## Bacterial Cell Walls

- Prokaryotes and Cell Walls
  - Outside plasma membrane
  - Provides shape and protects cell from osmotic breakdown
  - Exceptions: mycoplasma and certain archaea

- Bacteria and Cell Walls
  - Peptidoglycan makes the wall rigid
    - Only bacteria have peptidoglycan
  - Peptidoglycan structure
    - Glycan chains of disaccharides held together by tetrapeptide chains
    - Two sugars: NAG and NAM
    - Layers of spirals
    - Covalent bonds with peptide chains
  - Peptidoglycan Crosslinks
    - Contains D-isomers of amino acids and diaminopimelic acid (DAP)
    - Direct connection or peptide interbridges
    - site of action of Penicillin!
      - attacks peptidoglycan crosslinks to weaken the cell wall
      - kills bacteria

- Gram Positive vs Negative Cell Walls
  - Positive
    - Inner plasma membrane
    - Thick peptidoglycan
      - Around 30 sheets!
      - Permeable
    - Teichoic acids
      - Sugars attached to phosphate groups
      - Covalently attached to peptidoglycan via NAM
      - Gives peptidoglycan a negative charge
      - Triggers inflammation
  - Negative
    - Outer and inner plasma membrane
      - Outer membrane contains lipids, lipoproteins, and LPS
    - Thin peptidoglycan
    - Lipopolysaccharides (LPS)
      - Consists of O side chain (protection from host immune system), core polysaccharide (sugars that make cell surface negative), and Lipid A (embedded in membrane; acts as endotoxin)
  - Gram Staining
    - Difference occurs during decolorizer phase
      - Thick peptidoglycan of gram-positive bacteria prevents crystal violet dye from leaking out
      - Alcohol/alcohol acetone creates holes in the outer membrane of gram-negative bacteria, causing crystal violet dye to leak out

- Acid-Fast Bacteria
  - Atypical cell walls
    - Structure resembles Gram-positive bacteria but does not stain consistently
    - Waxy lipid outer layer
    - Contains mycolic acids
      - Long chain of fatty acids covalently bonded to peptidoglycan
      - Hydrophobic
  - Actinomycetes
    - Mycobacteria, diphtheriae, nocardia
  - Difficult to kill
    - Repels antibiotics, enzymes, and oxidizing chemicals

- Acid-Fast Stain
  - Step 1: Primary Dye
    - Carbofuchsin (pink)
    - Uses heat to stain (steam bath)
    - No mordant
      **Acid Fast Bacteria**
      **Non-Acid-Fast Bacteria**
  - Step 2: Decolorizer
    - Acid alcohol
    - Removes stain from non-acid-fast bacteria
      **Acid Fast Bacteria**
      **Non-Acid-Fast Bacteria**
  - Step 3: Counterstain
    - Methylene blue
    - Stains non-acid-fast bacteria
      **Acid Fast Bacteria**
      **Non-Acid-Fast Bacteria**



## Cellular Structures in Bacteria

- Overview
  - Nucleoid region, ribosomes, macromolecules, cytoplasm
  - No membrane enclosed organelles!

- Ribosomes
  - Consists of RNA and proteins
  - Site of protein synthesis
  - Small than eukaryotic ribosomes
    - Prokaryotic: 70S, Eukaryotic: 80S
    - Important because this is how scientists make drugs that specifically target prokaryotes and leave our eukaryotic cells alone!
    - Composed of 30s and 50s subunits
  - Target of antibiotics
    - Example: streptomycin

- Nucleoid Region
  - Most bacteria only have 1 chromosome
    - Closed, circular, double-stranded DNA
    - Coiled and looped
    - Contains nucleoid proteins
  - Composed of DNA, RNA, and proteins

- Plasmids
  - Small, circular, nonessential pieces of DNA
  - Genes on plasmids carry advantages
    - Example: drug resistance
  - Separate from the chromosome
  - Allows exchange of genetic info between different cells
  - Applications in biotech
    - Example: cloning

## Staining

- Staining
  - Staining = coloring key structures of a microbe using a dye
  - Smear = thin film containing a solution of microbes fixed and attached to a slide
    - Fixing the microbes usually kills them ☹
- Staining Basics
  - Simple Stain
    - Uses one basic stain, so the cells are all the same color
    - Increased contrast between cells and background
  - Steps for a Simple Stain
    - Smear -> air dry -> fixing (using heat) -> stain -> rinse -> dry -> examine
  - Basic Dyes
    - Positive charge
    - Bonds to negatively charged parts of the cell
  - Acidic Dyes
    - Negative charge
    - Bonds to positively charged parts of the cell
  - Mordant
    - Maintains the integrity of the stain
    - Enlarges specimen
- Shapes and Arrangements of Bacteria
  - Shape #1: Cocci (Coccus)
    - Can be singular, pairs, chains, tetrads, and clusters
    - Spherical
    - Example: Streptococcus (chain of spherical-shaped bacteria)
  - Shape #2: Bacilli (Bacillus)
    - Rod-shaped
      - Can also be coccoid shaped
      - Curved rods = vibrios
    - Singular or short chains
    - Example: Bacillus anthracis
  - Shape #3: Spirilla (Spirillum)
    - Spiral-shaped (like a corkscrew pasta noodle 😉)
    - Flexible helix = spirochete
    - Example: Heliobacter pylori
  - Arrangements
    - One plane of division = pairs and chains
      - Pairs = diplo (e.g. diplococci)
      - Chains = stepto (e.g. streptococci)
    - Two-three planes of division = tetrads and cubical packets
    - Random planes of division = clusters
      - Clusters = staphylo (e.g. staphylococcus)