The Gram stain works the way it does due to the differences in the structure of the bacterial cell wall.

- This cell wall is composed of sugars and amino acids – called peptidoglycan.
  - Its role is to protect the cell from lysis and so give the cell shape.
  - Peptidoglycan is a polymer of N-acetyl glucosamine and N-acetyl muramic with side chains of alternating D and L amino acids.
  - These D amino acids are useful targets for antibiotics.
  - Peptidoglycan is highly crosslinked meaning it will make the cell rigid.

- Gram positive cells contain large amount of another polymer named teichoic acid.
  - The right image is of a Gram positive cell wall.

- Gram negative cell walls contain a thin layer of peptidoglycan; this is acting as a permeability barrier.
  - The wall is also not highly cross-linked.
  - Once the bacteria are stained with crystal violet followed by iodine, an iodine-crystal violet complex is formed – it is thought that the addition of alcohol to decolourise results in pores of peptidoglycan to shrink and therefore remain a purple colour.
  - Gram negative cells also have lipopolysaccharides on the outer membrane of the cell.
  - The right image is of a gram negative cell wall/membrane.

- The Ziehl–Neelsen stain, also known as the acid-fast stain, is another staining technique used to identify acid-fast organisms such as Mycobacterium tuberculosis.
  - These acid-fast organisms have a waxy lipid in their cell walls, therefore they do not stain readily with the Gram stain.
  - The right image is an example of the Ziehl-Neelsen stain at 1000x magnification.

- The Auramine-Phenol stain is another stain used to identify acid-fast bacteria.
  - Although it is not as specific as the Ziehl-Neelsen, is more affordable and so is used more often as a screening tool.
  - The microbes fluoresce under a light microscope with this stain.
  - The image below shows the Auramine-Phenol stain at 40x magnification.