

TIME: A New Membrane Protein Extraction Detergent Screening Kit

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ABSTRACT

Membrane proteins require detergent solubilization for purification and crystallization. The choice of the proper detergent is typically restricted to a low number of readily available detergents and extraction experiments are hence often carried out sequentially. FSEC – fluorescence size exclusion chromatography has proven to be a useful tool in characterizing the extraction yield and behavior in detergent solution of fluorescently labeled membrane proteins (Kawate & Gouaux, 2006; Hattori *et al*, 2012). Here we describe a detergent kit (The Wizard TIME, total integral membrane protein extraction), consisting of 84 different formulations and its utility in identifying those detergent reagents that successfully extract a membrane protein from a membrane preparation. Each detergent formulation consists of a detergent at 2% (w/v) concentration, the stabilizing co-detergent cholesterolhemisuccinate and a buffer. We present an optimized combined ultracentrifugation / FSEC protocol and its application to a test membrane protein.

Background: Screening Detergents for Membrane Protein Extraction

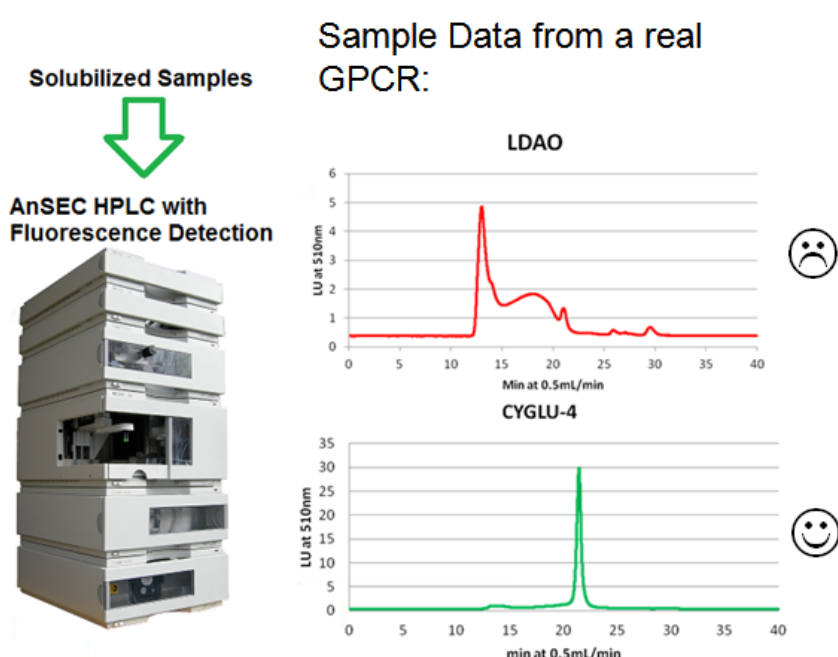
What is FSEC?

- Fluorescence-Detection Size Exclusion Chromatography

Use a fluorescent tag to see what detergents can be used to purify a membrane protein

Uses a small amount of material, 0.1mg of protein is enough to test our entire detergent library (84 commercially available detergents)

Wizard TIME Screen
• Total Integral Membrane-Protein Extraction



References

Kawate T and Gouaux E Fluorescence-detection Size Exclusion Chromatography for precrystallization screening of integral membrane proteins. *Structure* (2006) Vol. 14:4, 673-681

Hattori M, Hibbs RE, Gouaux E. A fluorescence-detection size-exclusion chromatography-based thermostability assay for membrane protein precrystallization screening. *Structure*. (2006) 20(8):1293-9

Tucker, J. and Grishammer, R. (1996) *Biochem. J.* 317, 891-899

Eshaghi, S., Hedrén, M., Nasser, M. I. A., Hammarberg, T., Thornell, A. and Nordlund, P. An efficient strategy for high-throughput expression screening of recombinant integral membrane proteins. *Protein Science*, (2005), 14: 676–683.

Gutmann, D. A.P., Mizohata, E., Newstead, S., Ferrandon, S., Henderson, P. J.F., van Veen, H. W. and Byrne, B. (2007), A high-throughput method for membrane protein solubility screening: The ultracentrifugation dispersity sedimentation assay. *Protein Science*, 16: 1422–1428.

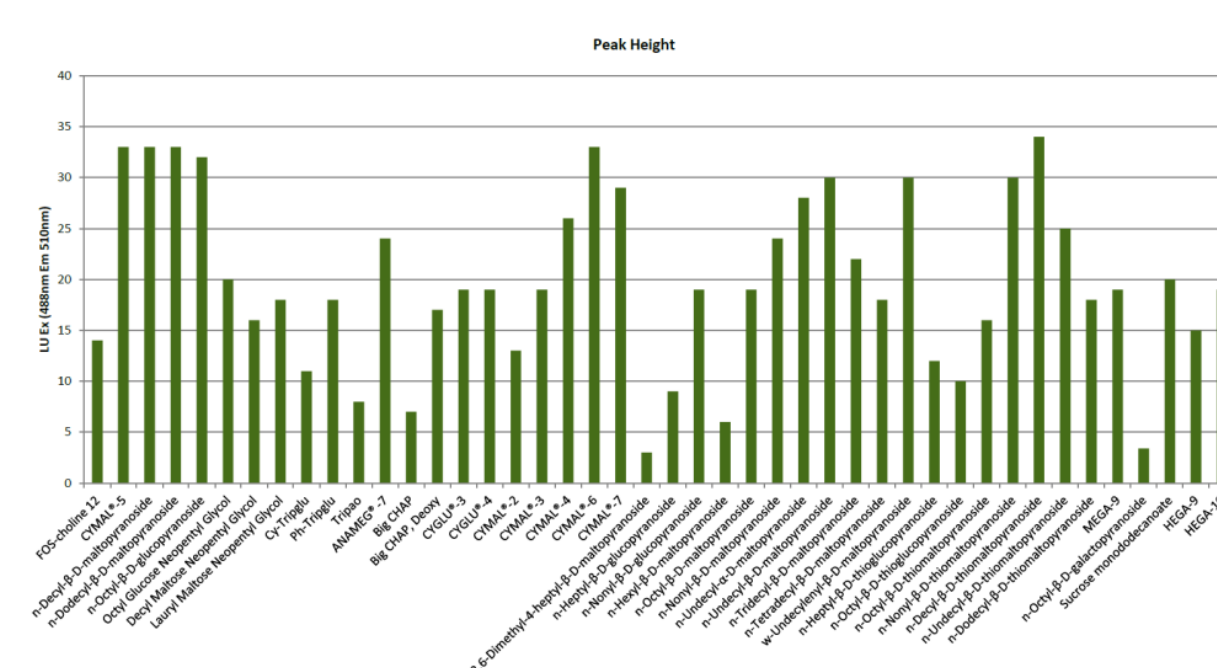
Vergis JM Purdy MD, Wiener MC A high-throughput differential filtration assay to screen and select detergents for membrane proteins *Analytical Biochemistry* (2010) 407:1, 1–11.

Discussion

The WIZARD TIME screen was used to identify detergents that successfully extract a GFP-labeled class 1 GPCR from an insect cell membrane preparation. Results from applying 84 different detergents:

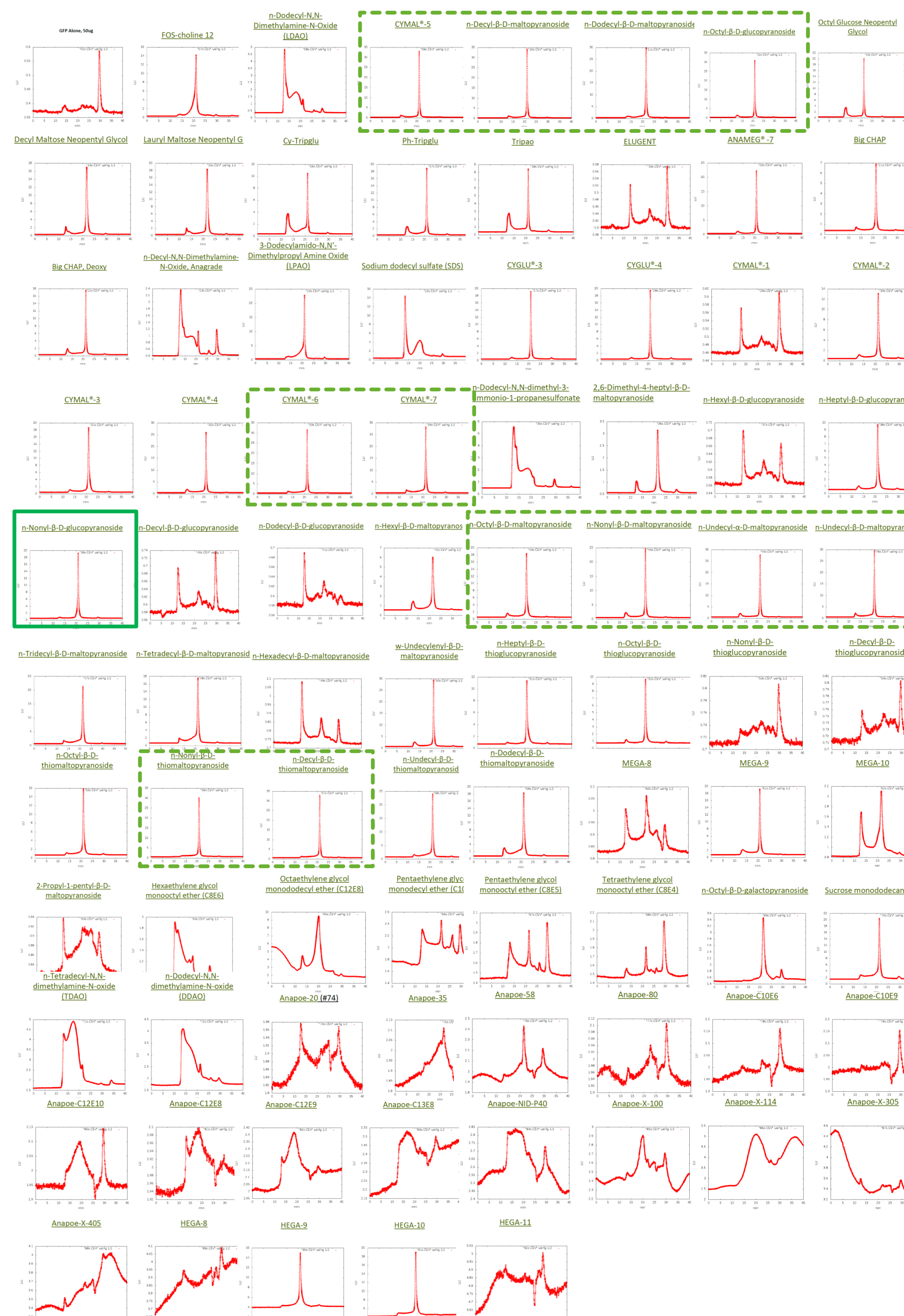
- several detergents (e.g. Nonyl-glucoside, emphasized with green boxes below yield single strong putative monomer peaks;
- certain pyranosides, maltosides and members in the Cymal series are **high-yield extraction reagents that keep this particular membrane protein as a monomer in solution.**

This information is instrumental in **quickly devising a GPCR extraction and purification procedure**, and points out the suitability of 44 detergents for crystalliation spiking experiments.



TIME Screen Extraction Screen Results

To validate the non-ionic detergent collection we extracted a GFP-labeled class 1 GPCR (overexpressed in insect cells) from membranes and applied it to an automated FSEC (Biosil column) experiment (DDM in mobile phase). Shown below are FSEC traces for 84 detergents.



Detergent Formulations

Detergent Name	Injection #	Detergent Name	Injection #
FOS-choline 12	A7	n-Undecyl-β-D-thiomaltopyranoside	E10
n-Dodecyl-N,N-Dimethylamine-N-Oxide (DDAO)	A8	n-Dodecyl-β-D-thiomaltopyranoside	E11
CYMAL*-3	A9	MEGA-8	E12
n-Decyl-β-D-maltopyranoside	A10	MEGA-9	F1
n-Octyl-β-D-glucopyranoside	A11	MEGA-10	F2
n-Octyl-β-D-glucopyranoside	A12	2-Propyl-1-pentyl-β-D-maltopyranoside	F3
Octyl Glucose Neopentyl Glycol	B1	Hexaethylene glycol monoethyl ether (C8E6)	F4
Decyl Maltose Neopentyl Glycol	B2	Octaethylene glycol monododecyl ether (C12E8)	F5
Lauryl Maltose Neopentyl Glycol	B3	Pentaethylene glycol monododecyl ether (C10E5)	F6
Cy-Tripoli	B4	Tetraethylene glycol monoethyl ether (C8E4)	F8
Ph-Tripoli	B5	n-Octyl-β-D-galactopyranoside	F9
Trisac	B6	Sucrose monododecanolate	F10
FLUGENT	B7	n-Tetradecyl-N,N-dimethylamine-N-oxide (TDDAO)	F11
ANAMEG*-7	B8	n-Dodecyl-N,N-dimethylamine-N-oxide (DDAO)	F12
Big CHAP	B9	Anapoe-20	G2
Big CHAP Docus	B10	Anapoe-35	G3
n-Decyl-N,N-Dimethylamine-N-Oxide, Anapoe-81	B11	Anapoe-58	G4
3-Dodecylamido-N,N'-Dimethylpropyl Amine Oxide (LPAO)	B12	Anapoe-80	G5
Sodium dodecyl sulfate (SDS)	C1	Anapoe-C10E5	G6
CYGLU*-3	C3	Anapoe-C12E10	G8
CYGLU*-4	C4	Anapoe-C12E8	G9
CYMAL*-1	C5	Anapoe-C12E6	G10
CYMAL*-2	C6	Anapoe-C13E8	G11
CYMAL*-3	C7	Anapoe-NID-P40	G12
CYMAL*-4	C8	Anapoe-X-100	H1
CYMAL*-5	C9	Anapoe-X-114	H2
CYMAL*-6	C10	Anapoe-X-305	H3
CYMAL*-7	C10	Anapoe-X-405	H4
propanesulfonate	C11	HEGA-8	H5
propanesulfonate	C11	HEGA-9	H6
propanesulfonate	C11	HEGA-10	H7
propanesulfonate	C11	HEGA-11	H8
n-Hexyl-β-D-glucopyranoside	D1		
n-Heptyl-β-D-glucopyranoside	D2		
n-Nonyl-β-D-glucopyranoside	D3		
n-Decyl-β-D-glucopyranoside	D4		
n-Dodecyl-β-D-glucopyranoside	D5		
n-Hexyl-β-D-maltopyranoside	D6		
n-Octyl-β-D-maltopyranoside	D7		
n-Nonyl-β-D-maltopyranoside	D8		
n-Undecyl-α-D-maltopyranoside	D9		
n-Undecyl-β-D-maltopyranoside	D10		
n-Tridecyl-β-D-maltopyranoside	D11		
n-Tetradecyl-β-D-maltopyranoside	D12		
n-Hexadecyl-β-D-maltopyranoside	E1		
w-Undecyl-β-D-maltopyranoside	E2		
n-Heptyl-β-D-thioglucopyranoside	E3		
n-Octyl-β-D-thioglucopyranoside	E4		
n-Nonyl-β-D-thioglucopyranoside	E5		
n-Decyl-β-D-thioglucopyranoside	E6		
n-Octyl-β-D-thiomaltopyranoside	E7		
n-Nonyl-β-D-thiomaltopyranoside	E8		
n-Decyl-β-D-thiomaltopyranoside	E9		

Detergents are supplied at a concentration of 2% (w/v) each, formulated with cholesterol-hemisuccinate as a co-detergent and stabilizing reagent (Tucker & Grishammer, 1996)

TIME Screen Availability

The Wizard: Total Integral Membrane Protein Extraction (TIME) screen aids in the identification of the detergent reagent that successfully extracts a membrane protein from a membrane preparation. Further analysis can be carried out by ultracentrifugation, Fluorescence detection SEC-HPLC (Kawate & Gouaux, 2006, Hattori *et al.*, 2006; Eshagi *et al.*, 2005, Gutmann *et al.*, 2007, Vergis *et al.*, 2010). One membrane protein target can be screened with one kit.

Wizard: TIME - 96 Well Block Plate; EB-TIME-B; \$445.00