# Biology for MX

MSE – 212

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Programmable Biomaterials Laboratory

**Course 8: Endocytosis**

# Course Content

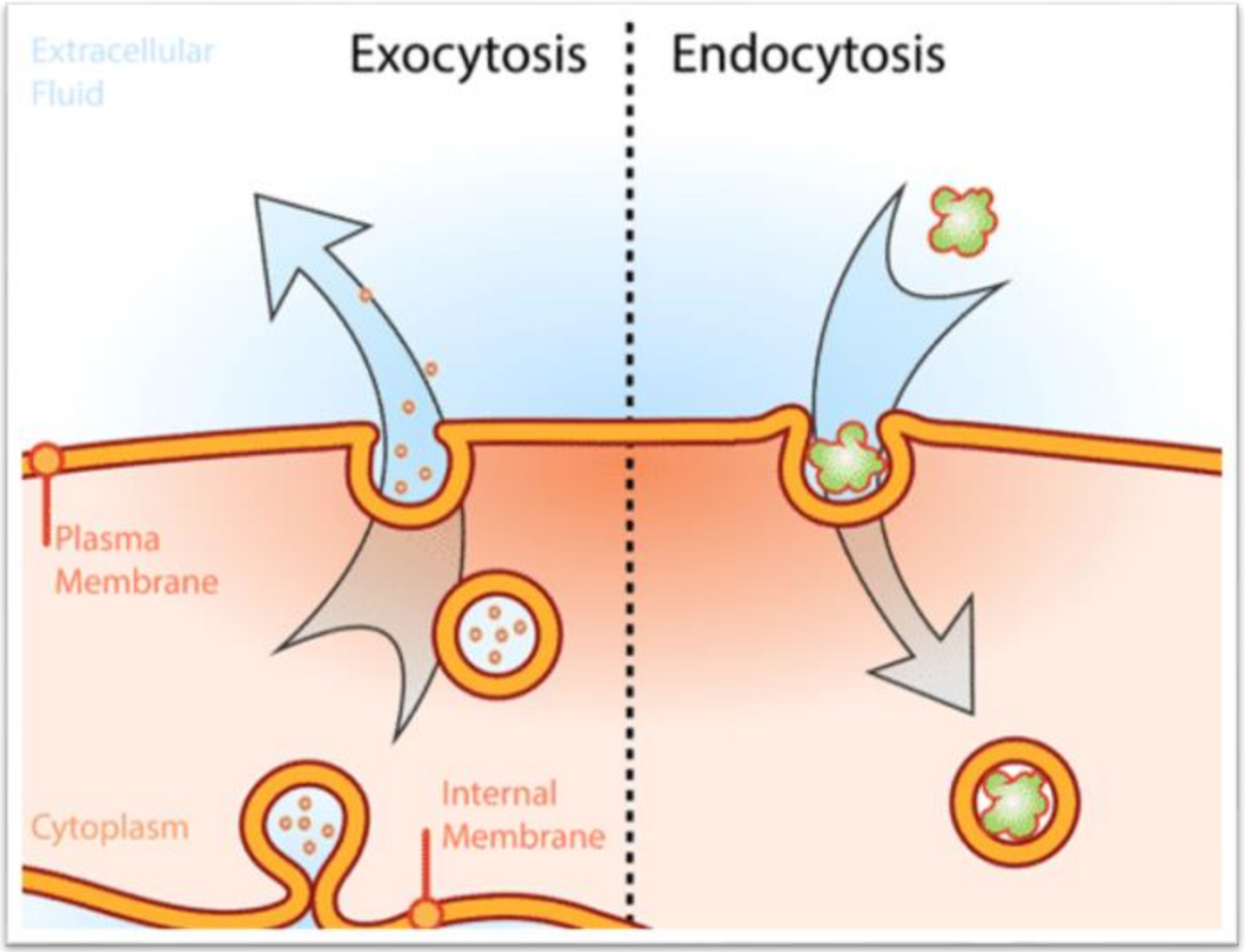
| | | |
|---|---|---|
| *Intro Ex.* | *Defining our starting point (online questionnaire & video)* | *(February 19)* |
| **BLOCK 1: Introduction and engineering with cellular components** | | |
| Lecture 1. | Intro to biology and cells | (February 26) |
| Lecture 2. | Proteins and protein based materials | (March 5) |
| Lecture 3. | DNA and DNA-based materials | (March 12) |
| *Exercise 1.* | *Proteins, peptides and DNA* | *(March 19)* |
| **BLOCK 2: Inter- and intracellular action** | | |
| Lecture 4. | ECM, adhesion and artificial matrices | (March 26) |
| Lecture 5. | Virus, antibodies and immune engineering | (April 2) |
| Lecture 6. | Bacteria | (April 9) |
| *Exercise 2.* | *Nanoparticles and Scaffolds* | *(April 16)* |
| **BLOCK 3: Physics of biological processes** | | |
| **Lecture 7.** | **Receptors and targeting** | **(April 30)** |
| Lecture 8. | Endocytosis | (May 7) |
| *Exercise 3.* | *Engineering functionality* | *(May 14)* |
| Lecture 9. | Signaling and communication | (May 21) |
| Lecture 10. | Revision and conclusion | (May 28) |

# Endocytosis

Endocytosis is a cellular process in which substances are **brought into the cell**.

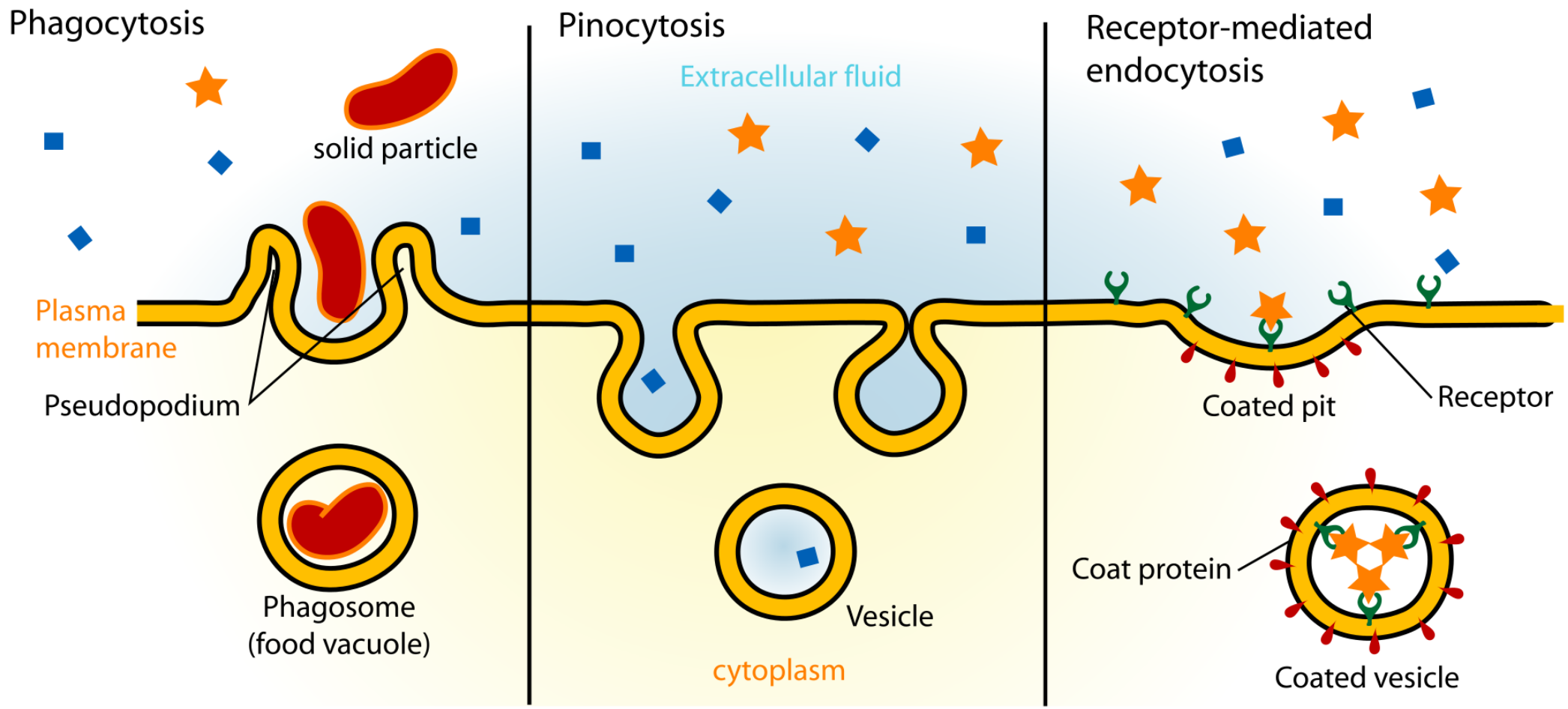
The material to be internalized is surrounded by an area of cell membrane, which then buds off inside the cell to form a vesicle containing the ingested material.

The opposite is called **Exocytosis**. In this process, an internal vesicle fuses with the outer cell membrane to release its content.



# Specialized Pathways

- Receptor-mediated endocytosis (clathrin-mediated endocytosis),
- Caveolae (not pictured),
- Pinocytosis (drinking),
- Phagocytosis (eating).



# Eating: Phagocytosis

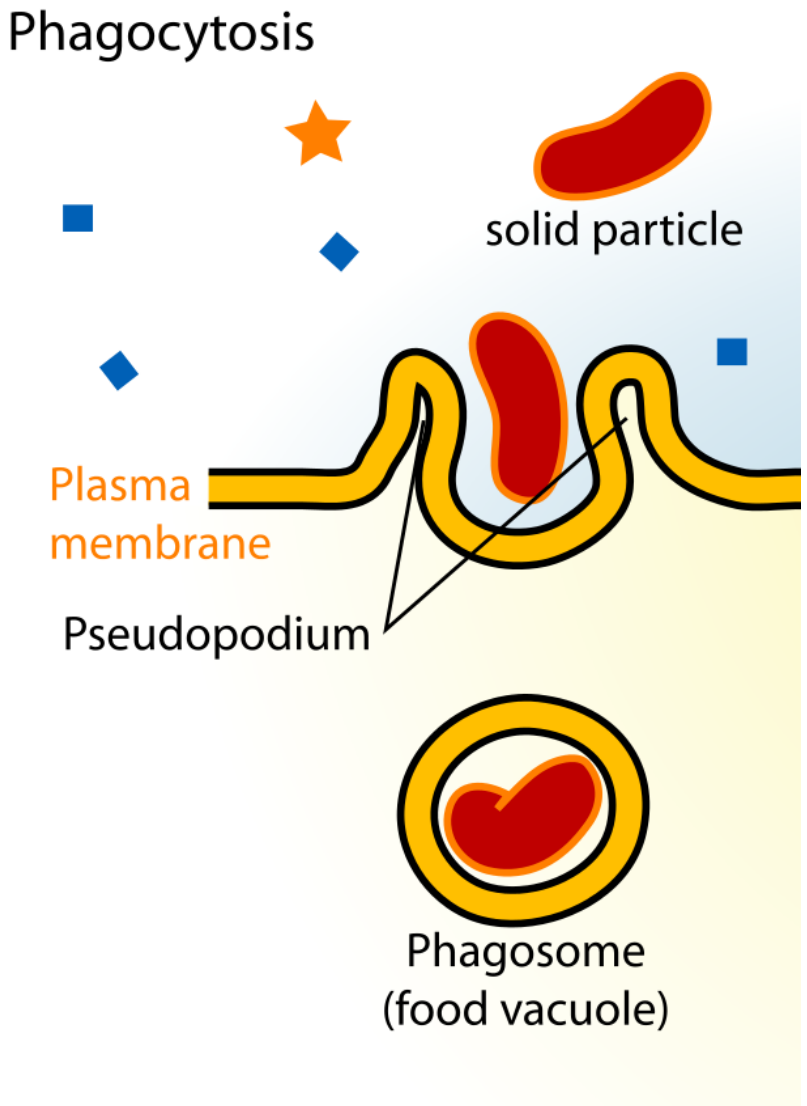
During phagocytosis, cells **engulf** large particles such as bacteria, cell debris, or even intact cells.

Binding of the particle to receptors on the surface of the phagocytic cell triggers the extension of **pseudopodia**—an actin-based movement of the cell surface.

The pseudopodia eventually surround the particle and their membranes **fuse** to form a **large intracellular vesicle** (>0.25 µm in diameter) called a **phagosome**.

The phagosomes then fuse with **lysosomes**, producing phagolysosomes in which the ingested material is **digested** by the action of lysosomal acid hydrolases.

During **maturation** of the phagolysosome, some of the internalized membrane proteins are **recycled** to the plasma membrane.




**The Cell: A Molecular Approach. 2nd edition**

# Eating: Phagocytosis

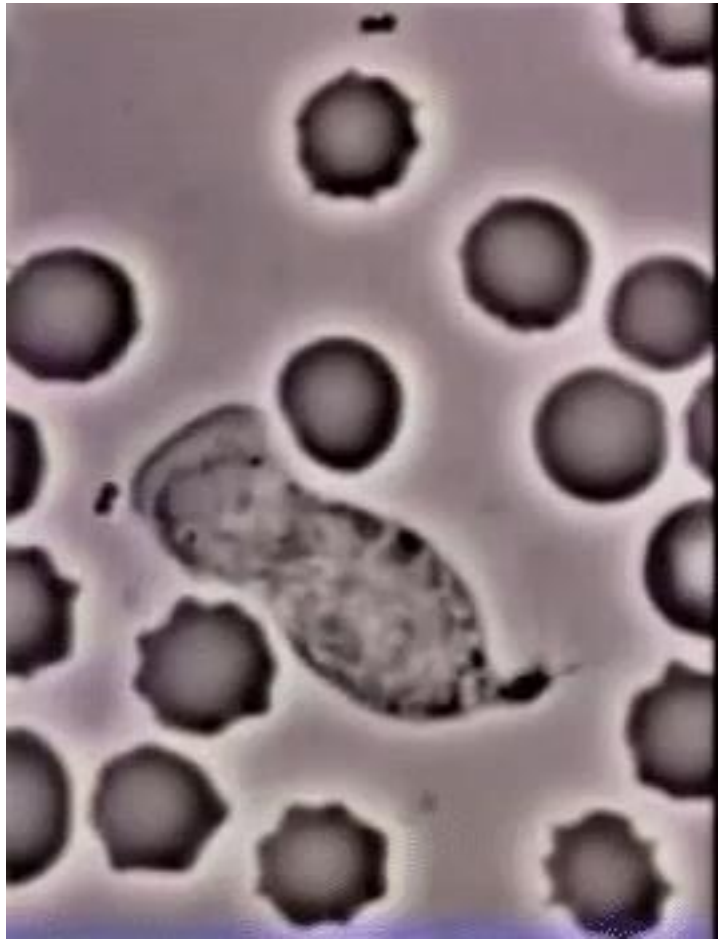
The ingestion of large particles by phagocytosis plays distinct roles in different kinds of cells. Many amoebas use phagocytosis to **capture food particles**, such as bacteria or other protozoans.

In multicellular animals, the major roles of phagocytosis are to provide a **defense against invading microorganisms** and to **eliminate aged or damaged cells from the body**.

In mammals, phagocytosis is the function of primarily two types of white blood cells, **macrophages and neutrophils**, which are frequently referred to as "**professional phagocytes**."

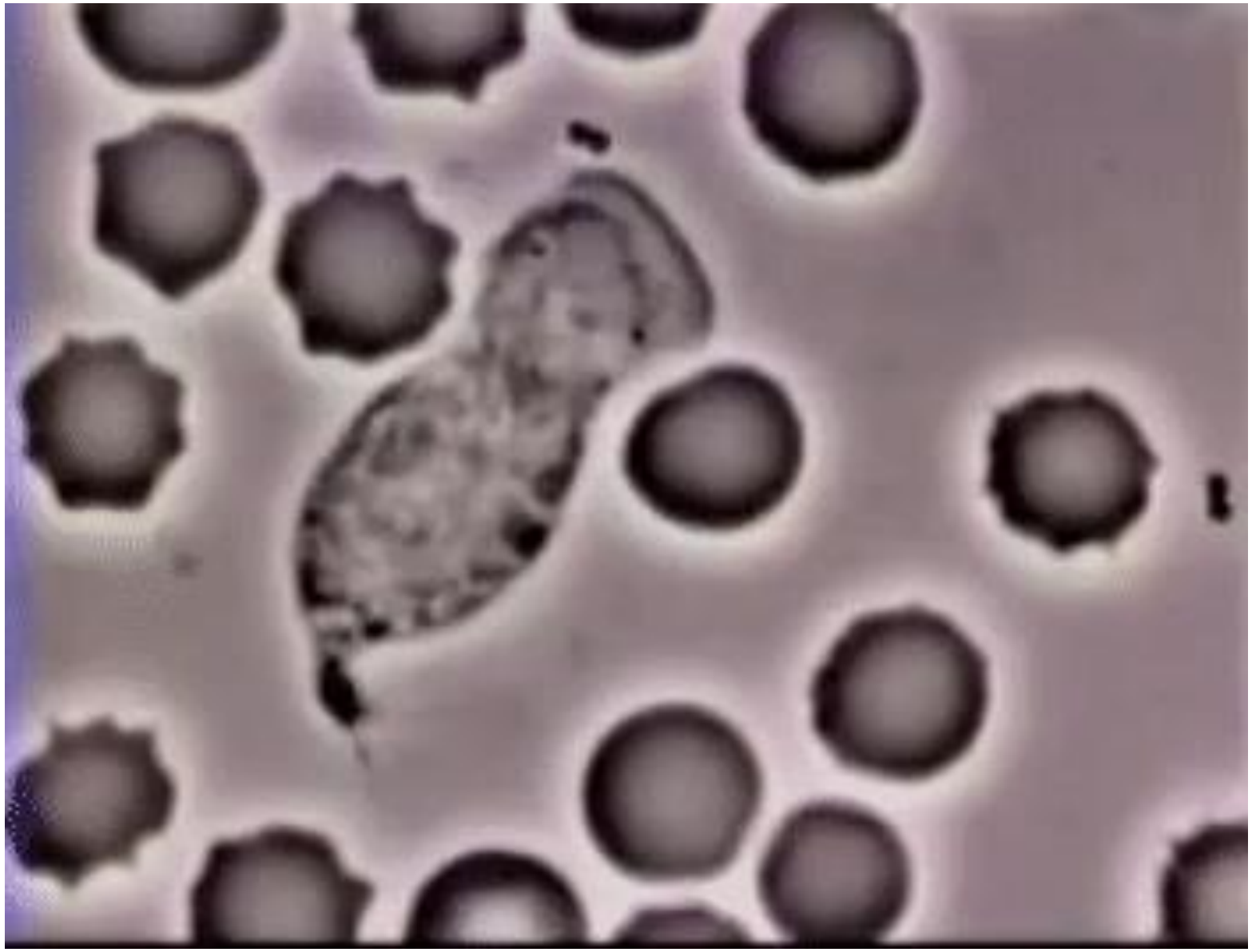
Both macrophages and neutrophils play critical roles in the body's **defense systems** by **eliminating microorganisms from infected tissues**. In addition, macrophages **eliminate aged or dead cells** from tissues throughout the body.

A striking example of the scope of this activity is provided by the macrophages of the **human spleen and liver**, which are responsible for the disposal of more than $10^{11}$ aged blood cells on a daily basis.



**The Cell: A Molecular Approach. 2nd edition**

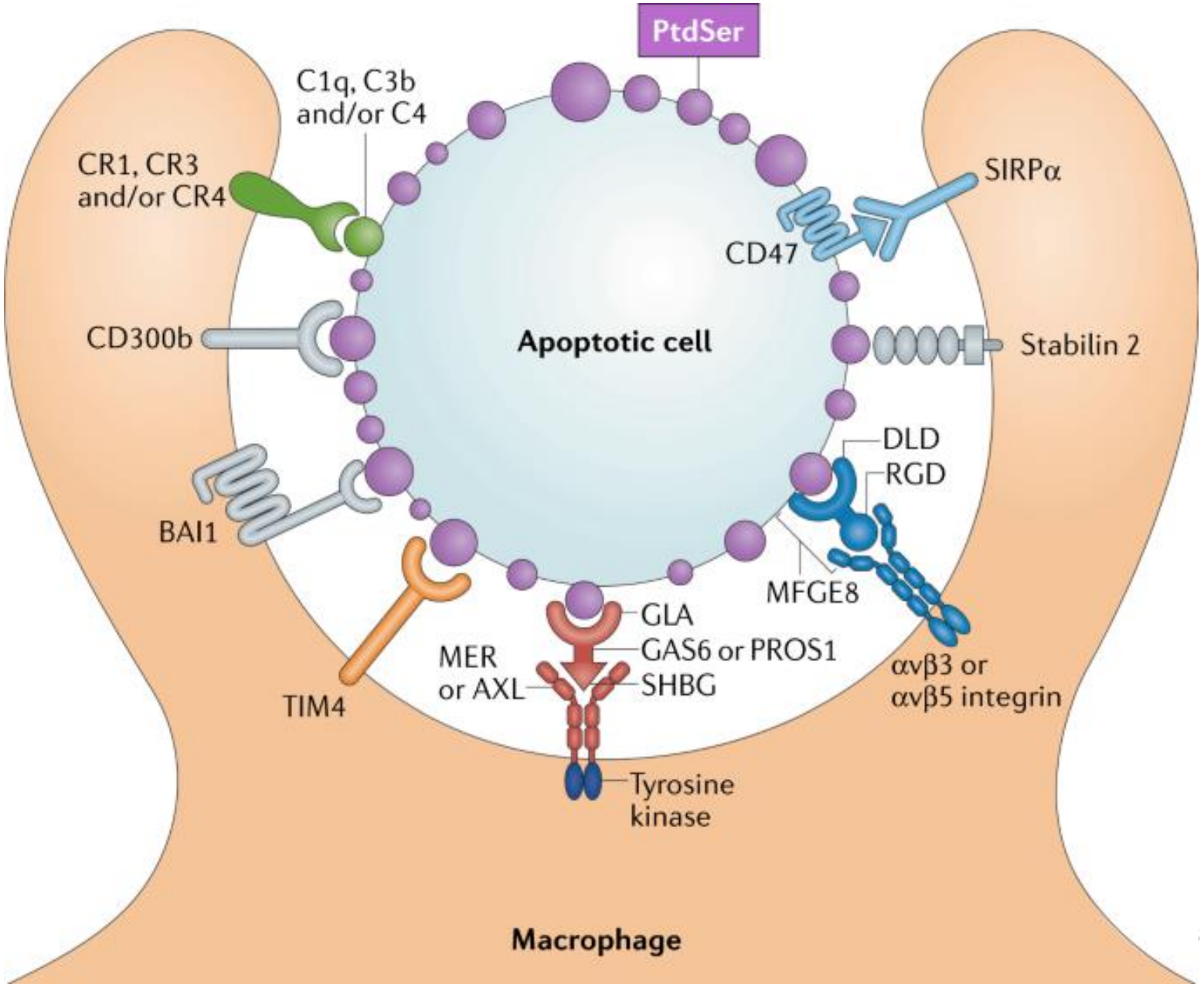
# Eating: Phagocytosis



**The Cell: A Molecular Approach. 2nd edition**

# Eating: Phagocytosis

Apoptotic cell surfaces are marked by a profusion of membrane blebs that universally **display externalized phosphatidylserine (PtdSer; purple)**. This is the most potent so-called eat-me signal for apoptotic cell phagocytosis — is recognized by macrophage receptors that directly bind this phospholipid.

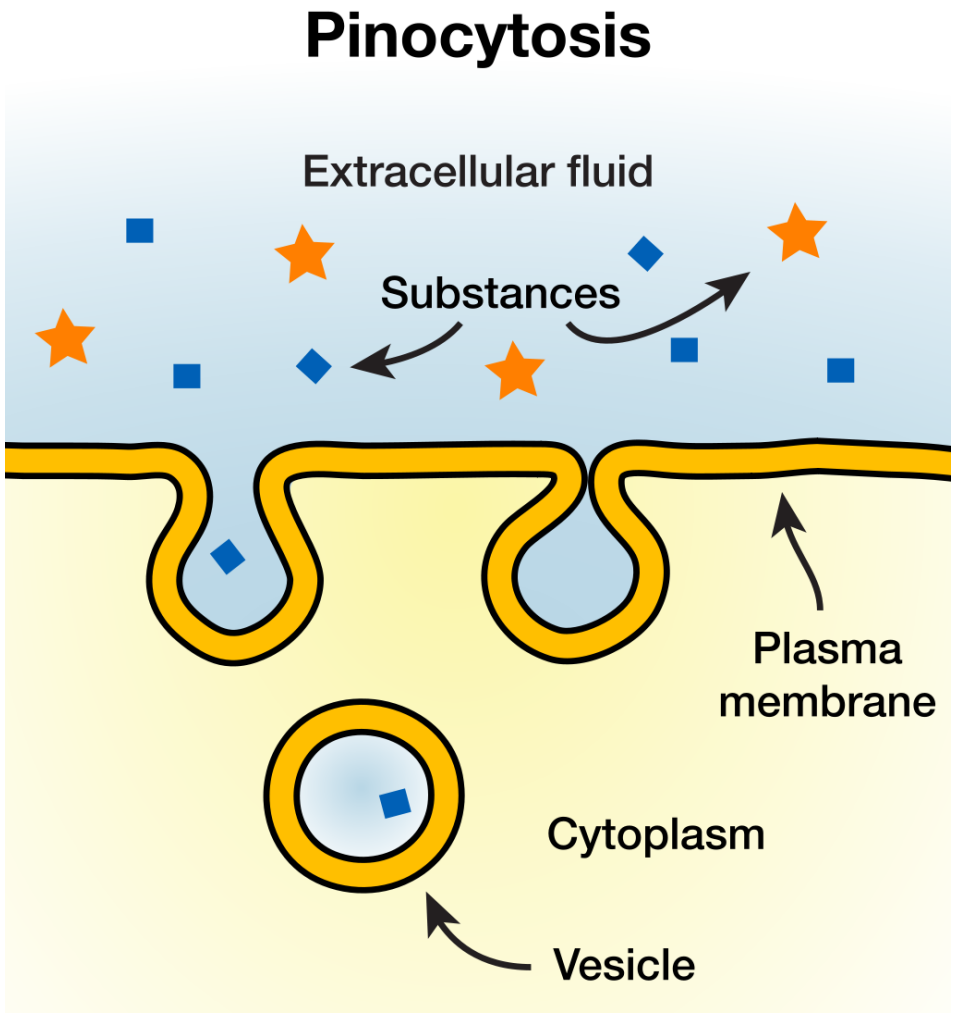



Nature reviews immunology
https://doi.org/10.1038/ s41577-019-0167-y

# Drinking: Pinocytosis

**Pinocytosis**, otherwise known as **fluid endocytosis** and **bulk-phase pinocytosis**, is a mode of endocytosis in which small particles suspended in extracellular fluid are brought into the cell through an invagination of the cell membrane, resulting in a suspension of the particles within a small vesicle inside the cell. These pinocytotic vesicles then typically fuse with early endosomes to hydrolyze (break down) the particles.

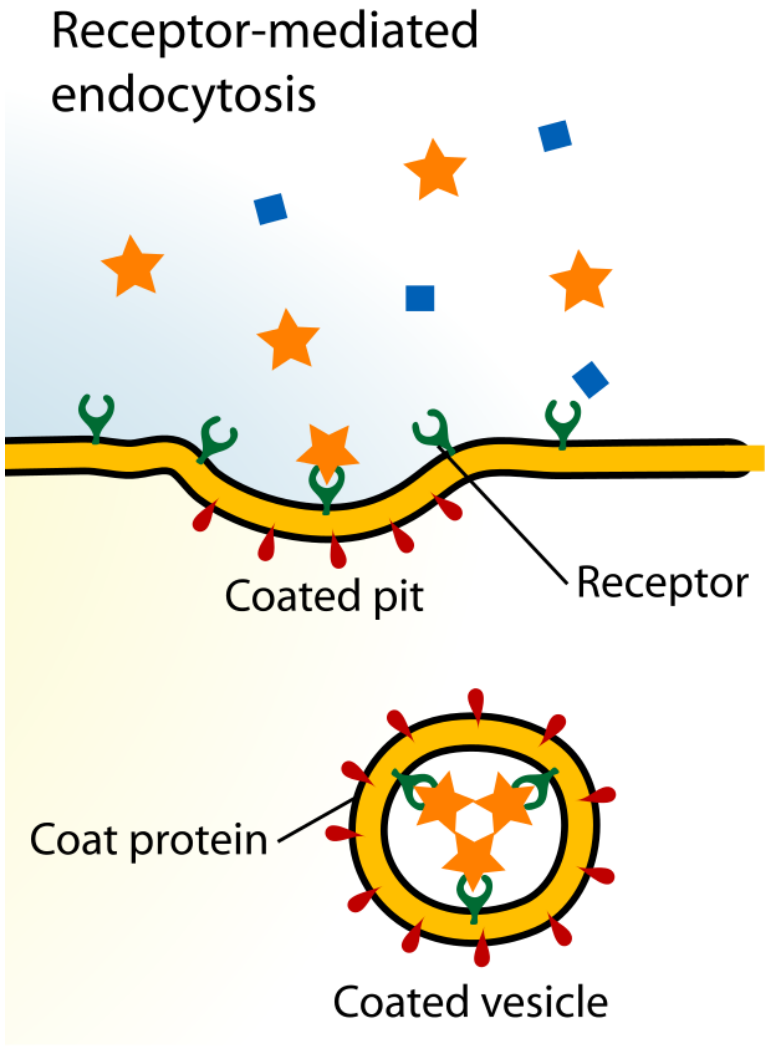
Pinocytosis is further segregated into the pathways macro-pinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, or clathrin- and caveolin-independent endocytosis, all of which differ by the mechanism of vesicle formation as well as the resulting size of these vesicles.



# Clathrin-mediated endocytosis

Receptor-mediated endocytosis (RME), also called clathrin-mediated endocytosis, is the main process by which cells absorb metabolites, hormones, proteins – and in some cases viruses – by the inward budding of the plasma membrane (**invagination**). This process forms vesicles containing the absorbed substances and is strictly mediated by receptors on the surface of the cell. **Only the receptor-specific substances can enter the cell through this process.**

Endocytosis generates **small (60–120 nm) membrane vesicles** that transport various cargo molecules from the plasma membrane of eukaryotic cells into the cytoplasm. **The cargo consists mainly of transmembrane proteins and their extracellular ligands**. These cargoes are involved in a broad range of physiological processes, including nutrient uptake, cell signalling, developmental regulation through morphogens, cell adhesion, etc.



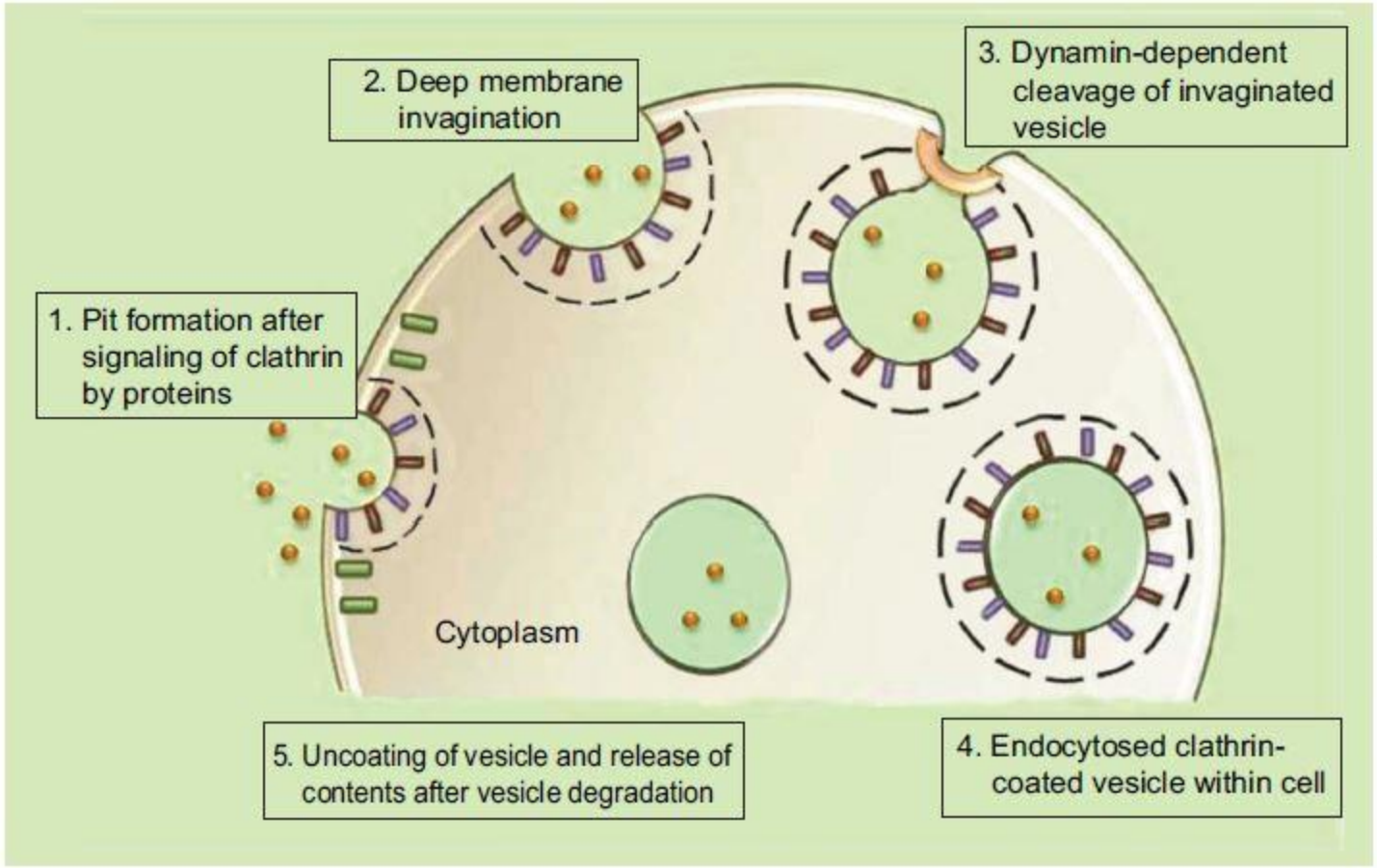

Grant, B. D. and Sato, M - http://www.wormbook.org/chapters/www_intracellulartrafficking/intracellulartrafficking.html

# Clathrin-mediated endocytosis

Upon binding of particles to extracellular receptors, clathrin monomeric protein modules driving the different steps of the endocytic process are **assembled sequentially from soluble cytosolic protein pools**.

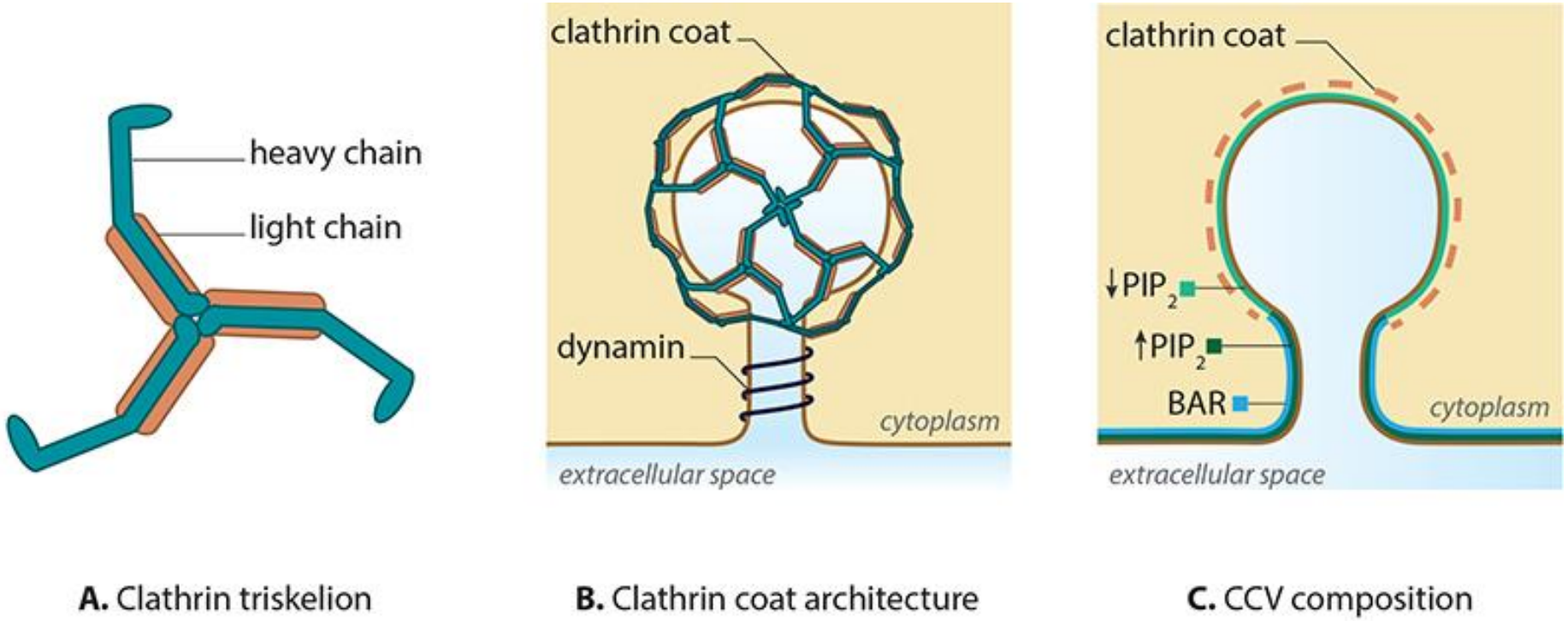
After initiation of the endocytic site, cargo is recruited to this site and the **membrane is shaped** into an invagination, which is finally separated from the plasma membrane by scission.

**Uncoating** releases the proteins of the endocytic machinery back to the cytosolic pool and releases a vesicle that can participate in intracellular membrane trafficking events




Nature Reviews Molecular Cell Biology 19, 313–326(2018)

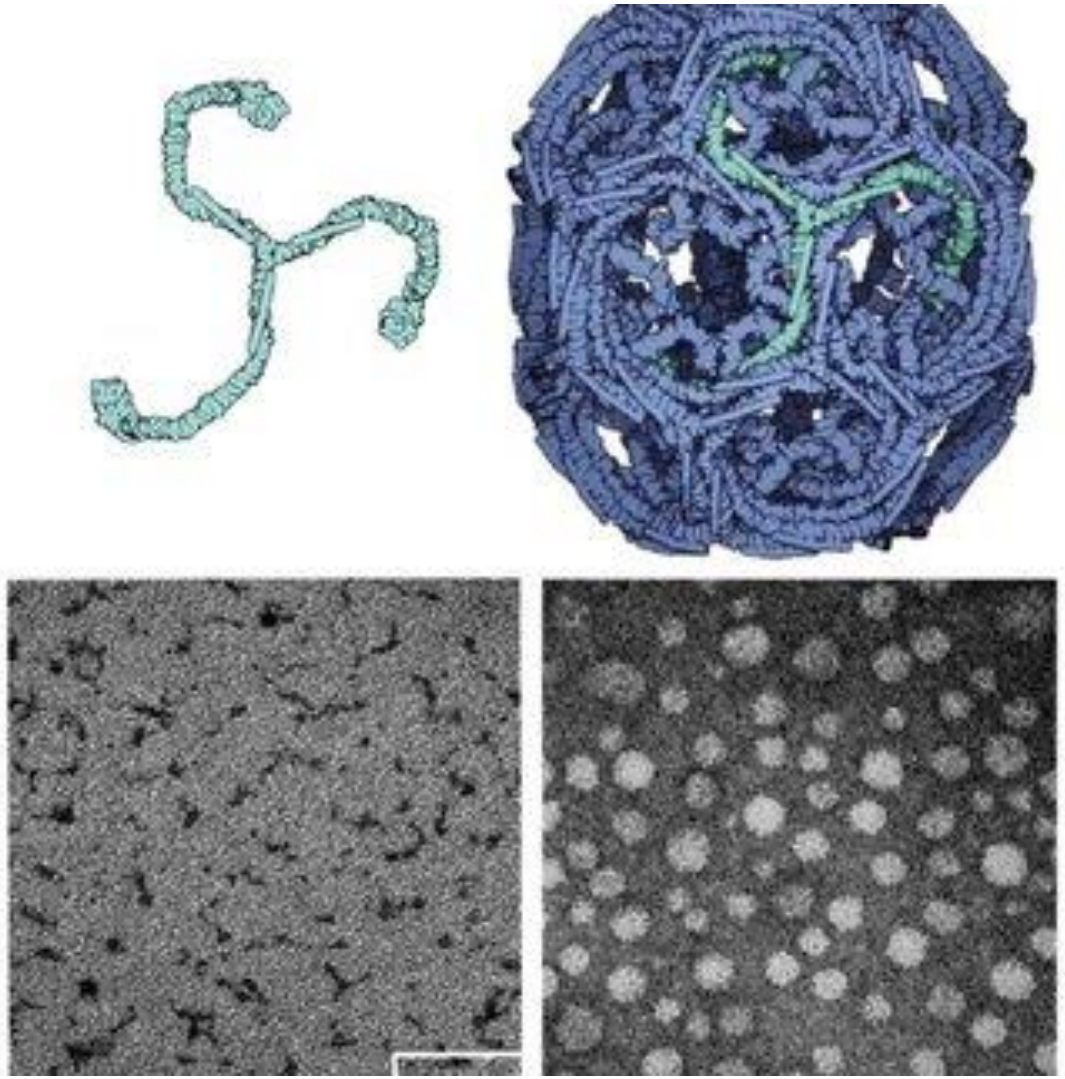
# Clathrin-mediated endocytosis

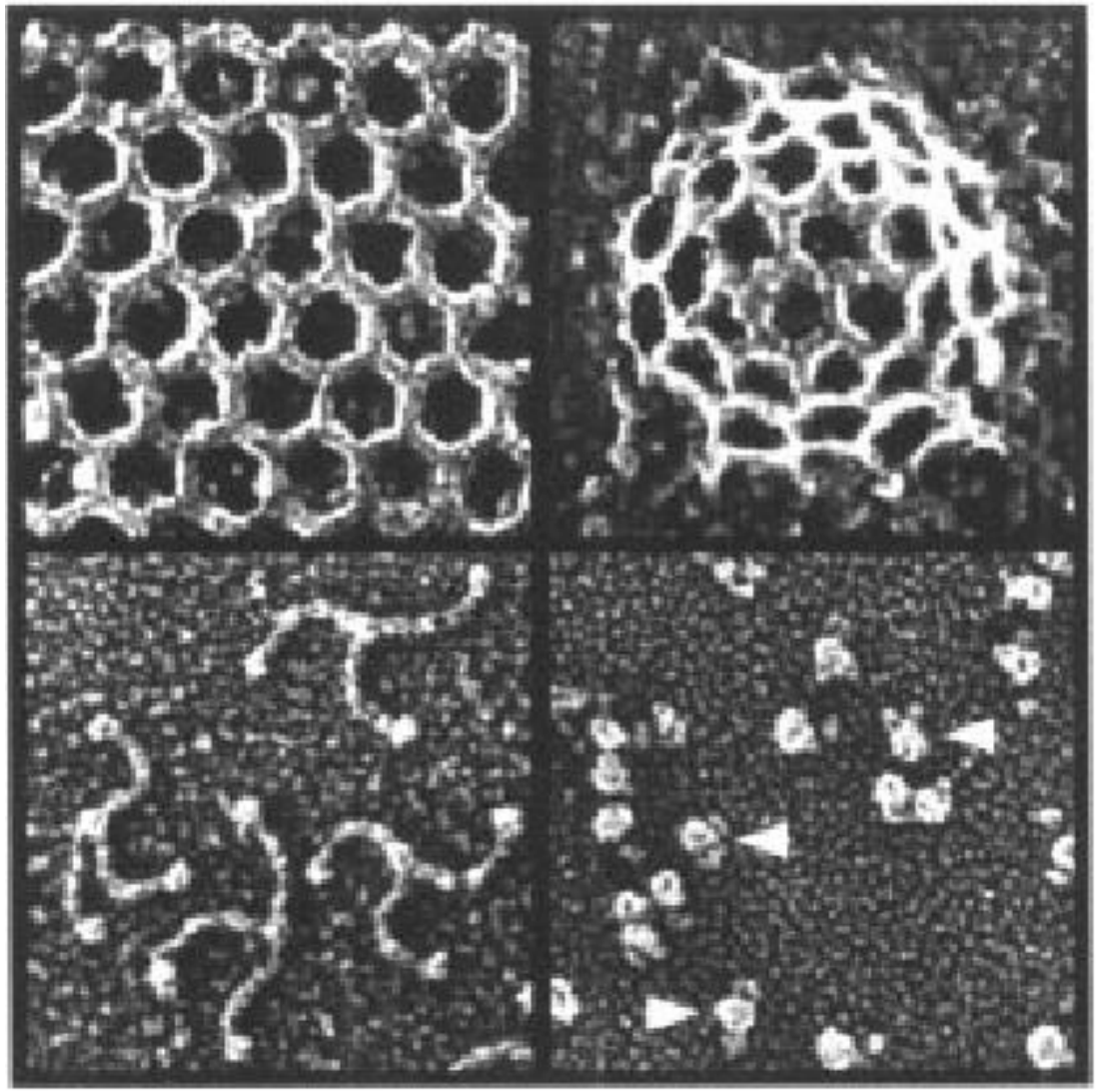



**A.** Clathrin triskelion **B.** Clathrin coat architecture **C.** CCV composition

A. Clathrin is a triskelion shaped scaffold protein. B. Clathrin assembles to form a coat around a mature vesicle. C. Formation of the clathrin coated vesicle requires changes in membrane curvature, which is driven by PIP2 levels and BAR protein binding.

# Clathrin-assembly





PLoS ONE 7(5):e35821
DOI:10.1371/journal.pone.0035821

# The Purpose of Endocytosis

**Taking in nutrients for cellular growth, function and repair**:
Cells need materials like proteins and lipids to function.

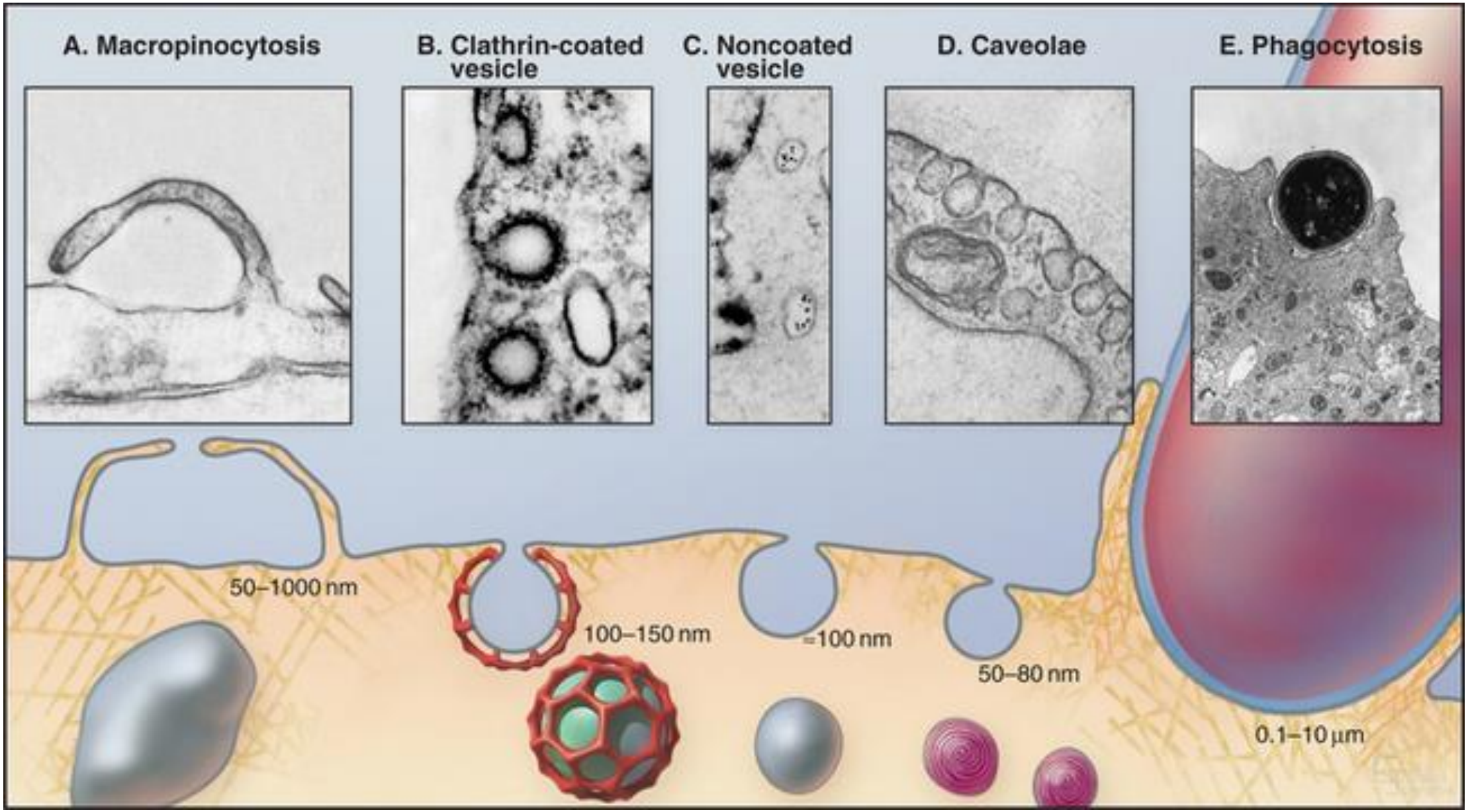
**Capturing pathogens or other unknown substances that may endanger the organism**:
When pathogens like bacteria are identified by the immune system, they are engulfed by immune cells to be destroyed.

**Disposing of old or damaged cells**: Cells must be safely disposed of when they stop functioning properly to prevent damage to other cells. These cells are eliminated through endocytosis.

# Video: Immune cell eating a bacterium

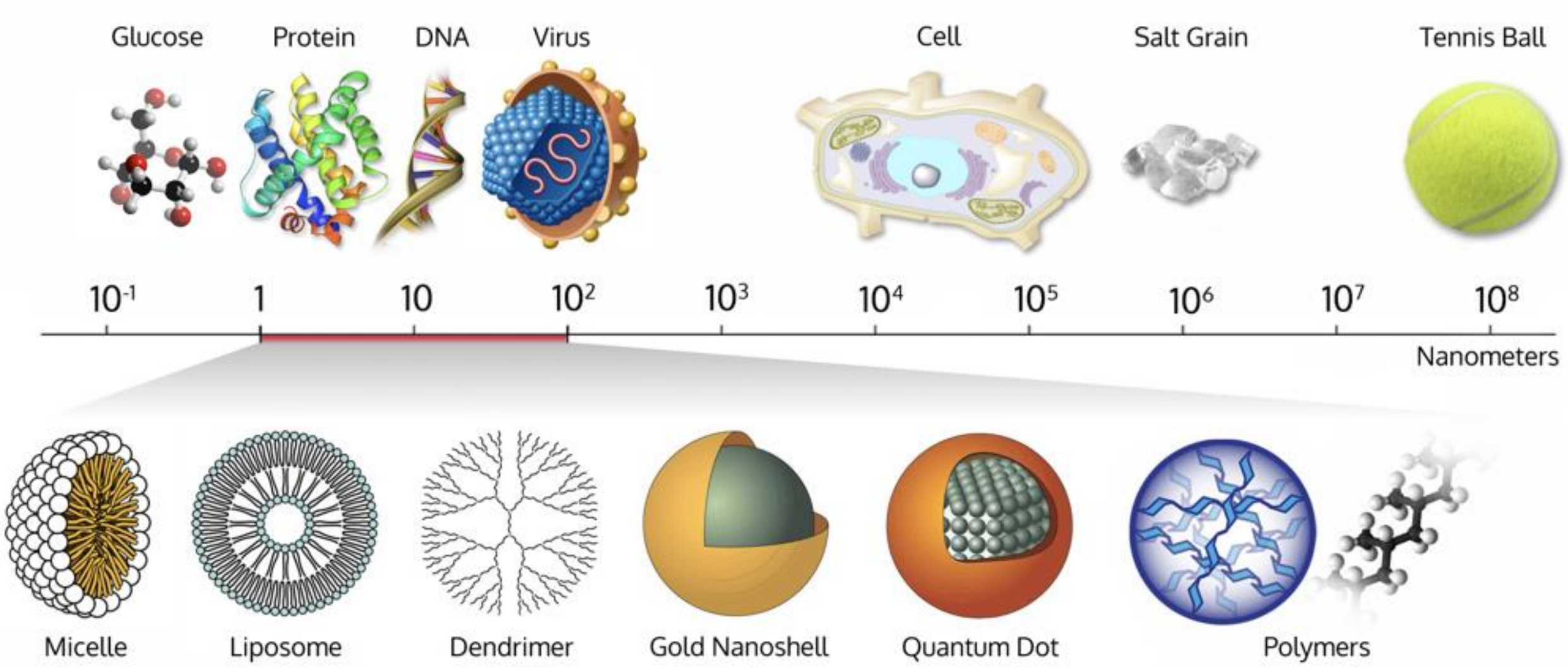

https://www.youtube.com/watch?v=iZYLeIJwe4w

# Size Dependence



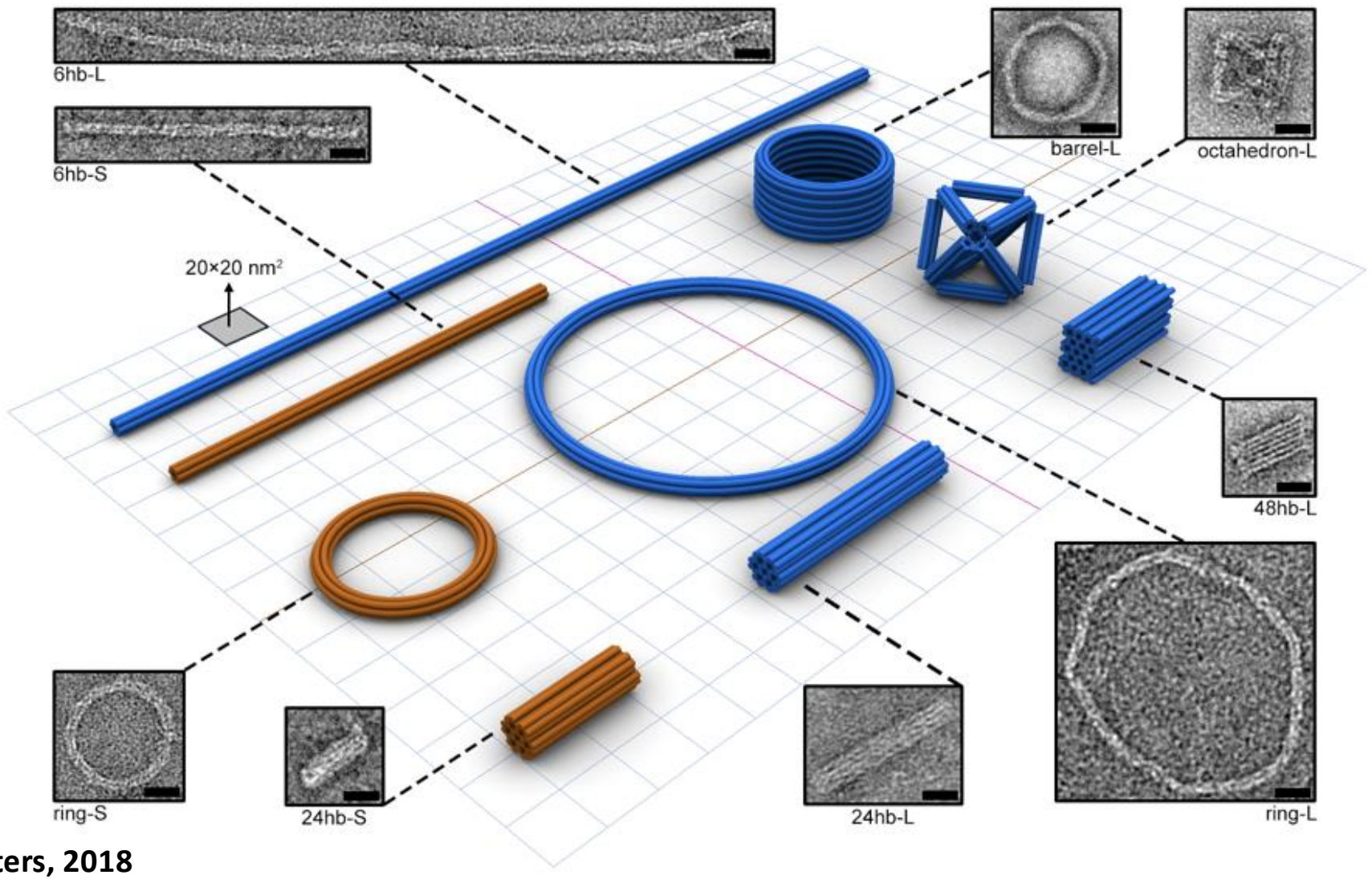

*(A and D, Courtesy of D. Fawcett, Harvard Medical School, Boston, Massachusetts. B, Courtesy of C-M Chang and S. Schmid, Scripps Research Institute, La Jolla, California. C, Courtesy of S. Hansen and B. van Deurs, University of Copenhagen, Denmark. E, Courtesy of Blair Bowers, National Institutes of Health, Bethesda, Maryland.)*

# Importance of size and shape



# Importance of Shape: DNA

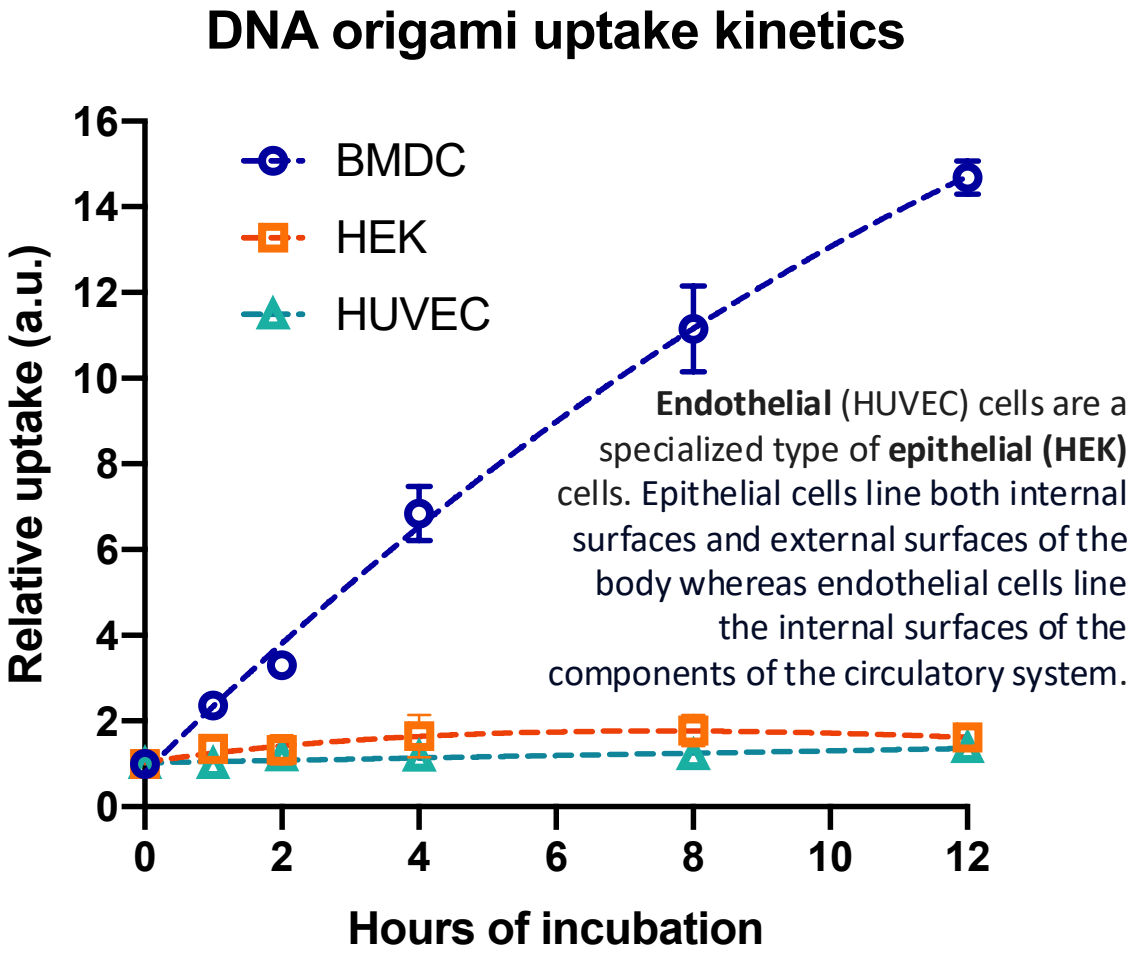
## Do cells react differently to DNA particles of varying geometries?



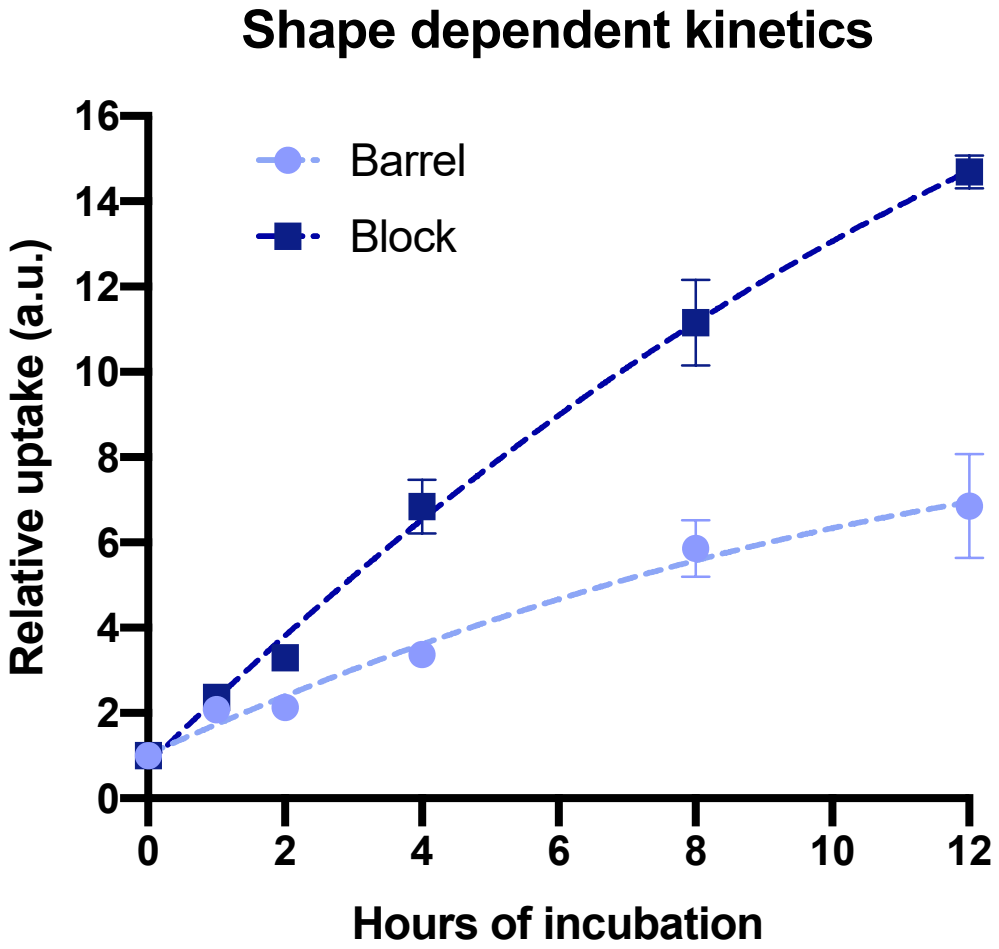

Bastings et al, NanoLetters, 2018

# Importance of Shape: Kinetics

## Immune cells take particles up much more, and this is shape dependent too



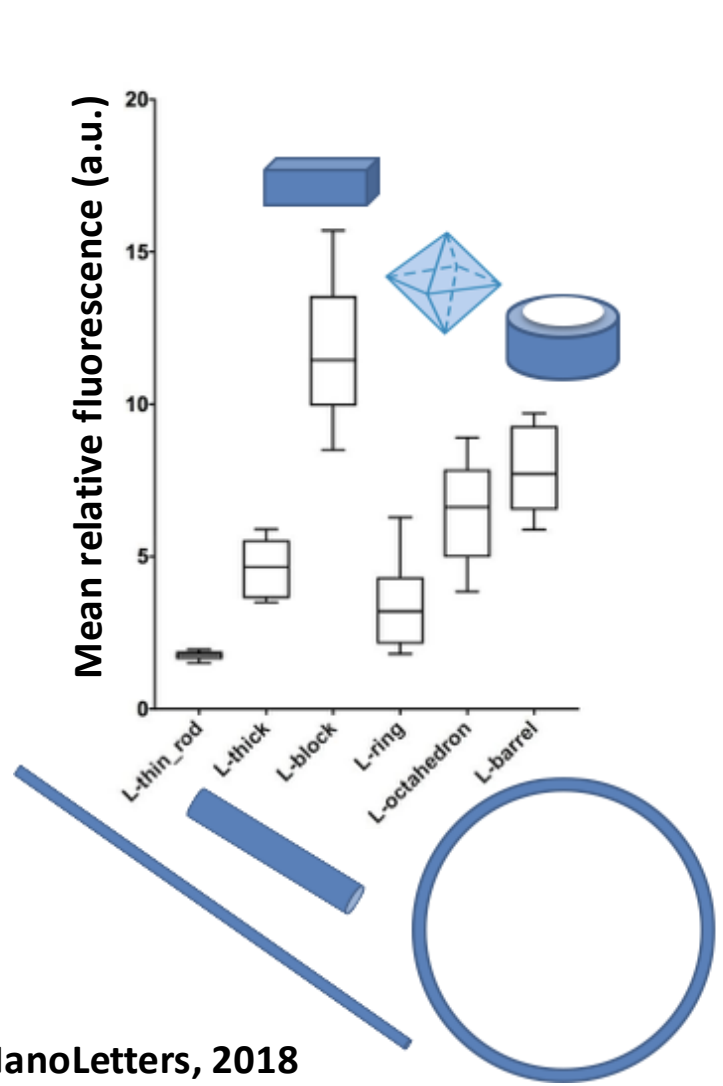

**Endothelial** (HUVEC) cells are a specialized type of **epithelial (HEK)** cells. Epithelial cells line both internal surfaces and external surfaces of the body whereas endothelial cells line the internal surfaces of the components of the circulatory system.

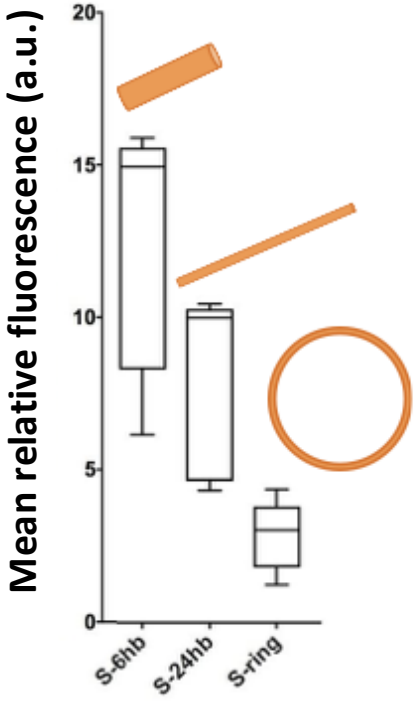


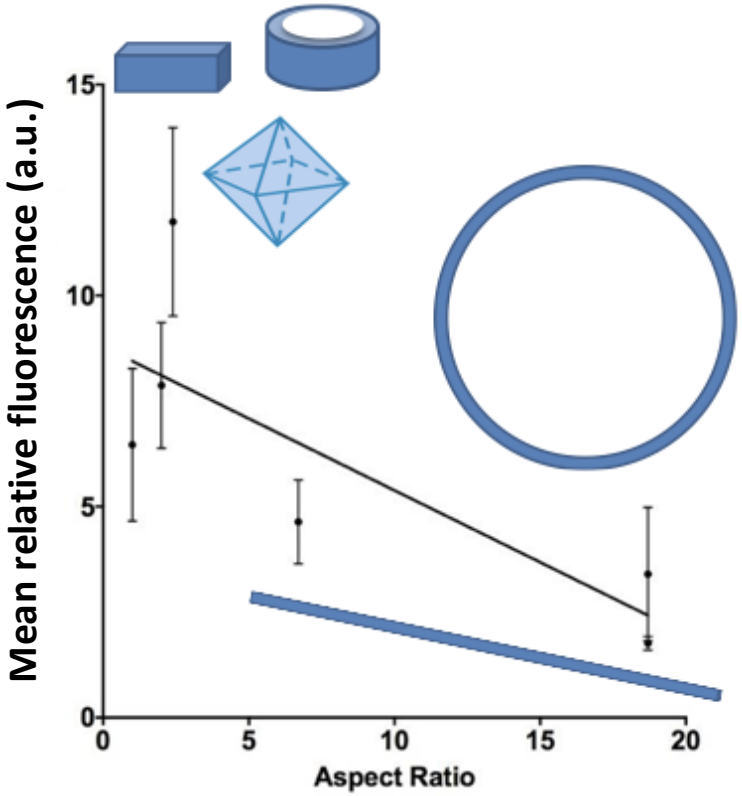

Bastings et al, NanoLetters, 2018

# Compact shapes are preferred

## Low aspect-ratio particles are taken up more dominantly

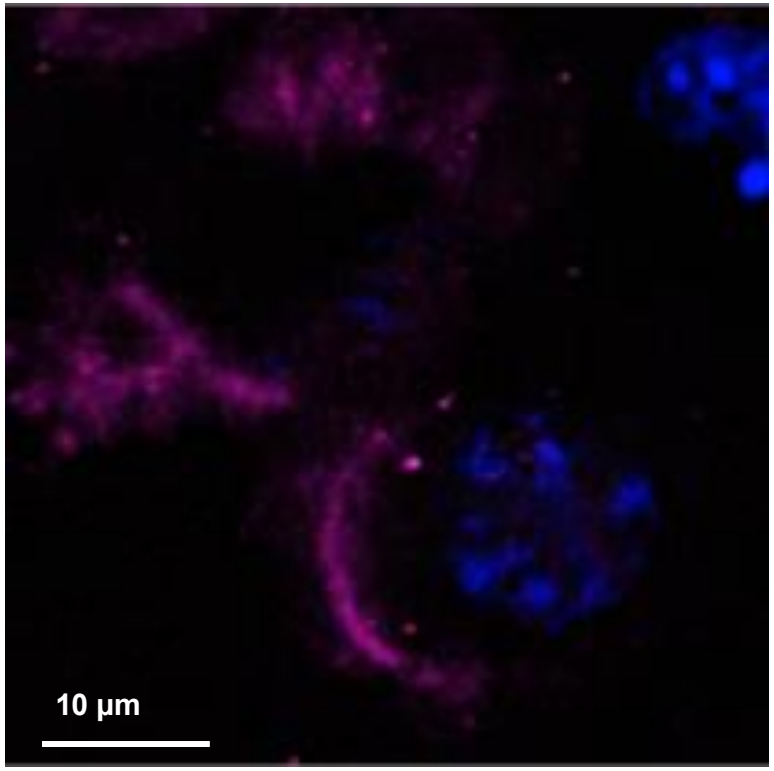


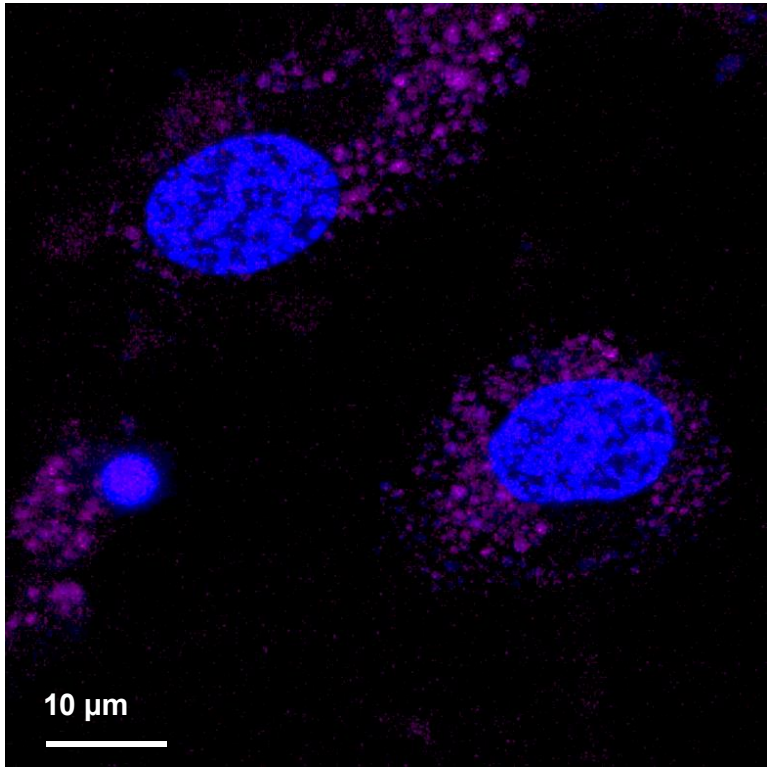




Bastings et al, NanoLetters, 2018

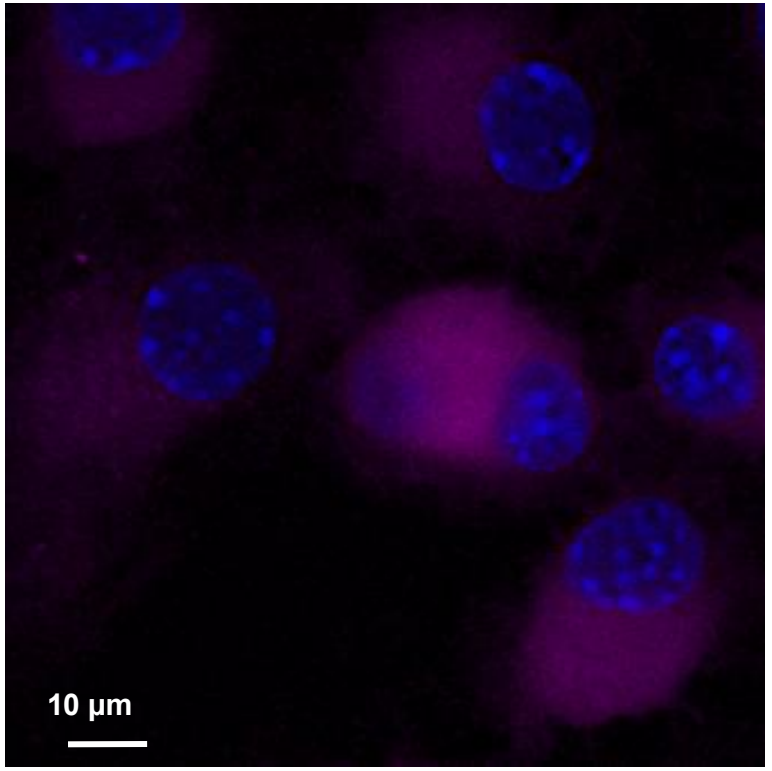
# Importance of Charge




(+) on cell membrane

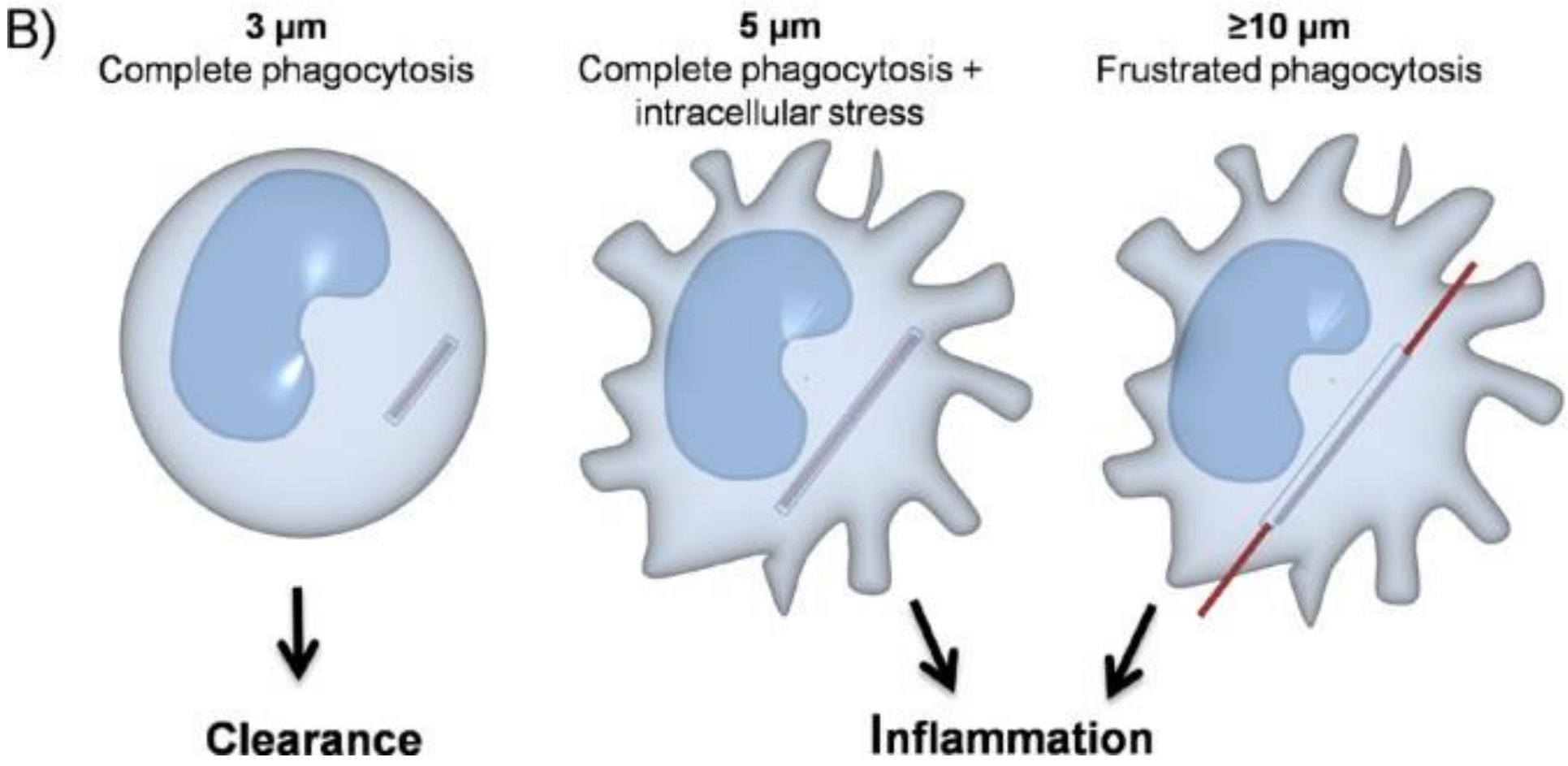



(o) in endosomes




Endosomal escape to cytoplasm

# Frustrated Phagocytosis




*Particle and Fibre Toxicology* **volume 9**,
Article number: 34 (2012)

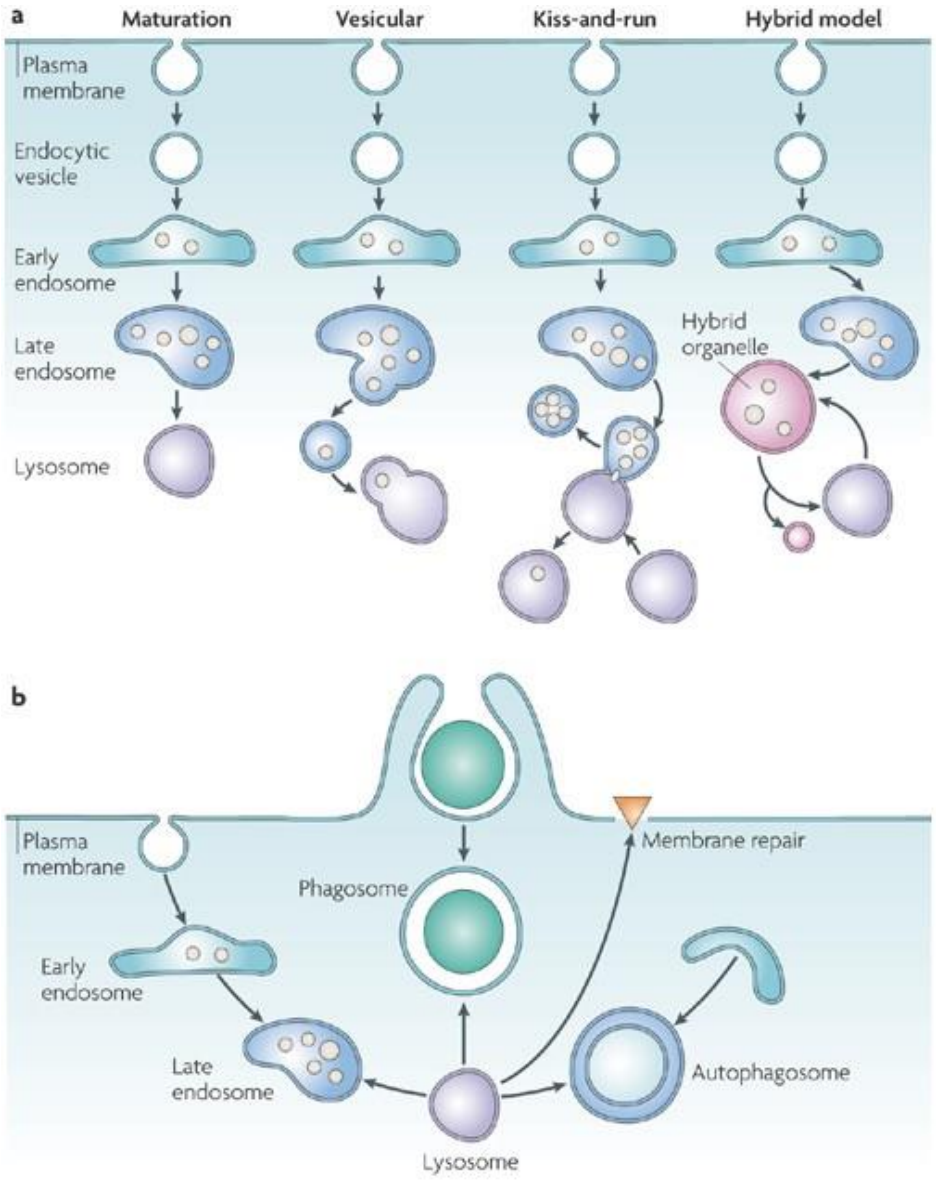
# When inside…

The primary function of endosomes relates to the transportation of extracellular material into the intracellular domain. Lysosomes, on the other hand, are primarily involved in the degradation of macromolecules.

**Endosome** A membrane-bound compartment (organelle) to which ligands, membrane components and fluid are delivered following internalization (endocytosis) from the cell surface.

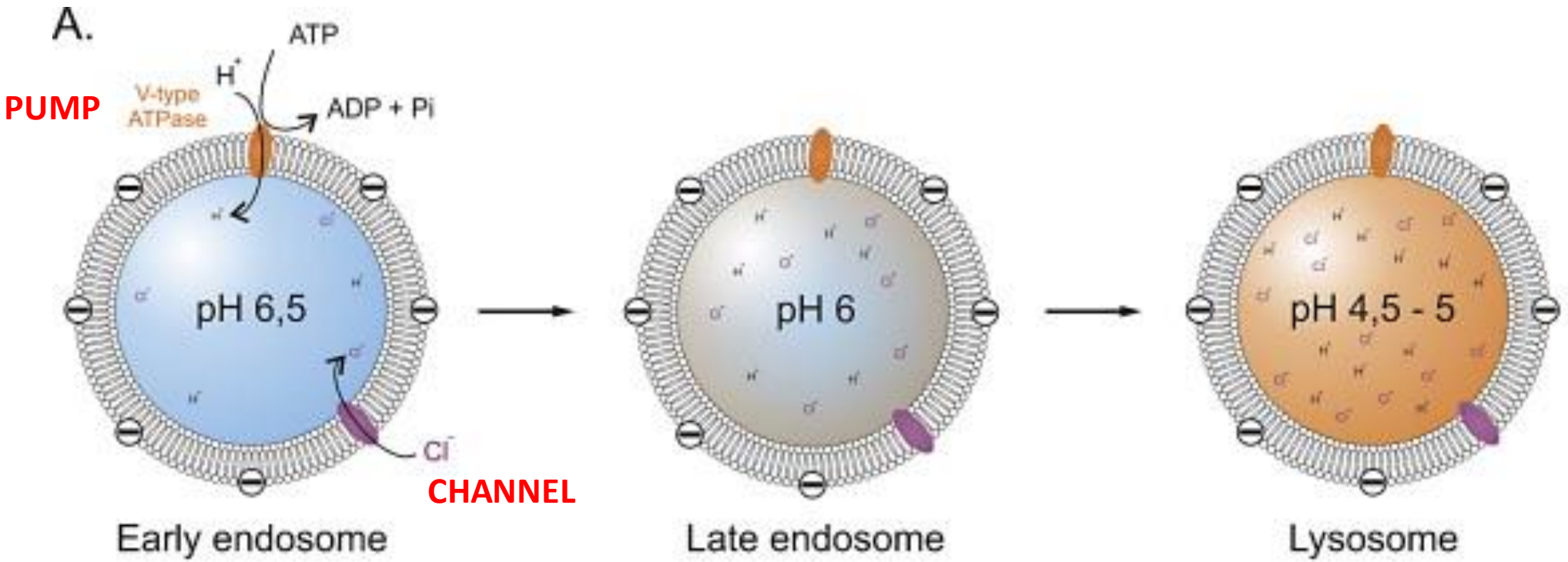
**Phagosome** A membrane-bound compartment containing particles such as bacteria, yeast or parasites that have been internalized from the cell surface by the process of phagocytosis.

**Lysosomes** Dynamic organelles that receive membrane traffic input from the secretory, endocytic, autophagic and phagocytic pathways. They can also fuse with the plasma membrane.



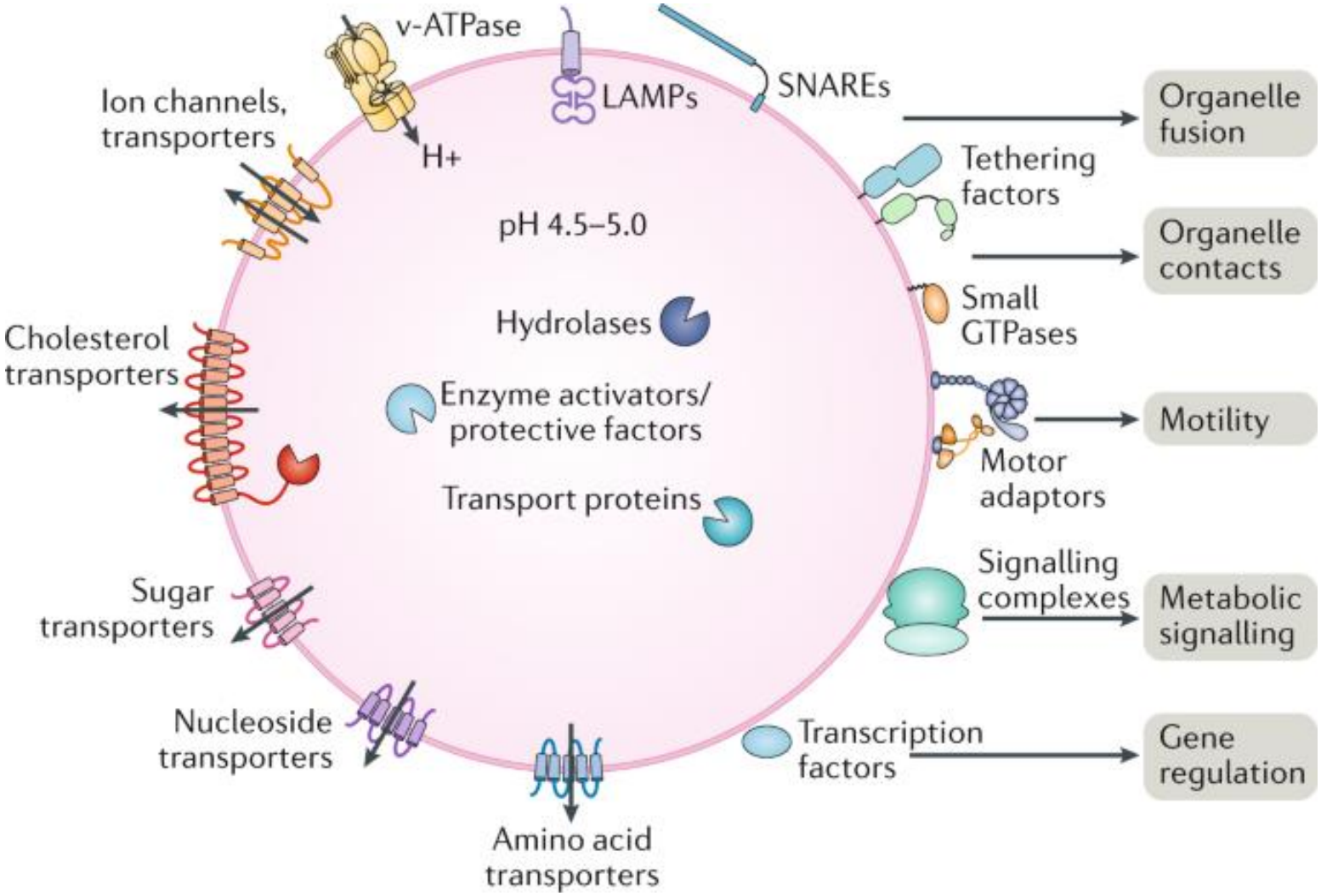

*Nature Reviews Molecular Cell Biology*
**volume 8**, pages 622–632 (2007)

# pH transition



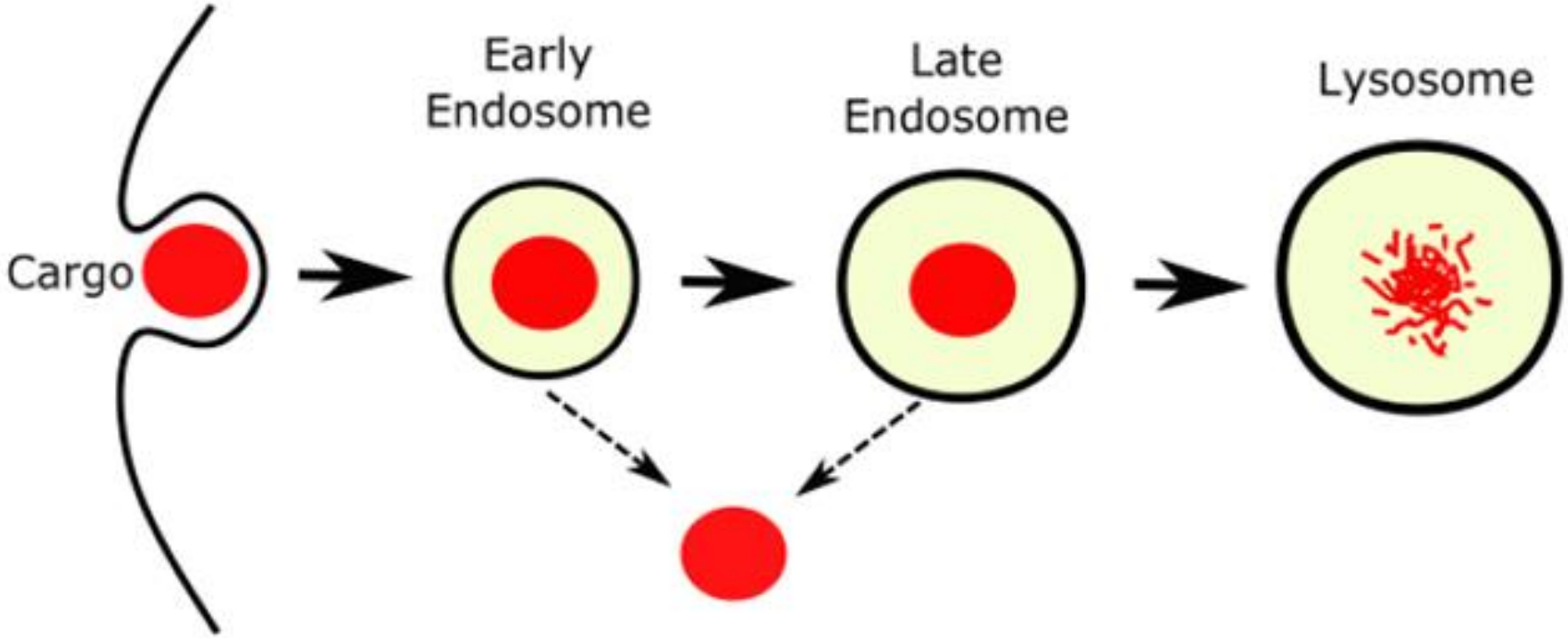
# In a Lysosome

Lysosomes contain portions of cytoplasmic components such as glycogen, mitochondria, or cisternae of the endoplasmic reticulum. Hydrolytic enzymes (phosphatases and proteases) provide intracellular digestion of worn-out cellular organelles and materials taken into the cell by endocytosis.

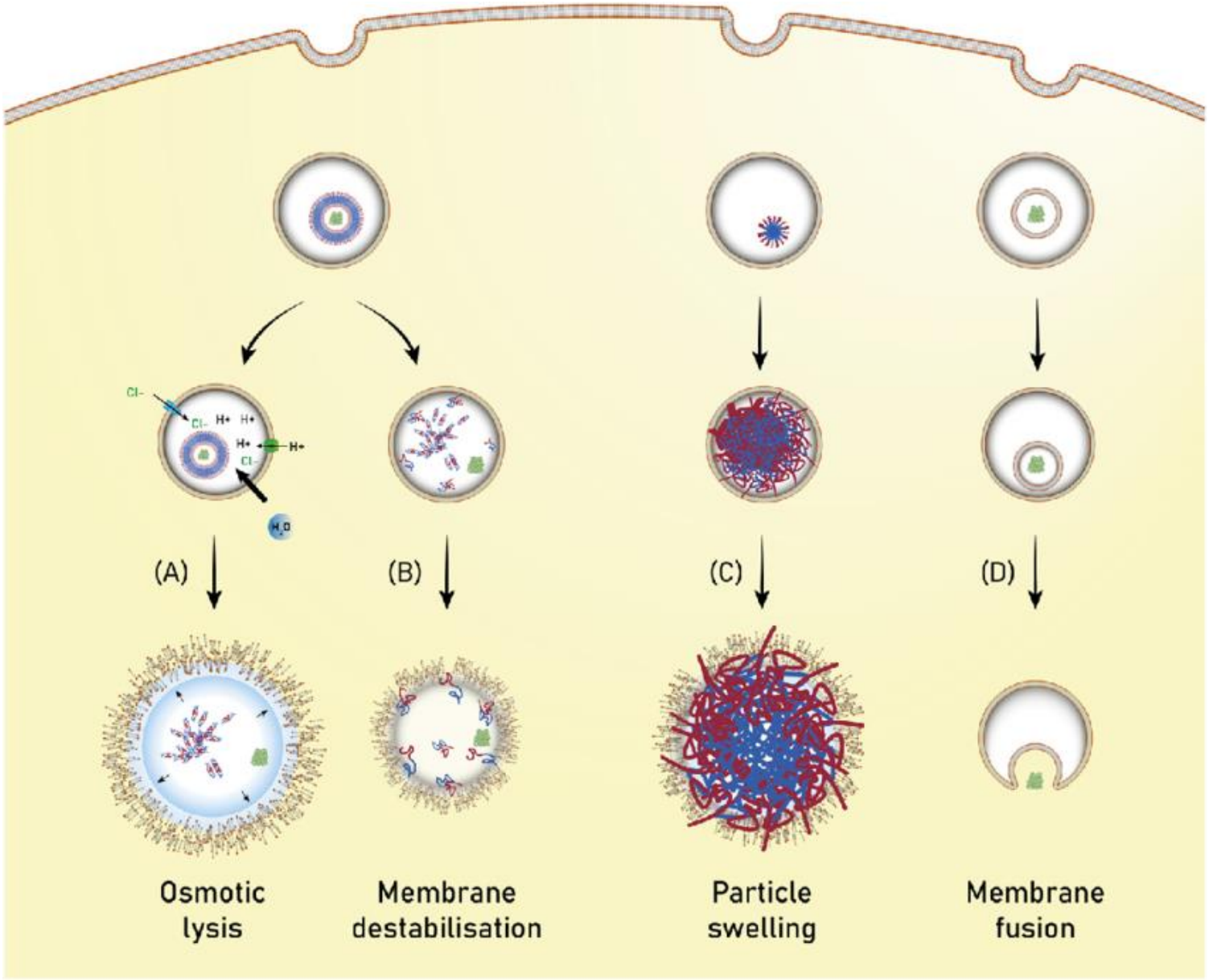



*Nature Reviews Molecular Cell Biology*
**volume 21**, pages 101–118 (2020)

# Need to escape



# Endosomal escape



# Endosomal escape

A)**Osmotic rupture:** It is also known as the “proton sponge effect”. The pH is continuously decreased by influx of protons during endosomal maturation. When a polymer with pH buffering capacity enters the endosome, it starts to resist the decrease of the pH inside the endosome, leading to further influx of protons for lowering the pH. This is followed by influx of chloride ions and subsequently water molecules, resulting in high osmotic pressure, leading to swelling and ultimately the rupture of the endosome.

B) **Membrane destabilization:** Membrane destabilization may be mediated by oxidative stress and resulting generation of reactive oxygen species due to the release of contents and breakdown of nanocarriers in response to the decreased pH inside an endosome.

C)**Swelling of nanocarriers:** Nanocarriers may expand and swell in response to reduction in pH due to proton influx and rupture the endosomal membrane by mechanical strain.

D) **Membrane fusion:** The nanocarrier fuses with and then ruptures the endosomal membranes.

# Cell penetrating peptides

Cell Penetrating Peptides are small peptide domains (generally less than 40 amino acids) that can easily cross cell membranes.

Multiple cell permeable peptides have been identified that facilitate cellular uptake of various molecular cargo, ranging from nanosize particles to small chemical molecules.

Cell penetrating sequences can be used as extensions to peptide sequences thereby making them more permeable to cell membranes, or cell penetrating peptide can be attached to other cargo molecules to enhance their cellular uptake.

https://www.pepscan.com/custom-peptide-synthesis/special-peptides/cell-penetrating-special-peptides/

Commonly used CPPs

| Name | Sequence |
|---|---|
| HIV-TAT | GRKKRRQRRRPQ |
| Oligo-Arginine | RRRRRRRR |
| MPG | Ac-GALFLGFLGAAGSTMGAWSQPKKKRKV-cya |
| PEP-1 | Ac-KETWWETWWTEWSQPKKKRKC-cya |
| EB1 | LIKLWSHLIHIWFQNRRLKWKKK |
| Transportan | GWTLNSAGYLLGKINLKALAALAKKIL |
| p-Antp | RQIKIWFQNRRMKWKK |
| hCT(18-32) | KFHTFPQTAIGVGAP-NH2 |
| KLA seq | KLALKLALKALKAALKLA |

A limitation of most of the CPPs is their lack of cell or tissue selectivity, which limits their use in clinical development

# Conclusion

Endocytosis is a cellular process in which substances are **brought into the cell**. The opposite is exocytosis. The most common pathway is clathrin-mediated uptake, in which specific receptors interact with the components that want to enter the cell.

The pathways are size dependent, some are dependent on specific ligand-receptor interactions.

The reason why cells endocytose is to obtain nutrients, to detect bacteria or invadors, and to dispose of damaged or dead cells.

To engineer materials to enter the cells, we need to control their size, shape and charge as all these parameters influence what happens when inside the cell.

We can add specific ligands to interact with surface receptors, as well as peptides that can help enter the cell. Inside the cell, these materials can either be degraded, accumulate, or be expelled through exocytosis.