Structure-function analysis of stomatin

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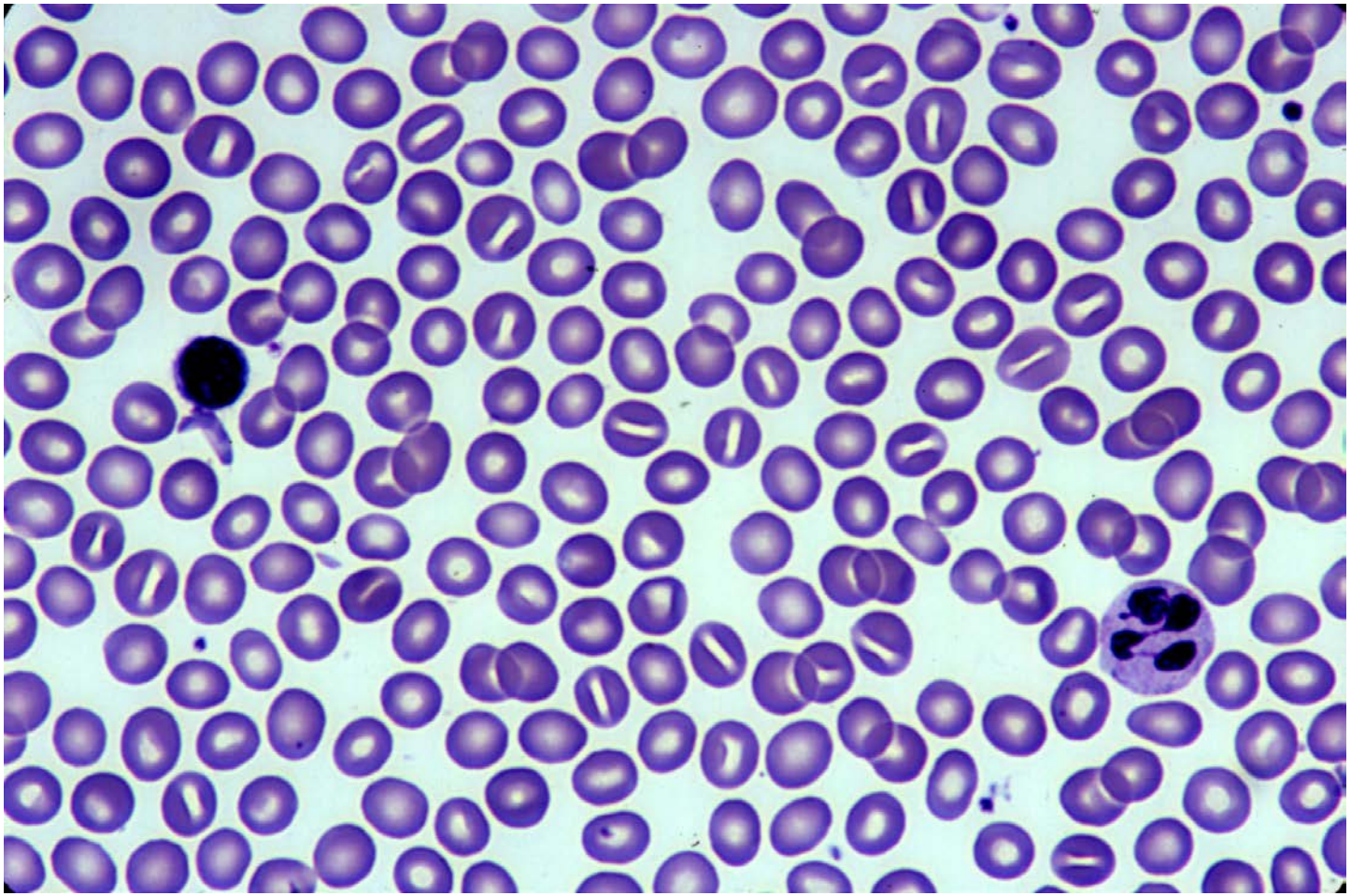
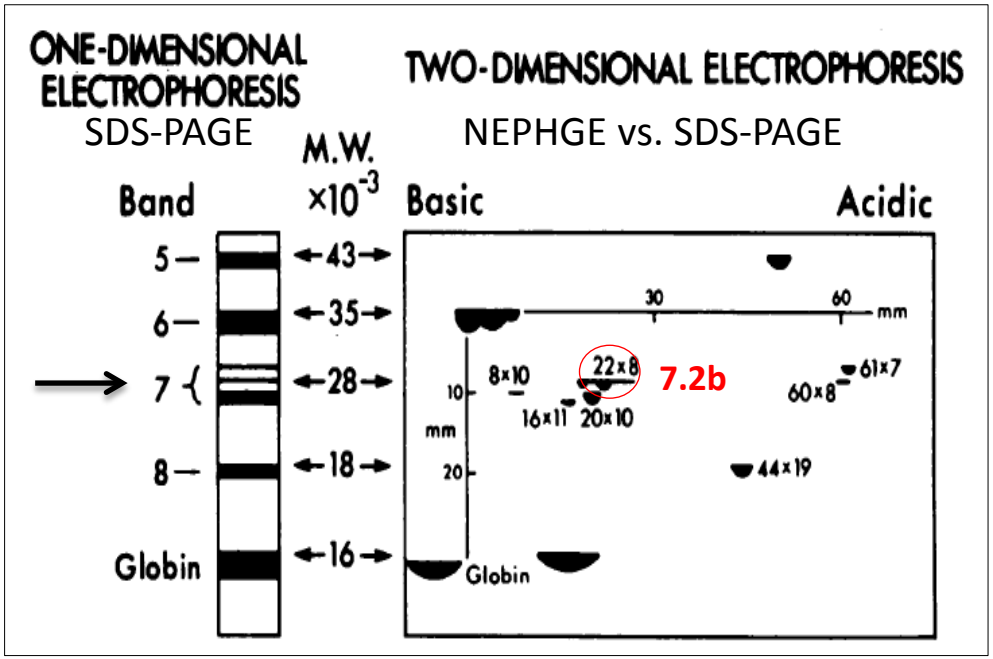
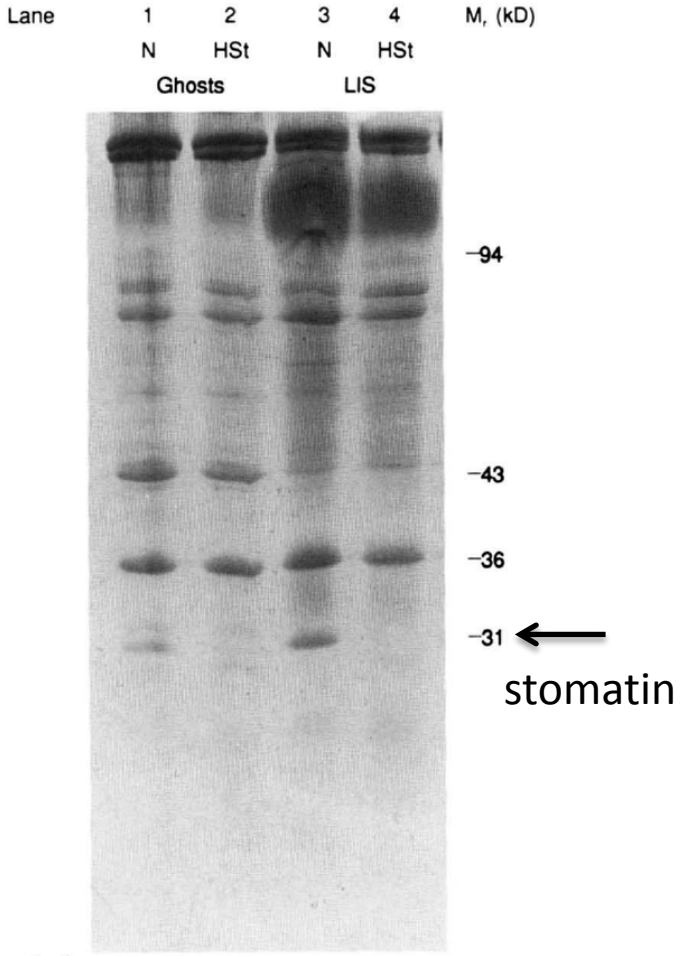
Overhydrated Hereditary Stomatocytosis



http://emedicine.medscape.com/article/955921-media

Missing band 7 membrane protein in OHSt

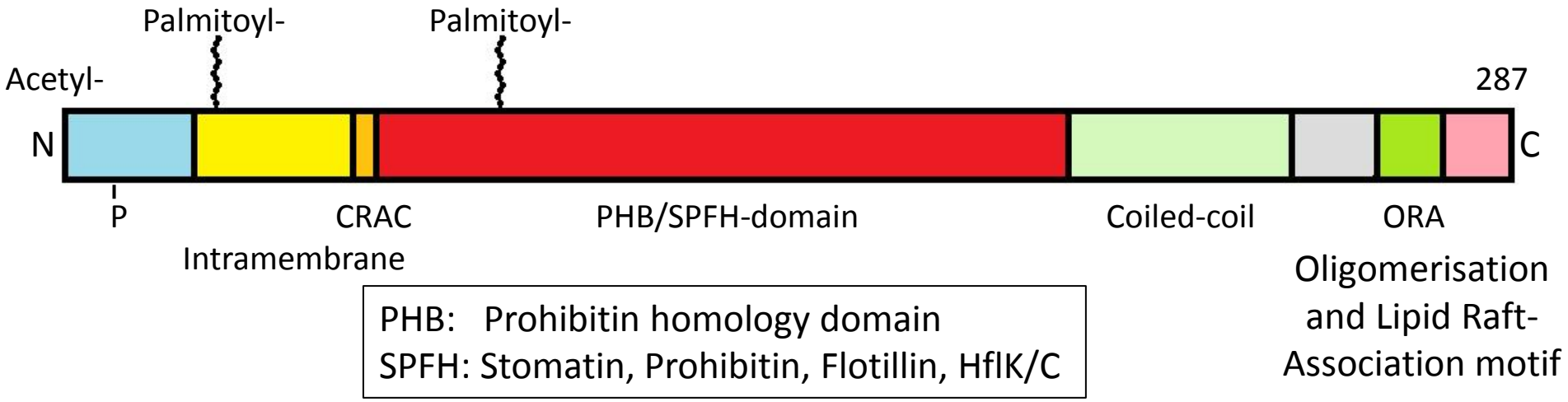




Lande et al. (1982) J.Clin.Invest. 70, 1273

Stewart et al. (1992) Blood 79, 1593

Schematic structure of stomatin

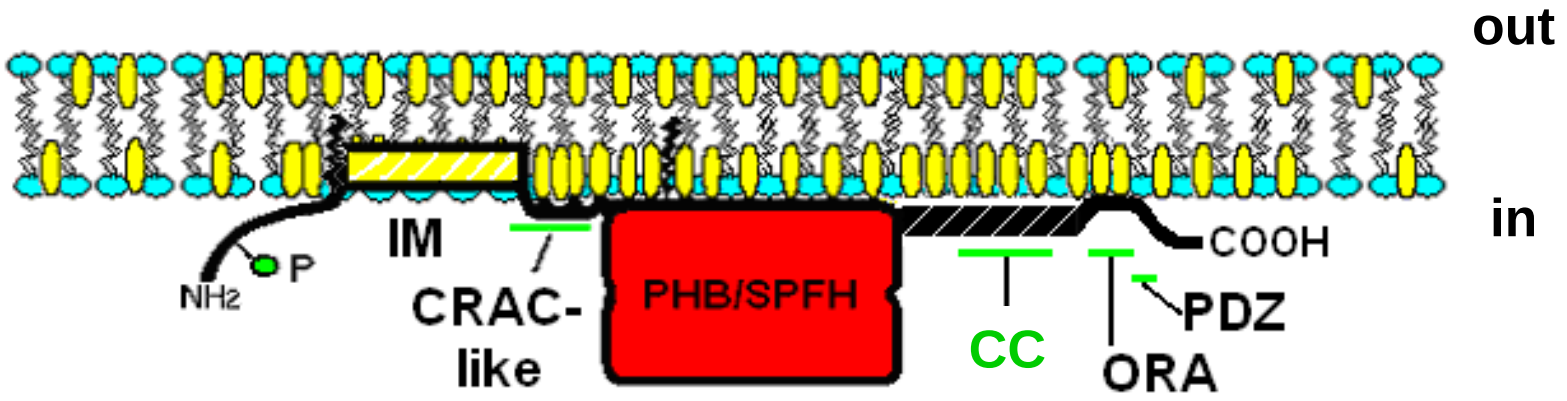


PHB: Prohibitin homology domain
SPFH: Stomatin, Prohibitin, Flotillin, HflK/C

<u>cDNA sequence:</u>
Hiebl-Dirschmied et al. (1991) BBA 1090, 123
Stewart et al. (1992) Blood 79, 1593

<u>Phosphorylation:</u>
Salzer et al. (1993) BBA 1151,149
<u>Palmitoylation:</u>
Snyers et al. (1999) FEBS Lett. 449, 101

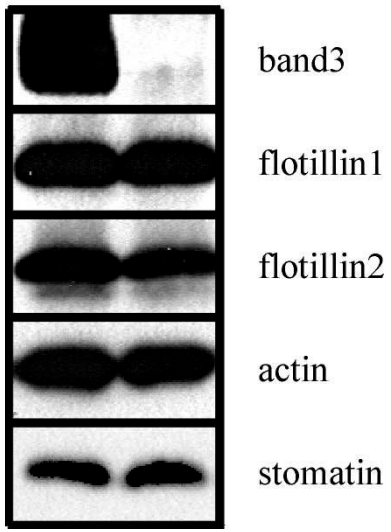
Schematic model of stomatin structure



cholesterol
phospholipid
palmitate

Intact human RBCs
+/- proteinase K

Stomatin and flotillins are monotopic membrane proteins

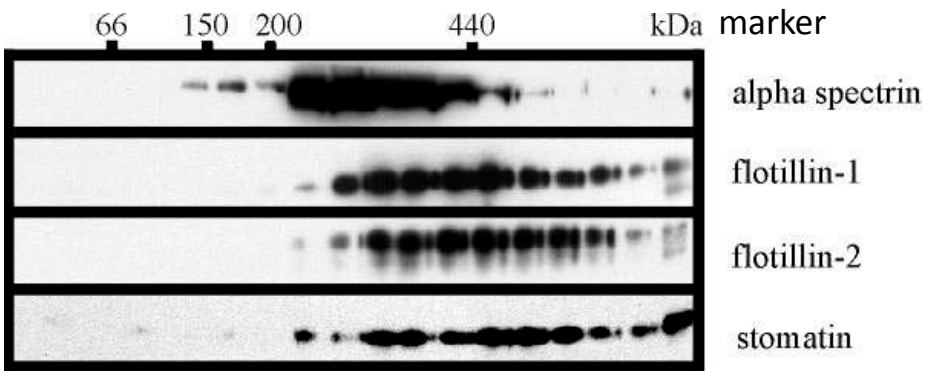


immunoblot

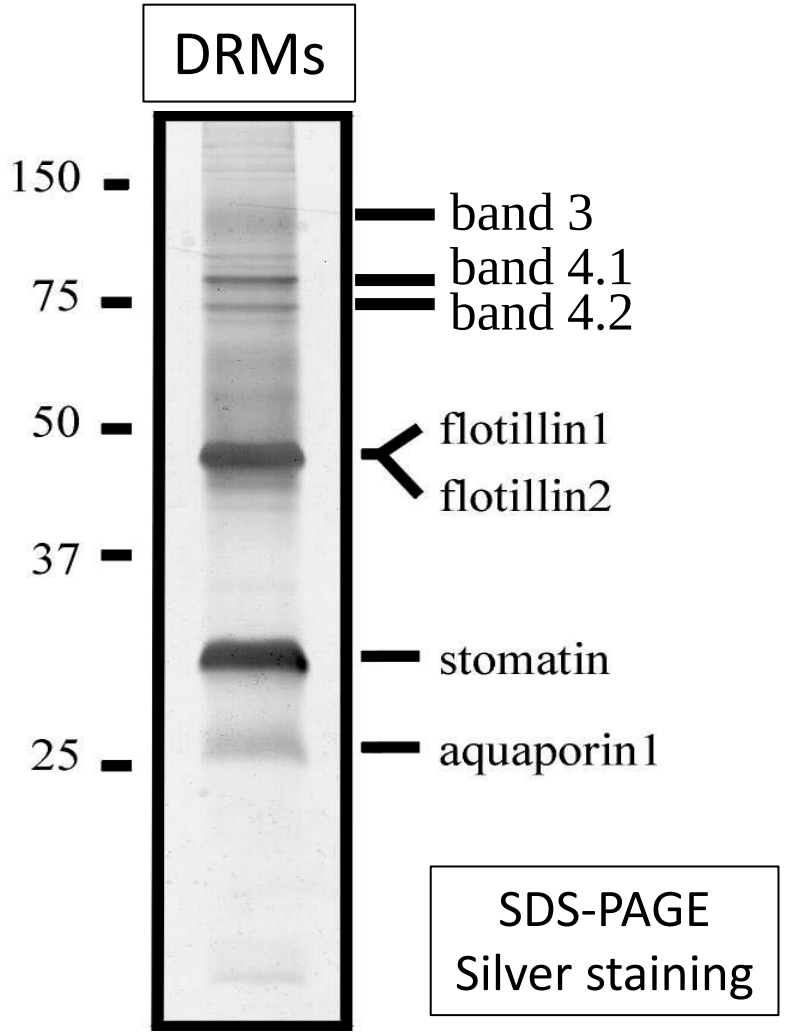
Ulrich Salzer

Stomatin and flotillins are oligomeric lipid raft (DRM) proteins

Sedimentation analysis



immunoblot



Salzer & Prohaska (2001) Blood 97,1141

Stomatin

- 31 kDa RBC integral membrane protein (band 7.2b) with a monotopic, oligomeric structure; associated with lipid rafts.
- Missing in OHSt RBCs (→ "K^+/Na^+ leak"). Regulator of an ion channel? RhAG mutation → Bruce et al. (2009) Blood 113, 1350. mRNA is present in OHSt erythroid precursors. Protein is made but degraded upon enucleation → Fricke et al. (2005) Br.J.Haematol. 131, 265.
- Widely distributed in mammalian tissues.
- Subcellular localisation: plasma membrane (apical), late endosomal compartment (MVBs, lysosomes), lipid droplets, phagosomes, neutrophil granules, platelet α-granules.
- K.o. mouse does not show stomatocytosis, nor another major phenotype → Zhu et al. (1999) Blood 93, 2404.
- Stomatin orthologues are present in all kingdoms of life.

The stomatin family

• Human stomatins

- Stomatin (*STOM,* EPB72) ►ubiquitous
- SLP1 (*STOML1*, hUNC24) ►brain, heart →LTP-D
- SLP2 (*STOML2*) ►ubiquitous
- SLP3 (*STOML3*, SRO) ►sensory neurons
- Podocin (*NPHS2*) ►kidney podocytes

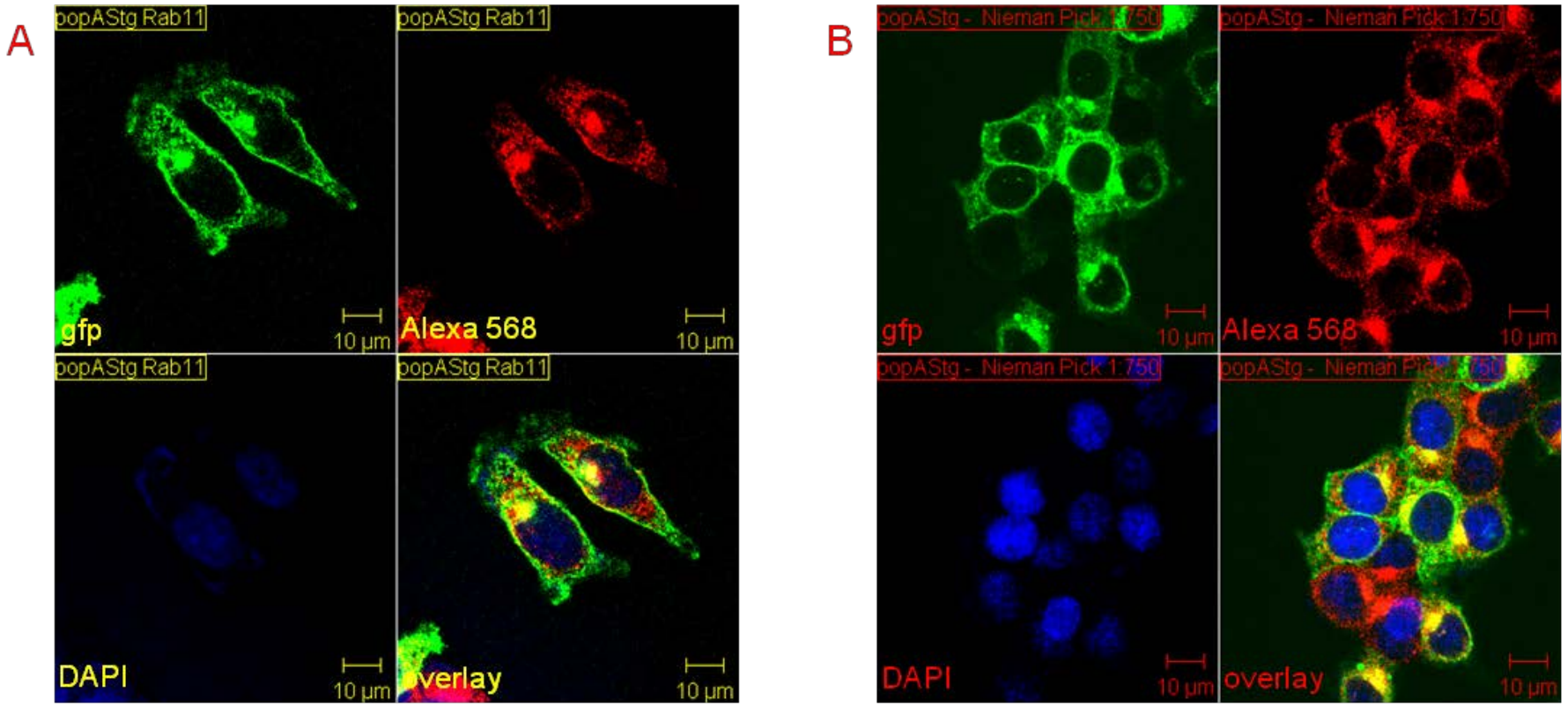
• *C. elegans* stomatins

- sto-1, sto-2, sto-3, sto-4, sto-5, sto-6, stl-1
- mec-2, unc-1, unc-24 ►neurons

Stomatin localisation to PM, recycling endosomes, and lysosomes

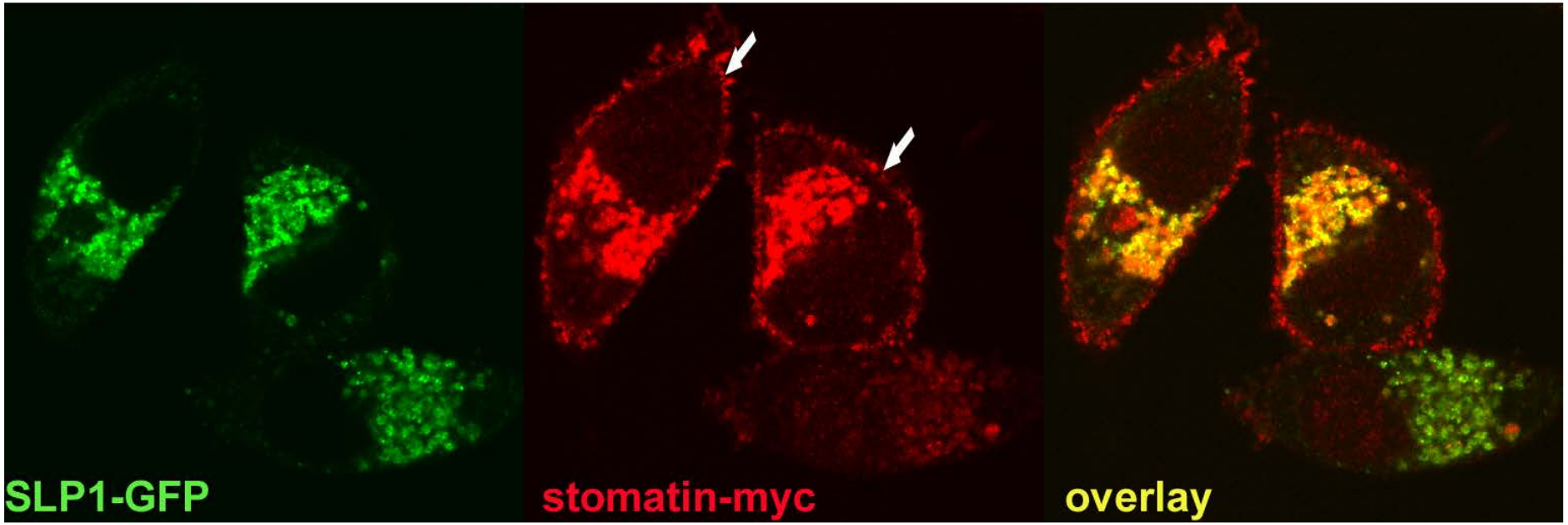
A431 carcinoma cells

Stomatin Rab11 Stomatin Nieman-Pick C1



Stefanie Rungaldier

Stomatin and SLP-1 co-localise to the late endosomal compartment



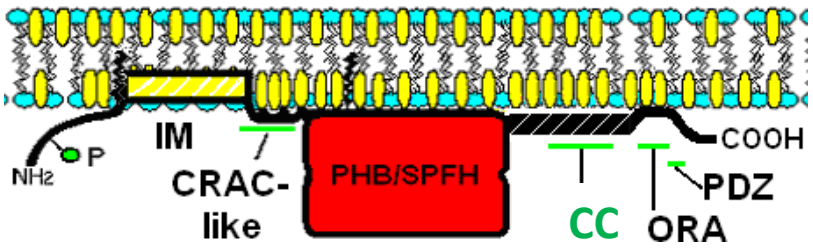
Stomatin is localised to the plasma membrane (arrows) and late endosomes of HeLa cells, whereas SLP-1 is exclusively localised to LEs

Mario Mairhofer

Localisation of stomatin mutants

(GFP-tagged)

out



in

Stefanie Rungaldier

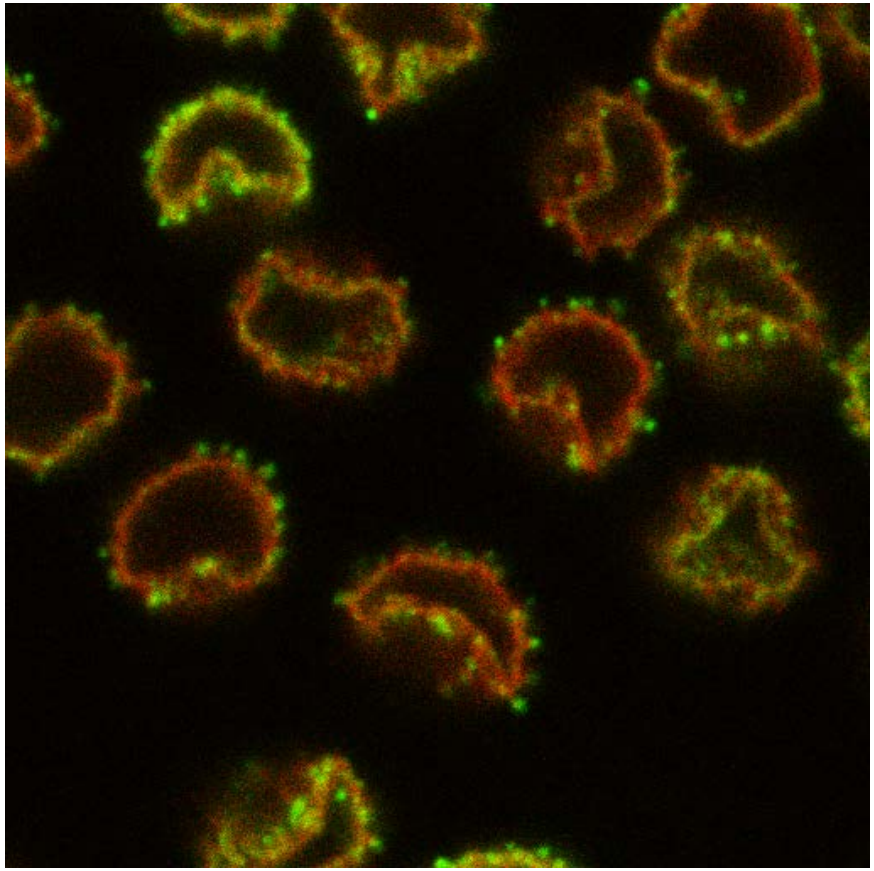
Stable transfectants in A431 carcinoma cells

	Mutation	CLSM localization	localization
Stom (1-288)	Wt	PM, LE	normal
Stom (22-288)	ΔN	PM, LE	normal
Stom (1-263)	ΔC	PM, LE	normal
Stom (C30S)	IM	PM, LE	normal
Stom (P47S)	IM	Cytoplasmic, ER	aberrant
Stom (I57A)	CRAC	PM, LE	normal
Stom (Y60A)	CRAC	Cytoplasmic	aberrant
Stom (R62A)	CRAC	PM, LE	normal
Stom (C87S)	PHB	Cytoplasmic	aberrant
Stom (D89A)	PHB	Cytoplasmic	aberrant
Stom (F91A)	PHB	PM, LE	normal
Stom (R97A)	PHB	Cytoplasmic	aberrant
Stom (K198A)	PHB	LE, cytoplasmic	aberrant
Stom (P200A)	CC	PM, LE, cytoplasmic	aberrant
Stom (Δ204-241)	ΔCC	PM, LE	normal
Stom (P245A)	CC	PM, LE, cytoplasmic	aberrant
Stom (F269A)	ORA	PM, LE	normal
Stom (P270A)	ORA	LE, cytoplasmic	aberrant

Stomatin in exovesicles

Ca^{2+}-induced vesiculation of red cells

actin
(TRITC-phalloidin)
stomatin
(mcAb GARP-50)

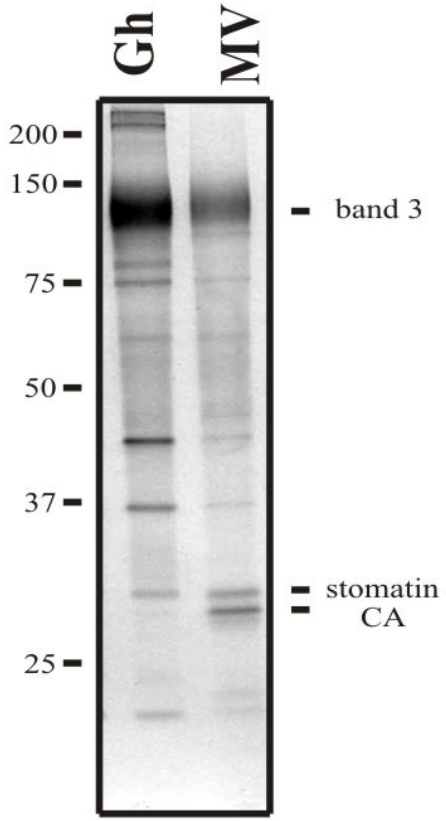


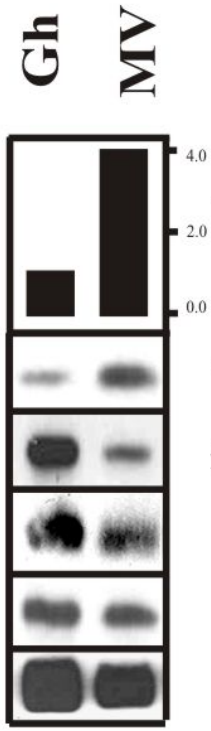
RBCs treated with Ca^{2+} and ionophore A23187

Ulrich Salzer

Segregation of stomatin and flotillins upon (micro-)vesiculation

Gh: Ghosts
MV: Microvesicles

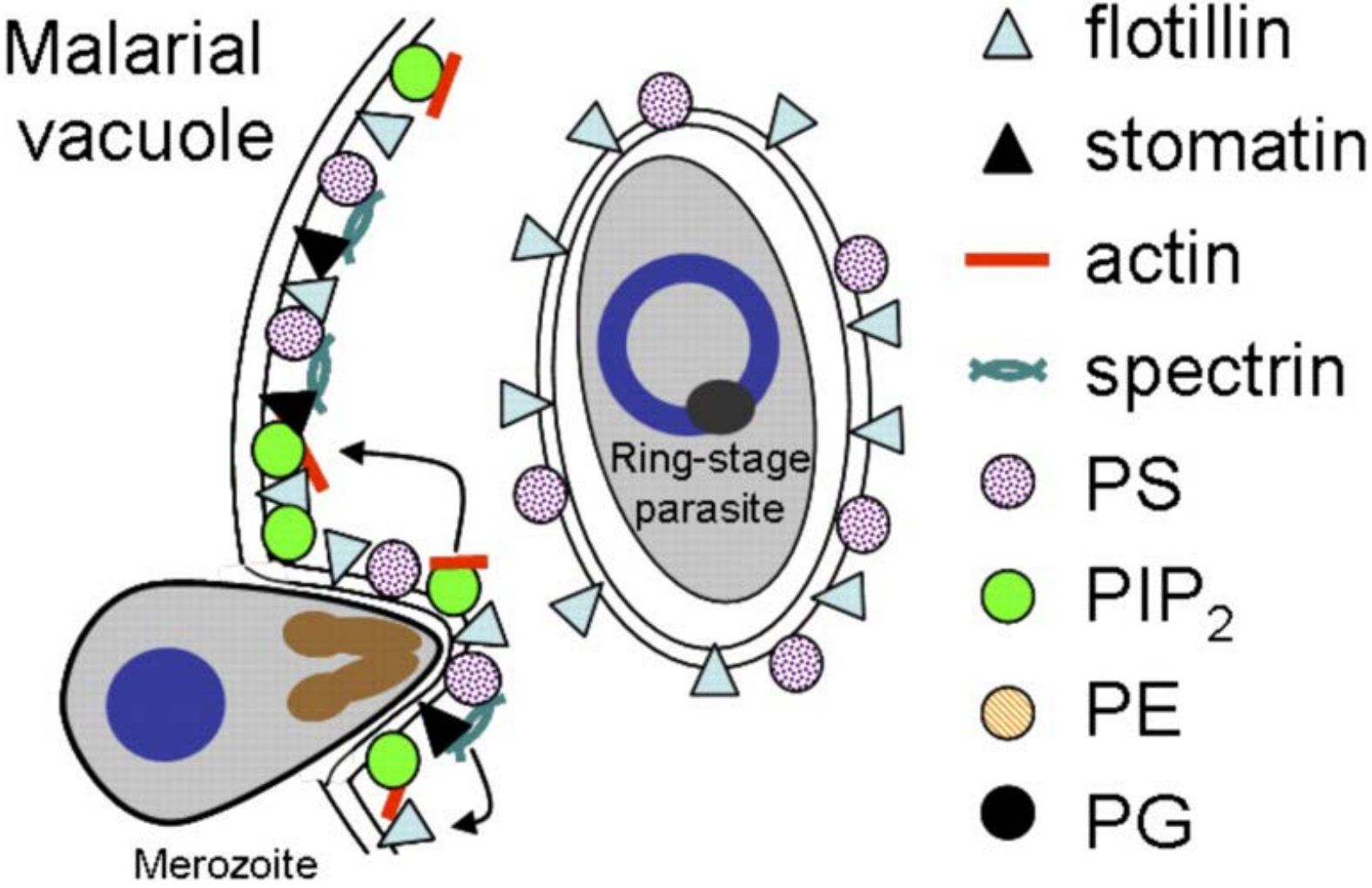




Protein Ratio **MV/Gh**
4.0
2.0
0.2
0.4
0.5
0.4

Ulrich Salzer

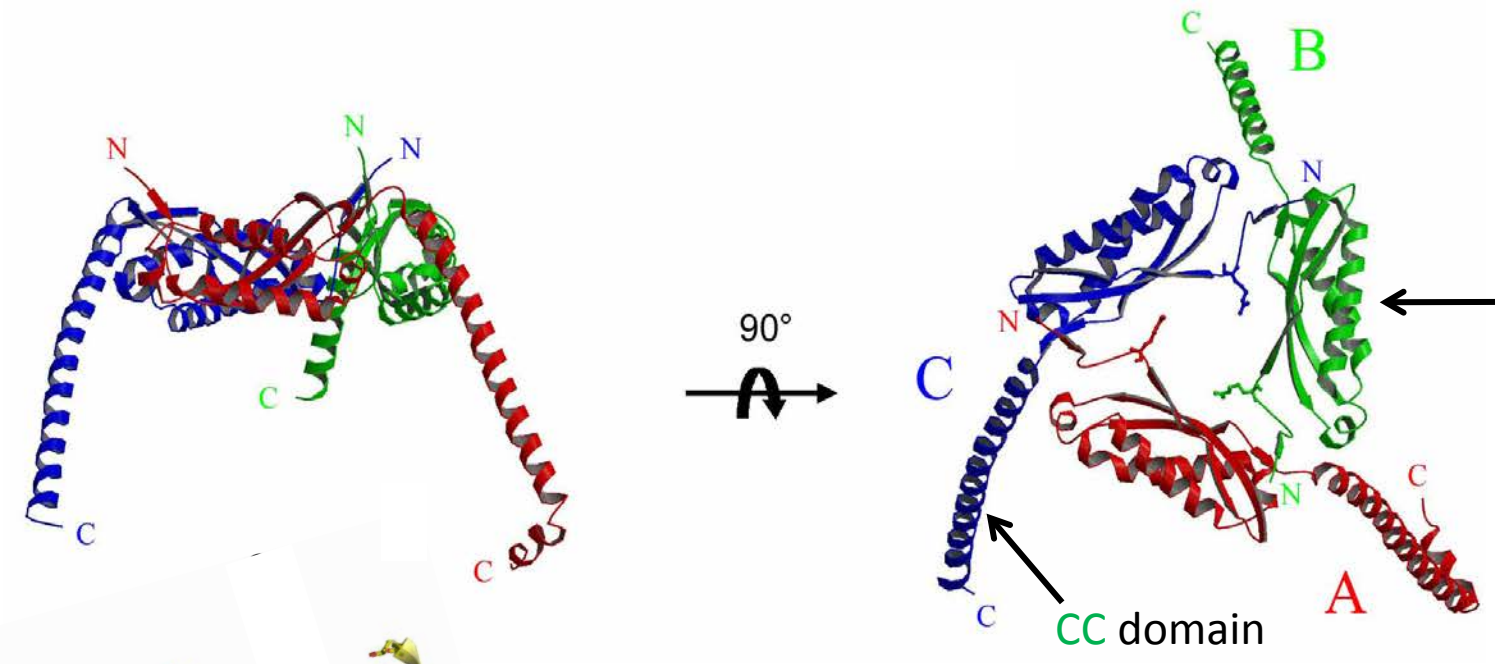
Model of lipid and protein uptake into the malarial vacuole

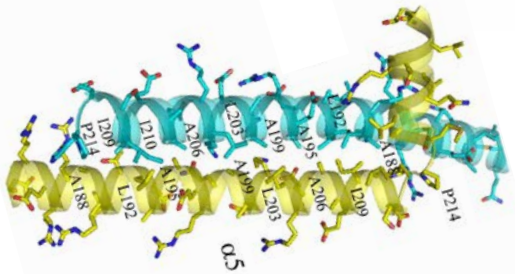


Murphy et al. (2007) Blood 110, 2132

Structural considerations

The crystal structure of the *P. horikoshii* stomatin core domain

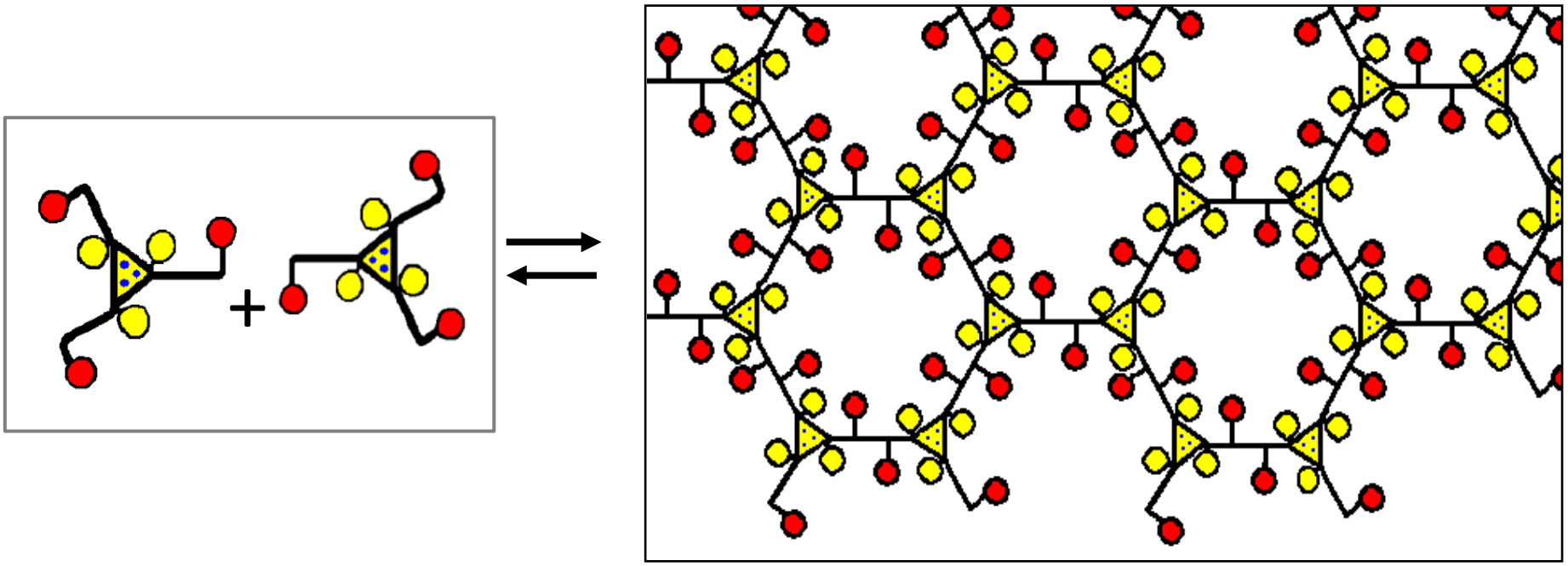




Anti-parallel coiled-coil interaction

Yokoyama et al. (2008) J.Mol.Biol. 376, 868

Oligomerisation?
Hypothetical model of stomatin multimers



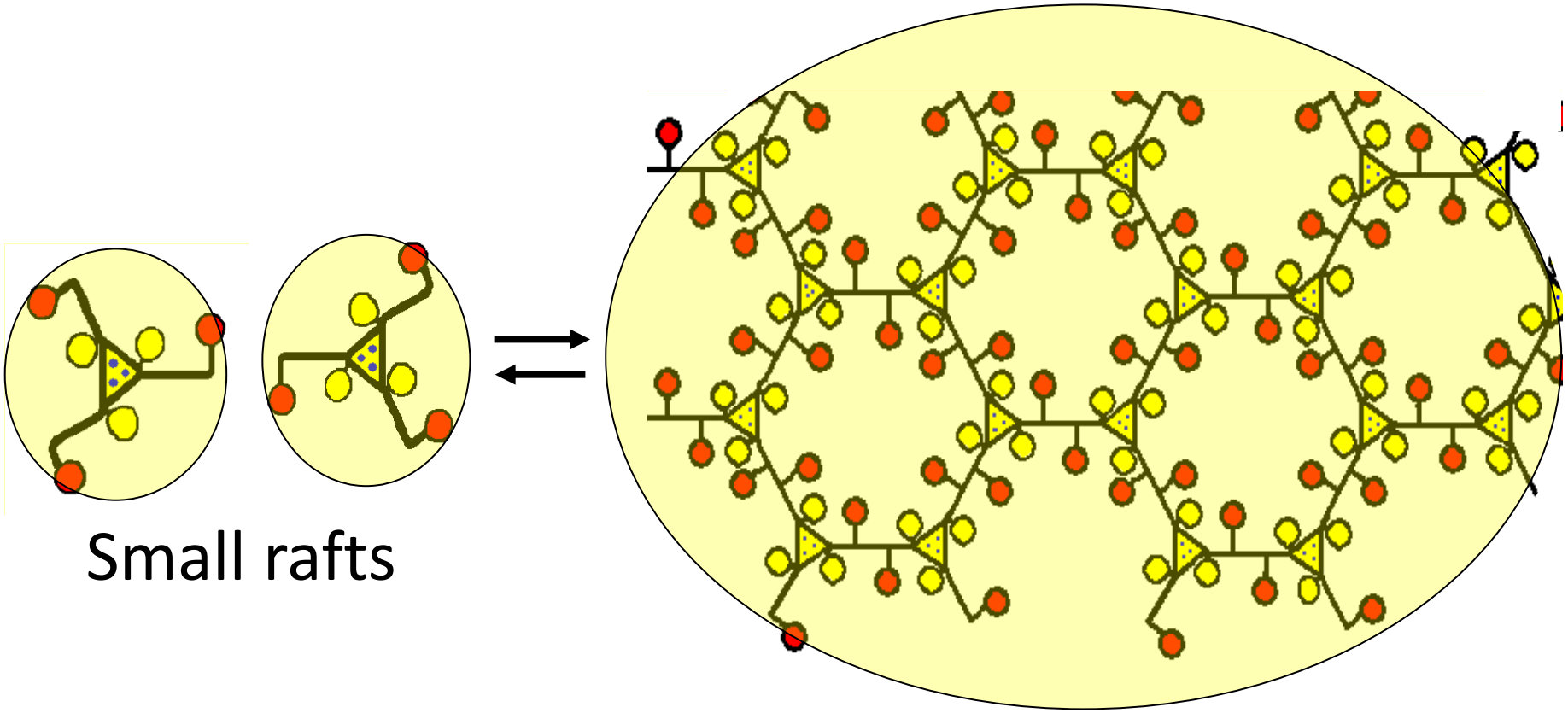
… N-terminal IM domain

… C-terminal ORA/PDZ domain

… PHB domains triangular platform

… kinked α-helices

Lipid raft association?
Model of stomatin-raft aggregation



Small rafts

Aggregated raft

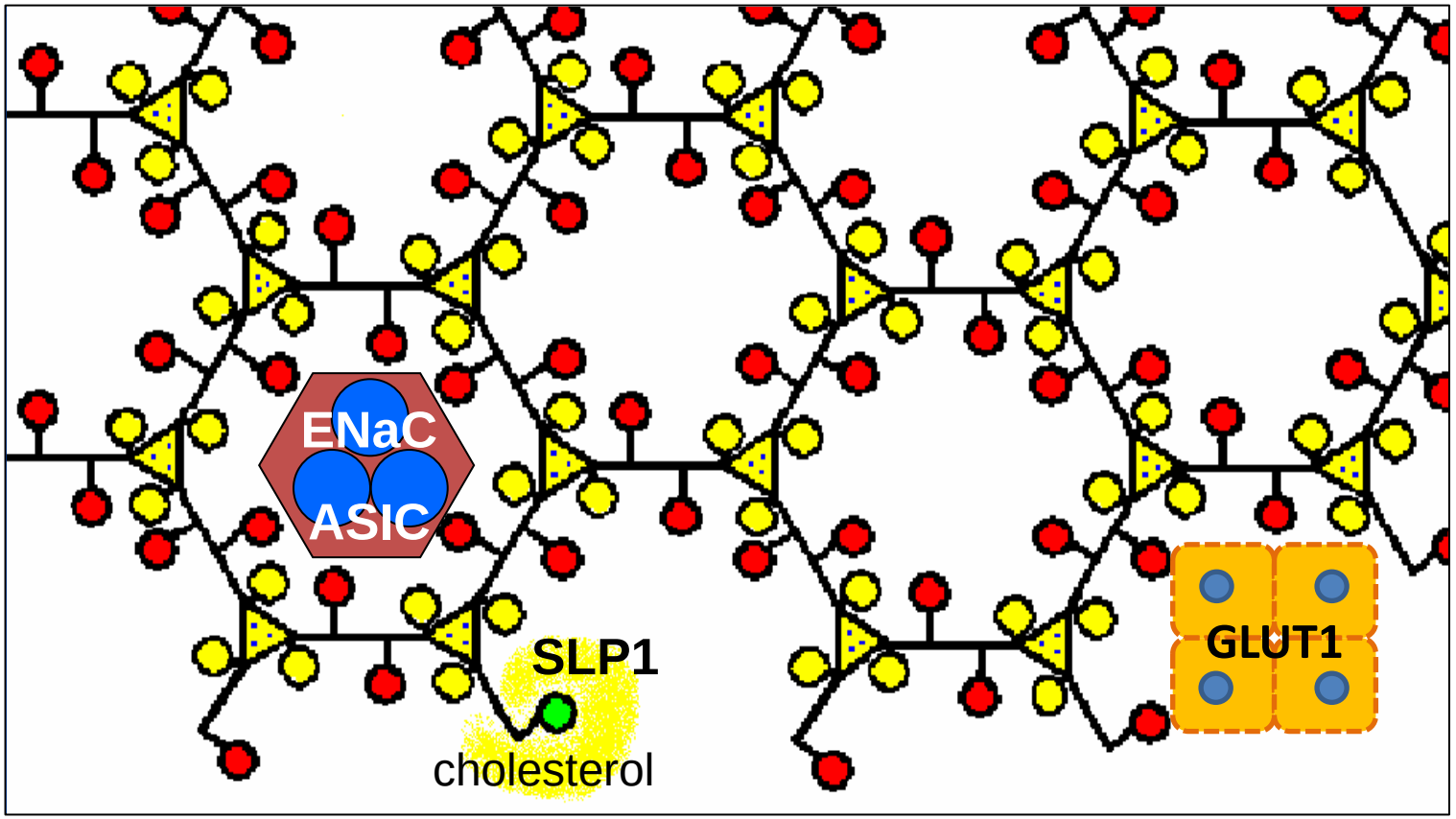
→ Scaffolding platform?

Stomatins interact with transporters and channels

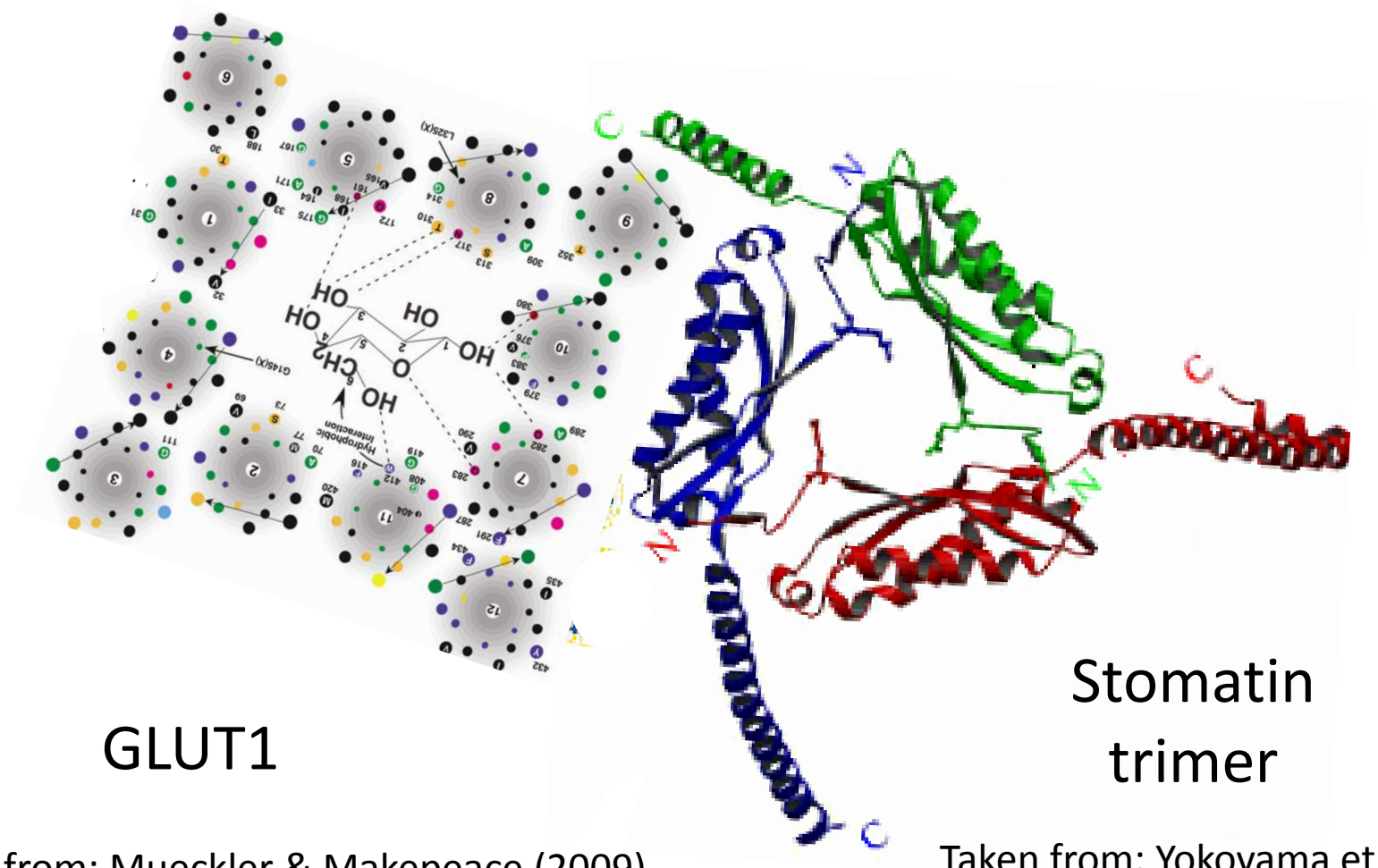
- MEC-2 / ENaC (Epithelial sodium channels)
- Stomatin / ASICs (Acid sensing ion channels)
- Podocin / TRPC6 (Transient receptor potential channel 6)
- UNC-1 / UNC-9 (Innexin gap junction)
- Stomatin / Pannexin-1 (gap junction)
- Stomatin / GLUT1 (Glucose and DHA transporter)

→ Integral scaffolding proteins?

Hypothetical model of transporter- or channel-stomatin-complexes



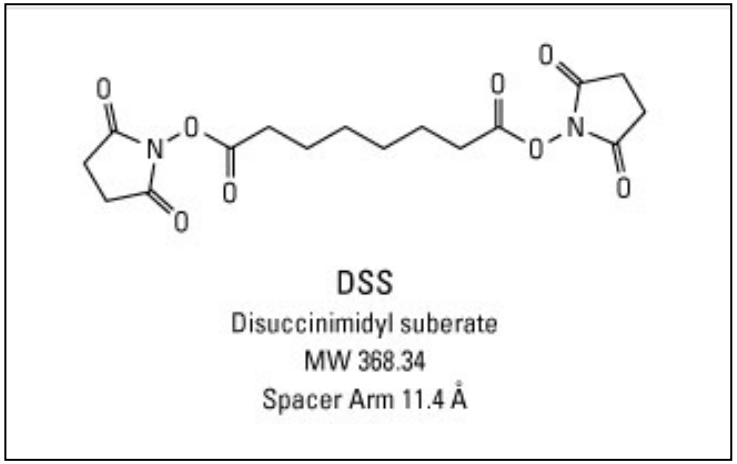
Hypothetical model of lateral GLUT1-stomatin interaction



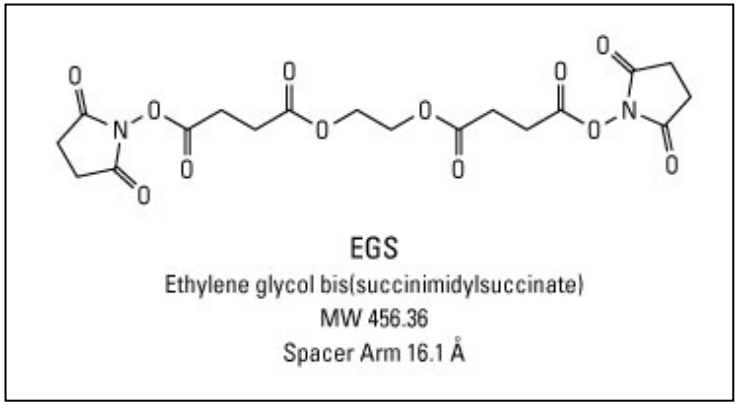
Taken from: Mueckler & Makepeace (2009) Biochem. 48, 5934

Taken from: Yokoyama et al. (2008) J.Mol.Biol. 376, 868

Crosslinking of RBC membrane proteins

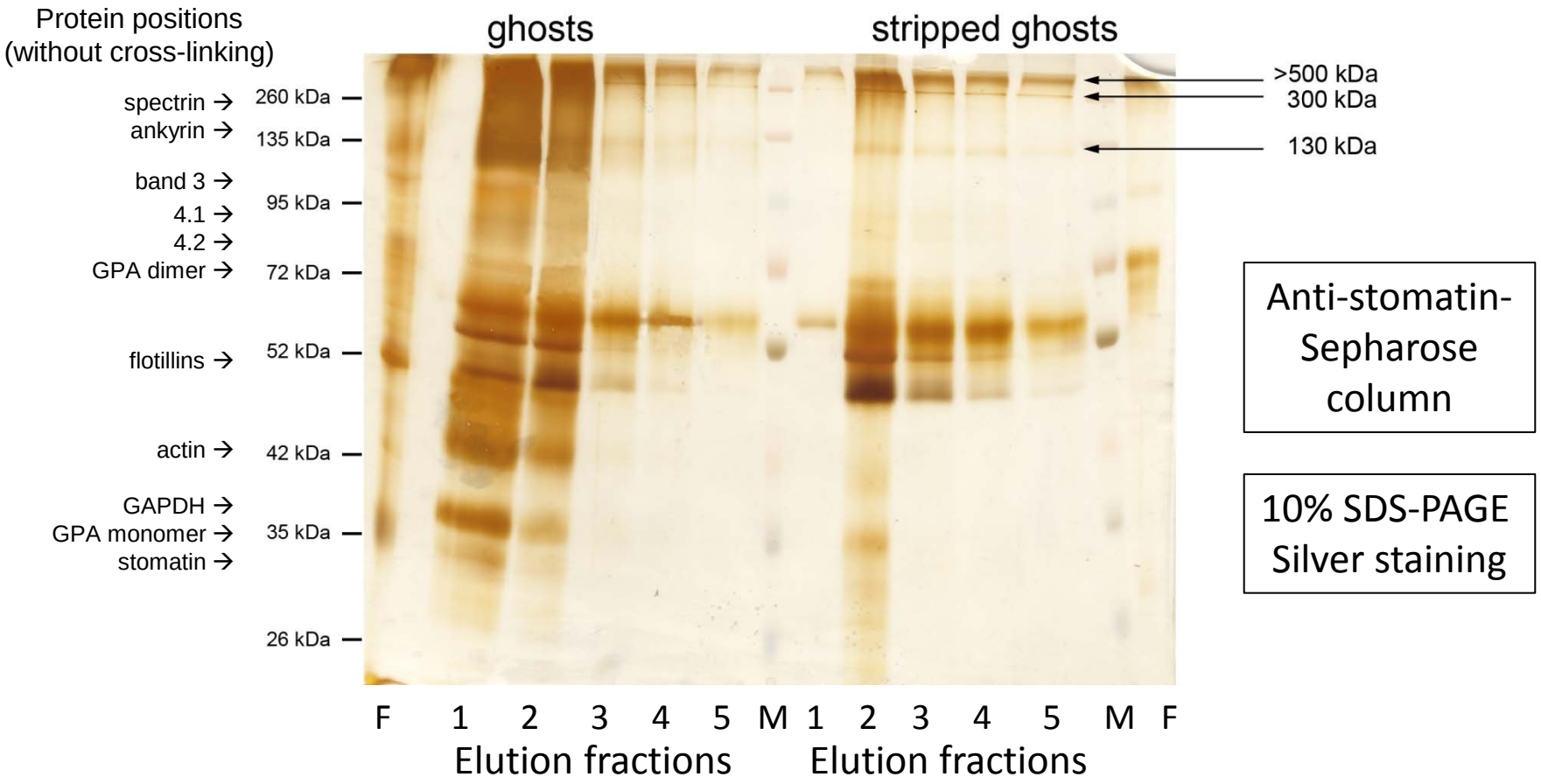


DSS
Disuccinimidyl suberate
MW 368.34
Spacer Arm 11.4 Å



EGS
Ethylene glycol bis(succinimidylsuccinate)
MW 456.36
Spacer Arm 16.1 Å

Isolation of EGS-cross-linked stomatin-complexes

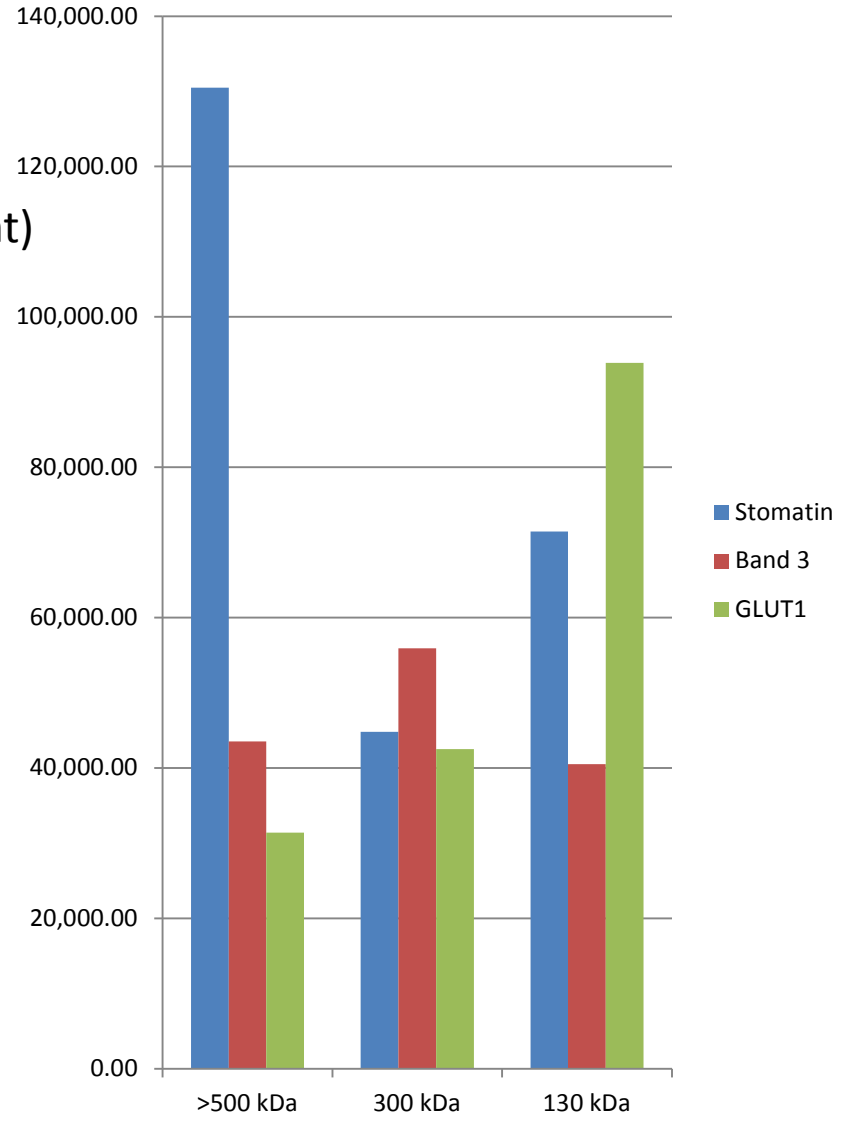


Rungaldier et al. (2013) BBA-Mem. 1828, 956

Semiquantitative MS/MS analysis of EGS-cross-linked stomatin complexes

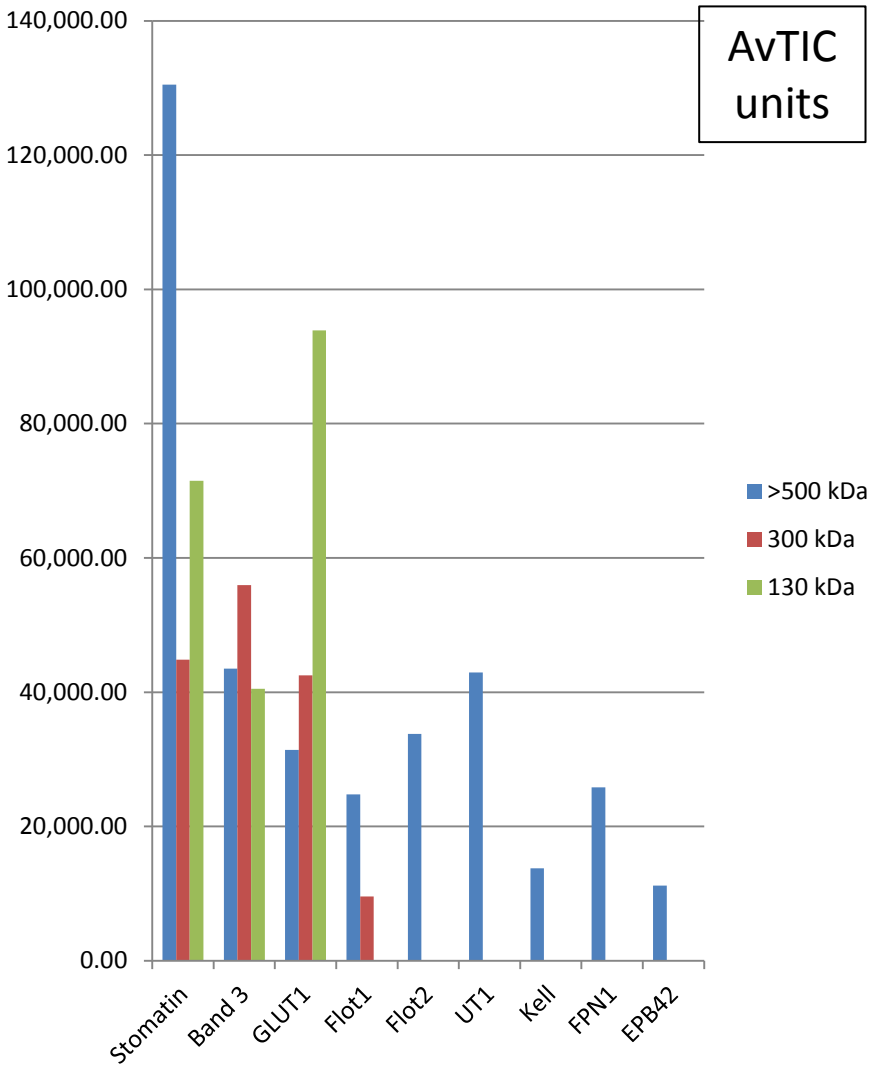
AvTIC units

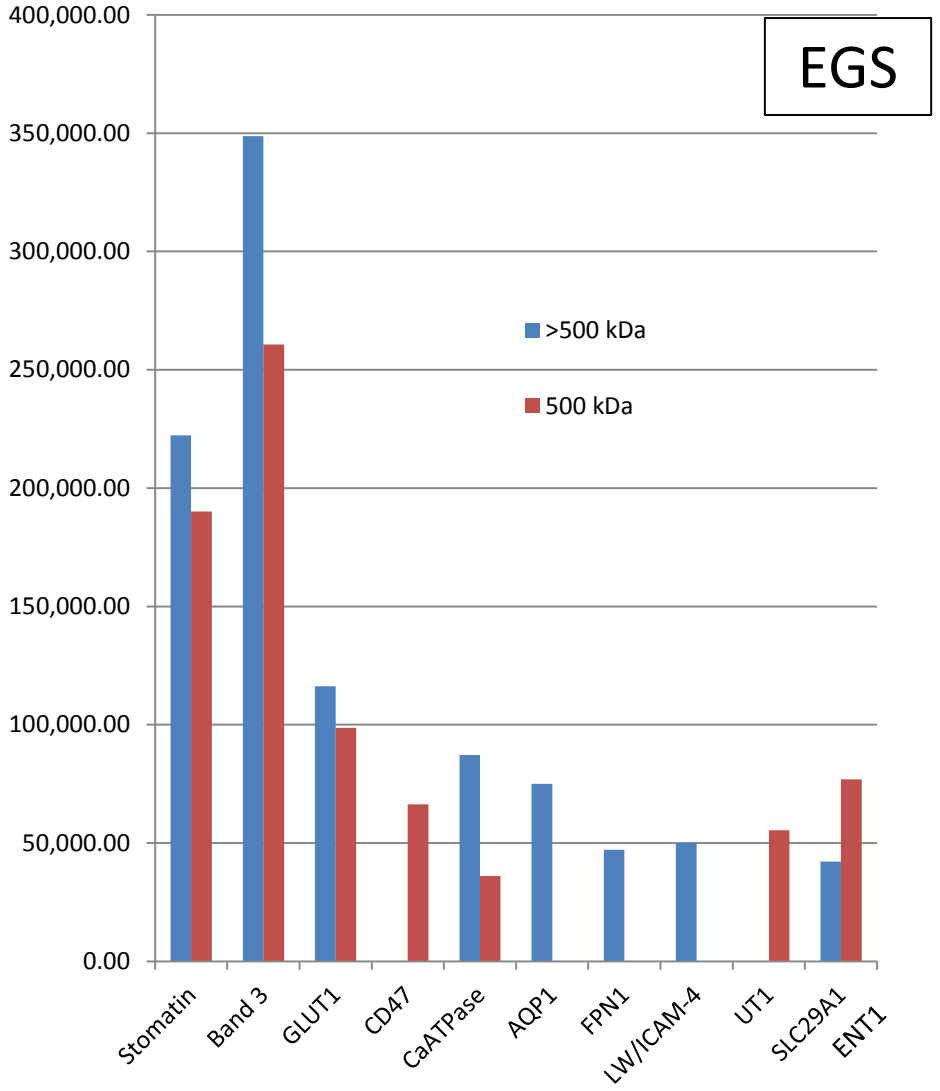
(Average Total Ion Current)



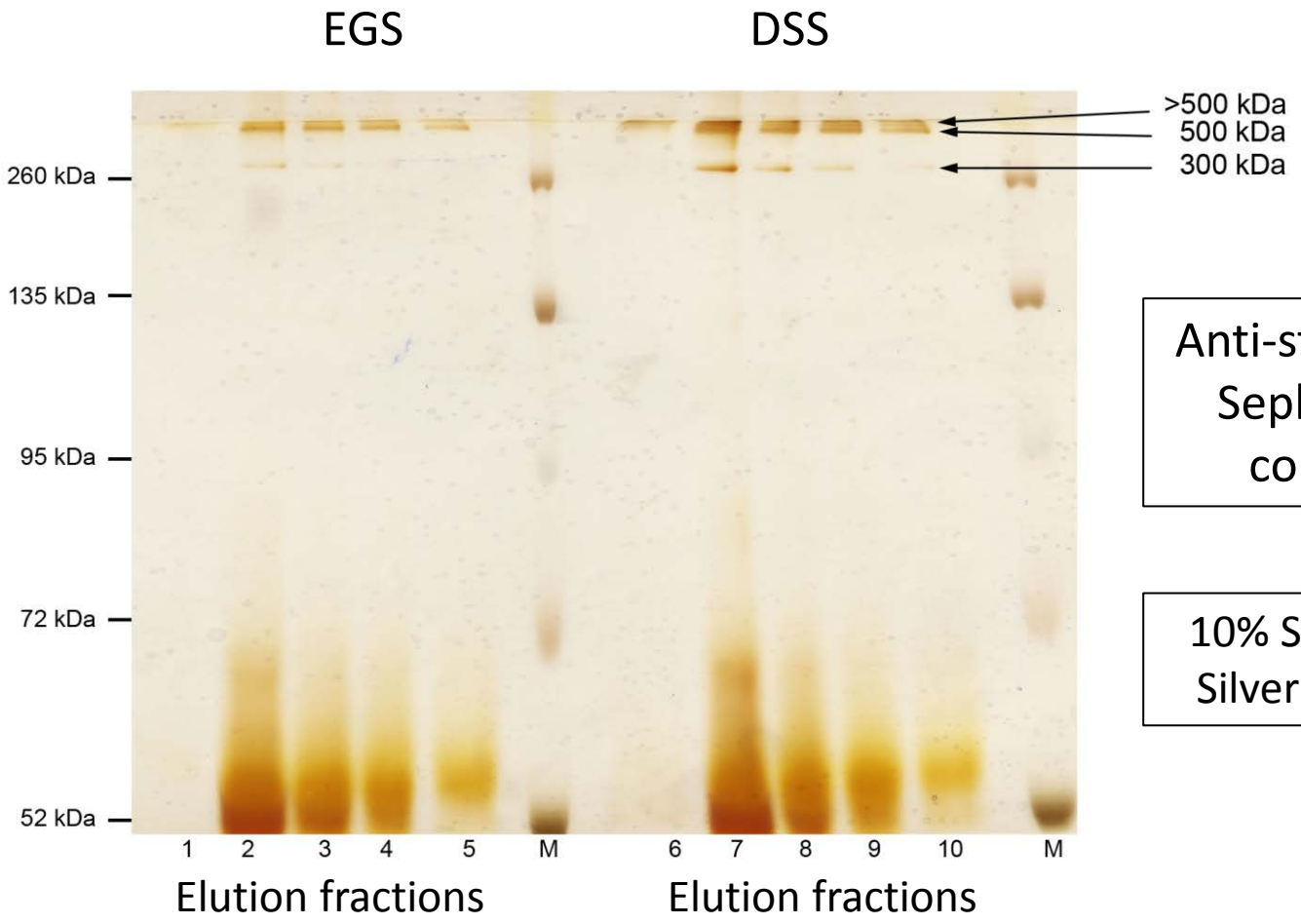
EGS

MS/MS identification of EGS-cross-linked stomatin-associated proteins





Isolation of DSS-cross-linked stomatin-complexes



Anti-stomatin-Sepharose column

10% SDS-PAGE
Silver staining

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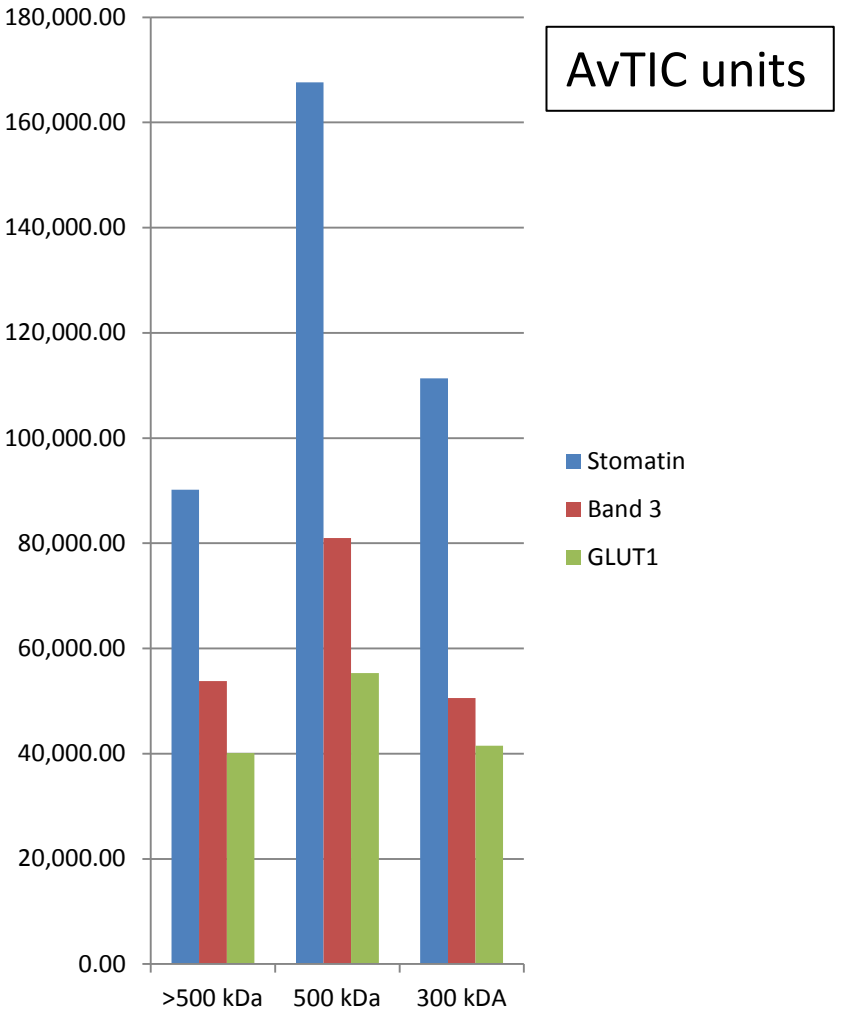
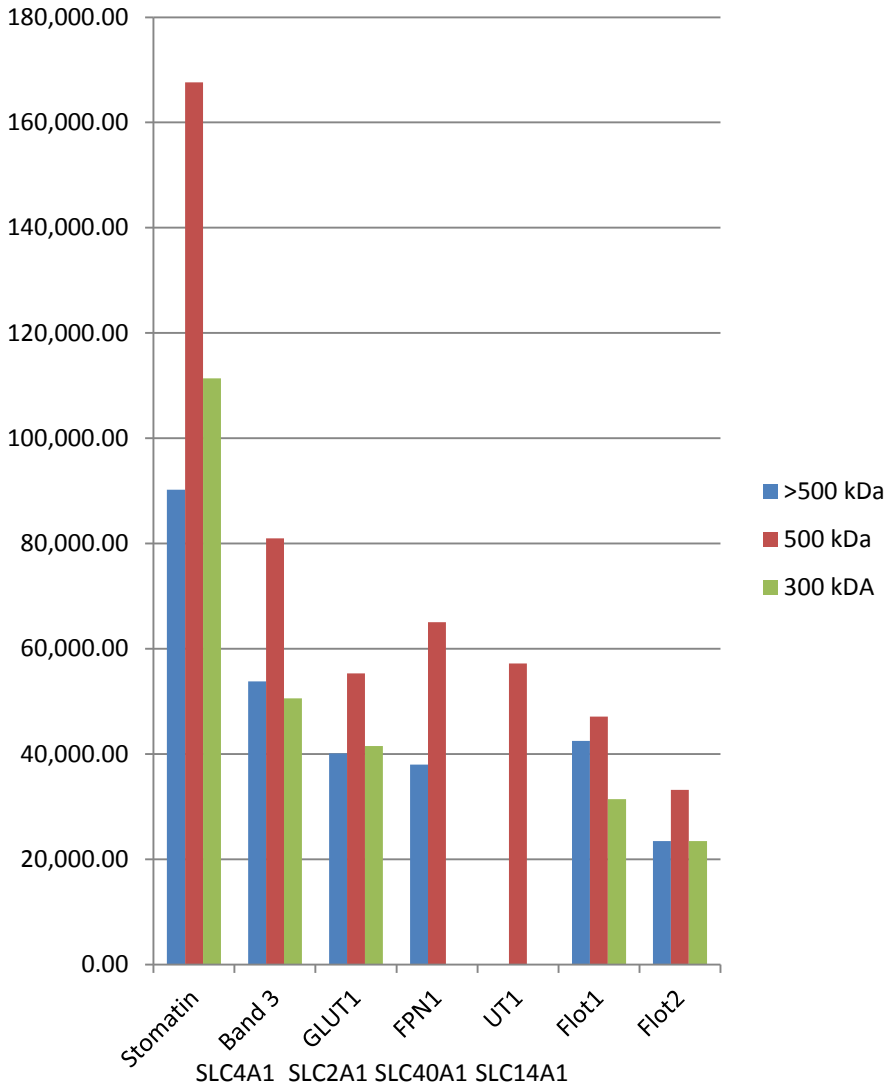
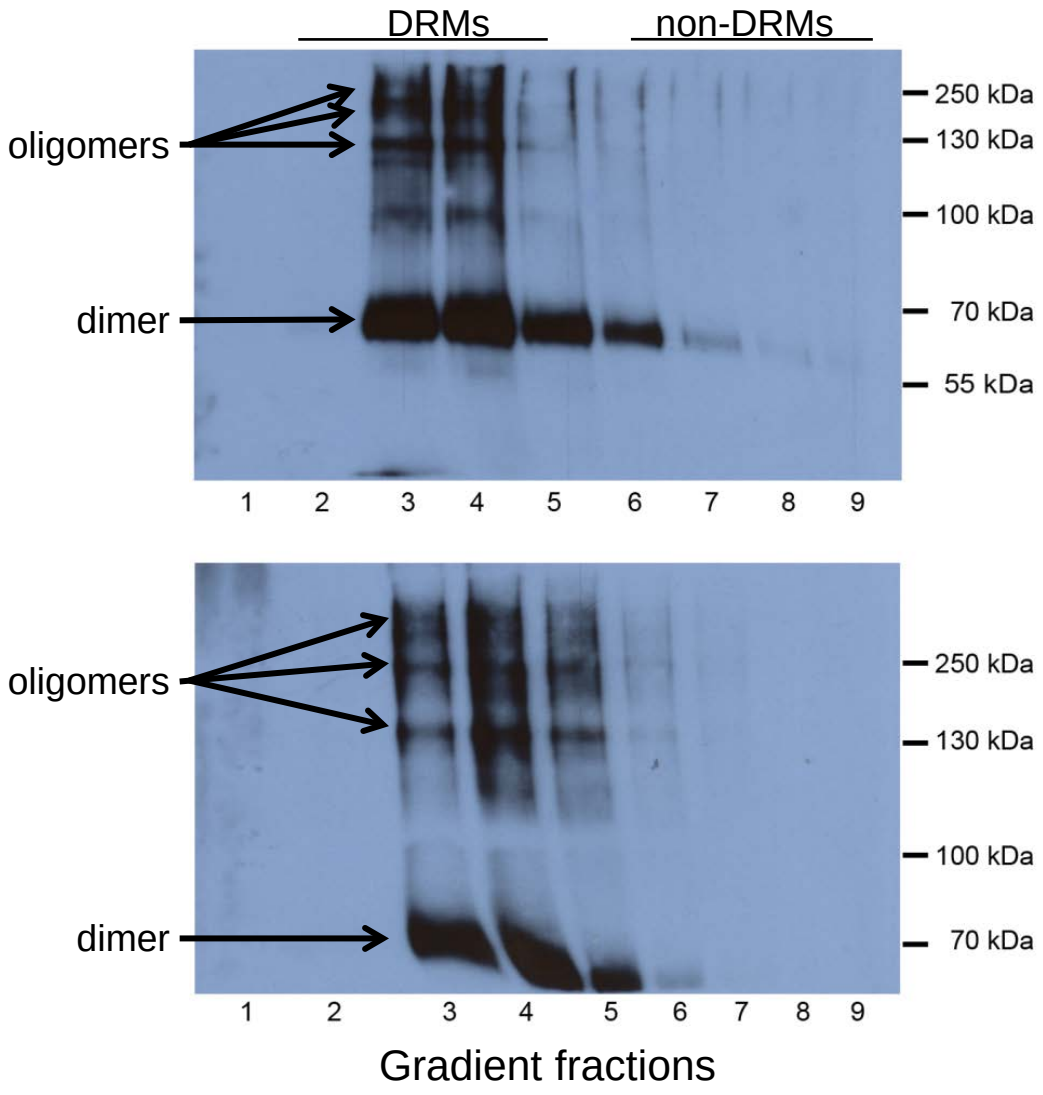
MS/MS analysis of DSS-cross-linked stomatin complexes





DRMs from cross-linked membranes



EGS

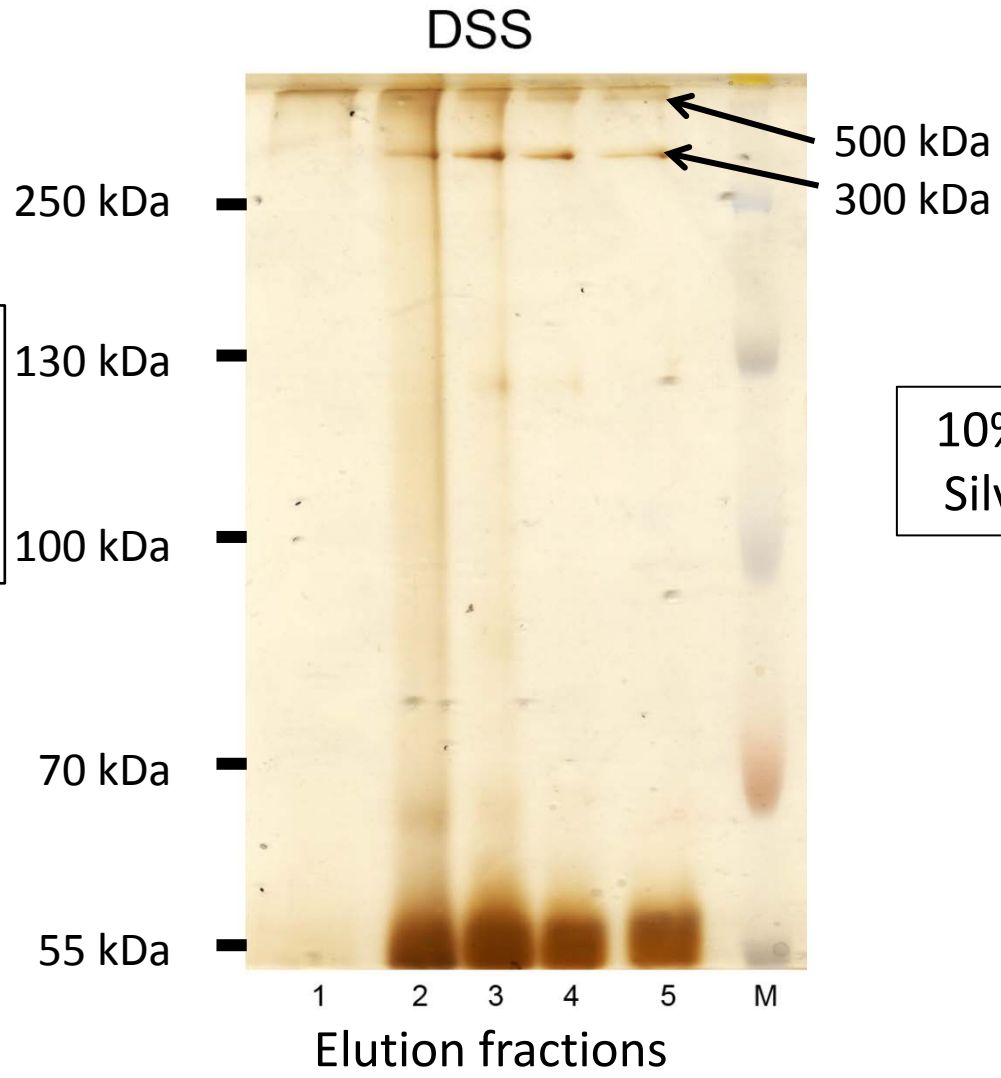
WB: stomatin

DSS

Sucrose density gradient ultracentrifugation

Isolation of DSS-cross-linked stomatin-complexes from DRMs

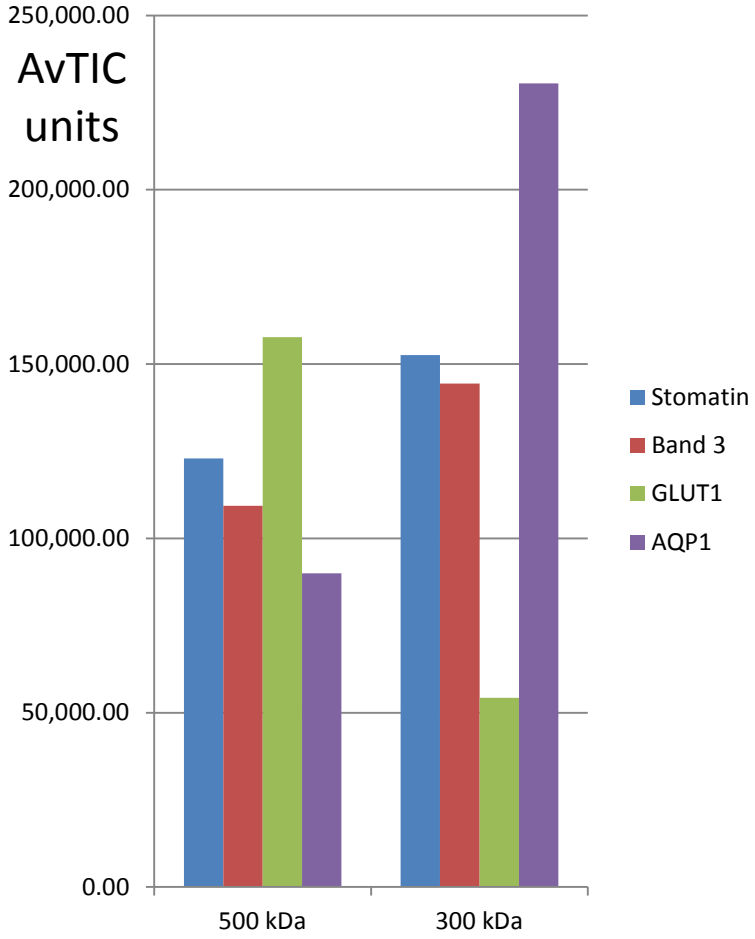
Immunoaffinity purification by anti-stomatin-Sepharose

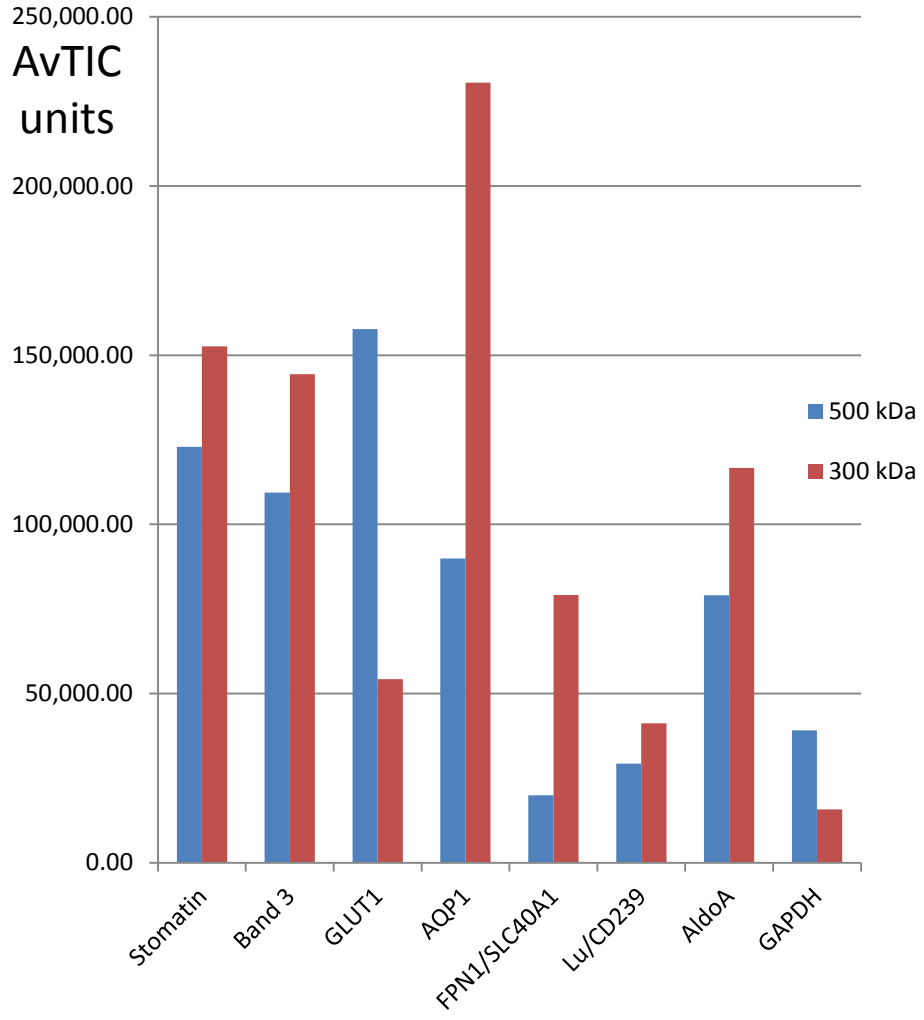


10% SDS-PAGE
Silver staining

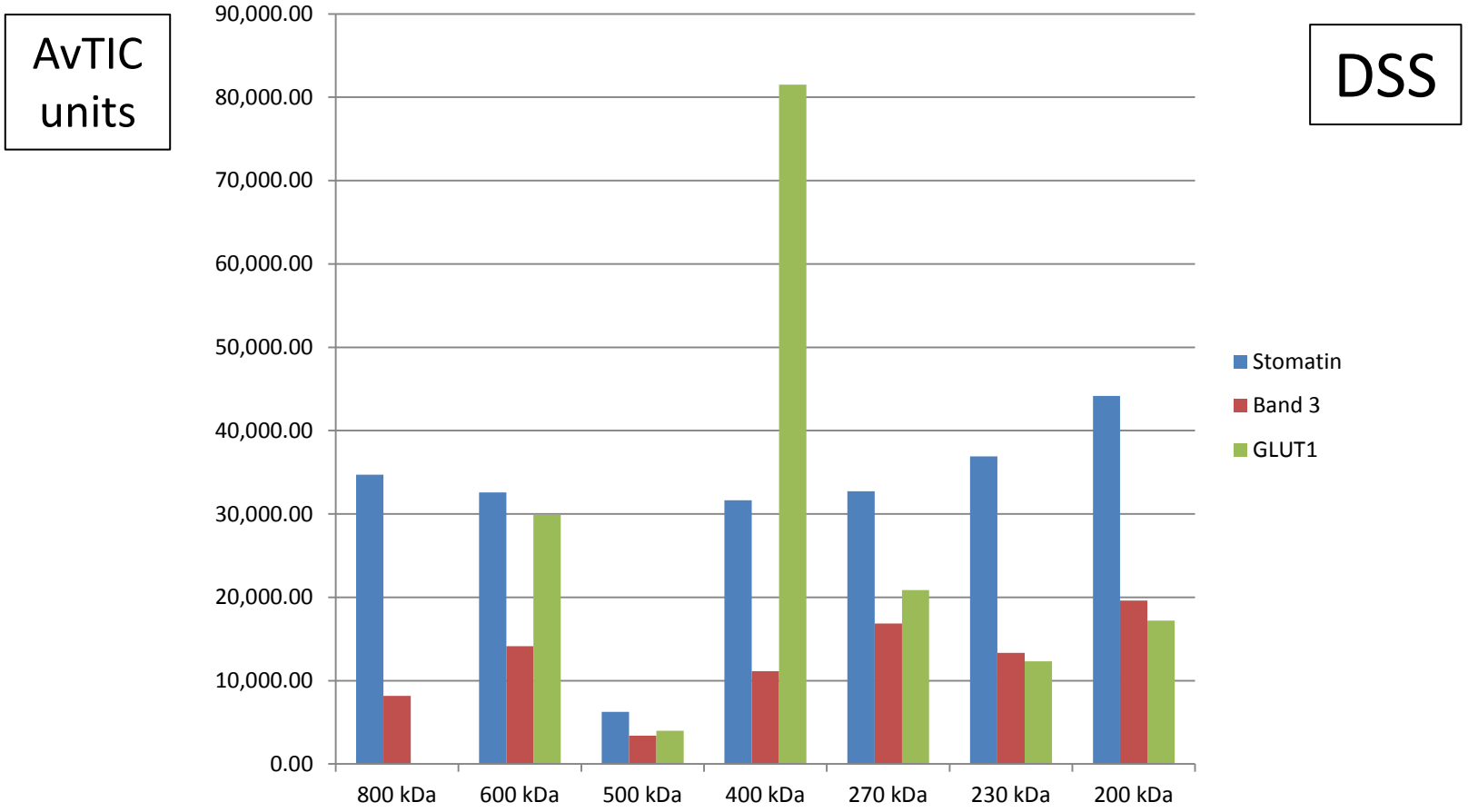
MS/MS-analysis of DSS-cross-linked stomatin-complexes from DRMs

Band 3, GLUT1, Aquaporin-1, Ferroportin-1, Lutheran-BG-GP/laminin receptor,
Aldolase A, Glyceraldehyde-3-phosphate dehydrogenase





MS/MS analysis of high-molecular stomatin-complexes from DRMs



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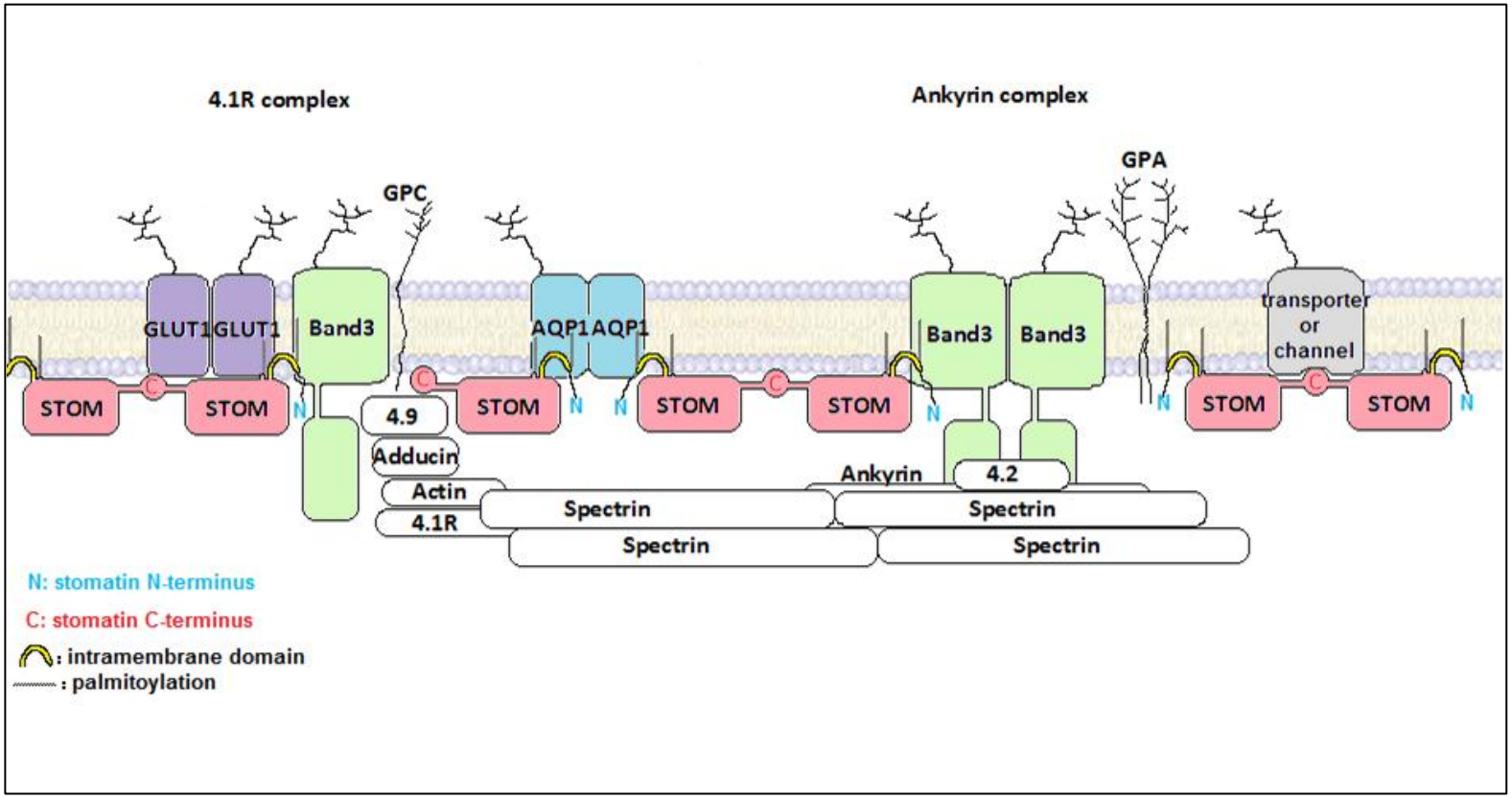
Hypothetical model of the RBC membrane



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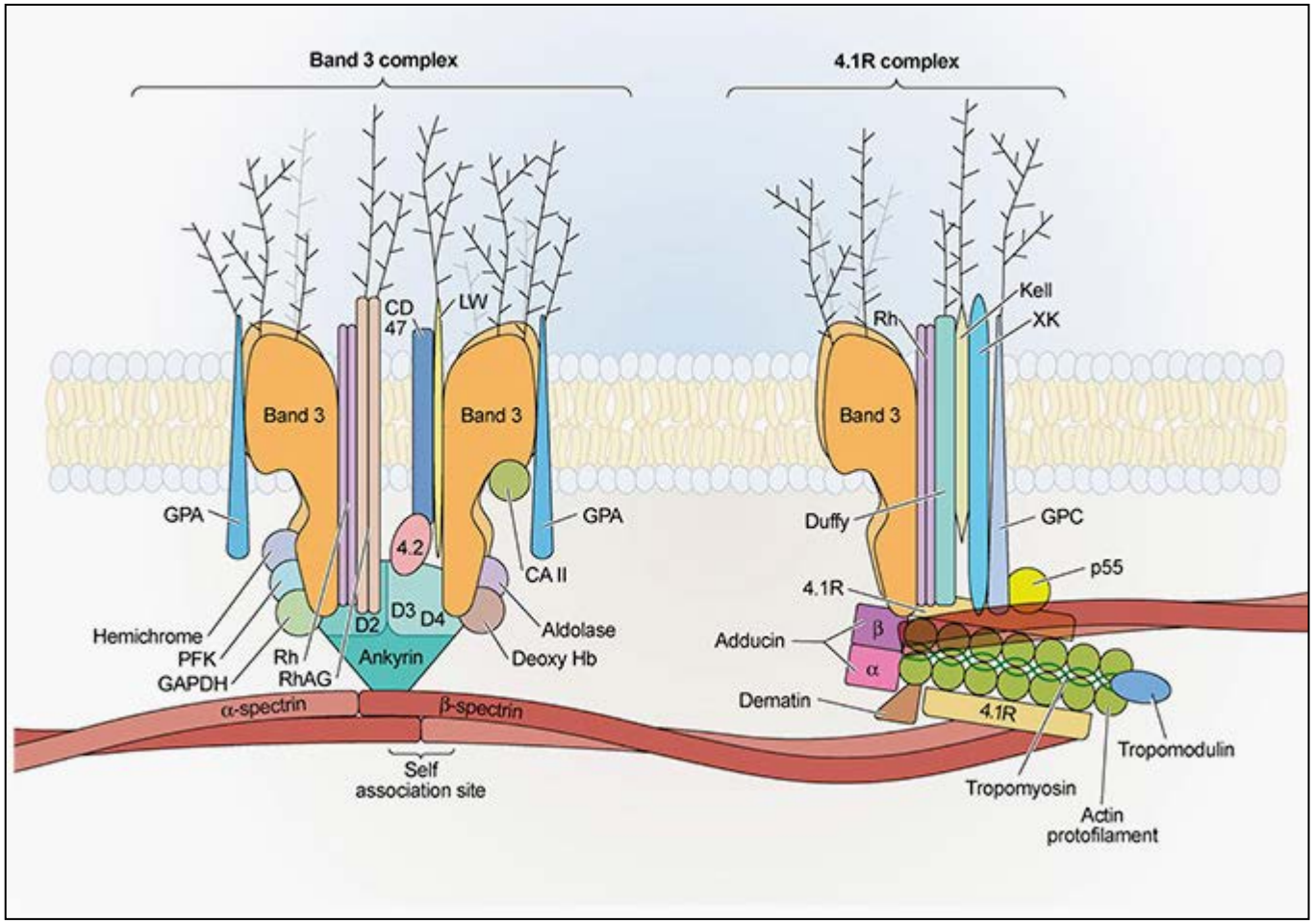
Multiprotein complexes in the red cell membrane



Salomao et al. (2008) Proc.Natl.Acad.Sci.USA 105, 8026

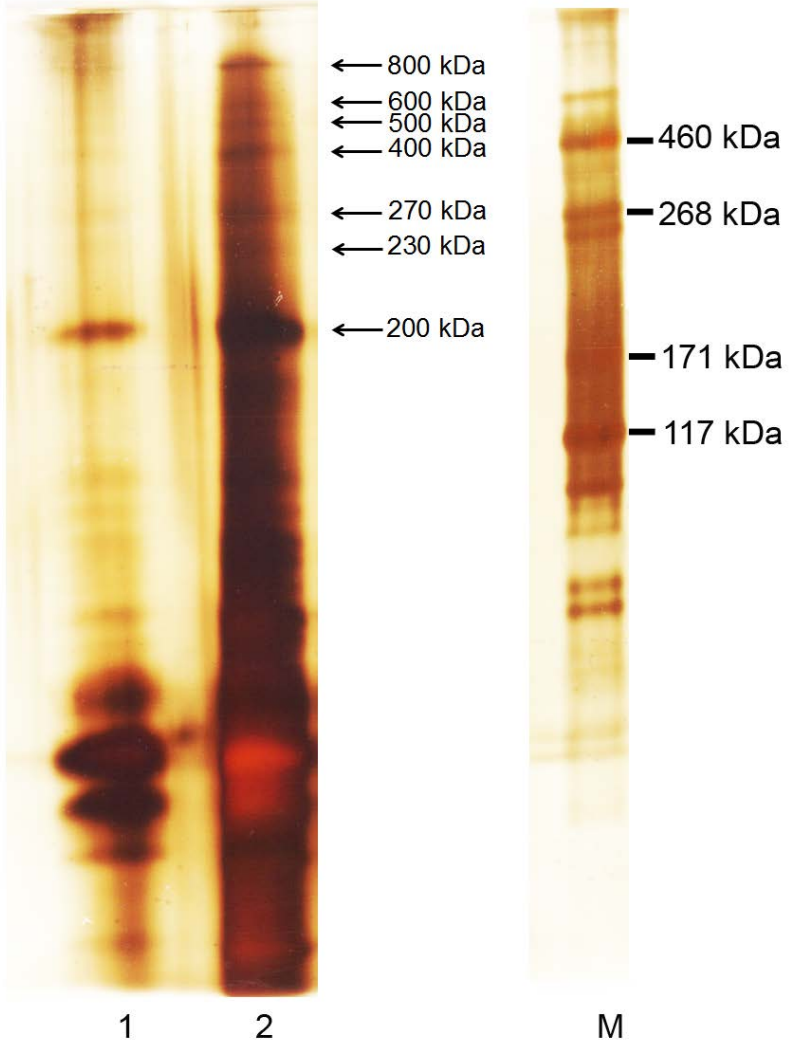
Isolation and analysis of cross-linked stomatin-complexes

- Cross-linking of RBC membranes (EGS or DSS)
- Quenching and solubilisation with TX-100 or SDS/TX-100
- ► +/- Isolation of DRMs
- Solution onto anti-stomatin affinity column
- Extensive washing, elution with glycine/HCl
- SDS-PAGE / MS-compatible silver staining
- Excision of bands, trypsinisation, LC-MS/MS

High-molecular stomatin-complexes from DRMs

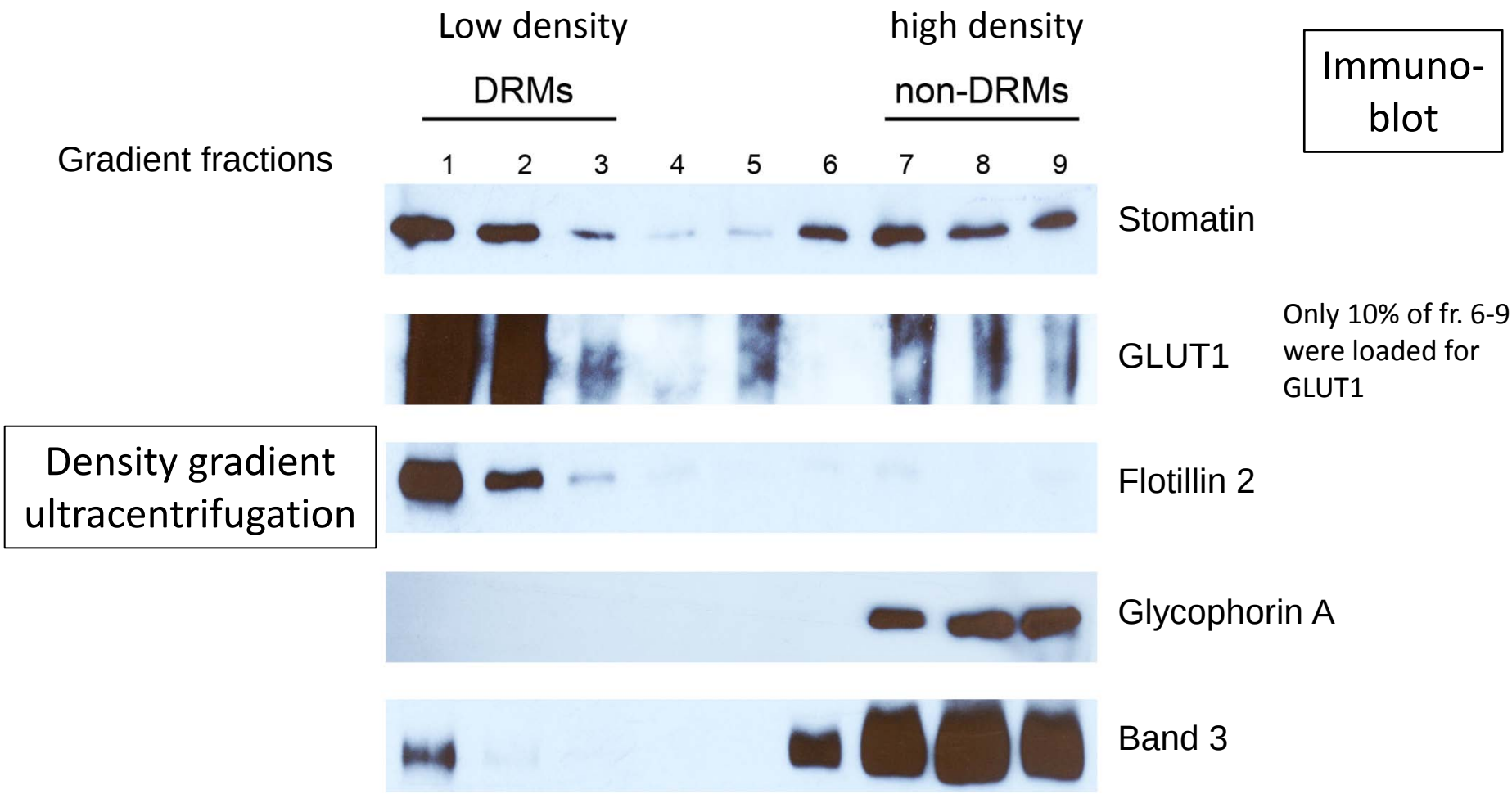
4–12% gradient
SDS-PAGE

Silver staining



Elution fractions

Erythrocyte DRMs



Rungaldier et al. (2013) BBA-Mem. 1828, 956

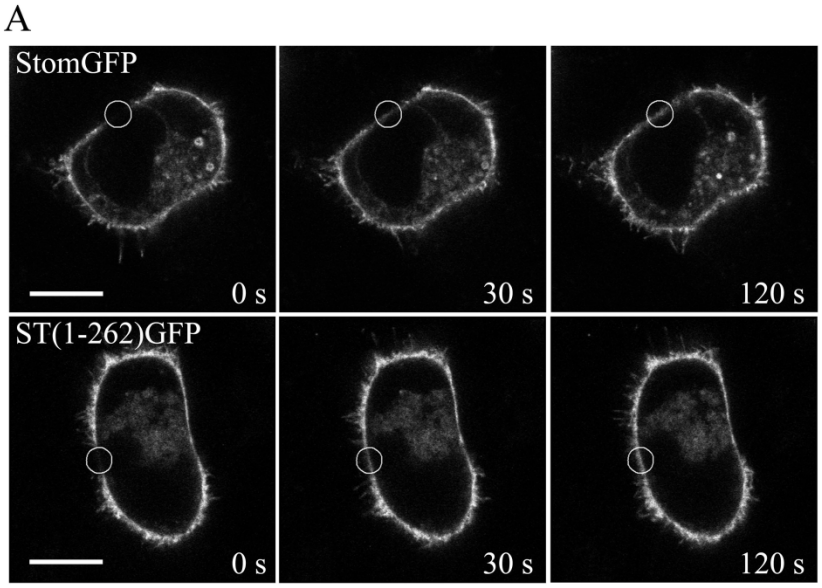
FRAP analysis of stomatin mobility

Wt Stom

oligomeric
DRM-protein

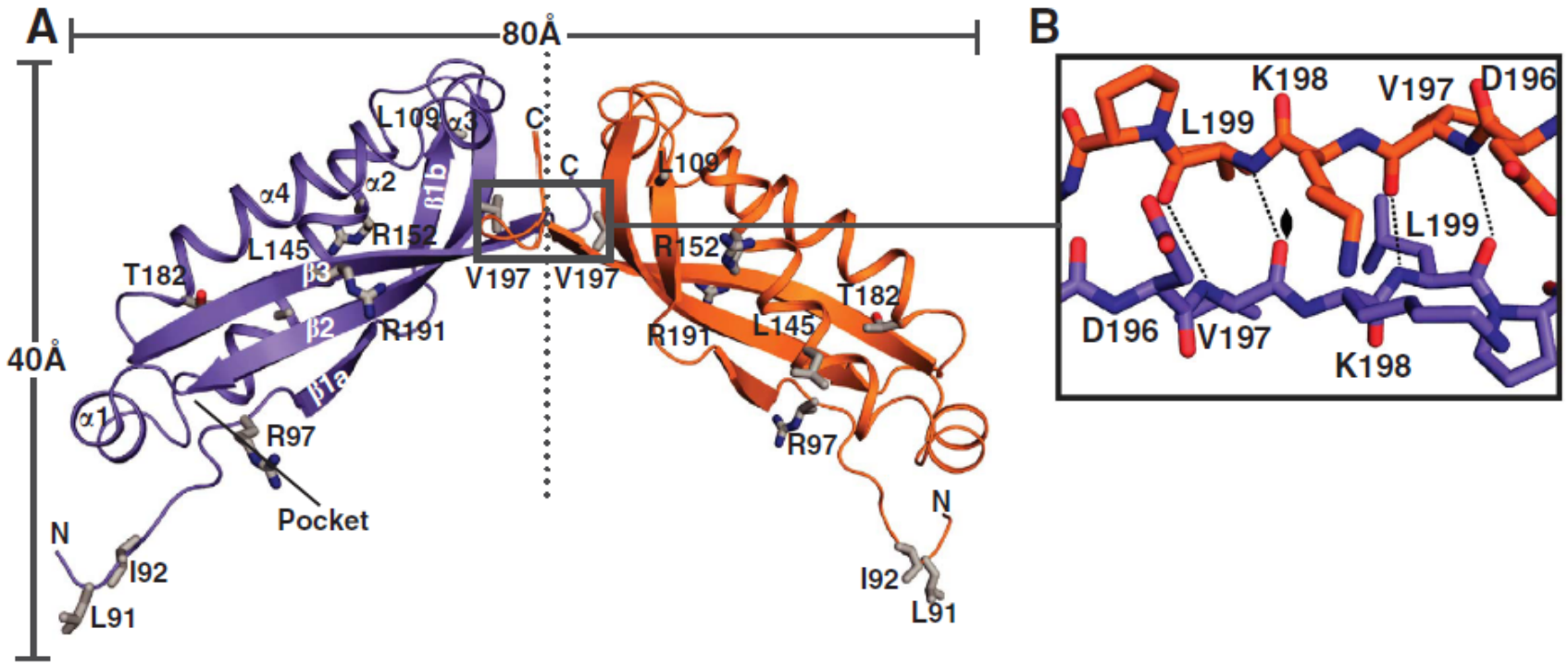
Stom(1-262)

monomeric
non-DRM protein



Umlauf et al. (2006) J.Biol.Chem. 281, 23349

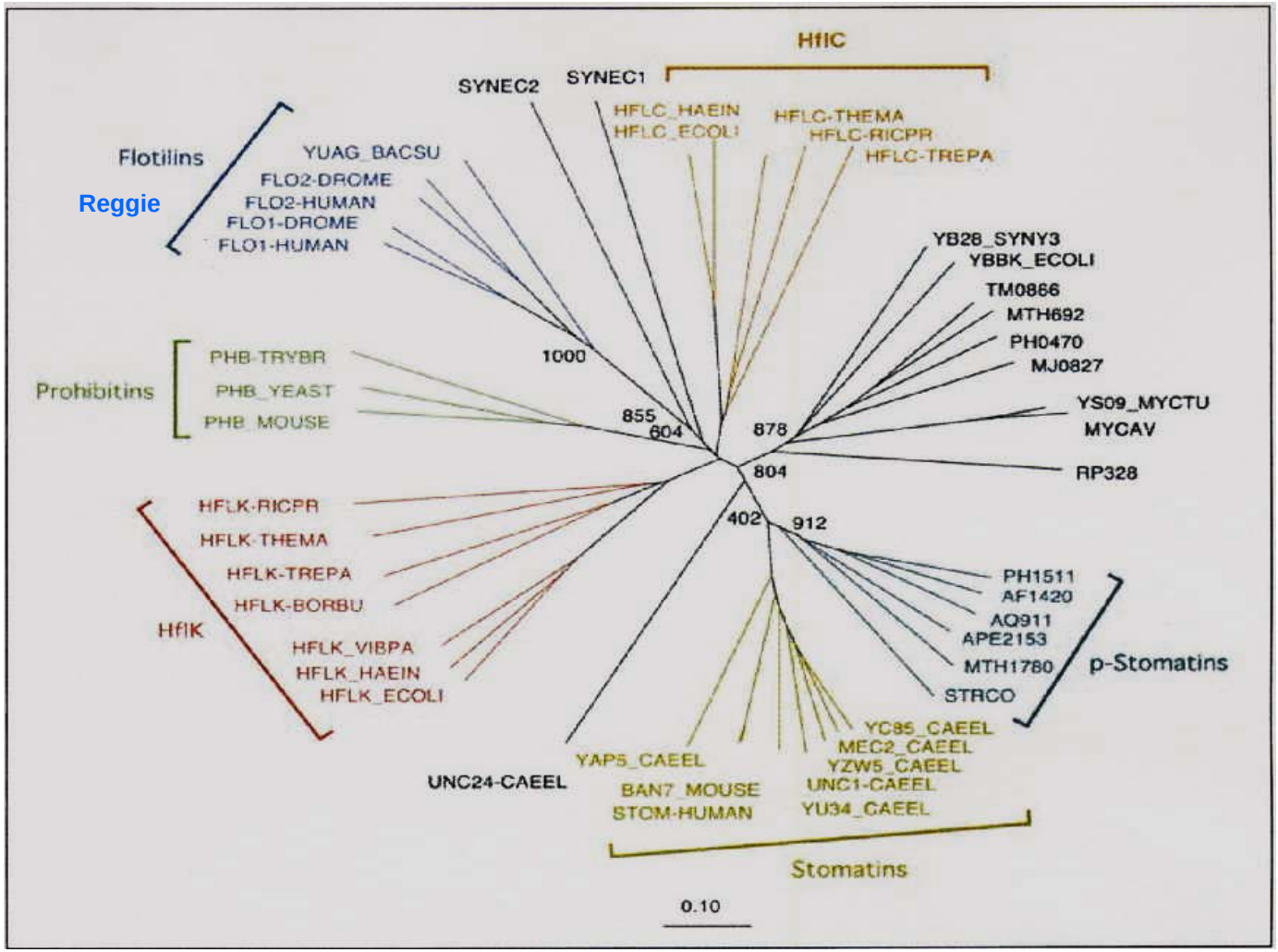
Stomatin domain dimer



Brand et al. (2012) EMBO J. 31, 3635

The SPFH/PHB-superfamily

Stomatin, Prohibitin, Flotillin, HflK/C



Tavernarakis et al. (1999) TIBS 24, 425