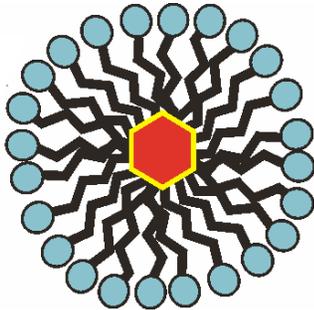


Membrane Proteins & Detergents



*Crystallization:
Focus on membrane proteins
NSLS, June 2007*

Pat Loll

pat.loll@drexel.edu

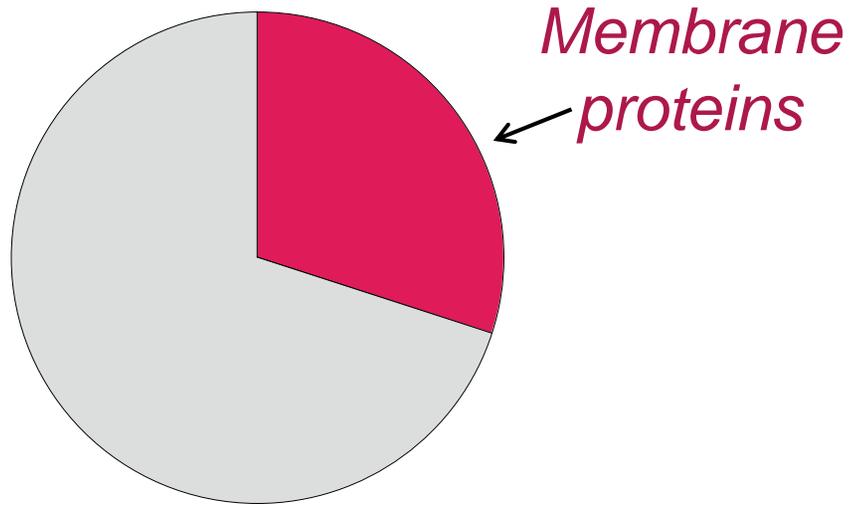




Drexel University College of Medicine

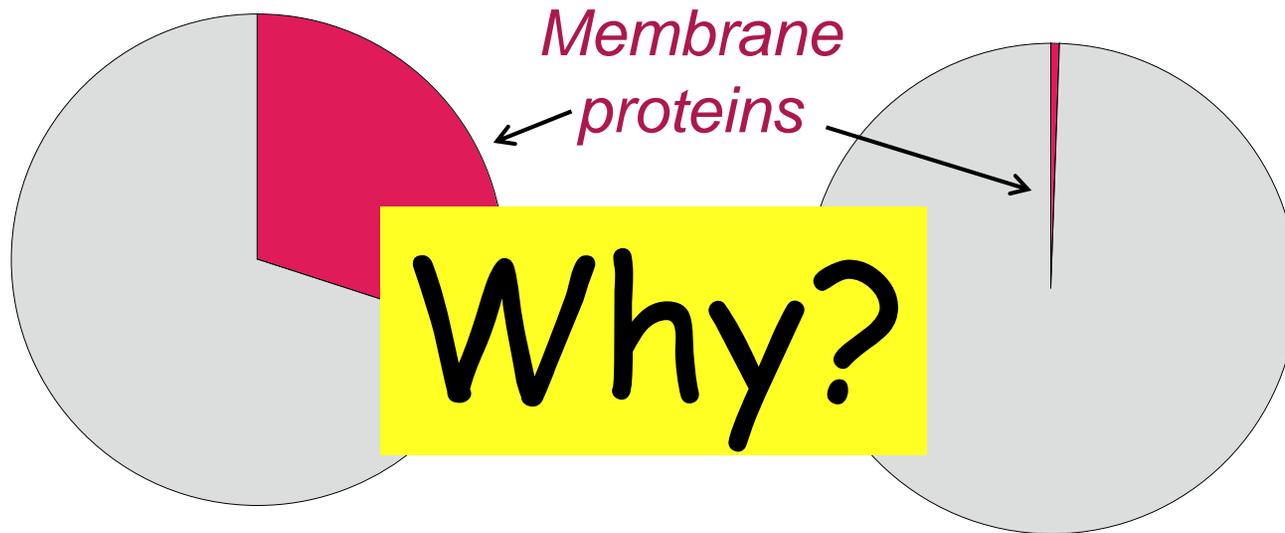
*In the tradition of the Medical College of PA and Hahneman
Medical College*





Proteins encoded
in the genome

- **Roughly 1 in 3 proteins is membrane bound.**



Proteins encoded
in the genome

Proteins of known
structure

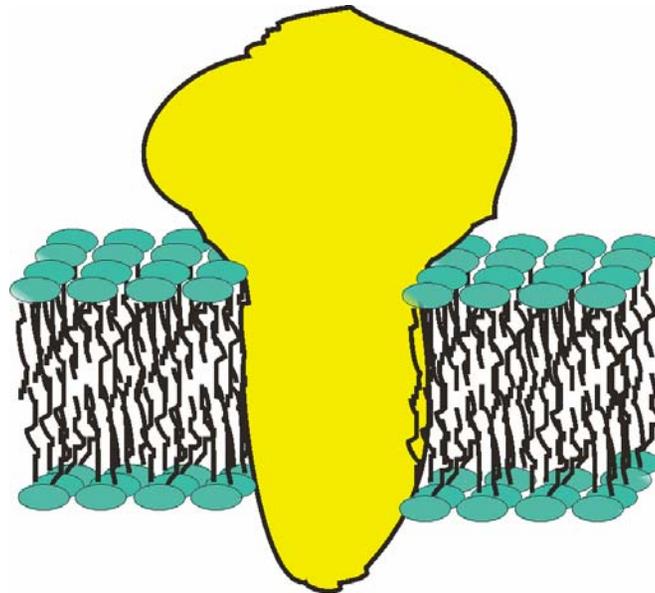
- Roughly 1 in 3 proteins is membrane bound.
- Roughly 1 in 300 proteins *of known structure* is membrane bound.

😊 You never hesitate to tackle the
most difficult problems. 😊

#1 Difficulty: Obtaining Suitable Samples

- Scarce starting materials
- Working outside the membrane environment
- Detergents add complexity

Membrane proteins have evolved to exist in a complex environment

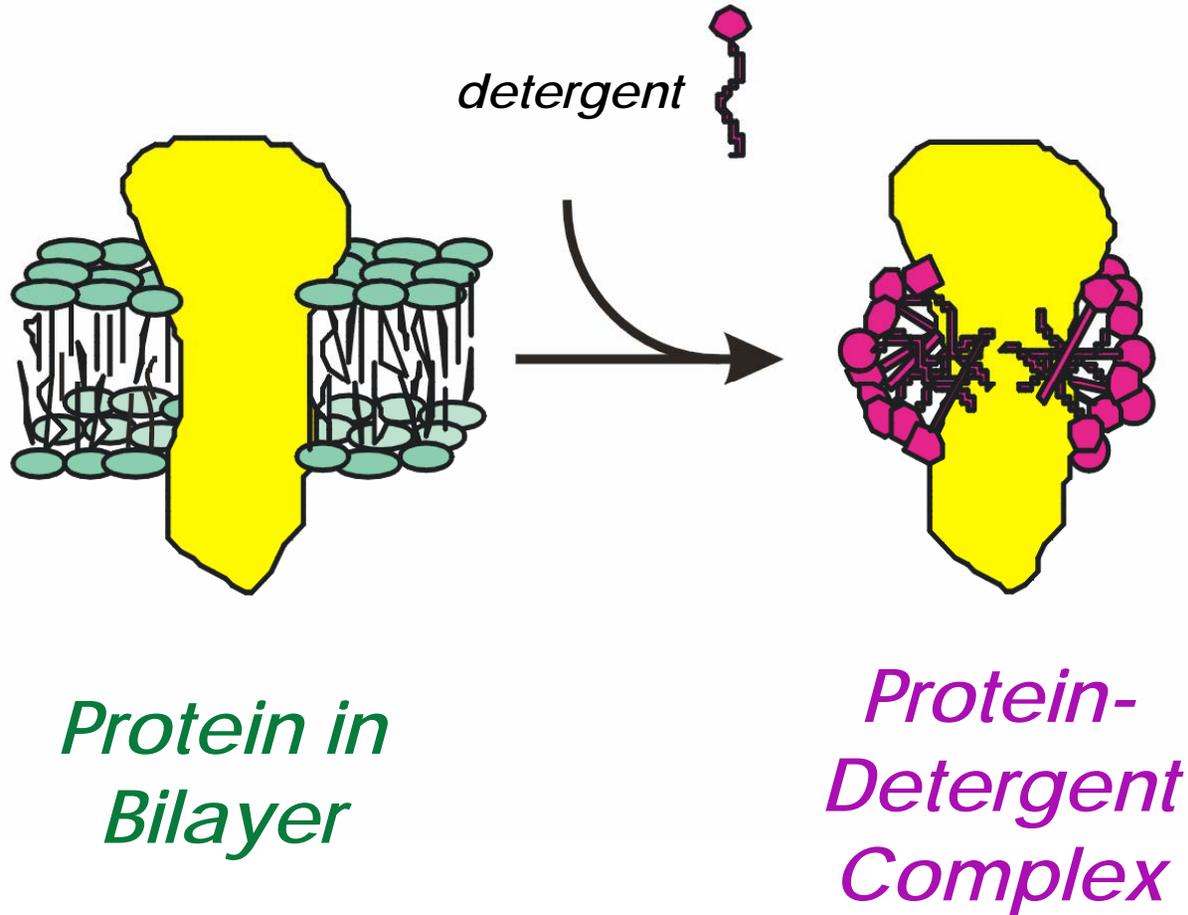


Membrane proteins are:

amphipathic

typically not soluble in any single solvent

Membrane Proteins are Solubilized as Protein-Detergent Complexes



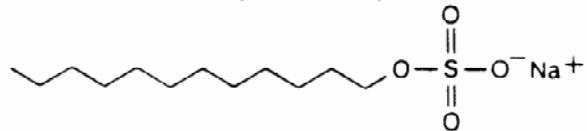
What makes a detergent?



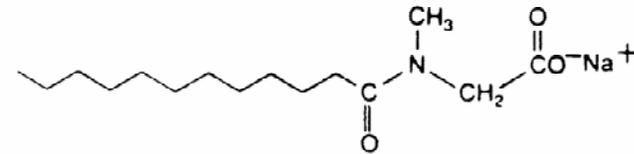
Detergents 101

ANIONIC

Sodium dodecyl sulfate
(Sodium lauryl sulfate)

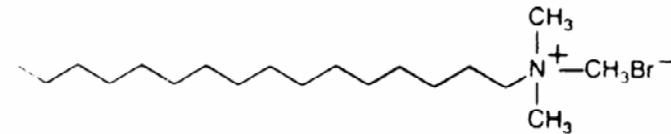


Sodium dodecyl-N-sarcosinate
(Sodium lauryl-N-sarcosinate)
(Sarkosyl L)



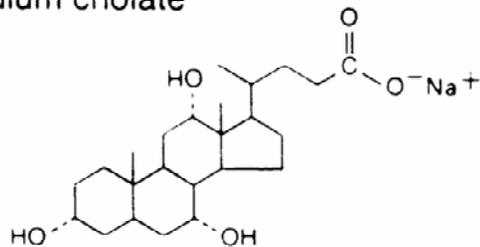
CATIONIC

Cetyl trimethylammonium bromide
(Hexadecyl trimethylammonium bromide)
(CTAB)

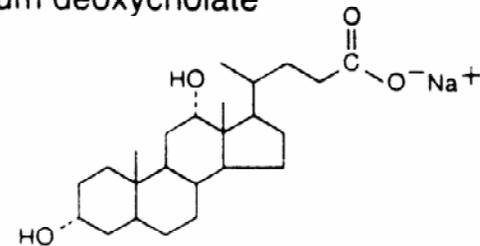


BILE SALTS

Sodium cholate

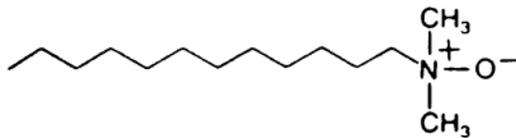


Sodium deoxycholate

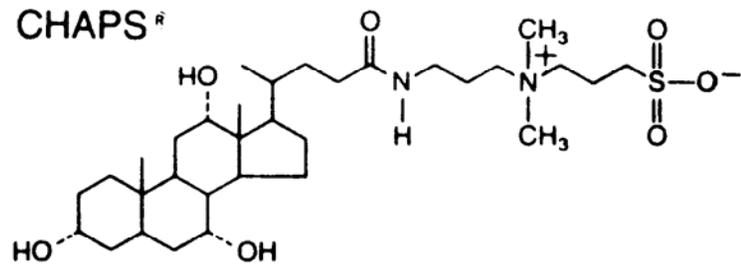
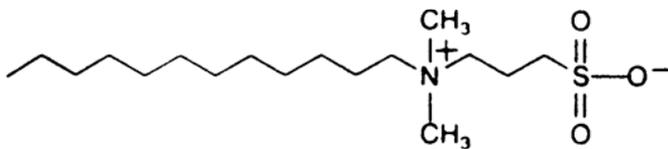


ZWITTERIONIC

Lauryldimethylamine oxide (LDAO)
(Dodecylamine N-oxide)



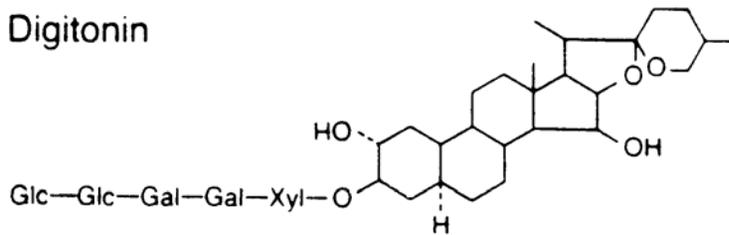
Sulfobetaines
(Zwittergent brand)



[®]Registered trademark, CalBiochem

UNCHARGED

Digitonin



Polyoxyethylene alcohols

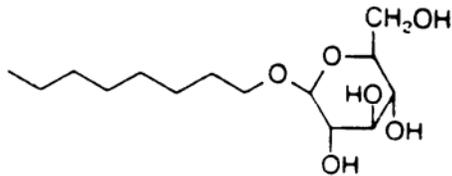
(denoted C_xE_n)

(1) Brij series

(2) Lubrol (WX,PX)

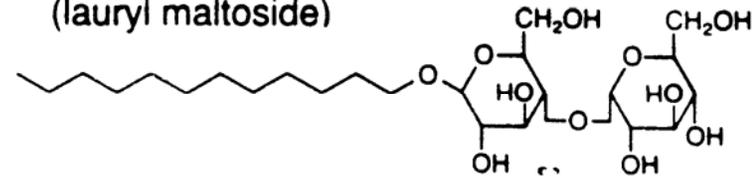


β -D-octylglucoside



β -D-Dodecylmaltoside

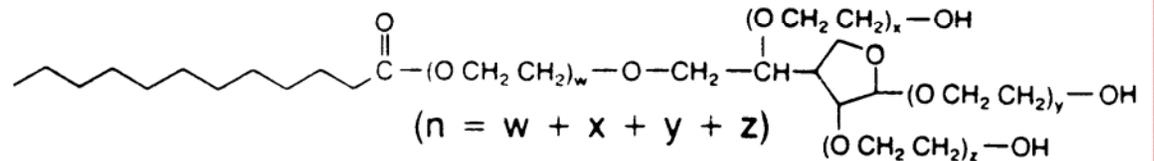
(lauryl maltoside)



Fatty acid esters of Polyoxyethylene sorbitan

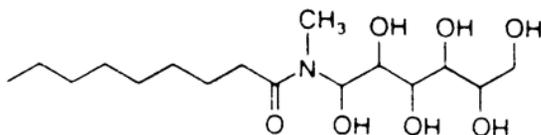
(denoted C_x -sorbitan- E_n)

Tween series



Alkyl-N-methylglucamides

(MEGA[®] brand)



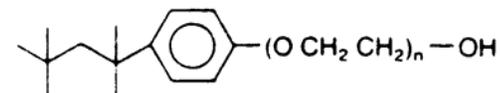
Polyoxyethylene *p tert* octylphenols

(denoted *tert* - $C_8 \emptyset E_n$)

(1) Triton X-100, $n = 9.6$

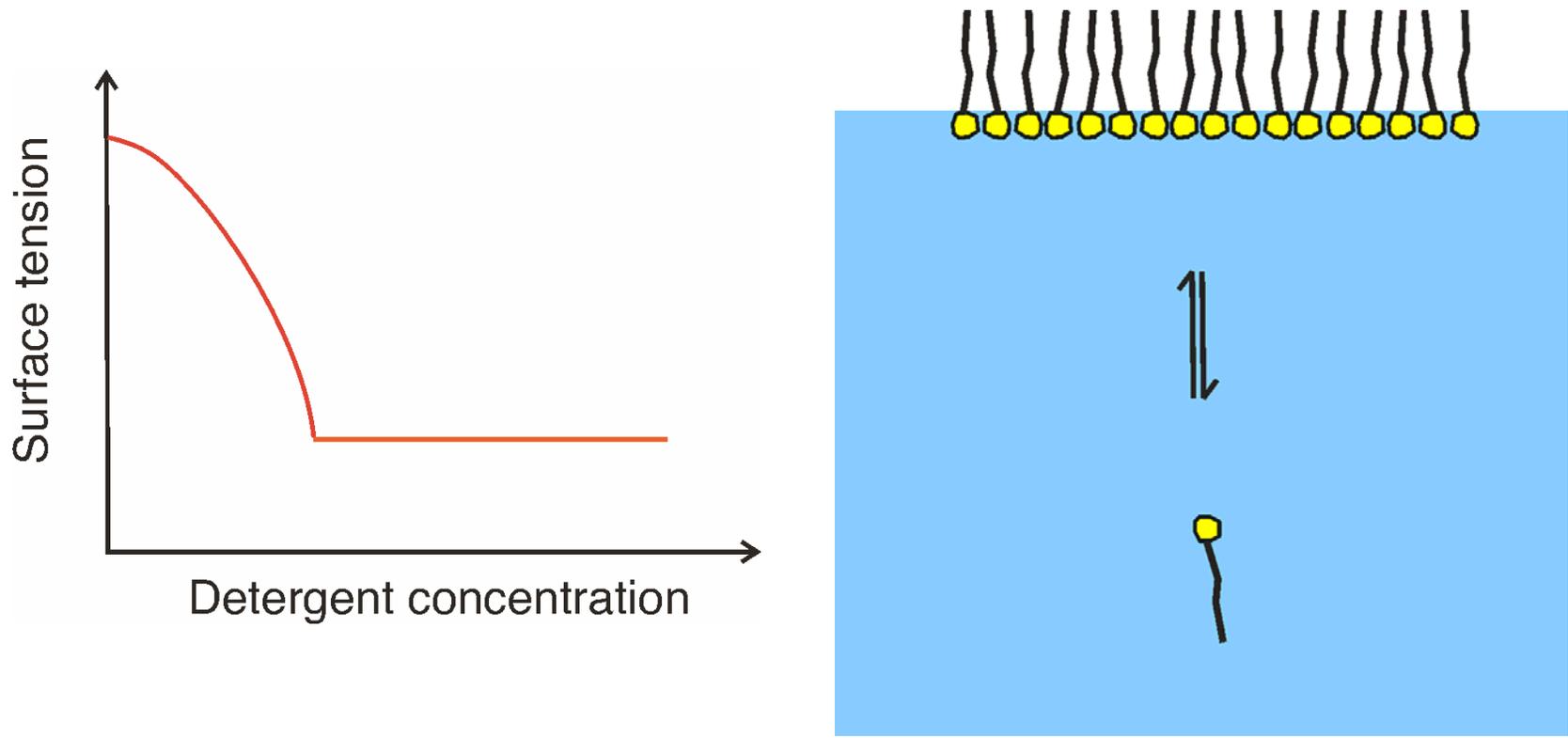
(2) Triton X-114, $n = 7-8$

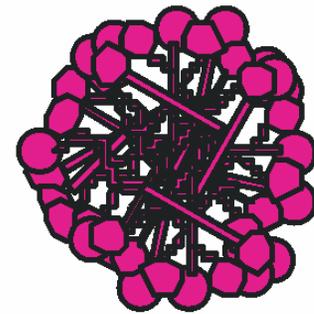
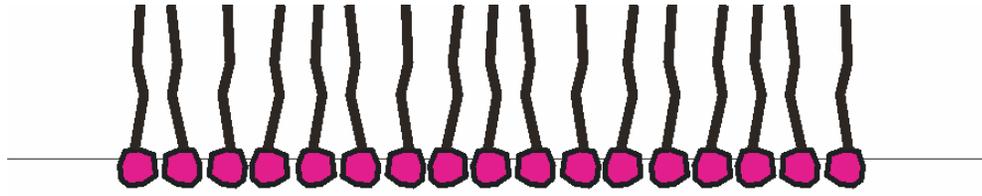
(3) Nonidet P-40, $n = 9$



1. Detergents are surface active

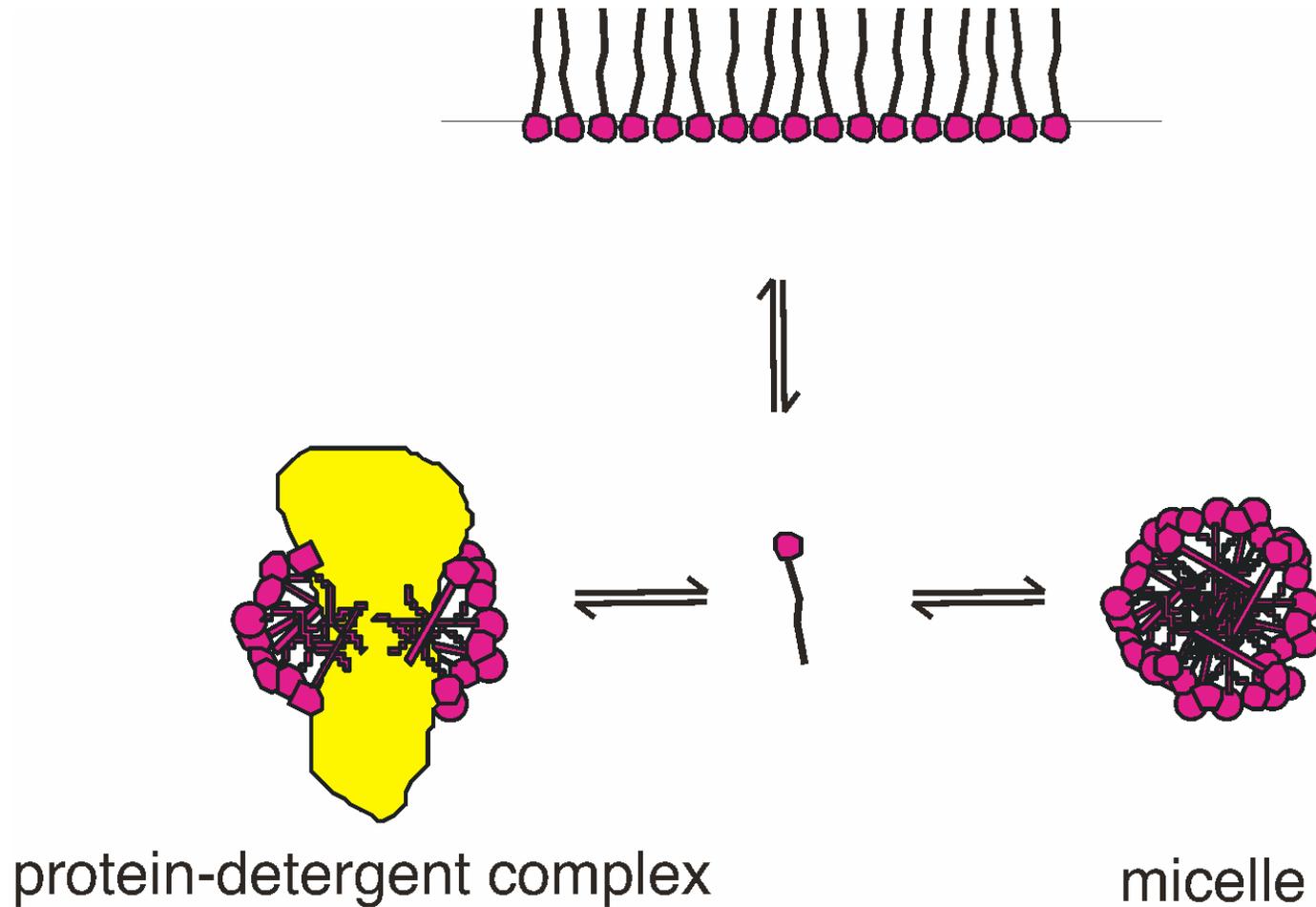
detergents = surfactants





micelle

Why should we care about the CMC?

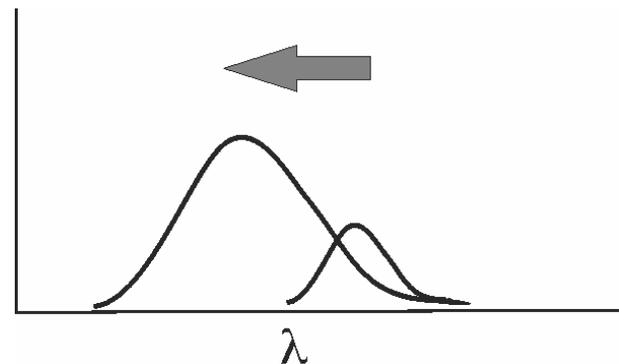
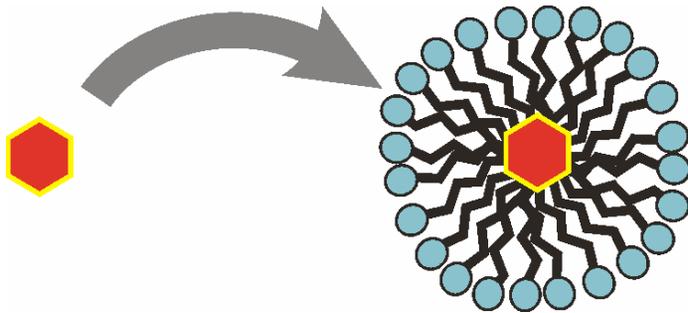


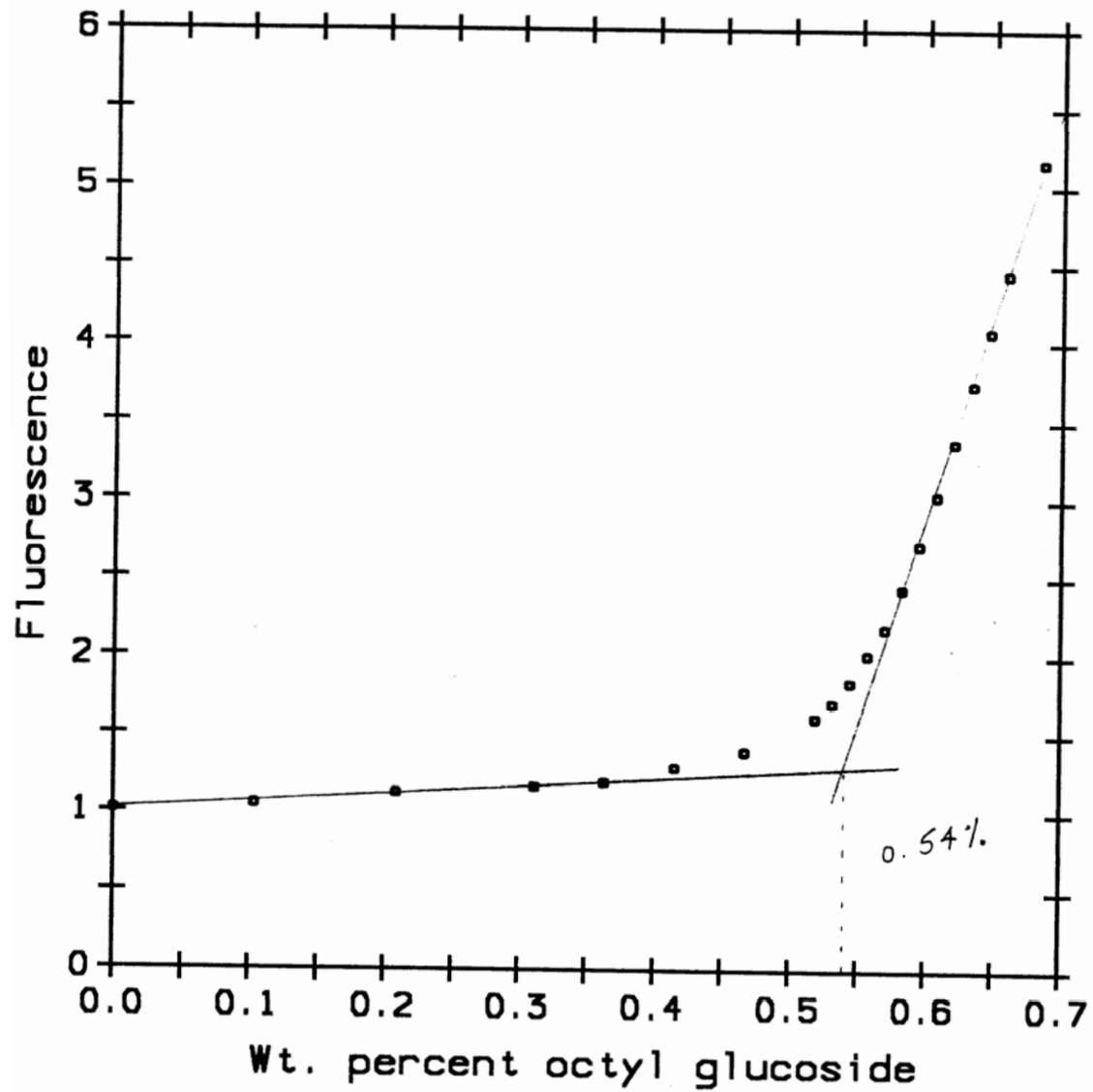
We almost invariably use detergent concentrations $>$ CMC when working with membrane proteins.

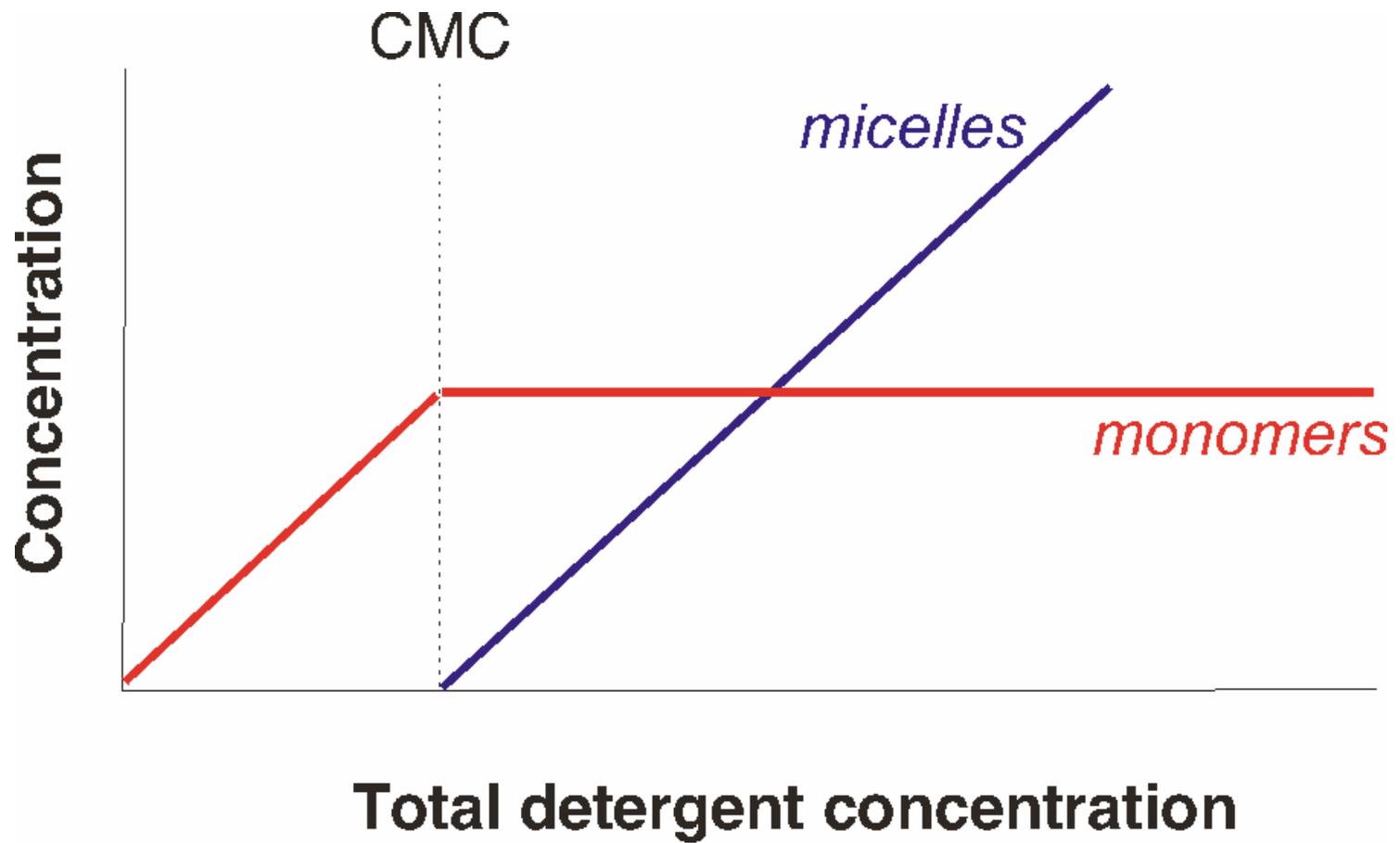
How determine the CMC?

Surface tension measurements

Dye partitioning







What controls CMC?

Solution conditions

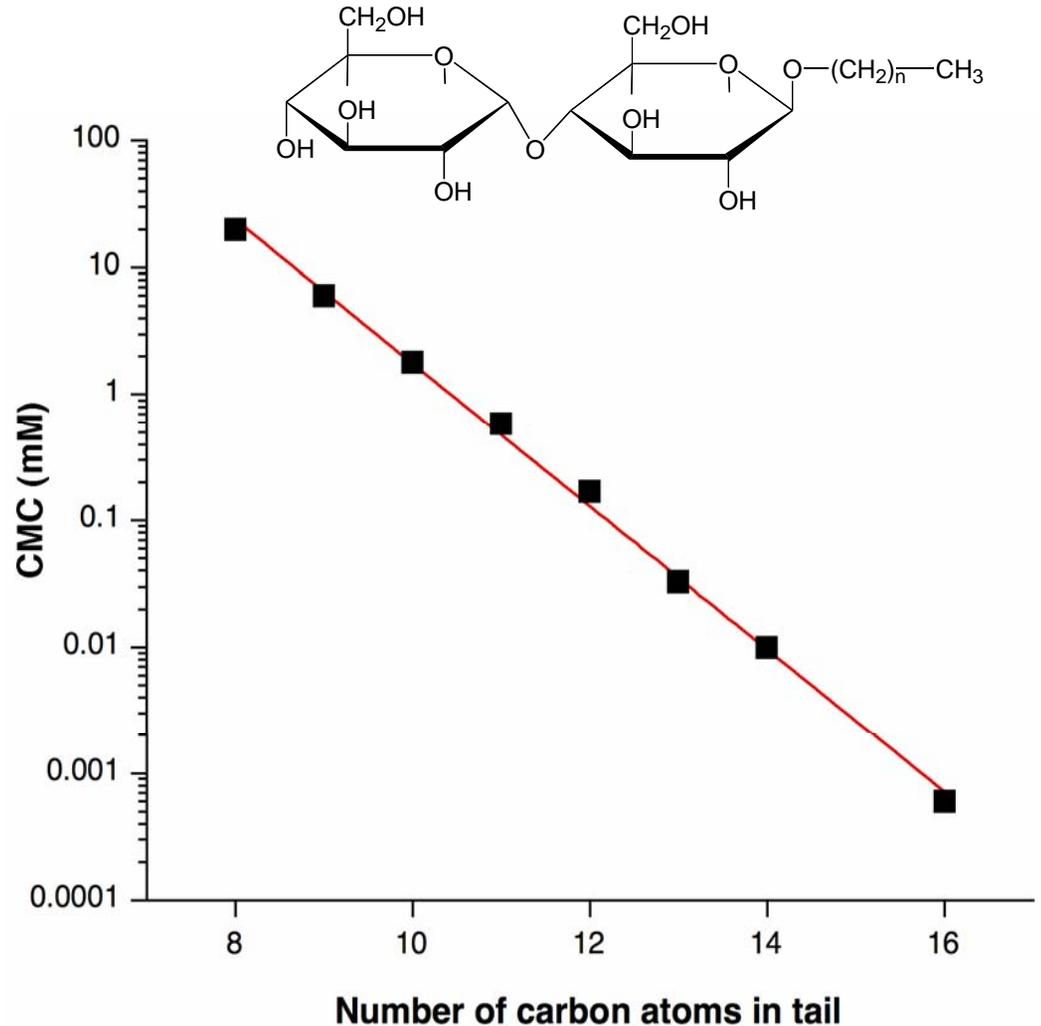
ionic strength

(significant for ionic;

less so for non-ionic)

Hydrophobic/hydrophilic
balance

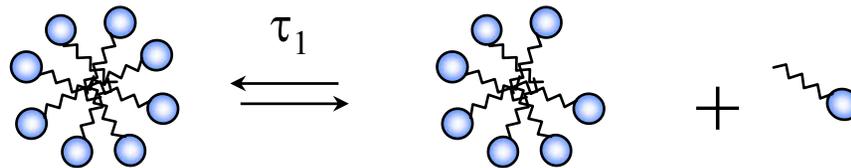
CMC values for the
alkyl maltoside series



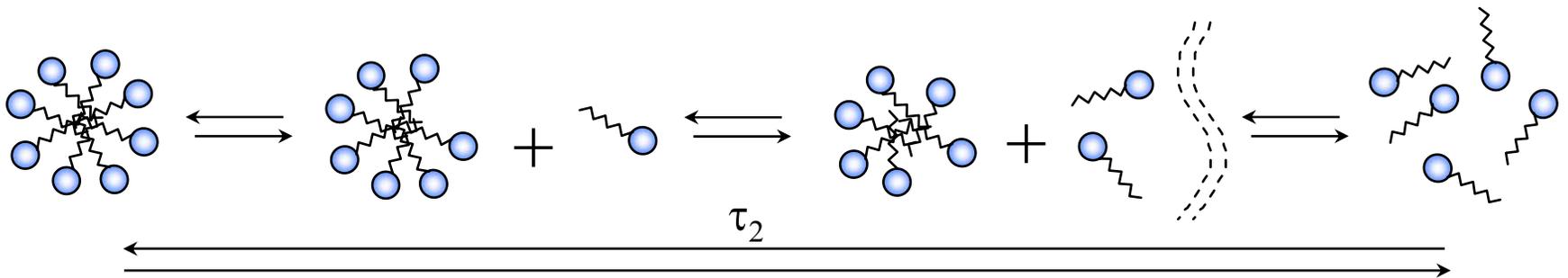
Micelles are:

Large. Aggregation number (no. monomers/micelle) is typically 50-100

Dynamic.



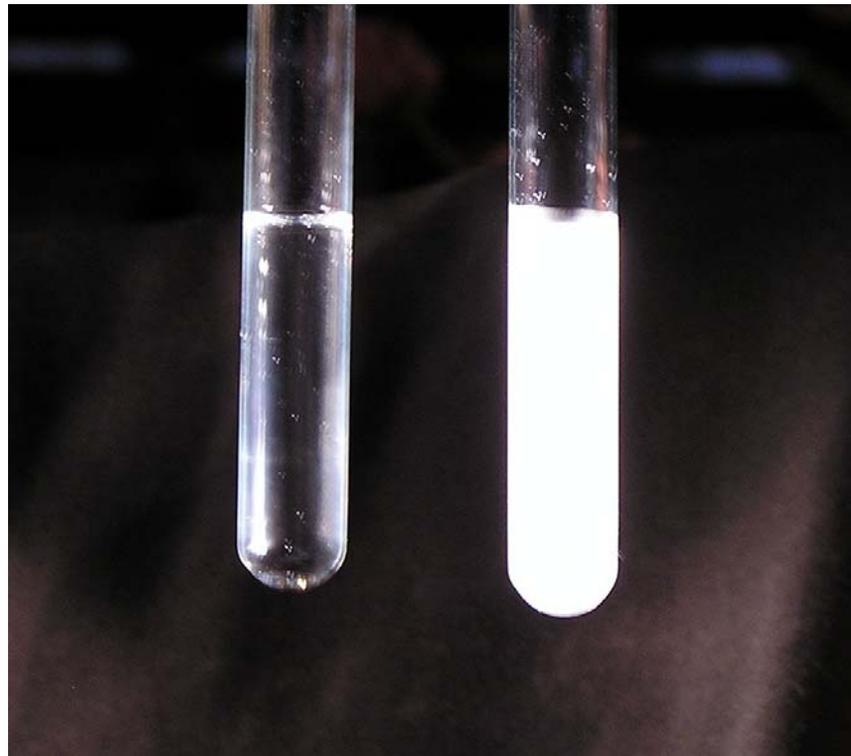
Fast relaxation time, microseconds



Slow relaxation time, milliseconds to minutes

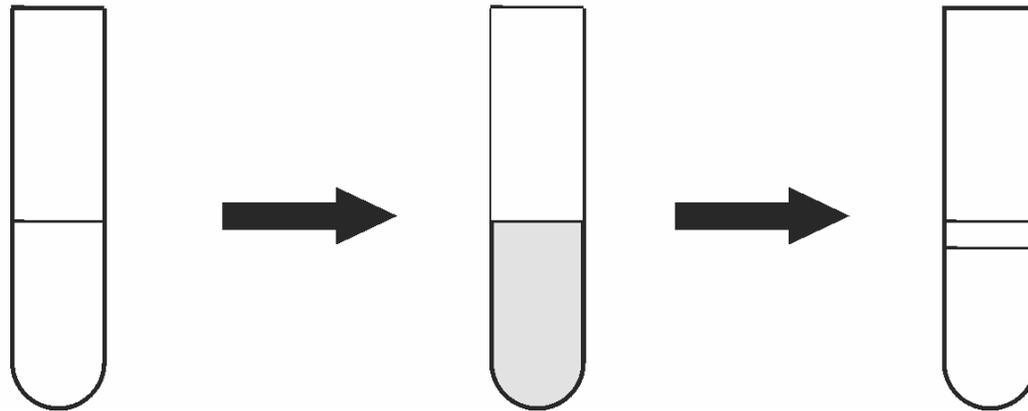
The cloud point:

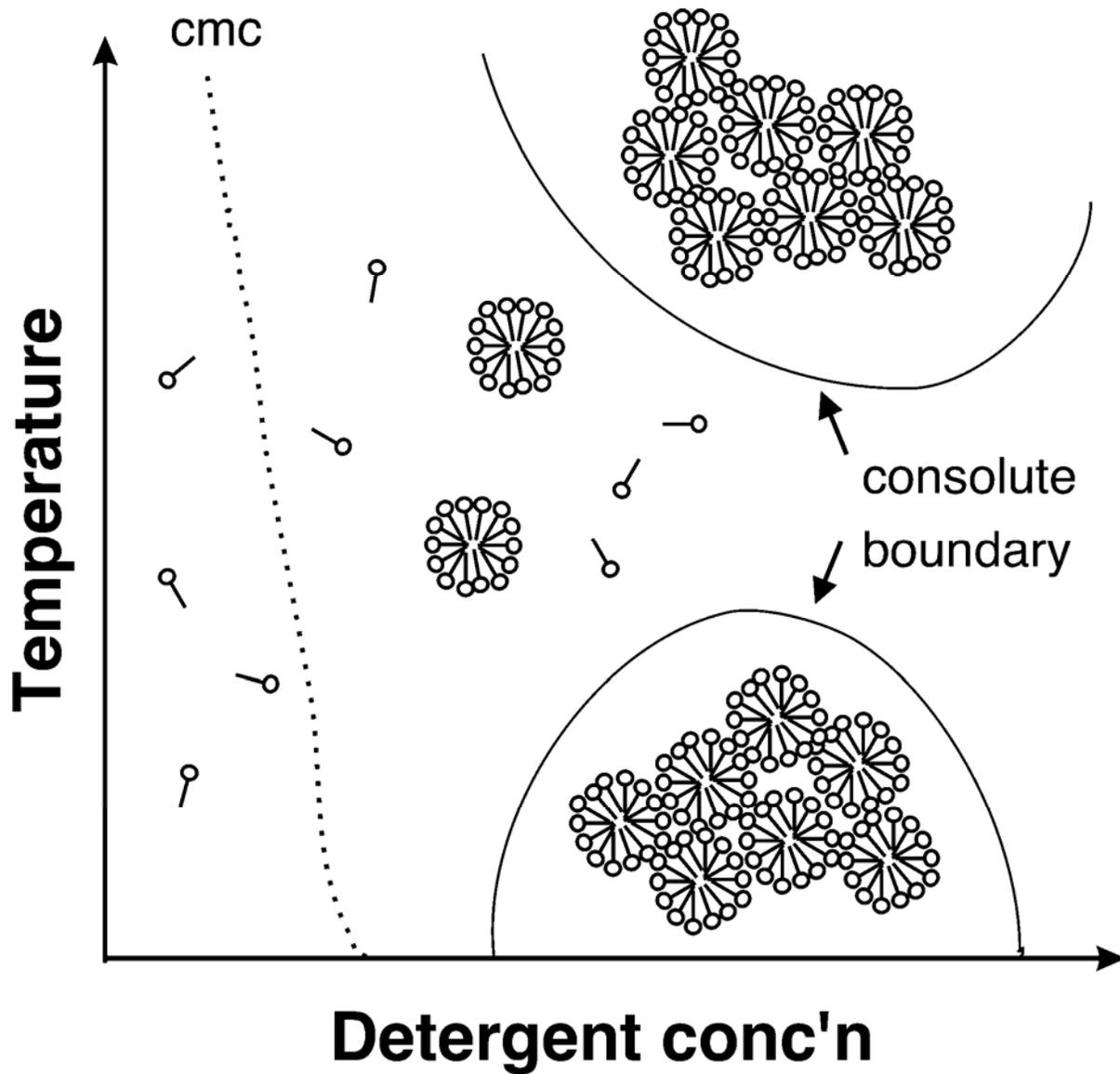
The *other* detergent phase transition



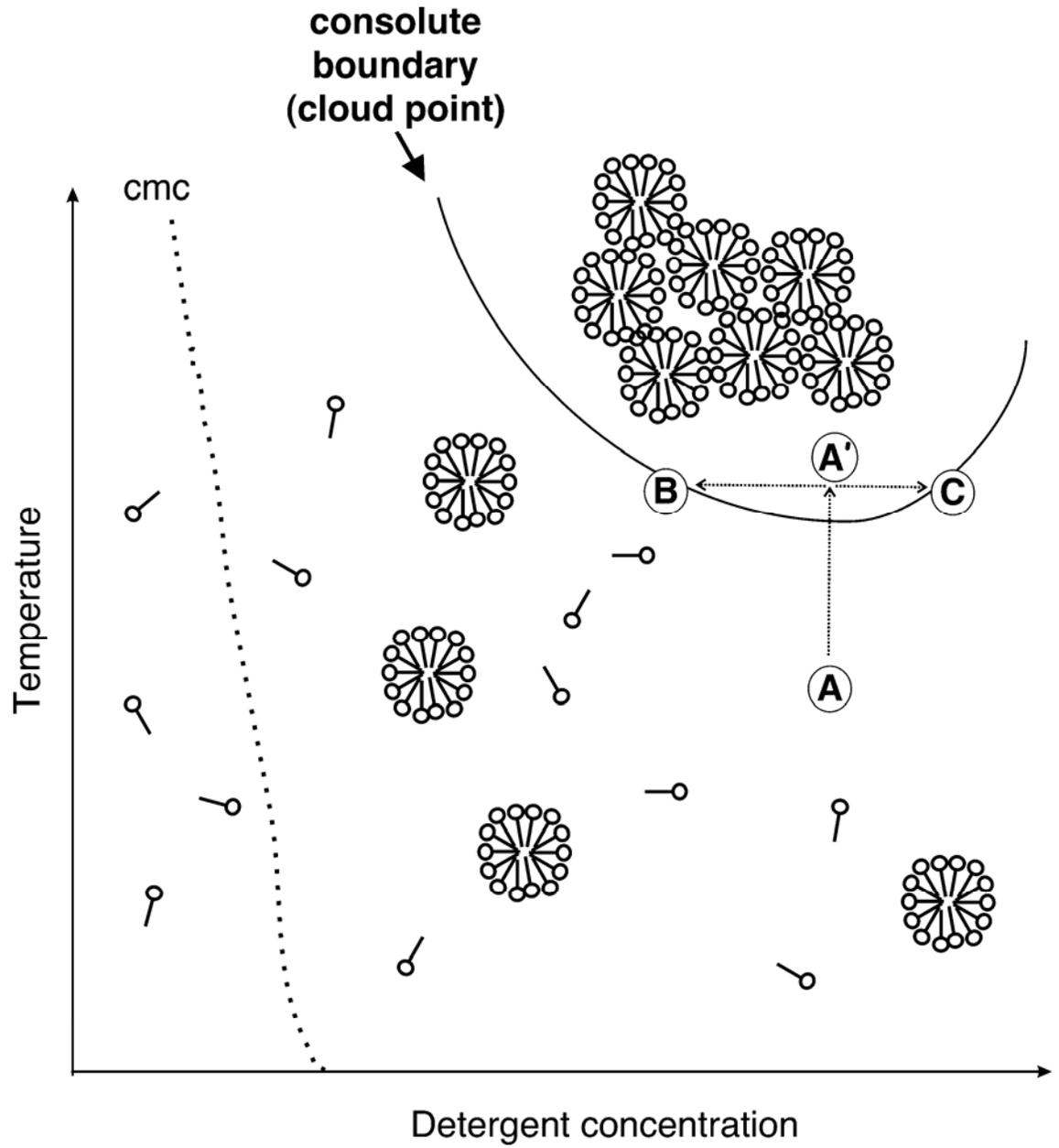
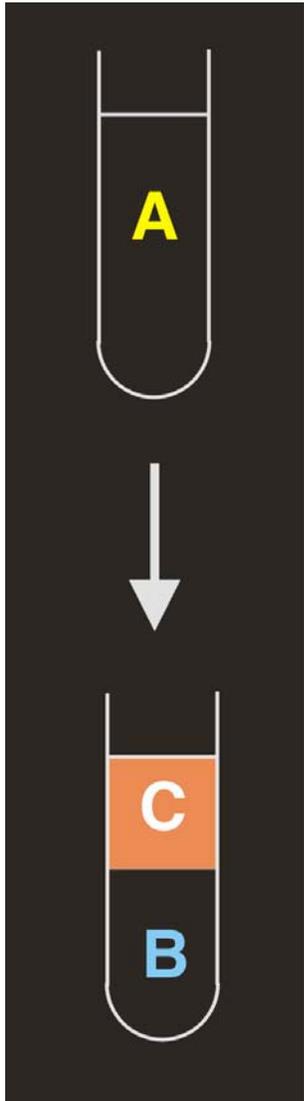
The cloud point:

The *other* detergent phase transition



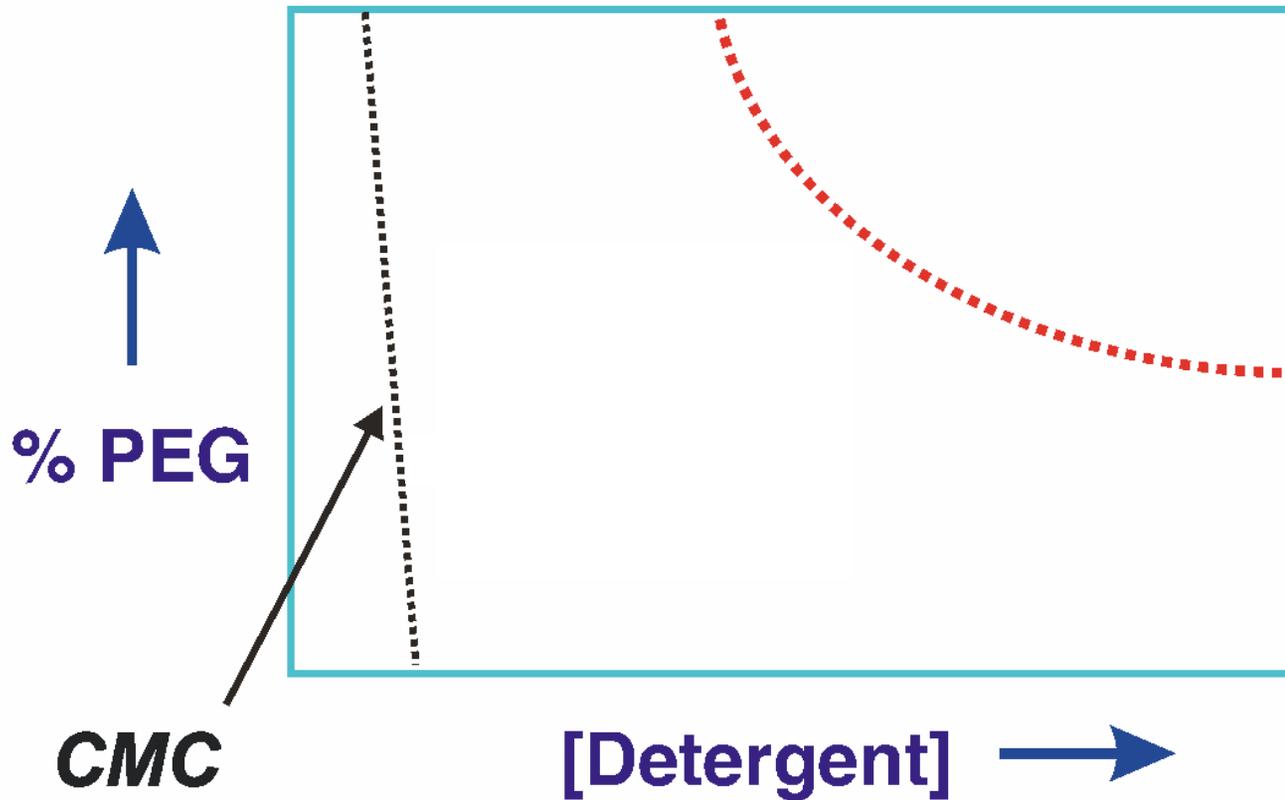


Note that any given detergent will display either an upper or a lower consolute boundary, but not both.



Cloud point (consolute boundary)

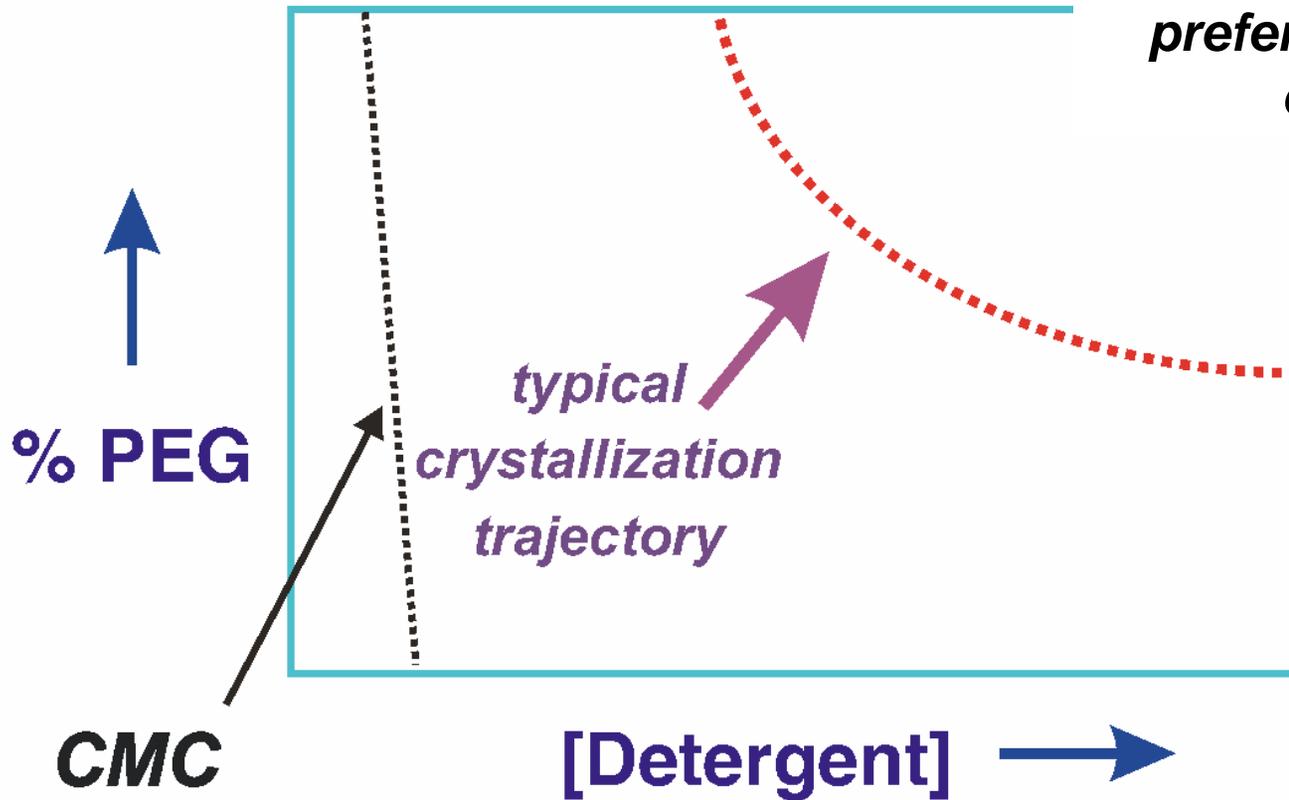
Some detergents (e.g. β OG) only display cloud point behavior in the presence of PEG. So let's re-draw the phase diagram as a function of PEG concentration.



**Cloud point
(consolute boundary)**

So why should we care about the cloud point?

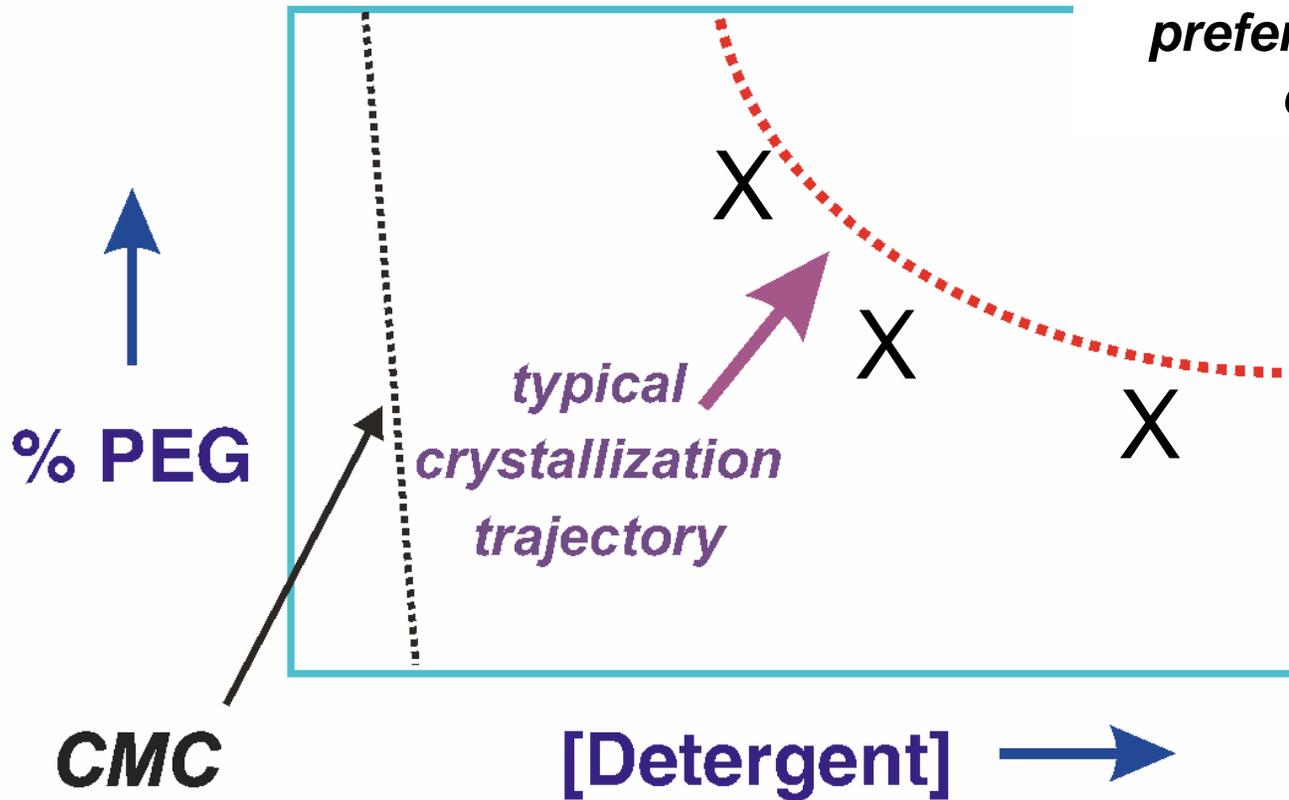
Membrane proteins appear to crystallize preferentially near the cloud point



**Cloud point
(consolute boundary)**

So why should we care about the cloud point?

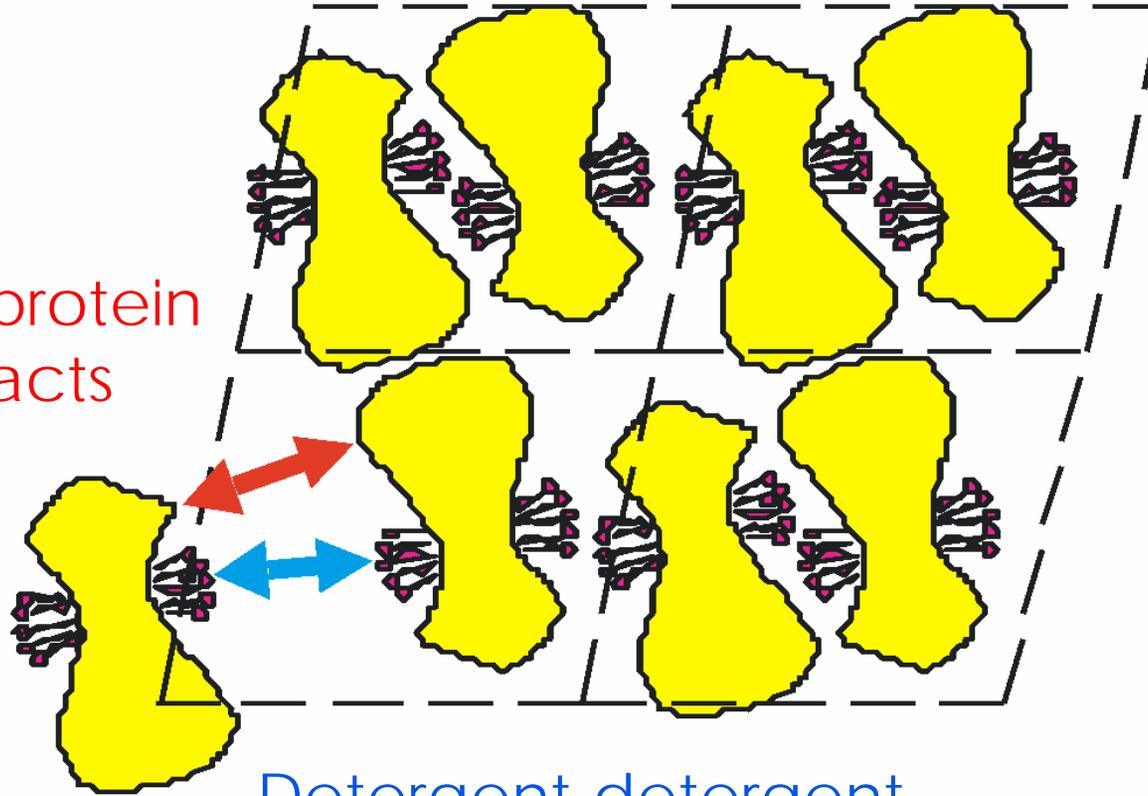
Membrane proteins appear to crystallize preferentially near the cloud point



How to rationalize the observed correlation between crystallization & proximity to the cloud point?

Micelle-micelle forces become attractive as we approach the cloud point

Protein-protein
contacts

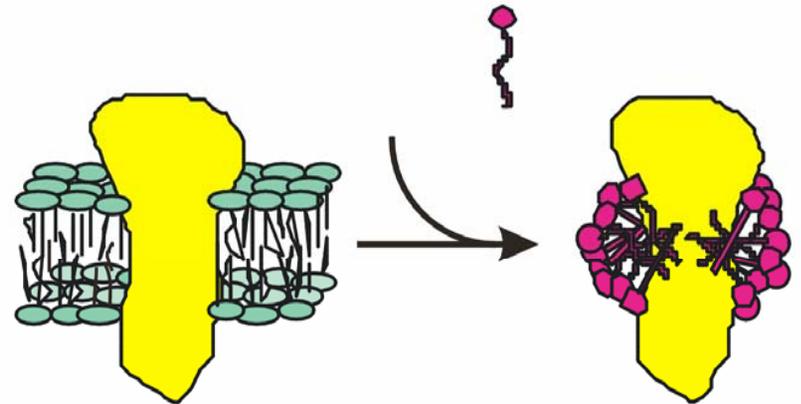


Detergent-detergent
contacts

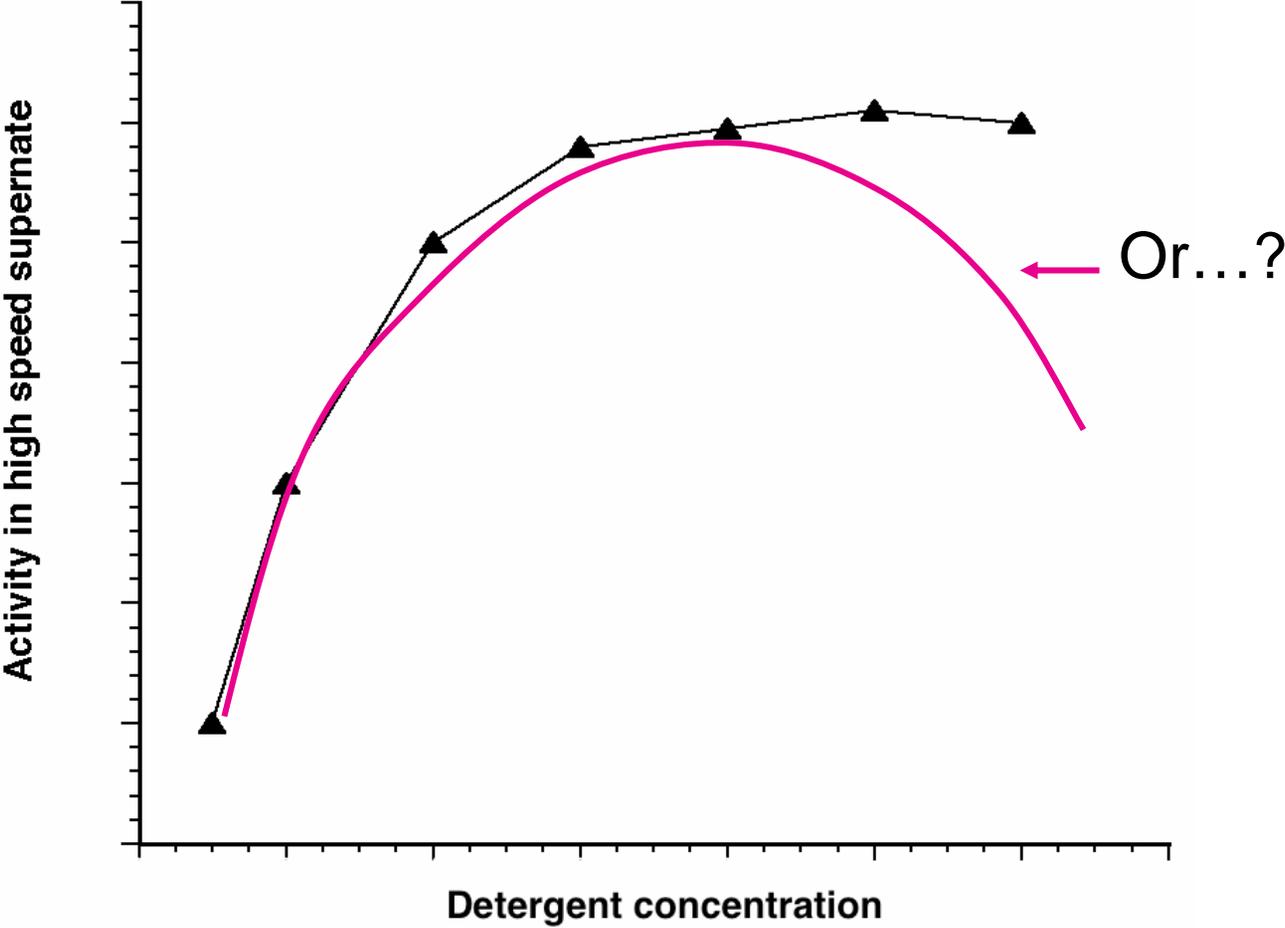
Detergents & membrane proteins: Practical considerations

Solubilization from membranes

- Need to mop up membrane lipids
- Requires high detergent concentrations ($> 10\times$ CMC, *higher than is required to maintain protein once it's solubilized*)
- Incubate with detergent (30 min-several hrs), then high speed spin ($200,000 \times g$).



Solubilization as function of detergent concentration: *Typical scenarios*



Choice of detergent

- Need screen different candidates
- Assess activity, aggregation state, folding
- Low CMC good for solubilization, purification
- High CMC convenient for crystallization
- Detergent exchange
 - Sizing, diafiltration, adsorption chromatography
 - Assess efficiency by TLC,...?

Other practical matters

- Small amphiphiles
 - Heptanetriol, benzamidine, NDSBs, etc. etc. etc.
 - Partition into micelles and alter their physical properties
 - Small amount of a 2nd detergent = a small amphiphilic additive
- Concentrating
 - Detergents will be concentrated along with the protein!
 - Methods for measuring detergent concentration need improvement
- Homogeneity

Issues relating to high throughput

- Different proteins “like” different detergents
 - Probably smart to test multiple detergents in parallel
 - If aiming for lipidic cubic phase, choose compatible detergent?
- Good to screen crystallization conditions for cloud point behavior.
 - Customize screens to detergents