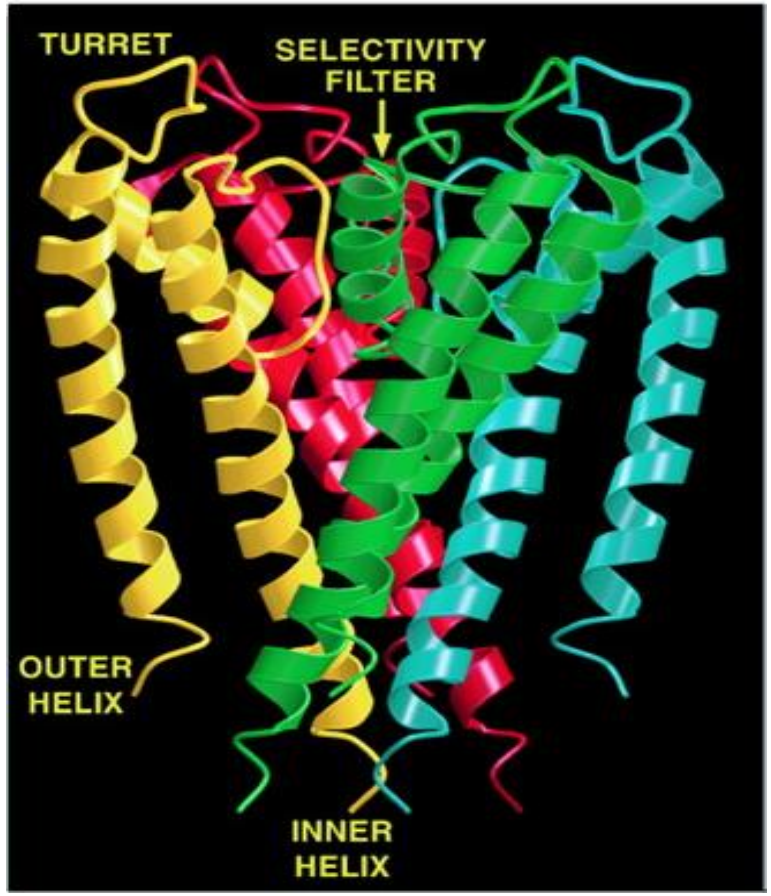
MacKinnon

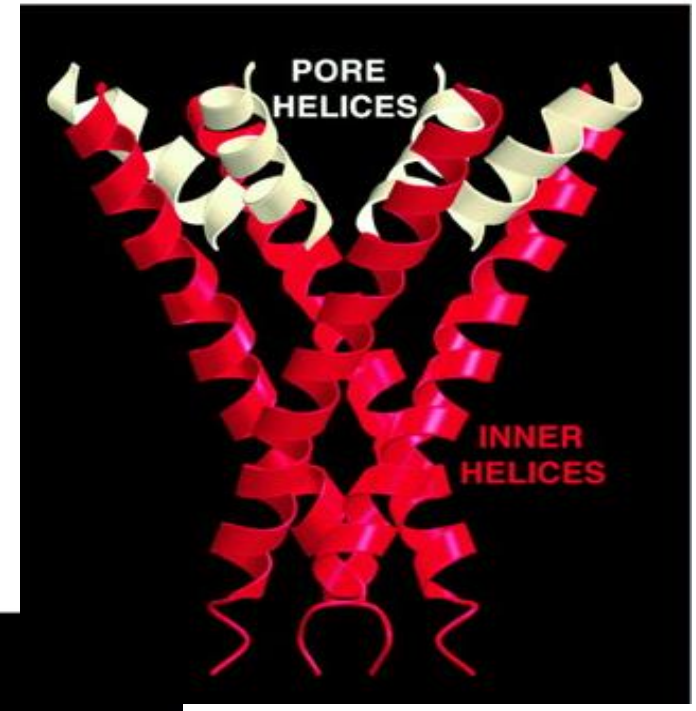
麥金農

Nobel Prize in 2003

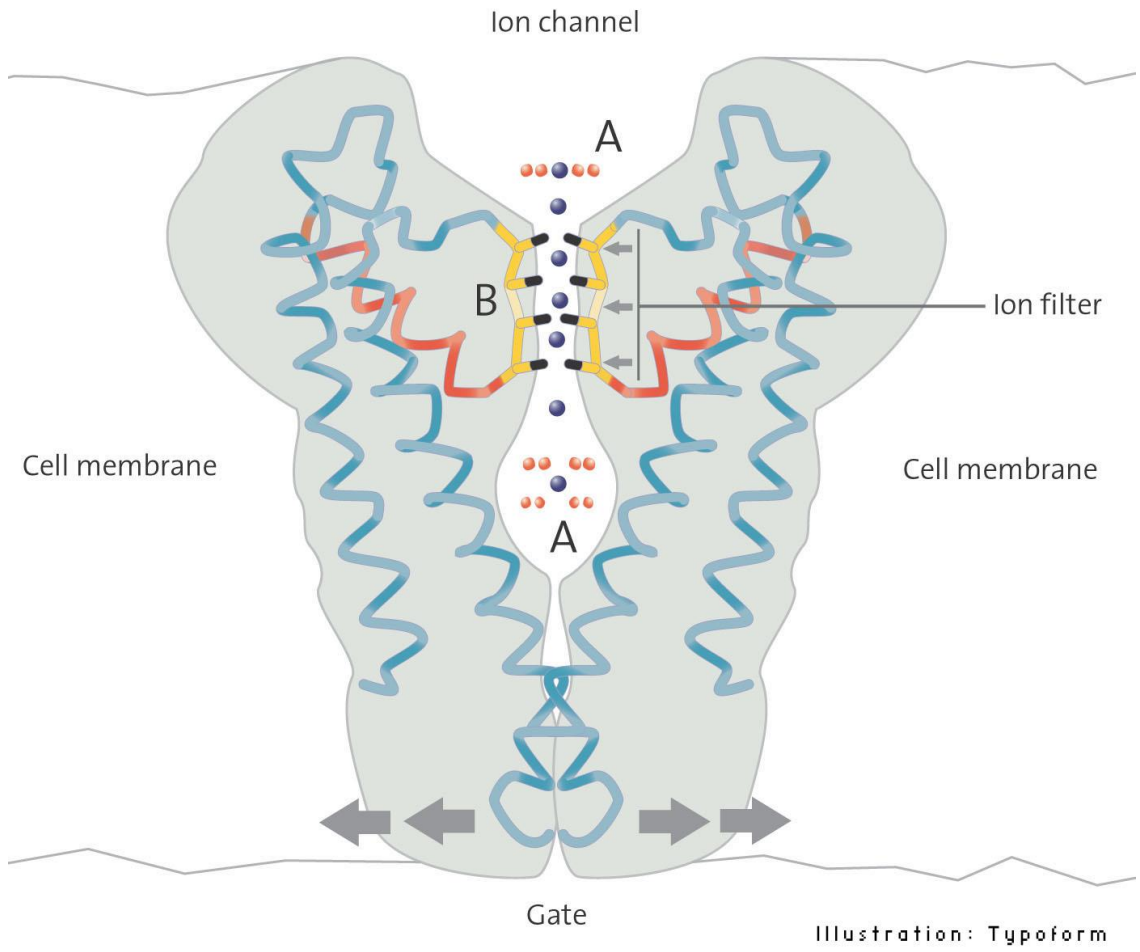
What they look like:

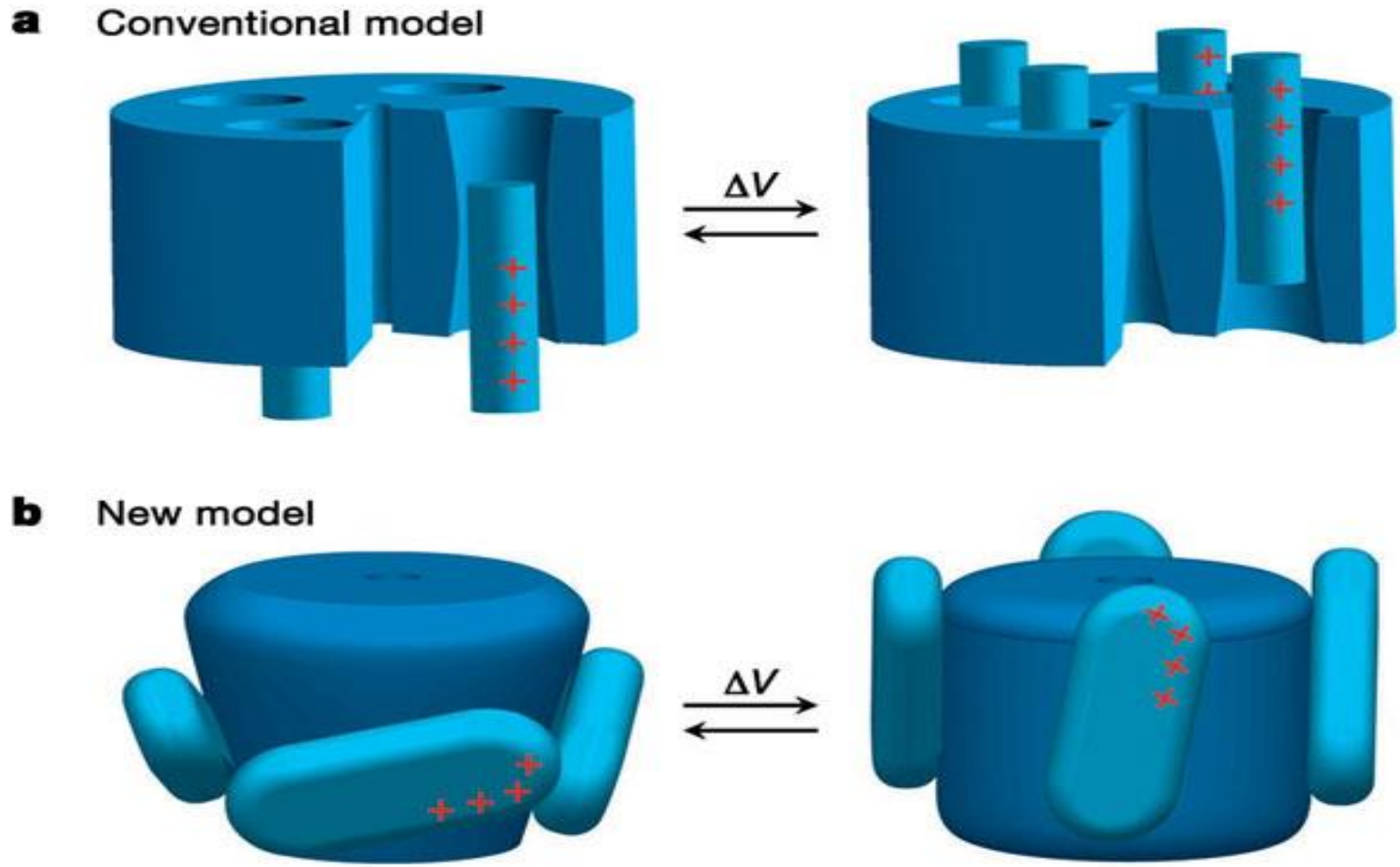
Bacterial KcsA channels





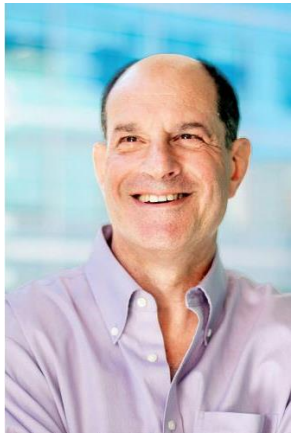
K^+ channel-鉀離子通道





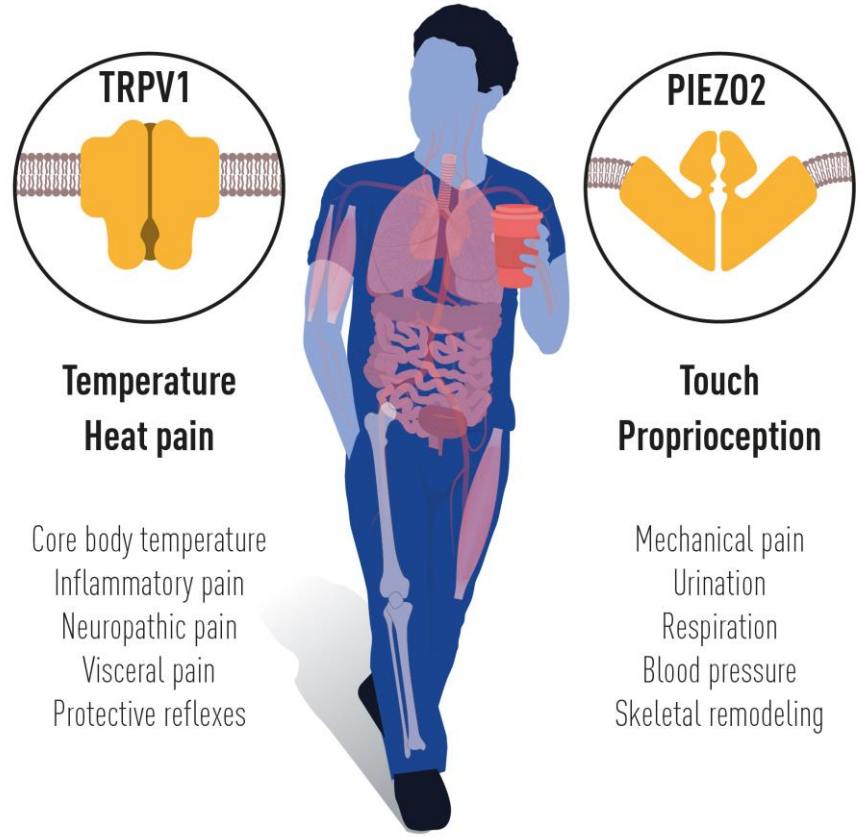
Jiang et al., Nature (2003)

The fourth Nobel Prize for ion channel research



David Julius

朱利葉斯

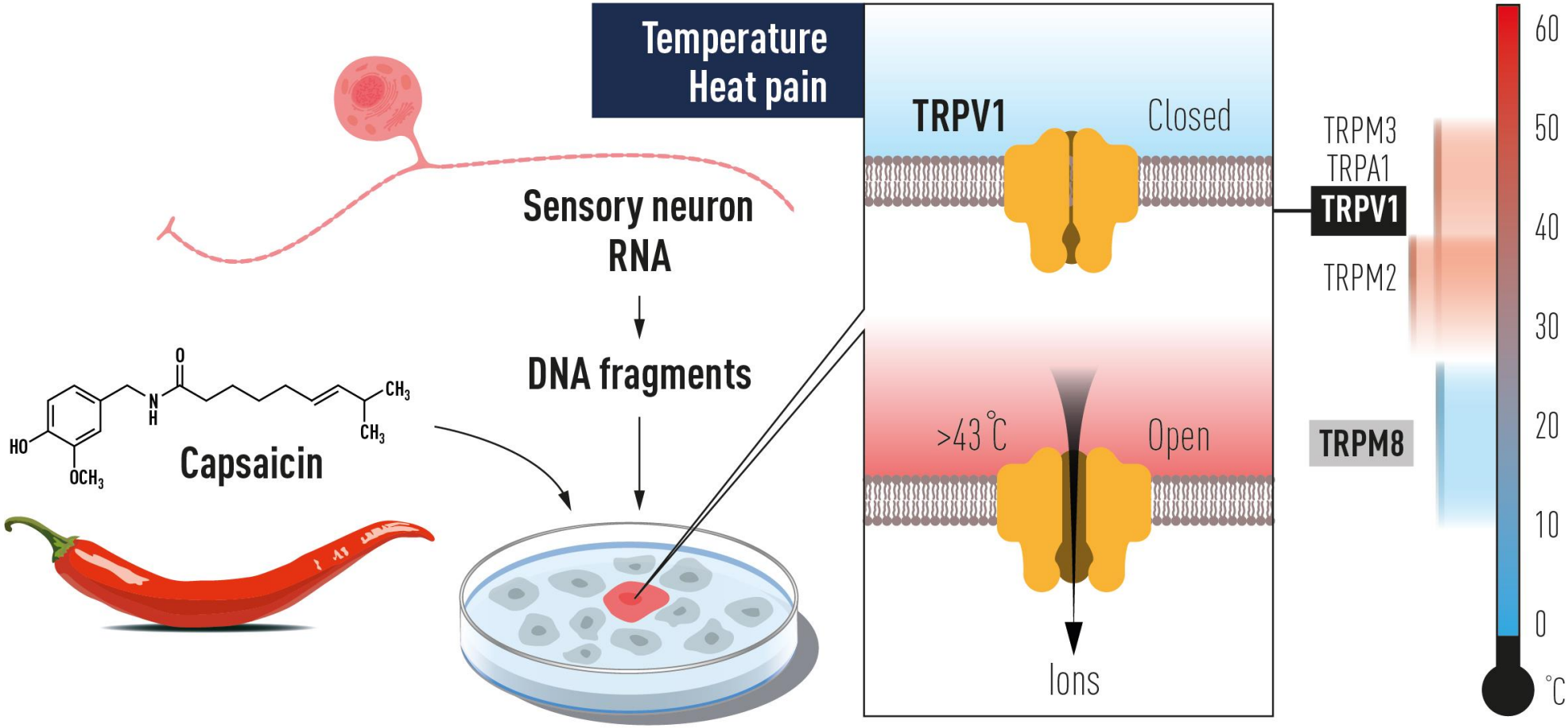


Ardem Patapoutian

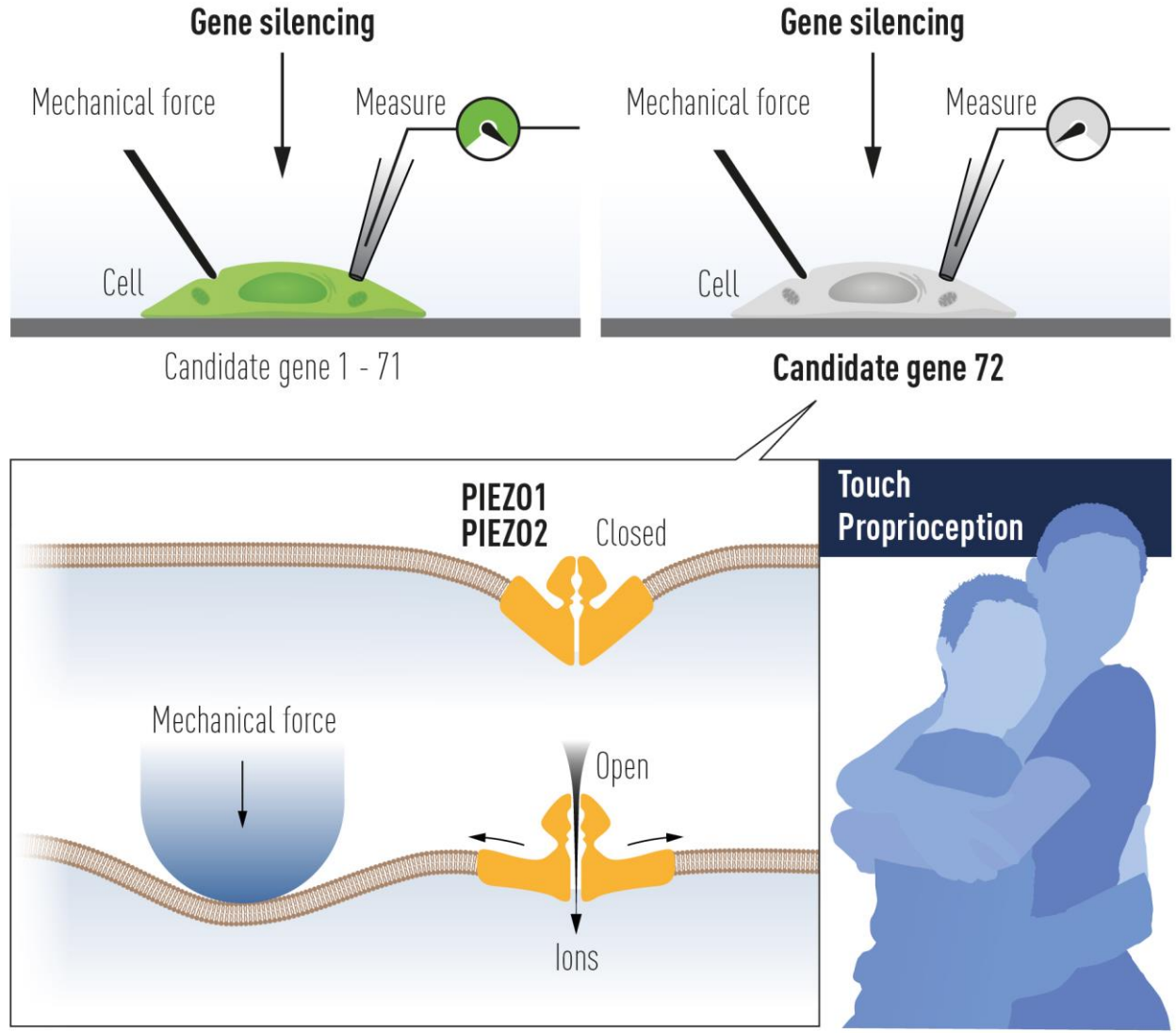
帕塔普蒂安

Nobel Prize in 2021

David Julius



Ardem Patapoutian



Ion Channelopathies
離子通道相關疾病

Cystic fibrosis (Cl^- channel; CFTR)
Thomsen Myotonia congenita(Cl^- channel; CLC-1)
Becker Myotonia congenita (Cl^- channel; CLC-1)
Hypercalciuric nephrolithiasis (Cl^- channel; CLC-5)
Bartter's syndrome type 1 (Cl^- channel; CLC-Kb)
Angleman / Prader-Willi (GABA channel; GABAAB3)
Low molecular weight proteinuria (CLCN5)
Bartter's syndrome type 3 (SLC12A3)
Startle disease (hyperexplexia) (glycine receptor)

Liddle's syndrome (ENaC; SCNN1A, SCNN1B)
Paramyotonia congenita (Na^+ channel; SCN4A)
Hyperkalemic periodic paralysis (Na^+ channel; SCN4A)
Myotonia Fluctuans (Na^+ channel; SCN4A)
Myotonia Permanens (Na^+ channel; SCN4A)
Acetzolamide-responsive myotonia (Na^+ channel; SCN4A)
Malignant hyperthermia (Na^+ channel; SCN4A)
Idiopathic ventricular fibrillation (Na^+ channel; SCN5A)
Long QT Syndrome (LQT3 Na^+ channel; SCN5A)
Epilepsy with febrile seizures (Na^+ channel; SCN1B immune)
Acute motor axonal neuropathy (Na^+ channel; immune)
Guillain-Barré & CIDP (Na^+ channel; immune)
Multifocal Motor Neuropathy (Na^+ channel; immune)

Nephrogenic diabetes incipidus (AQP-2)
Total color blindness (CNGA3)

Hypokalemic periodic paralysis (Ca^{2+} channel; CACNL1A3)
Malignant Hyperthermia (Ca^{2+} channel; CACNL1A3, RyR 1)
X-linked congenital night blindness (Ca^{2+} channel; CSNB2)
Muscular dysgenesis (rodent Ca^{2+} channel; CACNL1A3)
Episodic ataxia type-2 (Ca^{2+} channel; CACNL1A4)
Familial hemiplegic migraine (Ca^{2+} channel; CACNL1A4)
Spinocerebellar ataxia (Ca^{2+} channel; CACNL1A4)
Congenital myasthenic syndrome (nAChR)
Lambert-Eaton Myasthenic Syndrome (Ca^{2+} channel; immune)
Insulin-Dependent Diabetes (Ca^{2+} channel; immune)

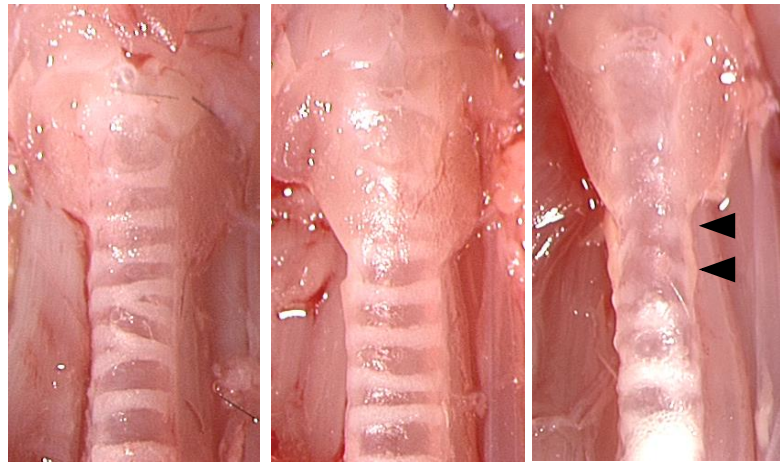
Antenatal Bartter's Syndrome type 2 (K^+ channel; KCNJ1)
Long QT Syndrome (LQT1; K^+ channel; KCNA8)
Long QT Syndrome (LQT2 K^+ channel; HERG)
Jervell & Lange-Nielsen Syndrome (K^+ channel; KCNE1, KCNQ1)
Episodic Ataxia / Myokymia Syndrome (K^+ channel; KCNA1)
Benign neonatal epilepsy (K^+ channel; KCNQ2, KCNQ3)
Schizophrenia (K^+ channel; KCNN3)
Retinitis pigmentosa (K_{NS} channel; CNGA1)
Rod monochromacy (K_{NS} channel; CNGA3)
Hyperinsulinism of Infancy (K^+ channel; SUR1)
Hyperinsulinism of Infancy (K^+ channel; Kir6.2)

Visceroatrial Heterotaxia (gap junction; CXA1)
CMT-X (gap junction; CXB1)
Non-syndromic deafness (gap junction; CXB2)

Abnormal Vocalization of $\alpha_{1H}^{-/-}$ Mice

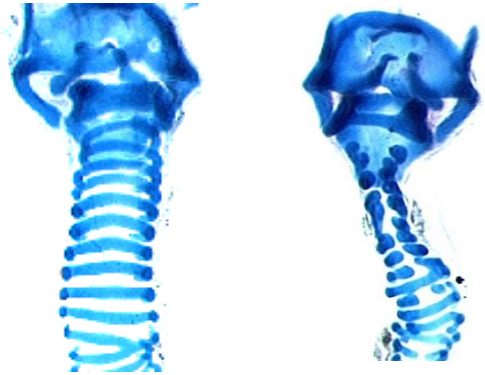
Abnormal Trachea Formation

+/+ +/- -/-



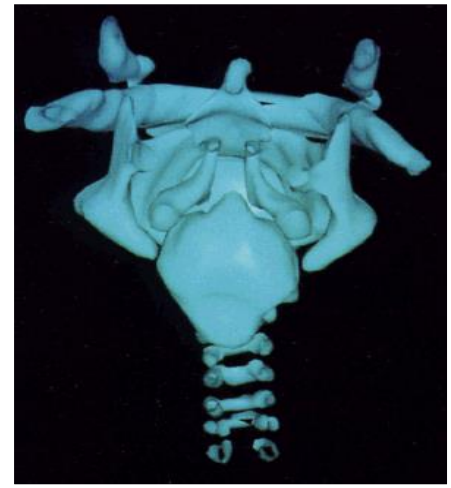
+/+ -/-

+/+ -/-

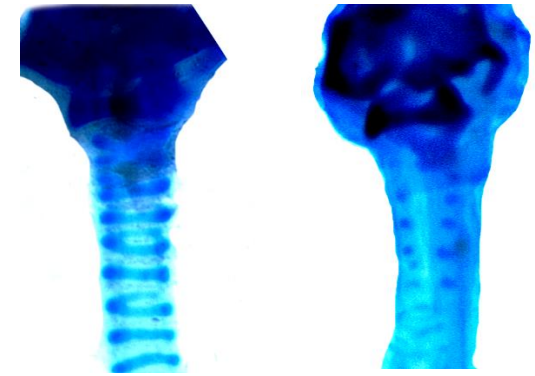


P1 P1

E16

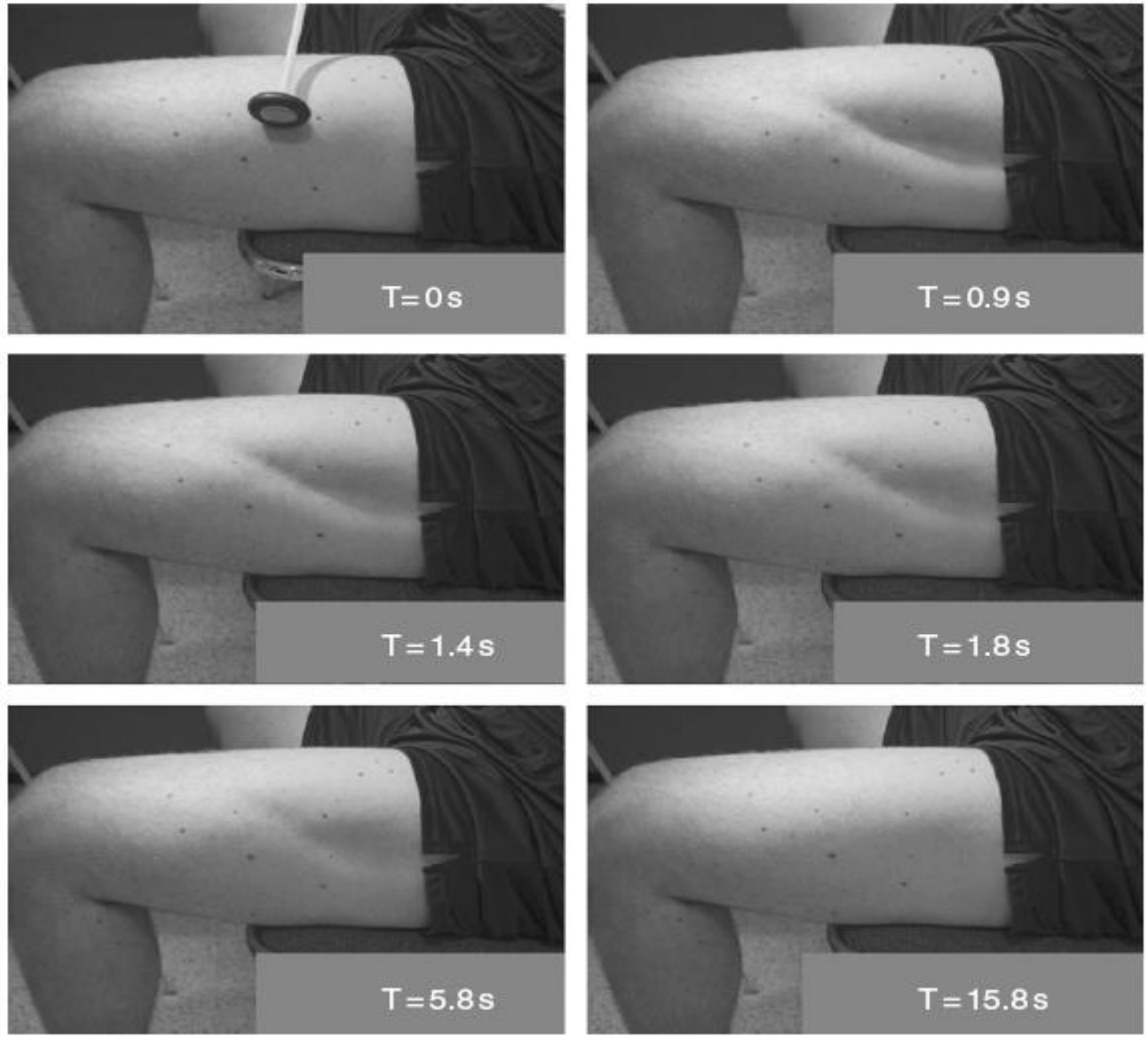


+/+ -/-



E15.5 E15.5

Becker myotonia



Advances in Genetics, Vol. 63

myotonia - 肌強直

There are two forms of the disorder: Becker-type (autosomal recessive), which is the most common form; and Thomsen's disease (autosomal dominant), which is a rare and milder form.

The disorder is cause[redacted]g off electrical excitation in

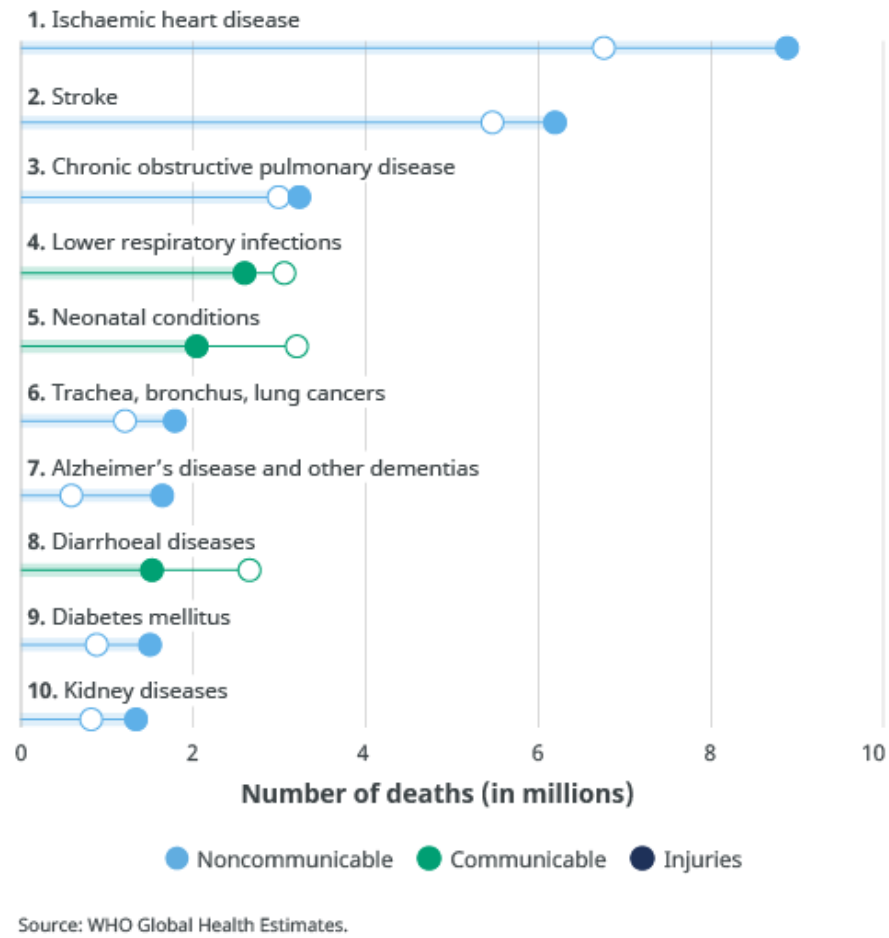
- 細胞，細胞膜，細胞膜蛋白質
- 研究離子通道的方法
- 離子通道的種類、功能和相關疾病種類
- 疼痛的研究

什麼是疼痛？

- 根據國際疼痛研究協會（IASP），疼痛的定義為：**「一種感覺及情緒上的不適經驗，可能與真實或潛在的組織損傷相關，或與其相似之不愉快感受與情感體驗」**

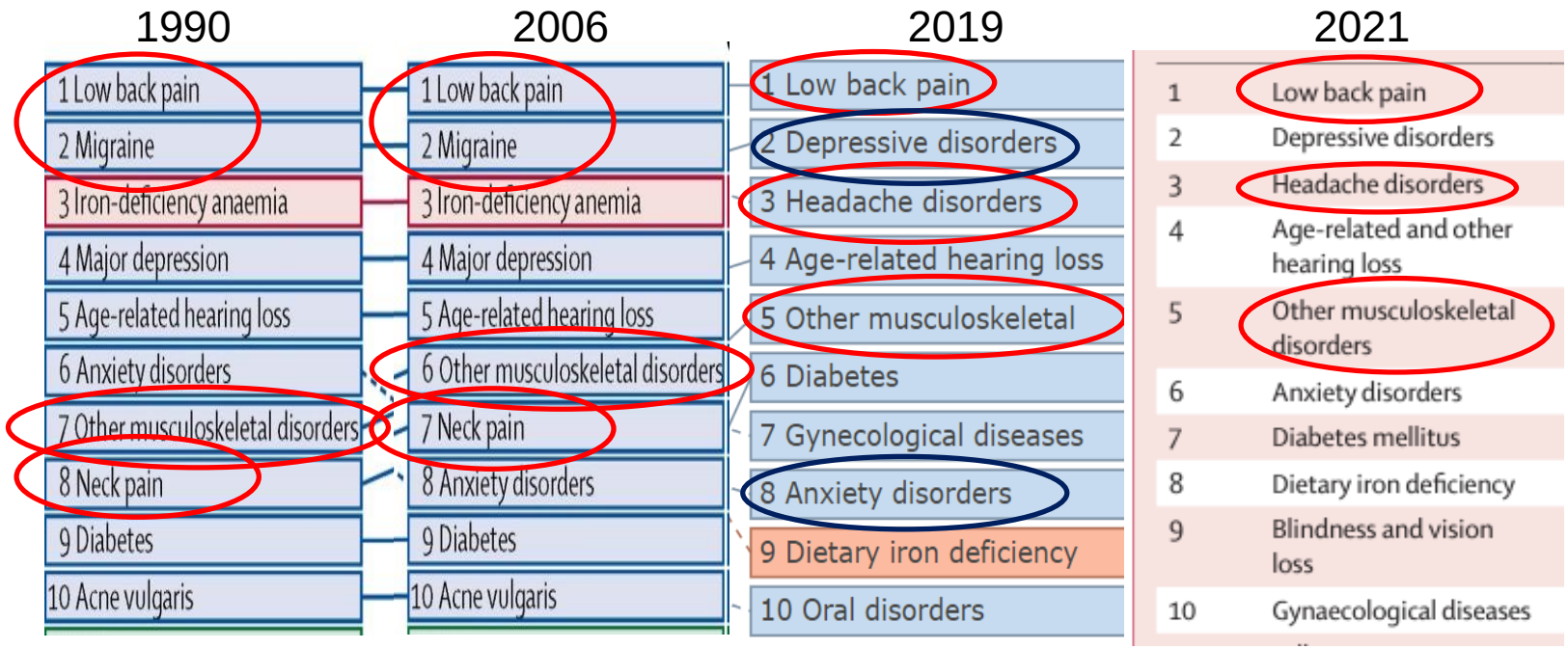
疼痛是主觀的，你覺得痛，就是痛


111年-國人十大死因
死亡人數/死亡人數年增率
癌症 51927 0.5%
心臟疾病 高血壓性疾病除外 23688 8.3%
COVID-19 14667 1556.9%
肺炎 14320 5.7%
腦血管疾病 12416 1.9%
糖尿病 12289 7.3%
高血壓性疾病 8720 10.6%
事故傷害 6953 2.4%
慢性下呼吸道疾病 6494 4.1%
腎炎、腎病症候群及腎病變 5813 6.3%
0 10000 20000 30000 40000 50000 6000


1. Ischaemic heart disease
2. Stroke
3. Chronic obstructive pulmonary disease
4. Lower respiratory infections
5. Neonatal conditions
6. Trachea, bronchus, lung cancers
7. Alzheimer's disease and other dementias
8. Diarrhoeal diseases
9. Diabetes mellitus
10. Kidney diseases
0 2 4 6 8 10
Number of deaths (in millions)
Noncommunicable
Communicable
Injuries
Source: WHO Global Health Estimates.

WHO report

慢性疼痛是全球性的問題



2-4 of the top 10 leading causes of years lived with disability are chronic pain from the Global Burden of Disease Study 2024.

~**40-50%** of the elder in Taiwan suffer chronic pain.
J. Pain Symptom Manag. 2010; 40(4): 575-581. J Occup Health. 2004, 46(1): 26-36.

慢性疼痛

- 慢性疼痛是指持續或反覆出現超過三個月的疼痛。它可能起源於最初的損傷，例如背部扭傷，或是由疾病引起。有時，疼痛的原因可能並不明顯。
- 全球約有 25% 的人口有慢性疼痛。
- 慢性疼痛還會影響情緒、睡眠、心理健康和生活質量。在臨床上，大約 40% 的慢性疼痛患者患有焦慮症，另外高達 50% 的患者合併有抑鬱症。

如果沒有痛覺，生活會很美好！？

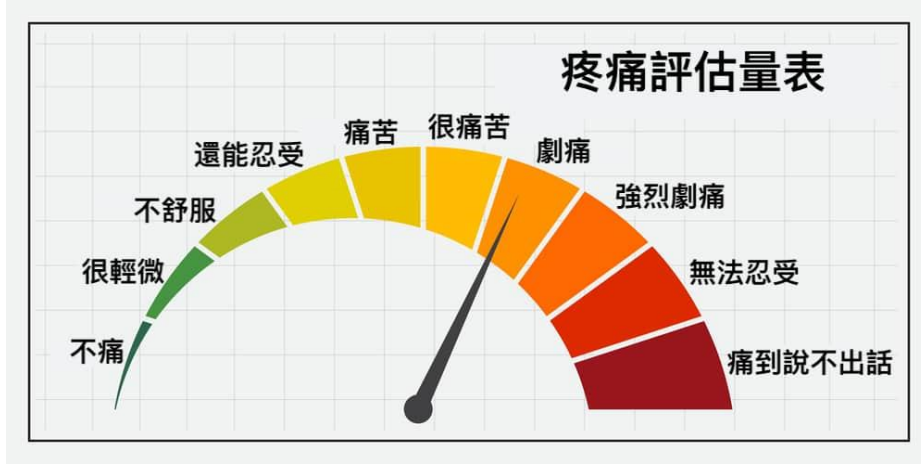
Congenital Insensitivity To Pain/先天性痛覺不敏感症

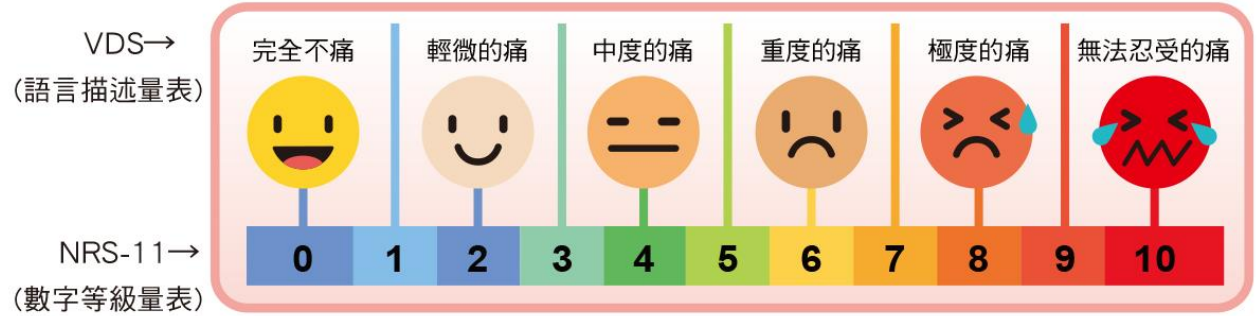
patients with a complete absence of pain

the absence of nocifensive behavior leads to an accumulation of painless injuries, bites, bruises, bone fractures, and a reduction of life expectancy.

14 CIP-inducing mutations (**Nav1.7, SCN9A**) identified so far introduce a stop codon which leads to premature protein truncation

如何測量疼痛？


疼痛評估量表
不痛
很輕微
不舒服
還能忍受
痛苦
很痛苦
劇痛
強烈劇痛
無法忍受
痛到說不出話

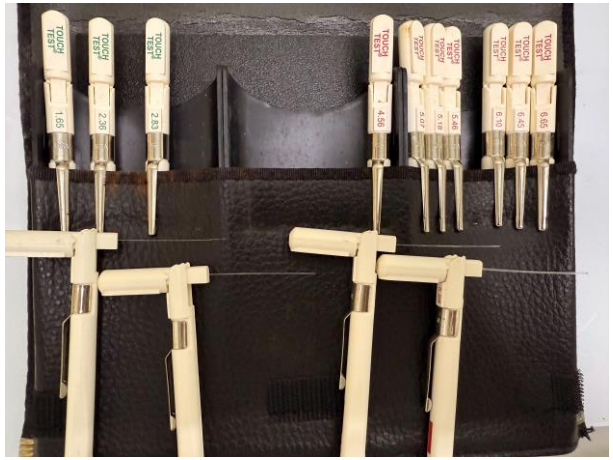

VDS→
(語言描述量表)
NRS-11→
(數字等級量表)
完全不痛
輕微的痛
中度的痛
重度的痛
極度的痛
無法忍受的痛
0
1
2
3
4
5
6
7
8
9
10

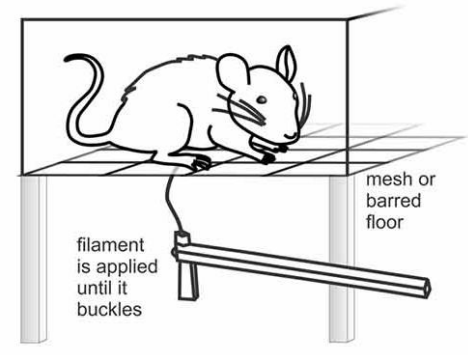
如何研究疼痛？

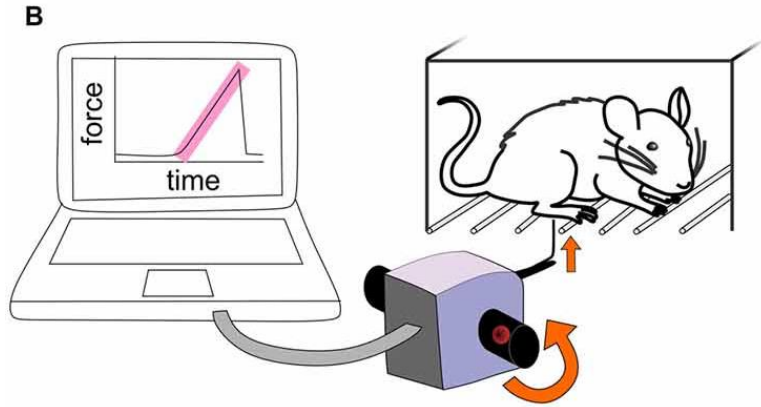
https://www.fairy-tales-inc.com/doc-mouse-patient-m-055-by-wee-forest-folk

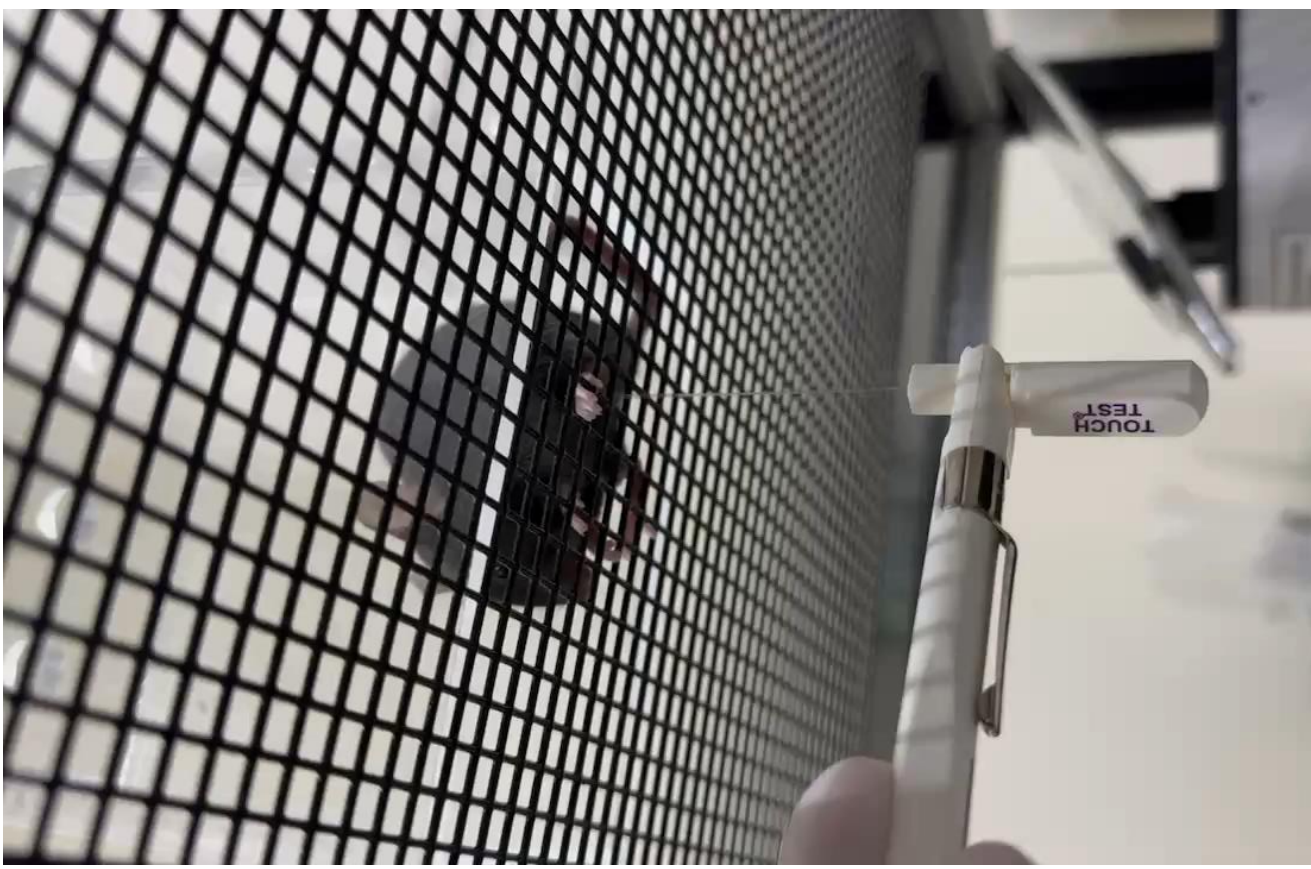
實驗小鼠行為測試

- 機械性 觸感的測量 **mechanical sensitivity**

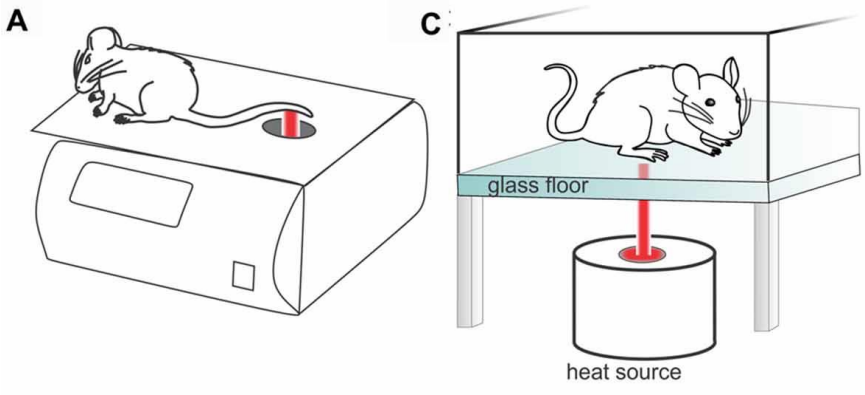


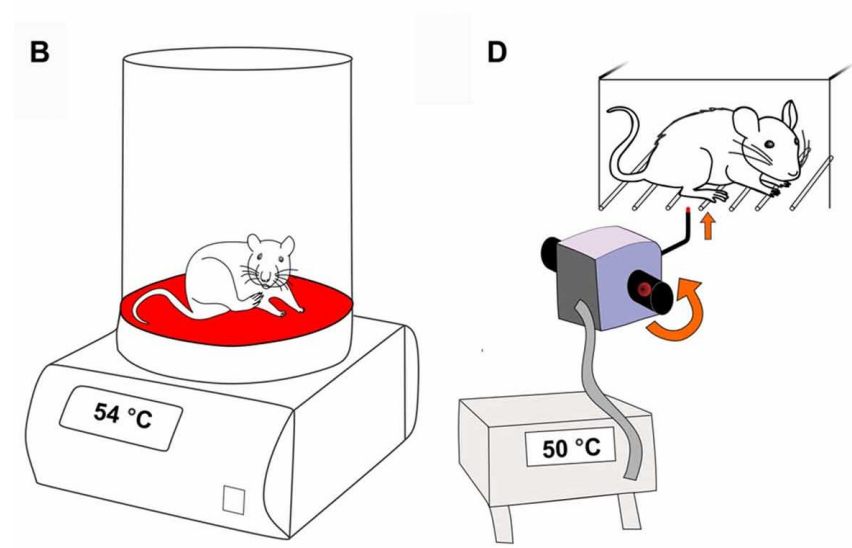




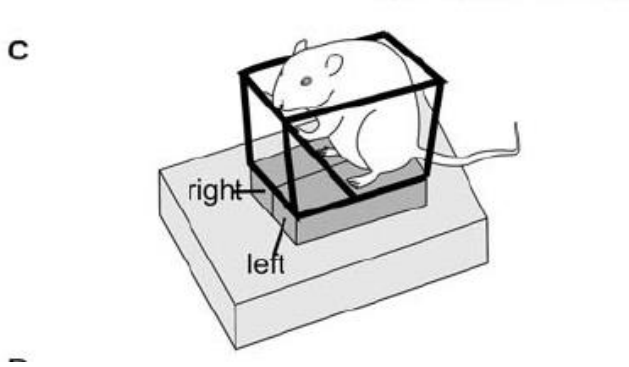

TOUCH
TEST

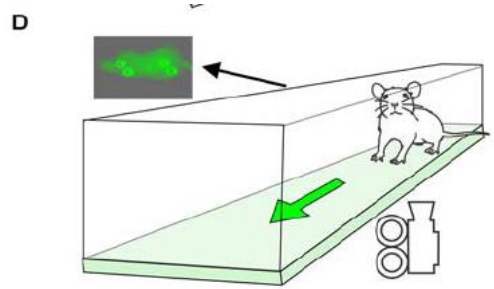
溫度感 **thermal sensitivity**

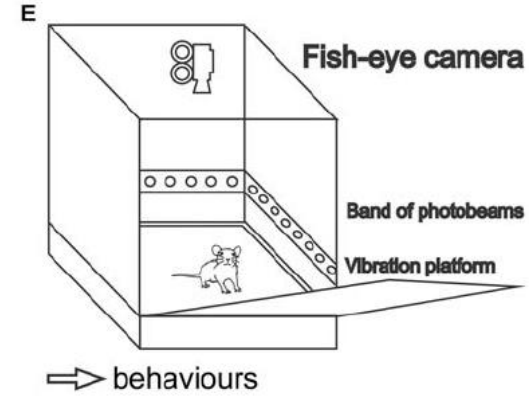




是否有較客觀的方法?

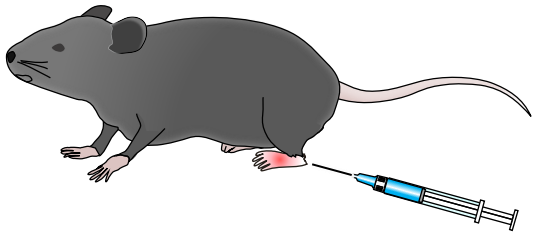






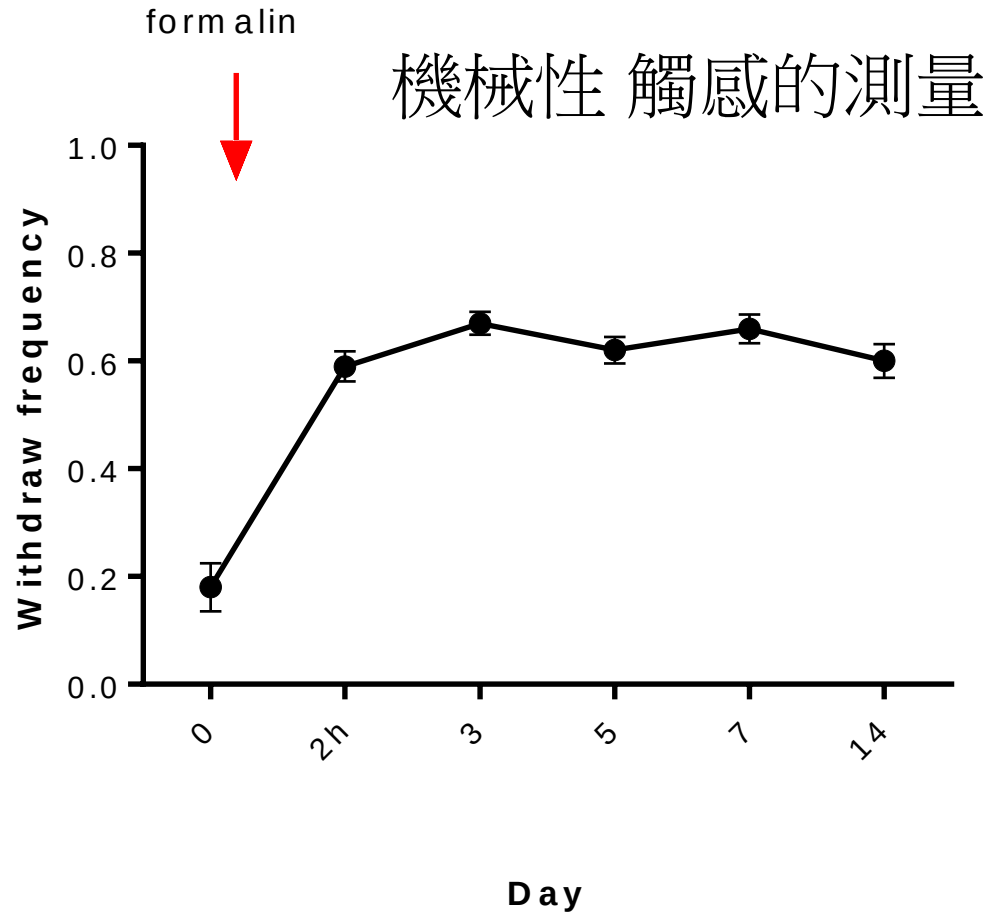
AI 能幫忙判斷實驗小鼠是否疼痛嗎？

福馬林造成的發炎疼痛



福馬林

機械性 觸感的測量

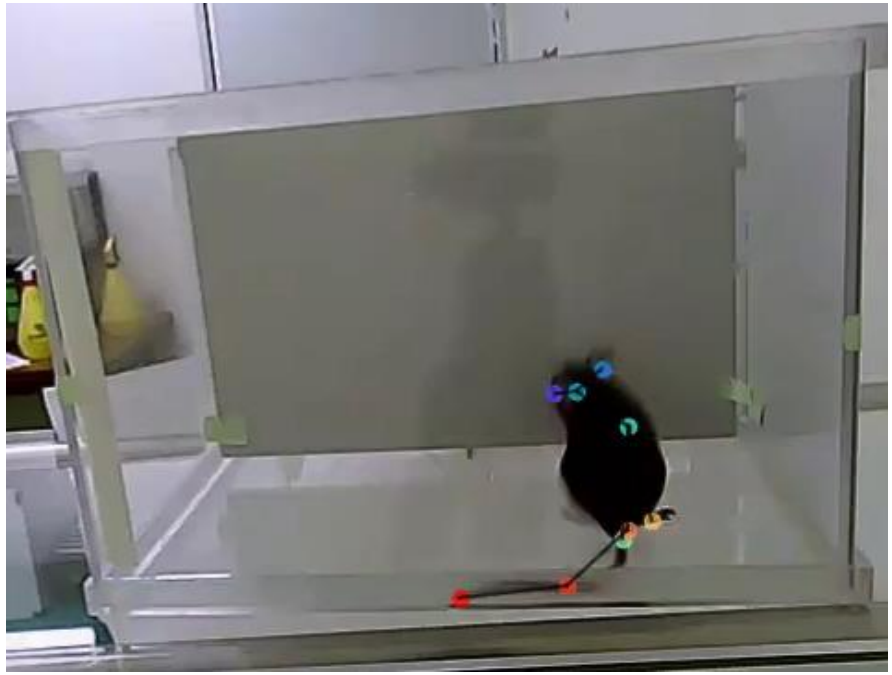


正常老鼠

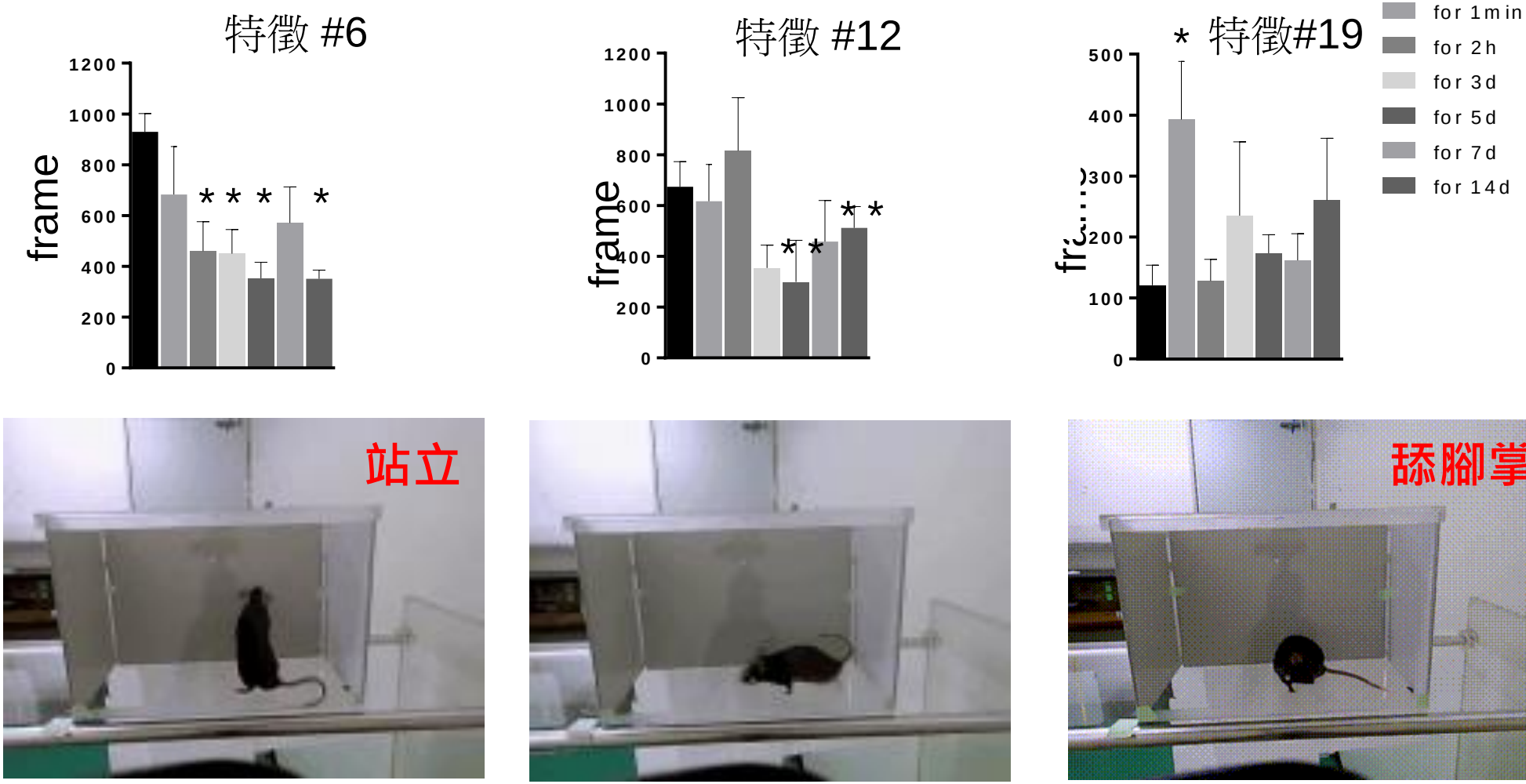
急性疼痛

慢性疼痛

標記小鼠身體12處部位 (DeepLabCut)

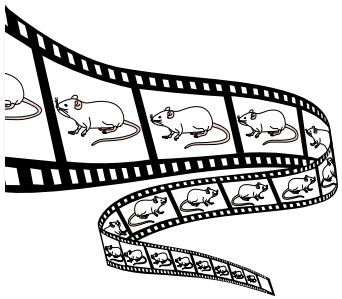


以機器學習來為小鼠行為分類：梳理毛髮、不動、舔腳掌、站立、行走

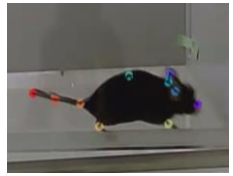


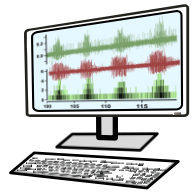
Collaborated with Drs. Mark Liao and Chien Yao Wang at Institute of Information Science

透過影片分析來分辨不同的小鼠疼痛模型

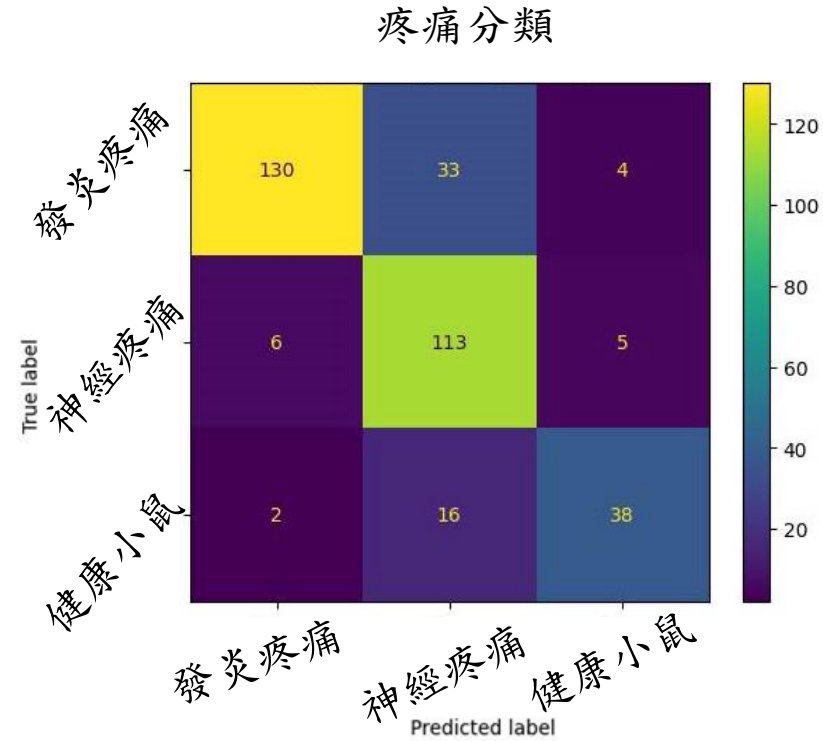


老鼠影片videos





特徵擷取



77%　91%　67%

平均準確率80%

陳建璋
中央研究院生物醫學科學研究所
N713
ccchen@ibms.sinica.edu.tw
2652-3522