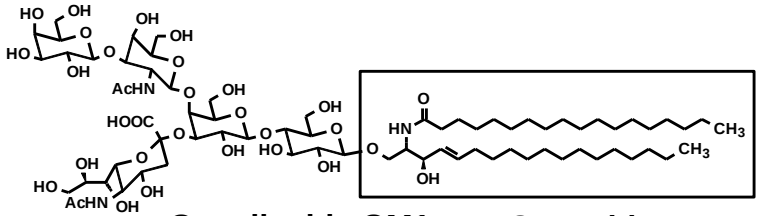
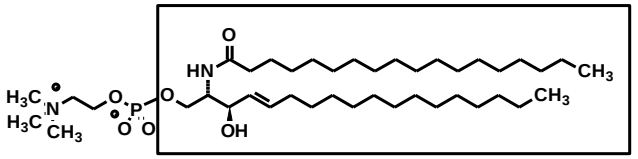
Membrane lipids regulate glycosphingolipid catabolism, its enzymes and lipid binding proteins

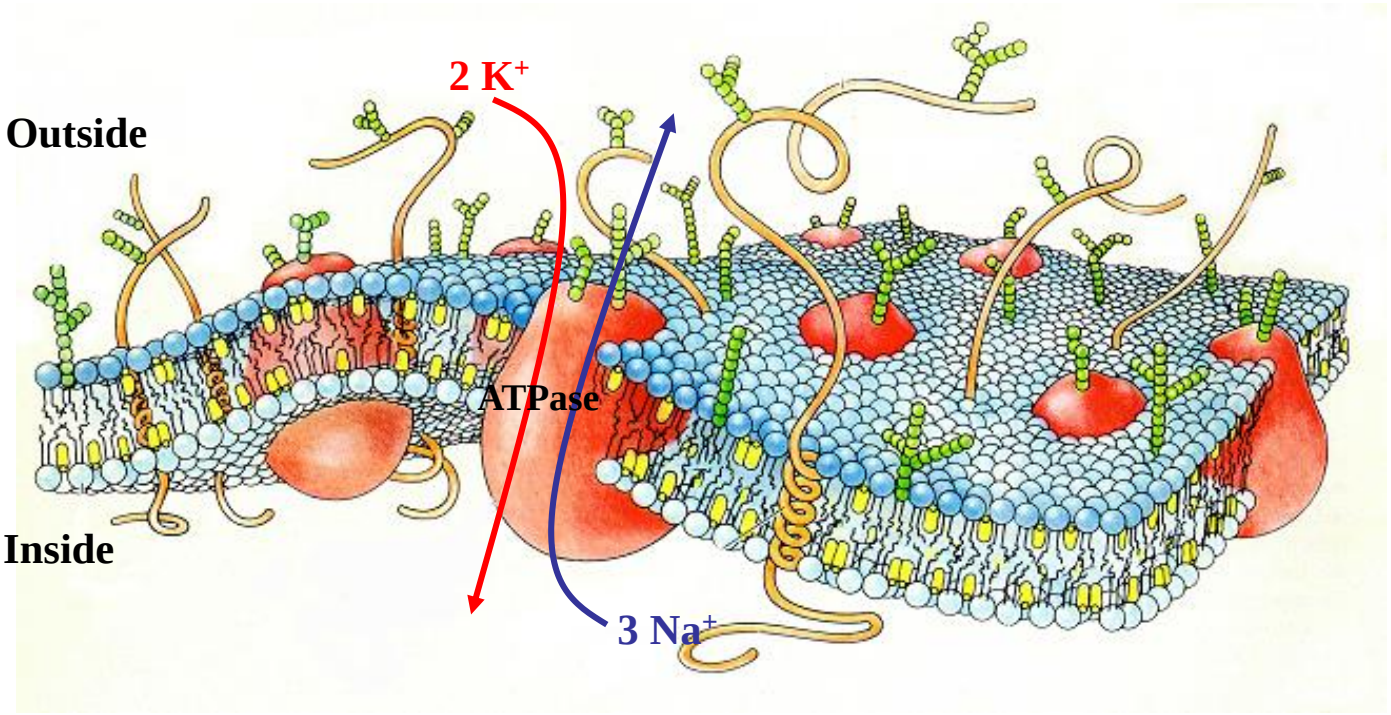
Philadelphia, Aug. 2015

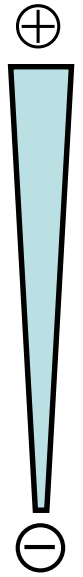


Ganglioside GM1 Ceramide



Sphingomyelin



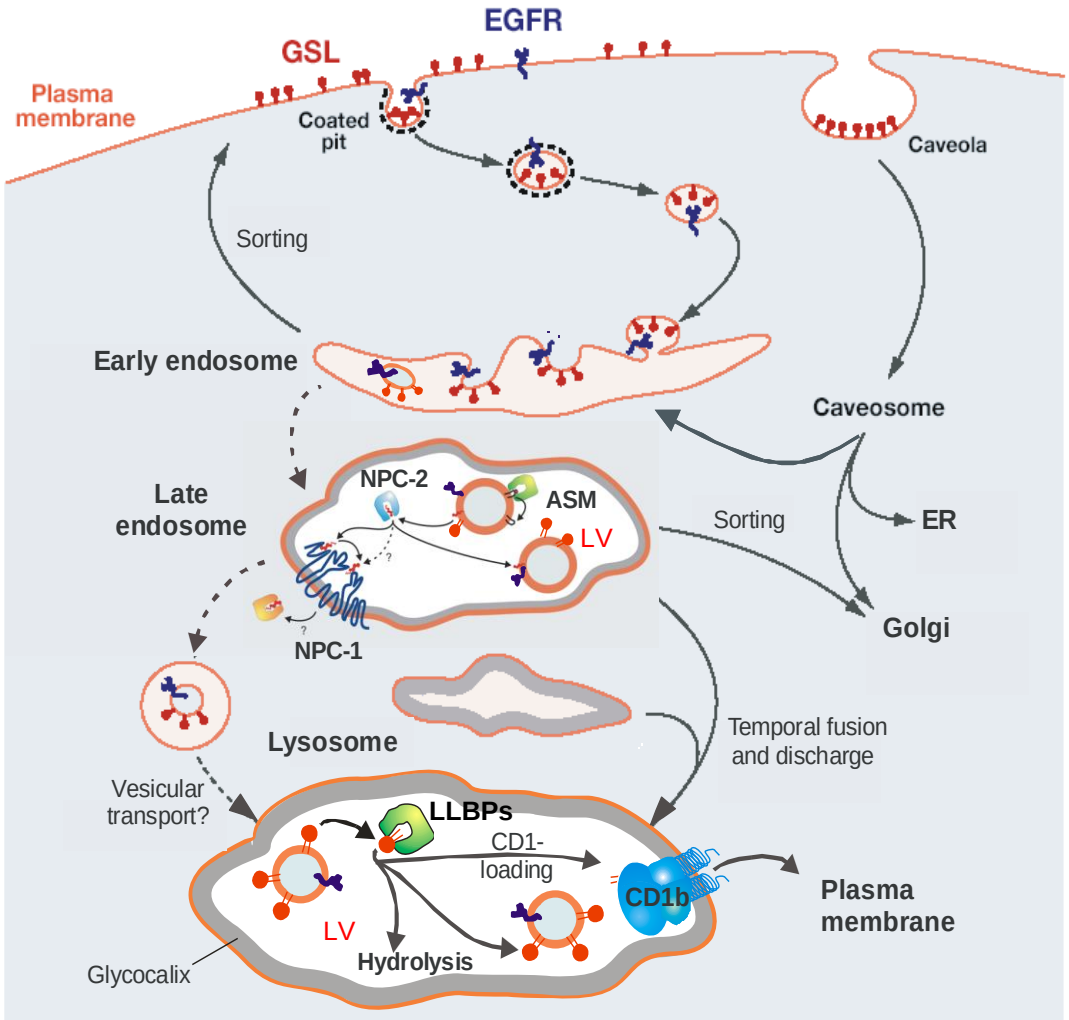


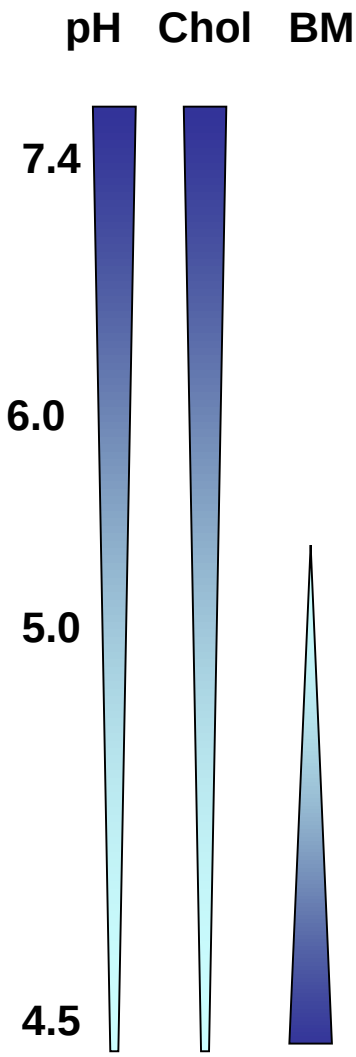
Electro-chemical potential, Gradients

Konrad Sandhoff
LIMES, Membrane Biology and Lipid Biochemistry Unit
Kekulé-Institut
Gerhard-Domagk-Str. 1, 53121, Bonn
Universität Bonn

-Gangliosides, SM & Chol. stabilize neuronal membranes.
-However, Chol. & SM inhibit lysos. catabolism of ganglioside GM2.-

Principles of lysosomal sphingolipid catabolism and membrane digestion





Luminal vesicles (LV) are platforms for SL, GSL- and membrane-degradation

1. Late endosomes:

Maturration of IMs by removal of Chol (NPC1, NPC2, NPC disease)

2. Lysosomes:

Degradation by enzymes and LLBPs (GM2AP, Sap A,B,C,D) :

Inherited defects cause fatal diseases, lipid and membrane storage,

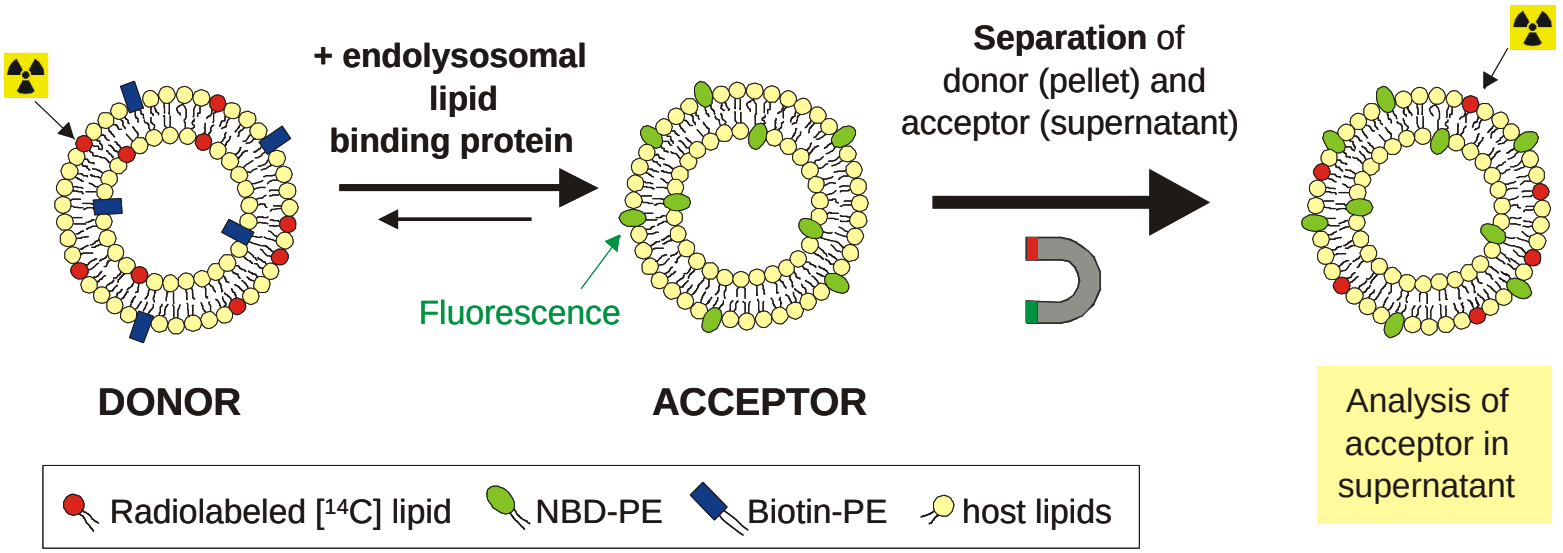
loss of permeability barrier in the skin,

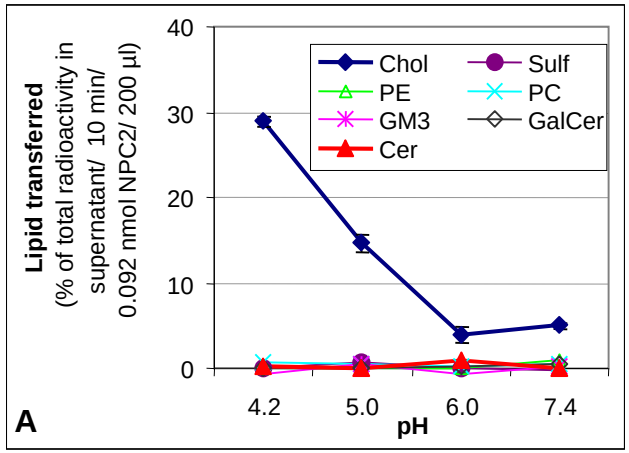
and affect CD1 loading

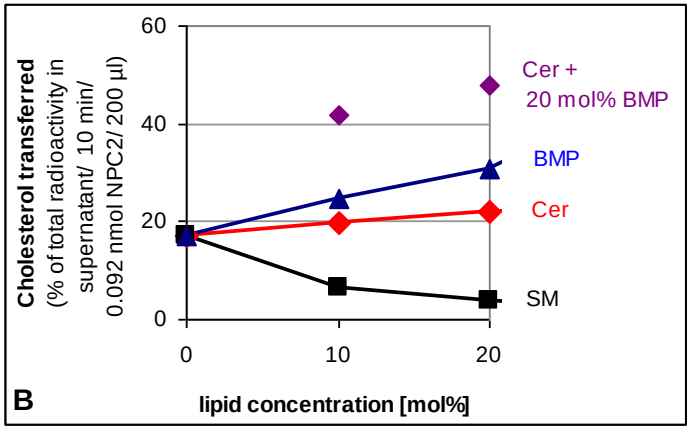
3. KO mice are available

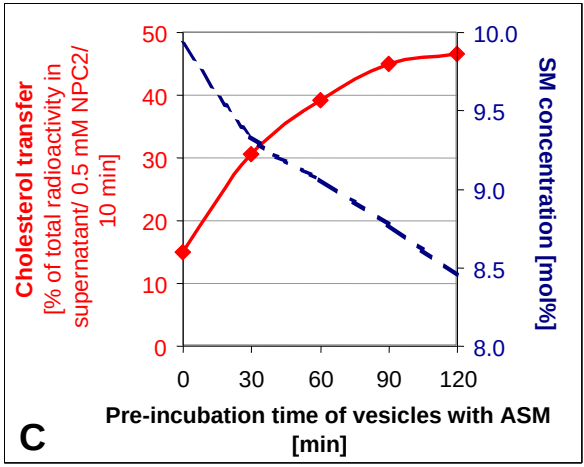
Gallala *et al*., J. Neurochem. 2011

At late endosomes: Membrane lipids & ASM regulate cholesterol transfer by NPC-2







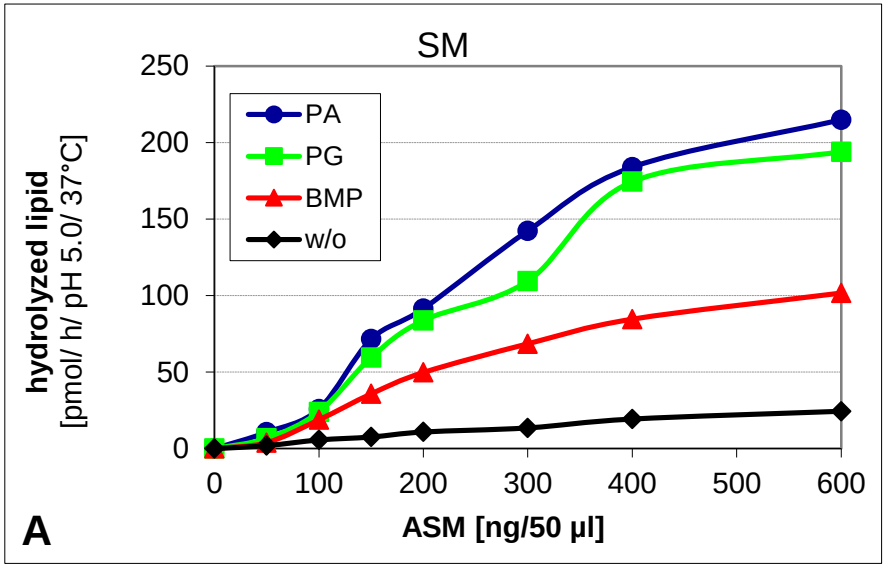


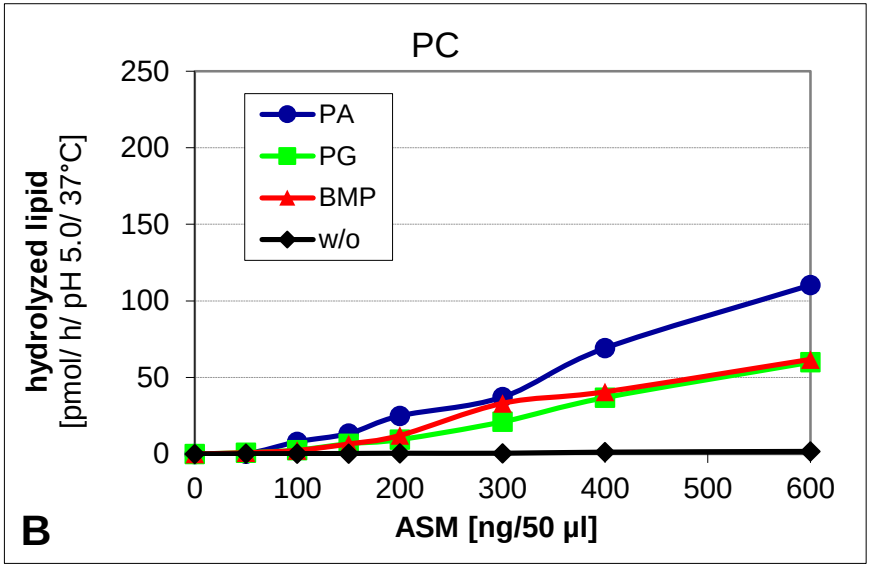
Abdul-Hammed *et al*., J. Lipid Res. 2010

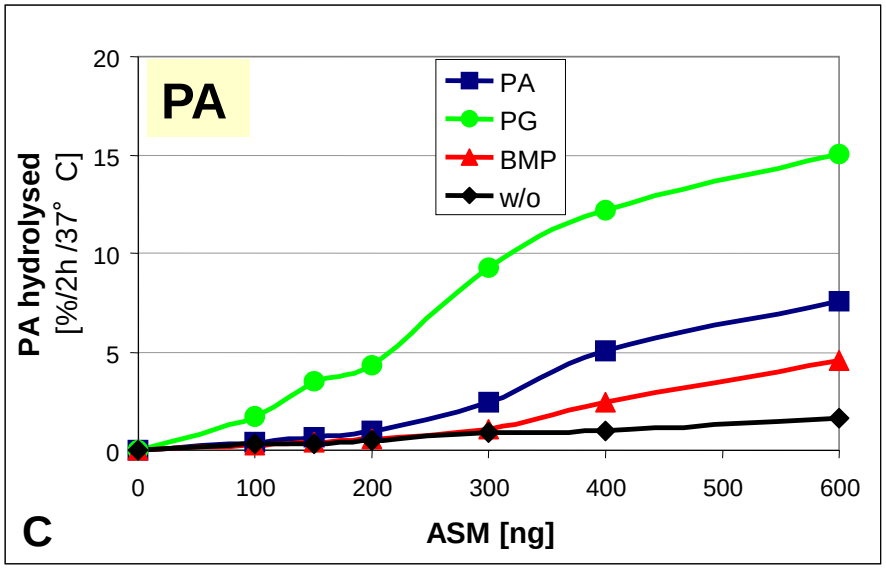
Oninla,Breiden, Sandhoff, 2014

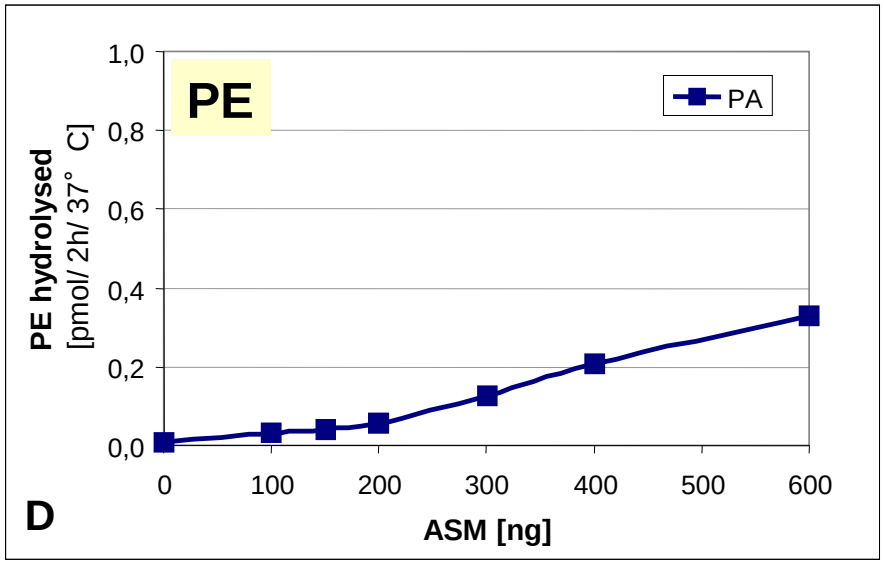
Chol.-storage in NPC disease triggers GM2 & GSL accumulation

ASM is regulated by membrane lipids







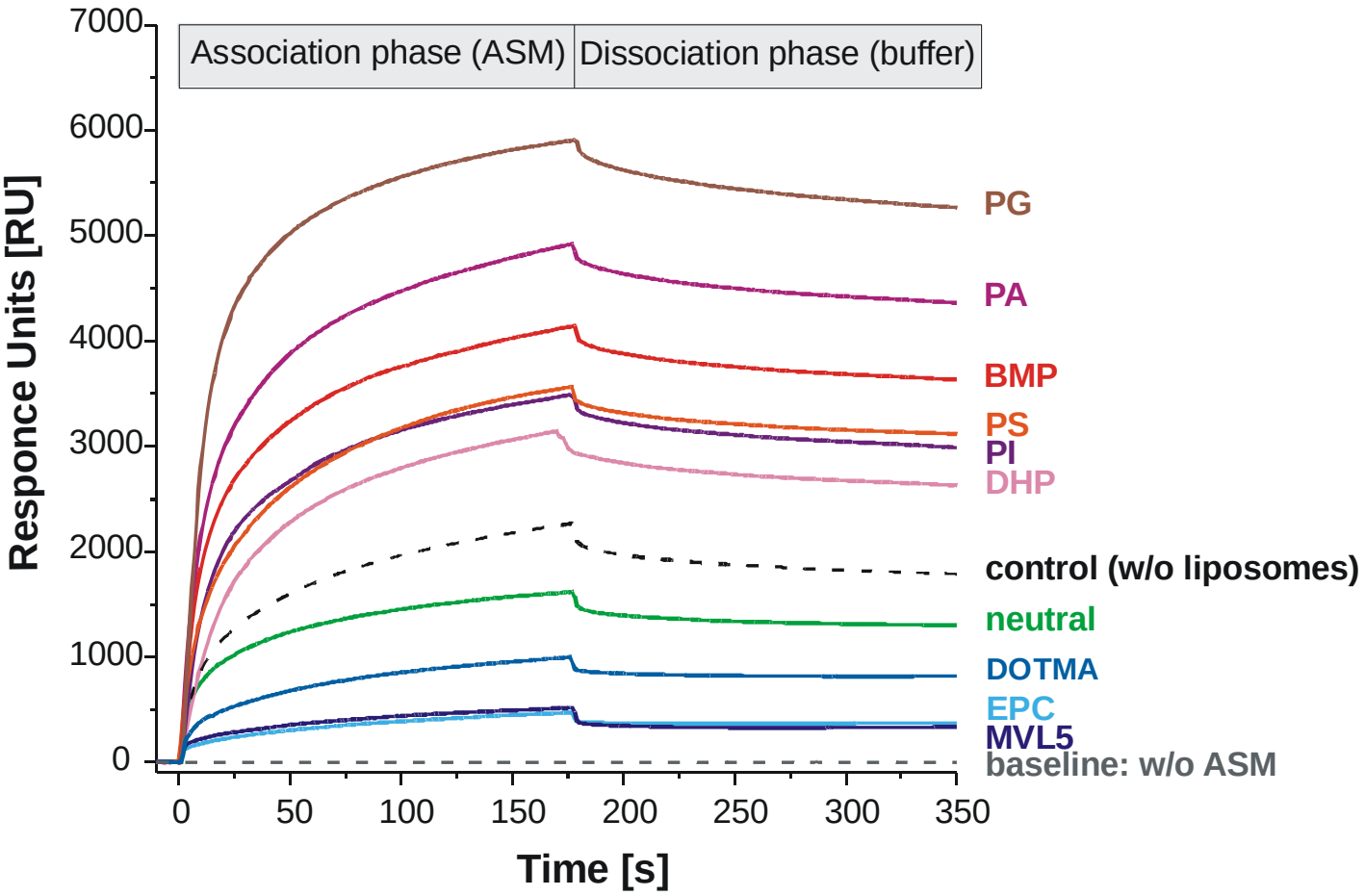


Liposomes: 10 mol% Cholesterol; 10 mol% SM ; 0 or 20 mol% anionic lipids ; 80 or 60 mol% DOPC

ASM: recombinat human ASM expressed in insect *Sf21* cells (Lansmann *et al*., 2000)

Oninla, Breiden et al. (2014) JLR

Electrostatic binding of ASM to liposomes

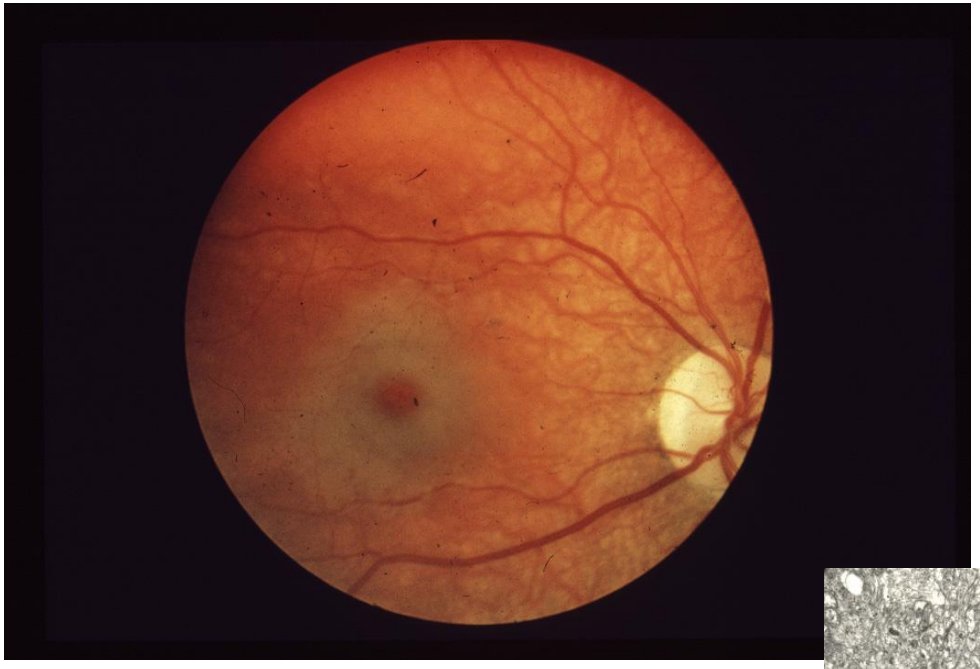


Zeta potential [mV] of liposomes	
PG	- 24.65
PA	- 24.48
BMP	- 26.53
PS	- 23.58
PI	- 21.50
DHP	- 22.45
Neutral (PC)	0.11
DOTMA	22.21
EPC	23.44

10 mol% Cholesterol
10 mol% SM
20 mol% **anionic** or **cationic** lipid
60 mol% DOPC

Oninla *et al.*, JLR 2014

TSD is a recessive and lethal ganglioside storage disease

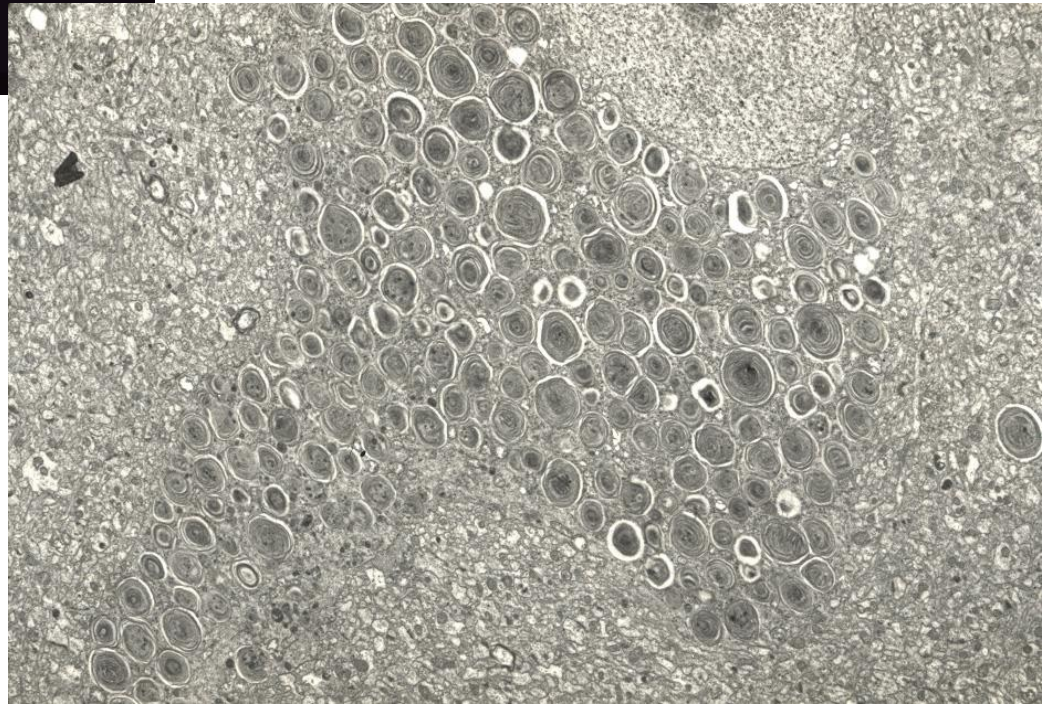


Infantile Amaurotic Idiocy:

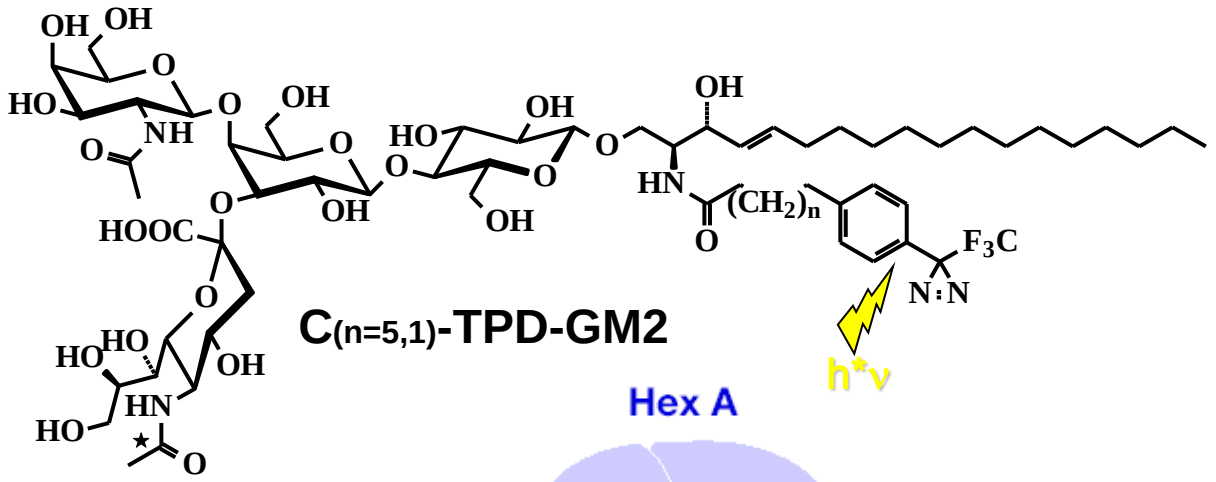
cherry red spot

Tay-Sachs Disease:

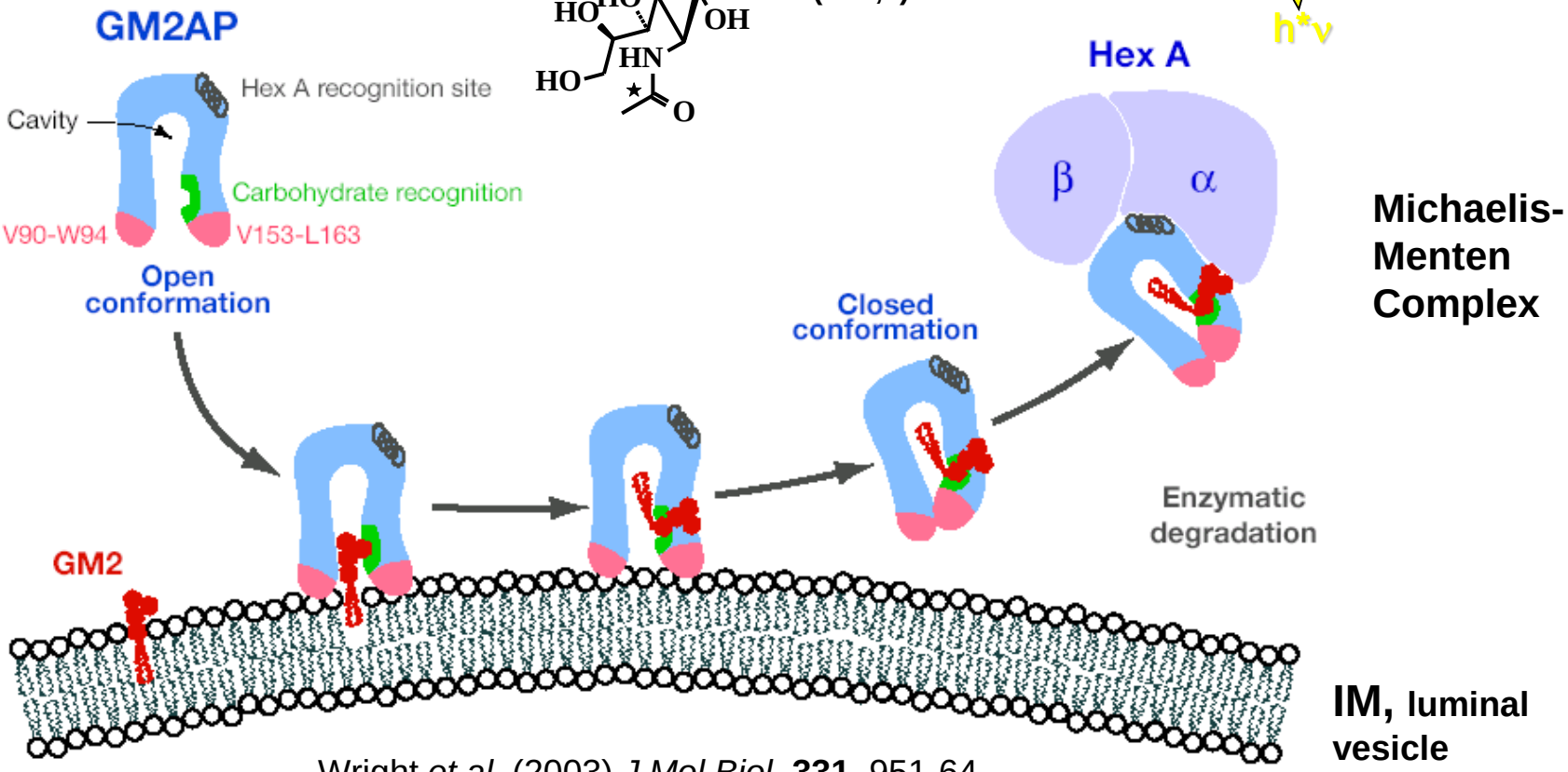
Neuronal MCBs, Lysosomal storage



Mechanism of GM2AP-Liftase



$C_{(n=5,1)}$-TPD-GM2

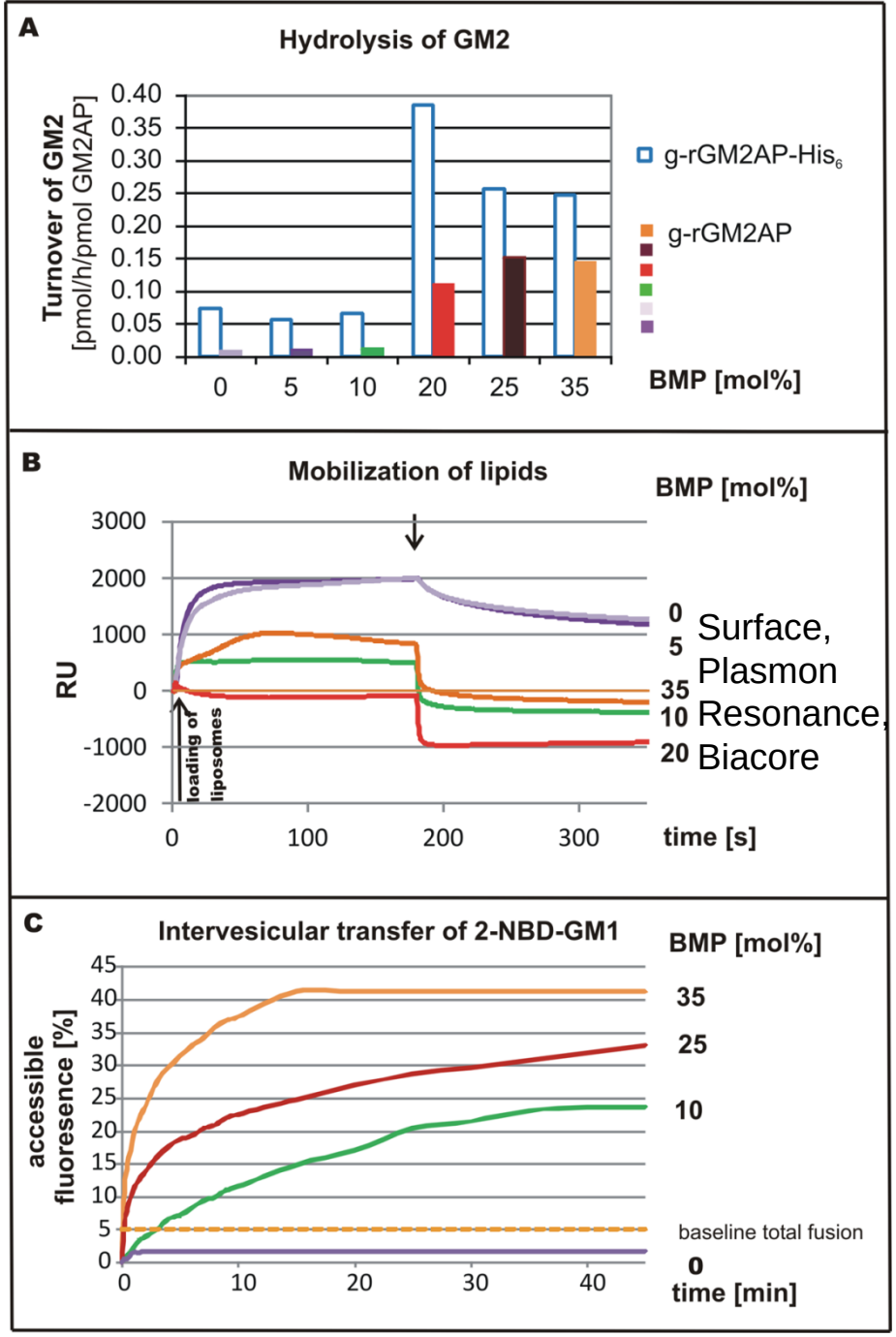


Michaelis-Menten Complex

IM, luminal vesicle

Wright *et al.* (2003) *J Mol Biol.* **331,** 951-64

Wendeler *et al.* (2004) *Eur. J. Biochem. 271:614-27*



BMP (PA&PI) stimulate hydrolysis of liposomal GM2 by Hex A and GM2AP

His-tag falsifies results

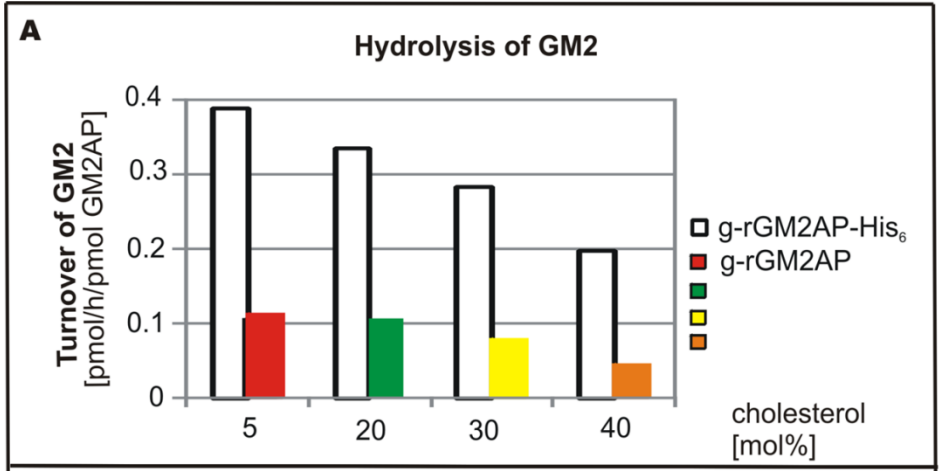
BMP mobilizes membrane lipids by GM2AP

BMP stimulates inter-vesicular lipid transfer by GM2AP

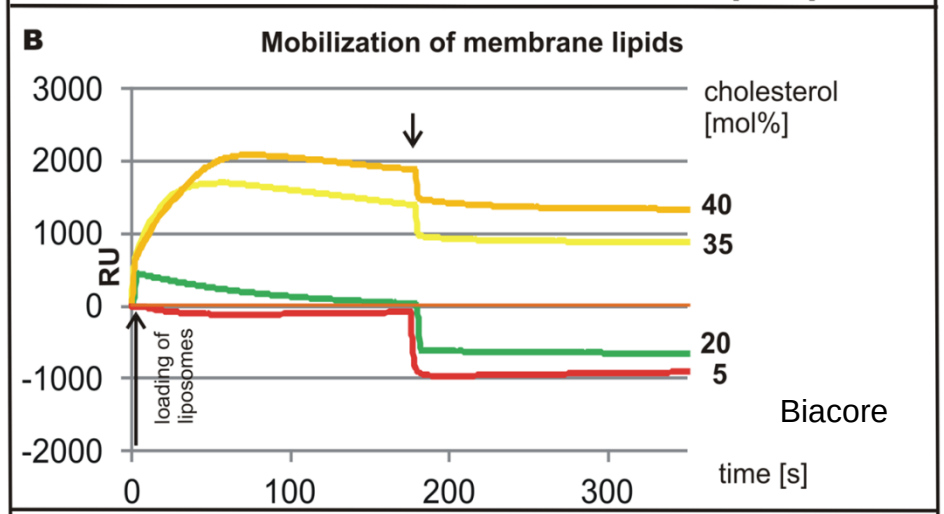
Anheuser et al.(2015), JLR

NPC: Lysosomal cholesterol accumulation inhibits lysosomal GM2 catabolism,e.g. In NPC disease

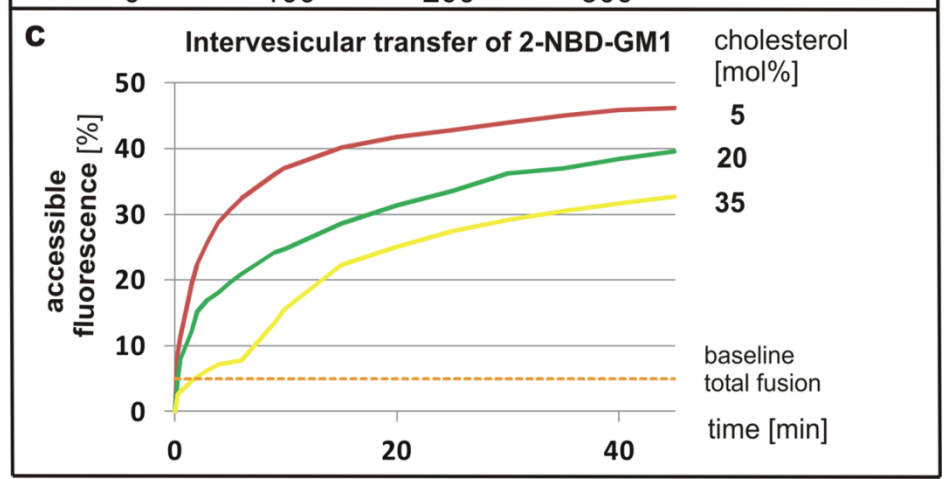
Q: Is solubilization of M-lipids by SAPs physiologically relevant?



Cholesterol inhibits hydrolysis of liposomal GM2 by Hex A & GM2AP



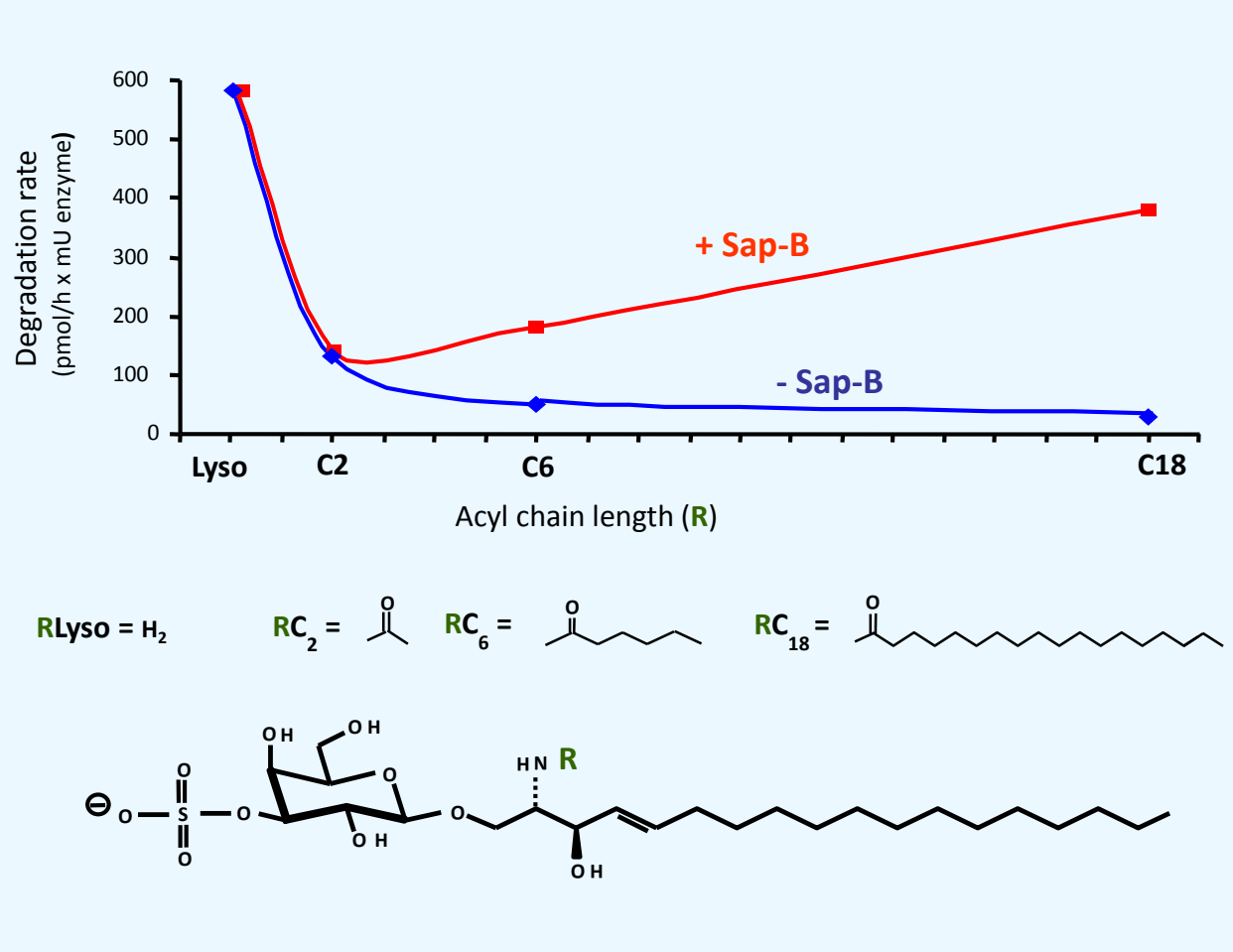
Cholesterol Inhibits solubilization of M-lipids by GM2AP



Cholesterol inhibits Intervesicular transfer M-lipids by GM2AP

Anhheuser et al., (2015) JLR

Lysosomes:
Lipid phase problem, sulfatide cleavage



(Also LUV-bound GlcCers by beta-Glcase, GM2 by Hex A, GM1 by beta-Galase)

Vogel *et al.* (1991) EJB **200**, 591-597

Glycosylation of Sap- B is essential for its physiological functions

	Sap-B	
	Glycosylated	**Unglycosylated**
Causing MLD (glycosylation site variants)	– [1,2]	+ [1,2]
Rescue of Sap-B deficient cells	+ [3,4]	– [4]
In vitro:		
Stimulation of sulfatide degradation (micellar/ liposomal assays)	+ [4]	++ [4]
Lipid transfer (sulfatide etc.)	+ [5]	(+) [6]
SPR (liposomes adsorbed on chip):		
Lipid binding	+ [4]	+ [4]
Lipid mobilization	++ [4]	– [4]

Lipid modifiers may contribute to clinical heterogeneity of lysosomal diseases

- **BMP & other anionic PLs enhance**
  a) cholesterol transfer by NPC2
  b) the hydrolysis of SM by ASM, of GM1 by ß-Galase, and of GM2 by Hex A
  c) the mobilization of membrane lipids by SAPs
  d) transfer of 2-NBD-GM1 by GM2AP
  e) the activities of GM2AP, Sap A & B etc.

- **Cholesterol levels inhibit**:
  Hydrolysis of GM2 by Hex A (in NPC2), PC by ASM, mobilization of lipids by Sap A,B & GM2AP, transfer of 2-NBD-GM1 by GM2AP

- **SM inhibits cholesterol transfer by NPC2 (in NPA&B)**, hydrolysis of GM2 by Hex A , and mobilization of membrane lipids by GM2AP

- **Turnover of GM2 by Hex A**, mobilization of membrane lipids and transfer of 2-NBD-GM1 by GM2AP are dependent on a narrow pH range of 4.2 +/- 0.4
  (Hex A range: pH 3 – 7)

- The His_6-tag changes capabilities of GM2AP :
  It stimulates of hydrolysis of liposomal GM2 by Hex A, but inhibits lipid mobilization completely. It triggers vesicle fusion.

Coworkers

Misbaudeen Abdul-Hammed

Bernadette Breiden

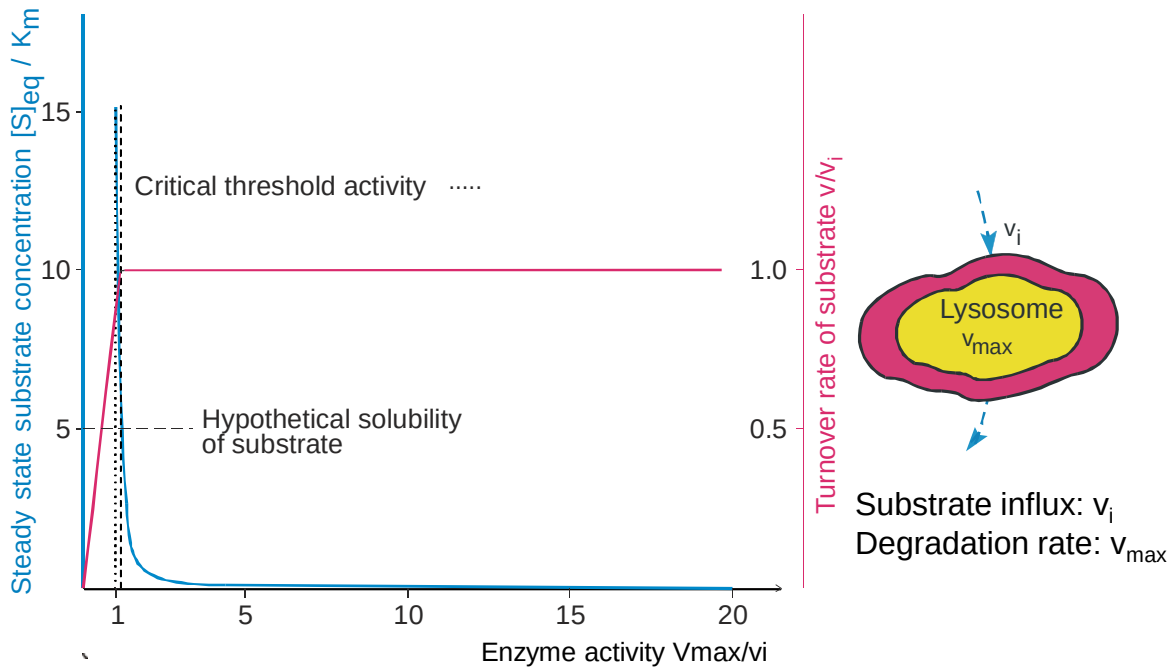
Susi Anheuser

Günter Schwarzmann

Threshold Theory

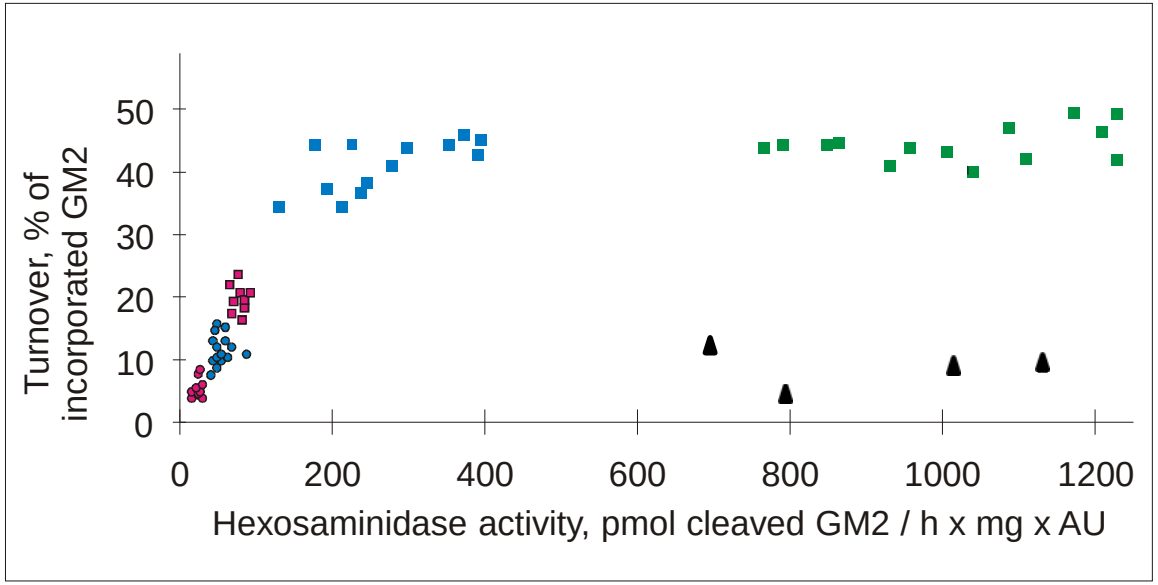
A

$[S]_{eq}/K_m = 1/[(v_{max}/v_i)-1]$



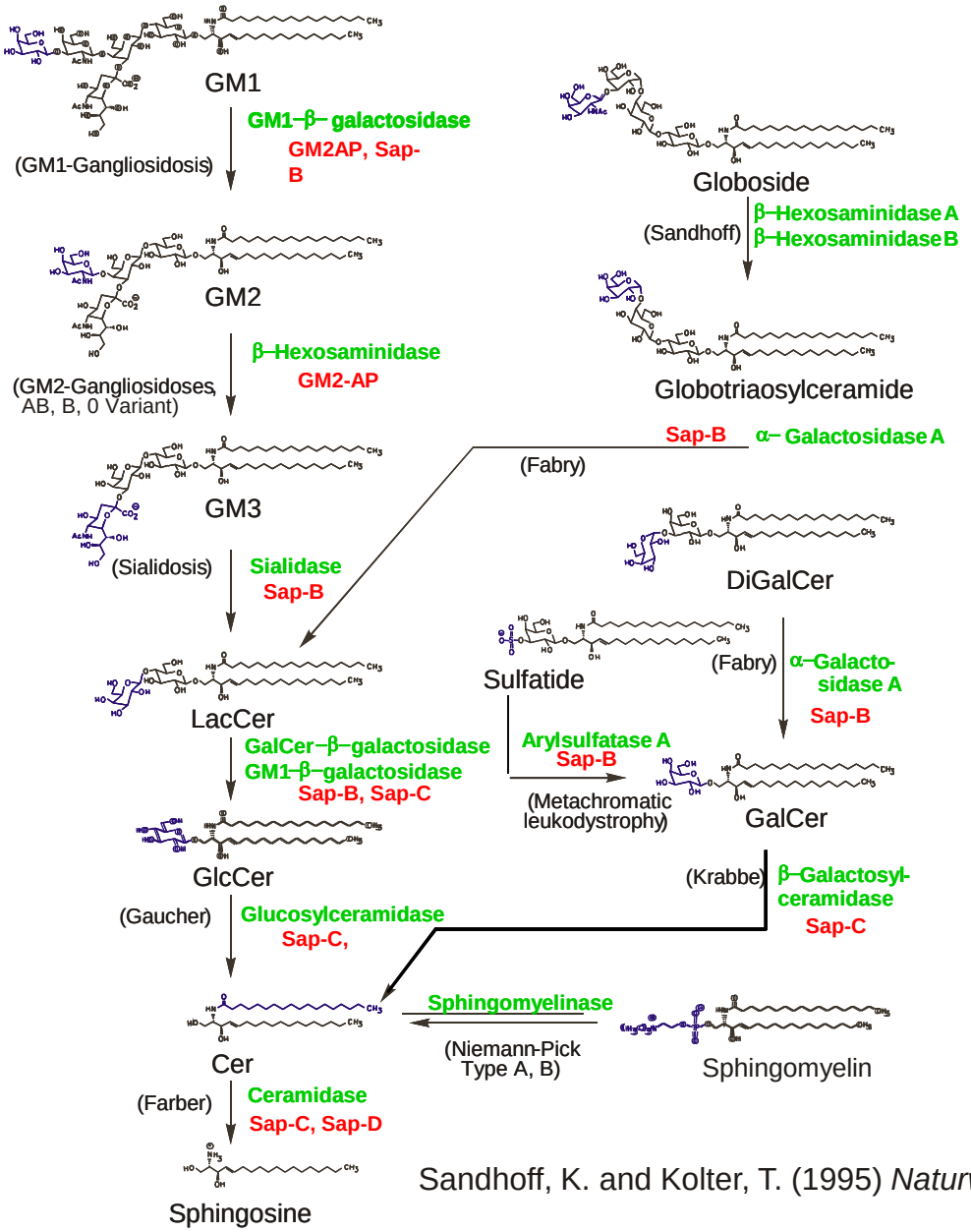
B

- Normal
- Heterozygotes
- Adult
- Juvenile
- Infantile
- GM2-Activator deficiency



1. Wrobe D, Henseler M et al. (2000) A non-glycosylated and functionally deficient mutant (N215H) of the sphingolipid activator protein B (SAP-B) in a novel case of metachromatic leukodystrophy (MLD)." J Inherit Metab Dis **23**(1): 63-76
2. Regis S., M. Filocamo, et al. (1999). "An Asn > Lys substitution in saposin B involving a conserved amino acidic residue and leading to the loss of the single N-glycosylation site in a patient with metachromatic leukodystrophy and normal arylsulphatase A activity." Eur J Hum Genet **7**(2): 125-30
3. Matzner , U., B. Breiden, et al. (2009). "Saposin B-dependent reconstitution of arylsulfatase A activity in vitro and in cell culture models of metachromatic leukodystrophy." J Biol Chem **284**(14): 9372-81
4. Remmel , N., S. Locatelli-Hoops, et al. (2007). "Saposin B mobilizes lipids from cholesterol-poor and bis(monoacylglycero)phosphate-rich membranes at acidic pH. Unglycosylated patient variant saposin B lacks lipid-extraction capacity." Febs J **274**(13): 3405-20
5. Vogel A., G. Schwarzmann, et al. (1991). "Glycosphingolipid specificity of the human sulfatide activator protein." Eur J Biochem **200**(2): 591-7
6. Abdul-Hammed, M., B. Breiden, et al. "Role of endosomal membrane lipids and NPC2 in cholesterol transfer and membrane fusion." J Lipid Res **51**(7): 1747-60

Lysosomal & extracellular GlcCer degradation: Protein & lipid modifiers



Konrad Sandhoff, LIMES, University of Bonn

Amaurotic idiocy: 5 genetic diseases:

GM1- and 4 forms GM2-gangliosidoses (Var AB, B, B1, 0)

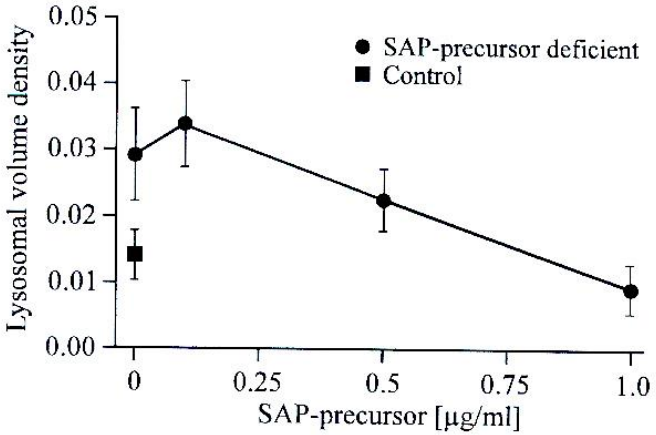
Hex A deficiency: various clinical courses (inf. juv. adult chron.), Threshold theory

Coworker:
Bernadette Breiden
Misbaudeen Abdul-Hammed
Günter Schwarzmann
Susi Anheuser

Sandhoff, K. and Kolter, T. (1995) *Naturw.,* **82, 403-413**

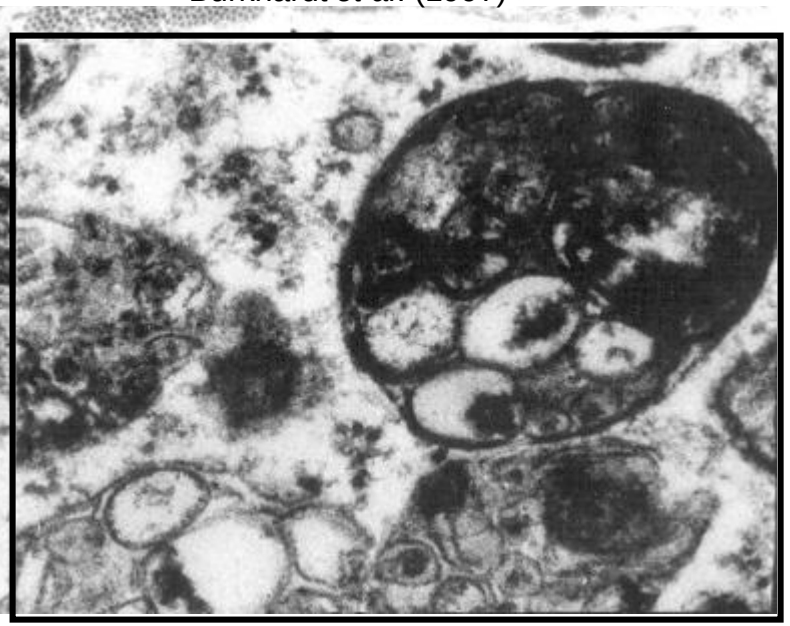
Skin biopsy of a pSap-deficient patient

(W. Roggendorf)

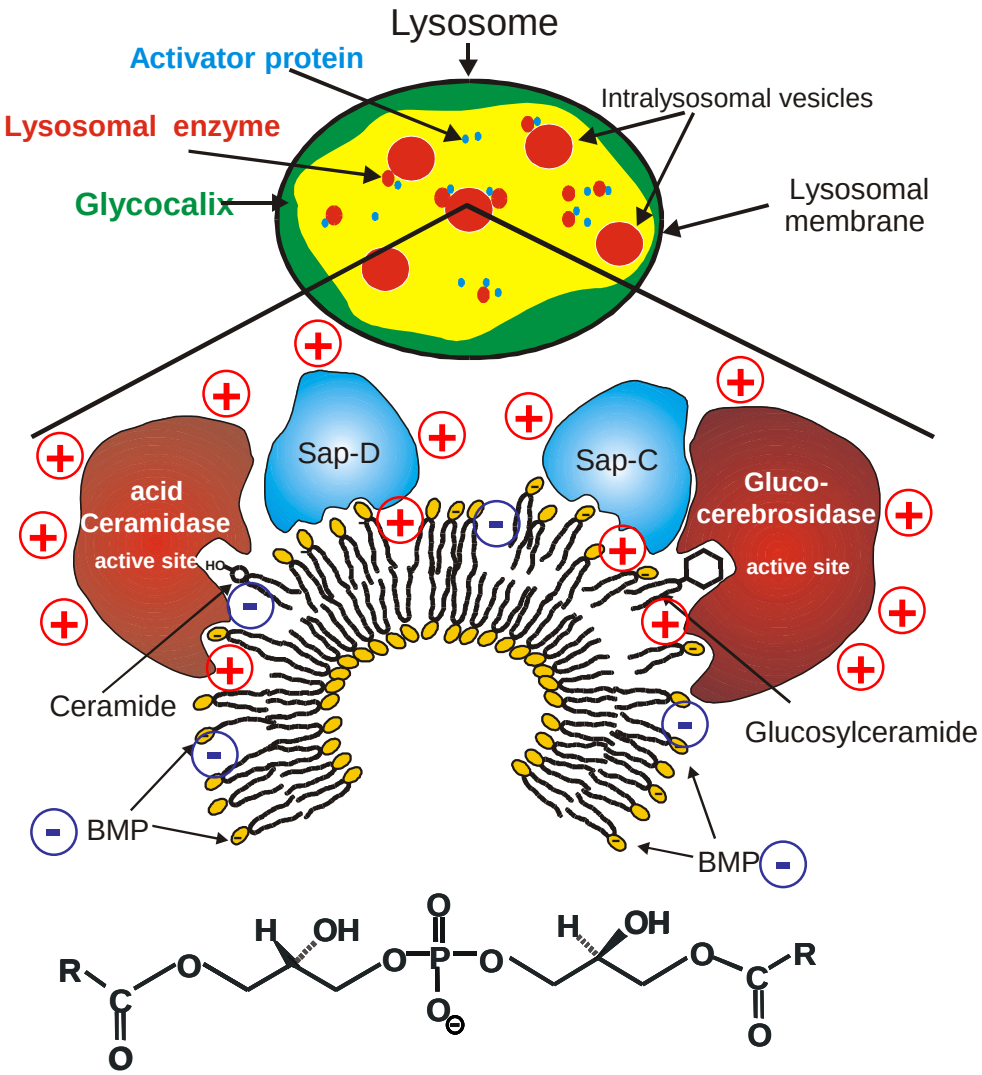


Feeding of pSAP reverses membrane storage

Burkhardt *et al*. (1997)



Sphingolipid degradation at intralysosomal membrane surfaces: Stimulation by SAPs and anionic lipids



Bis(monoacylglycero)phosphate (BMP, S-configuration, sn1)

- **Electrostatic interaction: Cationic proteins bind to anionic vesicles**
- **SAPs stimulate enzymatic hydrolysis of liposome-bound sphingolipids.**
- **BMP and other anionic PLs stimulate degradation of:**
  - Cer, GlcCer, SM, GM1, GM2:

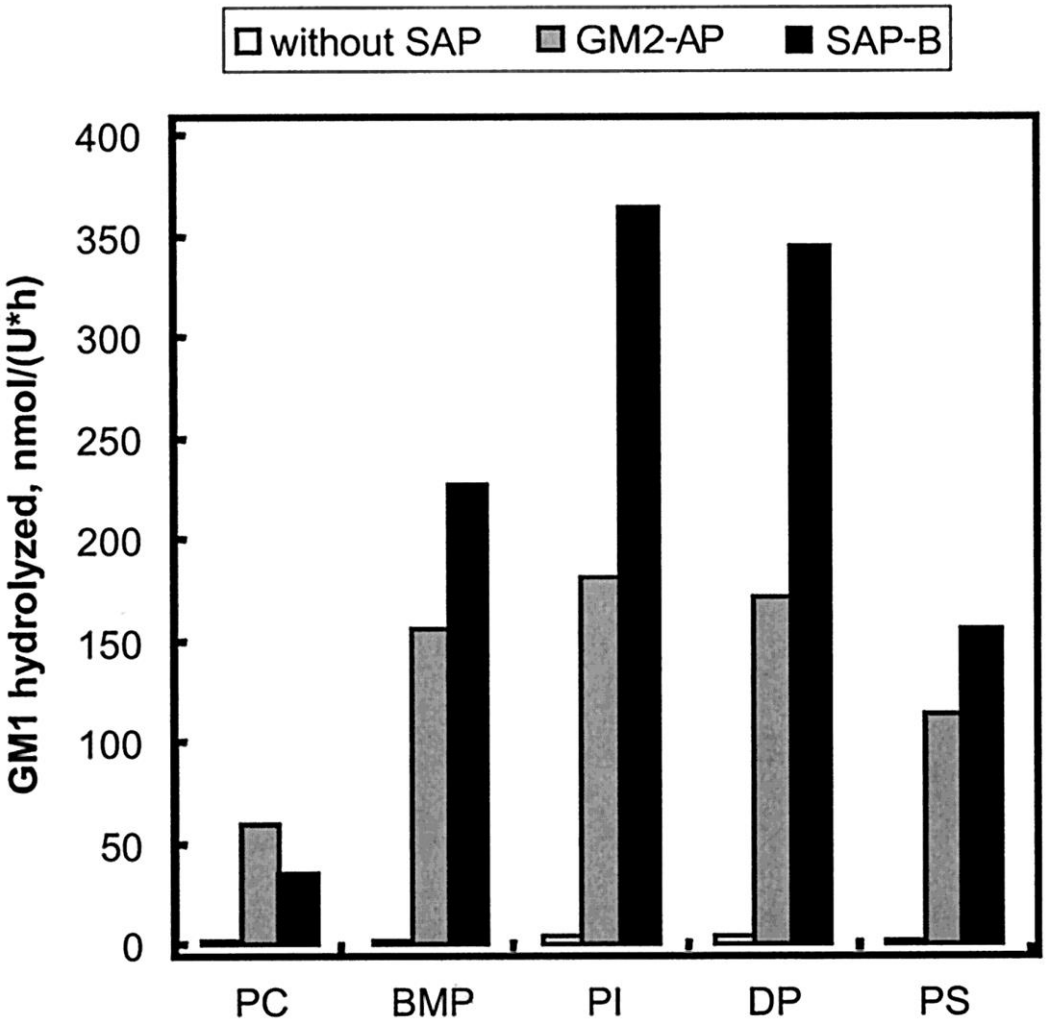
3-15 fold in the presence of SAPs.

(Sensitivity to CADs)

Sandhoff ,Proc. Jpn. Acad. Ser. B, Vol. 88, 554-82 (2012)

Wilkening, Linke, Sandhoff (1998) *J. Biol. Chem.* **273,** 30271-8

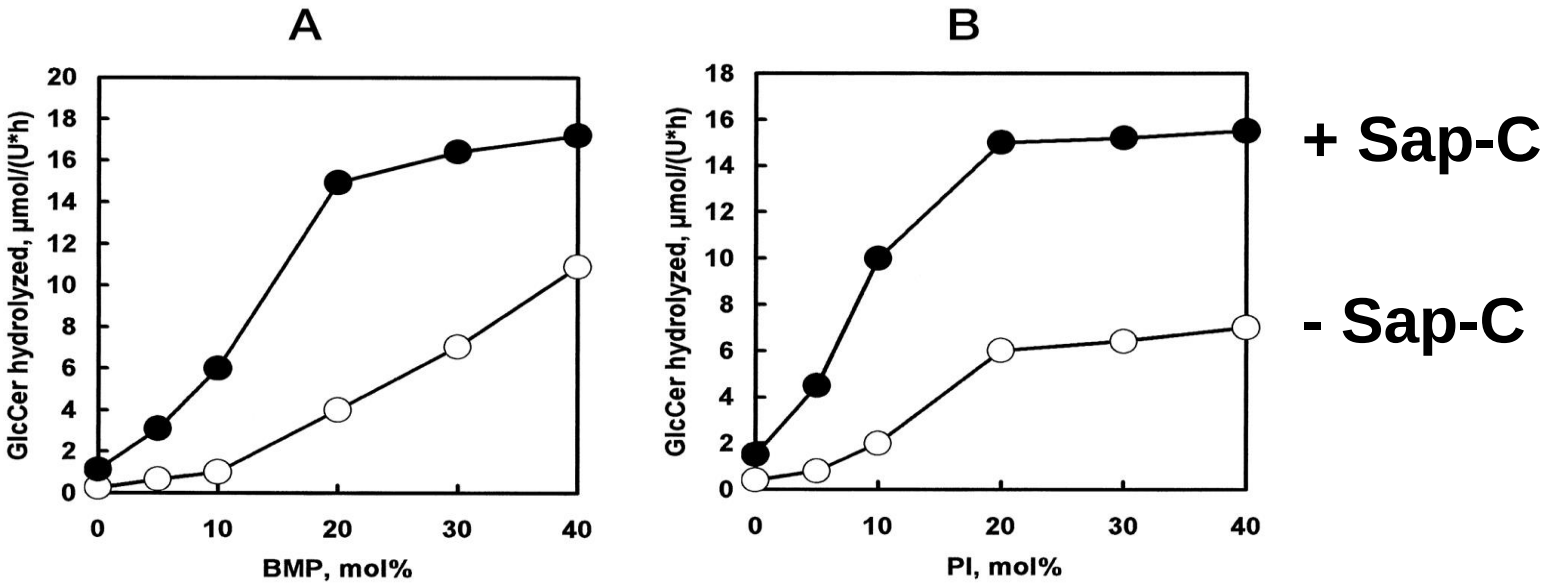
Lysosomal anionic lipids enhance the degradation of GM1 by β-galactosidase, but only in the presence of SAPs



Lysosomal anionic lipids (10 mol %) were incorporated in LUVs, composed of 10 mol % GM1, 20 mol % cholesterol, and 60 mol % PC. Assays in the absence of an activator protein and in the presence of 5 μm GM2-AP or 5 μm SAP-B were carried out.

Wilkening G *et al*. (2000) J. Biol. Chem. 275, 35814-35819

Lysosomal lipids in LUVs stimulate the enzymatic GlcCer hydrolysis in the presence and also in the absence of Sap-C.



A: Assays were conducted with GlcCer as substrate in the absence (○) and presence of Sap-C (2.5 µm) (•), using LUVs with various proportions of synthetic BMP (0–40 mol %).

B: Assays were carried out with varying concentrations of PI in LUVs, with (•) and without (○) the addition of 2.5 µm Sap-C, keeping the total lipid concentration in the assays constant.

Wilkening G et al. J. Biol. Chem. 1998;273:30271-30278

Conclusions

-- Several inherited diseases can generate the same clinical phenotype (amaurotic idiocy caused by GM1&GM2 gangliosidoses (TSD, SD, Var AB & B1)

-- Mutations in the same gene (e.g.Hex A) can cause different clinical courses (inf., juv., adult & chronic courses due to different levels of residual catabolic activities)

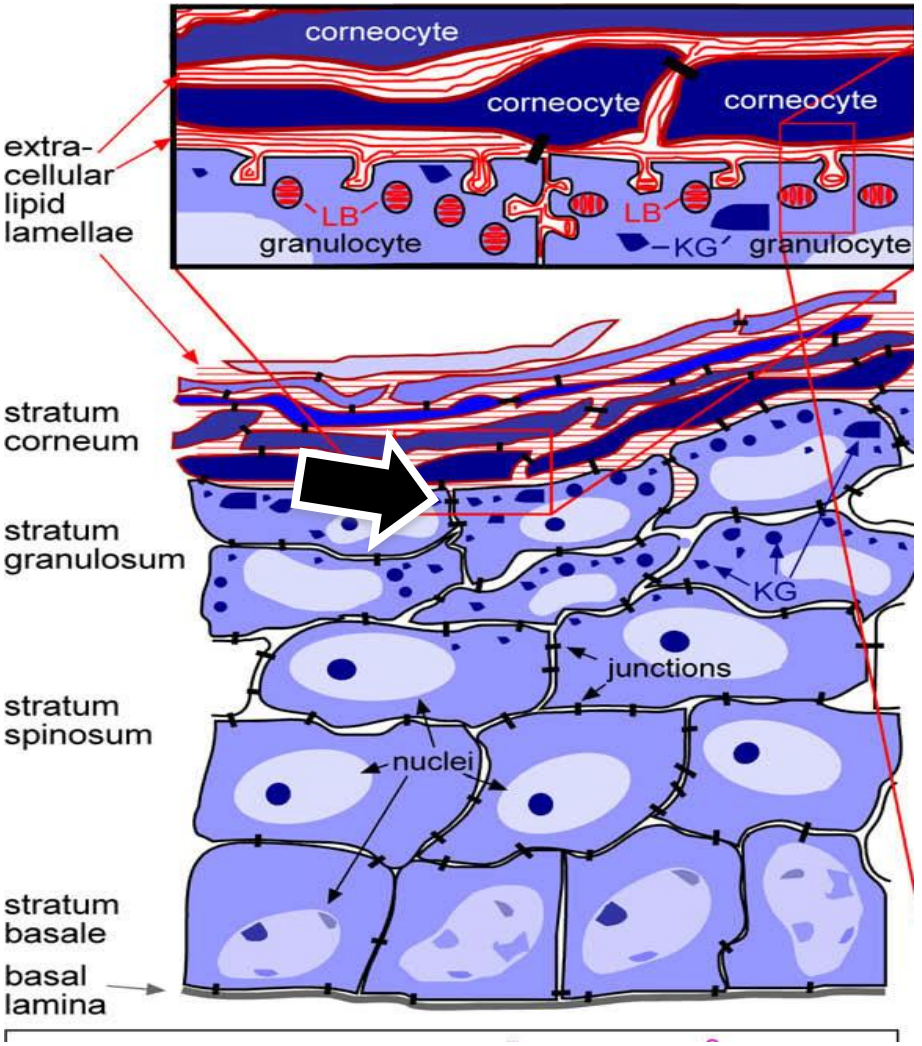
-- Catabolic activity of an enzyme (e.g. Hex A) is strongly modified by the microenvironment in which the enzymic reaction occurs, e.g. at the surface of liposomes (or in vivo at the surface of luminal lysosomal vesicles) (pH-value, ionic strength, LTPs or SAPs, lipid composition of the **GM2** carrying membrane: anionic M-lipids stimulate (up to 15 fold), cholesterol inhibits strongly

-- No direct correllation between gene mutations and the clinical course of a disease, the microenvironment of the enzymic reaction modifies the catabolic rate.

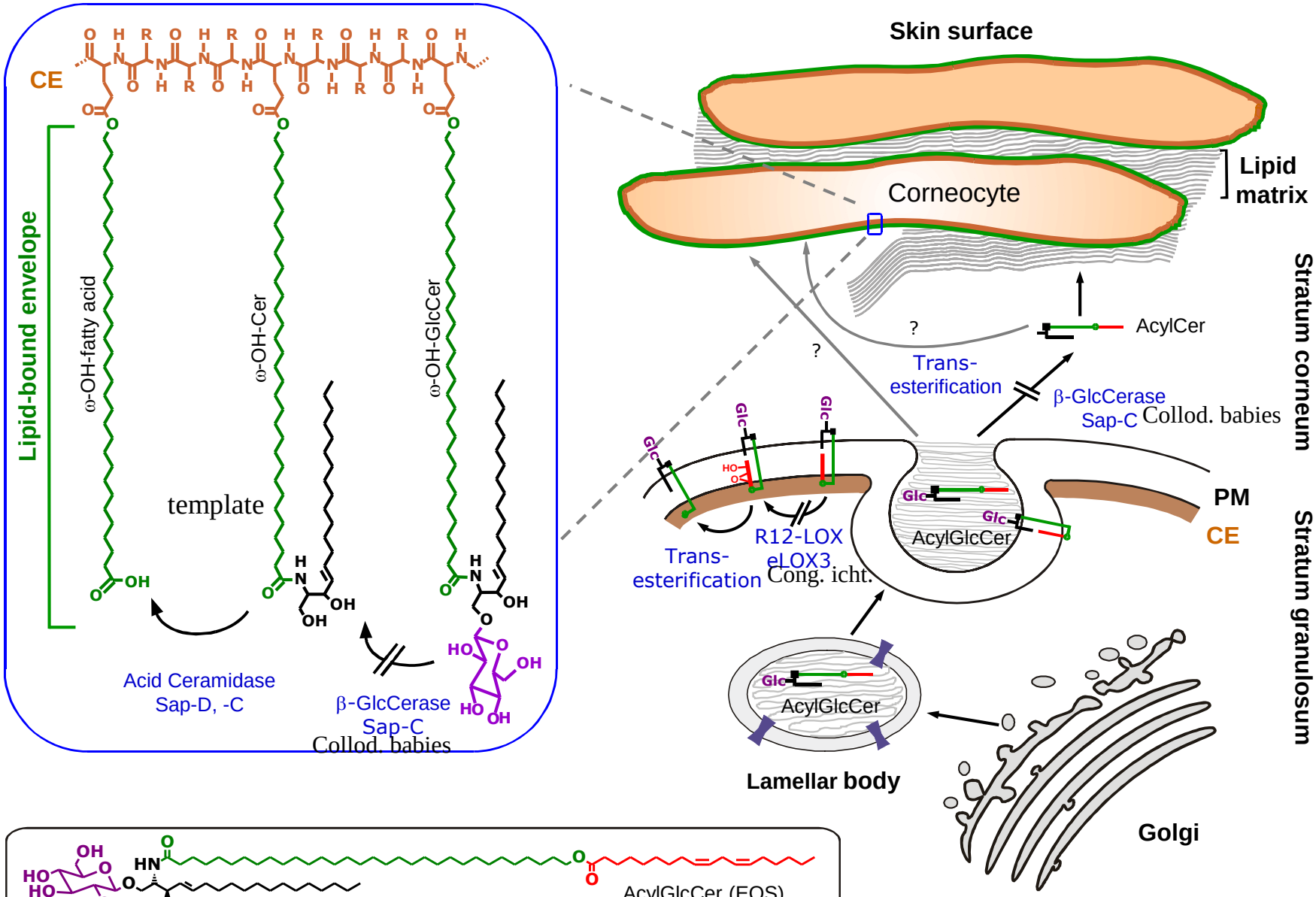
Ceramide metabolism: Key to skin barrier function

Epidermis is important
against desiccation and infections

Barrier : Extracellular lipid layers :ULC-Cers ,ULC-FA, Cholesterol

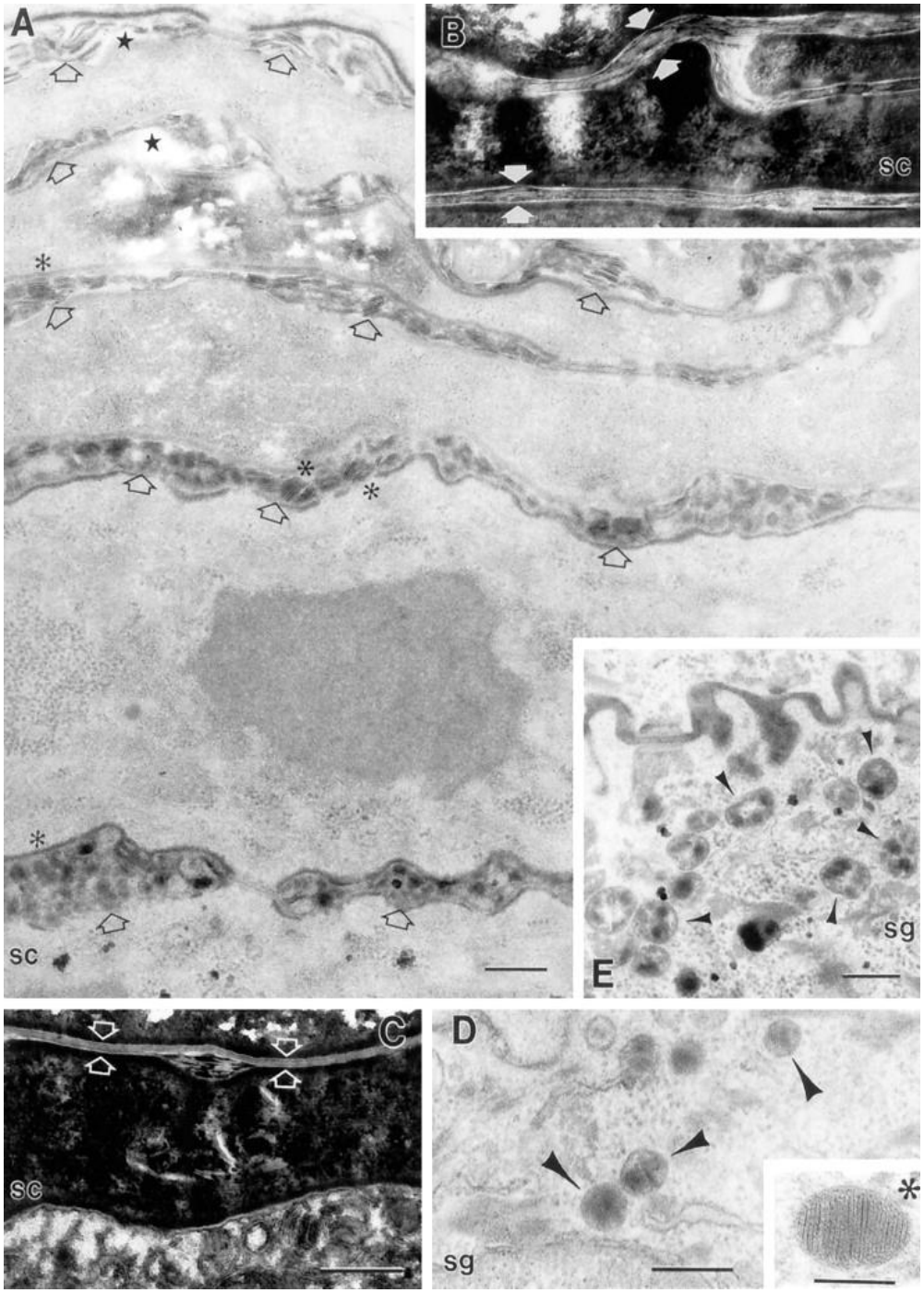


Permeability barrier of the skin



Breiden & Sandhoff, 2013

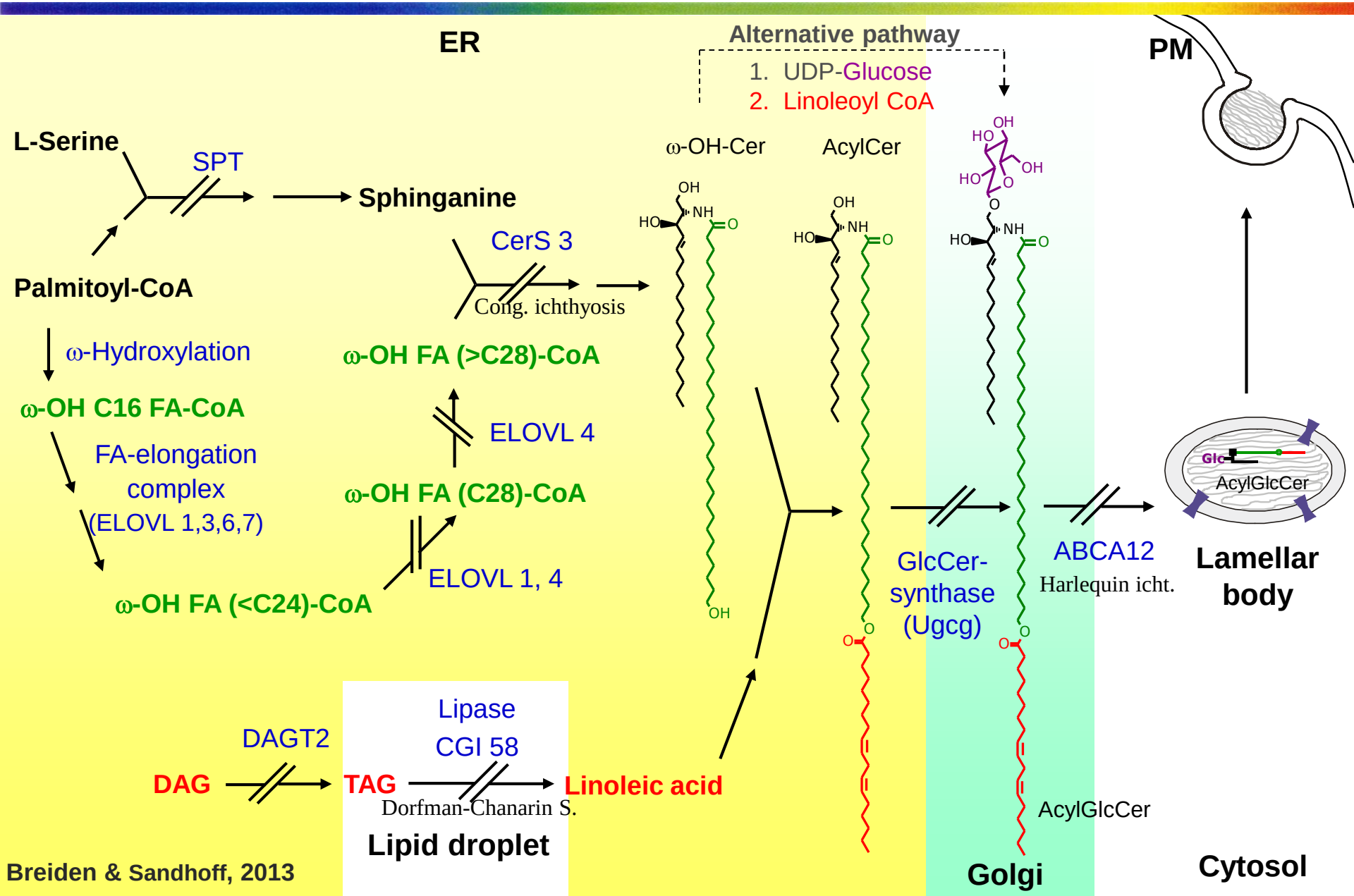
Ultrastructure of pSAP knockout epidermis



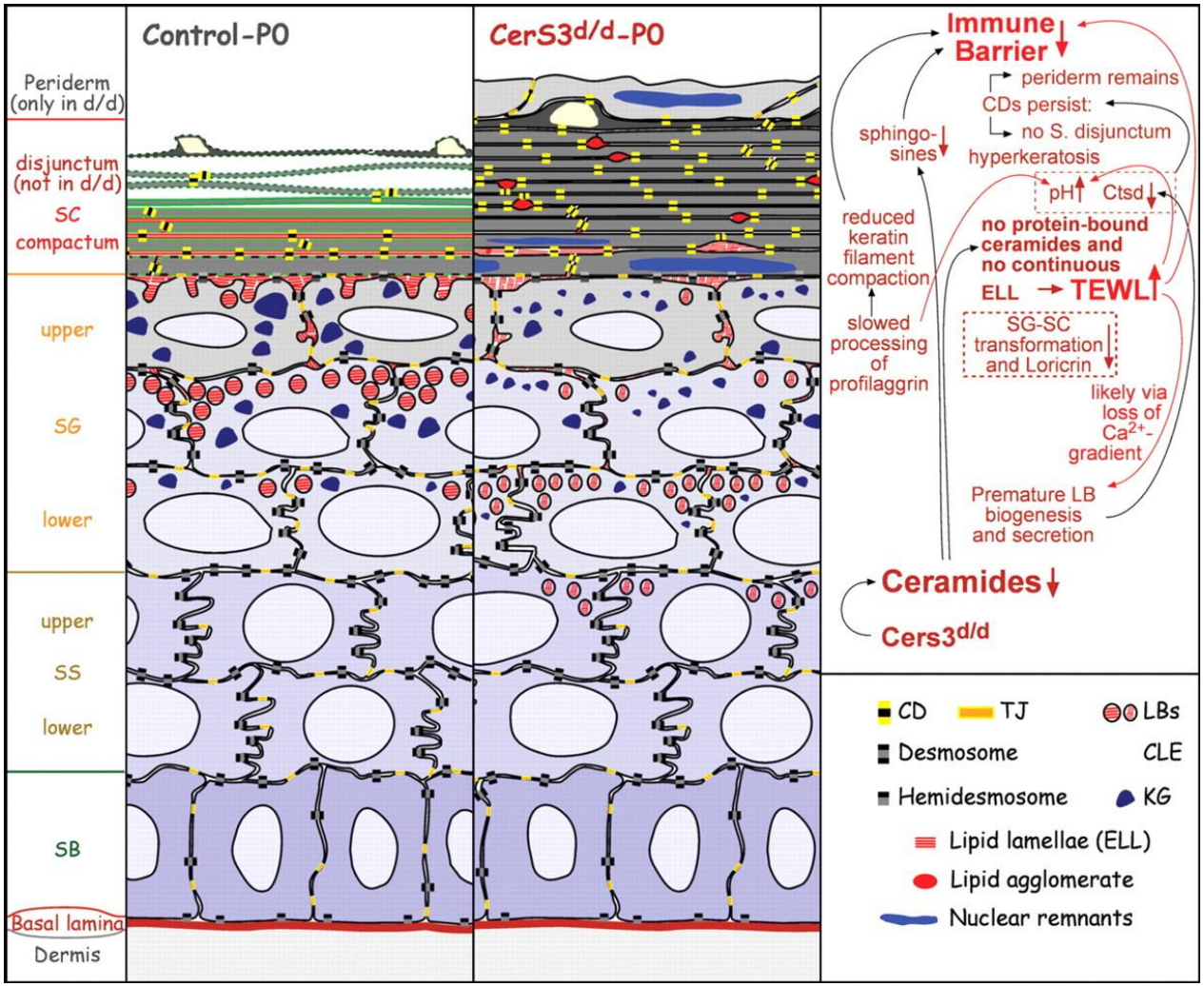
Ruthenium staining reveals that the pSAP-deficient interstices (*B, between arrows*) lack the regular, compact pattern of lamellar membranes observed in normal, pSAP-replete SC (*C, between open arrows*)..

Doering, T. et al. J Biol Chem, **274**, 11038-11045

Biosynthesis of AcylGlcCer



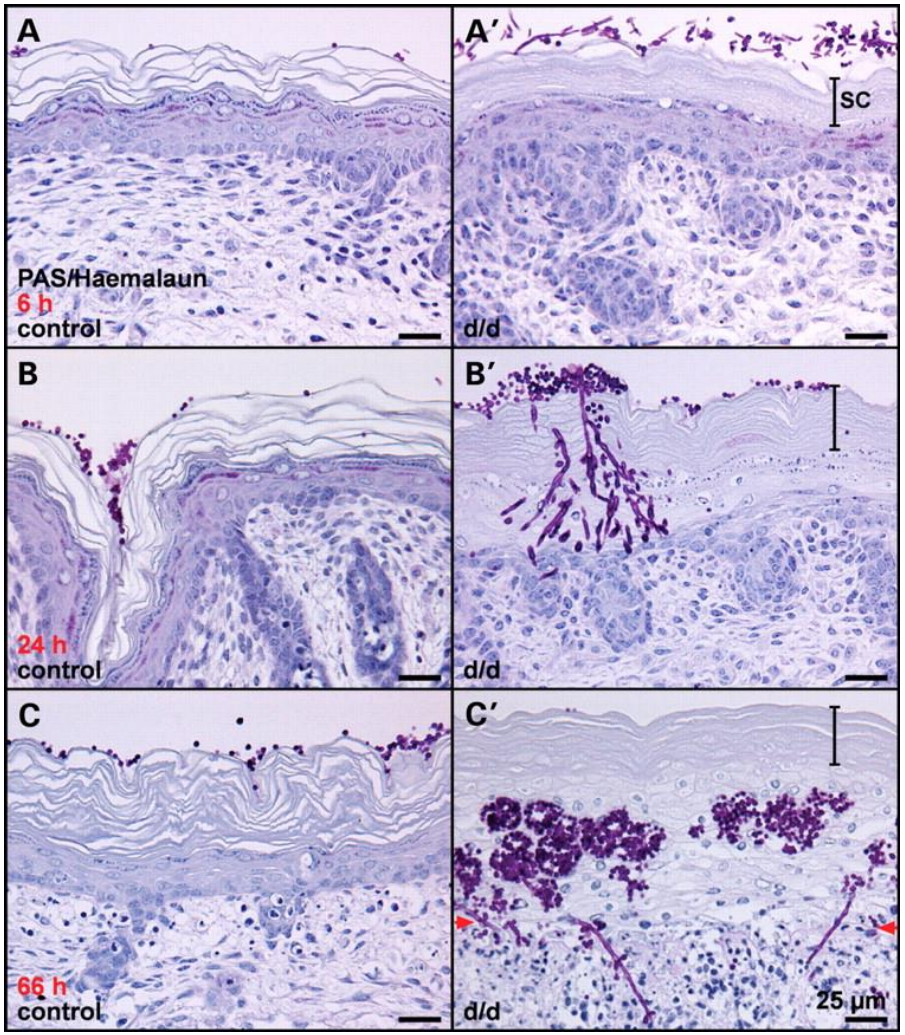
Schemes of CerS3d/d phenotypic alterations with proposed cascades of events.



- **CerS3 ko mice die shortly after birth from TEWL**
- **Loss of all SLs with acyl residues longer than 24 C-atoms, lack of protein-bound ceramides**
- Lack of continuous extracellular lipid lamellae, water loss, hyperkera tosis
- **High skin susceptibility to Candida infection**
- **SG –SC interface: absense of LB exocytotic figures and lamellar lipid-free domains**
- **Impaired processing of epidermal proteins: (pro) filaggrin, involucrin, loricrin.**

Jennemann R et al…..Sandhoff R, (2012) Hum. Mol. Genet.; 21:586-608

High skin susceptibility to Candida infection



Jennemann R et al. .Sandhoff R (2012) Hum. Mol. Genet. 21:586-608

(A–C′) Cultured skin samples of CerS3d/d mice reveal after inoculation with C. albicans increased microbial adhesion and growth after 6 h (A′), microbial invasion of all epidermal layers after 24 h (B′) and microbial colony formation within SS in parallel to migration of pseudohyphae into the dermis after 66 h (C′, the residual basement membrane, red arrowheads). Note the abundance of immune cells in mutant dermis after 66 h. PAS-hemalaun.

Principles of molecular and cellular pathology

- Cell type specificity of GSL- & SL- biosynthesis

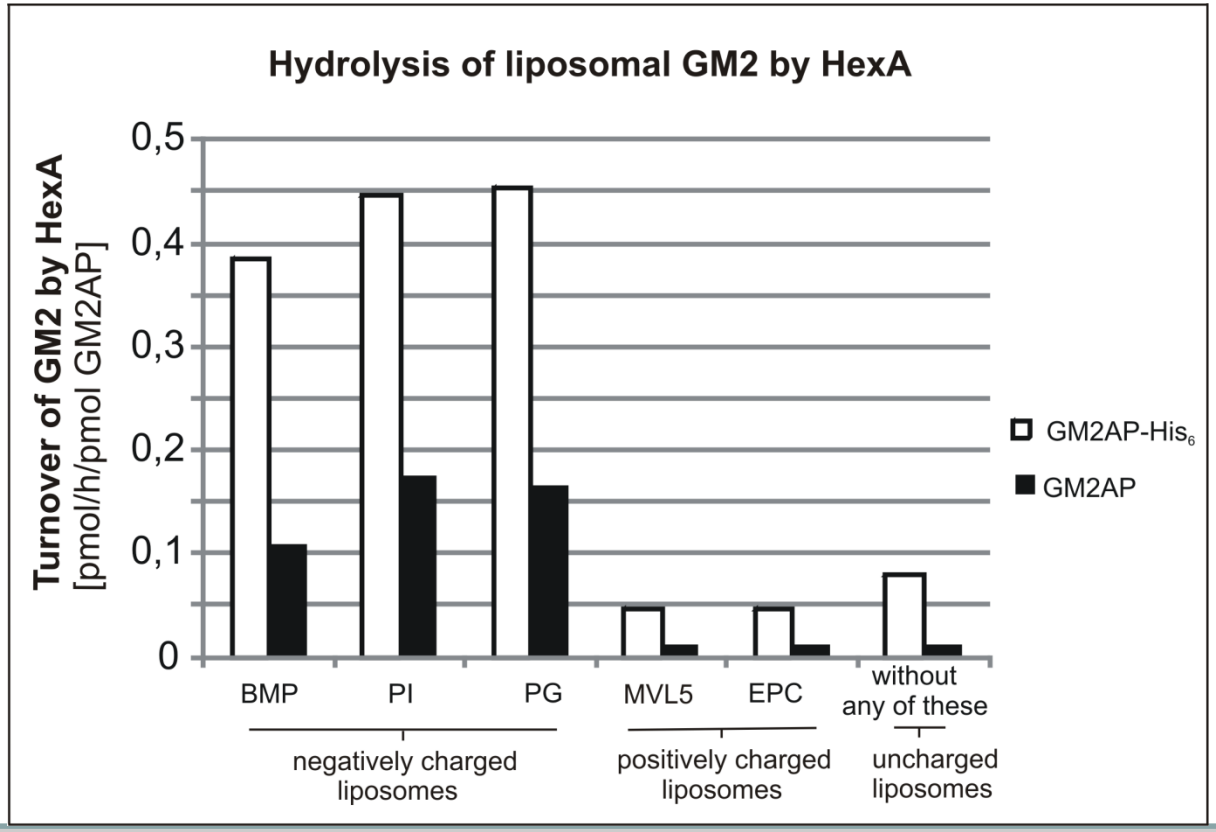
- Promiscuity (and redundancy) of hydrolases and SAPs

- SAPs are multifunctional proteins (lipid binding & mobilization, intermembrane lipid transfer and vesicle fusion)

- Membrane lipids are strong regulators of lipid degradation at vesicular surfaces

-Unknown transient levels of cholesterol & anionic lipids in luminal vesicles of lysosomes may contribute to clinical heterogeneiety of diseases

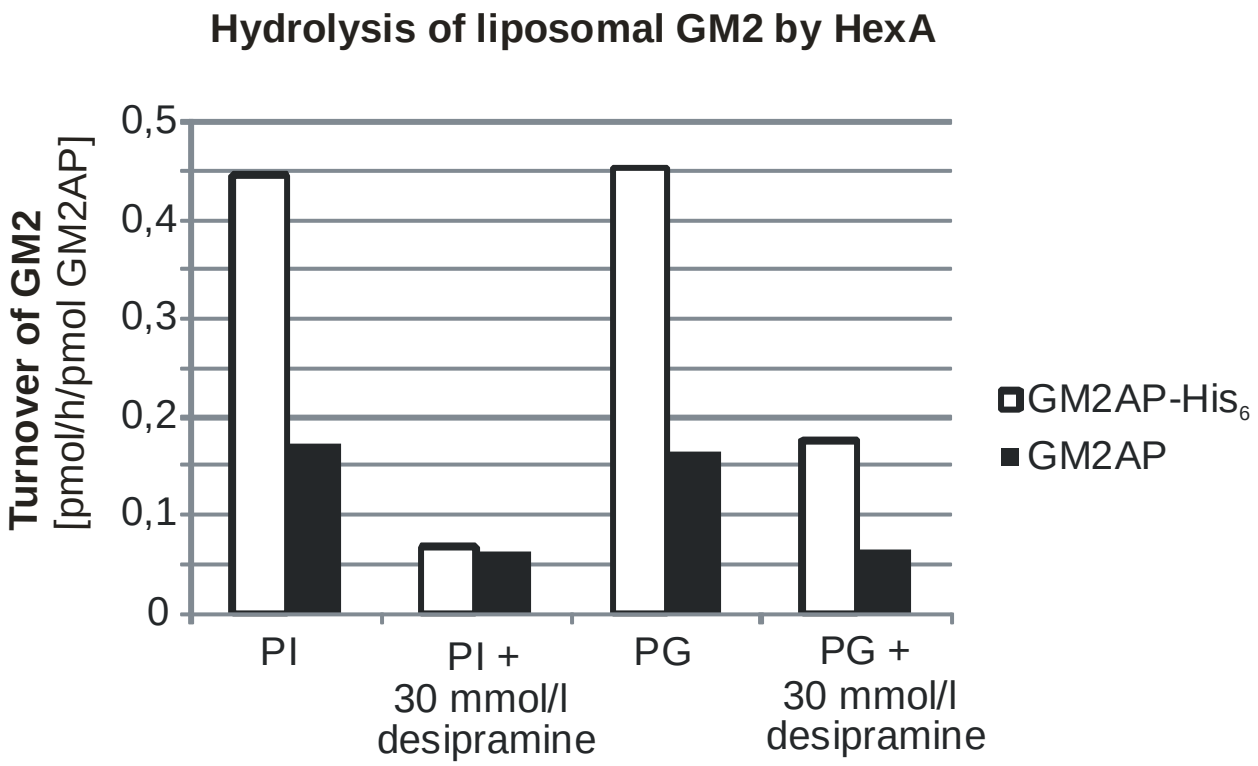
- ULC-Glc-Ceramides & ULC-Ceramides are key components of the water & immune barrier of mammalian skin. Defects in their metabolism can cause ichtiosys and are often fatal



Liposomal activity assay

- Uncharged liposomes contained: 5 mol% cholesterol, [^{14}C]-GM2 and PC as a host lipid
- negatively charged liposomes contained additional 20 mol% BMP, PI or PG, positively charged liposomes contained 20 mol% MVL5 or EPC

Adding negative charged lipids in the liposomal membranes resulted in a strong enhanced hydrolysis of of GM2 by HexA in presence of GM2AP. Using positively charged liposomes, the hydrolysis rate of GM2 was as low as that observed with unchaged vesicles.

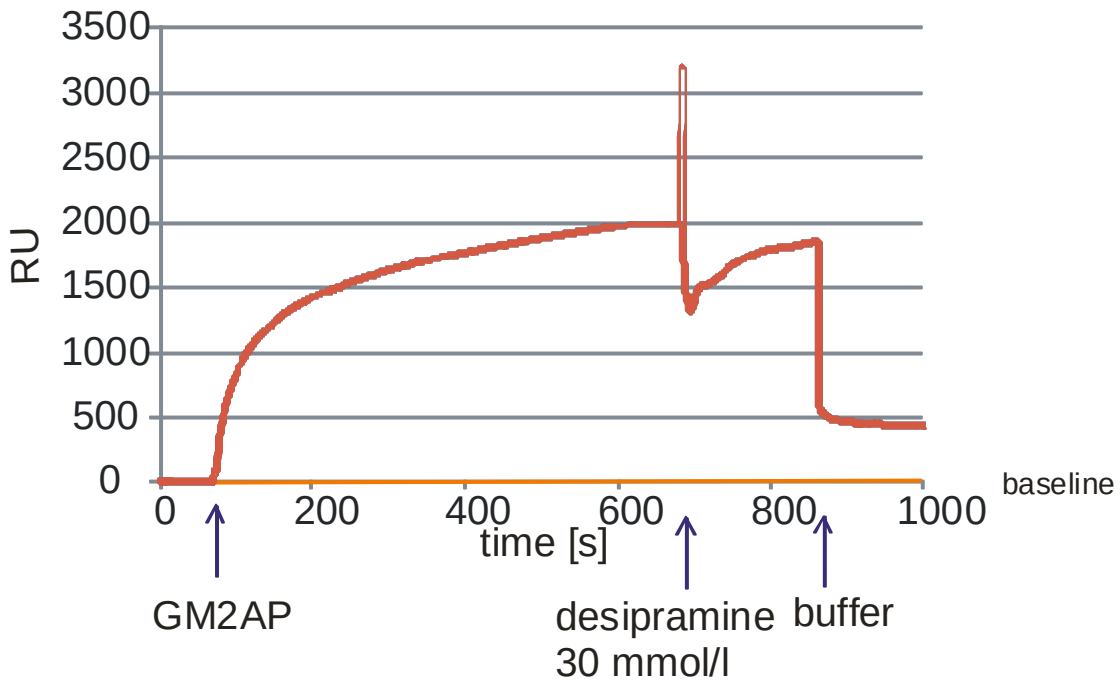


Liposomal activity assay

- The negatively charged liposomes contained 20 mol% PI or PG, 5 mol% cholesterol, [^{14}C]-GM2 and PC as a host lipid. Adding desipramine to the assay preparation (30 mmol/l final concentration), the hydrolysis of GM2 by HexA in presence of GM2AP was strongly reduced.

- The cationic anphiphilic drug desipramine is desturbing the interaction of GM2AP with the vesicle membrane, most likely by its positive netcharge.

Desipramine strongly reduces membrane binding of GM2AP

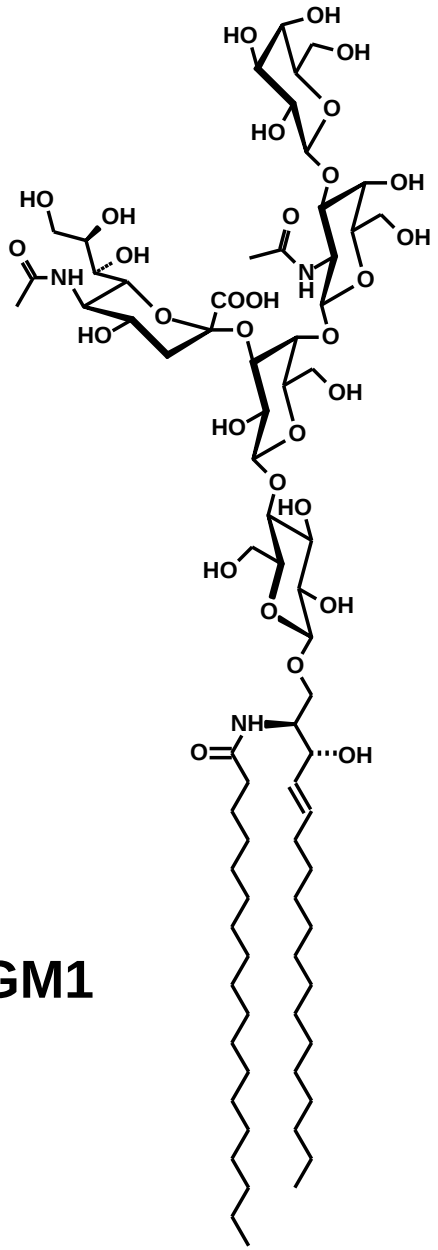


Surface plasmon resonance (SPR) study

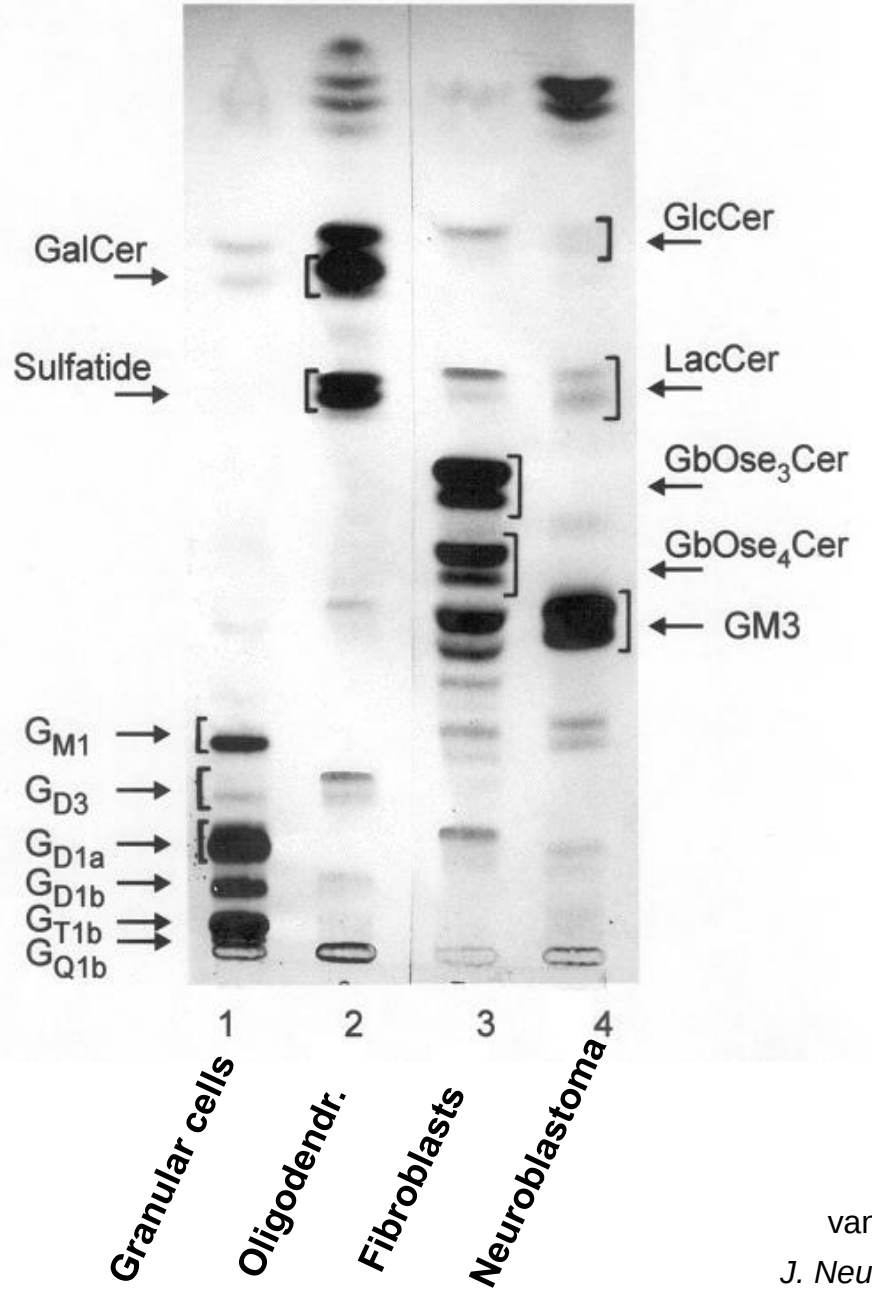
Negatively charged liposomes containing 5 mol% cholesterol, 5 mol% BMP and 10 mol% GM2 were immobilized on a Pioneer HPA-chip. Running buffer was 20 mM sodium citrate buffer (pH 4.2). Response signals measured after binding of membrane lipids were defined as zero (baseline: orange). GM2AP (0.2 mM) in running buffer was injected into the flow cells at a rate of 20 µl/min. Afterwards, desipramine (30 mmol/l, dissolved in water) was injected for 3 min (flow rate 20 µl/min), followed by buffer alone.

The cationic anphiphilic drug desipramine is desturbing the interaction of GM2AP with the vesicle membrane, most likely by its positive netcharge.

Cell type specific biosynthesis of glycosphingolipids

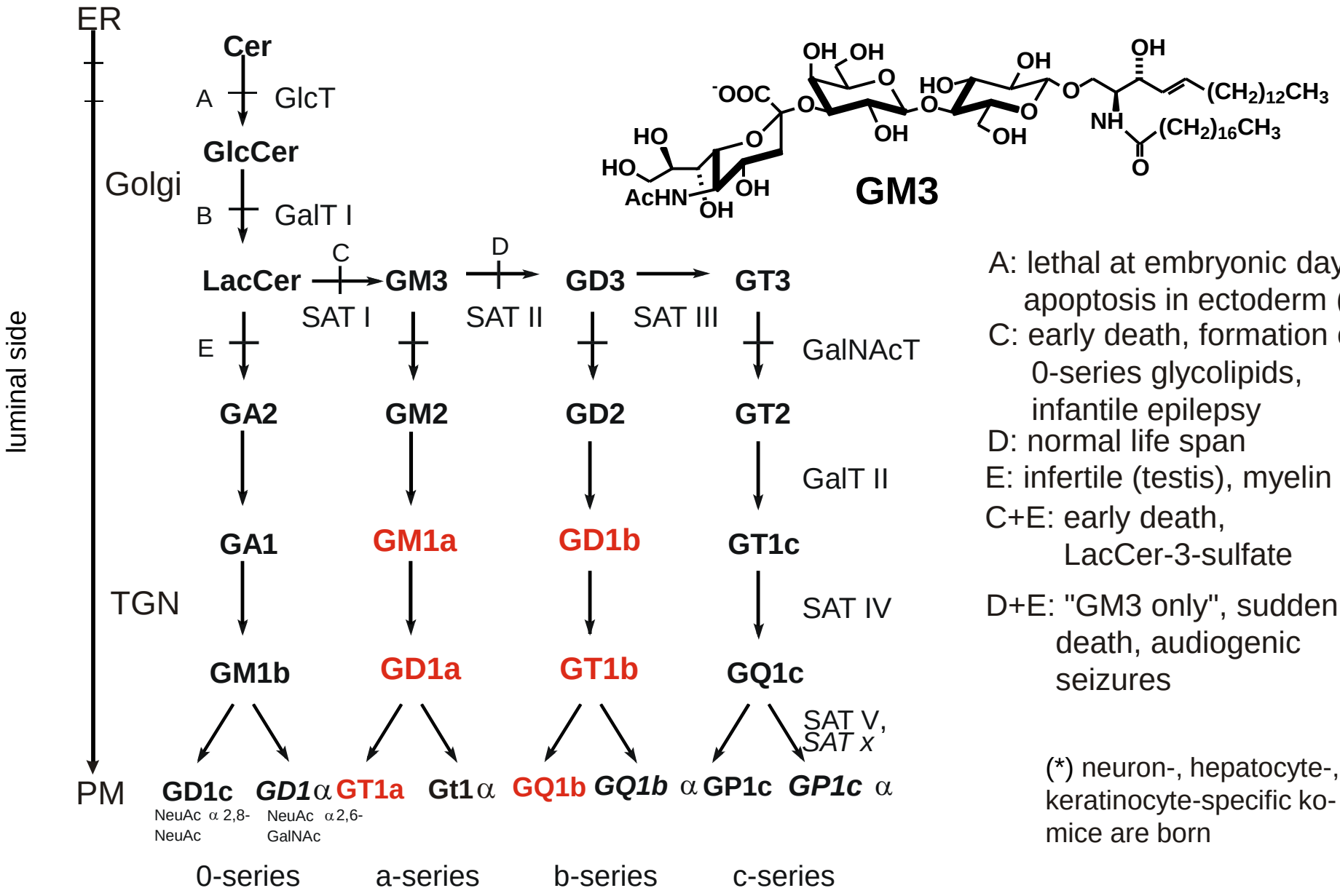


GM1



van Echten *et al.* (1989)
J. Neurochem. **52,** 207-214

Combinatorial Ganglioside Biosynthesis



A: lethal at embryonic day 6, apoptosis in ectoderm (*)

C: early death, formation of 0-series glycolipids, infantile epilepsy

D: normal life span

E: infertile (testis), myelin

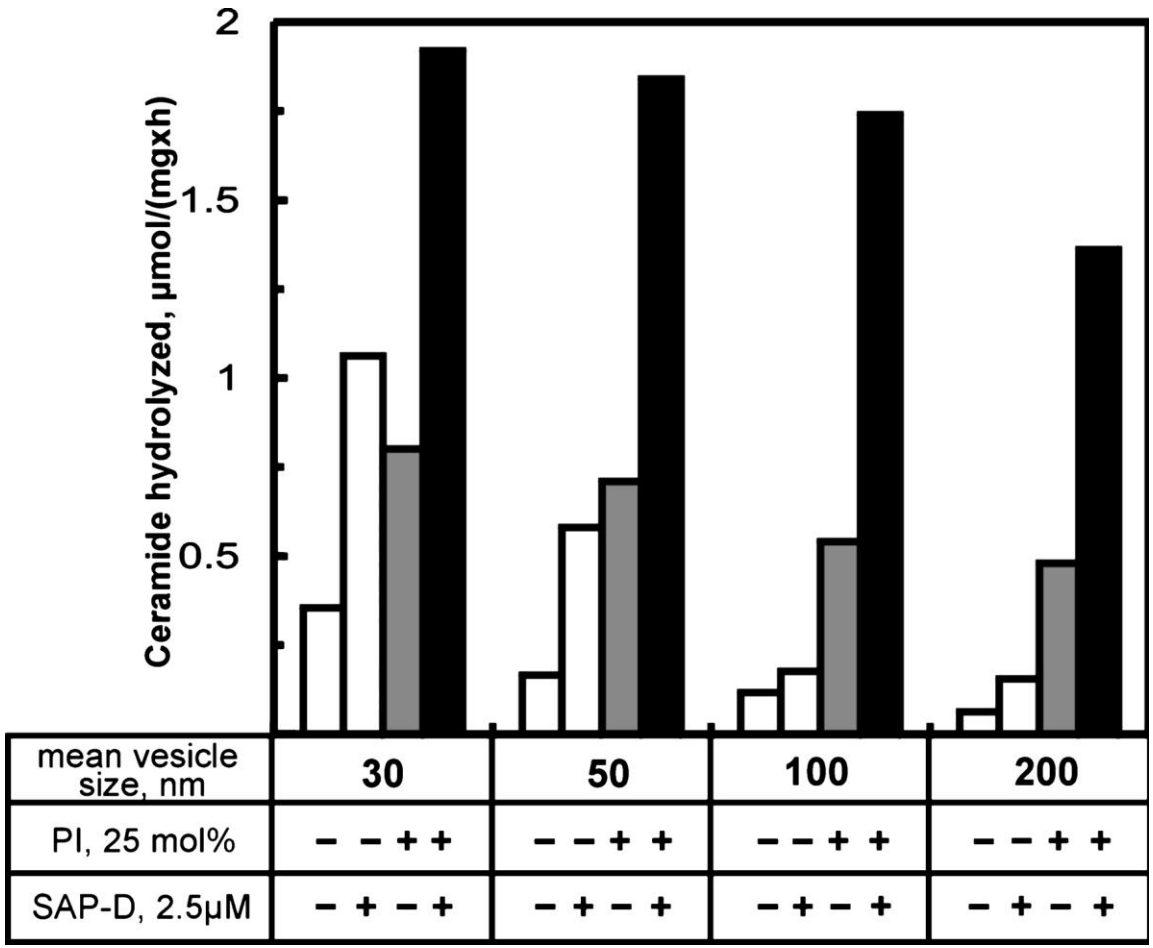
C+E: early death, LacCer-3-sulfate

D+E: "GM3 only", sudden death, audiogenic seizures

(*) neuron-, hepatocyte-, keratinocyte-specific ko-mice are born

Kolter, Proia, Sandhoff (2002) *J. Biol. C*hem. **277,** 25859-62

Sap-D, PI and increased membrane curvature enhance ceramide hydrolysis by aCerase

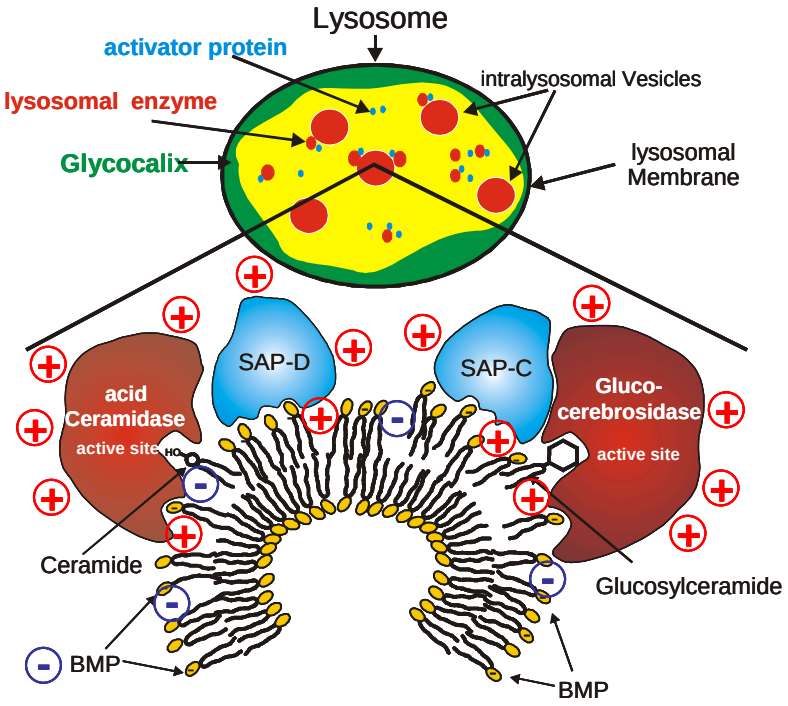


LUVs and SUVs with varying mean diameter either contained none or 25 mol % PI. In addition, incubation mixtures contained either none or 2.5 µm SAP-D.

Linke T et al. J. Biol. Chem. 2001;276:5760-5768

CADs: Cationic amphiphilic drugs (lipophilic) induce a phospholipidosis: the molecular view

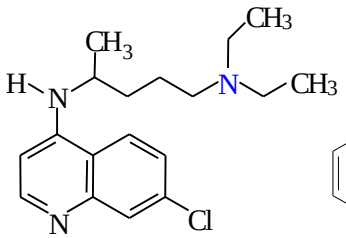
Cytoplasmic Inclusion body; x 78400
Chromaffin cell of a rat treated with
1-chloro-amitriptyline (120 mg/kg 10 wk)



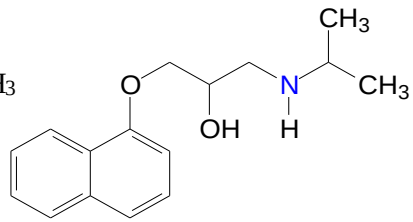
Wilkening, *et al*. (1998)

Many drugs are cationic amphiphilic lipids (CADs) :

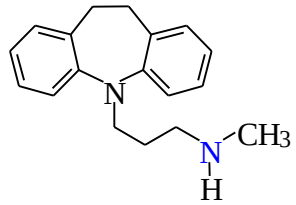
CADs are partially neutral at pH 7, penetrate membranes, and are protonated & trapped in lysosomes.



Chloroquine
(Antimalaria)



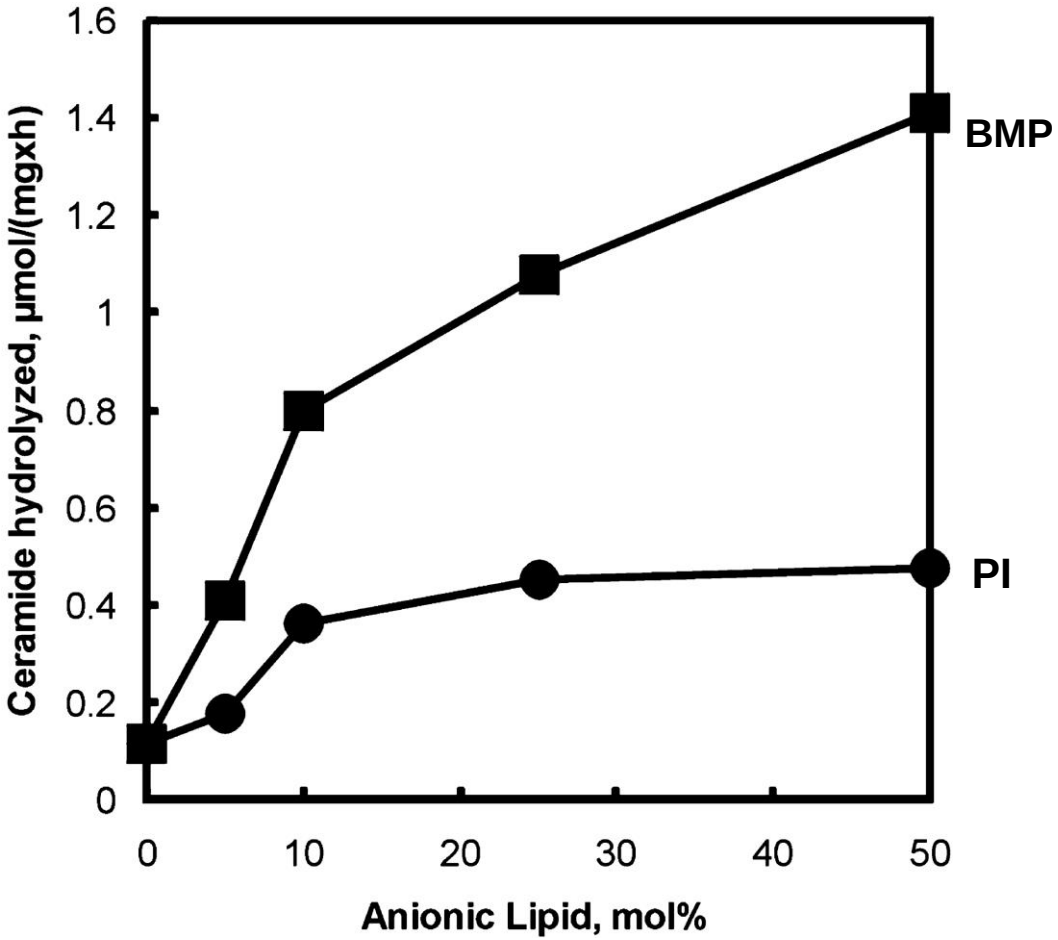
Propranolol
(β-Adrenoreceptor-antagonist)



Desipramine
(Antidepressant)

1. Induction of lipidoses in rats Lüllmann *et al*. (1978)
2. Detachment of acid sphingomyelinase from anionic liposomes Kölzer *et al*. (2004)
3. Proteolytic degradation of acid sphingomyelinase and other lysosomal enzymes by desipramine-treated cultured fibroblasts. Hurwitz *et al*. (1994)

BMP and PI stimulate the degradation of membrane-bound ceramide in the absence of SAPs



Rate increases with curvature of liposomes

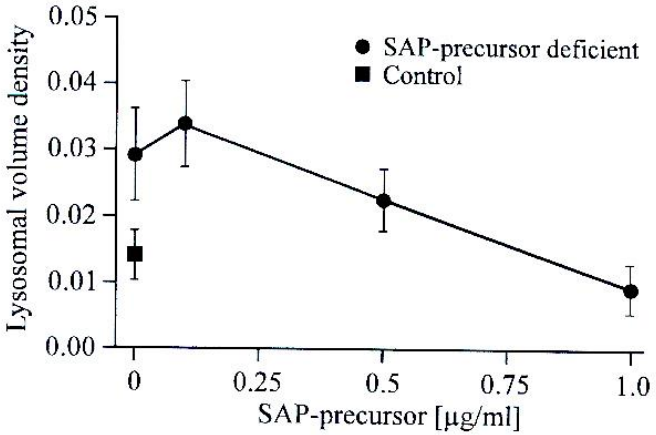
Sap-D stimulates up to 3fold

Acid ceramidase activity was measured in the presence of increasing concentrations of BMP (▪) and PI (●) in ceramide-bearing LUVs in the absence of SAPs. The data presented are the means of three determinations. All individual values were in the range of ±5 up to ±10% of the mean.

Linke T et al. J. Biol. Chem. 2001;276:5760-5768

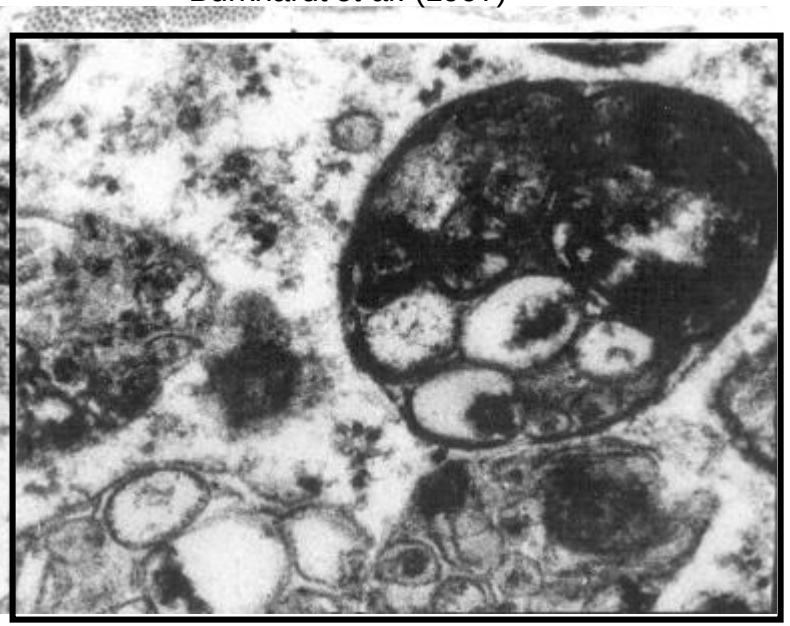
Skin biopsy of a pSap-deficient patient

(W. Roggendorf)

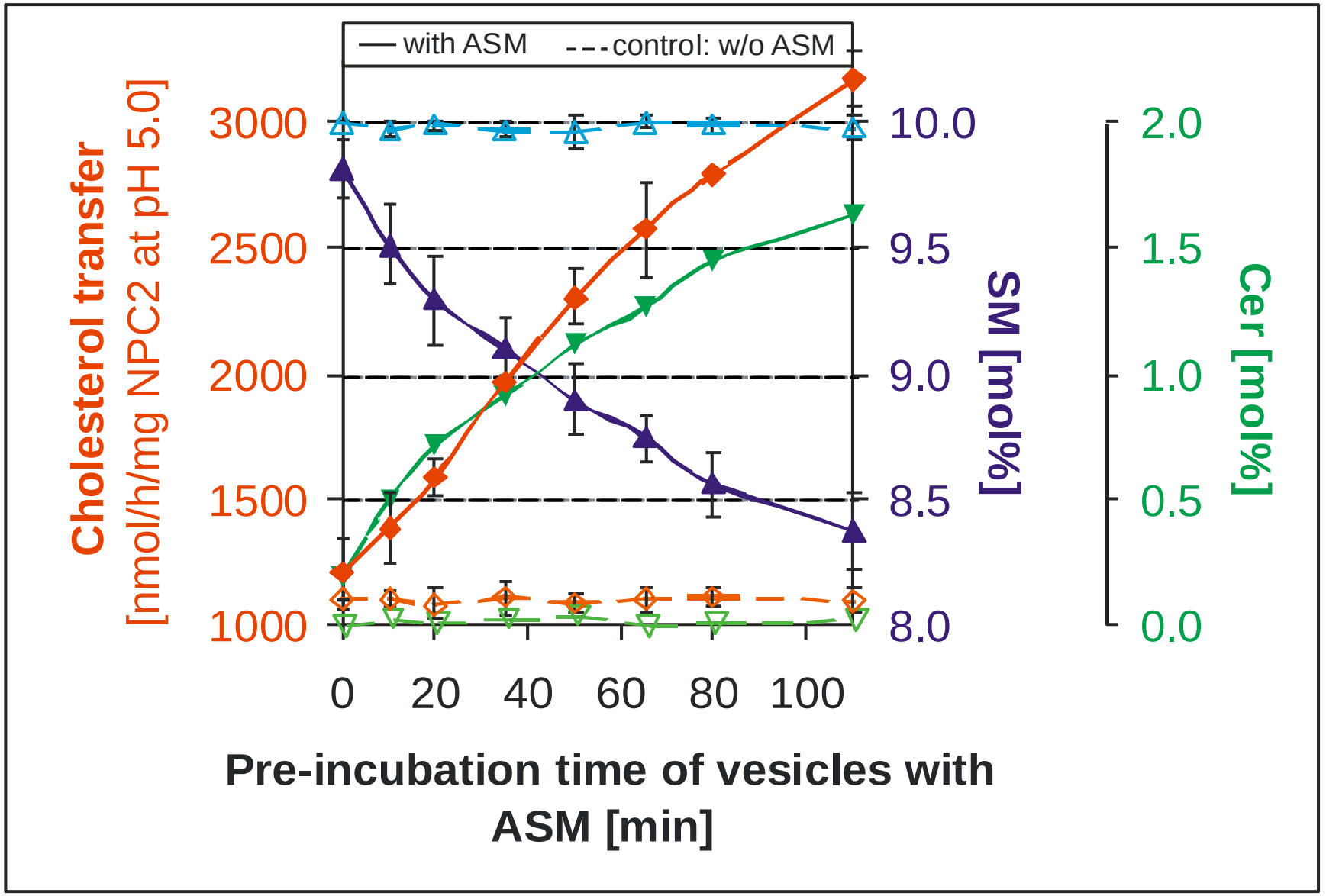


Feeding of pSAP reverses membrane storage

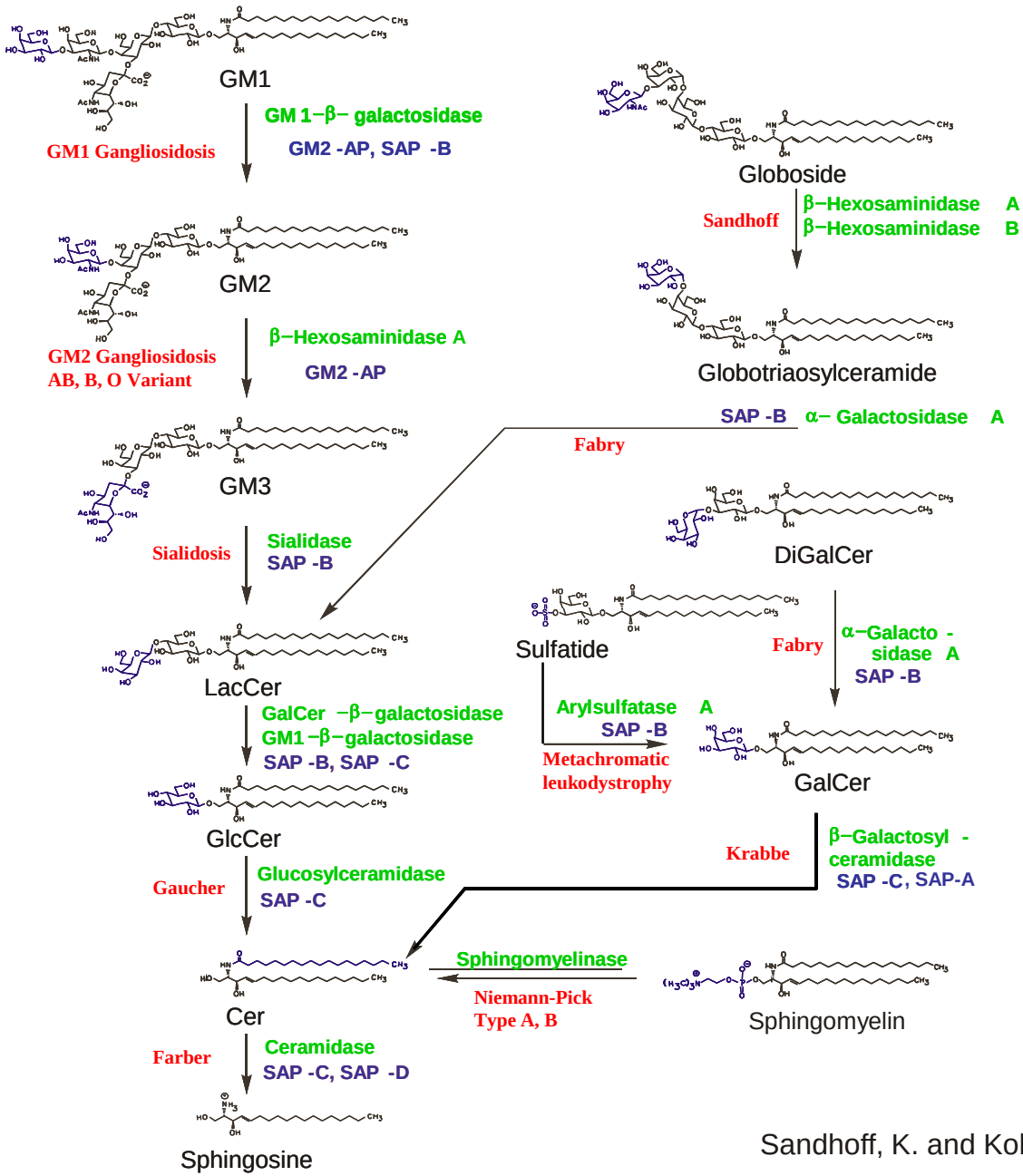
Burkhardt *et al*. (1997)



Degradation of SM by ASM stimulates intervesicular cholesterol transfer by NPC2



Lysosomal Sphingolipid Degradation

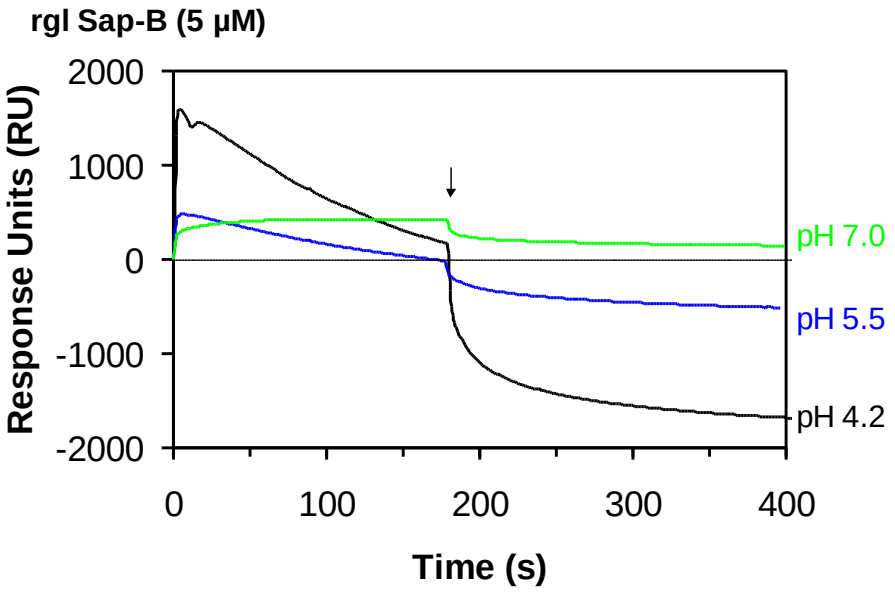


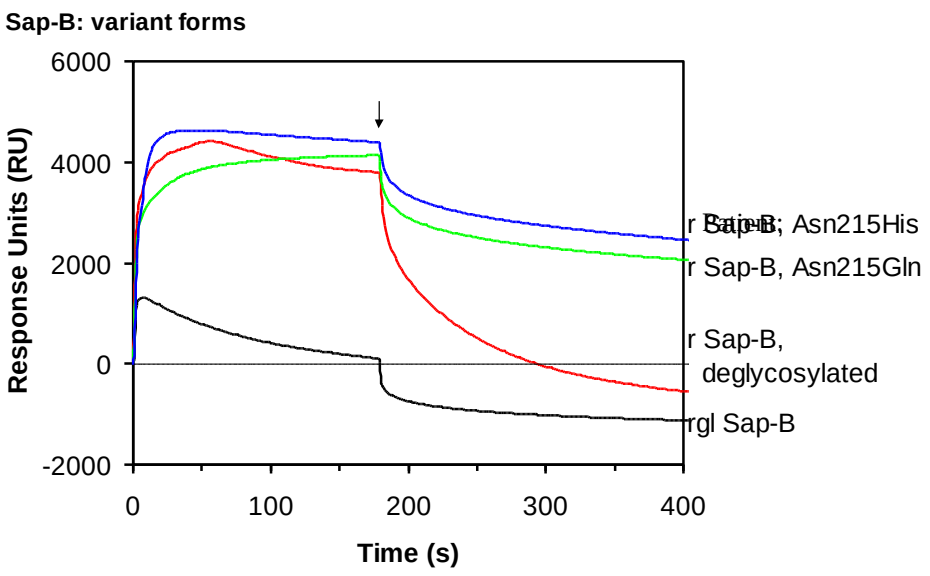
Coworkers:
Bernadette Breiden
Vincent Oninla
Günter Schwarzmann
Susi Anheuser

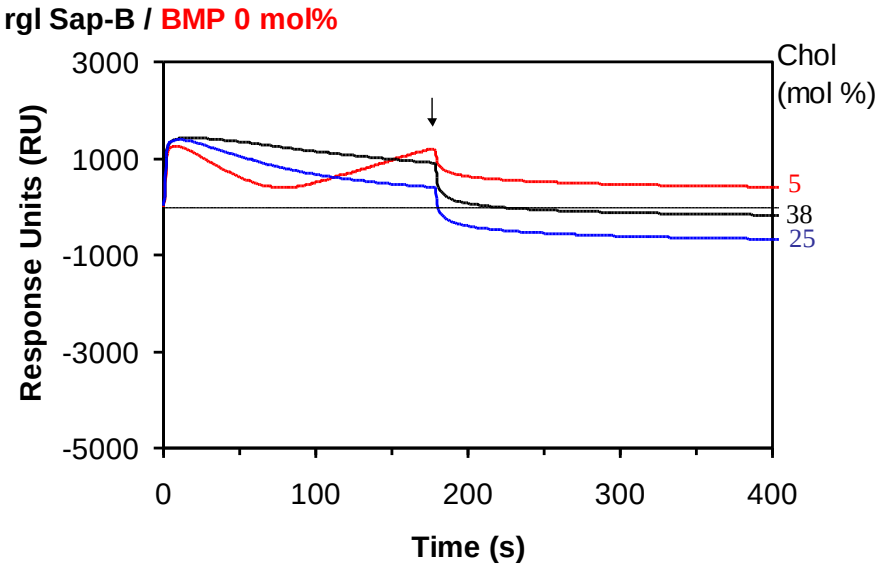
Previous Coworkers:
Misbaudeen Abdul-Hammed
Natascha Remmel
Silvia Locatelli-Hoops
Wiebke Möbius
Michaela Wendeler
Melanie Kölzer
Thomas Döring

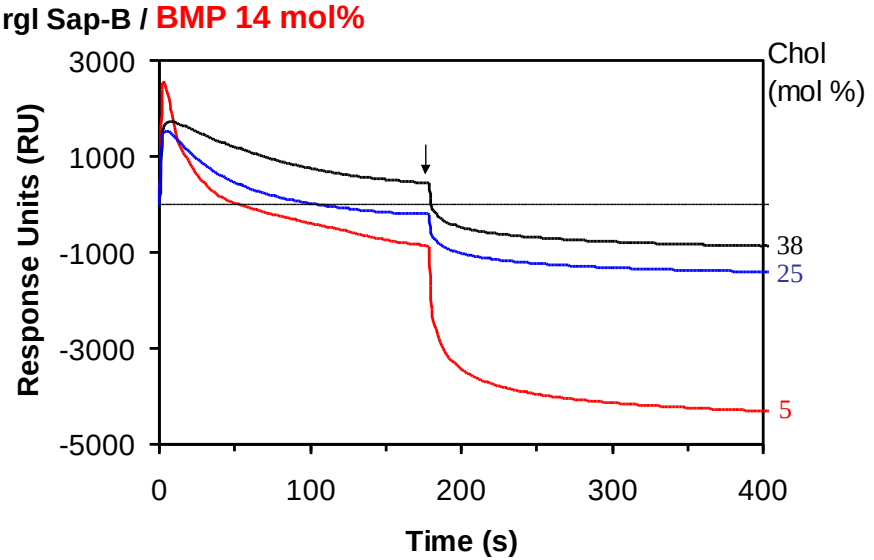
Sandhoff, K. and Kolter, T. (1995) *Naturwissenschaften,* **82, 403-413**

g-Sap-B mobilizes lipids from BMP rich and cholesterol poor membranes at acidic pH values









Surf. Plasmon resonance, Remmel, N. et al. (2006)

Localization of Biotin-GM1 on Cryosections:

Human fibroblasts were incubated with 10µM Biotin-GM1 for 72 h at 37° C.

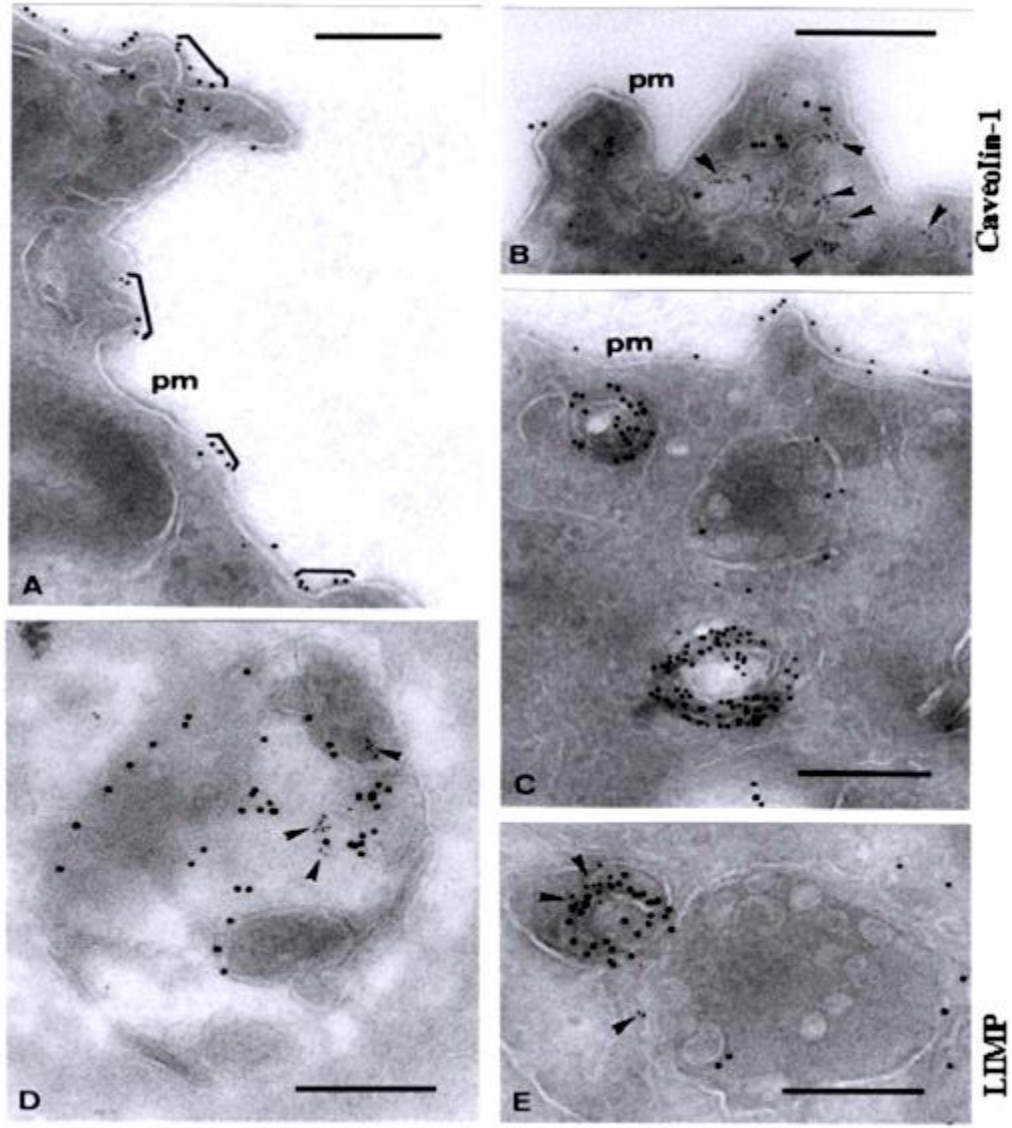
B: double labeling with anti-caveolin-1 (arrowheads) and anti-biotin (large gold)

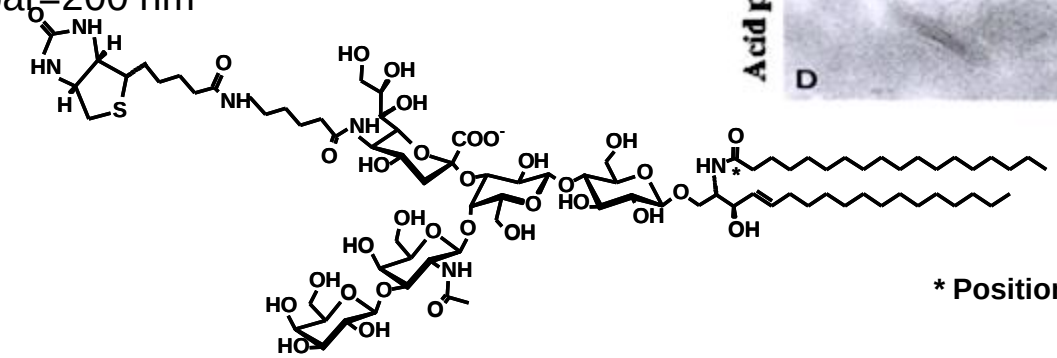
D: double labeling with anti-acid phosphatase (arrowheads) and anti-biotin (large gold)

E: double labeling with anti-LIMP (arrowheads) and anti-biotin (large gold)

Möbius, Herzog, Sandhoff, Schwarzmann (1999). J. Histochem. Cytochem. 7

bar=200 nm





*** Position of radiocarbon**

Perspectives for endocytosis and sphingolipid digestion

- **Cholesterol transfer** by NPC2 between vesicles (and cholesterol egress from late endosomes) is stimulated by BMP and other anionic lipids, and inhibited by SM down to 10%.
- **ASM** is stimulated by anionic lipids (PG, PA, BMP) up to 14 fold, but not inhibited by cholesterol.
- Results suggest a **sequential pathway of lipid degradation** at intraendosomal vesicles during endocytosis:

– At late endosomes PM derived anionic PLs (PG, PA) stimulate ASM to degrade SM, thereby releasing the block of NPC2 mediated cholesterol egress. ASM also degrades PG, PA, and other PLs, triggering loss of bilayer structure and barrier function of luminal membranes.

– Generation of BMP and reduction of cholesterol levels in intralysosomal vesicles activates SAPs (and hydrolases) needed for effective degradation GLS and ceramides.

– Cationic lipids are toxic and inhibit catabolic steps (GalSo (Krabbe), GlcSo (Gaucher), So, Sa). CADs trigger proteolysis of ASM & other hydrolases and induce a phospholipidoses.

Lysosomal lipid binding proteins (SAPs and NPC2)

LLBPs are small glycoproteins, generated from big precursors by proteolysis in endolysosomes. Their activity is regulated at acidic pH by membrane lipids:

Some (NPC2, Saps A, B, C) bind and lift membrane lipids from surfaces of liposomes.

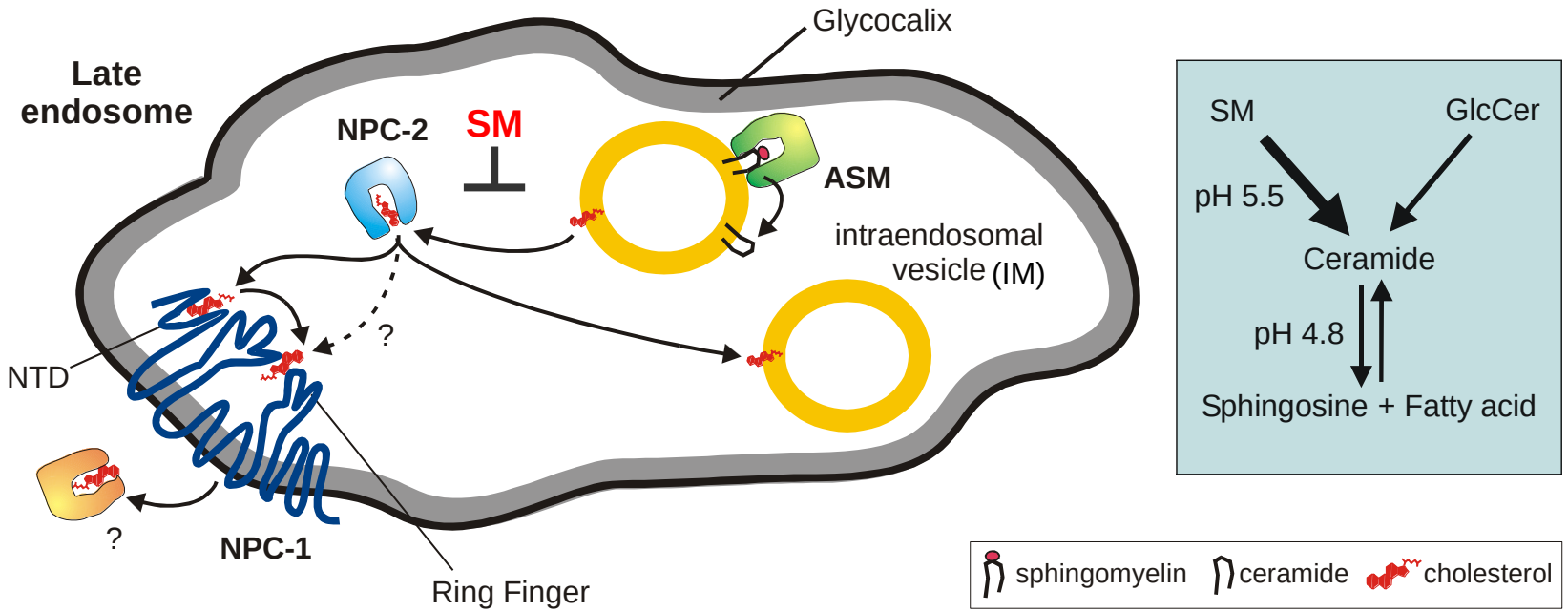
Some form soluble stochiometric lipid protein complexes (GM2 – GM2AP; sulfatides - $(Sap\text{-}B)_2$) (and Michaelis complexes with their catabolic hydrolases).SapA forms lipoprotein discs (2 SapA, 8-10 PL, Prive).

Inherited deficiencies of LLBPs (NPC-2, GM2AP, Sap A, B, C, D) cause fatal diseases (prosaposin (-/-) : SL and membrane storage, defective skin barrier).

LLBPs (GM2AP, Saps A,B,C…) are regulated by lipids and

- **transfer lipids from donor to acceptor vesicles,**
- **are fusogenic (GM2AP, Sap-D, Sap-C…..), and**
- **disintegrate BMP rich and cholesterol poor vesicles.**

IMs are adjusted for degradation, BMP is generated and cholesterol leaves inner vesicles of late endosomes



1. Membrane lipids regulate cholesterol transfer by NPC-2 (BMP… ↑, Cer ↑, SM ↓)
2. Digestion of SM(15%) by ASM enhances cholesterol transfer by NPC-2 (3fold)
3. ASM is a phospholipase C, cleaves PA, PG, PC, lyso-PC, SM and 17 other PLs
4. Anionic membrane lipids stimulate ASM: PA and PG 7-14fold; BMP 3fold
5. Diacylglycerol – a hydrolysis product of PLs by ASM- stimulates ASM activity
6. High cholesterol content inhibits PC, hydrolysis by ASM, but less SM cleavage

Pathologic mechanisms caused by lipid storage in late endosomes and lysosomes

•Expansion of lysosomal compartment at the expense of cytosol and other organelles

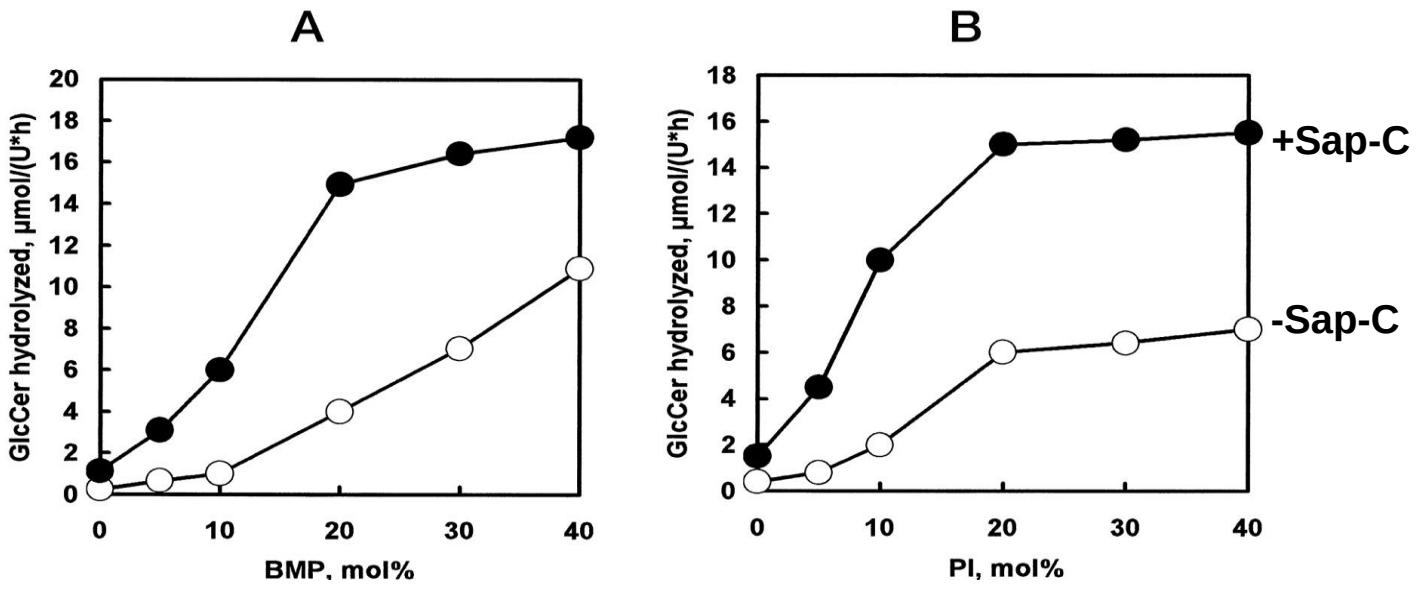
•Impaired digestion of macromolecules: Inhibition of catabolic enzymes and proteins (SM inhibits NPC-2 and lipid sorting, cholesterol inhibits Sap-A, -B etc., CADs inhibit catabolic steps and trigger proteolysis of ASM etc.)

•Starvation of neurons by impaired digestion and traffic jams in lysosomes (cellular stomachs reduce their nutrient supply: Fe^{++}, B12, FAs, AAs, CHO, sphingoid bases, salvage pathways).

•Further mechanisms:

- Accumulation of toxic cationic lipids; galactosylsphingosine (GalSo) (psychosine hypothesis, Krabbe), GlcSo, So, Sa.
- Release of cytokines,activation of microglia, influx of macrophages.
- CADs (Desipramine) impair lysosomal digestion, trigger proteolysis of ASM and other catabolic hydrolases.

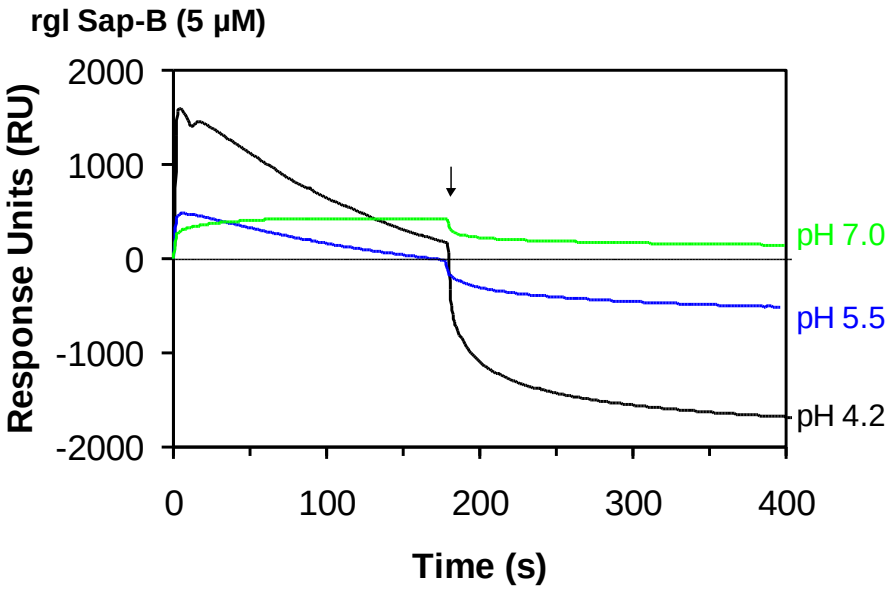
Lysosomal lipids in LUVs stimulate the enzymatic GlcCer hydrolysis in the presence and also in the absence of Sap-C.

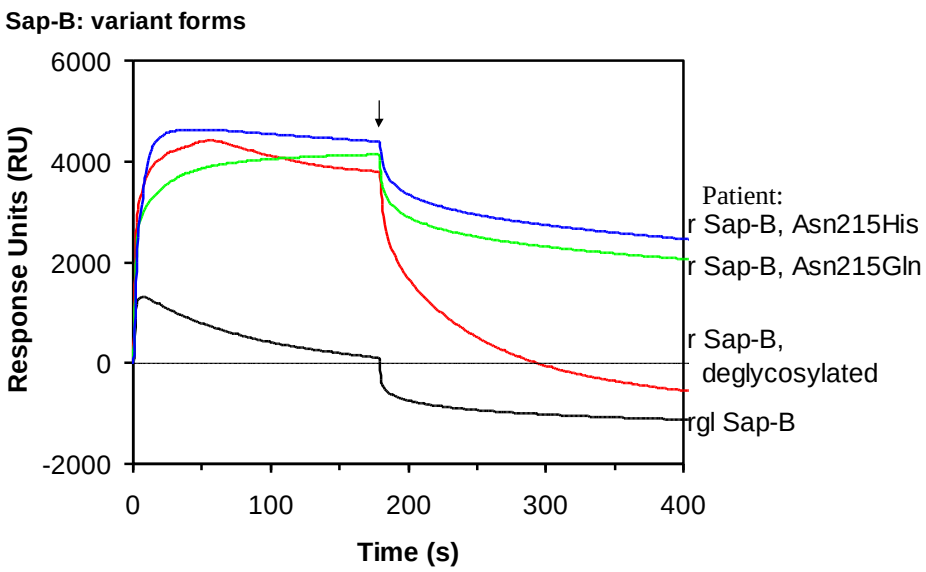


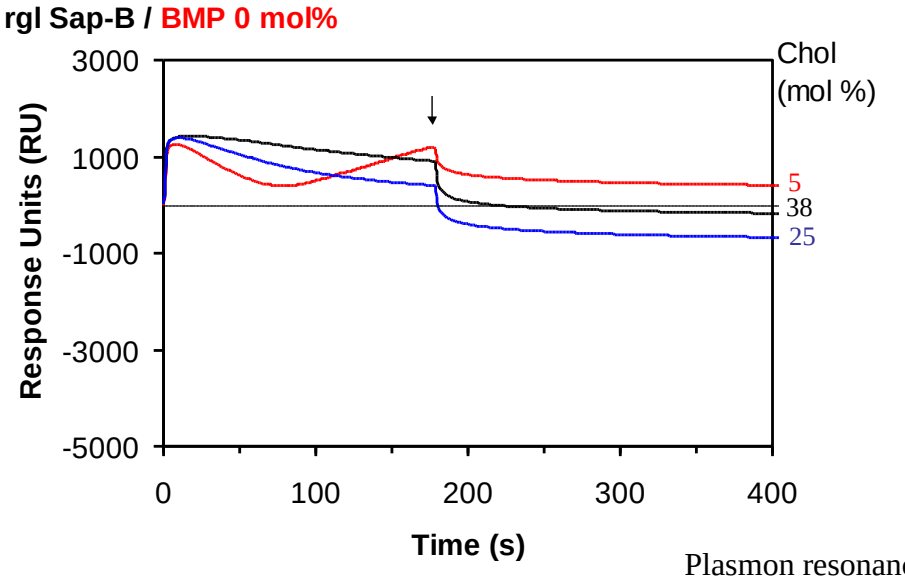
A, assays were conducted with GlcCer as substrate in the absence (○) and presence of Sap-C (2.5 µm) (•), using LUVs with various proportions of synthetic BMP (0–40 mol %). B, assays were carried out with varying concentrations of PI in LUVs, with (•) and without (○) the addition of 2.5 µm Sap-C, keeping the total lipid concentration in the assays constant.

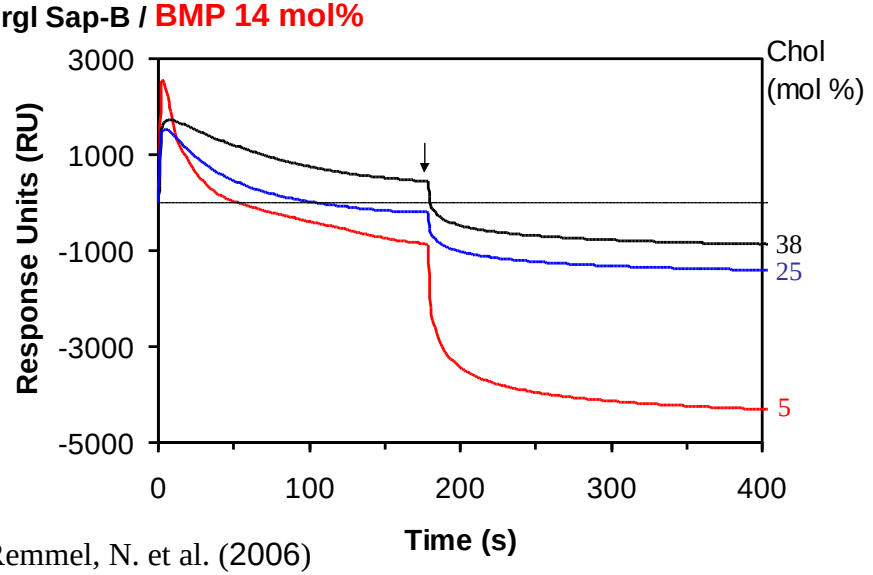
Wilkening G et al. J. Biol. Chem. 1998;273:30271-30278

gSap-B mobilizes lipids from BMP rich and cholesterol poor membranes at acidic pH values



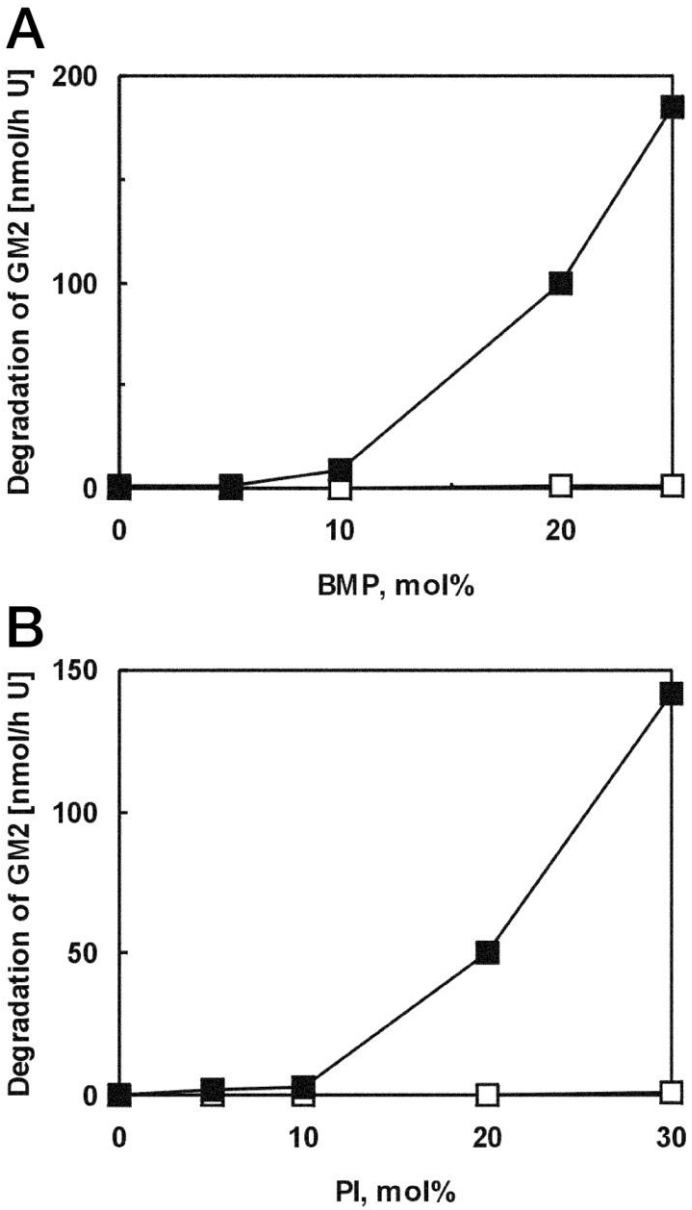






Plasmon resonance, Remmel, N. et al. (2006)

Lysosomal lipids in LUVs stimulate the enzymatic hydrolysis of ganglioside GM2 in the presence of GM2AP.

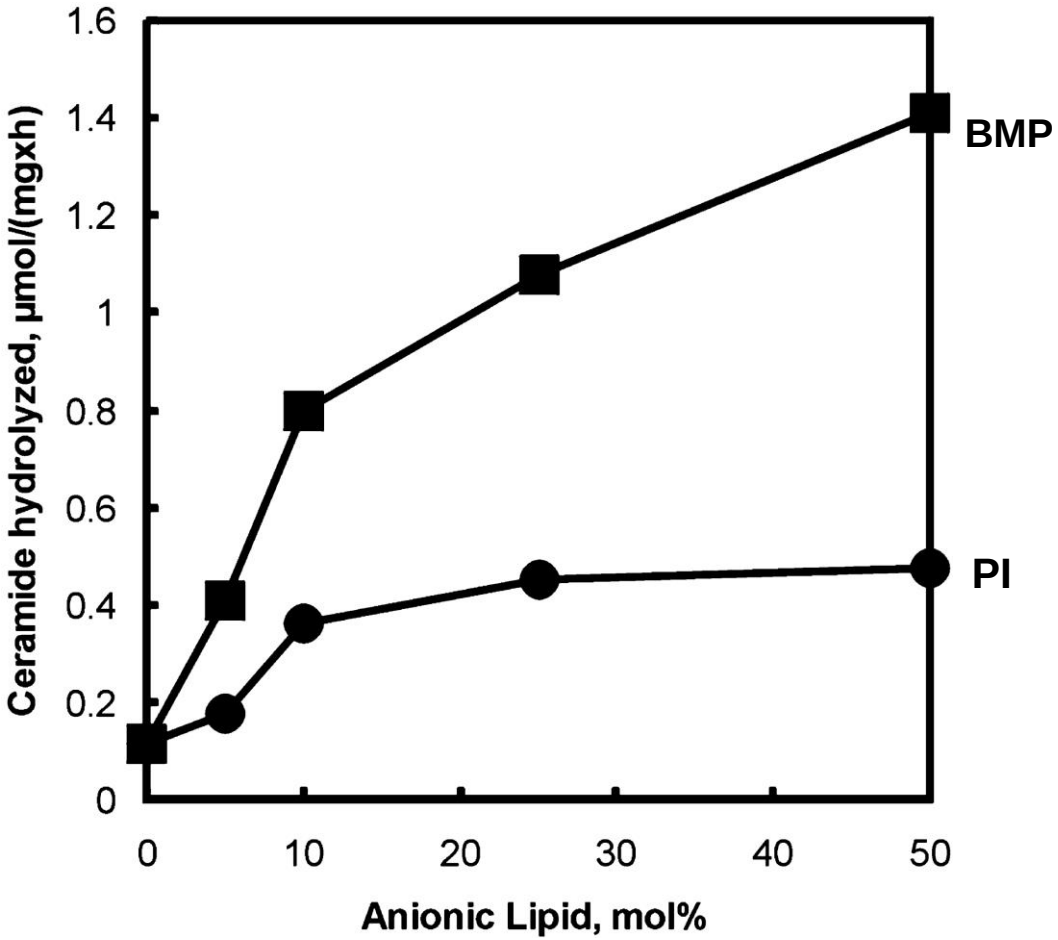


All assays were conducted with ganglioside GM2-carrying LUVs as substrates in the absence (□) and presence (■) of GM2AP (0.5 μm) using LUVs with various proportions of synthetic BMP (0–25 mol %) (A) or PI (0–30 mol %) (B).

PA, sulfatides and BMP plus Chol stimulate to a similar extent.

Werth N et al. J. Biol. Chem. 2001;276:12685-12690

BMP and PI stimulate the degradation of membrane-bound ceramide in the absence of SAPs



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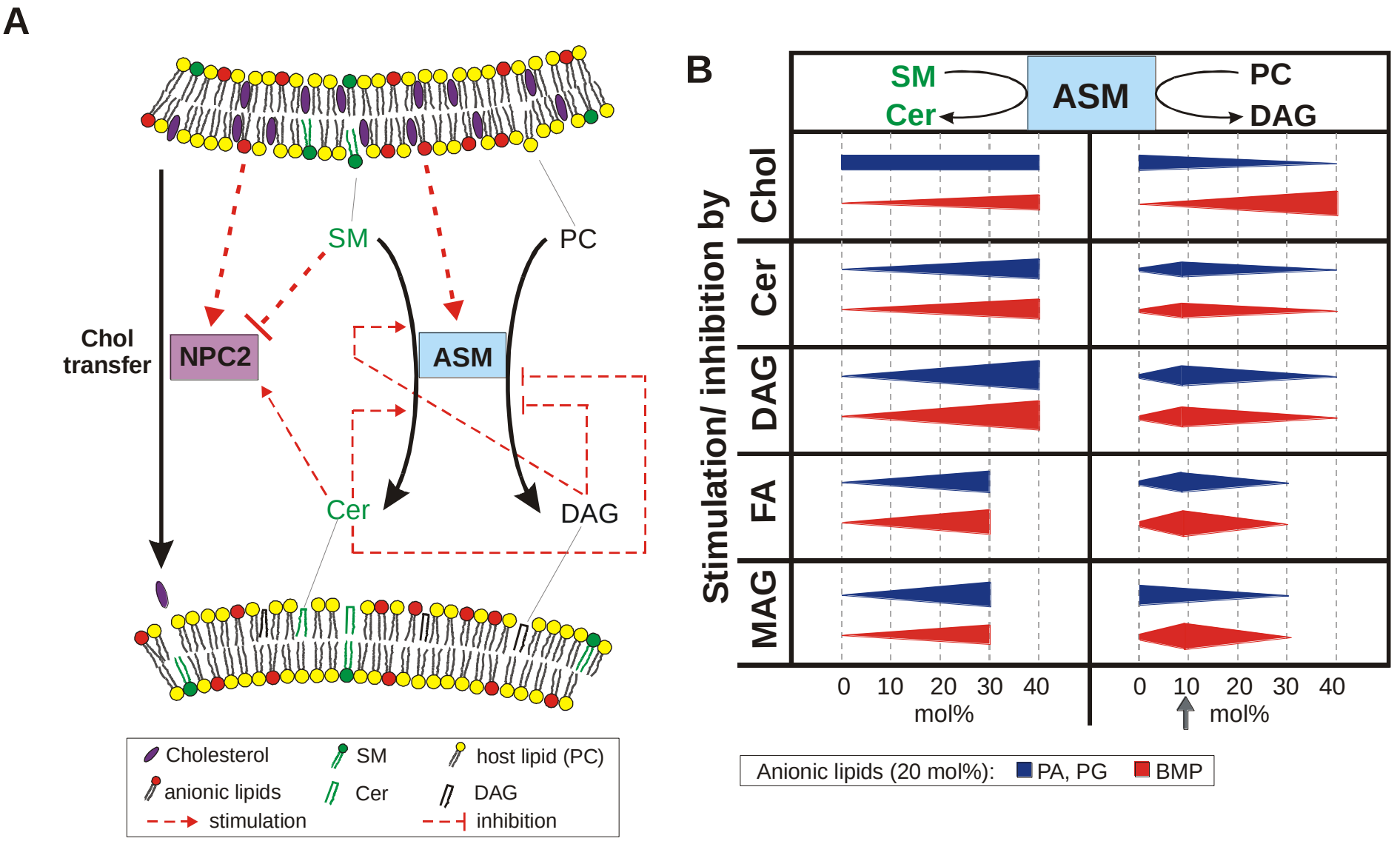
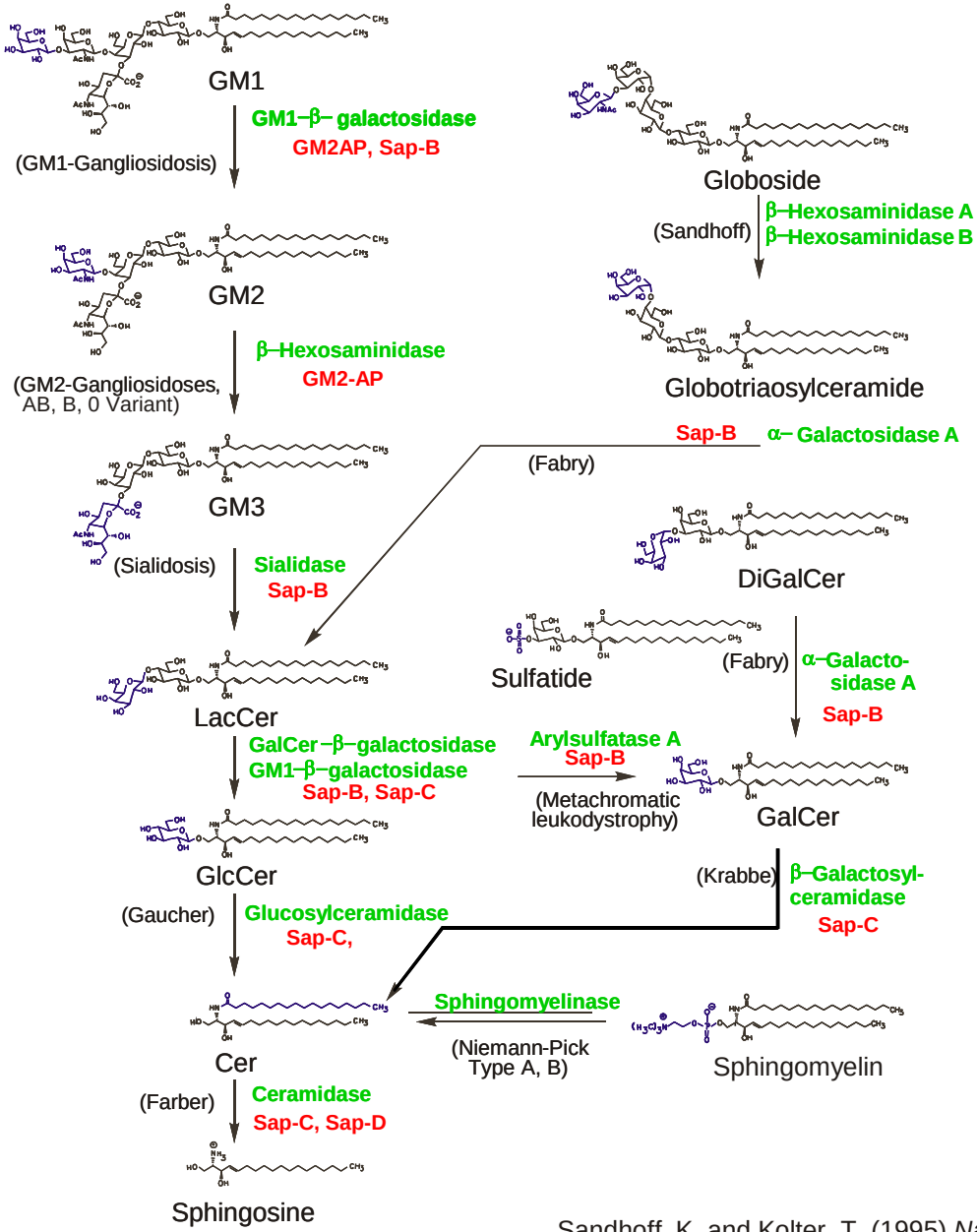
Linke T et al. J. Biol. Chem. 2001;276:5760-5768

Membrane lipids regulates ASM activity and cholesterol transfer by NPC2



Lysosomal sphingolipid degradation



Sandhoff, K. and Kolter, T. (1995) *Naturwissenschaften,* **82, 403-413**

Summary

- **NPC-2 is a cholesterol-binding and -transfer protein needed for secretion of cholesterol from intraluminal vesicles (IMs) of late endosomes (SM inhibits, PA, PG & BMP stimulate). NPC-2 deficiency causes NPC, ASM deficiency NPA & NPB disease**

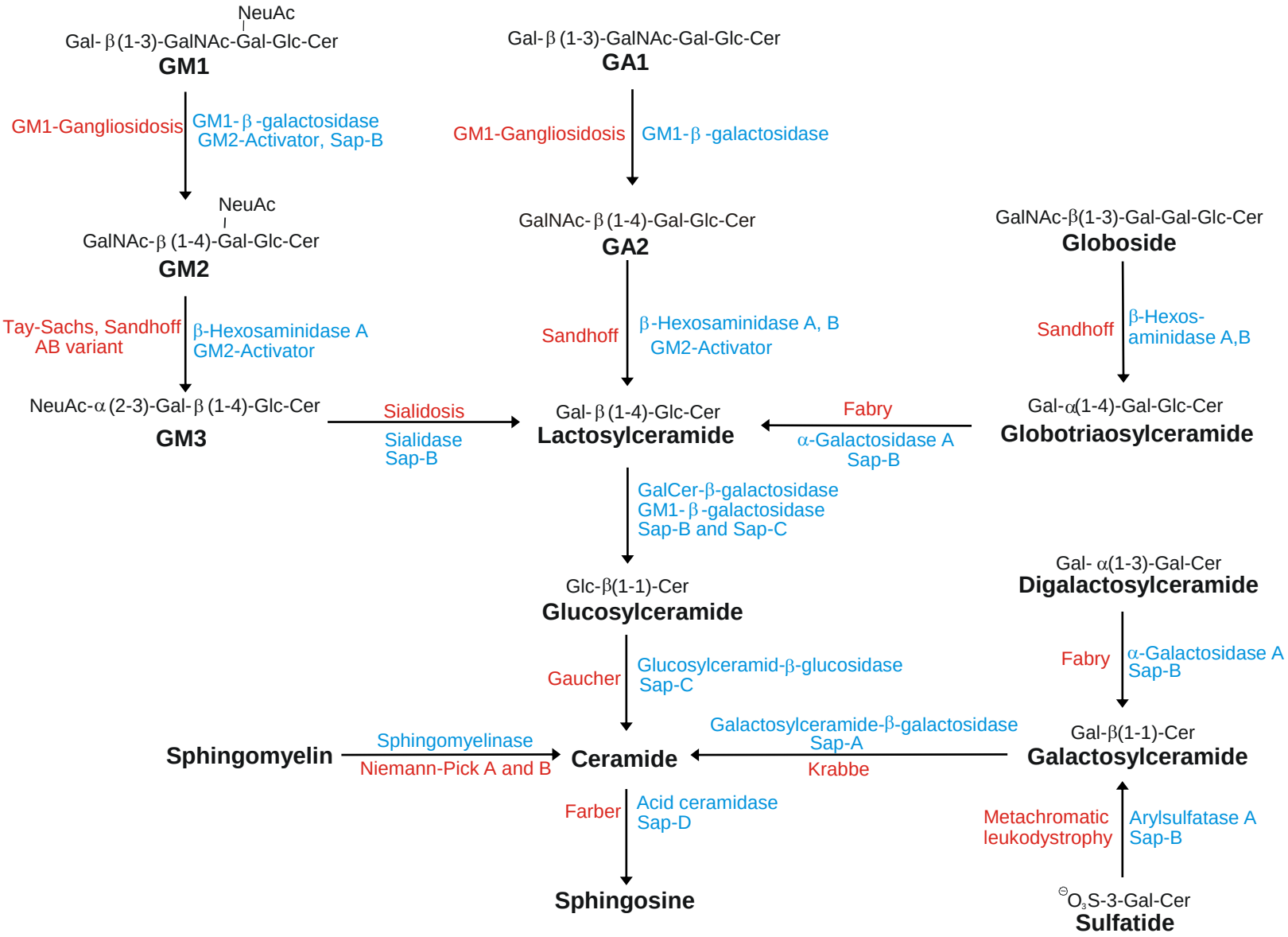
- **LLBPs (GM2AP, saposins A, B, C, D) are needed for sphingolipid- and membrane-degradation at intraluminal lysosomal membranes (lipid phase problem). They bind lipids, transfer lipids, some fuse vesicles, and disintegrate lipid vesicles at low pH.**

- **rgSap-A and rgSap-B solubilize liposomal lipids at low pH, low cholesterol and high BMP levels. Variant saposins of patients are inactive.**

- **Blocks in SL-catabolism cause fatal neurodegenerative lipid storage diseases. Prosaposin and ßGlcase(-/-):skin barier broken; Collodion babies; O-acyl-VLC-GlcCers up.**

- **Cationic lipids (GalSo in Krabbe, GlcSo in Gaucher, So&Sa)are cytotoxic. Desipramine (CAD, a lipid !) interferes with lipid catabolism and triggers proteolytic degradation of ASM and other hydrolases.**

Lysosomal sphingolipid degradation



Lipid substrate (*GM2) :

Its turnover in patient`s cells correlates with:

- Patient`s celluar Hex A & GM2AP (GM2 cleaving) activity and with the clinical course of the disease.

- But the clinical course hardly correlates with patient`s enzyme activity on soluble, artificial, synthetic substrates & the gene mutations.

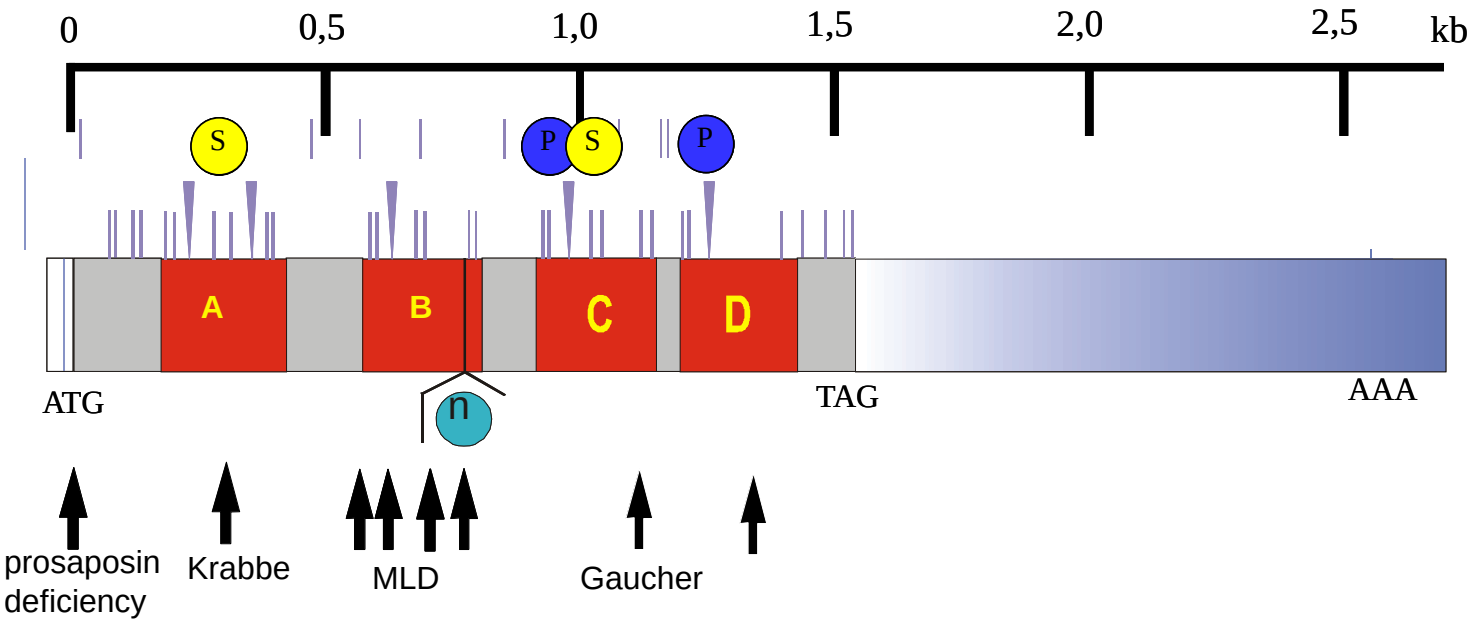
The latter concepts ignore the influence of :

- (Patient`s) enzyme specificity against lipids,
- Activity and specificity of essential, promiscuous and multifunctional SAPs,
- Extensive regulation of lipid turnover by membrane lipids at luminal vesicular surfaces.

Perspectives:
Membrane lipids regulate activity of endolysosomal proteins

- NPC-2 transfer of cholesterol between vesicles:
  - Is stimulated by BMP, PA, PG and inhibited by SM down to 10%.
  - PG, PA and BMP stimulate ASM up to 14 fold and release the block by SM.
- Results suggest a sequential pathway of lipid degradation at luminal IMs during endocytosis:
  - At late endosomes PM derived anionic PLs (PG, PA) stimulate ASM to degrade SM, thereby releasing the block of cholesterol egress by NPC-2 inhibition. ASM also degrades PG, PA, and other PLs, triggering loss of bilayer structure and barrier function of luminal membranes.
  - Generation of BMP and reduction of cholesterol levels in IMs activates SAPs (and hydrolases) needed for effective degradation GLS and ceramides.
  - Cationic lipids are toxic and inhibit catabolic steps (GalSo (Krabbe), GlcSo, So, Sa). CADs trigger proteolysis of ASM & other hydrolases and induce a phospholipidoses.

Human prosaposin



protein domain

precursor coding region

noncoding region

P phosphorylation of CHO

S sulfatation of CHO

cysteine residues

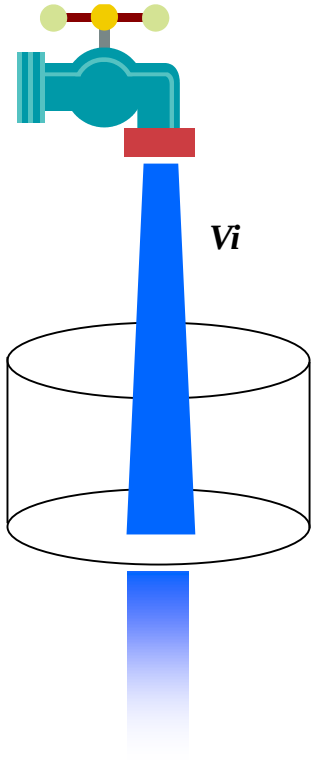
glycosylation sites

n insertion of 0, 6, 9 bp of Exon 8, 8‘

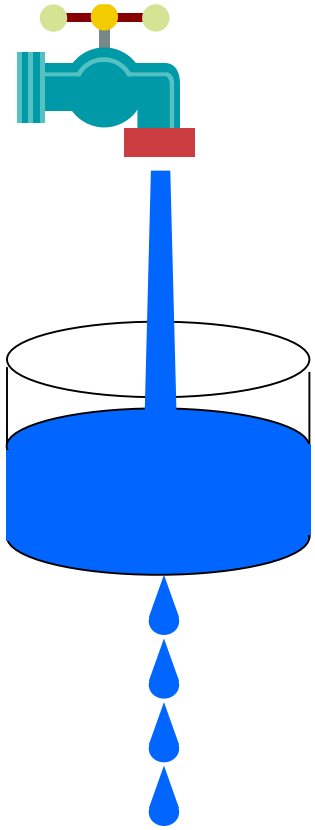
disease causing mutations

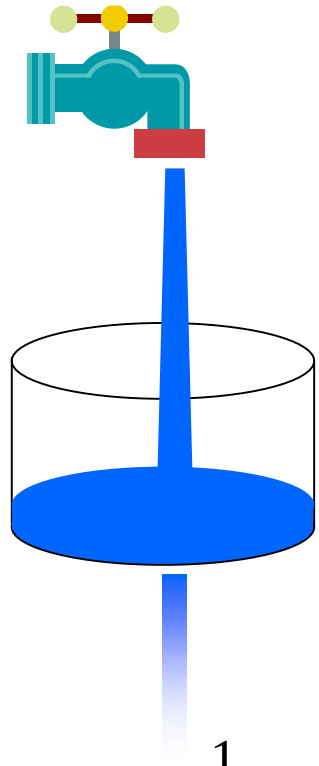
Sandhoff et al (2001) in: ***The Metabolic and Molecular Bases of Inherited Disease***

Lipid Storage and Threshold Theory



Lysosome





$$\frac{[S]_{eq}}{Km} = \frac{1}{[\frac{V\,\max}{Vi} - 1]}$$

Normal

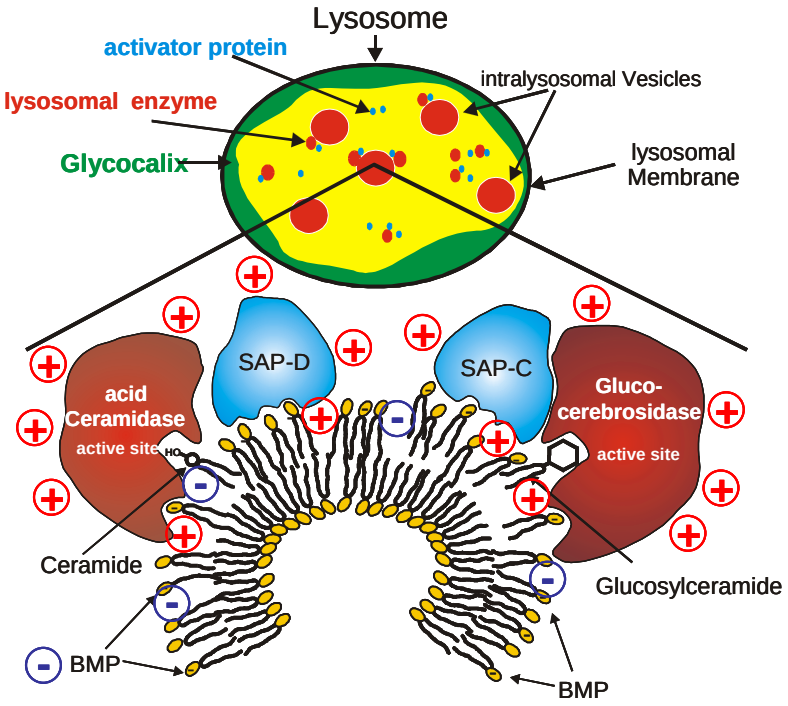
Patient (Infantile)

Residual catabolic activity (juvenile)

Conzelmann and Sandhoff (1983/84), Dev. Neurosci., 6, 58-71

CADs: Cationic amphiphilic drugs(lipids!) induce a phospholipidosis: the molecular view

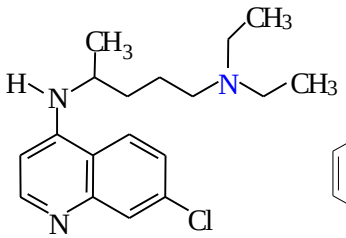
Cytoplasmic Inclusion body; x 78400
Chromaffin cell of a rat treated with
1-chloro-amitriptyline (120 mg/kg 10 wk)



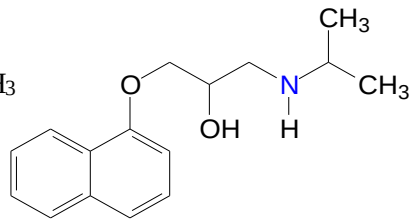
Wilkening, *et al*. (1998)

Cationic Amphiphilic Drugs (CADs):

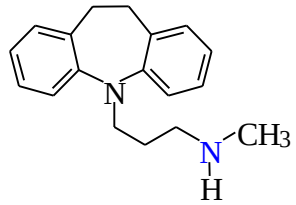
- Many drugs are CADs
- CADs are neutral at pH 7 and penetrate membranes
- They are protonated and trapped in lysosomes



Chloroquine
(Antimalaria)



Propranolol
(β-Adrenoreceptor-antagonist)

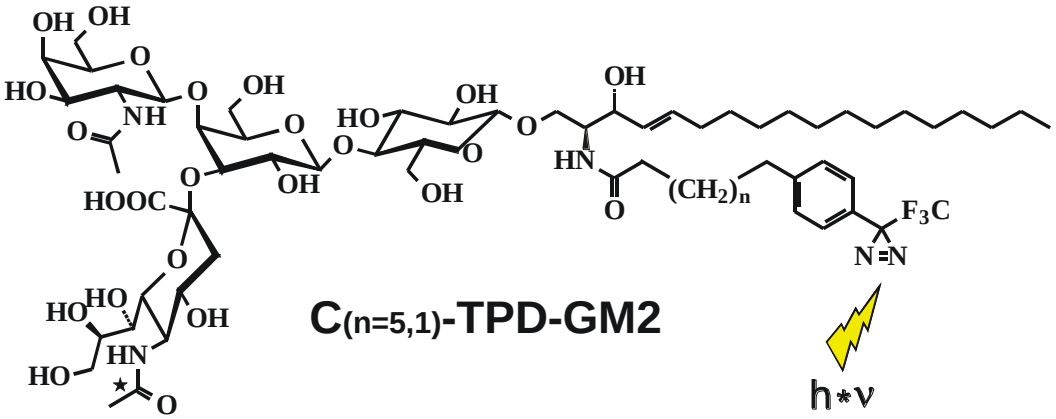


Desipramine
(Antidepressant)

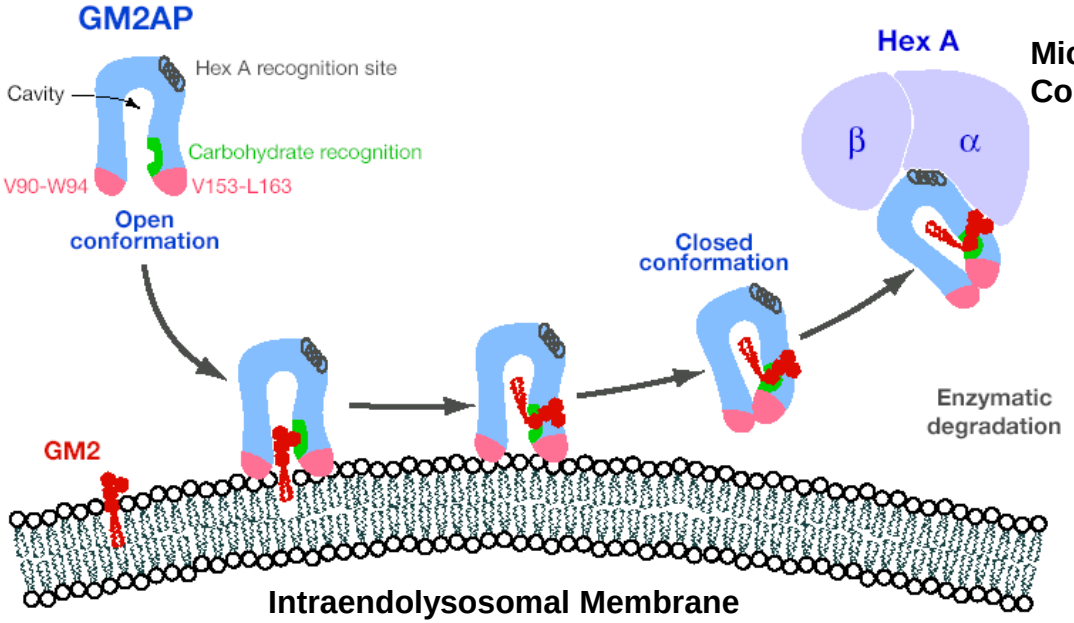
1. Induction of lipidoses in rats Lüllmann *et al*. (1978)
2. Detachment of acid sphingomyelinase from anionic liposomes Kölzer *et al*. (2004)
3. Proteolytic degradation of acid sphingomyelinase and other lysosomal enzymes by desipramine-treated cultured fibroblasts. Hurwitz *et al*. (1994)

Mechanism of GM2AP-liftase

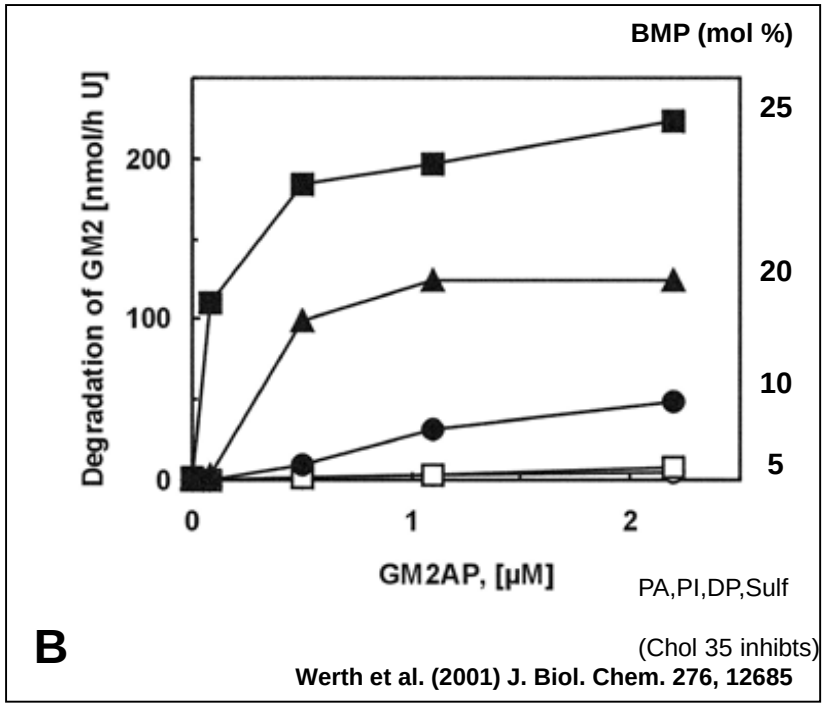
A



$C_{(n=5,1)}$-TPD-GM2



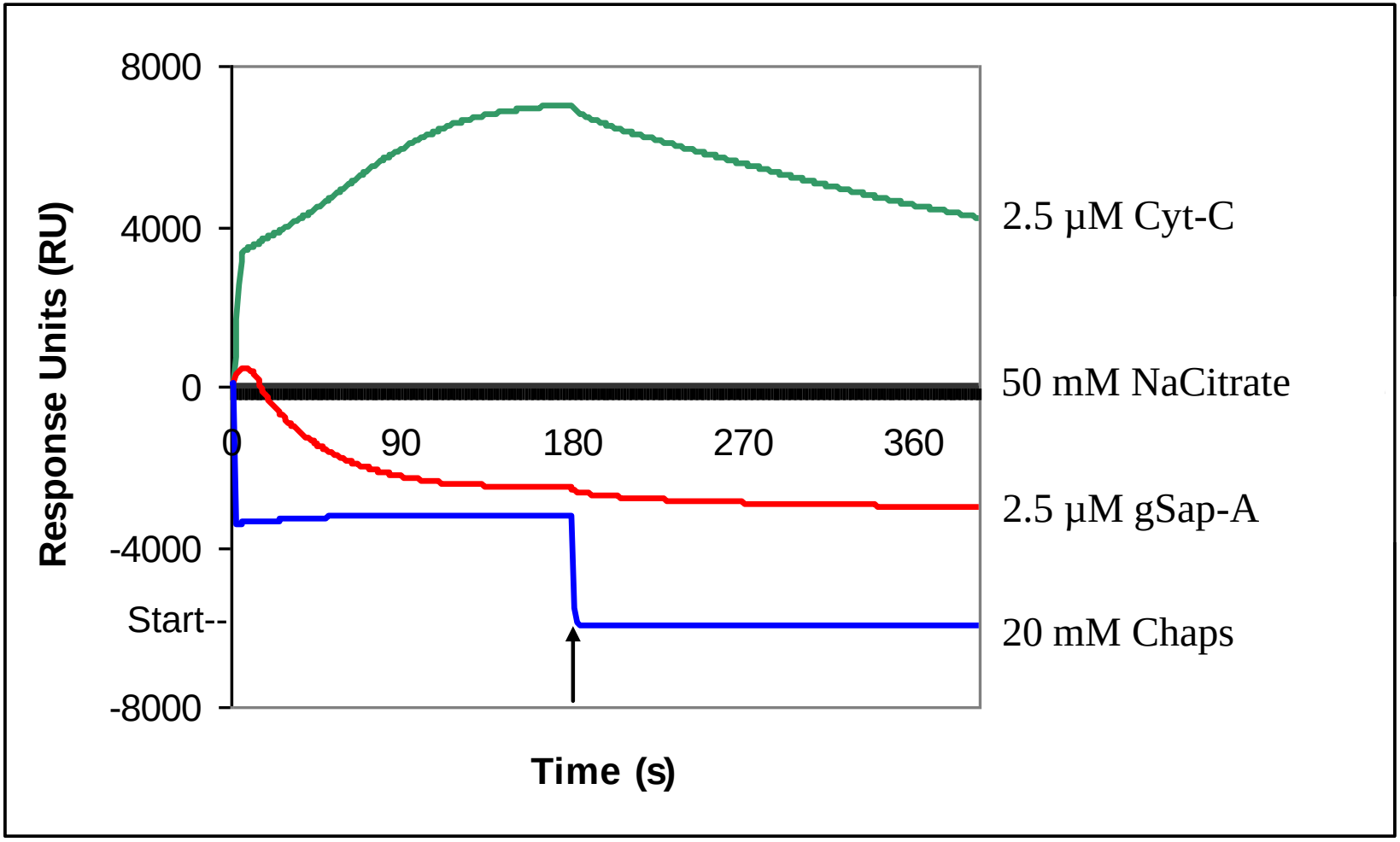
Michaelis Menten Complex



Werth et al. (2001) J. Biol. Chem. 276, 12685

Wright et al. (2003), J. Mol. Biol. 331, 951-64
Wendeler et al. (2004),Eur. J.Biochem. 271, 614-27

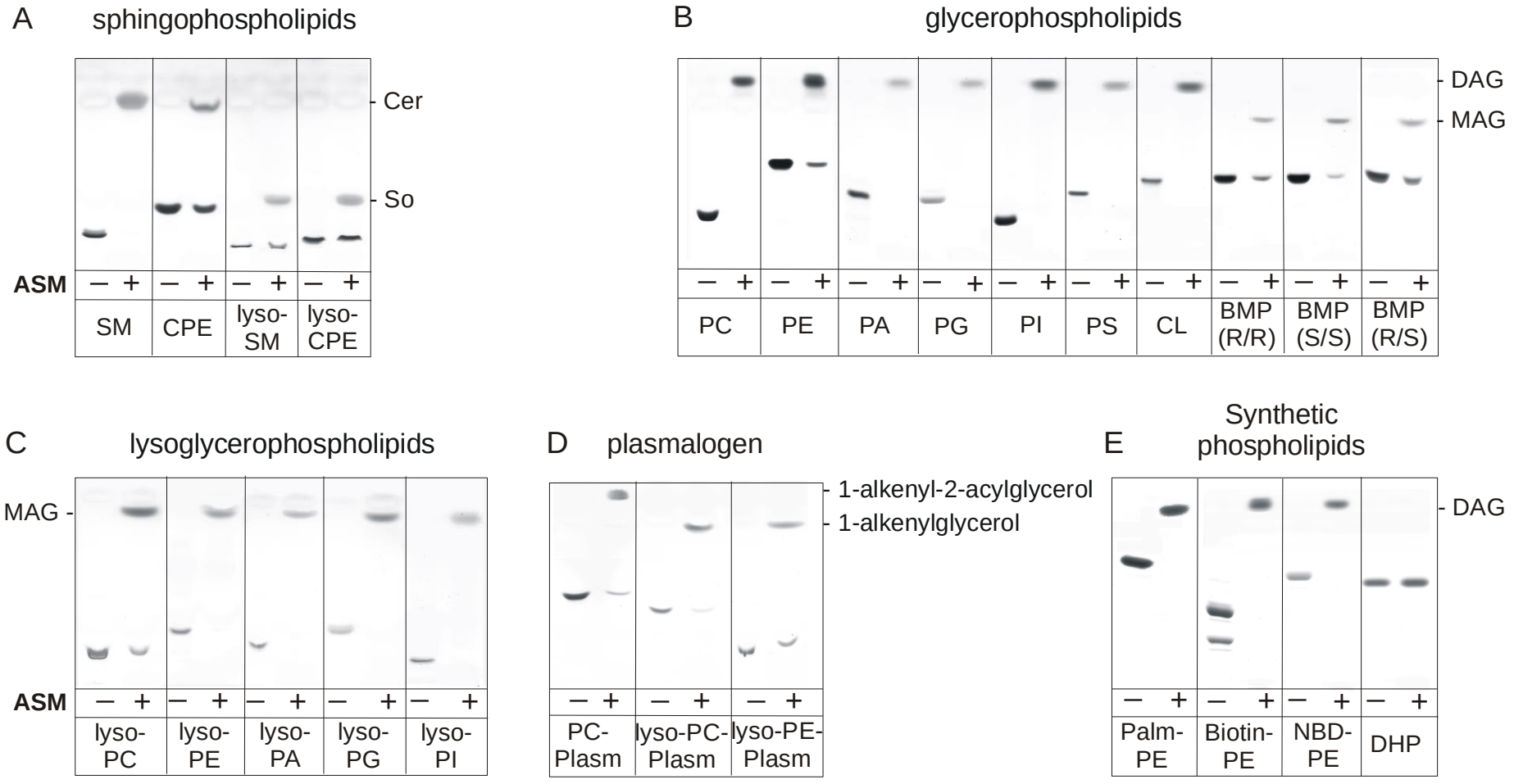
Lipid solubilization: Sap-A releases lipids from liposomes bound to a sensor chip



Plasmon resonance studies: Liposomes were reversibly bound to the hydrophobic surface of the sensor chip (dextran matrix derivatized with alkyl chains) and can be totally removed by detergent (Chaps). Proteins that are not membrane active (cytochrome-C) bind to liposomes. Their curves reach a plateau value and do not fall below the base line.

Locatelli Hoops, S. et al. JBC (2006)

ASM is a nonspecific phosoholipase C

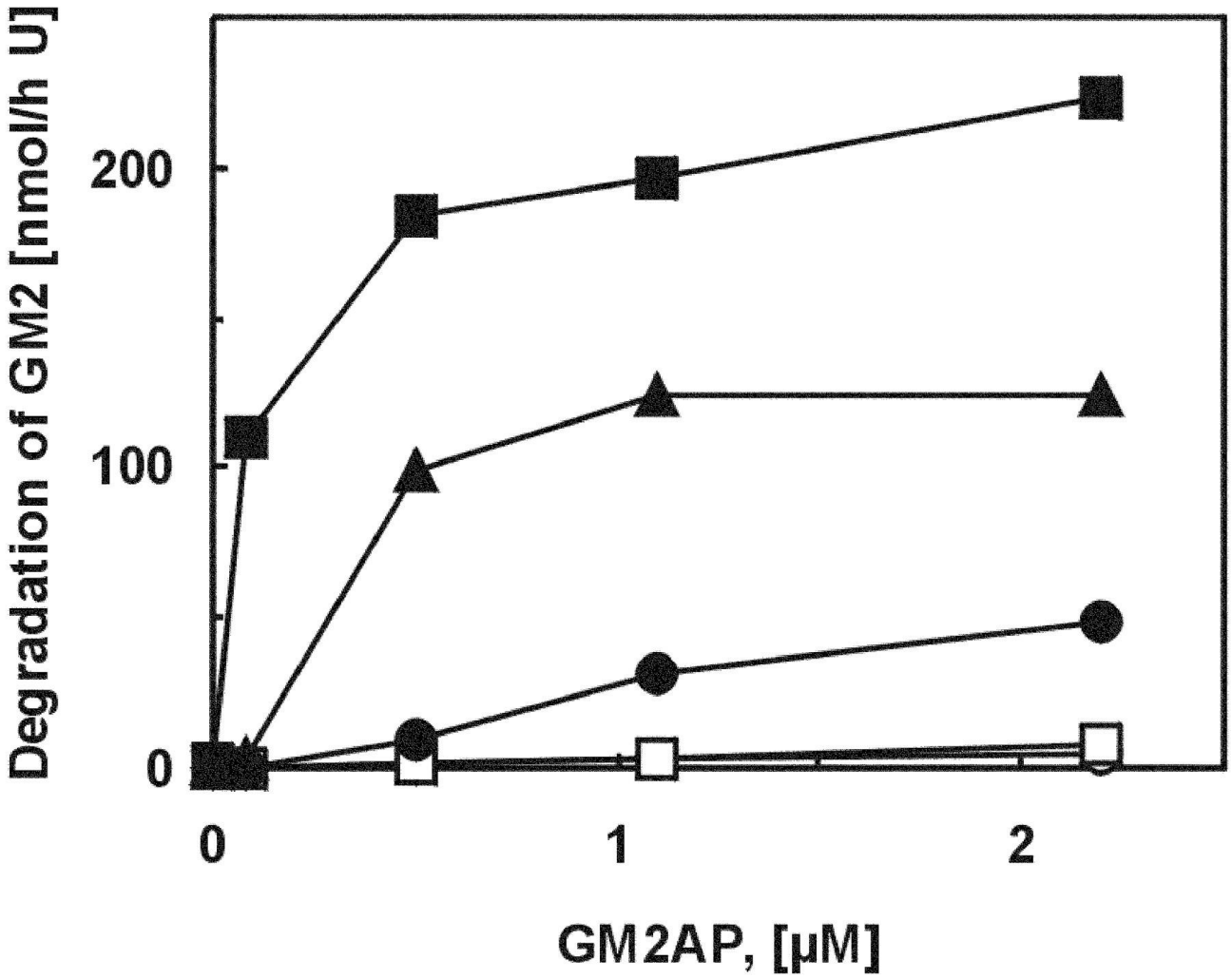


Micellar assay: 15 µg lipid/10 µg ASM/ 24 h at 37˚ C pH 4.5

ASM: recombinat HUMAN ASM expressed in insect *Sf21* cells (Lansmann *et al*., 2000)

Breiden, Oninla, Sandhoff, 2012

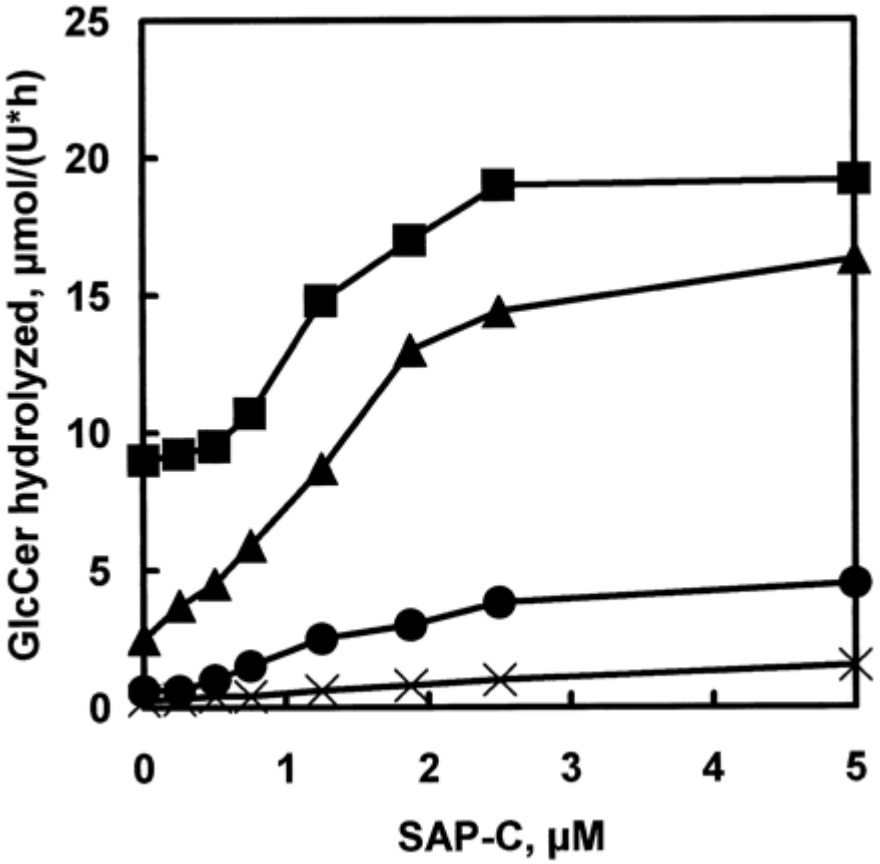
BMP and GM2AP stimulate the hydrolysis of LUV-bound ganglioside GM2.



BMP and GM2AP stimulate the hydrolysis of LUV-bound ganglioside GM2. The degradation of ganglioside GM2 inserted into LUVs doped with 0 mol % BMP (○), 5 mol % BMP (■), 10 mol % BMP (●), 20 mol % BMP (▲), or 25 mol % BMP (▪) was measured in the presence of increasing concentrations of GM2AP (0–2.2 μm).

Werth N et al. J. Biol. Chem. 2001;276:12685-12690

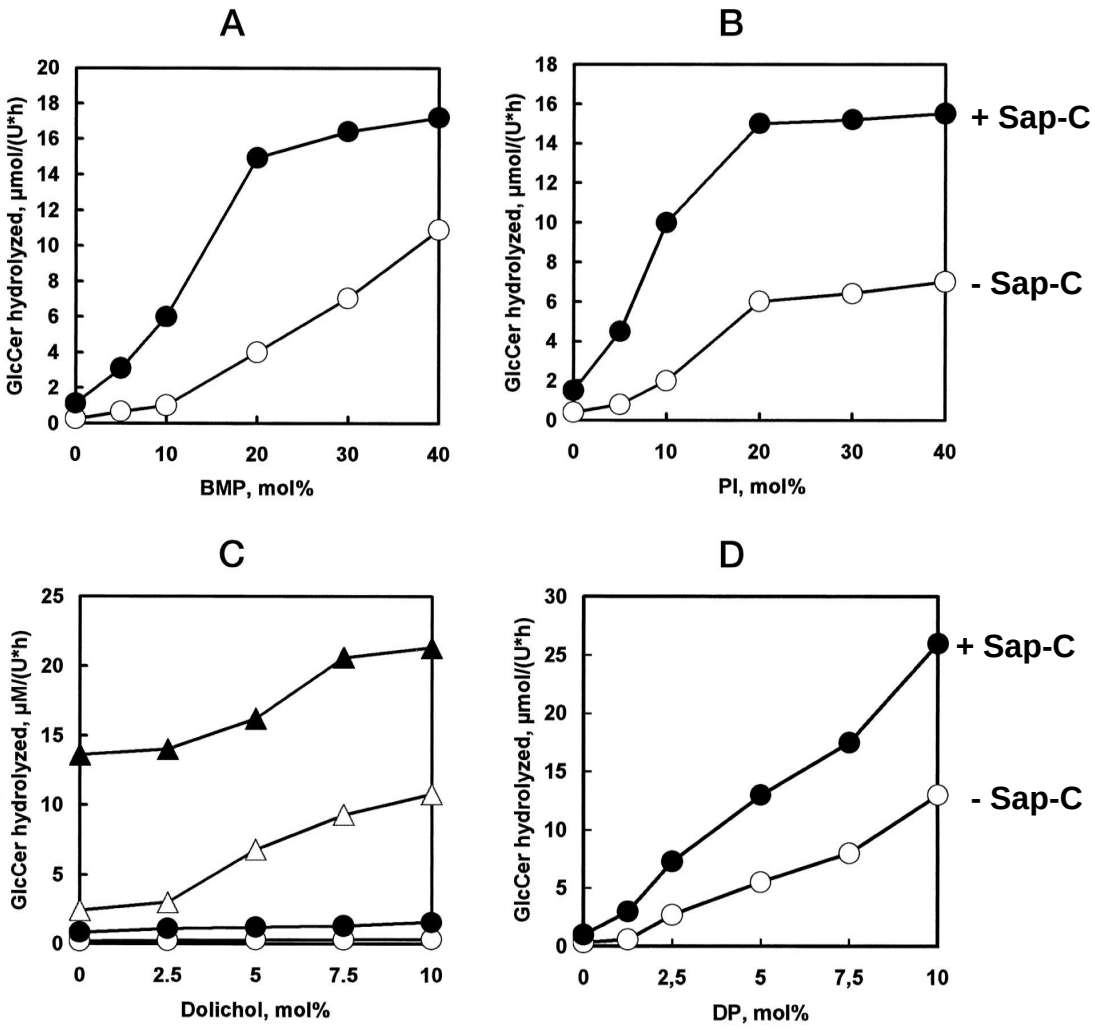
PA and SAP-C stimulate hydrolysis of LUV-bound glucosylceramide.



The degradation was measured with LUVs doped with 0 mol % PA (×), 5 mol % PA (•), 10 mol % PA (▲), or 20 mol % PA (▪) and increasing concentrations of SAP-C. All assay mixtures were prepared as described under "Experimental Procedures" and contained increasing proportions of PA in LUVs.

Wilkening et al, 2000, JBC 273, 30271-8

Anionic lipids in LUVs stimulate the enzymatic GlcCer hydrolysis in the presence and absence of SAP-C.



A, assays were conducted with GlcCer as substrate in the absence (○) and presence of SAP-C (2.5 µm) (•) using LUVs with various proportions of synthetic BMP (0–40 mol %). **B**, assays were carried out with varying concentrations of PI in LUVs, with (•) and without (○) the addition of 2.5 µm SAP-C, keeping the total lipid concentration in the assays constant. **C**, GlcCer carrying LUVs with or without PA were doped with increasing proportions of dolichol (0–10 mol %) and assayed for enzymatic GlcCer hydrolysis as follows: without SAP-C (○), with 2.5 µm SAP-C (•), with 10 mol % PA (△), and with 10 mol % PA and 2.5 µm SAP-C (▲).**D**, assays were performed with varying concentrations of dolichol phosphate (DP) (0–10 mol %) with 2.5 µm SAP-C (•) or no SAP-C (○).

Wilkening G et al. J. Biol. Chem. 1998;273:30271-30278

Lysosomal sphingolipid degradation