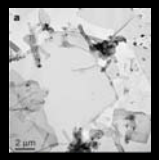
ELECTRON CRYSTALLOGRAPHY WORKSHOP DAVIS 2008

Membrane

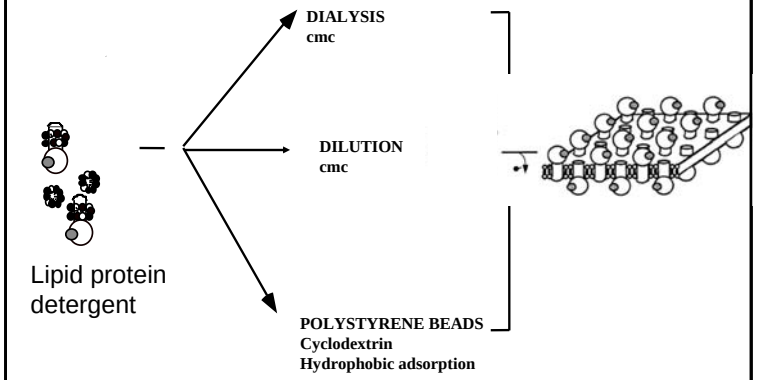
2D crystallization

2D crystal
(Aqp0, 2005)



Michael Landsberg (detergent)
Catherine Venien-Bryan (lipid)
Thomas Walz (dialysis)
Daniel Lévy (BB & monolayer)
Hervé Remigy (Cyclo)

DETERGENT-MEDIATED RECONSTITUTIONS



DETERGENTS AND THEIR PROPERTIES

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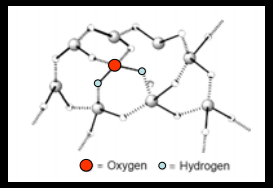
THE UNIVERSITY OF QUEENSLAND AUSTRALIA | IMB Institute for Molecular Bioscience | ammrf

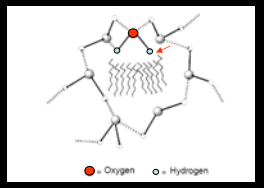
OVERVIEW

1. Hydrophobicity
2. Detergents and membrane proteins
3. Classes of detergents
4. Detergent phase diagrams
5. Properties of detergents and relevance to crystallisation
6. Alternative amphipaths
7. Quantification of detergents

WATER, AMPHIPHILES AND HYDROPHOBIC INTERACTIONS

1. Water forms a highly ordered network of intermolecular hydrogen bonds.



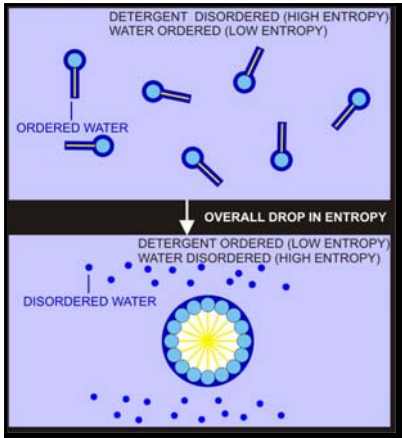


2. It is the strength of all of the hydrogen bonds combined that give water its liquid properties.

3. Polar or hydrophilic substances dissolve in water because they form hydrogen bonds and electrostatic interactions with water molecules.

4. Non-polar or hydrophobic substances are unable to form these interactions and consequently are immiscible with water.

5. Addition of non-polar substances to water disrupts intermolecular hydrogen bonds of water molecules and creates a cavity which is devoid of water. At the surface of the cavity, water molecules rearrange in an orderly manner.

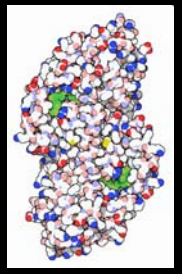


6. This results in a thermodynamically unfavourable decrease in entropy (decrease in disorder).

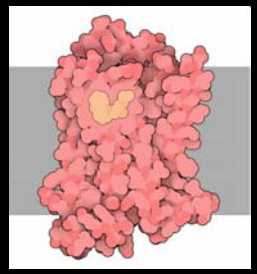
7. To compensate for this loss of entropy, water molecules force the hydrophobic molecules to cluster and thus occupy the minimum space.

8. This effect is known as the hydrophobic effect and the forces between hydrophobic regions (e.g. membrane spanning domains of membrane proteins) are called hydrophobic interactions.

HYDROPHOBIC INTERACTIONS, PROTEIN FOLDING AND SOLUBILITY



SOLUBLE



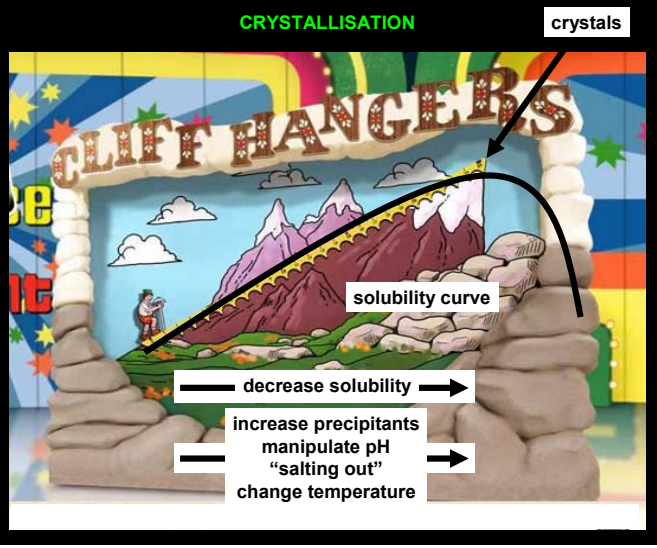
MEMBRANE

THE FOLDING OF SOLUBLE PROTEINS IS DRIVEN BY THE HYDROPHOBIC EFFECT, WHICH CLUSTER THE HYDROPHOBIC SIDE CHAINS AT THE CORE OF THE STRUCTURE SO THEY ARE BURIED

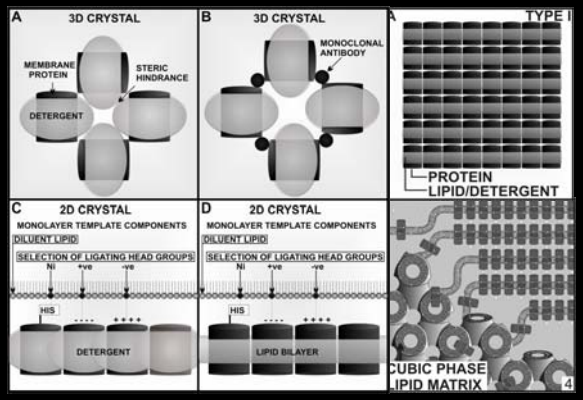
TRANS-MEMBRANE PROTEINS HAVE SOLVENT-EXPOSED HYDROPHOBIC DOMAINS, USUALLY PROTECTED BY LIPID MEMBRANES *IN VIVO*, AND MUST BE PROTECTED BY AMPHIPATHS WHEN EXTRACTED *IN VITRO*

Images from PDB.org

CRYSTALLISATION



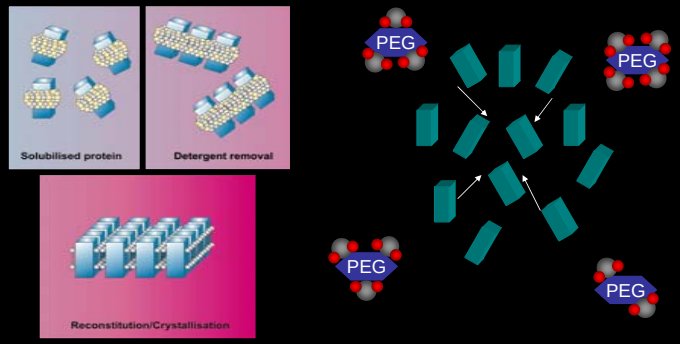
AMPHIPATHS PLAY A CRUCIAL ROLE IN MEMBRANE PROTEIN CRYSTALLISATION



PROTEIN SOLUBILITY CONTROLS CRYSTALLISATION

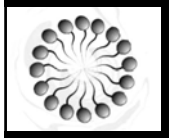
- **In membrane protein crystallisation, the solubility of the protein is controlled by amphipaths rather than precipitants.**

Membrane proteins *Soluble proteins*



DETERGENT DEFINITION

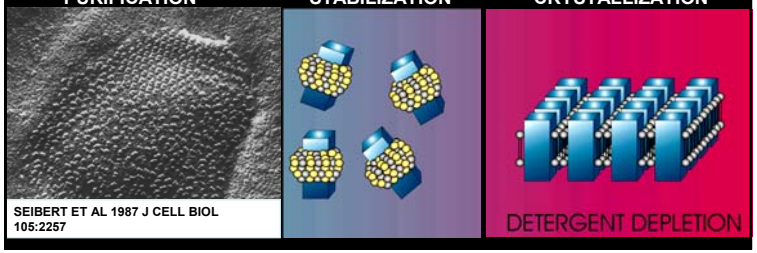
DETERGENTS ARE AMPHIPATHIC MOLECULES THAT CONTAIN BOTH A POLAR (HEADGROUP) AND HYDROPHOBIC REGIONS (TAIL).



1. **POLAR HEADGROUPS FORM HYDROGEN BONDS WITH WATER.**
2. **HYDROPHOBIC TAILS DO NOT FORM H-BONDS WITH H_2O….THEREFORE AGGREGATE INTO MICELLES.**
3. **DETERGENTS REPLACE LIPIDS DURING SOLUBILIZATION AND STABILIZE TRANS-MEMBRANE DOMAINS IN SOLUTION.**

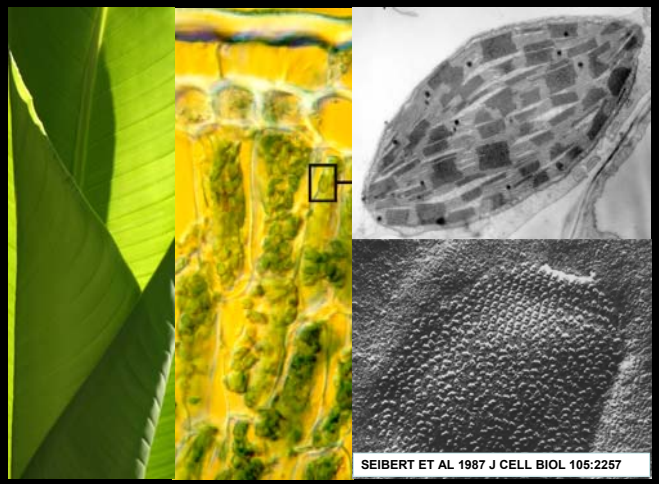
PLAY AN IMPORTANT ROLE IN:

PURIFICATION **STABILIZATION** **CRYSTALLIZATION**



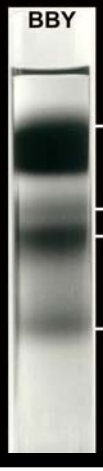
SEIBERT ET AL 1987 J CELL BIOL 105:2257

FROM TISSUE TO PROTEIN



SEIBERT ET AL 1987 J CELL BIOL 105:2257

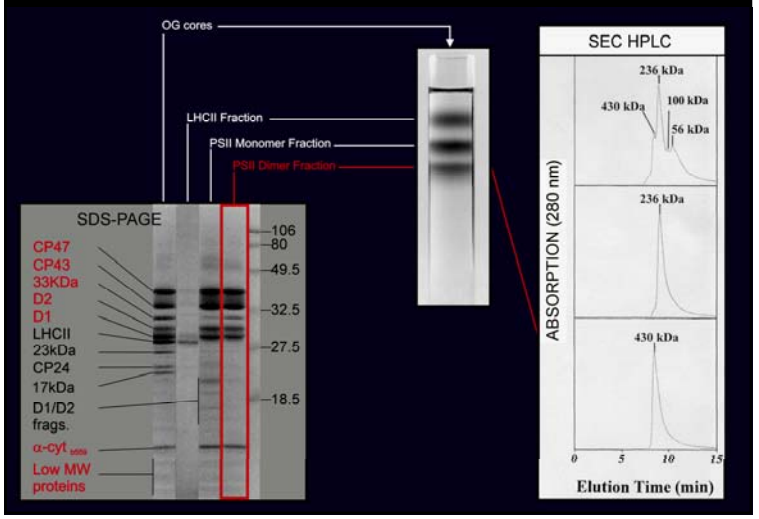
PROTEIN PURIFICATION: SUCROSE GRADIENT CENTRIFUGATION



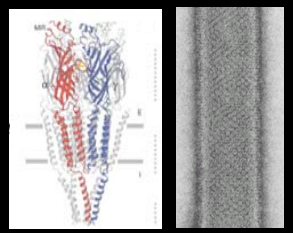
IMPORTANT PROPERTIES OF DETERGENTS TO CONSIDER FOR PURIFICATION

1. PURITY (e.g. α and β DDM)
2. ABILITY TO SOLUBILIZE LIPIDS
3. PROTECT AGAINST DENATURATION
4. LOW CONCENTRATIONS / CMC (COST)
5. ABILITY TO EXCHANGE AND REMOVE

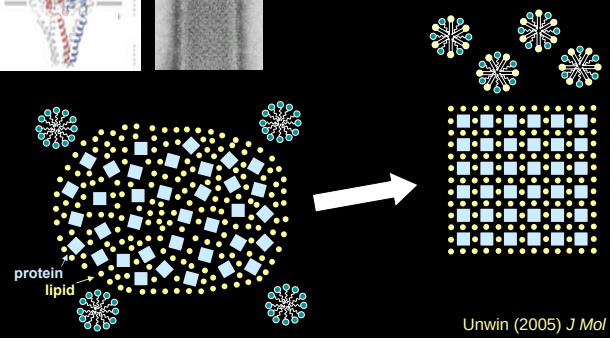
PURIFICATION: SUBUNIT COMPOSITION AND OLIGOMERIC STATE



SOMTIMES MILD SOLUBILISATION OF NATIVE MEMBRANES YIELDS CRYSTALS

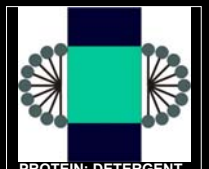


- Crystals of Nicotonic Acetylcholine Receptor and gap junction Connexin form under conditions of mild detergent solubilisation



Unwin (2005) *J Mol Biol* **346**:967

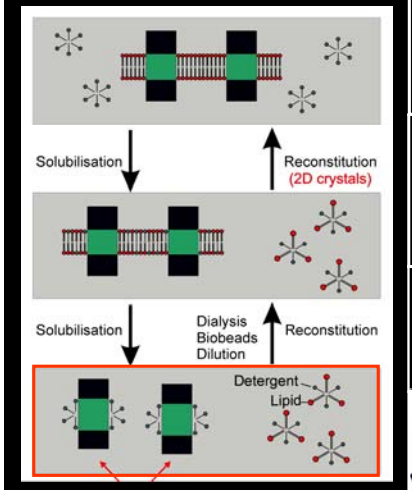
DETERGENTS: PROTEIN STABILIZATION



IMPORTANT PROPERTIES OF DETERGENTS TO CONSIDER FOR STABILIZATION

1. PURITY
2. ABILITY TO PROTECT AGAINST SUBUNIT DISSOCIATION
3. ABILITY TO PROTECT AGAINST SUBUNIT UNFOLDING
4. PREVENT EXPOSURE OF TRANSMEMBRANE DOMAIN
5. LOW CMC / CONCENTRATIONS (COST)
6. EXCHANGABILITY

RECONSTITUTION OF MEMBRANE PROTEINS INTO 2D ARRAYS



PROTEIN: DETERGENT MICELLE

PROTEIN:DETERGENT COMPLEX(NATIVE LIPID)

PROTEIN: DETERGENT COMPLEX (ADDED LIPID)

BICELLE: PROTEIN COMPLEX

MP EMBEDDED IN A PLANAR LIPID DETERGENT BILAYER

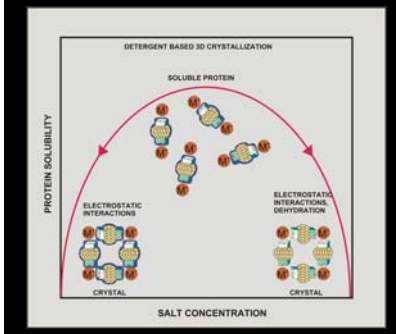
SMALL VESICLES

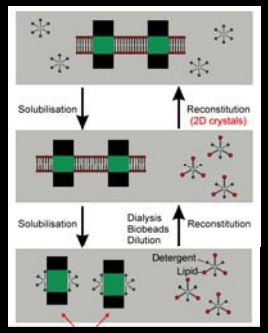
DENATURED PROTEIN

DETERGENTS: CRYSTALLIZATION

IMPORTANT PROPERTIES OF DETERGENTS TO CONSIDER FOR CRYSTALLIZATION

1. PURITY
2. EASE OF REMOVAL BY DILUTION, DIALYSIS OR BIOBEADS
3. IN MONOLAYER CRYSTALLIZATION – EFFECT ON MONOLAYER STABILITY.
4. EFFECT OF TEMPERATURE ON HEADGROUP BEHAVIOUR (E.G. TRITON X-114)
5. EFFECT OF COUNTERIONS ON HEADGROUP (IONIC DETERGENTS)
6. LONGER CHAIN LENGTHS CAN BE USED THAN FOR 3D



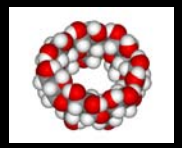


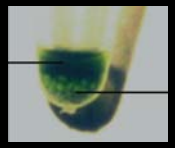
DETERGENT EXCHANGE

1. HYDROPHOBIC ADSORPTION (EG. BIOBEADS, CALBIOSORB)
2. DIALYSIS
3. DILUTION
4. GEL CHROMATOGRAPHY
5. ION EXCHANGE CHROMATOGRAPHY

METHODS REVIEWED IN:
A GUIDE TO THE PROPERTIES AND USES OF DETERGENTS IN BIOLOGY AND BIOCHEMISTRY CALBIOCHEM.


microdialysis


cyclodextrin

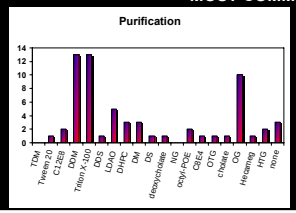

biobeads

NOT ALL METHODS WORK EQUALLY FOR ALL DETERGENTS

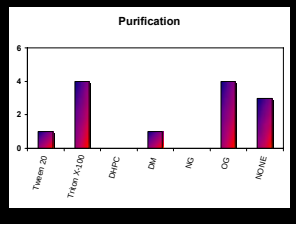
DETERGENT STATISTICS

140 PUBLICATIONS | 51 PROTEINS OR COMPLEXES | 79 STRUCTURES

MOST COMMONLY USED DETERGENTS



6Å OR BETTER: ACh receptor, AQP-0, AQP-1, LHC-II, AQP-2, AQP-4, hR, connexin, GST,



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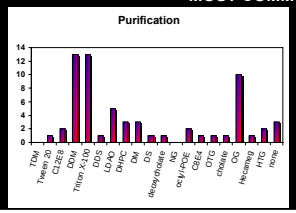
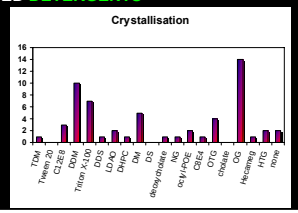
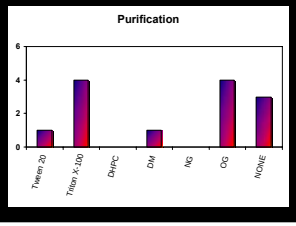
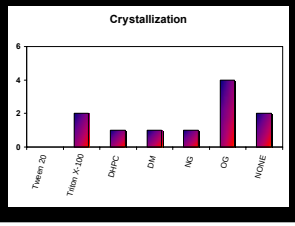


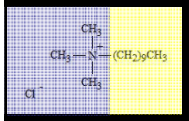


6Å OR BETTER: ACh receptor, AQP-0, AQP-1, LHC-II, AQP-2, AQP-4, hR, connexin, GST,



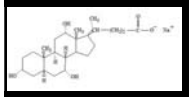


MAIN CLASSES OF DETERGENTS



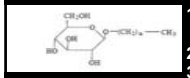
IONIC DETERGENTS

1. -VE CHARGE = ANIONIC (E.G. SDS)
2. +VE CHARGE = CATIONIC (CTAB)
3. EFFECTIVE AT BREAKING PROTEIN: PROTEIN INTERACTIONS
4. THEREFORE OFTEN DENATURING
5. SIMILARLY CHARGED HEADGROUPS REPEL
6. THEREFORE MICELLE SIZE DEPENDENT ON:
   A. COUNTERION TYPE/CONC- NEUTRALIZATION = REDUCED CURVATURE = INCREASED MICELLE SIZE.
   B. CHAIN LENGTH & SHAPE OF HYDROPHOBIC REGION



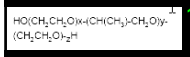
BILE ACID SALTS

1. ANIONIC DETERGENTS CONTAINING RIGID STEROIDAL RING
2. NO CLEARLY DEFINED HEADGROUP (POLAR/APOLAR FACE) DUE TO HYDROXYLATION OF THE STEROIDAL RINGS
3. KIDNEY SHAPED MICELLES-SIZE DEPENDENT ON COUNTERIONS
4. DUE TO LOW pKa (5-6) AND LOW SOLUBILITY OF UNCONJUGATED BILE SALTS ONLY USEFUL IN ALKALINE RANGE. CONJUGATED BILE SALTS CAN BE USED OVER A BROADER pH RANGE.

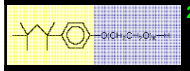


NON-IONIC DETERGENTS

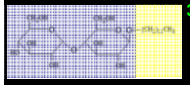
1. NON-IONIC DETERGENTS ARE GOOD AT BREAKING LIPID:LIPID AND LIPID:PROTEIN CONTACTS
2. NOT SO GOOD AT BREAKING PROTEIN:PROTEIN CONTACTS
3. THEREFORE PROTECT PROTEIN FUNCTION
4. MICELLE SIZE NOT GREATLY EFFECTED BY COUNTERIONS
5. MICELLE SIZE IS EFFECTED BY SALT CONCENTRATION



1. POLYOXYETHELENE HEADGROUPS (E.G. BRIJ)
   - CAN FORM RANDOM COIL STRUCTURES
   - SHORT CHAIN POLYOXYETHELENES FORM AGGREGATES AND VISCOUS SOLUTIONS IN WATER AT ROOM TEMPERATURE
   - LONG CHAIN POLYOXYETHELENES DO NOT AGGREGATE



2. POLYOXYETHELENE HEADGROUPS COUPLED TO PHENOL RINGS (E.G. TRITON)
   - HIGH ABSORPTION AT 280NM
   - TRITON CAN OFTEN CONTAIN PEROXIDE CONTAMINATION
   - OFTEN USED DURING PURIFICATION (CHEAP, LOW CMC)



3. SUGAR HEADGROUPS (E.G. MALTOSIDES, GLUCOSIDES)
   - HOMOGENEOUS STRUCTURE (but check α and β conformations)
   - SELECTION OF HYDROPHOBIC CHAIN LENGTHS (C_6-C_{12}) STRAIGHT, AS WELL AS CYCLIC) AND HEADGROUPS (MALTOSE, SUCROSE, GLUCOSE)

IMPORTANT PROPERTIES OF NONIONIC DETERGENTS:
PURE, WELL DEFINED MICELLES, PROTECT AGAINST DENATURATION

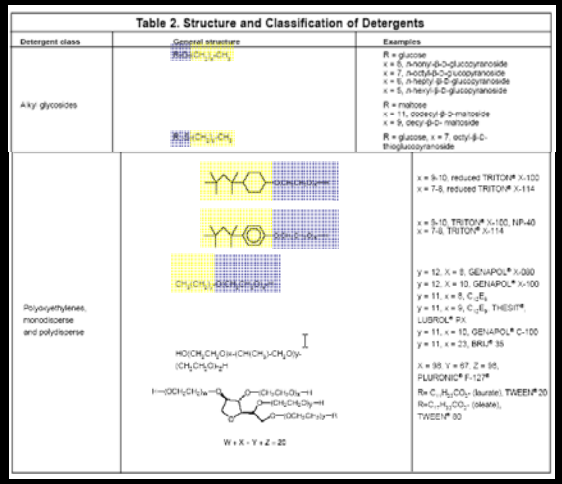
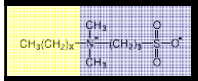


Table 2. Structure and Classification of Detergents

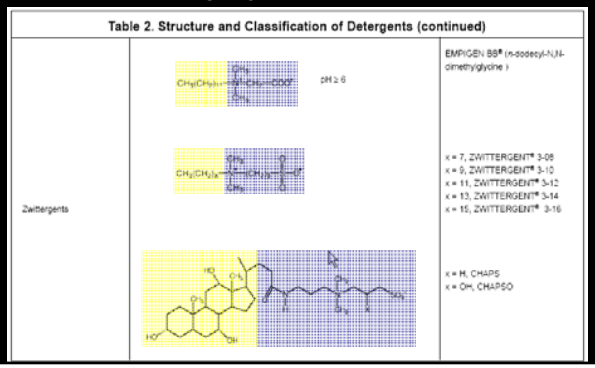
Detergent class	General structure	Examples
Alkyl glycosides		R = glucose x = 6, N-heptyl-β-D-glucopyranoside x = 7, N-octyl-β-D-glucopyranoside x = 8, N-nonyl-β-D-glucopyranoside x = 5, N-hexyl-β-D-glucopyranoside R = maltose x = 11, decyl-β-D-maltoside x = 9, decyl-β-D- maltoside R = glucose, x = 7, octyl-β-D-thioglucopyranoside
Polyoxyethylenes, monodisperse and polydisperse		x = 9-10, reduced TRITON® X-100 x = 7-8, reduced TRITON® X-114 x = 9-10, TRITON® X-100, NP-40 x = 7-8, TRITON® X-114 y = 12, X = 8, GENAPOL® X-080 y = 12, X = 10, GENAPOL® X-100 y = 11, x = 5, $C_{12}E_5$ y = 11, x = 9, $C_{12}E_9$, THESIT®, LUBROL® PX y = 11, x = 10, GENAPOL® C-100 y = 11, x = 23, BRIJ® 35 X = 98, Y = 67, Z = 98, PLURONIC® F-127® R= $C_{11}H_{23}CO_2$- (laurate), TWEEN® 20 R=$C_{17}H_{33}CO_2$- (oleate), TWEEN® 80



ZWITTERIONIC DETERGENTS

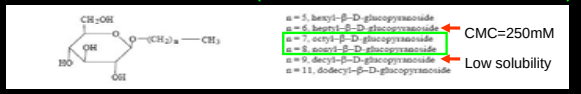
OFFER COMBINED PROPERTIES OF IONIC AND NON-IONIC DETERGENTS.

1. **NO NET CHARGE = LACK CONDUCTIVITY, ELECTROPHORETIC MOBILITY AND ABILITY TO BIND TO ION EXCHANGE RESIN.**
2. **BUT LIKE IONIC DETERGENTS GOOD AT BREAKING PROTEIN:PROTEIN CONTACTS.**
3. **RIGID STEROID RING OF CHAPS THOUGHT TO MAKE IT LESS DENATURING**

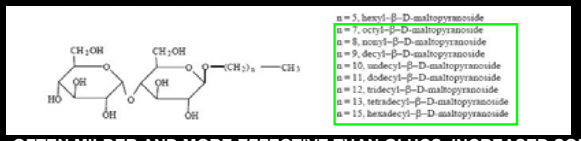


NON-IONIC DETERGENTS IN MORE DETAIL

ALKYL GLUCOPYRANOSIDES (THIO-GLYCOSIDES ARE LESS POLAR)

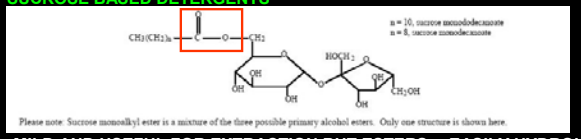


ALKYL MALTOPYRANOSIDES



OFTEN MILDER AND MORE EFFECTIVE THAN GLUCS. INCREASED SOLUBILITY

SUCROSE BASED DETERGENTS

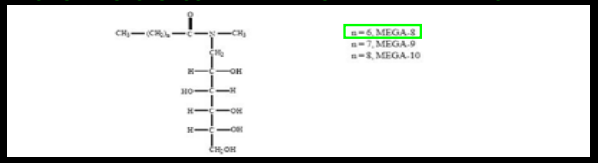


MILD AND USEFUL FOR EXTRACTION BUT ESTERS …EASILY HYDROLYSED, ALSO A MIXTURE OF ISOMERS

ANATRACE CATALOGUE

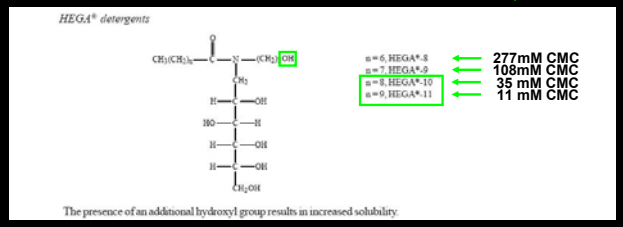
NONIONIC DETERGENTS IN MORE DETAIL

MEGA SERIES: GLUCOSE DERIVATIVES WITH AMIDE LINKAGE



CHEAP, LOW SOLUBILITY EXCEPT FOR MEGA 8

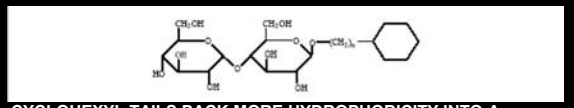
HEGA SERIES: GLUCOSE DERIVATIVES WITH AMIDE LINKAGE, PLUS ADDITIONAL OH



INCREASED SOLUBILITY

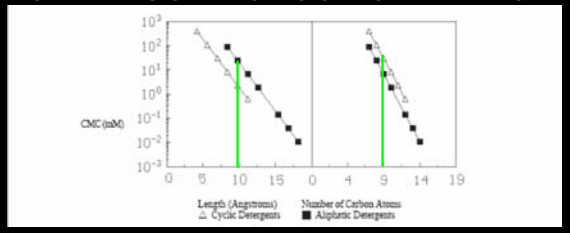
ANATRACE CATALOGUE

CYMAL: CYCLIC MALTOSIDE



CYCLOHEXYL TAILS PACK MORE HYDROPHOBICITY INTO A SHORTER EFFECTIVE CHAIN LENGTH.

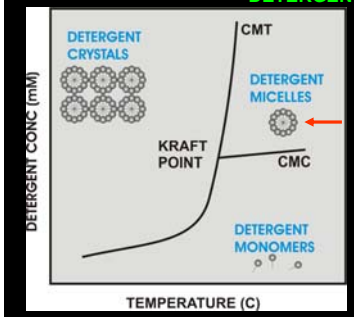
HIGER CMC VALUES THAN CORRESPONDING LINEAR DETERGENT



HARLAN, GARAVITO

ANATRACE CATALOGUE

DETERGENT PHASE DIAGRAMS



THE PHASE TO BE IN: FOR PURIFICATION, STABILIZATION AND AT THE START OF 2D CRYSTALLIZATION.

1. AT LOW TEMPERATURE, DETERGENTS LARGELY IN CRYSTALLINE PHASE
2. AS TEMPERATURE RISES DETERGENT REACHES AN EQUILIBRIUM BETWEEN CRYSTALLINE AND MONOMER PHASE
3. AT THE KRAFT POINT, SOLID, MONOMER AND MICELLES ARE IN EQUILIBRIUM
4. CRITICAL MICELLE CONCENTRATION (CMC) THE LOWEST DETERGENT CONCENTRATION ABOVE WHICH MONOMERS CLUSTER INTO MICELLES.
5. CRITICAL MICELLE TEMPERATURE (CMT) THE TEMPERATURE AT WHICH MICELLES FORM FROM CRYSTALS.

CLOUD POINT

A PARTICULAR TEMPERATURE ABOVE THE CMT, AT WHICH NON-IONIC DETERGENTS UNDERGO PHASE SEPARATION TO YIELD A DETERGENT RICH AND A DETERGENT POOR AQUEOUS PHASE.

CAUSED BY DECREASED HYDRATION OF THE HEADGROUP.

E.G.
TRITON X-100 CLOUD POINT = 64°C
TRITON X-114 CLOUD POINT = 22°C

USES: MEMBRANE PROTEINS CAN BE SOLUBILIZED AT 0°C, BEFORE BEING PURIFIED FROM SOLUBLE PROTEINS BY WARMING TO 30°C, SO THAT TWO PHASES ARE FORMED, WITH THE MP BEING ENRICHED IN THE DETERGENT RICH PHASE.

AGGREGATION NUMBER

$$\text{AGGREGATION NUMBER} = \frac{\text{MICELLAR MOLECULAR WEIGHT}}{\text{MONOMER MOLECULAR WEIGHT}}$$

[MICELLE] = ([DET]-CMC)/AGG. No.

The aggregation number represents the number of detergent molecules in a micelle

for more information see...

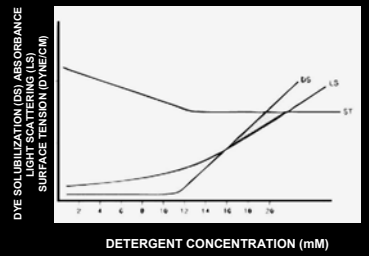
BHAIRI, S.M. (2001) A GUIDE TO THE PROPERTIES AND USES OF DETERGENTS IN BIOLOGY AND BIOCHEMISTRY. AVAILABLE ONLINE FROM THE CALBIOCHEM WEBSITE.

CRITICAL MICELLE CONCENTRATION (CMC)

The critical micelle concentration indicates the concentration above which the detergent will transition from a monomer phase into a micellar phase.

Low CMC		High CMC	
Tween-20	0.06 mM	CHAPS	4-7 mM
C12E8	0.09 mM	OTG	11.3 mM
DDM	0.17 mM	OG	20 mM
Triton X-100	0.2 mM	Cholate	14 mM
		HTG	30 mM

CRITICAL MICELLE CONCENTRATION (CMC)



Traditional approaches to measuring the CMC include dye solubilization (DS), light scattering (LS) and surface tension (ST) measurements. The point of inflection indicates a change in phase from detergent monomers to a micellar phase.

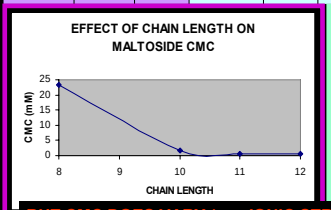
PROPERTIES OF DETERGENTS
--- A GENERAL SUMAMRY ---

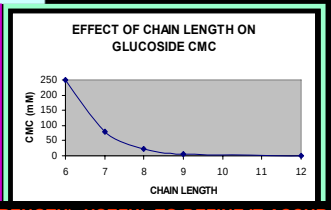
CMC	*Low*	*High*
C_n chain	*Longer*	*Shorter*
Binding	*Stronger*	*Weaker*
Membrane Extraction	*Harsh*	*Mild*
Dialysis	*Difficult*	*Easier*
Costs	*Cheap*	*Expensive*

EFFECT OF CHAIN LENGTH ON CMC

MALTOSIDES			GLUCOSIDES		
CHAIN LENGTH	CMC (mM)	AGG NO.	CHAIN LENGTH	CMC (mM)	AGG NO.
C6	-	-	C6	250	-
C7	-	-	C7	79	-
C8	23.4	84	C8	20-25	84
C9	-	-	C9	6.5	-
C10	1.6	-	C10	-	-
C11	0.59	-	C11	-	-
C12	0.1-0.6	98	C12	0.13	200

1. CMC DECREASES WITH INCREASED CHAIN LENGTH
2. CMC INCREASES WITH DESATURATION
3. ADDITIVES THAT BREAK UP WATER STRUCTURE (EG. UREA) INCREASE CMC.
4. LOW CMC DESIREABLE FOR REDUCED COST OF PURIFICATION
5. HIGH CMC DESIRABLE FOR DETERGENT REMOVAL
6. LONG CHAIN LENGTHS PROTECT BUT STERIC HINDRANCE IN 3D XTALS.





BUT CMC DOES VARY (e.g. IONIC STRENGTH): USEFUL TO DEFINE IT ACCURATELY

HYDROPHILE-LIPOPHILE BALANCE (HLB)

1. HLB IS A MEASURE OF HYDROPHOBIC CHARACTER OF THE DETERGENT
2. THE CLOSER THE HLB is TO 0, THE MORE HYDROPHOBIC THE DETERGENT
3. HLB VALUE OF A DETERGENT CORRELATES WITH ITS ABILITY TO SOLUBILIZE MEMBRANE PROTEINS.
4. DETERGENTS WITH A HLB OF 12 TO 20 ARE PREFERRED FOR NON-DENATURING SOLUBILIZATION OF MEMBRANE PROTEINS.
5. DETERGENTS WITH A HLB IN THE UPPER END OF THE ABOVE RANGE ARE PREFERRED FOR SOLUBILIZATION OF EXTRINSIC MEMBRANE PROTEINS.

HLB = 20(1- ML/MT)

WHERE ML = MW OF THE HYDROPHOBIC PART OF THE MOLECULE
AND MT = TOTAL MW

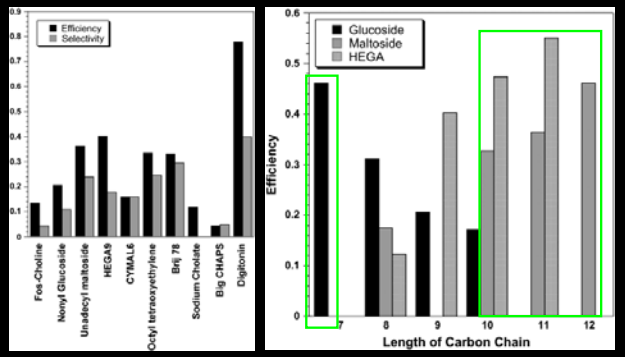
most hydrophobic
denaturing

least hydrophobic
non-denaturing solubilisation

Relating Surfactant Properties to Activity and Solubilization of the Human Adenosine A3 Receptor Berger BW et al 2005 Biophys J. 89: 452-462

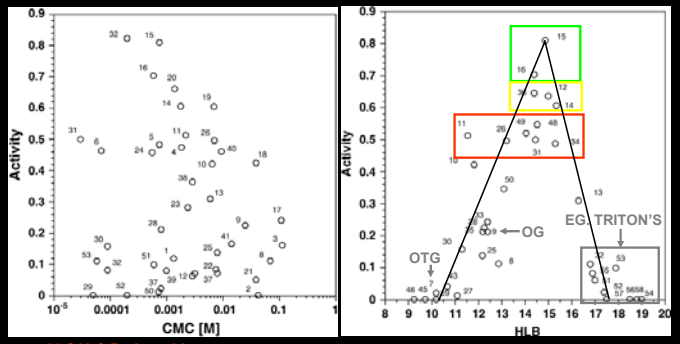
1 Fos-Fenth
2 Nonopol-Fos
3 Fos-choline
4 Fos-choline
5 Fos-choline
6 Fos-choline
7 C8-β-D-thioglucoside
8 C7-β-D-glucoside
9 C8-β-D-glucoside
10 C9-β-D-glucoside
11 C10-β-D-glucoside
12 C8-β-D-thiomaltoside
13 C8-β-D-maltoside
14 C10-β-D-maltoside
15 C11-β-D-maltoside
16 C12-β-D-maltoside
17 HEGA8
18 HEGA9
19 HEGA10
20 HEGA11
21 CYMAL3
22 CYMAL4
23 CYMAL5
24 CYMAL6
25 C8E4
26 C8E5
27 C10E4
28 C10E5
29 C12E4
30 C12E5
31 C12E10
32 Brij 35
33 Brij 76
34 Brij 78
35 Brij 93
36 Brij 96
37 CHAPS
38 Big CHAPS
39 DDAO 26
40 Sodium cholate
41 Sarkosyl
42 Digitonin
43 C7-β-D-thioglucoside
45 C9-β-D-thioglucoside
46 C10-β-D-thioglucoside
48 C9-β-D-thiomaltoside
49 C10-β-D-thiomaltoside
50 C12-β-D-thiomaltoside
51 Surfonyl 485
52 Triton X-305
53 Triton X-405
54 Triton X-705
55 Tween 20
56 Brij 700
57 PEG 4000 distearate
58 PEG 6000 distearate

Relating Surfactant Properties to Activity and Solubilization of the Human Adenosine A3 Receptor Berger BW et al 2005 Biophys J. 89: 452-462



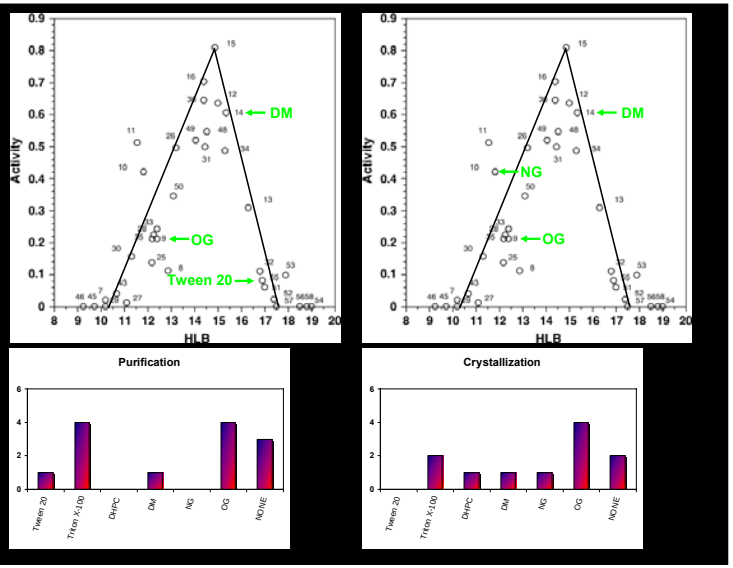
EFFICIENCY AND SELECTIVITY OF PURIFICATION LINKED
EFFICIENCY OF PURIFICATION LINKED TO CHAIN LENGTH

Relating Surfactant Properties to Activity and Solubilization of the Human Adenosine A3 Receptor Berger BW et al 2005 Biophys J. 89: 452-462

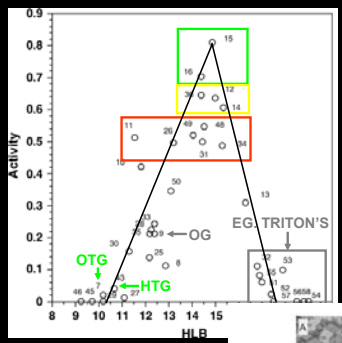


11 C10-β-D-glucoside
12 C8-β-D-thiomaltoside
13 C8-β-D-maltoside
14 C10-β-D-maltoside
15 C11-β-D-maltoside
16 C12-β-D-maltoside

26 C8E5
31 C12E10
34 Brij 78
36 Brij 96
48 C9-β-D-thiomaltoside
49 C10-β-D-thiomaltoside



TYPICAL FACTORS OPTIMIZED:DETERGENT



11 C10-β-D-glucoside
12 C8-β-D-thiomaltoside
14 C10-β-D-maltoside
15 C11-β-D-maltoside
16 C12-β-D-maltoside
26 C8E5
31 C12E10
34 Brij 78
36 Brij 96
48 C9-β-D-thiomaltoside
49 C10-β-D-thiomaltoside

CHAMI ET AL 2001 JSB:133:64-74

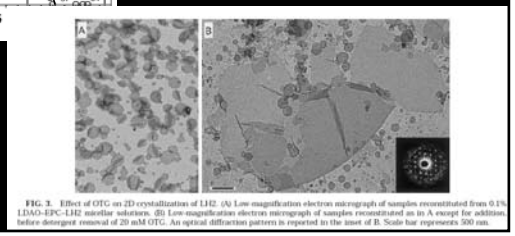
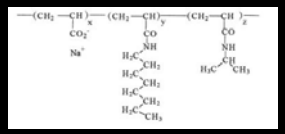


FIG. 3. Effect of OTG on 2D crystallization of LH2. (A) Low-magnification electron micrograph of samples reconstituted from 0.1% LDAO-EPC-LH2 micellar solutions. (B) Low-magnification electron micrograph of samples reconstituted as in A except for addition, before detergent removal of 20 mM OTG. An optical diffraction pattern is reported in the inset of B. Scale bar represents 500 nm.

NEW CLASSES OF AMPHIPATHIC MOLECULES

SCHAFMEISTER ET AL 1993

- A 24-AMINO ACID PEPTIDE DESIGNED TO SOLUBILIZE INTEGRAL MEMBRANE PROTEINS.
- DESIGNED AS AN AMPHIPATHIC ALPHA HELIX WITH A "FLAT" HYDROPHOBIC SURFACE THAT INTERACTS WITH A TRANSMEMBRANE DOMAIN AS A DETERGENT.
- WHEN MIXED WITH PEPTIDE, 85 PERCENT OF BACTERIORHODOPSIN AND 60 PERCENT OF RHODOPSIN REMAINED IN SOLUTION OVER A PERIOD OF 2 DAYS IN THEIR NATIVE FORMS.

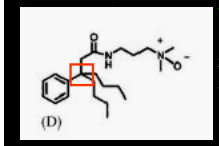


TRIBET ET AL 1996 PNAS 93:15047

1. AMPHIPOLS = AMPHIPHILIC POLYMERS
2. HYDROPHILIC BACKBONE
3. GRAFTED HYDROPHOBIC CHAINS
4. FAVOURABLE PHASE TRANSITIONS FOR CRYSTALLIZATION(?)

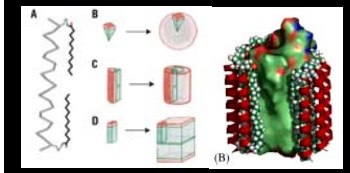
GOOD REVIEW ON DETERGENTS:
P. Nollert 2005 Progress in Biophysics and Molecular Biology 88:339–357

NEW CLASSES OF AMPHIPATHIC MOLECULES



YU ET AL 2000 PROT SCI 9:2518

1. POLAR PORTION CONTAIN AMIDE AND AMINE OXIDE
2. DESIGNED TO DISPLAY GREATER RIGIDITY (TETRA-SUBSTITUTED C).
3. INCREASED RIGIDITY PROPOSED TO INCREASE ABILITY TO CRYSTALLIZE



MCGREGOR ET AL 2003 NAT BIOTECH 21:171-176

1. SYNTHETIC LIPOPROTEINS.
2. MIMIC BILAYER
3. SELF ASSEMBLE INTO A CYLINDRICAL MICELLE
4. RIGID OUTER SHELL (PROTECTIVE)
5. FLEXIBLE INNER LIPID COMPONENT
6. DISPERSE PHOSPHLIPID MEMBRANES
7. GENTLE (PRESERVE MEMBRANE PROTEIN STRUCTURES)

REVIEW: P. Nollert 2005 Progress in Biophysics and Molecular Biology 88:339–357

QUANTIFICATION OF DETERGENTS

Accurate measurements of detergent concentration are desirable, but current methods are slow, unreliable and/or wasteful

- Radiolabelling
- Colorimetric assays of e.g. sugar head groups
- TLC
- Surface tension
- etc. etc.

WHY QUANTIFY DETERGENTS?

1. PROTEINS USUALLY PURIFIED IN EXCESS DETERGENT
2. DETERGENTS WITH LARGE MICELLES (e.g. DDM) ENRICHED DURING PROTEIN CONCENTRATION
3. PROLONGED EXPOSURE REDUCES STABILITY
4. HIGH CONCENTRATIONS INCREASE CRYSTALLIZATION TIME
5. OVERSIZED MICELLES CAN INTERFERE WITH CRYSTAL CONTACTS

A NOVEL METHOD FOR DETERGENT CONCENTRATION DETERMINATION
KAUFMANN ET AL 2006 BIOPHYS J. 90:1310-317 ($US 2500-4000)

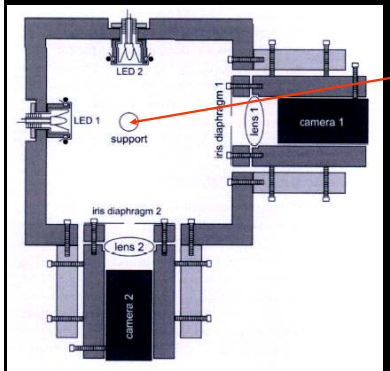
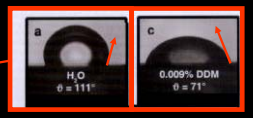


FIGURE 1 Schematic drawing showing the setup of the contact angle measuring device.



PRINCIPLE:

1. PUT DROPLET ON PARAFILM.
2. DET. MOLECULES AT SURFACE LESS ENERGETICALLY FAVOURABLE THAN THOSE IN INTERIOR.
3. THUS SYSTEM TRIES TO REDUCE SURFACE AREA.
4. ADSORPTION OF DET TO SURFACE DISTURBS THE ORDERING OF WATER.
5. THIS LEADS TO A DROP IN SURFACE TENSION AND A SPREADING OF THE DROPLET (20µl).
6. SYSTEM STABLE AFTER 30s.
7. THIS SPREADING IS MEASURED BY THE YOUNG CONTACT ANGLE.

SURFACE ACTIVE COMPOUNDS (GLYCEROL/PEG/LIPIDS) INTERFERE:CALIBRATE

A NOVEL METHOD FOR DETERGENT CONCENTRATION DETERMINATION
KAUFMANN ET AL 2006 BIOPHYS J. 90:1310-317

RAW IMAGES

THRESHOLD APPLIED

DROPLET CONTOURS

CONTACT ANGLES & MEAN VOLUME

FIGURE 2 Image analysis procedure. (*a–c*) Raw images of the droplet series for DDM. (*d* and *g*) Pictures *a* and *c* respectively, with applied threshold. (*e* and *h*) Extracted droplet contours. (*f* and *i*) Output file from GNUPLOT displaying contact angles and mean volume. Note: In *f*, the contour has been rotated by 90° with respect to *e*.

SUMMARY

THE CHOICE OF DETERGENT IS A CRITICAL DETERMINANT OF SUCCESS IN 2D MEMBRANE PROTEIN CRYSTALLISATION

DETERGENTS INFLUENCE SOLUBILISATION OF THE PROTEIN, STABILITY OF THE EXTRACTED PROTEIN AND ULTIMATELY RECONSTITUTION

IT IS DIFFICULT (PERHAPS IMPOSSIBLE) TO PREDICT WHICH DETERGENT WILL BE BEST FOR A PARTICULAR TARGET PROTEIN

A REASONABLE STRATEGY WOULD INVOLVE SAMPLING DETERGENTS COVERING A RANGE OF BIOPHYSICAL PROPERTIES (i.e. HLB, CMC)

ACCURATE DETERGENT QUANTITATION HAS BENEFITS IN AVOIDING EXCESSIVE DETERGENT SOLUBILISATION AND CONSISTENCY ACROSS PREPS

NOVEL AMPHIPATHS ARE BEING DEVELOPED WITH SUPERIOR SOLUBILISATION AND CRYSTALLISATION PROPERTIES