4.6: Non-Membrane Lipid Assemblies (Micelles)

Amphipathic compounds (detergents) are a unique set of molecules with the ability of manipulation (distortion or formation) of the hydrophobic-hydrophilic interactions in biological samples. Detergents in an aqueous solution self-associate to colloid particles. The formation of the colloidal aggregates by detergents are termed micelles[1]. In research micelle-forming detergents provide an amphipathic environment that can mimic lipid bilayers, be used to lyse cells (release soluble proteins), solubilize membrane proteins and lipids, and control protein crystallization.

Detergents and Critical Micelles Concentration

As mentioned above detergents are amphipathic molecules (Figure 4.6.1), which are composed of a hydrophilic head group (polar) and a hydrophobic tail (non-polar). The hydrophilic head group detergents can be either ionic (charged anion or cation), nonionic (uncharged), or zwitterionic (containing both positive and negative charged components to have a net zero charge).

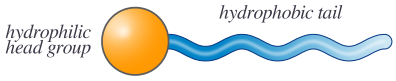

Figure 4.6.1: Amphipathic molecule. (CC BY-NC; Ümit Kaya)

The hydrophobic tail is an acyl chain (a long chain of aliphatic hydrocarbons, aka a carbon chain with hydrogen side groups). Its amphipathic properties allow detergents to dissolve both water and oil soluble compounds, such as soap [2]. In soap, particles insoluble in water (oil/fat) become associated with the hydrophobic tail of a micelle (Figure 4.6.2), shielding the insoluble particles from water, making it soluble. Detergents at low concentration in aqueous solution form a monolayer at the air-liquid interface. The detergents monomers can then begin to self-assemble into aggregates at and above the critical micelle concentration[3] (CMC) into structures called micelles (Figure 4.6.2). The viscosity of micelle core was found to be 17-50cP (cP: centipoise) by depolarization study of a fluorescence probe[4]. Thus, the interior is liquid like. But in some case, the interior is found to be more solid-like, with viscous rising to 151 cP. Basic micelles are generally described as roughly spherical (Figure 4.6.2A) with a separation of hydrophilic and hydrophobic parts.

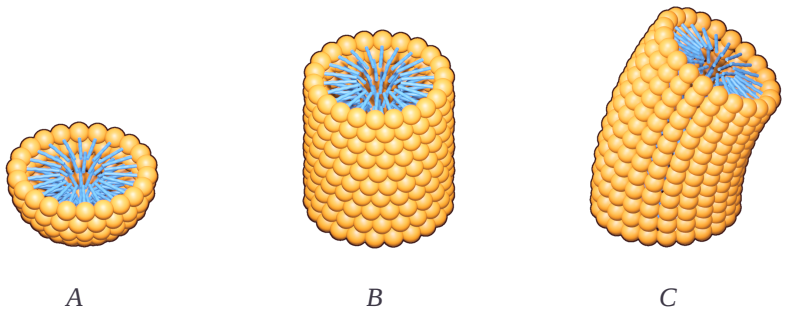

Figure 4.6.2: Ideal structure of a detergent micelle (A) spherical, (B) cylindrical, (c) worm-like. (CC BY-NC; Ümit Kaya)

Depending on the structure and physicochemical conditions, viz,. temperature, the presence of electrolytes, the self-aggregates structures can also be cylindrical (Figure 4.6.2B) or worm-like (Figure 4.6.2C). The detergent monomers bury the nonpolar tails by orienting them inward to avoid water contact and orienting the polar head groups outward to interact with the water. In detergents with more than one hydrophobic chain, similar to natural occurring lipids, the formation of cylindrical micelle may occur. The cylindrical micelle can then span into a two dimensions forming a bilayer.

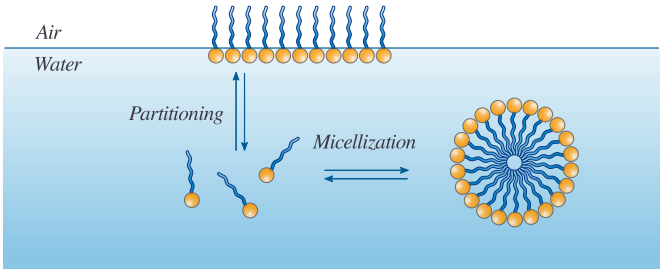

Figure 4.6.3: Schematic presentation of the equilibrium between detergents at a surface, free detergents, and in micelles. (CC BY-NC; Ümit Kaya)

Below the CMC, an increase of detergent will lower the surface tension while raising the osmotic pressure of the solution (Figure 4.6.4)[5]. This correlates to the lipids in the solution gathering almost exclusively at the air-water interface as shown below. At the CMC surface tension has been minimized to the greatest possible extent corresponding to the lipids fully saturating the air-water interface so that the entire surface is now a continuous lipid layer. Above the CMC, with an increase of detergent, the surface tension will stay constant because it is fully saturated. Thus the excess lipids will be forced into the water and will form micelles to protect their hydrophobic tails, which will then be able to dissolve previously insoluble compounds (like grease). The surface saturation process follows Langmuir (saturation) kinetics and can be described using a Langmuir isotherm.

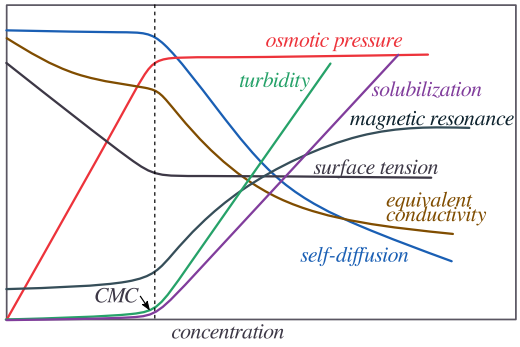

Figure 4.6.4: Changes in the concentration dependence of a wide range of physico-chemical quantities in the neighborhood of the critical micelle concentration. (CC BY-NC; Ümit Kaya)

Micelle formation can be described by the Gibbs adsorption isotherm [6]. The number of monomer detergents "n" to form micelles is called aggregation number and is characteristic of the surfactant molecule. Therefore, micellization can be described by an equilibrium (Figure 4.6.5) expression. With an equilibrium constant, $K_m=[Z_m]/[Z]^m$. A unitary free energy ΔG_m° for transfer of a single amphiphile from aqueous solution to a micelle of size *m*. The free energy expression can be calculated by replacing $RT\ln K_m$ by $-m\,\Delta G_m^\circ$, with concentrations expressed in mole fraction units. Therefore,

$$\ln K_m = \frac{-m\,\Delta G_m^o}{RT} + m\ln X_1 + \ln m \tag{4.6.1}$$

Where X_1 is the mole fraction of amphiphile in monomeric form and X_m is the mole fraction incorporated in micelles of size *m*, i.e, $X_m=m\,[Z_m]$. Equation 1 can be considered to be micelle size distribution function, giving X_m as a function of m at any given value of X_1. The cooperatively of micelle formation is reflected in the term $m\ ln\ X_1$, and limits the value of X_1 to a narrow range close to the CMC if reasonable values of X_m are to be obtained.

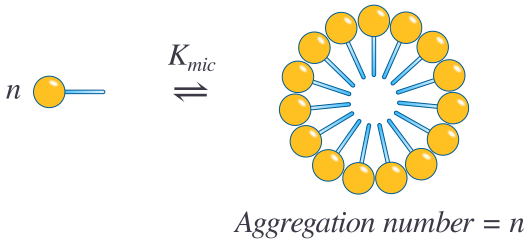

Figure 4.6.5: Micellization can be described by an equilibrium. (CC BY-NC; Ümit Kaya)

Over 70 types of methods have been deployed to determining the CMC from 1962-2010. Such as spectroscopic measurements (fluorescence probe, absorbance dye), electrochemical measurement (electrophoresis, capillary electrophoresis, conductimetric), surface tension measurements (contact angle measurements), optical measures (light scattering, optical fibers, refractro-metric), and many others[7]. Yet, the most common method is surface tension. This method determines the surface tension of solution towards several different concentrations. The turning points of a plot of the surface tension against log[C] (C: concentrations) graph is the CMC. Surface tension method commonly used because it's simple and convenient.

Research Applications

Detergents are crucial for the solubilization, purification, and crystallization processes needed for membrane protein structure determination[6]. Detergents are often used to solubilize important membrane proteins by generating a water-soluble protein-detergent-lipid complex (Figure 4.6.6 a). The detergents mimic the lipid membrane by surrounding the hydrophobic region of the integral membrane protein and leaving the hydrophilic region oriented toward the water. Maltosides and glucosides two of the most common used detergents for membrane protein crystallography, although there are is still a large variety of classes of detergents. Therefore, it's common to do a screening to determine the most suitable detergent for the desired target protein. For each detergent, varieties of hydrocarbon tail length are commonly available, allowing for fine tuning the size and stability of the protein-detergent-lipid complex.

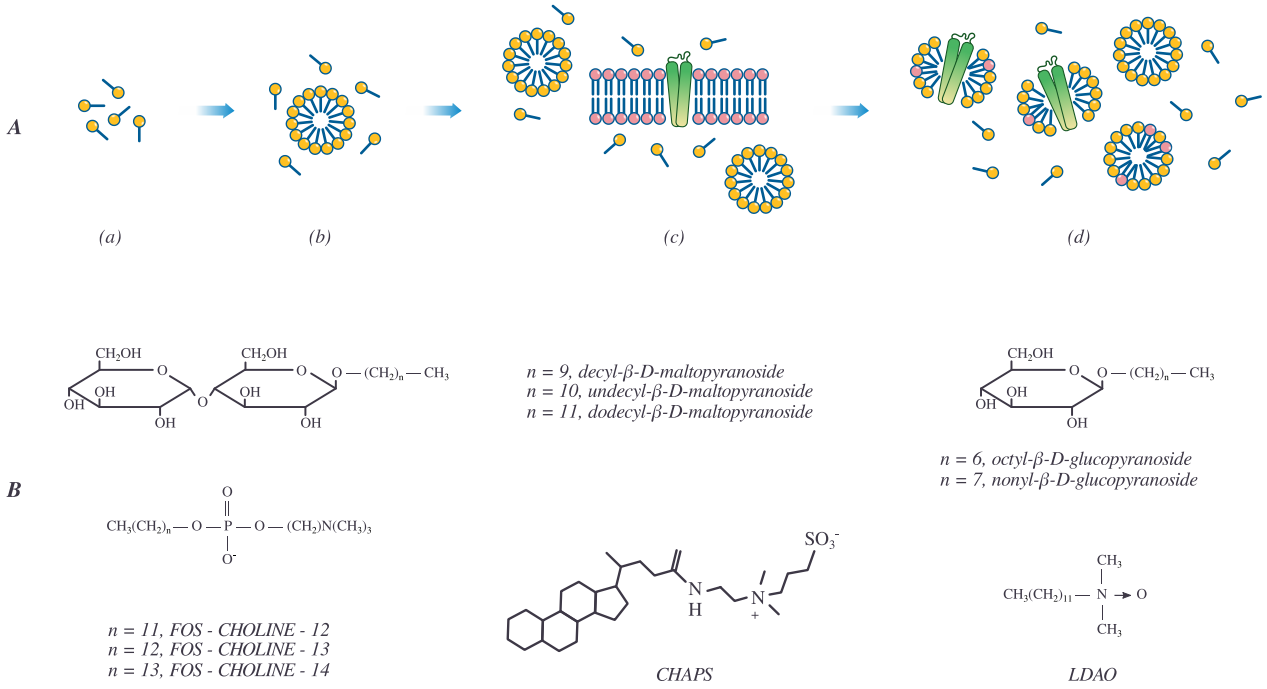

Figure 4.6.6: (a) schematic of the solubilization process. From left to right: free detergent monomers, detergent micelles above the CMC, a mixture of membrane and micelles, micelles extract membrane proteins from lipid bilayer to make a protein-detergent-lipid complex. (b) Some common detergents used in solubilization, purification, and crystallization of membrane proteins. (CC BY-NC; Ümit Kaya)

Once the integral membrane protein is solubilized and purified, it's necessary to concentrate (via. centrifugation and dialysis) to a supersaturated solution for crystallization to be successful. While concentrating it's important to maintain the CMC to ensure free

detergent micelles and retain the protein-detergent-lipid complex. If CMC is not retained, the integral membrane protein will aggregate out of solution. The detergent used during solubilization may be the same used while crystallization but is most commonly changed or an additive detergent is added. There are several physicochemical parameters to consider besides detergents for the crystallizing integral membrane proteins, such as buffers, pH, precipitants, salts, additives, and many others[8]. Over the past few years there has been an exponential increase of integral membrane protein structures published in the Protein Data Bank and many unique insights have been provided into successful conditions for membrane protein crystallization (Figure 4.6.7).

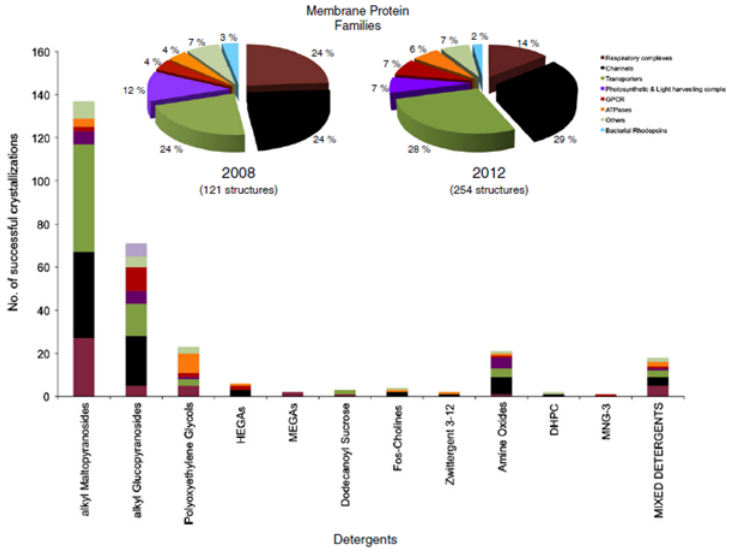

Figure 4.6.7: Alpha-helical membrane protein crystallization currents trends of successful detergents used[8].

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