AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 4.

Ultrastructure of bacteria. Flagella and capsule. Investigation of bacterial motility ("crushed and hanging" drop methods, vital staining). Detection of the capsule by Gins-Burry stain

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

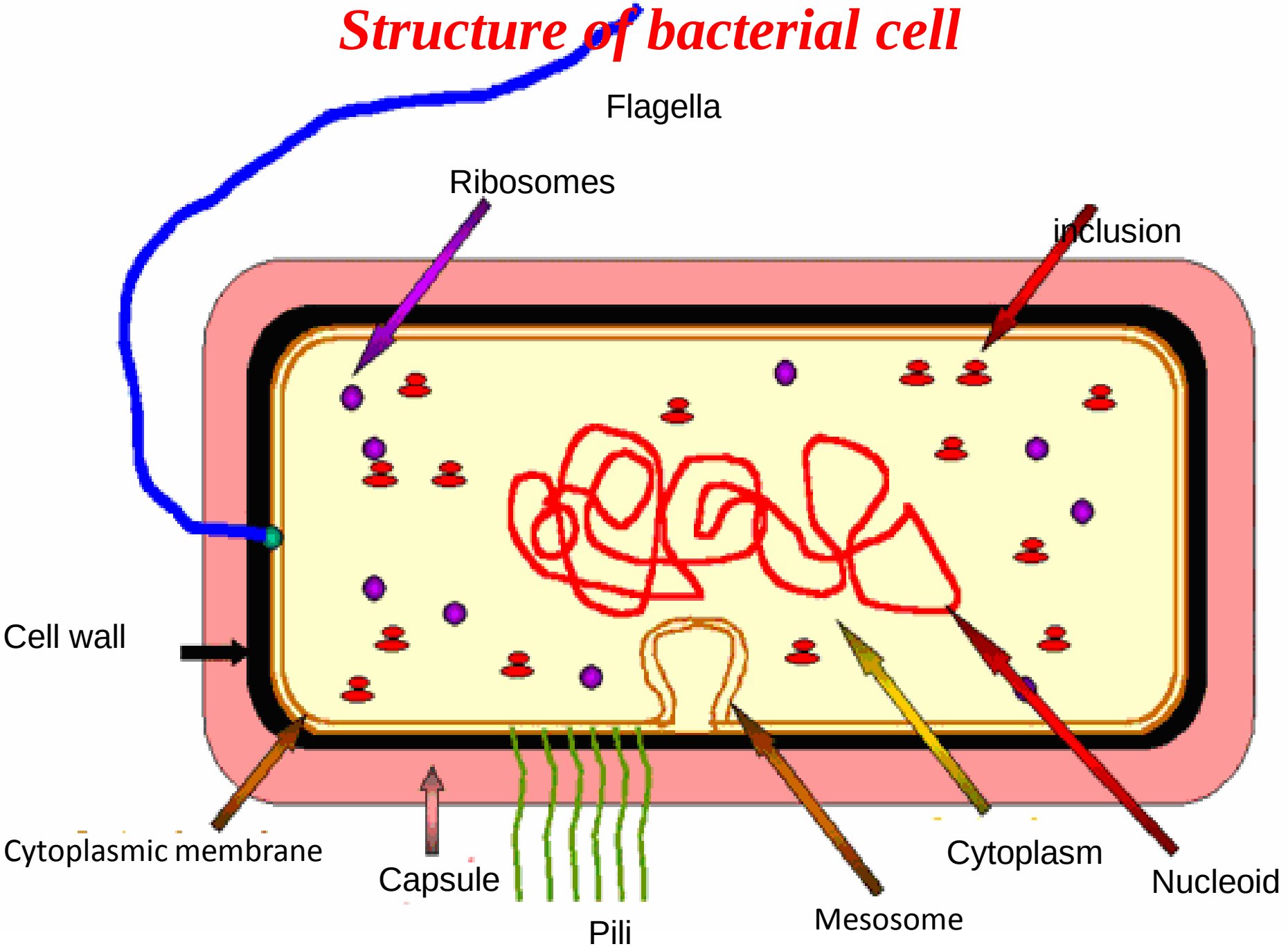
Discussed questions:

- The bacterial cell structure (capsule, glicocalix, flagella, pili)
- The motile bacteria. The structure, function and location of flagella.
- To movement study of microbes prepeared by «crushed and hanging» drop methods.
- Vital staining method.
- The encapsulated bacteria, chemical composition, structure and importance of the capsule
- The detection of capsule by Gins-Burry method

Purpose of the lesson:

- To explain to the students the structure, location, chemical composition and function of flagella, the movement organ of bacteria, the methods used to study the movement of bacteria and their role in diagnostics. To explain to them the capsule, its chemical composition and function, detection by the Gins-Burry method and the role of this method in diagnostics.

Structure of bacterial cell



Flagella

✓*Flagella is a movement organ, composed of the flagellin protein*

✓*It is mainly a movement (reptile, floating) organelles of rod- and spiral bacteria shapes.*

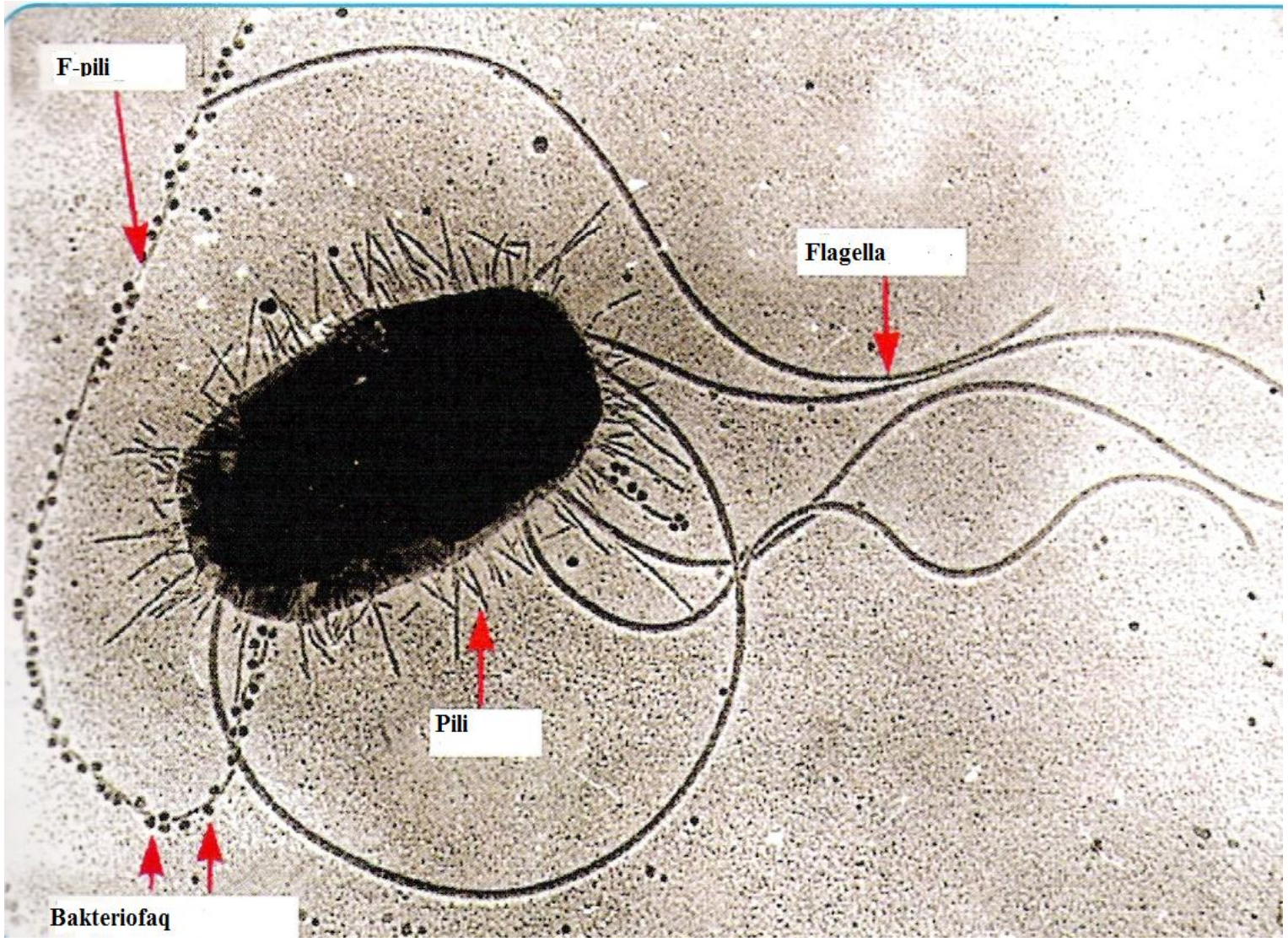
✓*It connects to the cytoplasmic membrane with the basal body (blepharoplast).*

✓*The basal body joins the layers of the cell membrane with a pair of helical rings. Gram-positive organisms have two of these basal body rings, one in the peptidoglycan layer and one in the plasma membrane. Gram-negative organisms have four such rings*

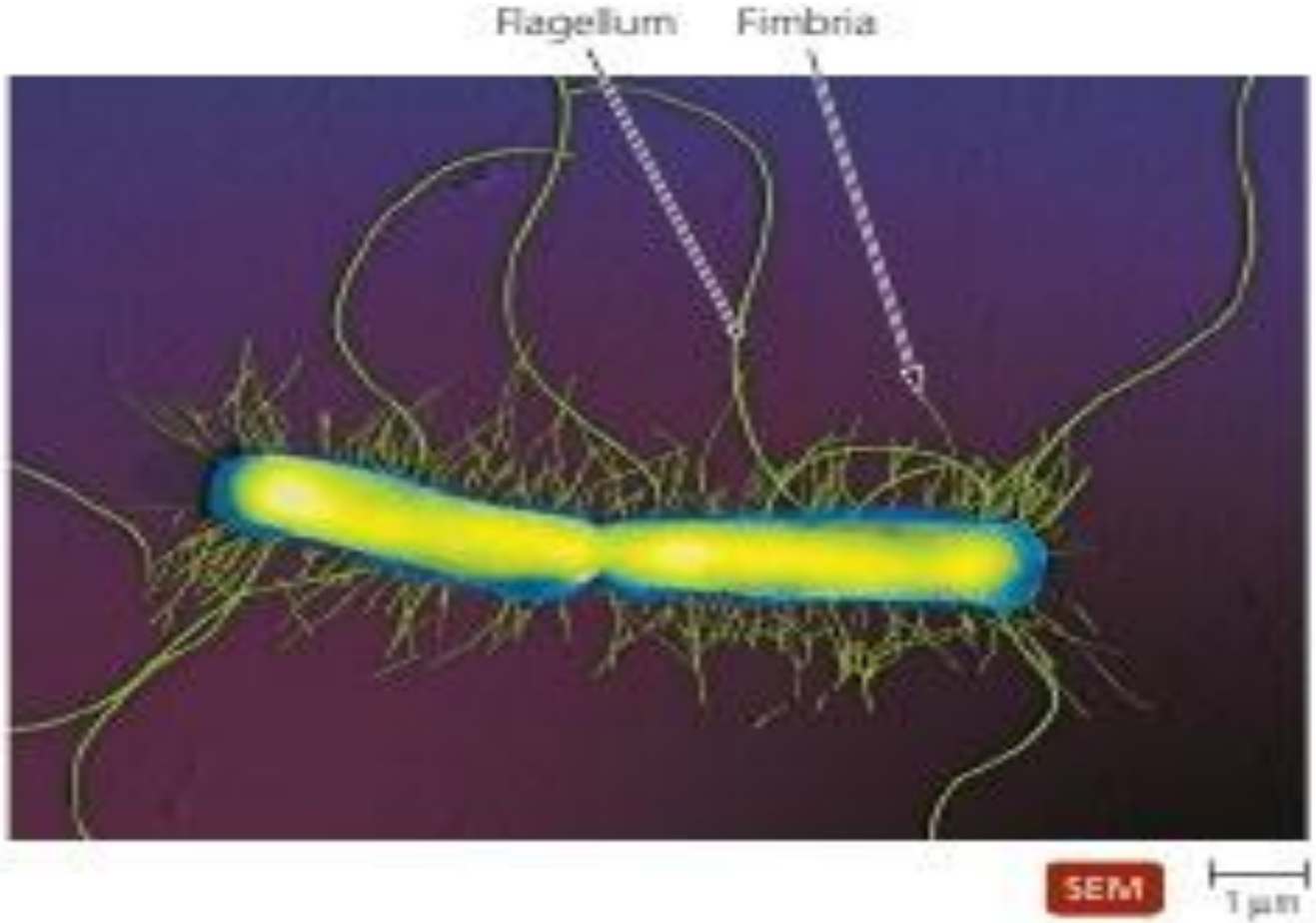
✓*Flagellin contains several thousand protein molecules (H antigen)*

✓*Spirochetes contain poles called axial filaments instead of flagella (endoflagella)*

Bacterial flagella and fimbria



Bacterial flagella and fimbria

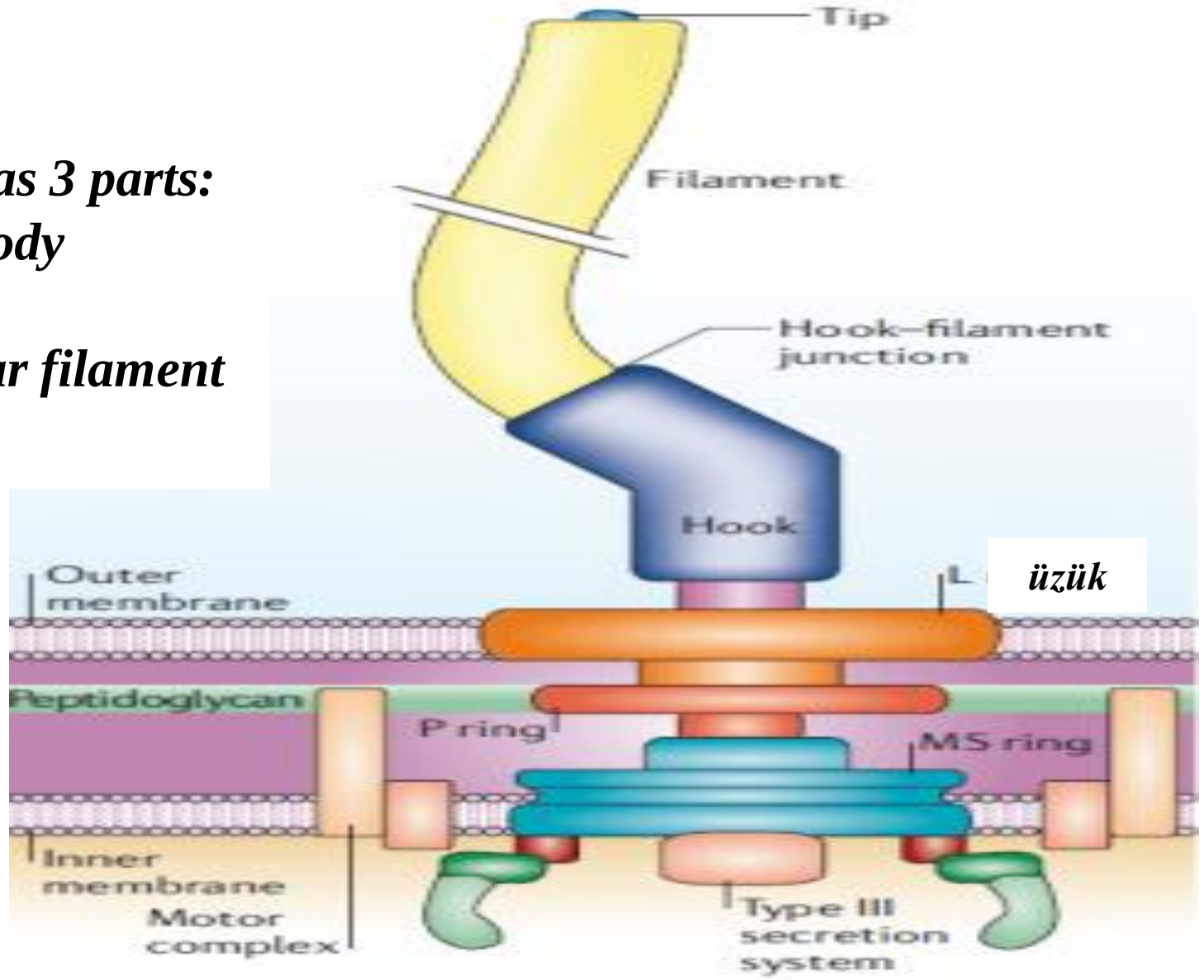


▲ **Figure 3.10 Fimbriae.** *Proteus vulgaris* has flagella and fimbriae.

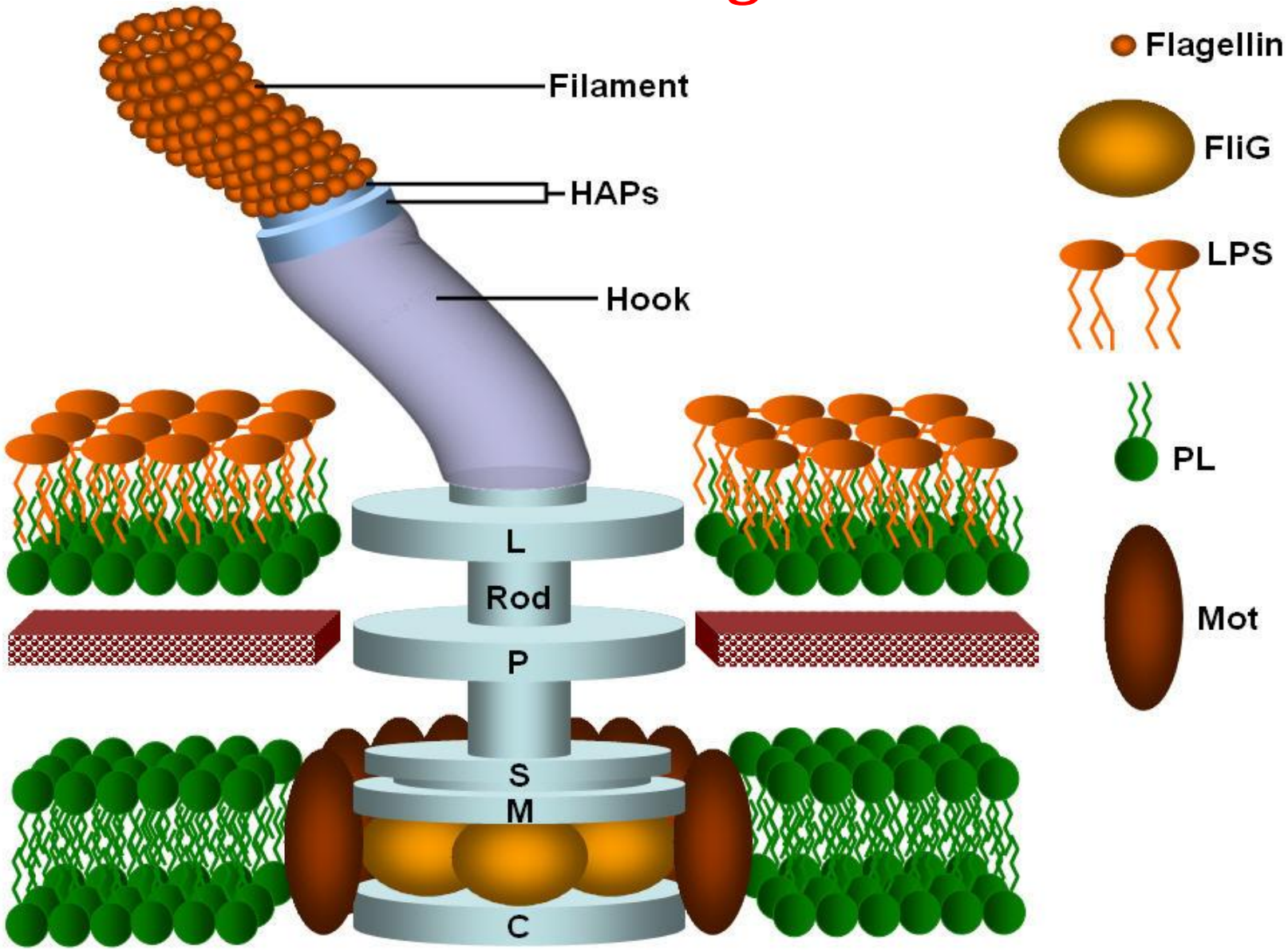
Structure of bacterial flagella

Flagella has 3 parts:

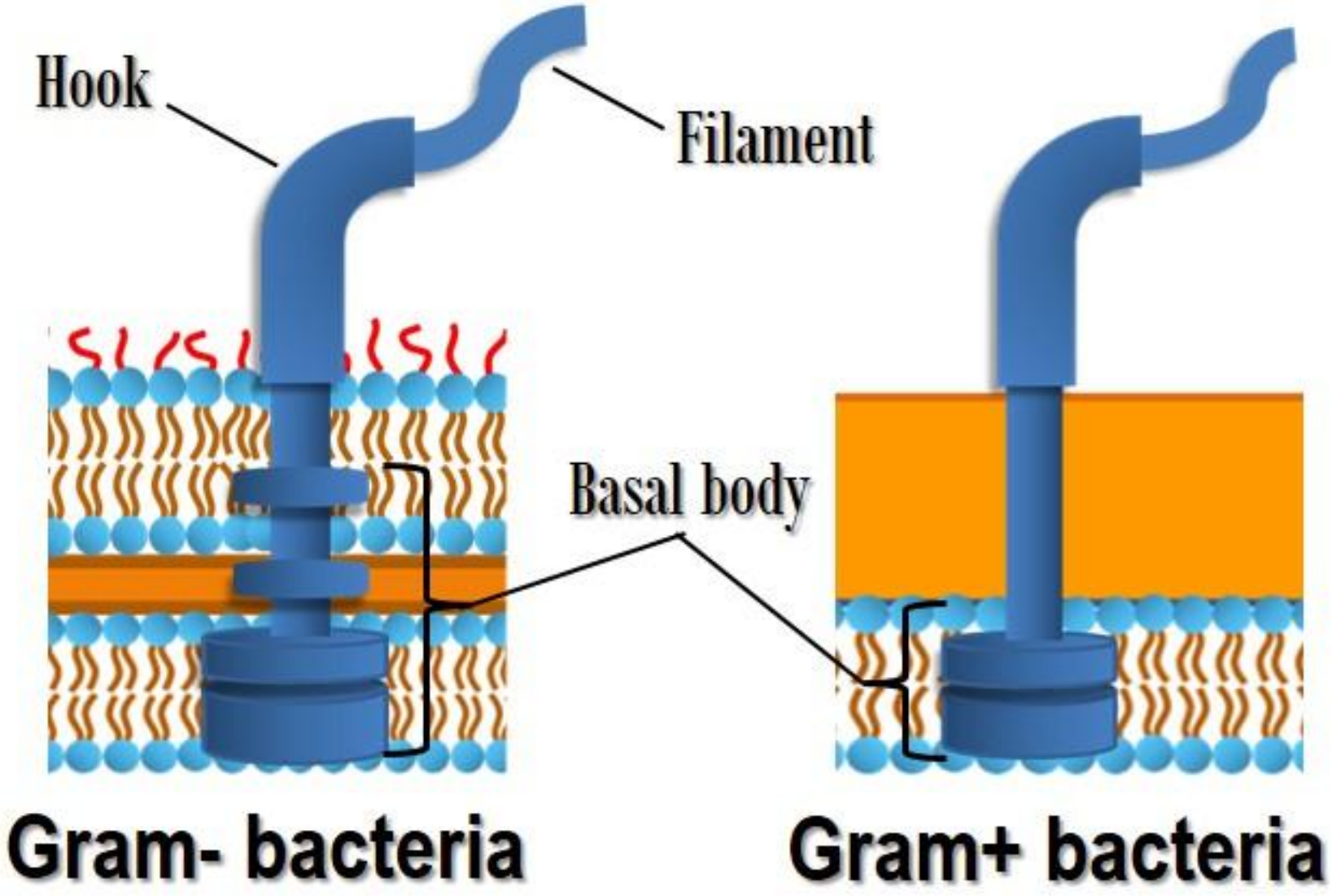
1. ***Basal body***
2. ***Hook***
3. ***Flagellar filament***



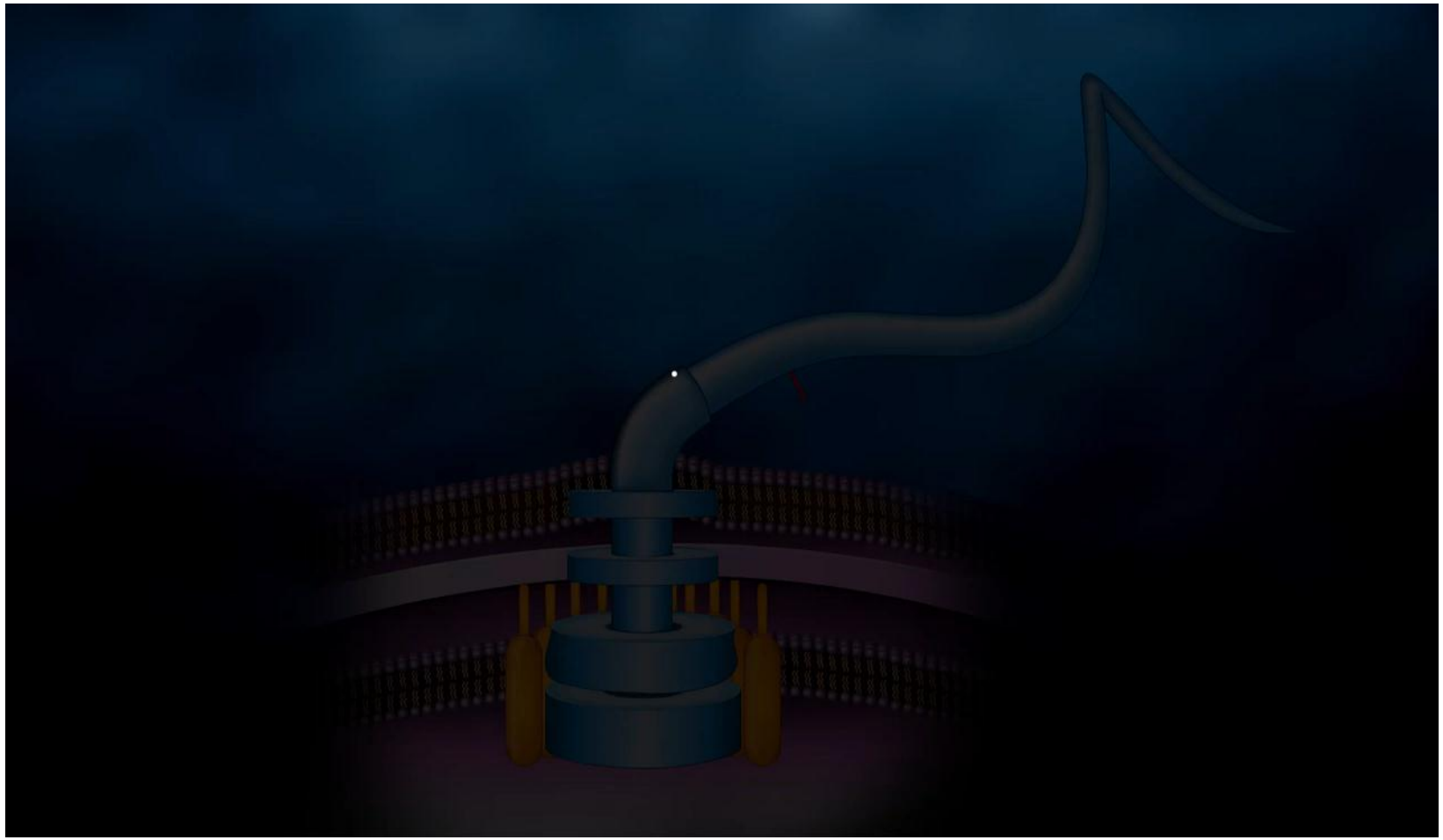
Structure of bacterial flagella



Structure of flagella of Gram (+) and Gram (-) bacteria



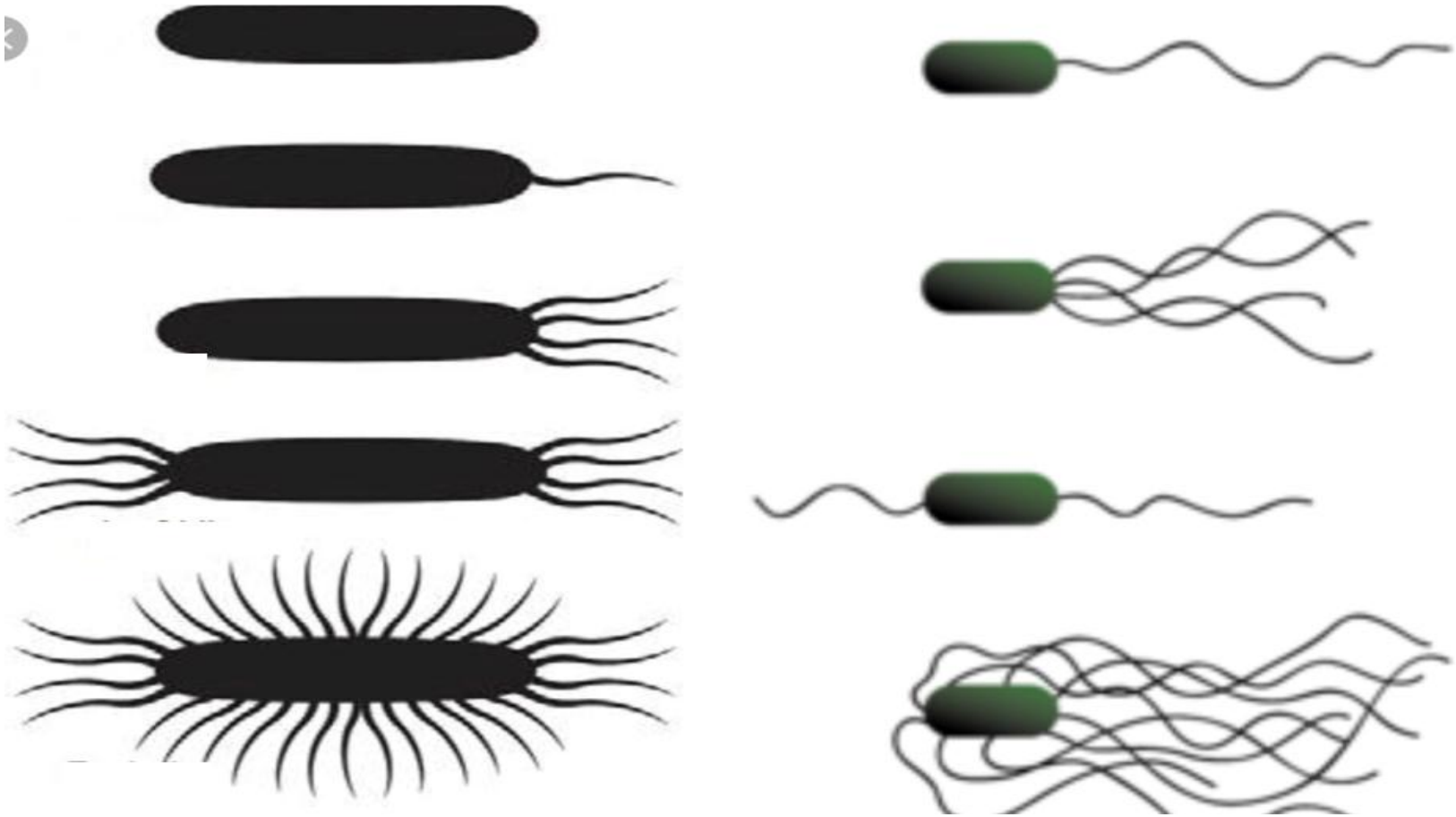
Structure of flagella of Gram (+) and Gram (-) bacteria



Different species of bacteria have different numbers arrangements of flagella

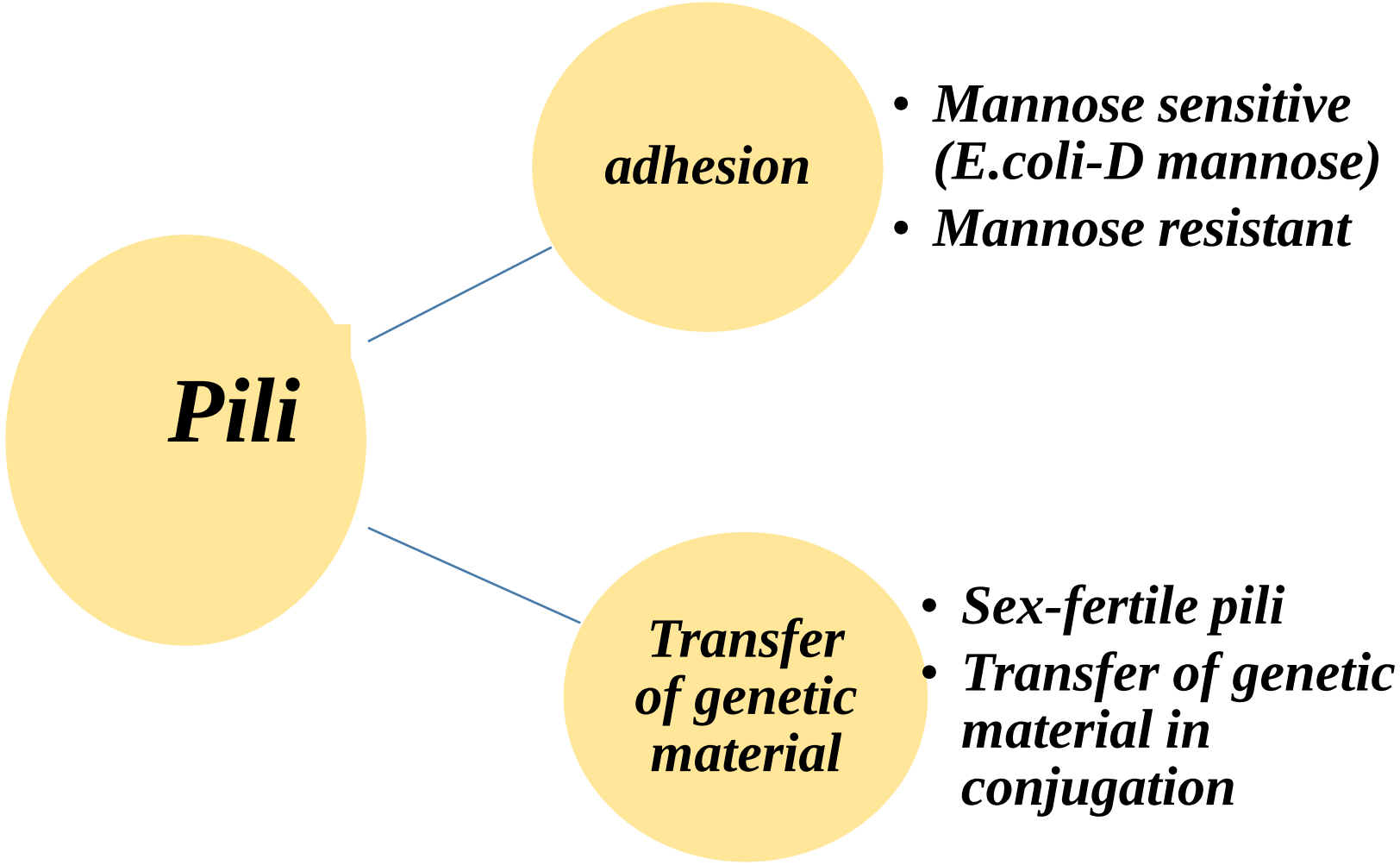
Atrichous	***Shigella, Klebsiella, Acinetobacter***
Monotrichous	***Campylobacter, V.cholera, Pseudomonas***
Lophotrichous	***Helicobacter***
Amphitrichous	
Peritrichous	***E.coli, Proteus, Salmonella***

different numbers arrangements of flagella

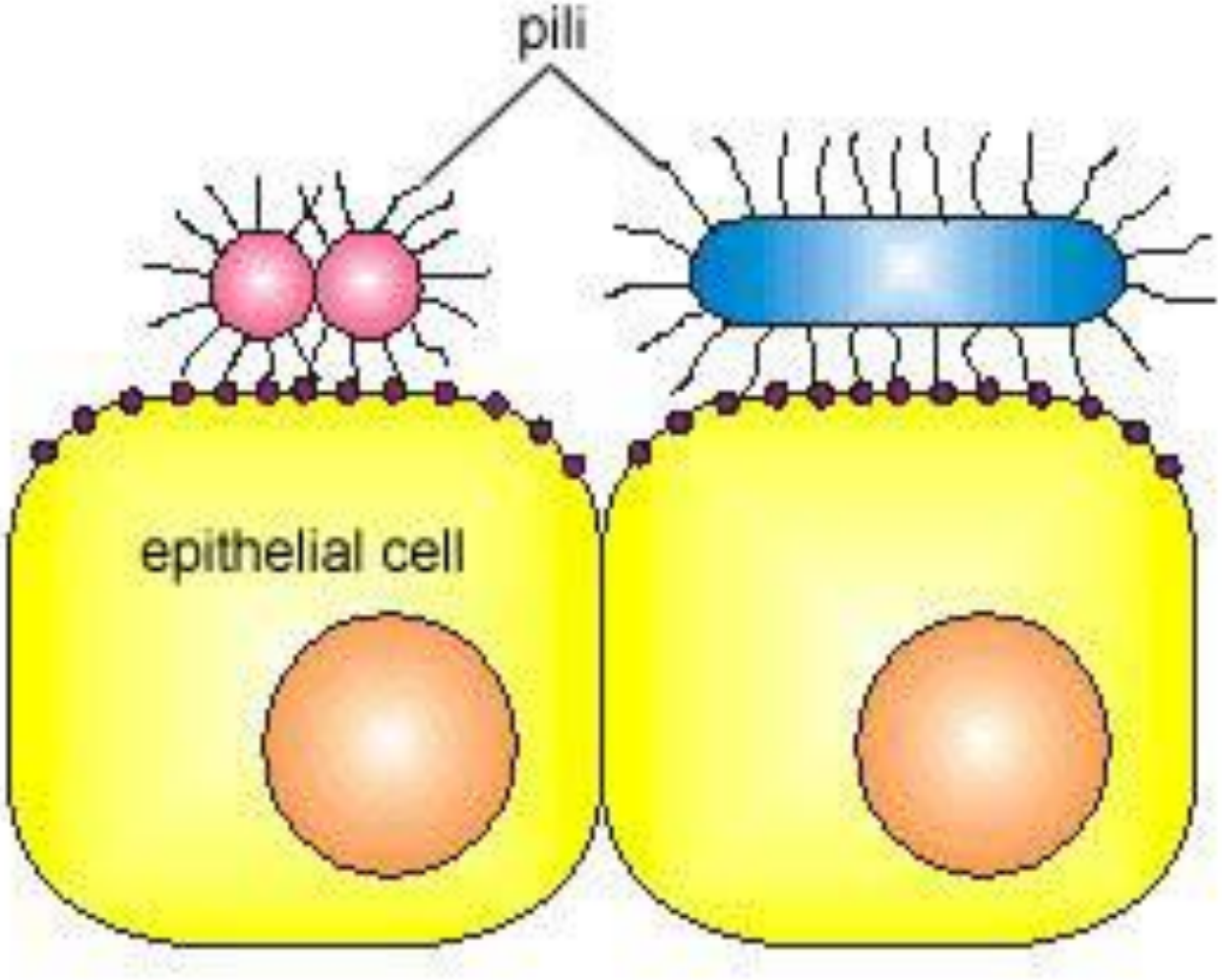


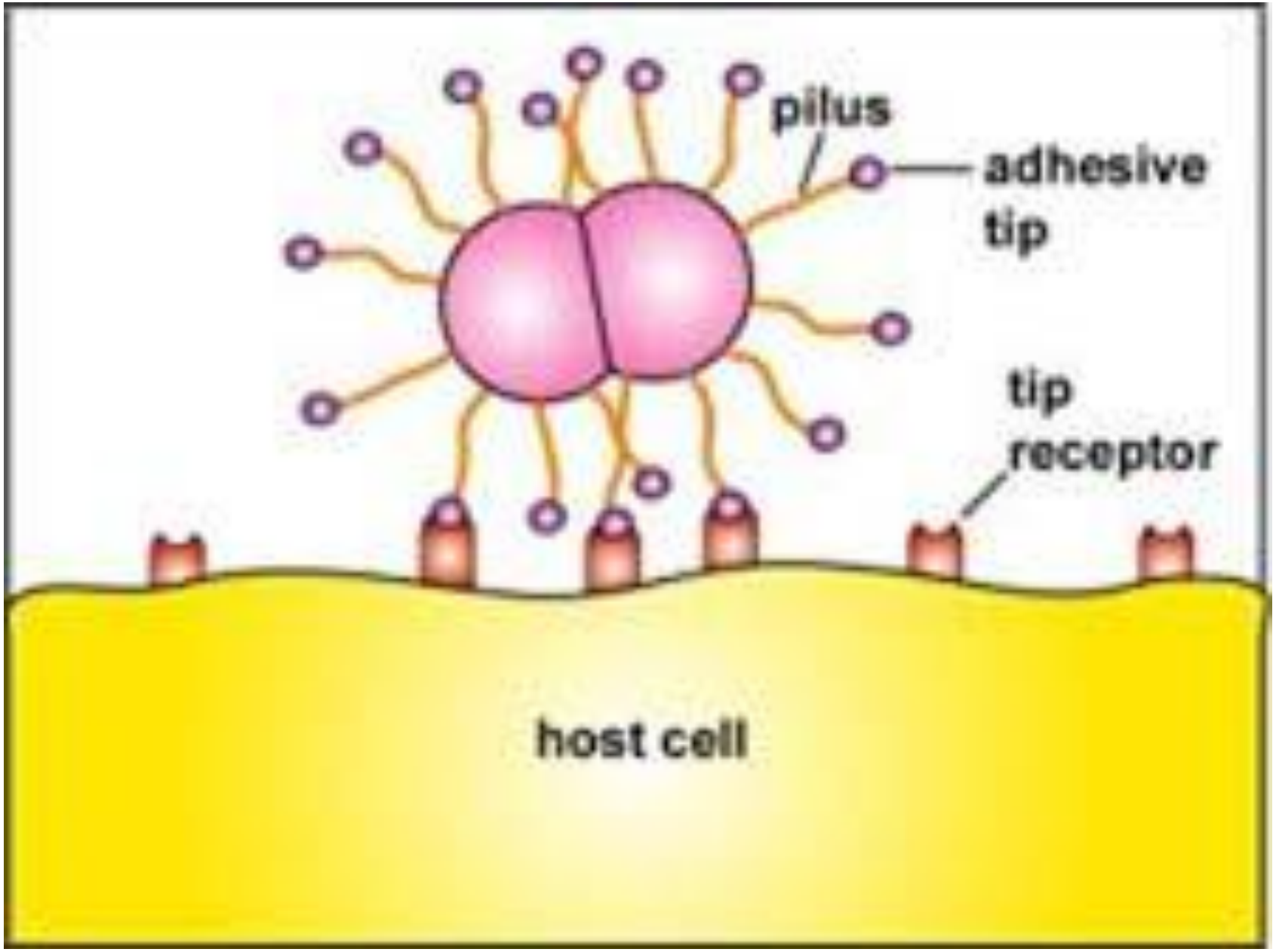
Pili (Fimbriae)

- ***Pili is composed of pilin protein***
- ***Pili begins from the cytoplasmic membrane***



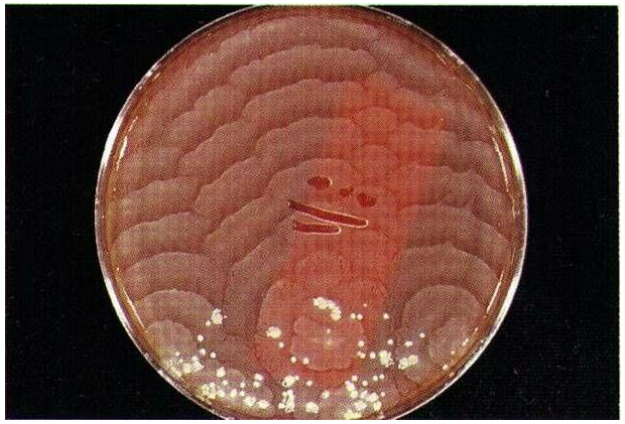
Adhesive pili as a factor of pathogenicity




pilus
adhesive tip
tip receptor
host cell

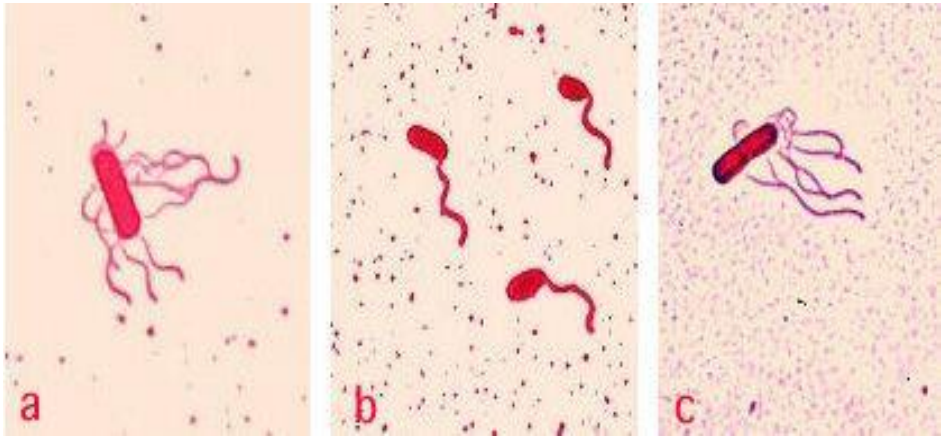
Determination of bacterial motion

- ***With naked eye → "collective motion"***



Proteus spp.

1. ***"crushed and hanged" drop preparations***
2. ***Vital staining***
3. ***Stains - working with tannins - can be detected by the Lefler method***



B. cereus V. cholerae B. brevis

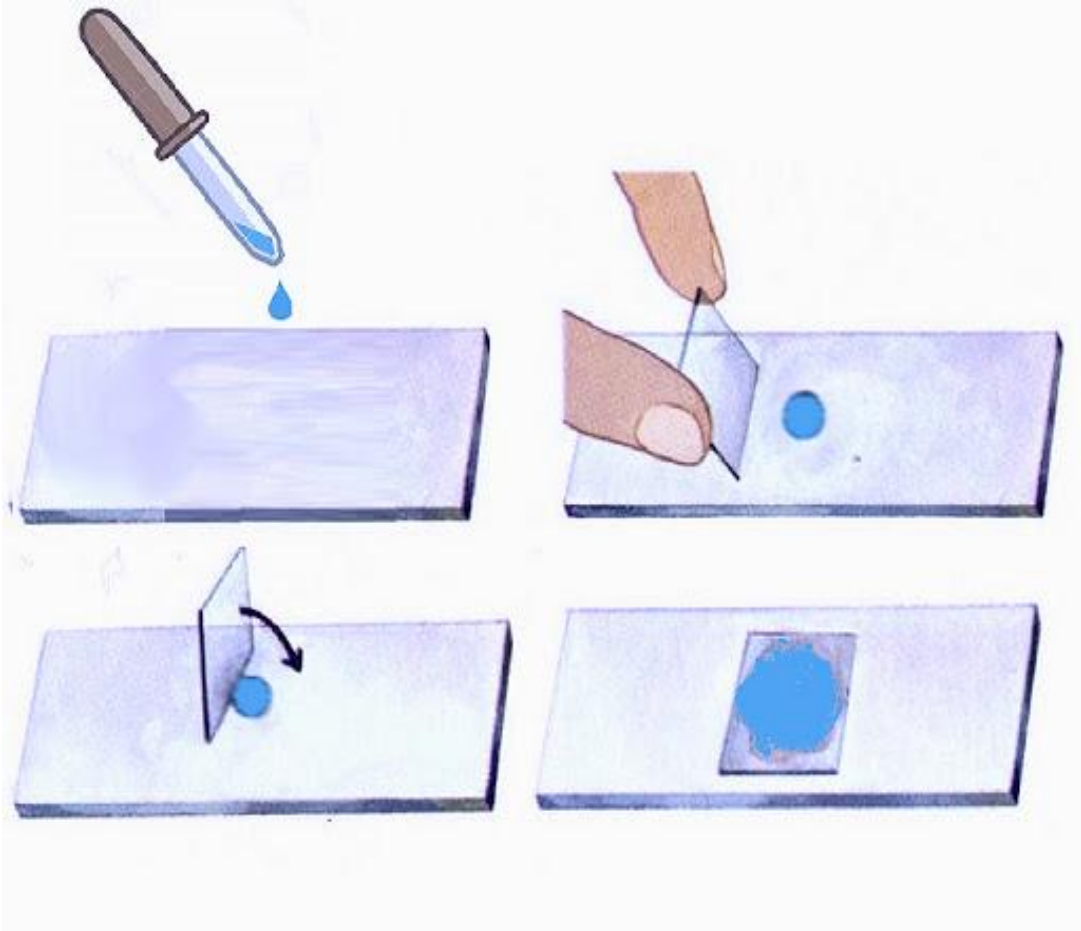
- ***Flagella staining***

“Crushed ”drop preparations

Microbial motion is studied with the " crushed“ drop preparations

1 drop of microbial suspension is placed in the center of the slide and covered with cover glass

Microscopy is performed on a dark-field microscope

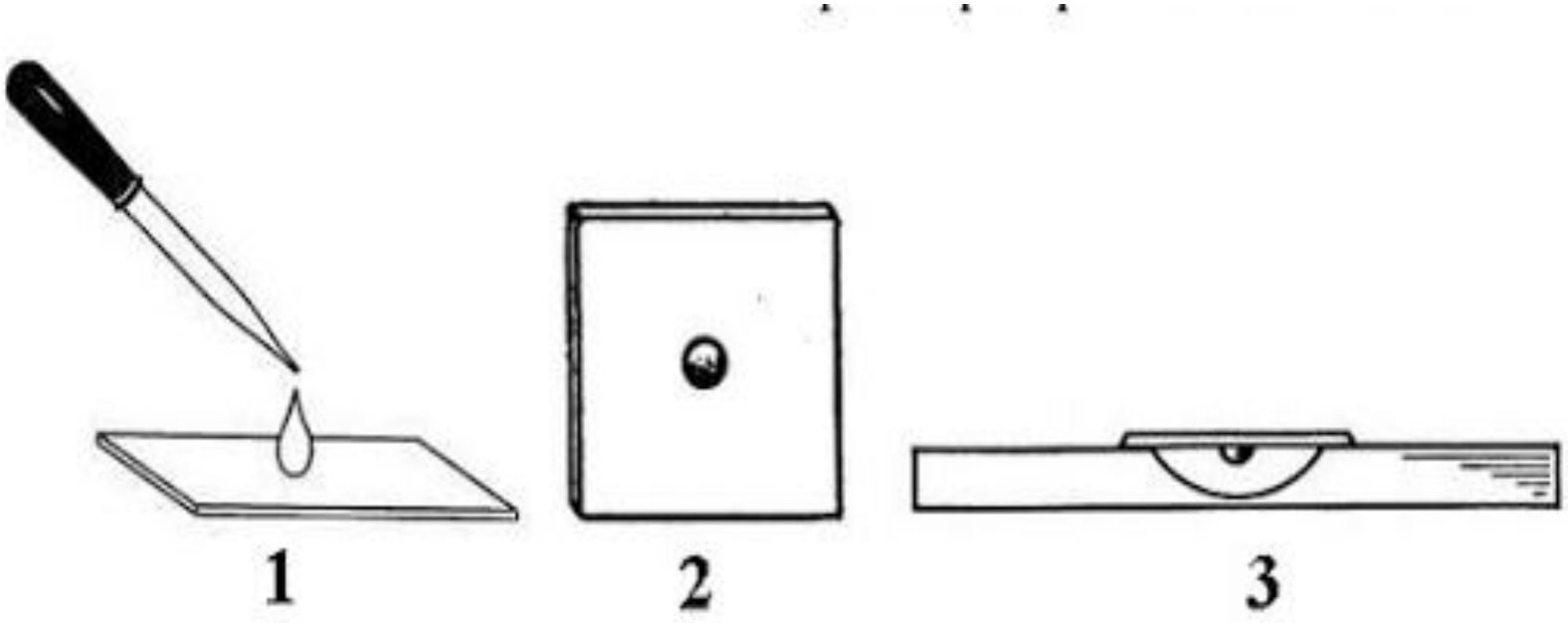


Microscopic view of "crushed" drop preparations

“Hanging” drop preparation

1-2. The microbe suspension is put on the cover glass.

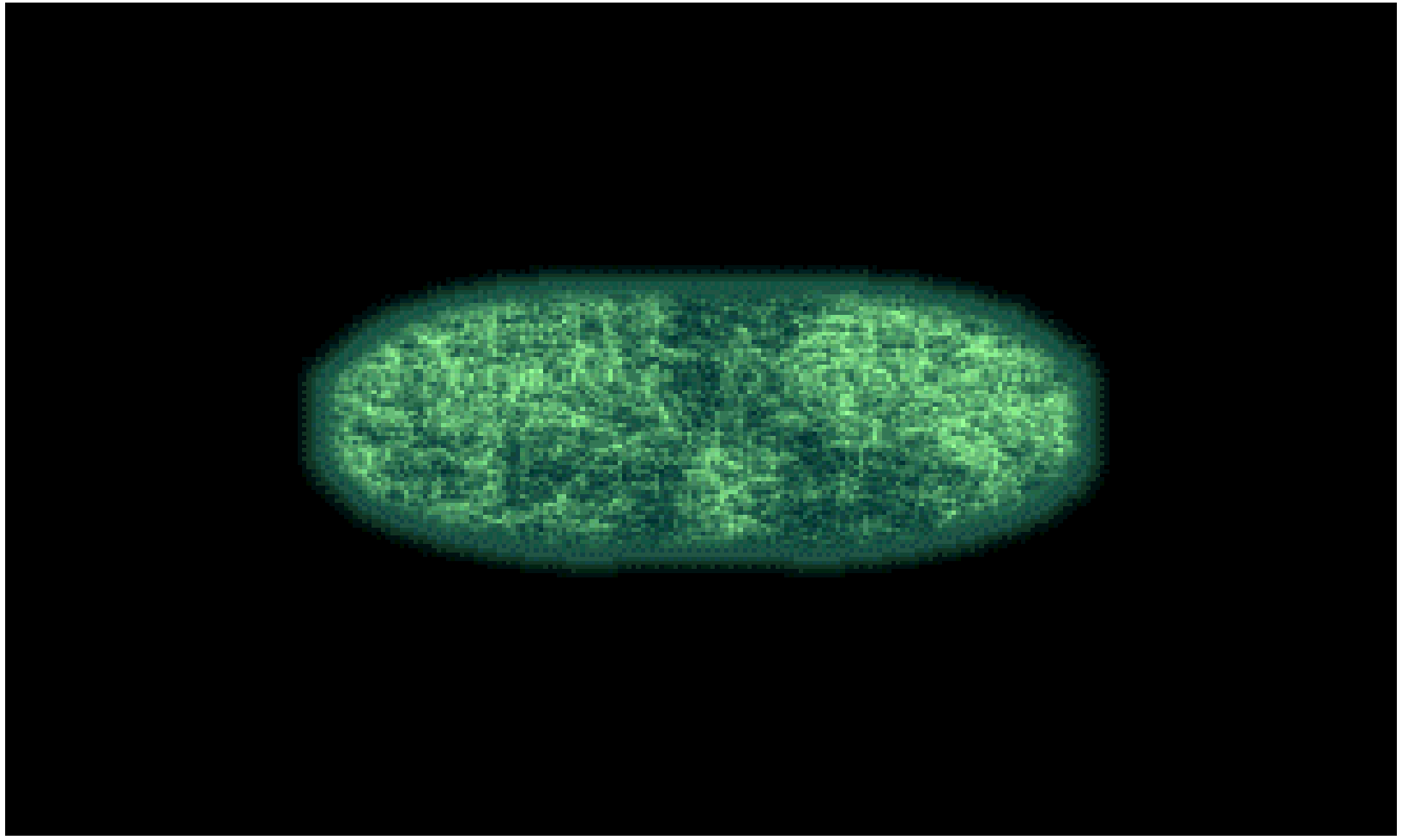
3. The slide with hole in the middle is placed over the cover glass and immediately returned in the opposite direction. At this point, the drop is attached to the center of the hole

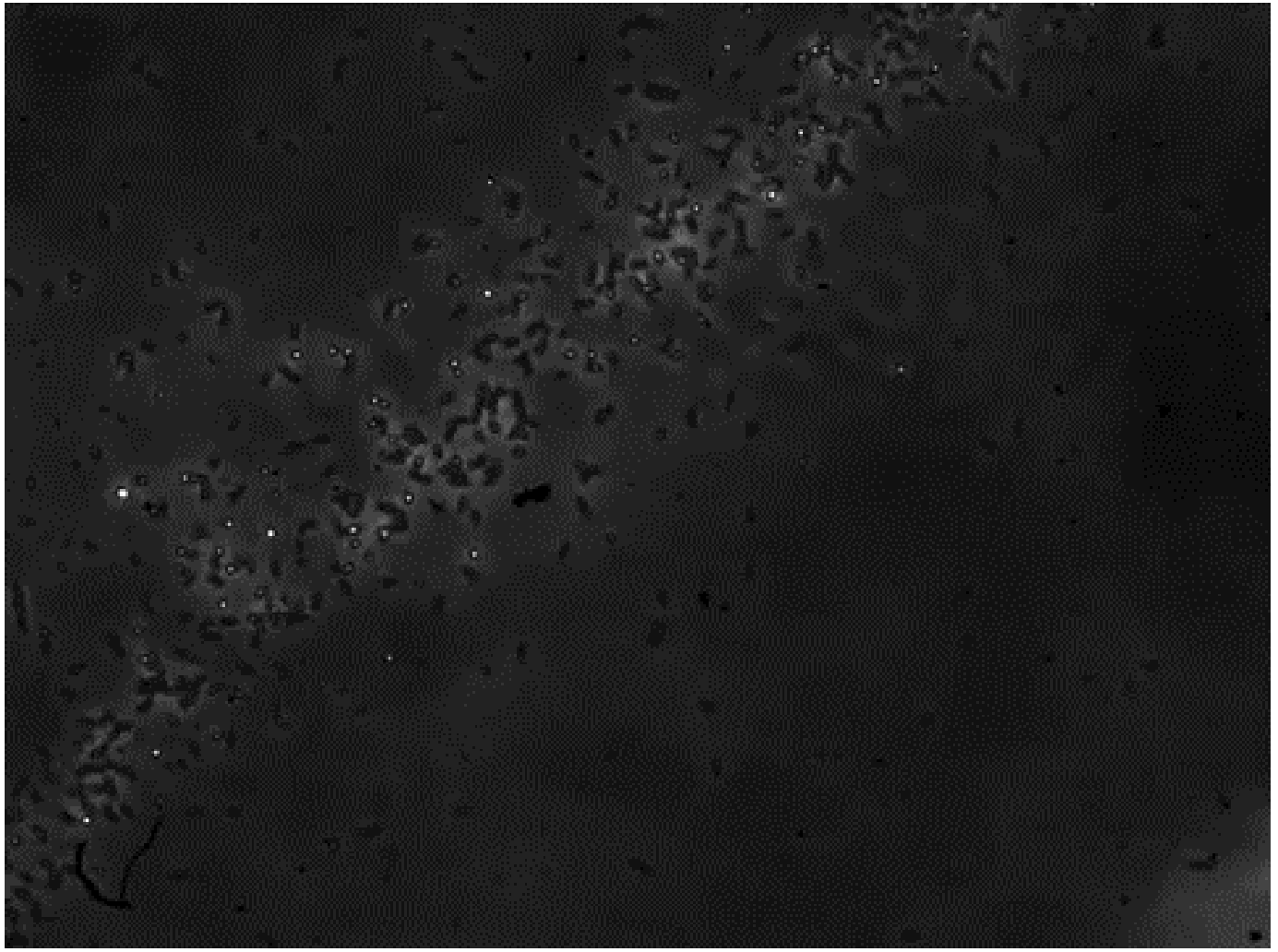


Vital Staining

- *Vital staining is used to study bacteria while alive.*
- *Reproduction of microorganisms*
- *Spore formation*
- *Influence of physical and chemical factors*
- *This method uses 100,000 times dilution of methylene sucker, neutral red solution*

Bacterial division– vital staining





Capsule

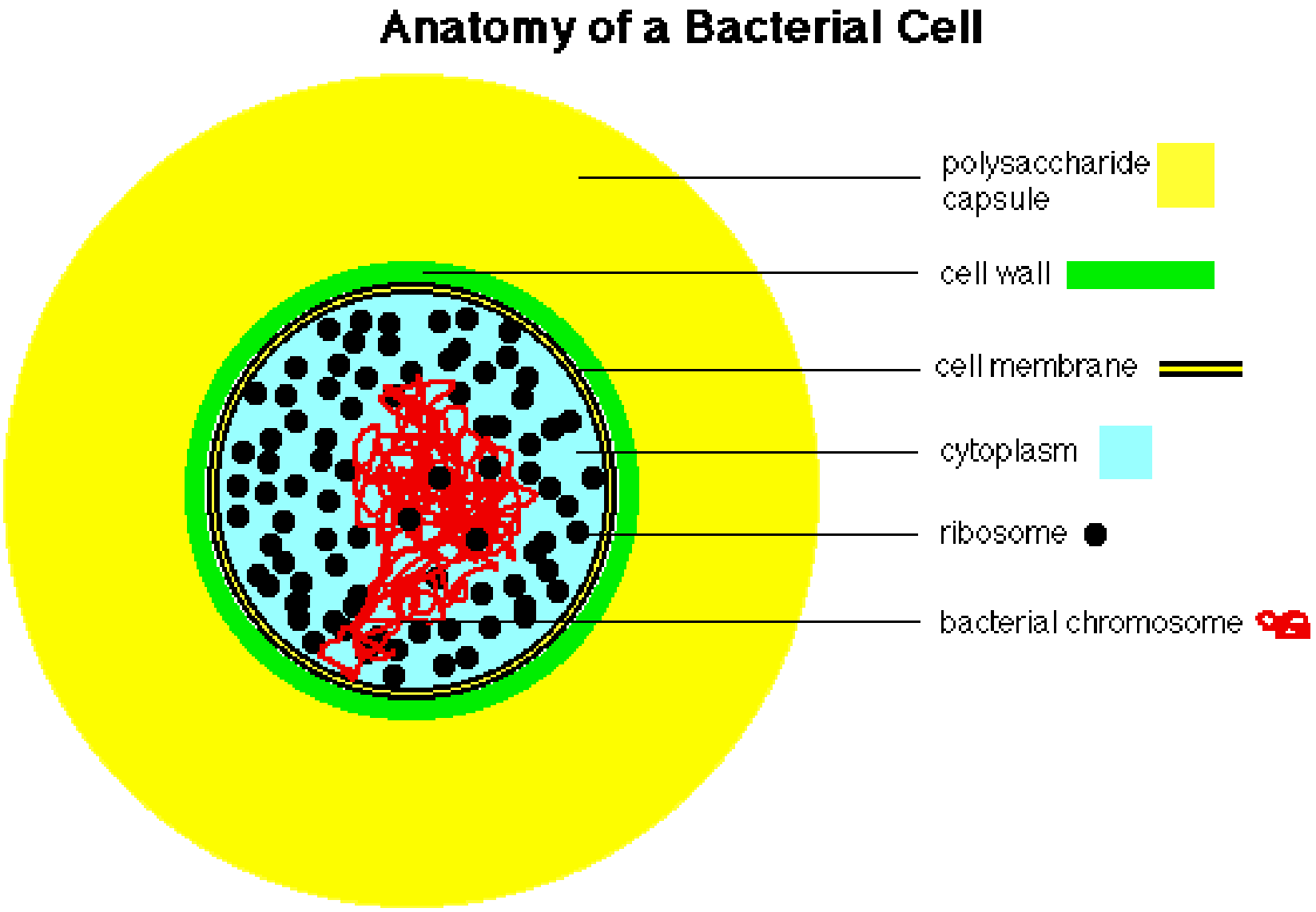
✓*The bacterial cell is coated with a mucous membrane (viscous layer) from the outside – glycocalyx*

✓*Capsule protects bacteria from environmental damage – dryness*

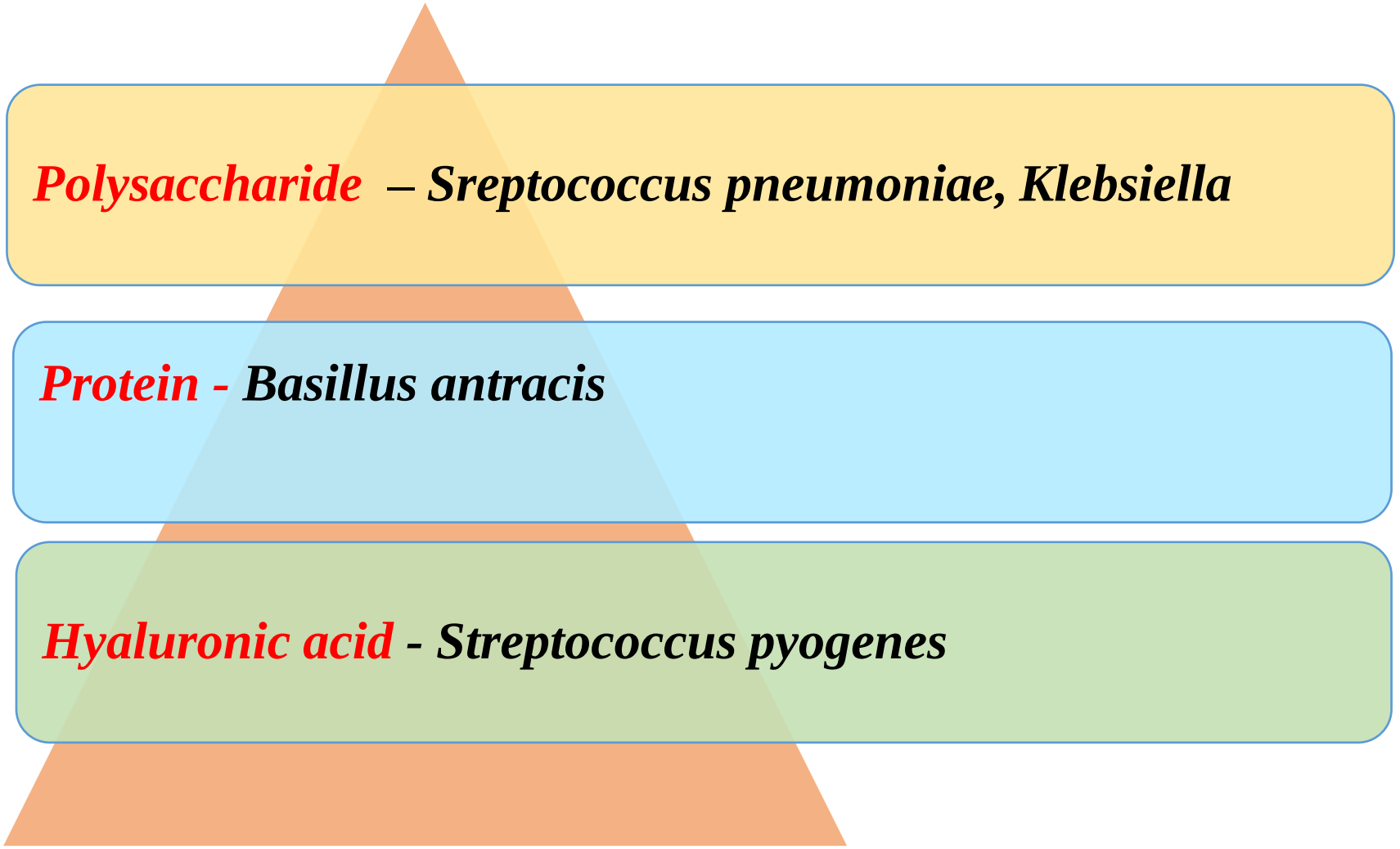
✓*The capsules that bacteria produce in the human and animal organism protect them from the effects of phagocytes (antibodies)*

✓*Since the capsule is antigenic in nature (K-antigens), antibodies are formed in the body against it*

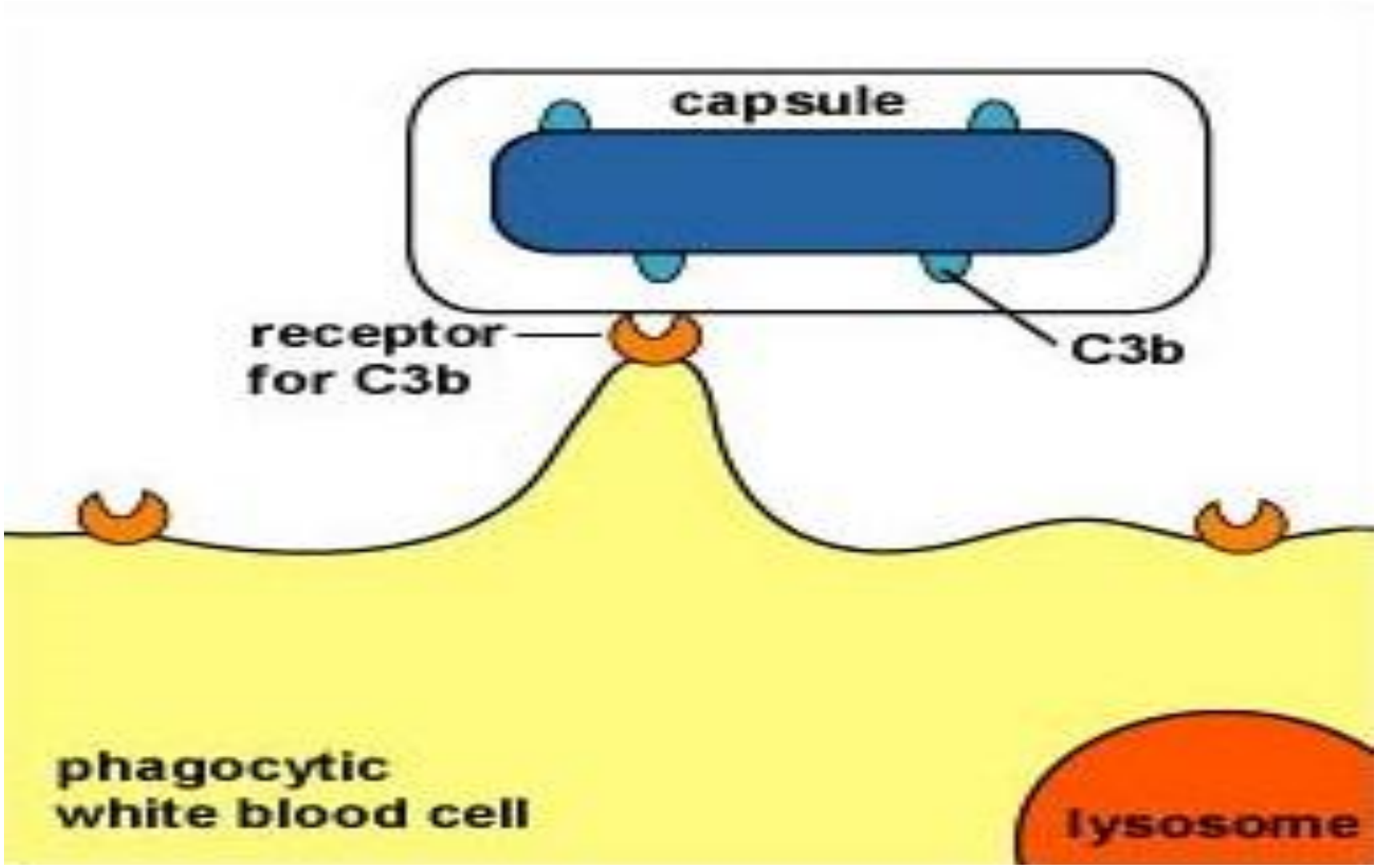
Structure of Capsule



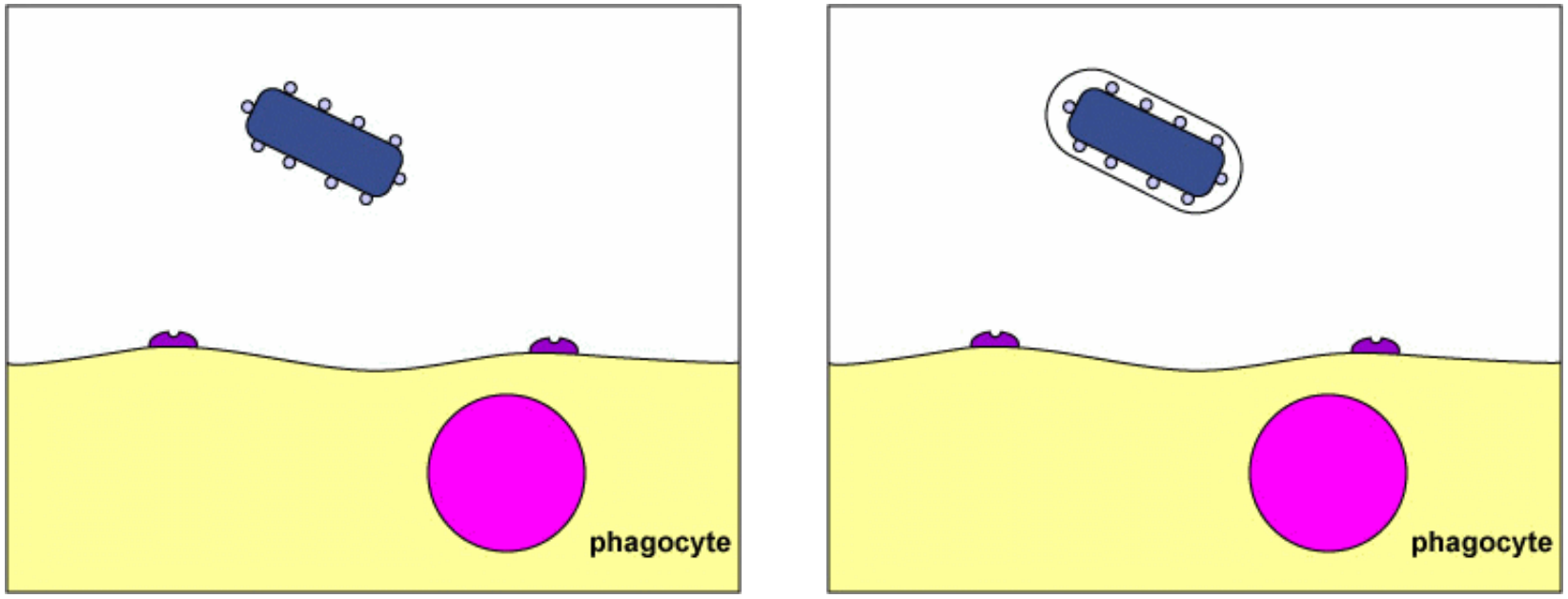
Chemical composition of the capsule



Capsule as a factor of pathogenicity



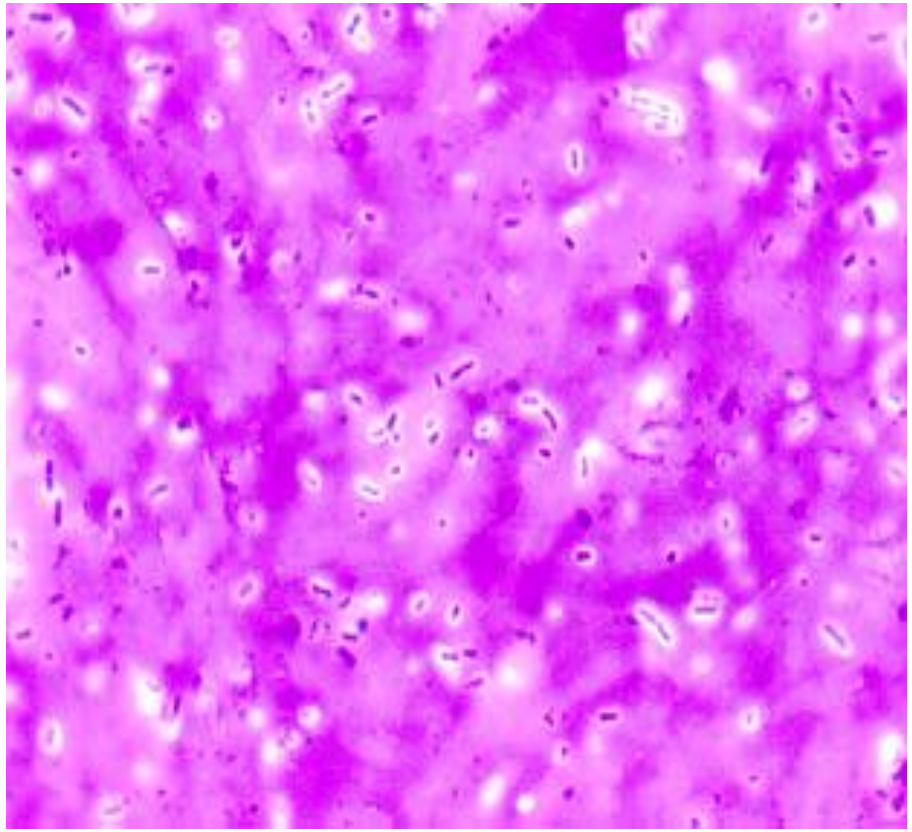
Capsule Protects Against Phagocytosis

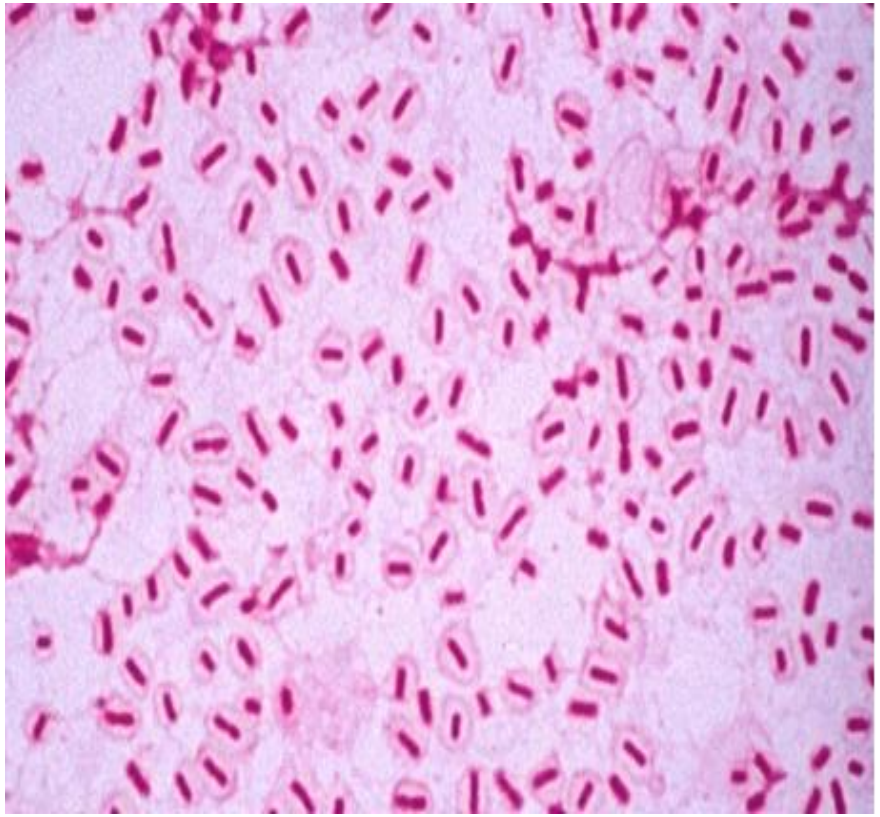


Gins-Burry staining for detection capsule

- Due to the poor perception of color solutions, the capsule can be distinguished by a special staining method - Gins-Burry
  A drop of 1:9 drops of india ink and bacterial suspension are added to one side of the slide. Take another clean slide, and holding at an angle of about 45 deg., touch the smear with one end of the slide so the smear runs along the edge of the slide
  Allow the slide to dry in air and fixated using a chemical method (Nikifirov solution)
- Then add the Pfeiffer fuchsin, wait 3-5 minutes, wash, dry and view smear under the microscope

Gins-Burry staining






Negative
Stain