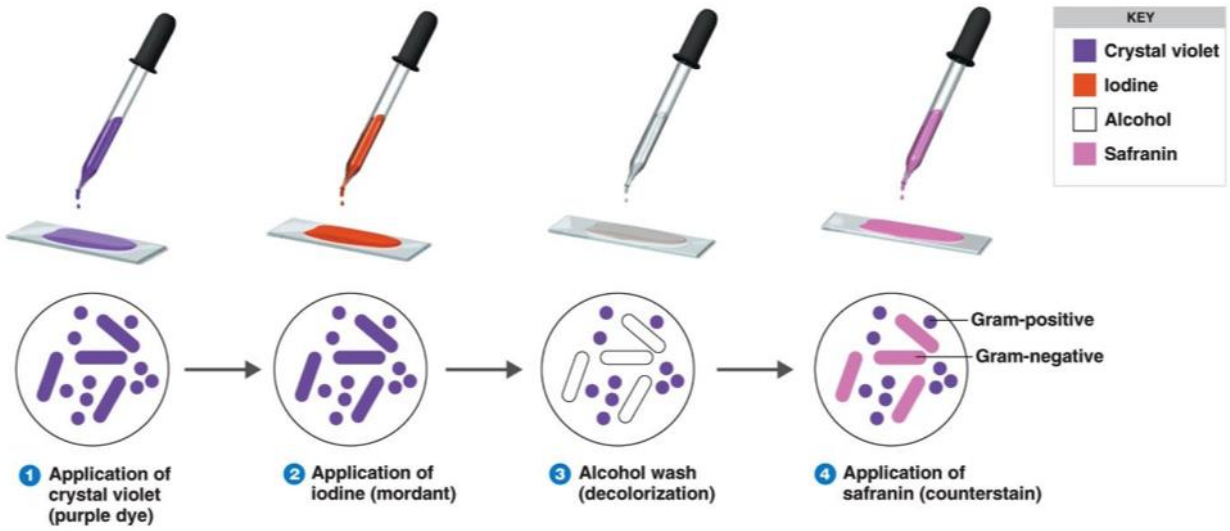
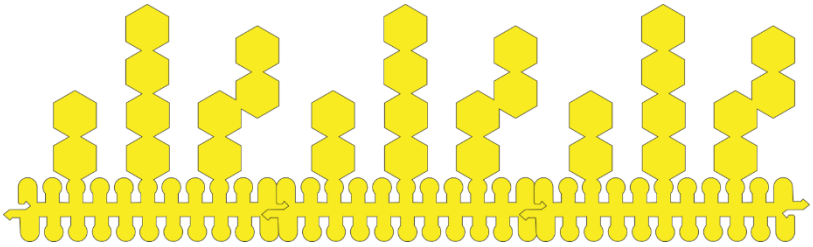
# Bacterial Membrane Modeling Kit....

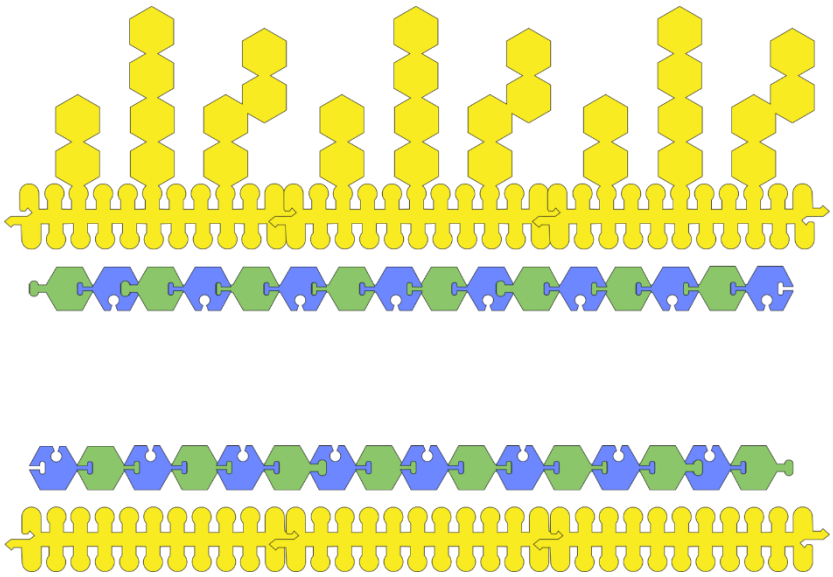
The bacterial membrane modeling kit has been designed to show the difference between gram-positive and gram-negative bacteria. Based on feedback from the Cohort I group; some new pieces have been developed to simplify the story.



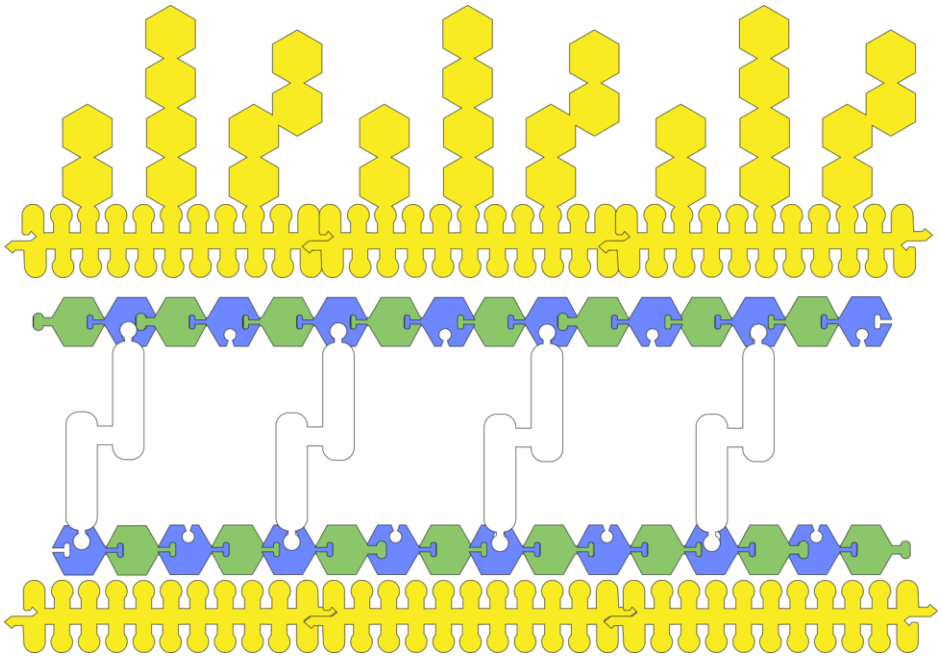

## Gram-negative, Simplified Quick Setup.



Begin by assembling the cell membrane (CM) and the Lipopolysaccharide (LPS) layers. The LPS components now have different sugar structures to differentiate them from the phospholipid bilayer of the cell membrane.

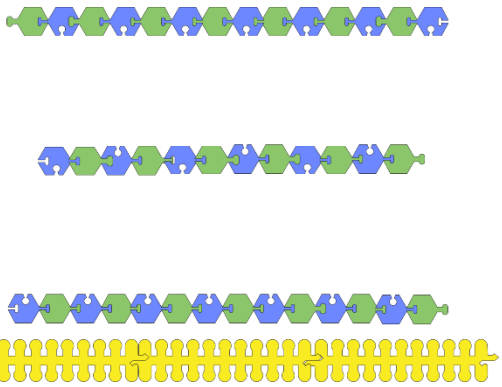


Begin to add the Peptidoglycan layer by adding the alternating sugars NAG *N-acetylglucosamine* (Green) and NAM *N-acetylmuramic Acid* (Blue).

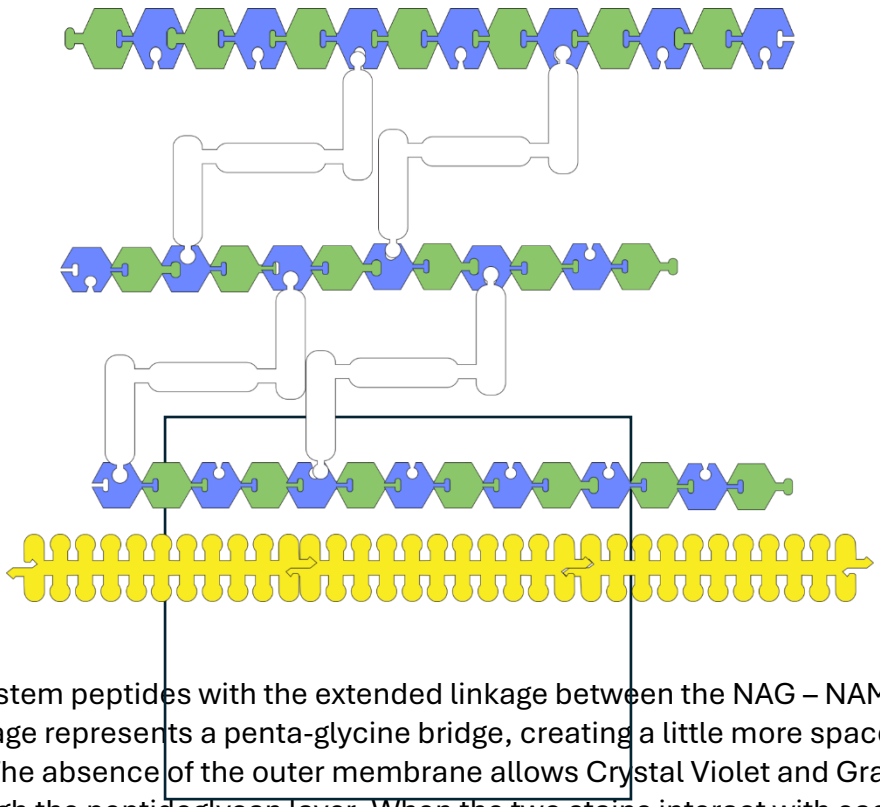


Add the Stem peptides with the **direct linkage**. You can discuss with your class that these linkages are formed by a PBP that is tethered to the inner membrane. During a gram stain, the LPS reduces the amount of Gram's Iodine and Crystal Violet that makes it to the Peptidoglycan layer. When alcohol is applied, the outer membrane is disrupted, allowing the safranin to penetrate the cell, causing it to appear pink under a microscope.

## Gram-positive, Simplified Quick Set-up:



Add your sugars NAG *N-acetylglucosamine* (Green) and NAM *N-acetylmuramic Acid* (Blue). Note that the middle layer has the NAMs alternating the peptide binding site.

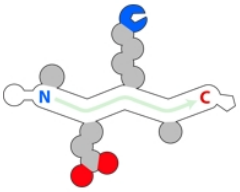


Next, add the stem peptides with the extended linkage between the NAG – NAM layers. The extended linkage represents a penta-glycine bridge, creating a little more space between the linkages. The absence of the outer membrane allows Crystal Violet and Gram's Iodine to move through the peptidoglycan layer. When the two stains interact with each other, they form a larger complex that cannot move out through the peptidoglycan layer. Alcohol causes the stem peptides to" collapse" as water moves out of the cell, further trapping the crystal violet / iodine complex. Safranin stain is then applied, but the dark purple color of the stain complex is all that can be seen through the microscope.

X20 – NAM – *N*-acetylmuramic acid - Carbohydrate

X20 – NAG – *N*-acetylglucosamine – Carbohydrate

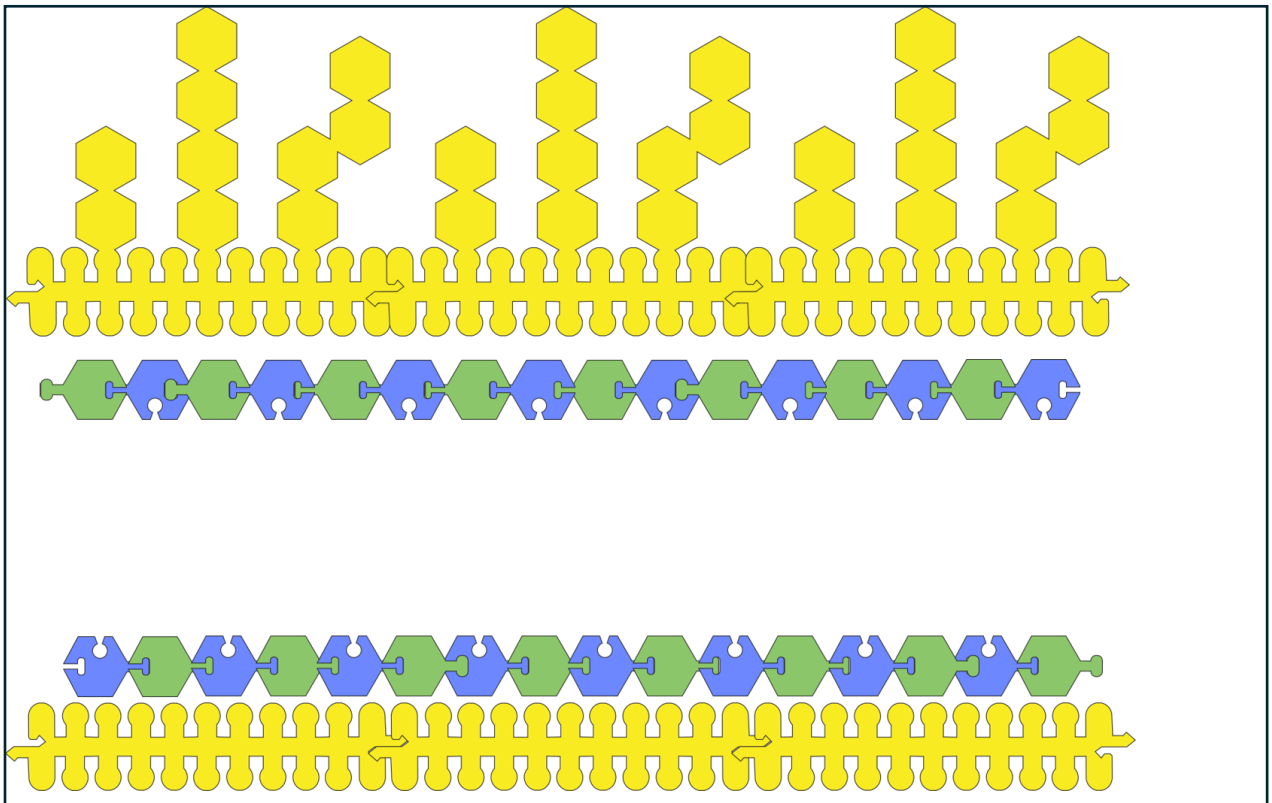

X8 – D-Alanine




X8 – Peptide stem (A-E-K-A or D or L -ala, L-Glu, L-Lys, D-ala)

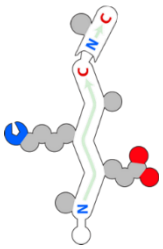

X4 – Pentaglycine bridge

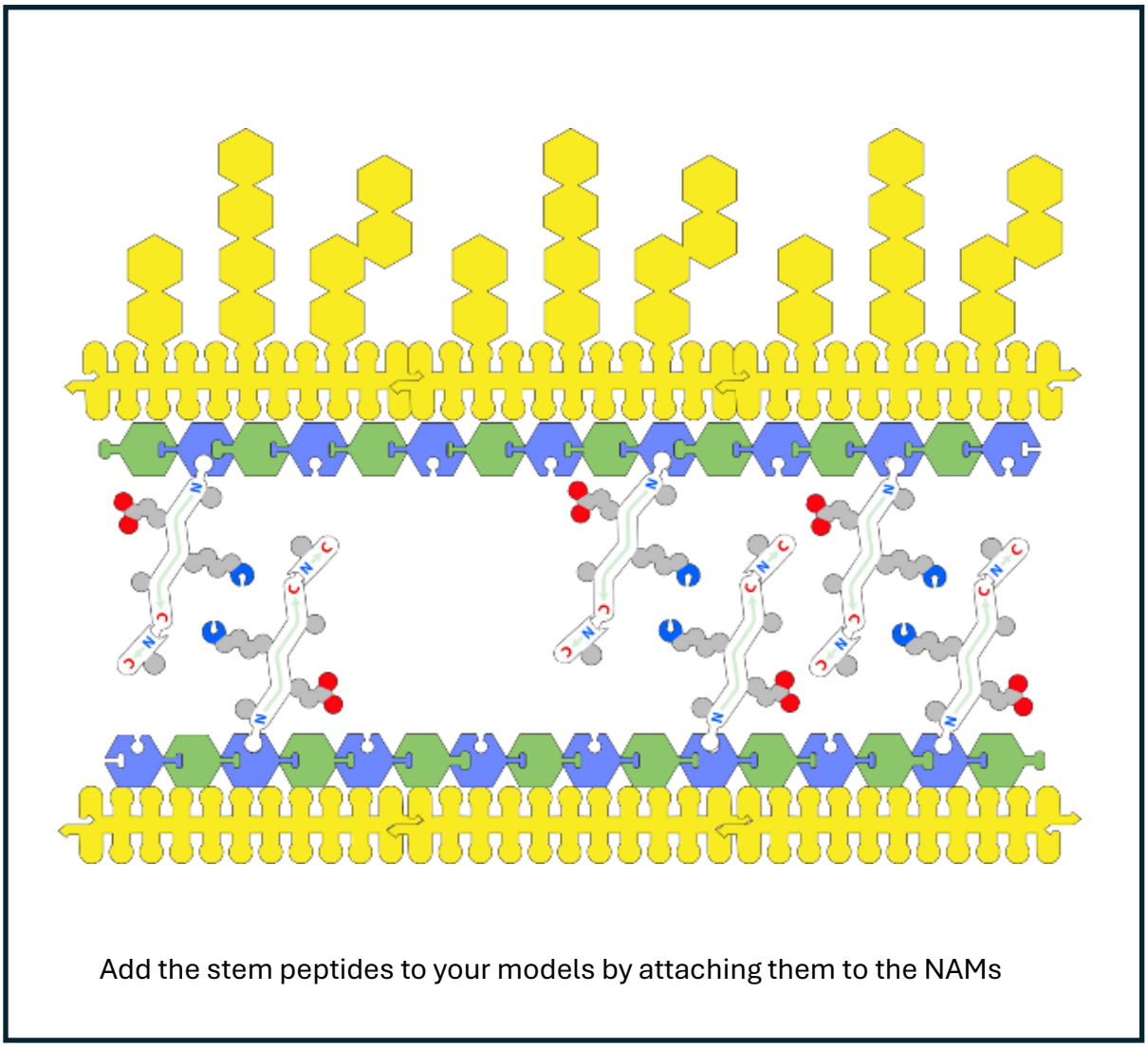
## Gram-negative – Advanced Modeling



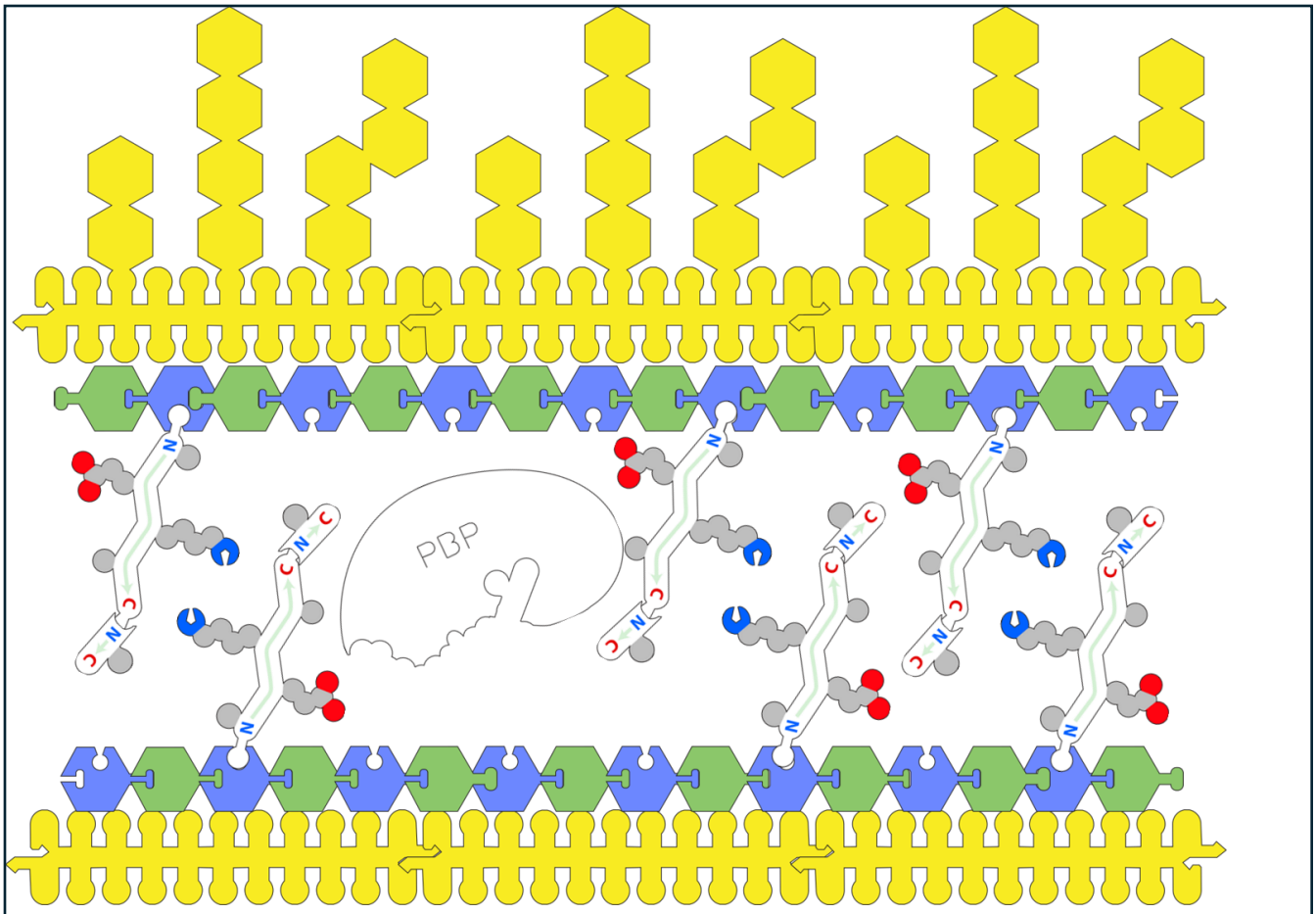
Follow the instructions above to model the CM, LPS, and start of the peptidoglycan layers.
* Note that the NAG-NAM sugars are moved across the membrane, linked together in pairs, and added to a growing strand of alternating NAG-NAMs.

Next, have your students build four stem peptides.
The two amino acids at the C-terminus of the peptide stem are D-Ala, D-Ala.



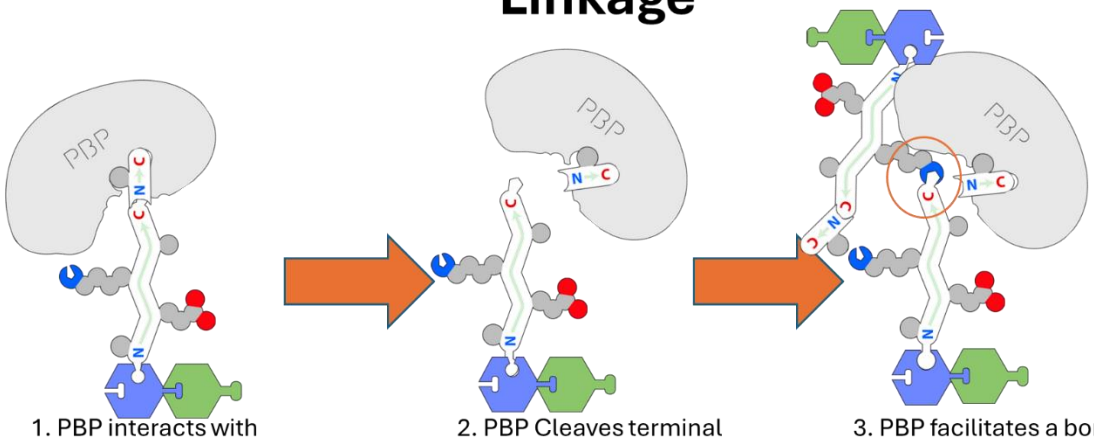


Add the stem peptides to your models by attaching them to the NAMs




Add your Penicillin Binding Protein (PBP)

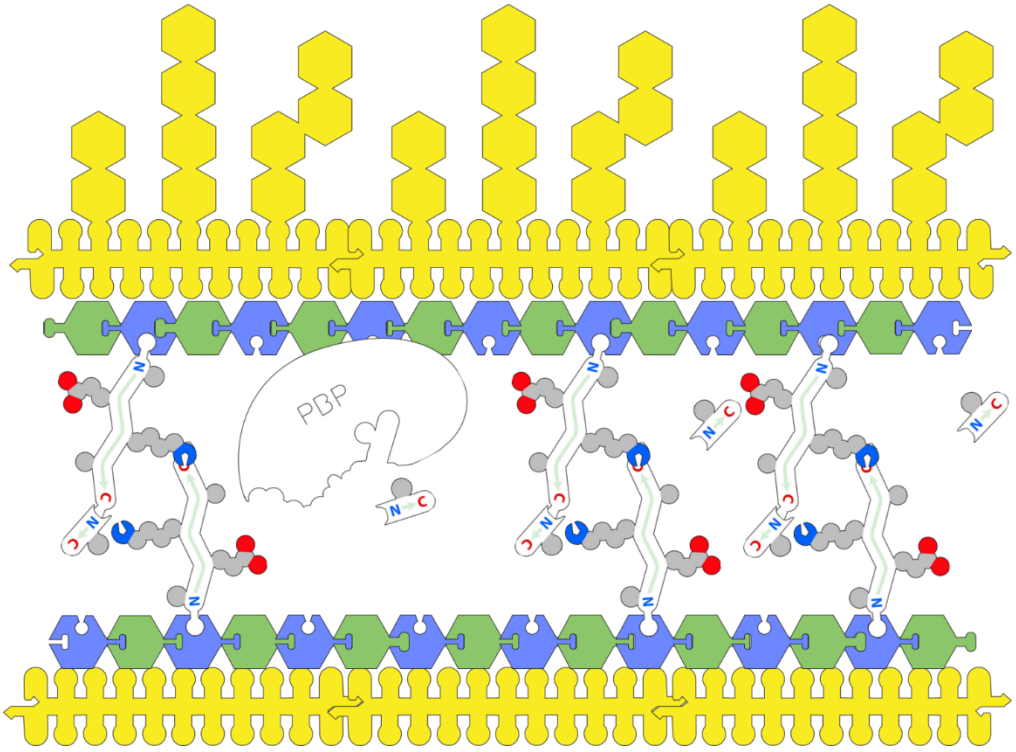
# Linkage




1. PBP interacts with the terminal D-ALA of Pentapeptide

2. PBP Cleaves terminal D-ALA, leaving a Carboxyl group

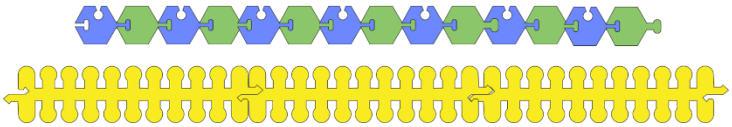
3. PBP facilitates a bond between the Amine group of 3rd residue (LYS) on the pentapeptide from another NAM to the exposed Carboxyl on the tetrapeptide side.




The D-Ala D-Ala transpeptidase domain of the Penicillin Binding Protein facilitates the Lysine sidechain in position three of one stem peptide to form a peptide bond with the new carboxyl end of the cleaved stem peptide. Alternatively, students could model all three states of the stem peptides.

Challenge your students to model the staining process.

## Gram-positive – Advanced Modeling



Start with a membrane and three layers of NAG-NAM components. Notice that the middle layer has NAM with the stem peptide attachment point alternating up and down.

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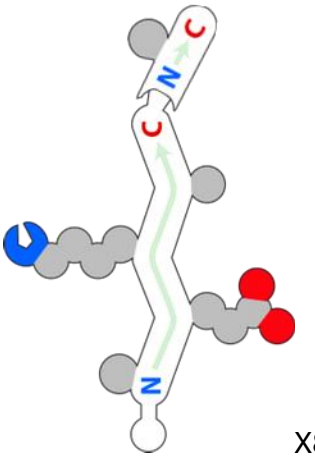


X8

Have your students build eight stem peptides.

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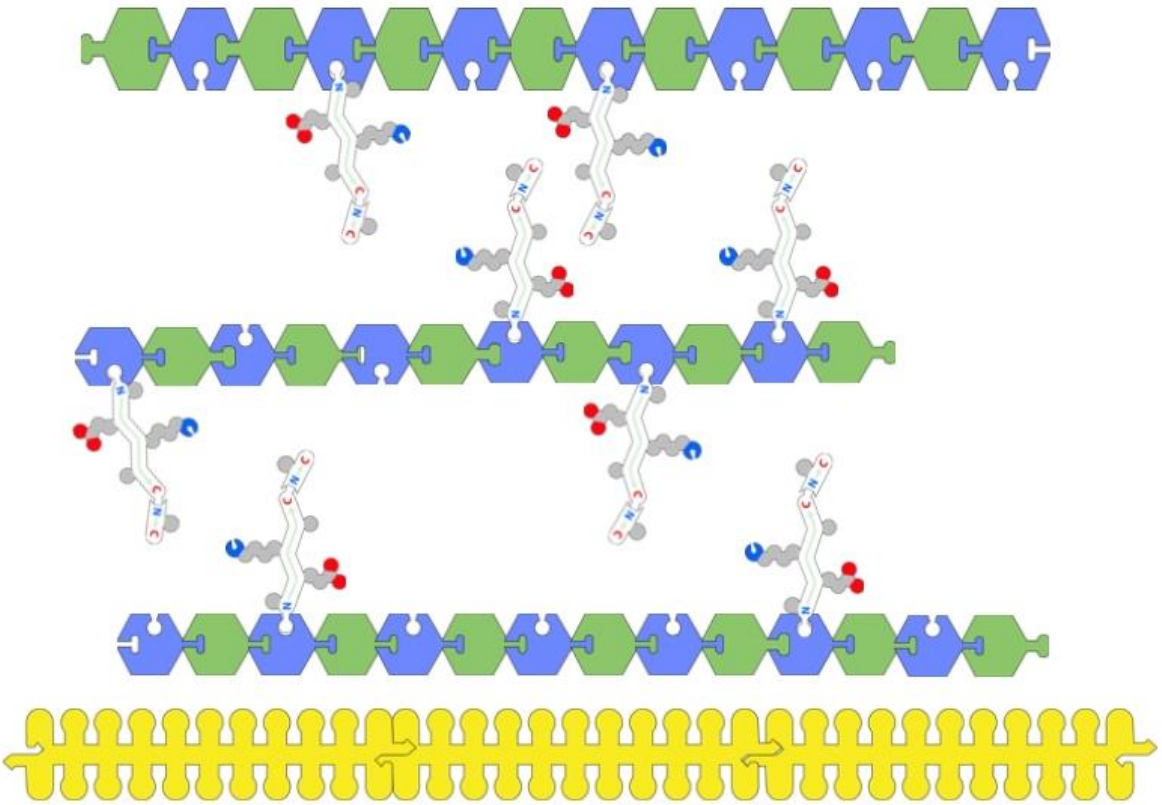
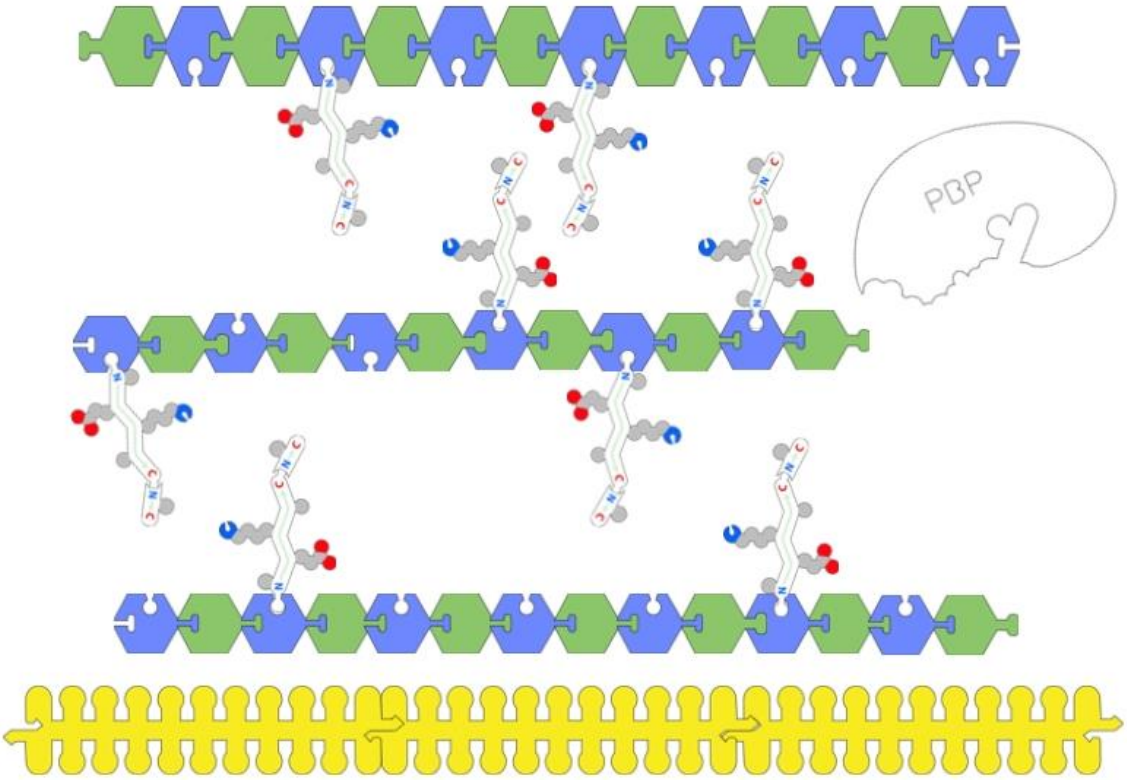


Connect the stem peptides to the NAM components of the peptidoglycan layer.




Add the PBP (Penicillin Binding Protein). Note: PBP is tethered to the cell membrane.

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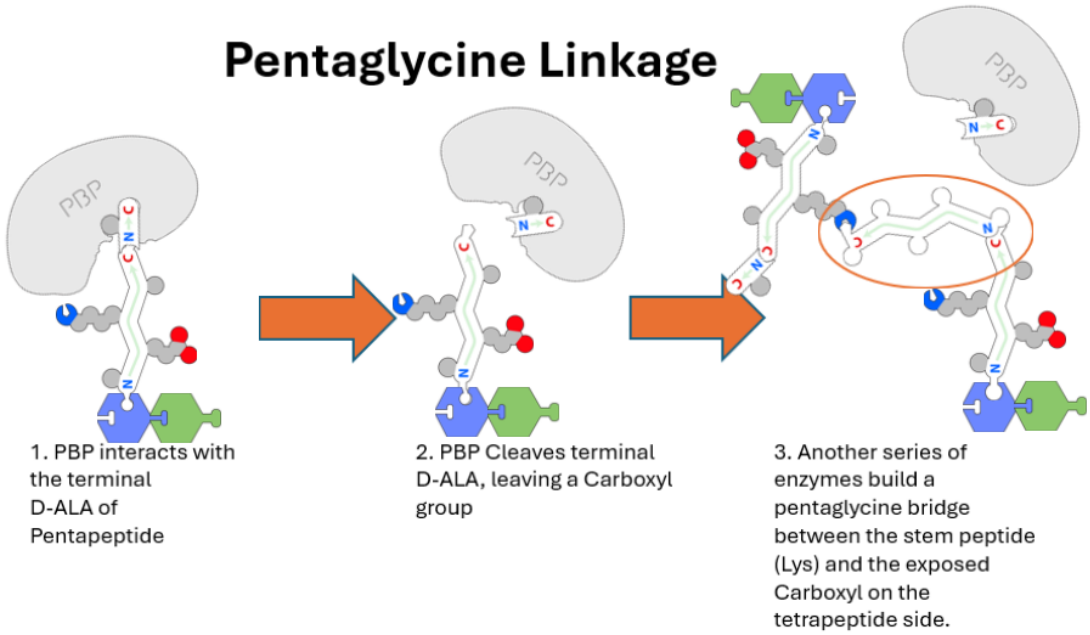

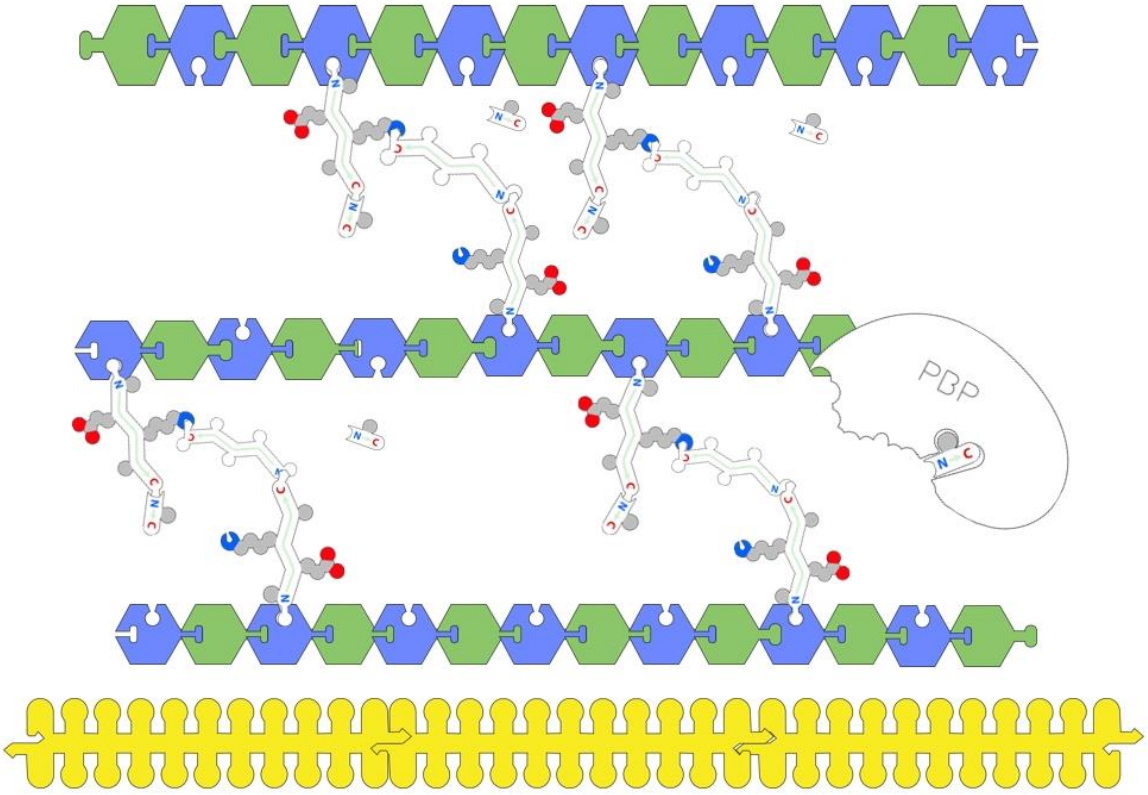


PBP will cleave the terminal D-Ala from the stem peptides, exposing a new carboxyl group. Another series of enzymes will build a pentaglycine bridge between the Lysine on an adjacent stem peptide to the newly exposed carboxyl group on the cleaved peptide.

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Challenge your students to explain the process of gram staining and incorporate what they now know about the bacterial membrane structure.