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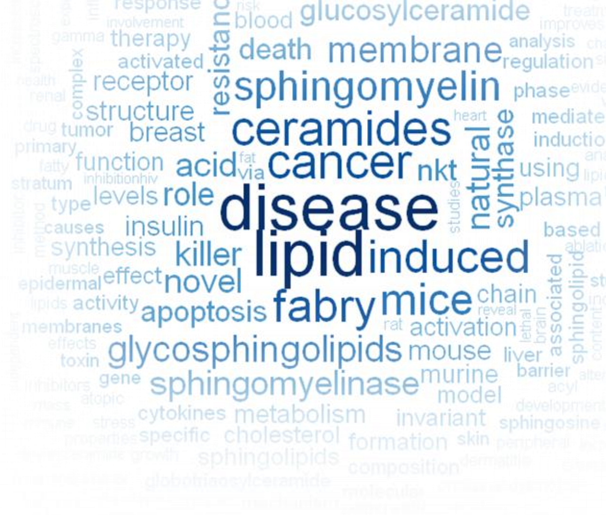

Glycosphingolipid-mediated interaction of Shiga toxin with the human endothelium: status quo of receptor research

Ivan U. Kouzel, Andreas Bauwens, Helge Karch and Johannes Müthing

Institute for Hygiene, University of Münster, Germany

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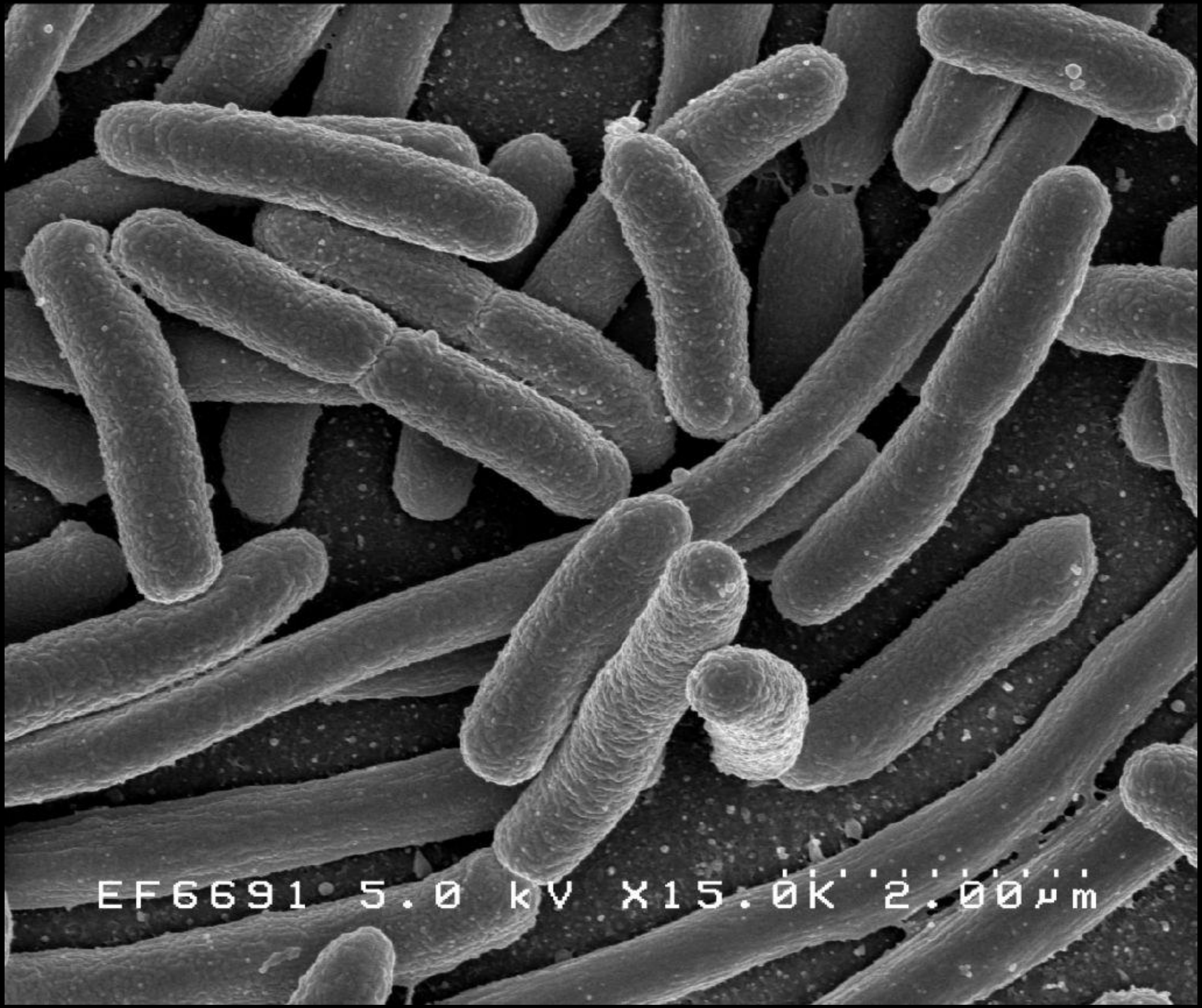
Outline

- Introduction
- Shiga toxin (Stx) glycosphingolipid (GSL) receptors of human endothelial cells
- *lipid raft* association of Stx GSL receptors
- Stx-mediated damage of human endothelium and blood brain barrier
- Perspectives

E. coli



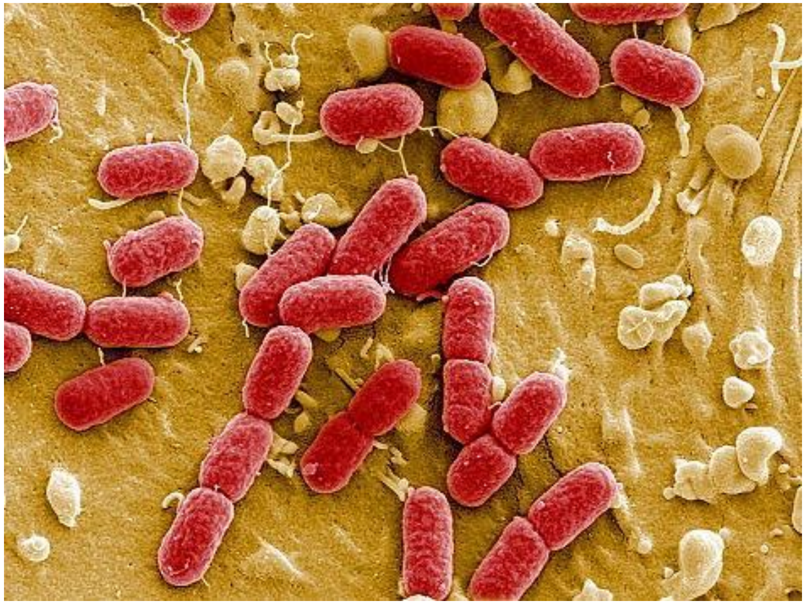
http://www.niaid.nih.gov/

introduction

EHEC outbreaks worldwide				
EHEC strain	Country	Year	Cases	Transmission
O157:H7	USA	1982	42	ground beef
O26:H11	Japan	2003	449	food borne
O157:H7	USA	2006	183	spinach
O104:H4	Germany	2011	3078 (54 deaths)	fenugreek seeds
O104:H4	Sweden	2011	35	food borne
O157:H7	Japan	2011	189	japanese rice cakes
O26:H-	Japan	2012	115	food borne

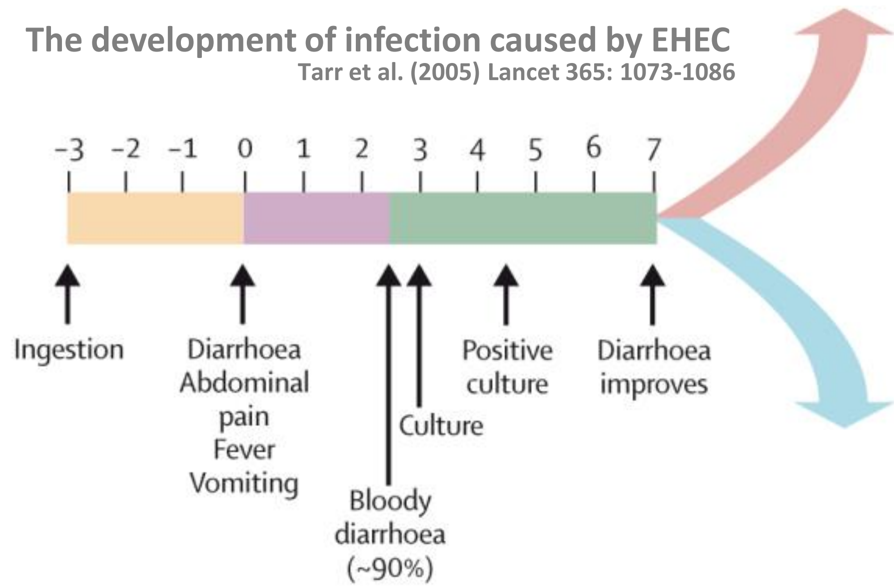
Terajima et al. (2014); Karch et al. (2012); Riley et al. (1983); http://www.cdc.gov/; http://www.euro.who.int/

EHEC and hemolytic uremic syndrome (HUS)

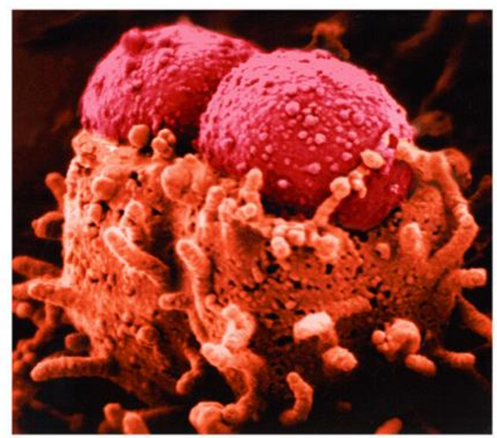


Electron micrograph of EHEC
Manfred Rohde, Helmholtz-Zentrum für Infektionsforschung

The development of infection caused by EHEC
Tarr et al. (2005) Lancet 365: 1073-1086

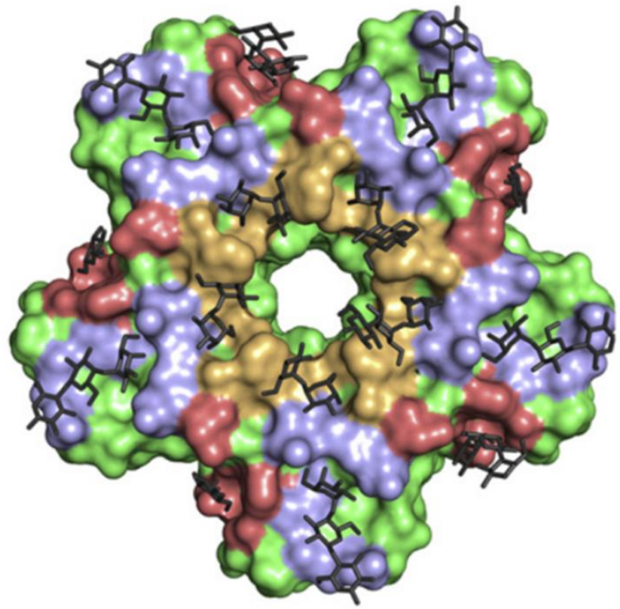


EHEC virulence factors	intimin
	EHEC hemolysin
	…
	Stx



Formation of pedestal-like structures by EHEC
Kaper et al. (2004) *Nat Rev Microbiol.* 2: 123-140

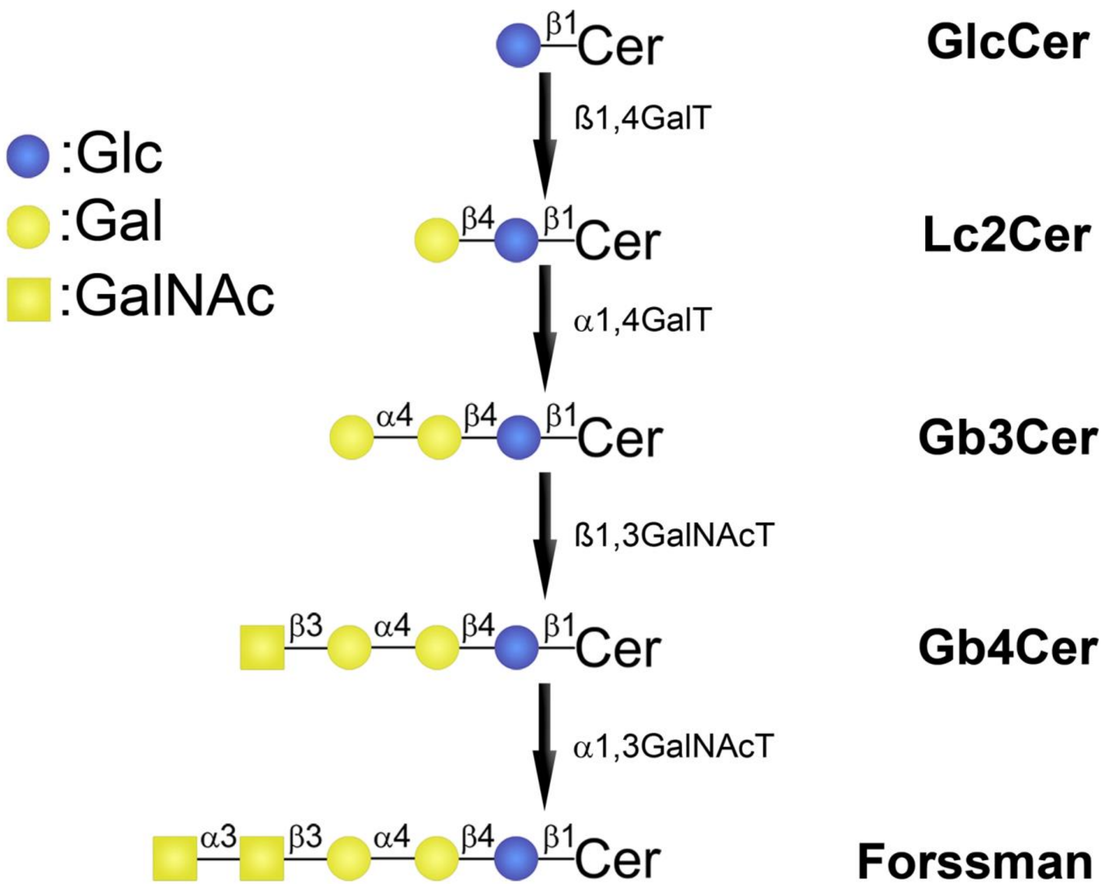
Shiga toxins (Stxs) – ribosome inactivating proteins (RIPs)



- one catalytically active **A subunit** (32.2 kDa): **rRNA *N*-glycosidase activity**
- five identical **B subunits** (7.7 kDa): **binding to GSL receptors**
- two main types: **Stx1** and **Stx2**

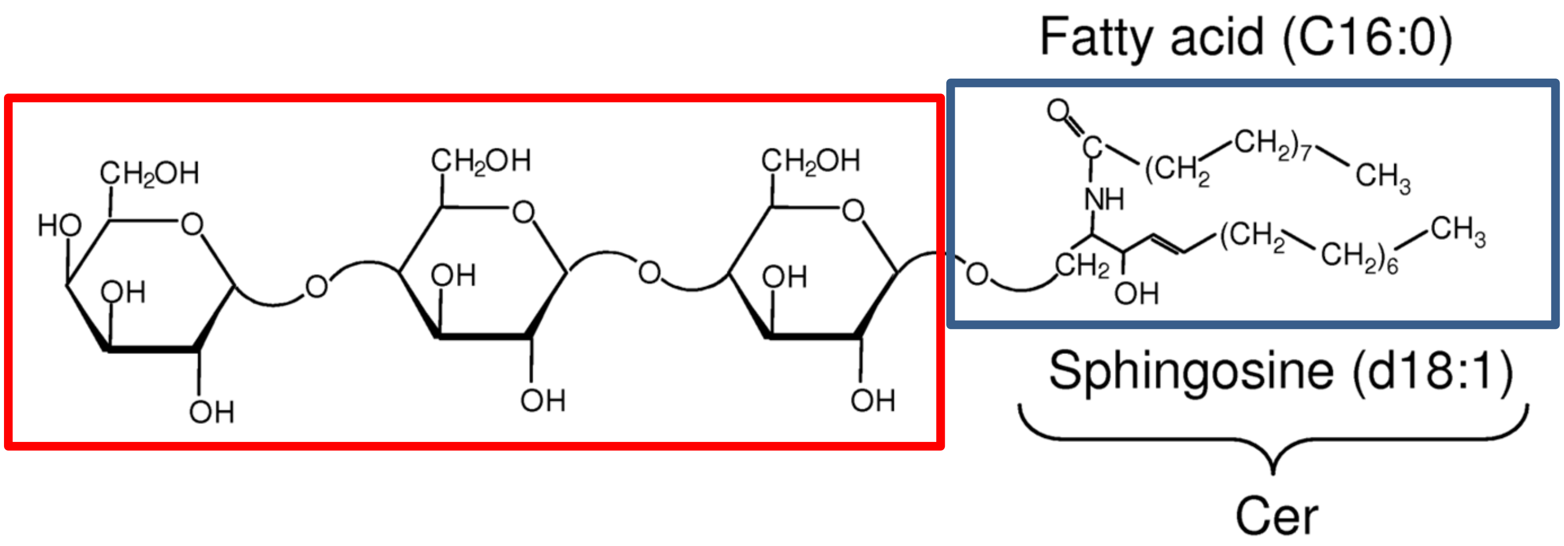
Bergan et al. (2012) *Toxicon.* 60: 1085–1107

Biosynthesis of Stx GSL receptors



Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

GSLs – amphipathic molecules



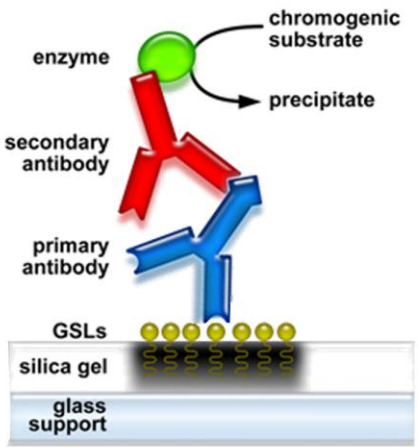
Galα4Galβ4Glcβ1Cer (d18:1, C16:0)

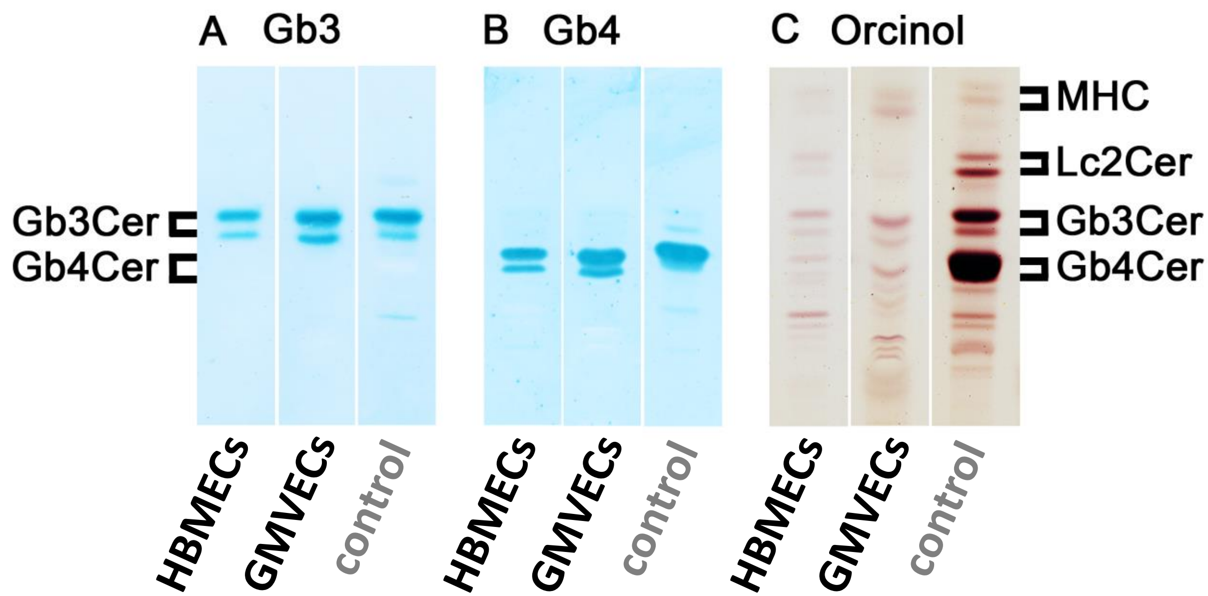
Globotriaosylceramide (Gb3Cer)

consist of a hydrophilic oligosaccharide chain and a hydrophobic ceramide moiety

Stx GSL receptors of human endothelial cells

HBMECs and GMVECs express GSL receptors Gb3Cer and Gb4Cer



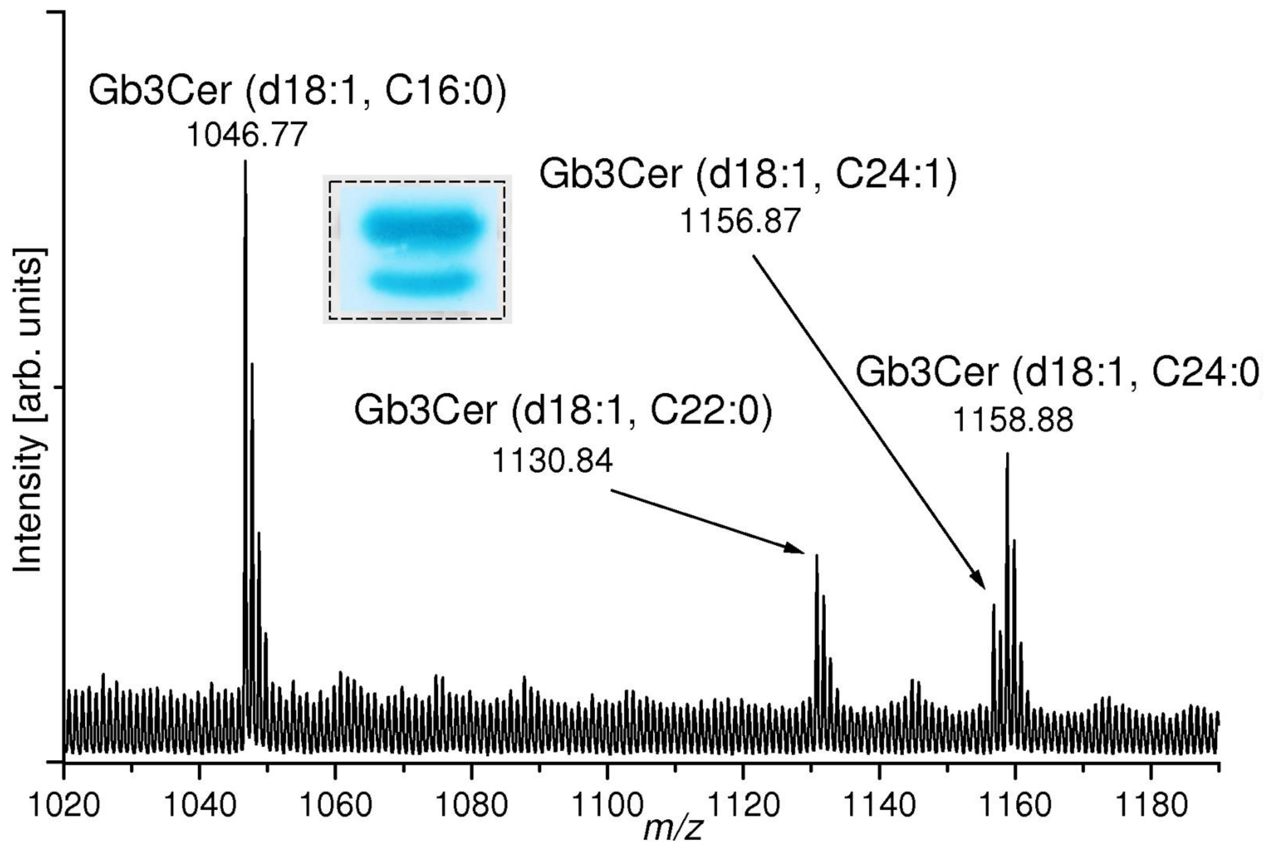


Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

HBMECs human brain microvascular endothelial cells
GMVECs glomerular microvascular endothelial cells

antibody-mediated detection of globo-series neutral GSLs in HBMECs and GMVECs

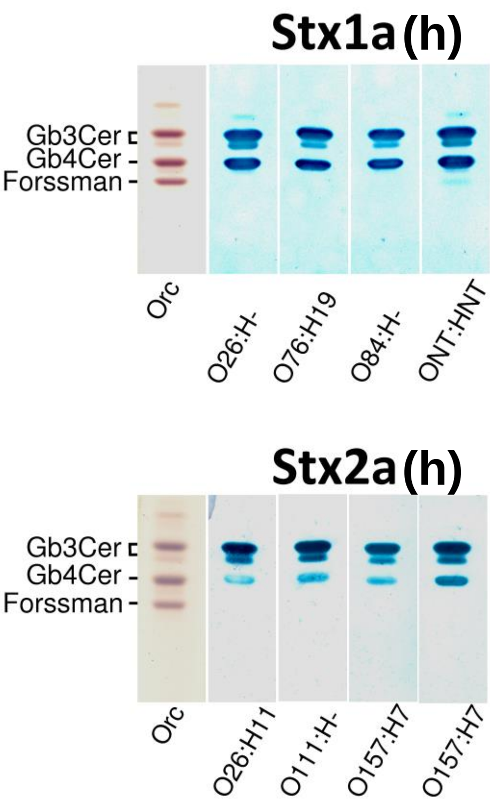
Structural diversity of **Stx** receptors in **GMVECs**

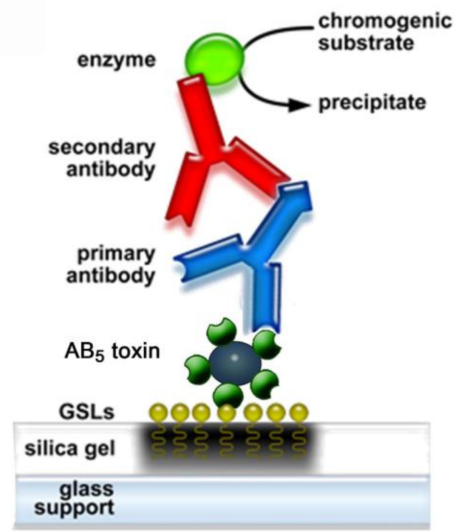


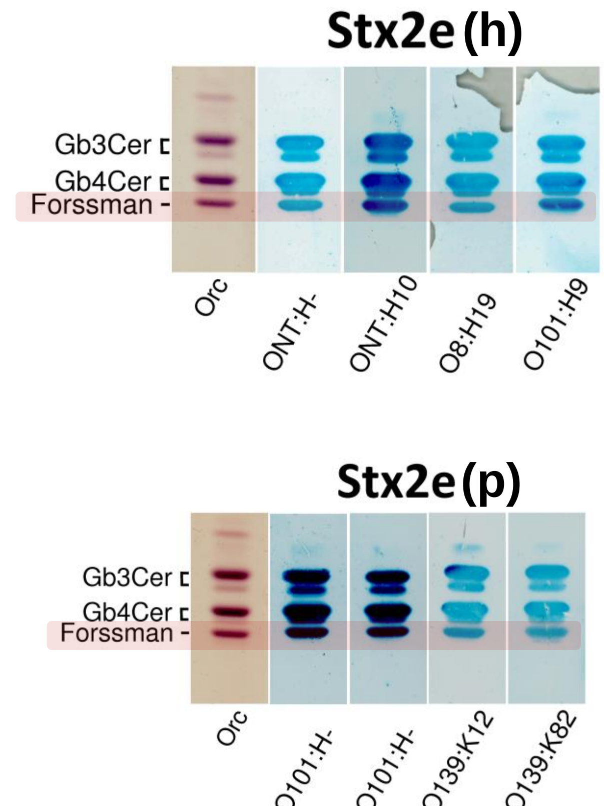
ESI-Q-TOF MS^1 spectra of antibody-detected Gb3Cer species

Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

Distinct binding of Stx1a, Stx2a, and Stx2e to GSL receptors





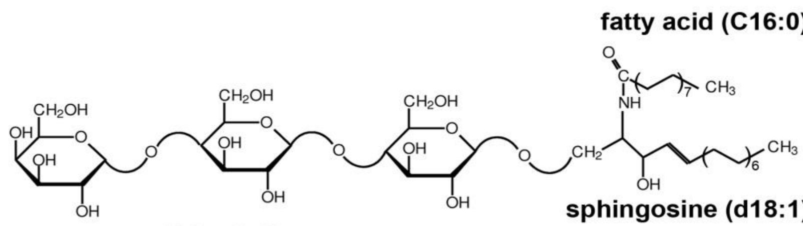


Only Stx2e recognizes Forssman

(h) human isolates
(p) porcine isolates

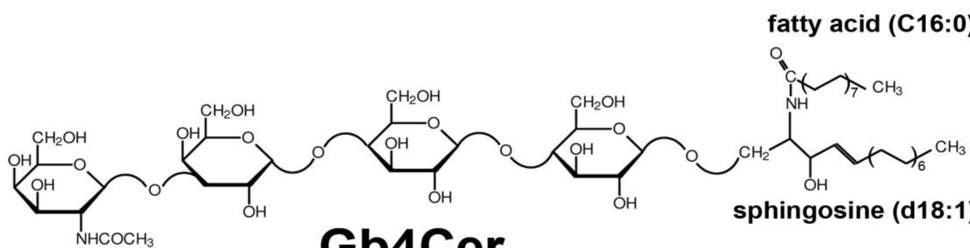
Müthing et al. (2012). *Glycobiology* 22: 849–862

GSLs involved in Stx binding



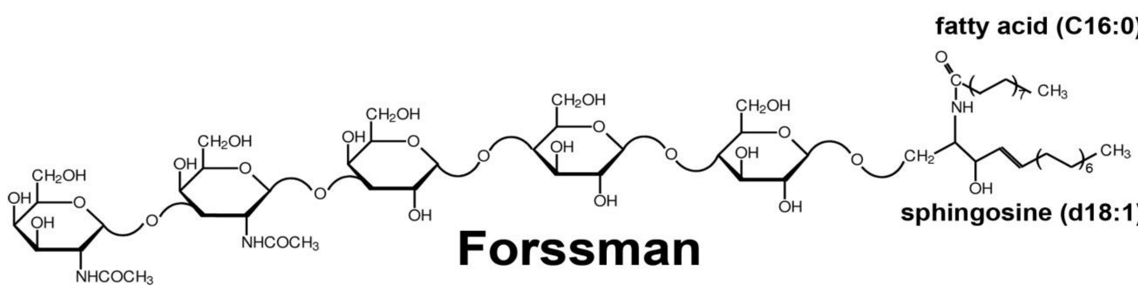
Gb3Cer

Stx1a +++
Stx2a +++
Stx2e ++



Gb4Cer

Stx1a ++
Stx2a +
Stx2e +++



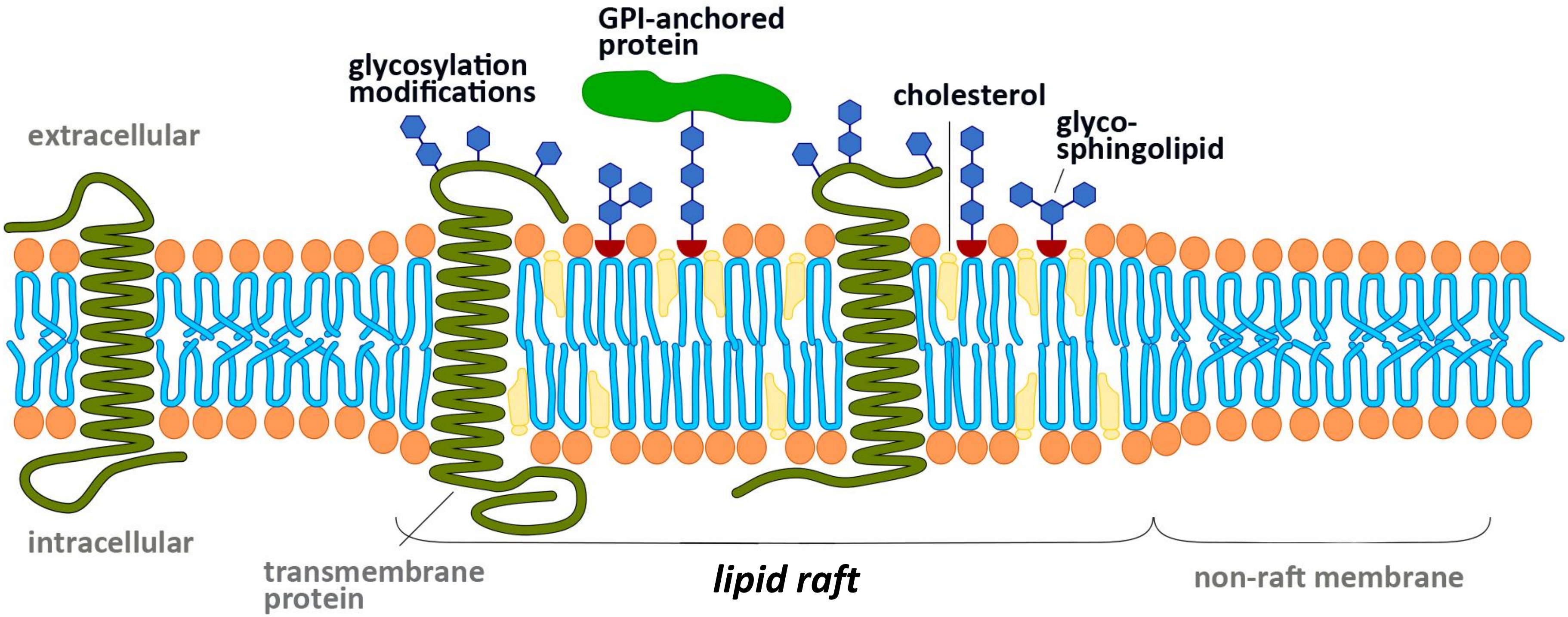
Forssman

Stx1a -
Stx2a -
Stx2e ++

Müthing et al. (2012) *Glycobiology* 22: 849–862

lipid raft **association of Stx GSL receptors**

Lipid rafts: platforms for entry of pathogens and their toxins

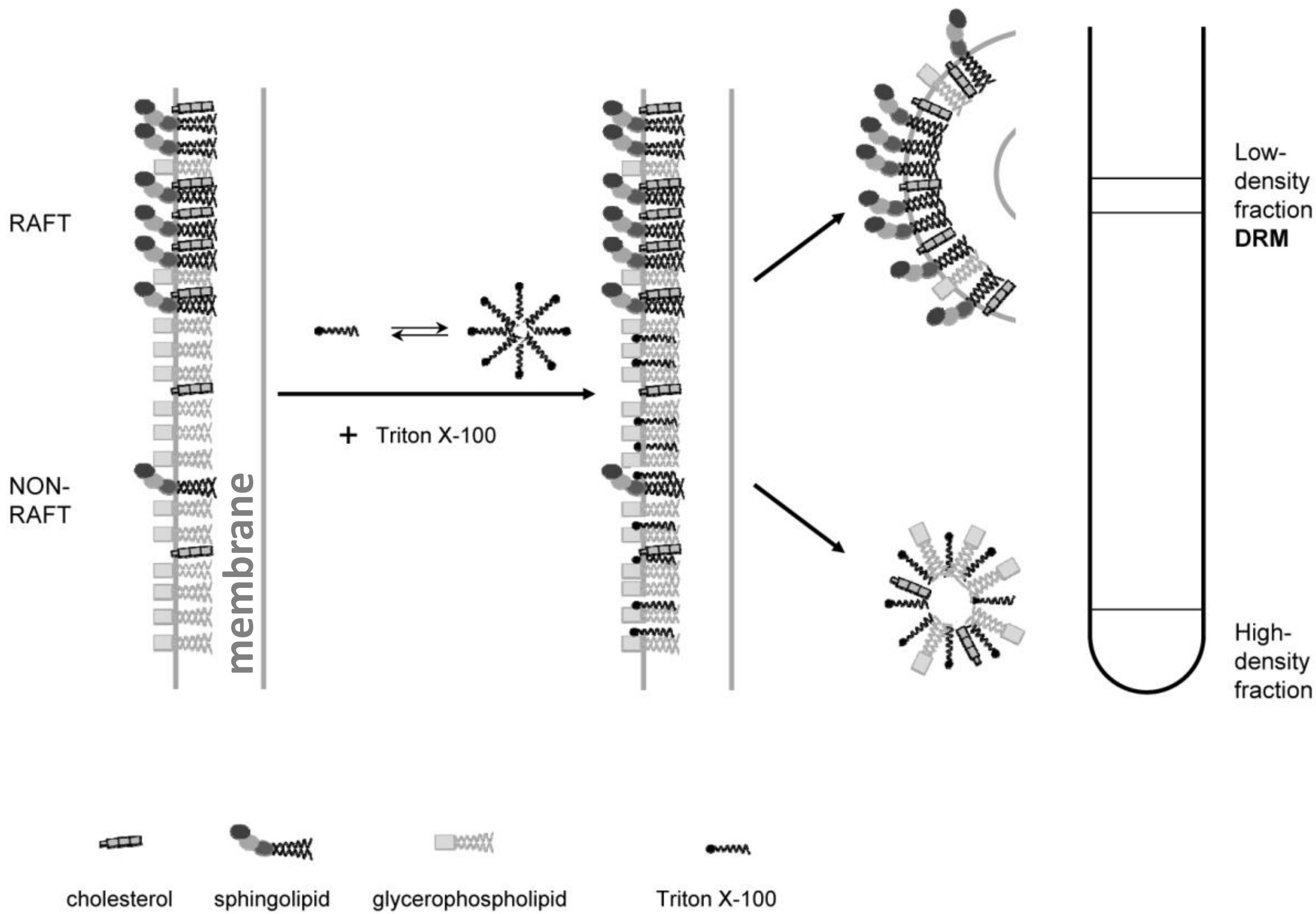


***lipid raft* association** of Stx GSL receptors –

prerequisite for retrograde transportation to the intracellular targets

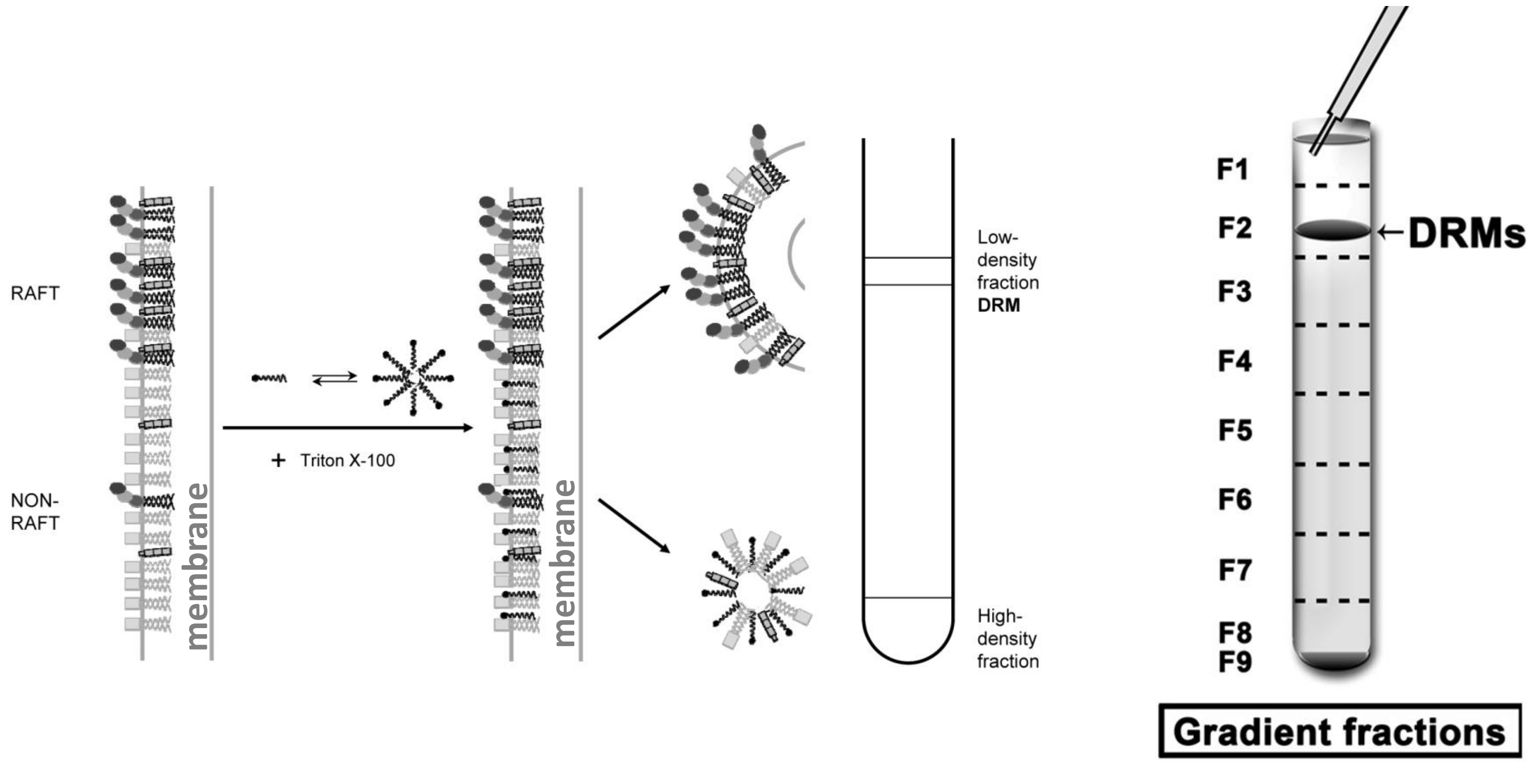
modified from Meisen et al. (2011) *Biochim. Biophys. Acta.* 1788: 875-896

Preparation of detergent-resistant membranes (DRMs): key method in lipid raft investigations



Sonnino and Prinetti (2013) *Curr Med Chem.* 20: 4-21

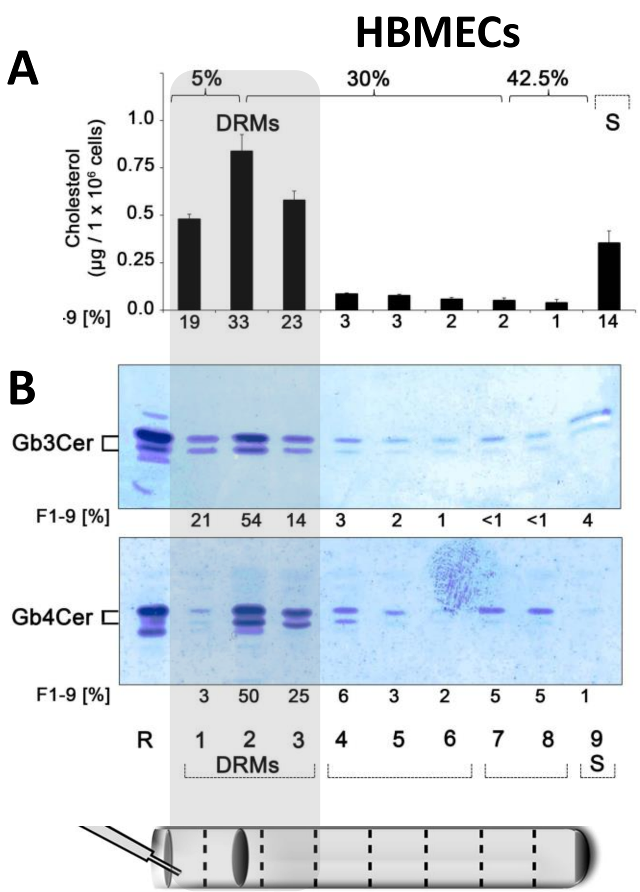
Preparation of detergent-resistant membranes (DRMs):
key method in ***lipid raft*** investigations

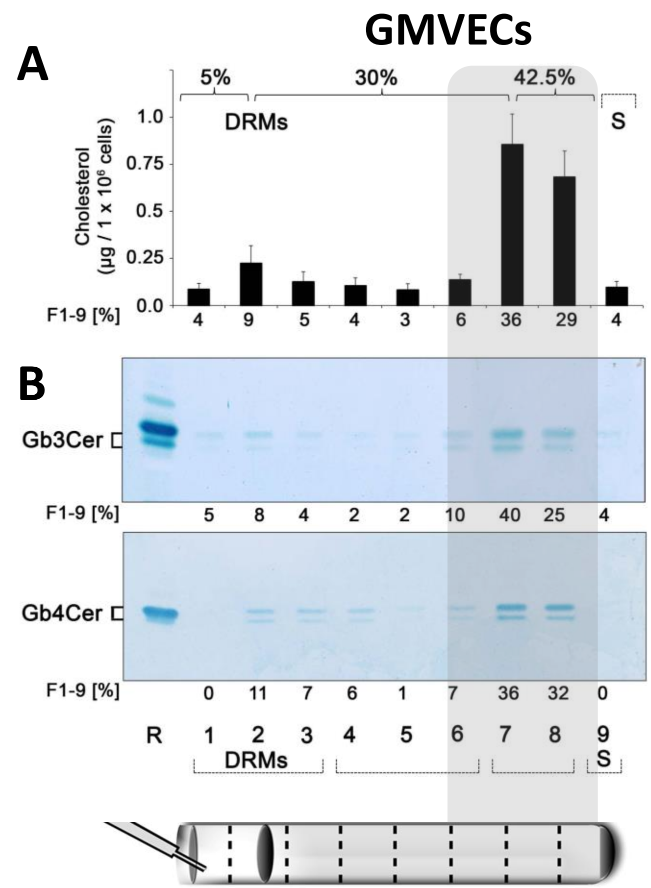


9 fractions	
F1-F3	DRM fractions
F4-F8	nonDRM fractions
F9	sediment (S)

subsequent analysis

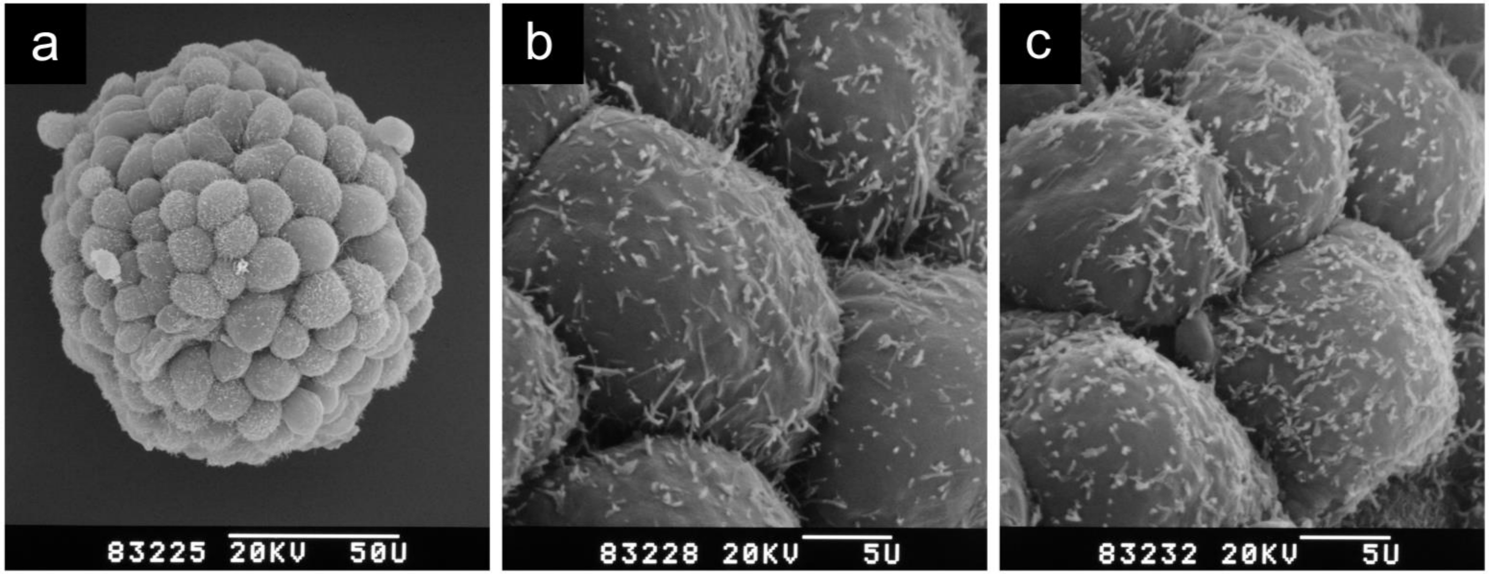
Distinct microdomain association of Stx GSL receptors in HBMECs and GMVECs





Stx-mediated damage of human endothelium

Intact HBMECs growing on microcarriers

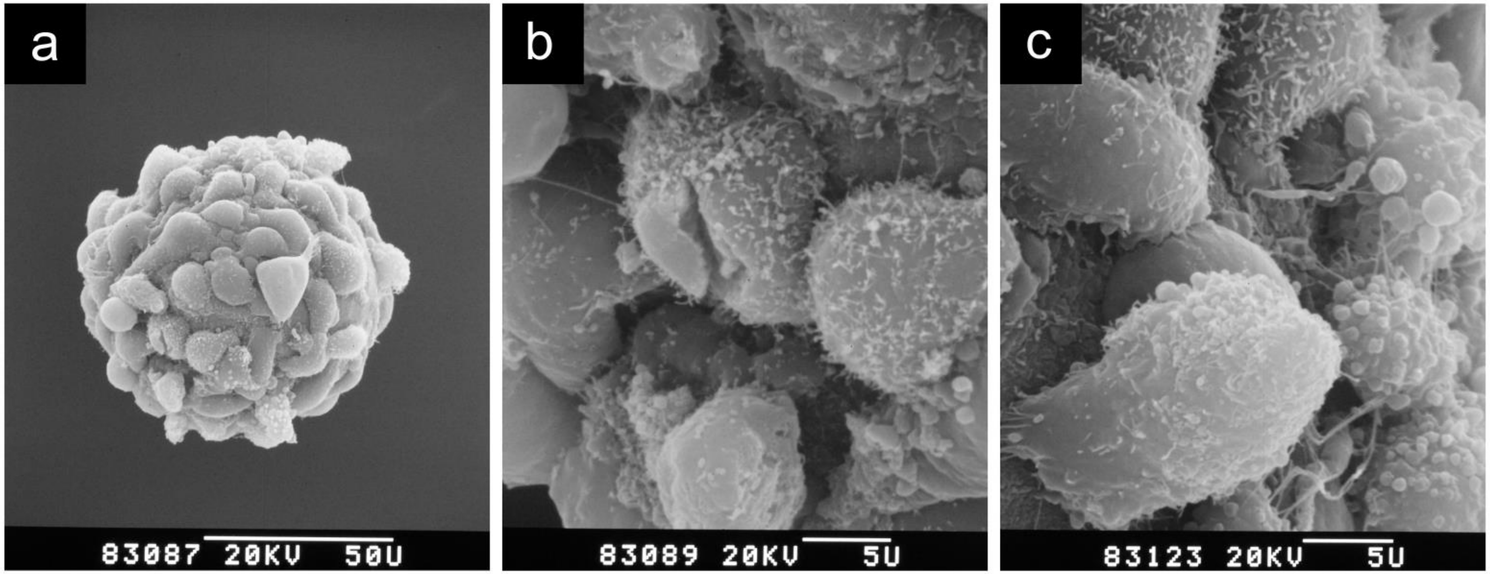


scanning electron micrographs of **untreated HBMECs:**

- **typical cobblestone pattern**
- **strict contact inhibition of cells**

Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

Stx1a induces necrosis and apoptosis in HBMECs

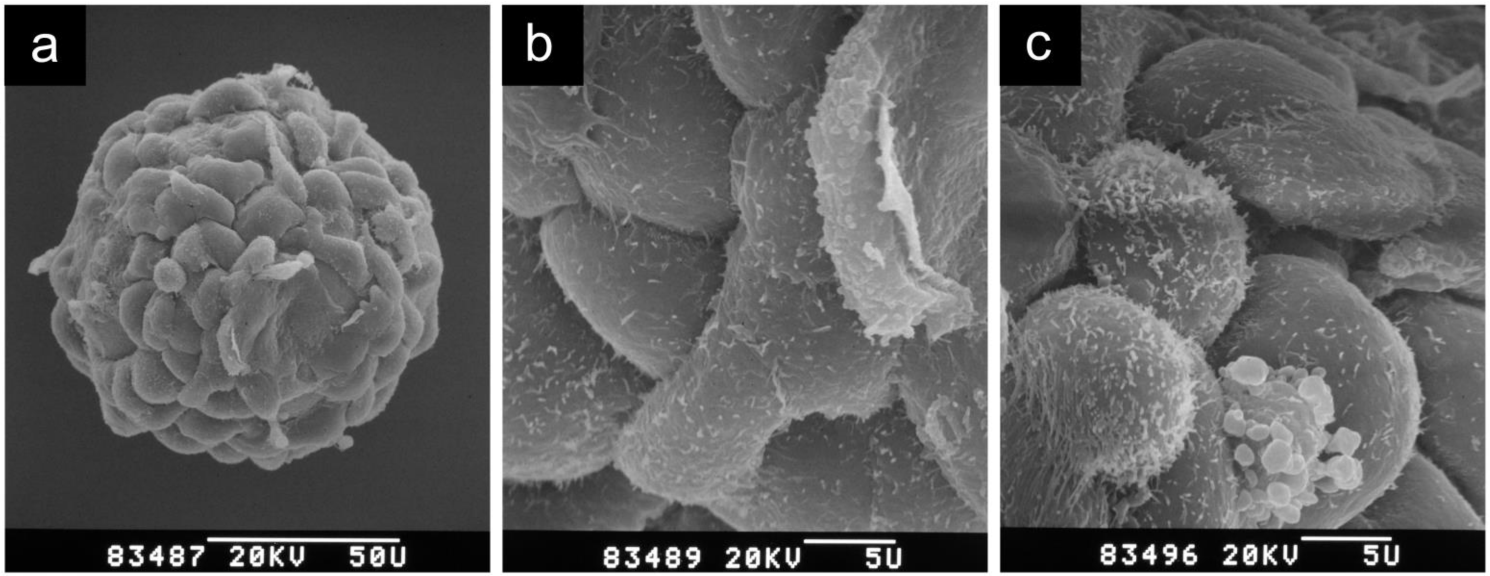


scanning electron micrographs of **HBMECs treated with Stx1a:**

- **irregular cell shape, plasma membrane lesions**
- **membrane blebbing, gaps between the cells**
- **disrupted cells, severe monolayer damage**

Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

Stx2a induces mostly apoptosis in HBMECs

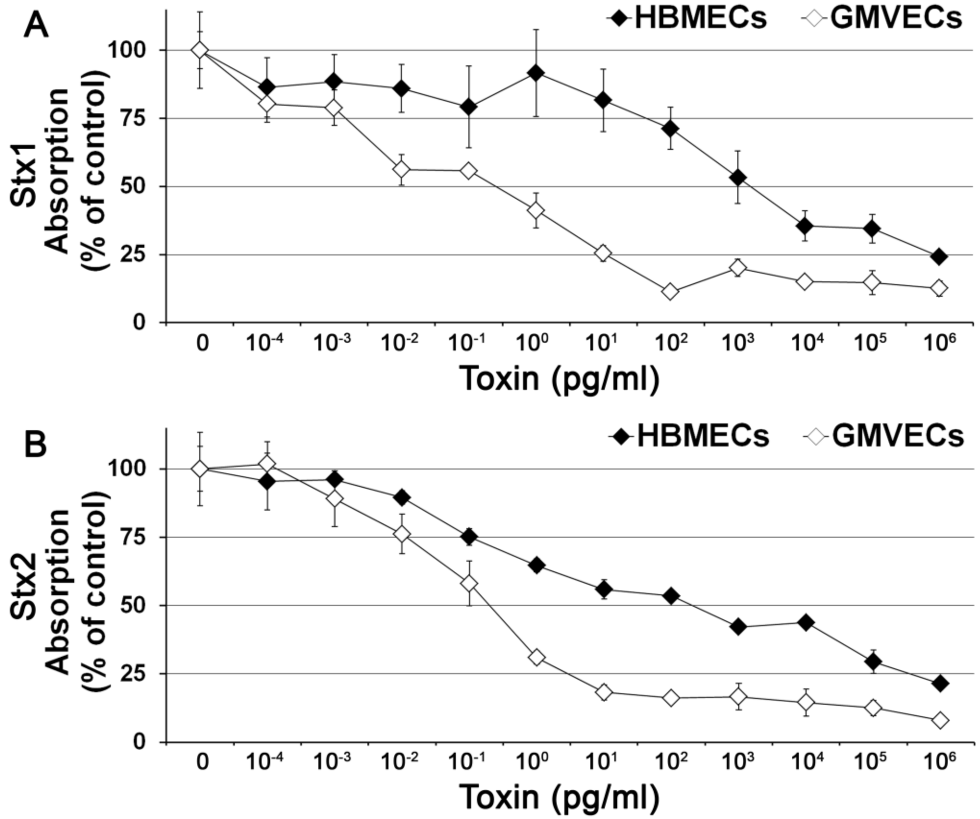


scanning electron micrographs of **HBMECs treated with Stx2a:**

- **only membrane blebbing**
- **no plasma membrane lesions and cell detachment**

Bauwens et al. (2013) *Cell. Mol. Life Sci.* 70:425–457

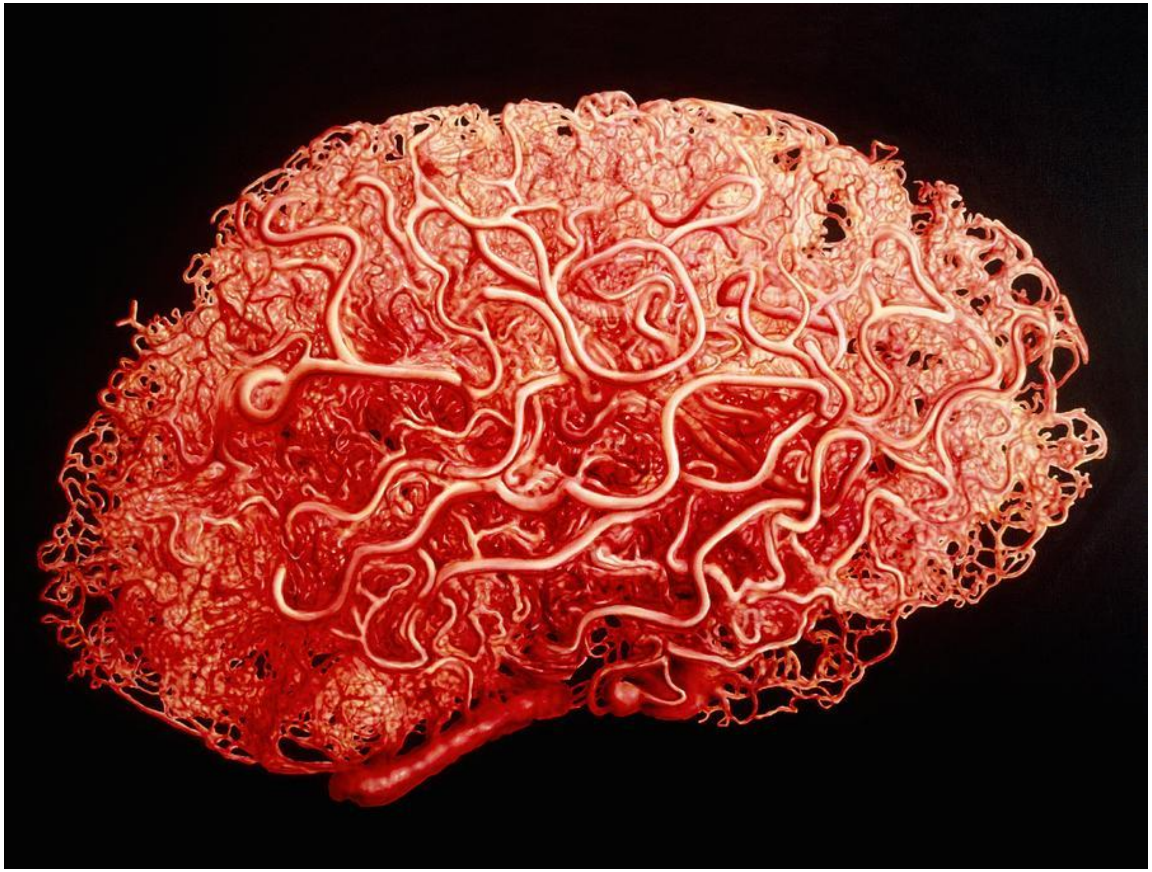
Differential susceptibility of HBMECs and GMVECs toward Stx1a and Stx2a



HBMECs human brain microvascular endothelial cells
GMVECs glomerular microvascular endothelial cells

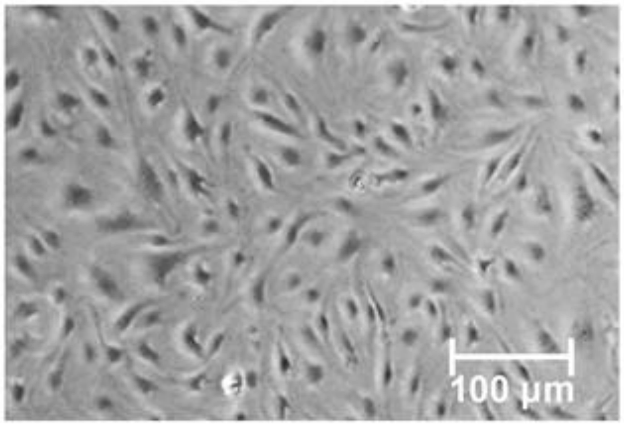
GMVECs are about 1000 times more sensitive (CD_{50}) to Stx1a and Stx2a than HBMECs

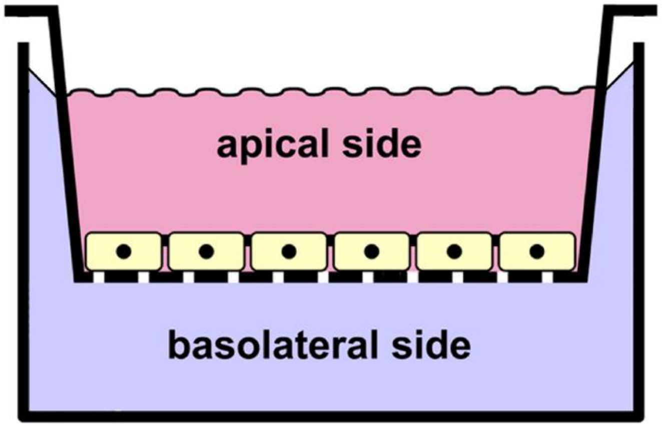
Vascularization of the brain



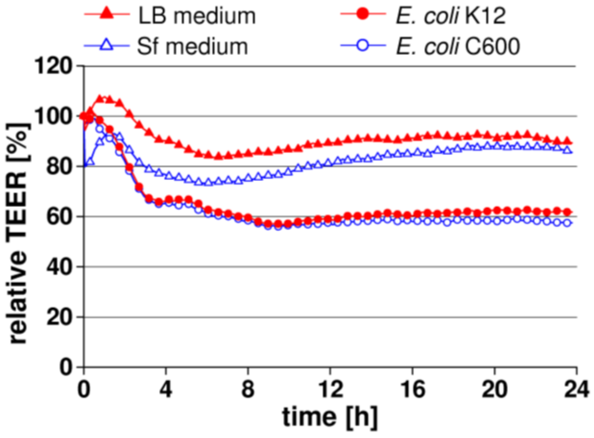
http://images.fineartamerica.com

Loss of blood brain barrier integrity upon exposure to Stx2e-containing STEC supernatants

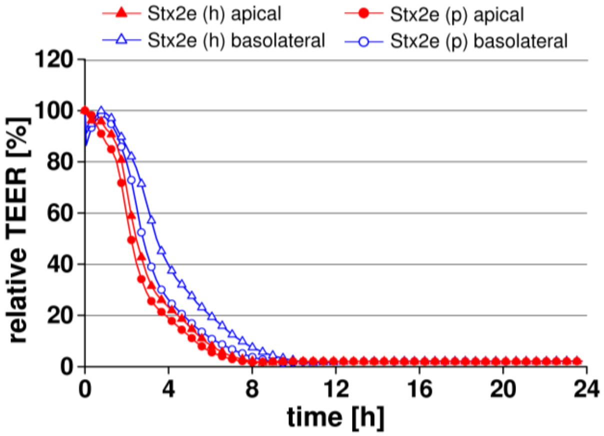




Controls



Stx2e assays



Meisen et al. (2013) *Glycobiology* 23: 745–759

Perspectives

- **investigating the functional role of different lipoforms of Stx GSL receptors**
- **unravelling the role of plasma membrane microenvironment on Stx binding and internalization**
- **defining exact molecular mechanisms by which Stxs interact with their cellular targets**

Developing new strategies aimed at preventing toxin binding and internalization

New complex specific therapeutics and protective measures for EHEC-mediated diseases

Acknowledgements

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all members of the working group






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