Alberts • Johnson • Lewis • Raff • Roberts • Walter

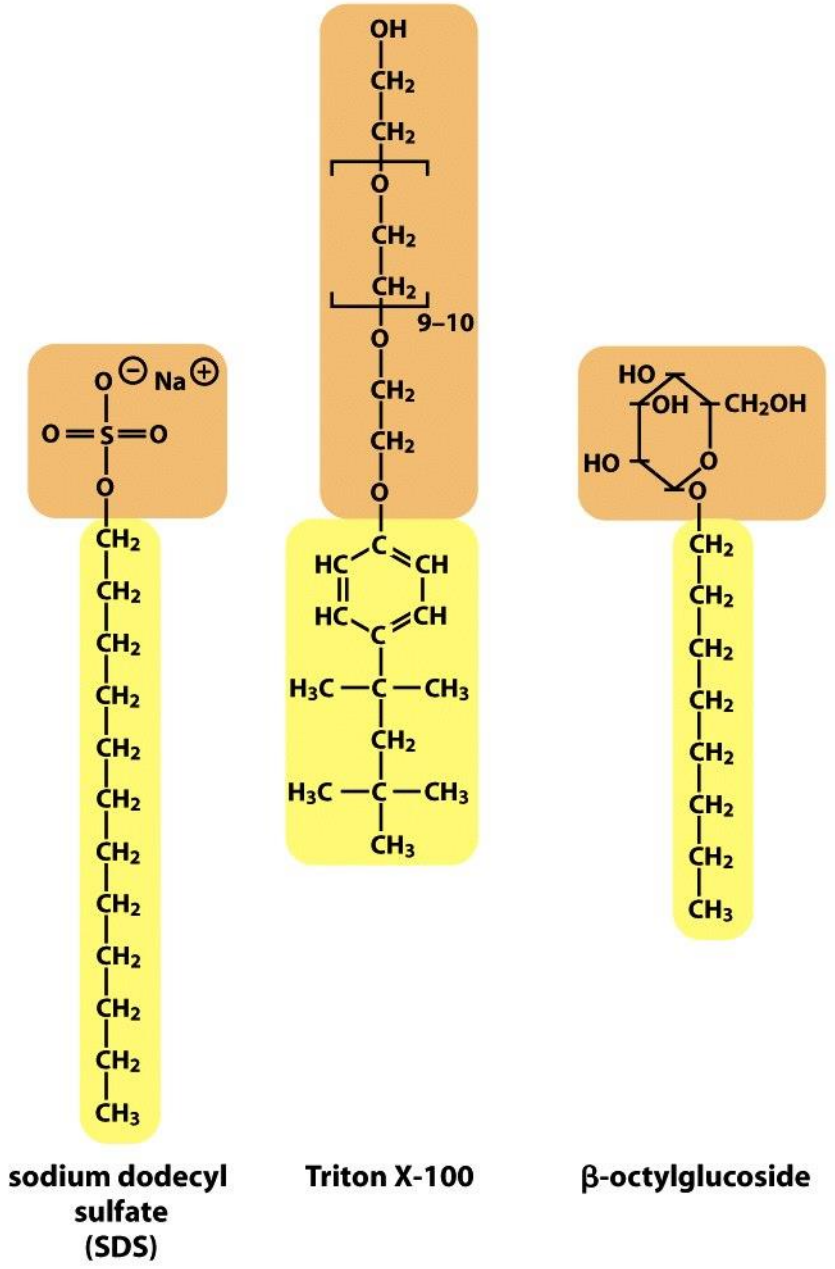
Molecular Biology of the Cell

Fifth Edition

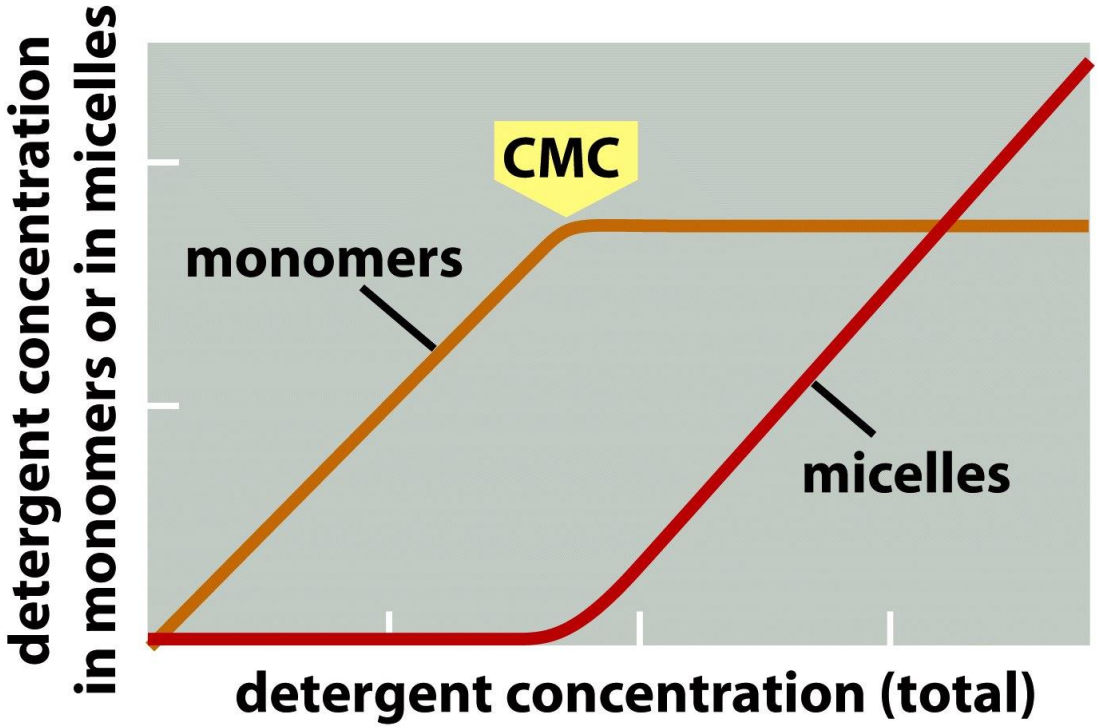
Chapter 10

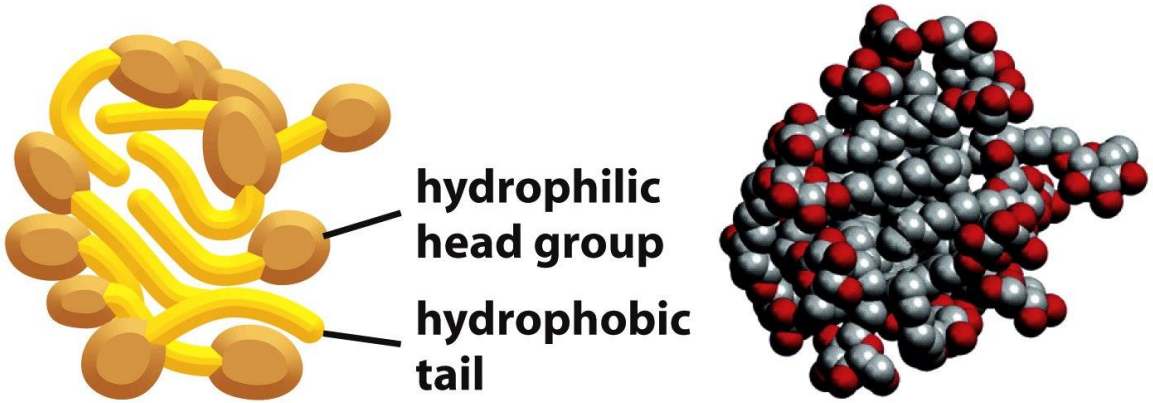
Membrane Structure

Detergents

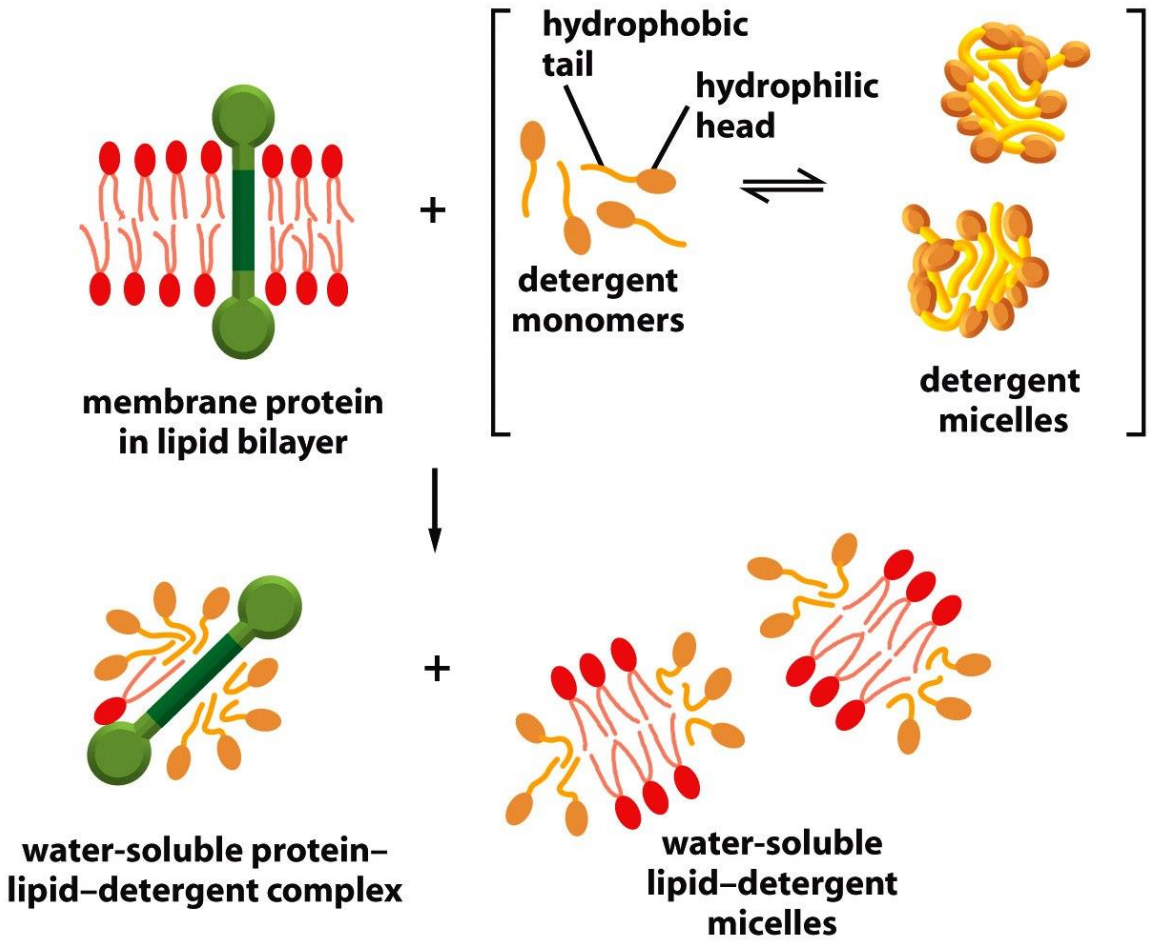


- In general, only agents that disrupt hydrophobic associations and destroy the lipid bilayer can **solubilize membrane proteins**.
- The most useful of these for the membrane biochemist are **detergents**, which are small amphiphilic molecules of variable structure.
- Detergents are much more soluble in water than lipids.
- Their polar (hydrophilic) ends can be either charged (ionic), as in sodium dodecyl sulfate (SDS), or uncharged (nonionic), as in octylglucoside and Triton





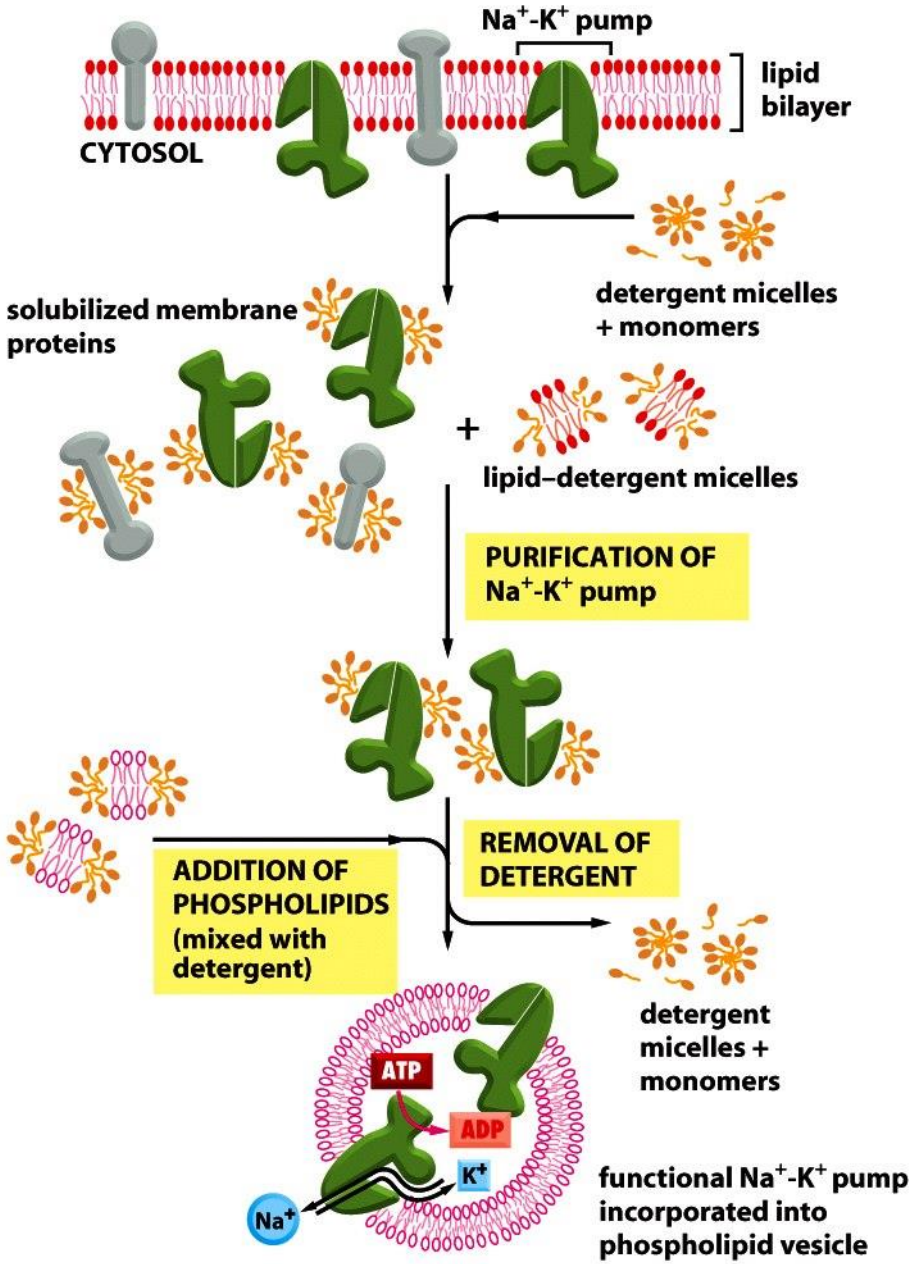
- At low concentration, detergents are monomeric in solution, but when their concentration is increased above a threshold, called the **critical micelle concentration (CMC),** they aggregate to form micelles.
- Above the CMC, detergent molecules rapidly diffuse in and out of micelles, keeping the concentration of monomer in the solution constant, no matter how many micelles are present.
- Both the CMC and the average number of detergent molecules in a micelle are characteristic properties of each detergent, but they also depend on the temperature, pH, and salt concentration.
- Detergent solutions are therefore complex systems and are difficult to study.



When mixed with membranes, the hydrophobic ends of detergents bind to the hydrophobic regions of the membrane proteins, where they displace lipid molecules with a collar of detergent molecules.

Since the other end of the detergent molecule is polar, this binding tends to bring the membrane proteins into solution as detergent–protein complexes.

Usually, some lipid molecules also remain attached to the protein.



- Strong ionic detergents, such as SDS, can solubilize even the most hydrophobic membrane proteins. This allows the proteins to be analyzed by SDS polyacrylamide-gel electrophoresis.
- Such strong detergents, however, unfold (denature) proteins by binding to their internal "**hydrophobic cores**," thereby rendering the proteins inactive and unusable for functional studies.
- Nonetheless, proteins can be readily separated and purified in their SDS-denatured form.
- In some cases, removal of the SDS allows the purified protein to renature, with recovery of functional activity.

- Many membrane proteins can be solubilized and then purified in an active form by the use of mild detergents. These detergents cover the hydrophobic regions on membrane-spanning segments that become exposed after lipid removal but do not unfold the protein.
- If the detergent concentration of a solution of solubilized membrane proteins is reduced (by dilution, for example), membrane proteins do not remain soluble. In the presence of an excess of phospholipid molecules in such a solution, however, membrane proteins incorporate into small liposomes that form spontaneously.
- In this way, **functionally active membrane protein systems** can be reconstituted from purified components, providing a powerful means of analyzing the activities of membrane transporters, ion channels, signaling receptors, and so on.