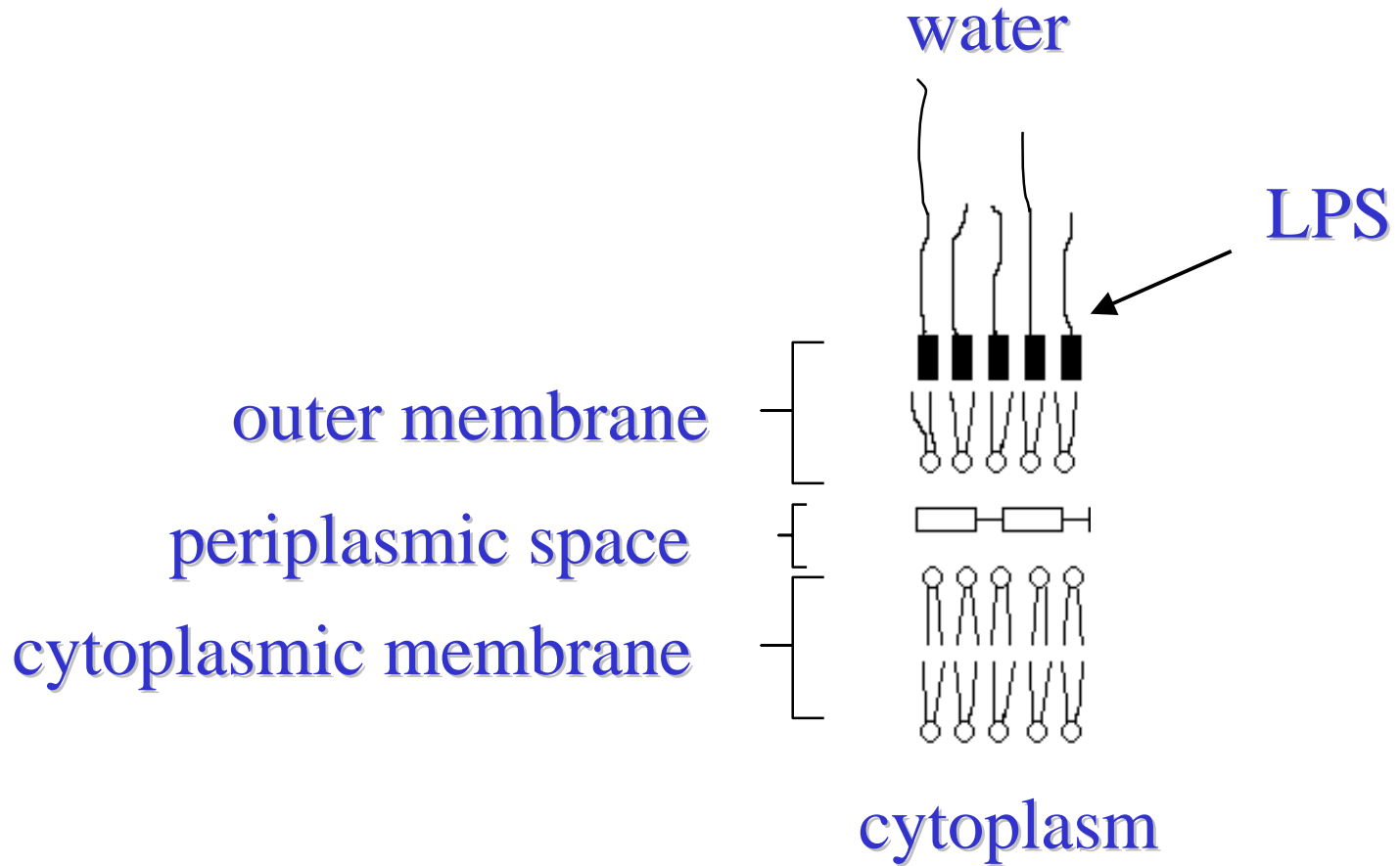


## Endotoxin analysis (LAL test)

- Limulus Amebocyte Lysate (LAL) test for Gram-negative bacterial endotoxins (lipopolysaccharides)
- Enzymatic reaction to detect a bacterial cell component
- Measure of rate of increase in turbidity - sensitivity is 0.001 Endotoxin Units/ml (approximately 0.1 pg/ml)
- Titer a sample to an endpoint indicated by gelation of the reaction mixture - sensitivity is 0.03 EU/ml

# Gram-Negative Cell Wall



# Lipopolysaccharide

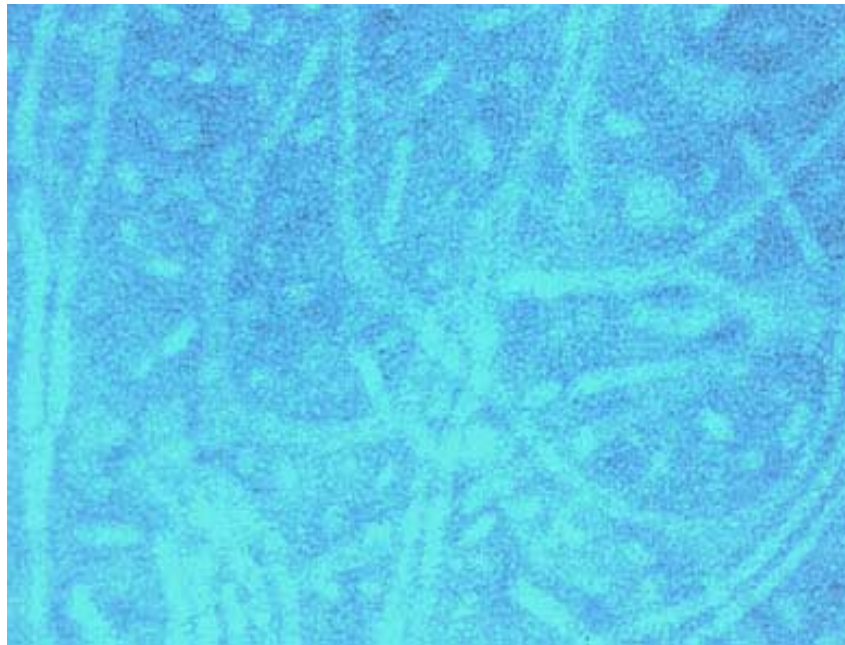
- Integral part of cell wall
- Pieces of wall or outer membrane released during normal growth
- Wall fragments released on cell death and lysis
- Design experiment to measure total endotoxin per mL of water, or to measure cell-associated concentration

# Lipopolysaccharide (LPS)

- Amphipathic (hydrophobic & hydrophilic)
- Hydrophobic end is negatively charged
- Is an organic molecule
- Therefore, will adsorb to surface materials and to particles (biofilm)
  - % in free-flowing water?
  - Rate of adsorption to collection container?

## How Big are LPS Molecules?

- in water, will be in membranes
- $10^6$  molecular weight



Electron micrograph of purified endotoxin

## LPS vs Total Count?

- Roughly 4 femptograms LPS/cell in highly purified water
- Roughly 40 femptograms LPS/cell in nutrient medium
- Somewhere in between in source water?
- Bacterial cell size fluctuates with nutrient concentration; size can change before cell numbers increase, but this is probably not detectable with present test sensitivities.

# Numbers, LPS, TOC

- Total counts of less than 10 cells/liter.
  - concentrate sample
  - statistically valid volume to concentrate?
- Most sensitive LAL method detects LPS from 30 cell/milliliter.
  - concentrate sample
  - assume detectable measurement is indicative of system failure
- TOC measurements of 3-4 ppb in product water
  - typically “less than” values
  - response time to detect change?

## Old Issues Still Here

- Sampling - biggest issue?
- Detection of very low numbers
- Detection of some cellular component in very low concentration
- Cause and effect