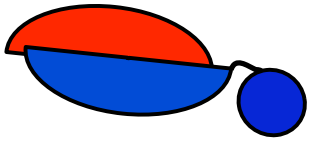
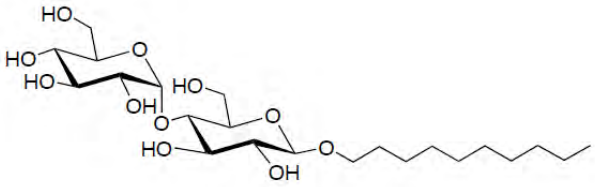


DETERGENTS: BIOPHYSICAL PROPERTIES & USES IN 2D CRYSTALLISATION



Michael Landsberg

Electron Crystallography Workshop
C-CINA, Basel, 1-7 Aug 2010

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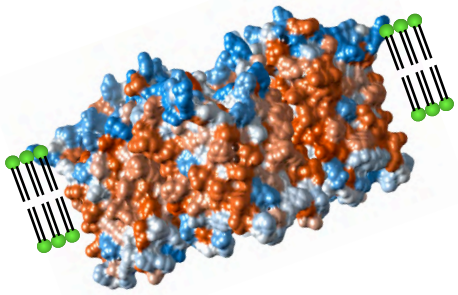


THE UNIVERSITY OF QUEENSLAND AUSTRALIA | IMB Institute for Molecular Bioscience | ammrf

Membrane Proteins (circa 2008)

FROM SWISSPROT
230,000+ protein sequences
~45,000 annotated as membrane proteins (20%)

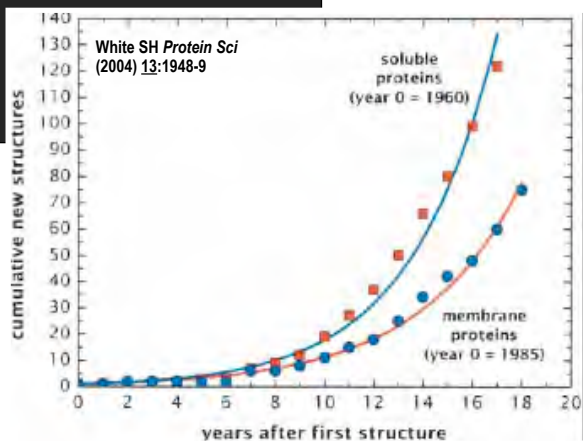
...AND GROWING
>3,400,000 non-redundant sequences in protein information resource
429/2175 completed/in progress genome sequencing projects

PROTEIN DATA BANK

~37,000 atomic models	~67000
~200 membrane proteins (0.5%)	~671

MEMBRANE PROTEIN STRUCTURES

~90% XRD	(~60%)
~3% NMR	(~3%)
~7% EM	(~37%)

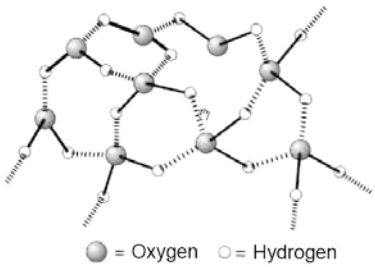


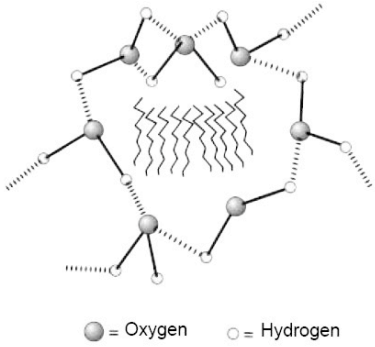
Membrane Proteins

- **The rate of membrane protein structure determination lags behind that of soluble proteins.**
- **Why?**
  - **Difficult to crystallize**
  - **Crystals don't diffract**
  - **Low/poor solubility**
  - **Unstable**
  - **Structural heterogeneity**
- **Structural studies of membrane proteins hampered by the fact that proteins have low solubility/stability outside their native environment**

Hydrophobicity

- **Hydrophobic residues are disruptive to the ordered H-bonding structure of water**

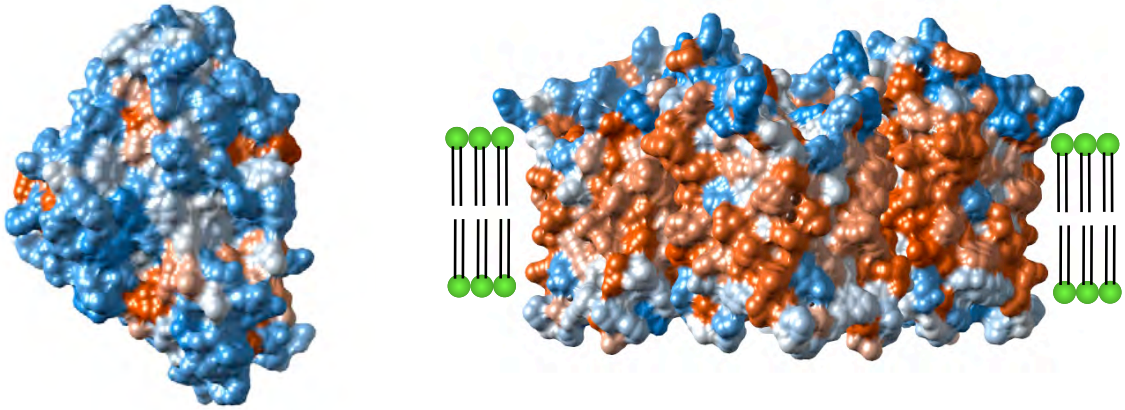




- **The "hydrophobic effect" drives e.g.**
  - **Protein folding**
  - **Detergent micelle formation**
  - **The formation of lipid membranes**
- **Aggregation of hydrophobic surfaces offsets the thermodynamically unfavourable effects of disrupting the H-bond network of water**

Soluble vs membrane proteins

- **Trans-membrane proteins are involved in critical cellular functions (e.g. channels, gates, signal transducers) which require that they span lipid membranes**
- **By having solvent exposed hydrophobic regions, these proteins are effectively held in the hydrophobic lipid bilayer.**

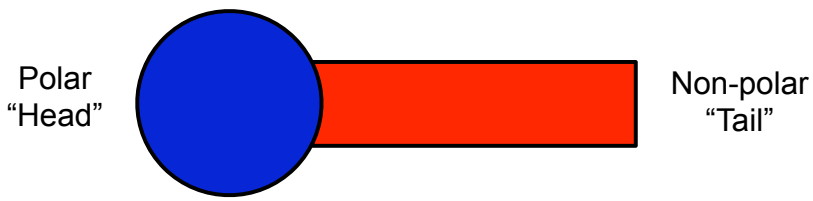


Amphihiles

- **a.k.a. amphipaths/surfactants/lipids(?)**
- **By definition**

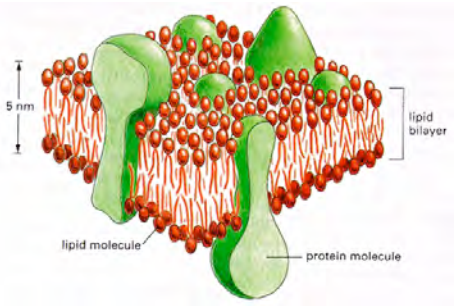
"Molecules which possess both hydrophillic and lipophillic properties simultaneously"

- **Critical to studies of membrane protein structure, function.**
  - **They are able to interact with membrane proteins and stabilise them outside of their native membrane environment**



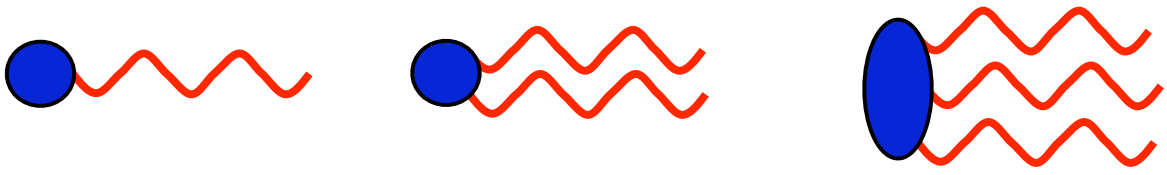
Amphihiles

Detergents Lipids Fats & Oils



Amphihiles

Detergents Lipids Fats & Oils



Water soluble Water insoluble Water insoluble
Form micellar structures Triglycerides

Overview

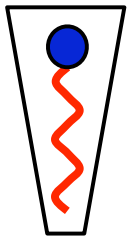
- Membrane proteins
- Amphiphiles
- Detergents
- Properties of detergents
- Classes of detergents
- Use of detergents in 2D crystallisation
- Working with detergents
- Novel detergent-like molecules

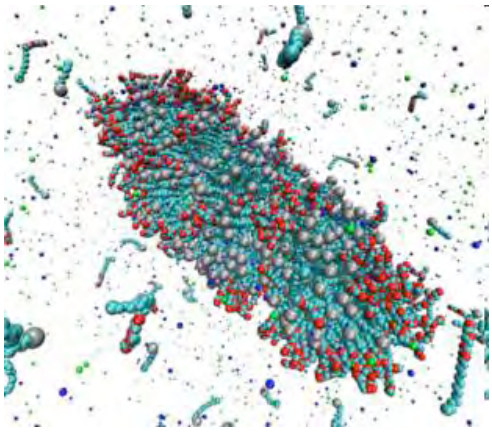
Detergent properties

- Critical micelle concentration
- Hydrophile/lipophile balance (HLB)
- Critical micelle temperature
- Kraft point
- Cloud point
- Aggregation number

Micelles

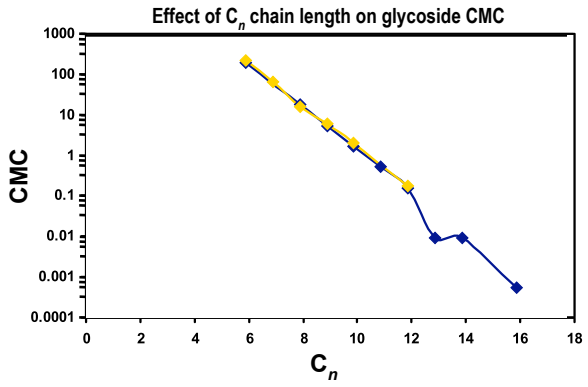
- Above the critical micelle concentration (CMC), hydrophobic effect drives formation of detergent micelles
- Cone shaped structure is basis for micelle formation
  Head group >>> tail group
- Use the CMC as a guide when working with membrane proteins
  - 2-3x CMC stabilises
  - up to 10x CMC for membrane extraction



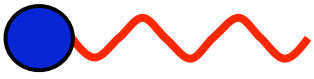


A. Jusufi et al. *J. Phys. Chem.* (2008) B112:13783

General properties relating to CMC



- Micelles form because hydrophobic tail-tail interactions overcome head-head repulsion
- Longer chains have lower CMC
  - Each CH_3 halves the CMC of ionics and decreases zwitter/non-ionics by 80%
  - Branched CH_3 has half the effect
  - Phenyl ring ~3.5 CH_3
  - CF_3 < CH_3
  - Saturations increase the CMC; cis>trans
  - -O or –OH increases CMC
- Generally, CMC decreases with increasing electrolytes
- Largely related to effects on micelle packing
  - Tighter packing = ⇩CMC



Summary of detergent properties

Lower	**CMC**	*Higher*
Longer	**Hydrocarbon chain**	*Shorter*
Stronger	**Binding**	*Weaker*
Tighter	**Micelle packing**	*Looser*
Milder	**Membrane extraction**	*Harsher*
Better	**Stabilising effect on protein**	*Worse*
Longer	**Dialysis time**	*Shorter*
Cheaper	**Cost**	*Expensive*