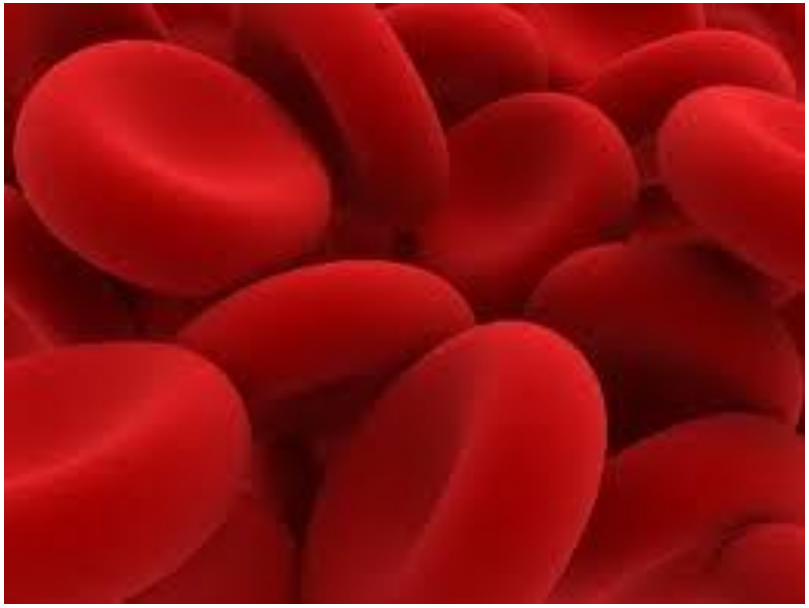
ΕΙΔΙΚΑ ΚΕΦΑΛΑΙΑ ΚΥΤΤΑΡΙΚΗΣ ΒΙΟΛΟΓΙΑΣ

«ΠΡΟΤΥΠΗ ΒΙΟΛΟΓΙΚΗ ΜΕΜΒΡΑΝΗ: Η ΜΕΜΒΡΑΝΗ ΤΟΥ ΕΡΥΘΡΟΚΥΤΤΑΡΟΥ»



Μαρτάννα Χ. Αντωνέλου, *Ph.D.*

Λέκτορας Τμήματος Βιολογίας, Πανεπιστημίου Αθηνών

2014

ΙΣΤΟΡΙΚΗ ΑΝΑΔΡΟΜΗ

- **1668: 1η παρατήρηση/περιγραφή Jan Swammerdam (Dutch biologist / microscopist)**

- **1675: Antonie van Leeuwenhoek, *Philosophical Transactions of the Royal Society*, χαρακτηριστικά, σχήμα, ελαστικότητα**

- **1862: George Gulliver, *Blood of Vertebrata*, membranous, ιξώδες**

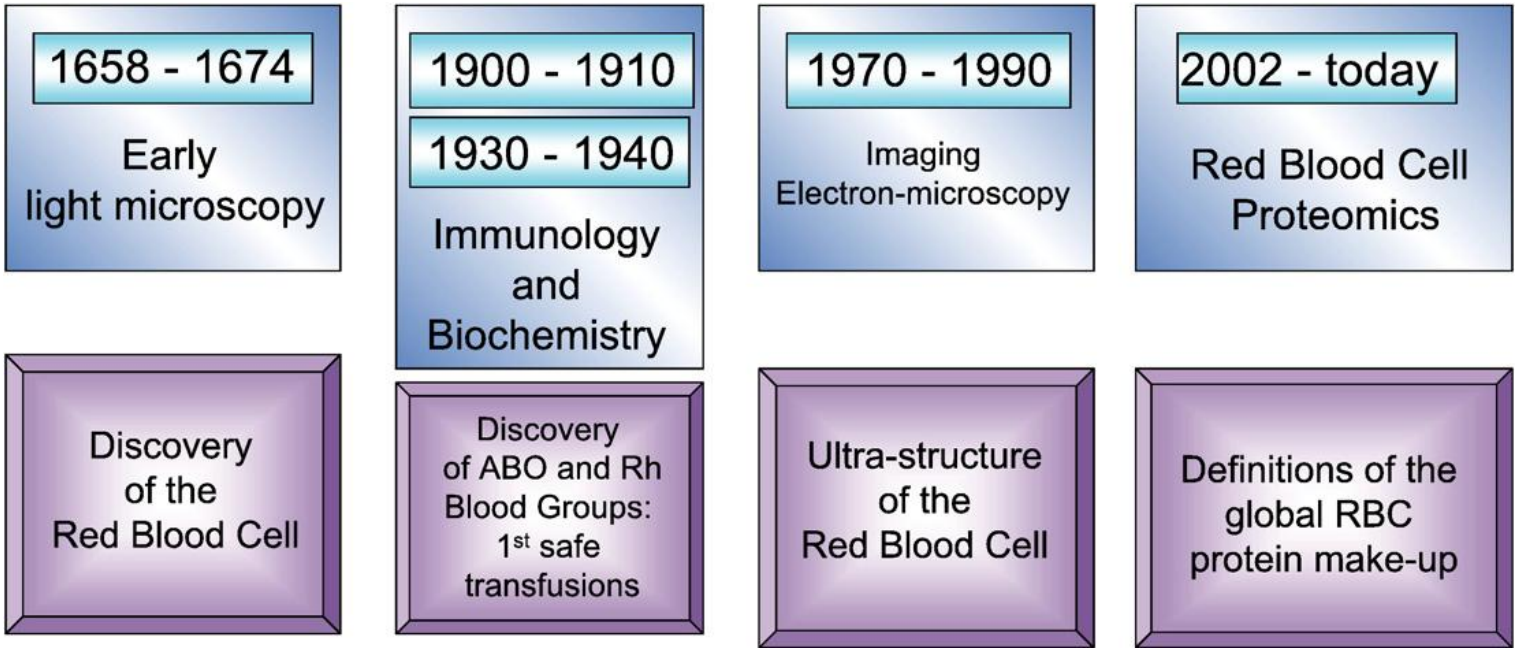
- **1925: Gorter & Grendel, *J Exp Med*, "bimolecular layers of lipids"**

- **1968: Marchesi & Steers, "Selective solubilization of a protein component of the red cell membrane", Science 159:203.**

- **1971: Steck, Fairbanks, Wallach, topology of RBC membrane proteins, Biochemistry, 10:2617.**

- **1972: Singer & Nicolson, "The fluid mosaic model of the structure of cell membranes", *Science*, 175:720.**

ΙΣΤΟΡΙΚΗ ΑΝΑΔΡΟΜΗ



Technological developments and advances in RBC research

ΙΣΤΟΡΙΚΗ ΑΝΑΔΡΟΜΗ

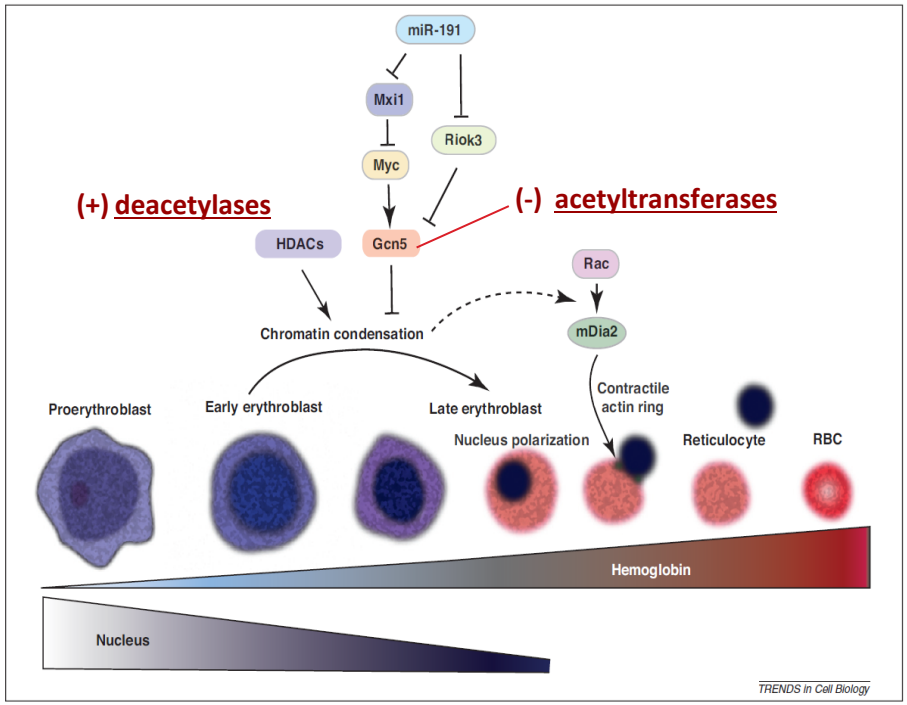
- Isolation of membranes, protein component analysis, SDS-PAGE, mass spectrometry, imaging technologies, microscopy….

- Peter Agre
- Jane Barker
- Daniel Branton
- Vann Bennett
- Jean Delaunay
- Bernard Forget
- Joseph Hoffman
- Philip Low
- Walter Gratzer
- Samuel Lux
- Vincent Marchesi
- Jon Morrow
- Jiri Palek
- Theodore Steck
- Lukas H. Margaritis
- Issidora S. Papassideri

ERYTHROPOIESIS

mature red cells are generated from hematopoietic stem cells

(Ji et al., Trends in Cell Biology; 2011, Vol. 21)

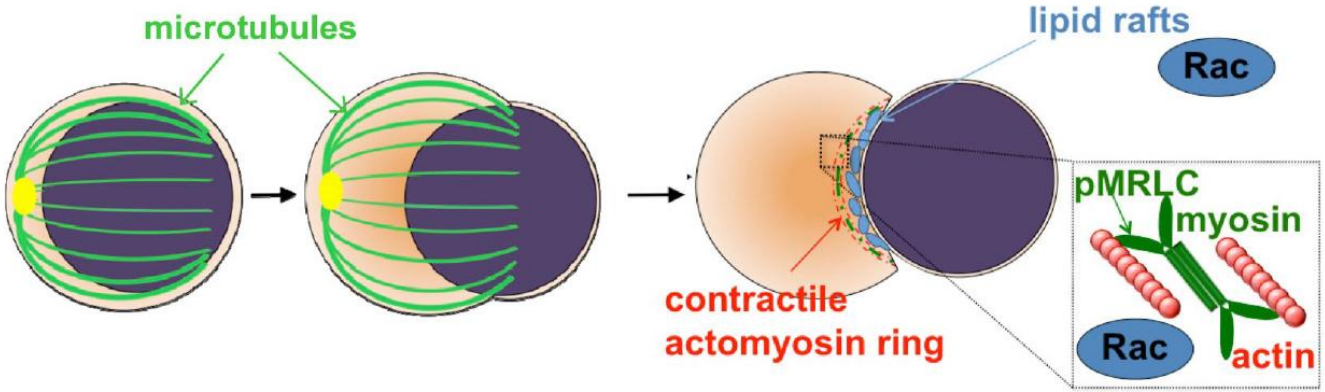


Το τελευταίο γεγονός στην εξέλιξη των θηλαστικών (εξειδίκευση κυτταρικών τύπων)

Critical physiological and evolutionary significance:

1) it allows an elevation of **Hb levels** in the blood
2) gives RBCs their **flexible biconcave** shape

Αποπυρήνωση
Vertebrates vs. mammals



(Konstantinidis et al., BLOOD 119(25):6118; 2012)

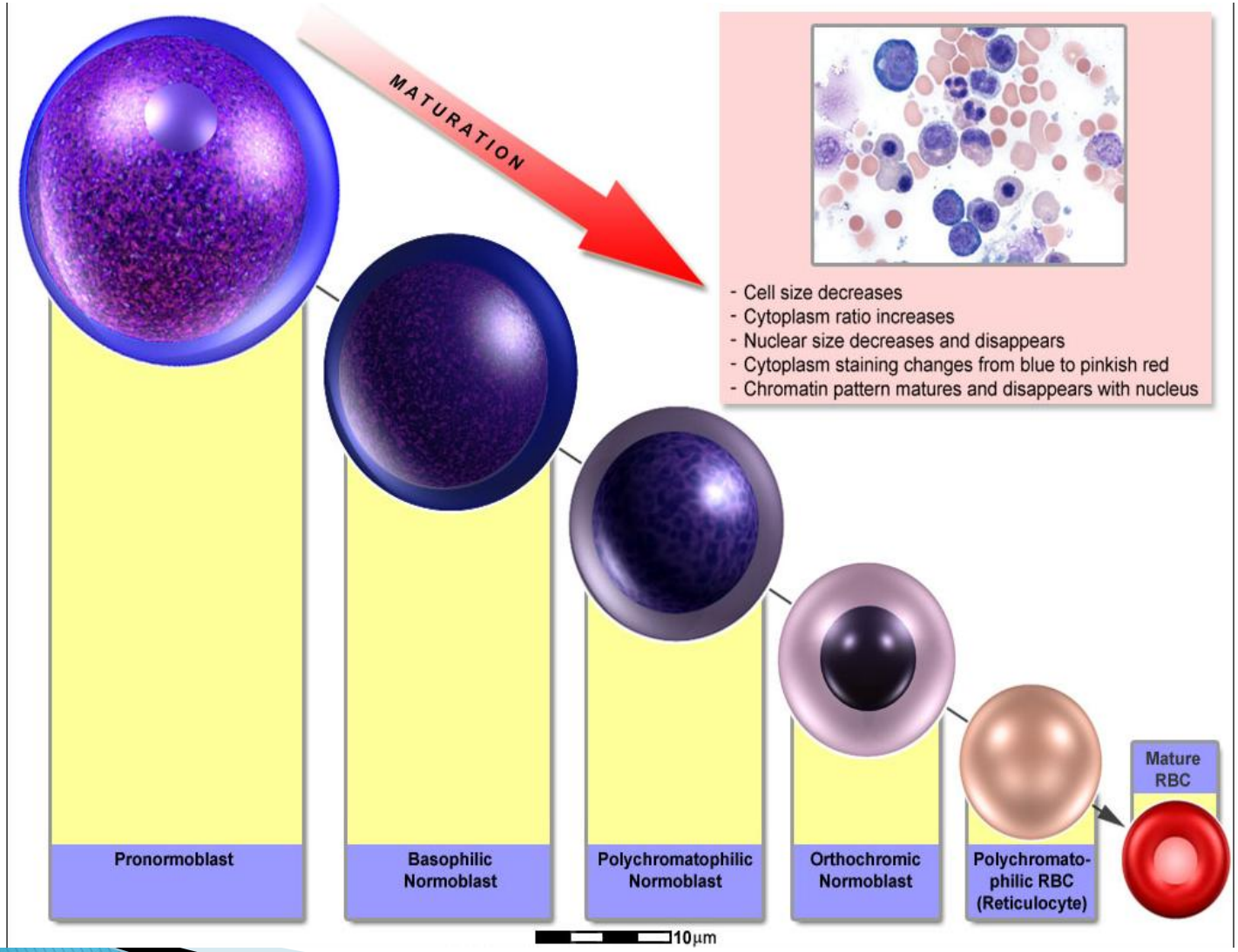
Working model of the erythroblast enucleation process:

Microtubules assist in the establishment of polarity in orthochromatic erythroblasts. Actin, under the control of Rac GTPases, assembles with myosin to form an actomyosin ring in the "cleavage furrow" between nucleus and incipient reticulocyte.

The phosphorylation of MRLC (myosin regulatory light chain) allows the actomyosin complex to contract.

Lipid rafts, containing and coordinated by Rac GTPases, coalesce in the cleavage furrow serving possibly to position the actomyosin ring properly and to target secretory vesicles toward creation of new membrane and separation of the nucleus.

ERYTHROPOIESIS



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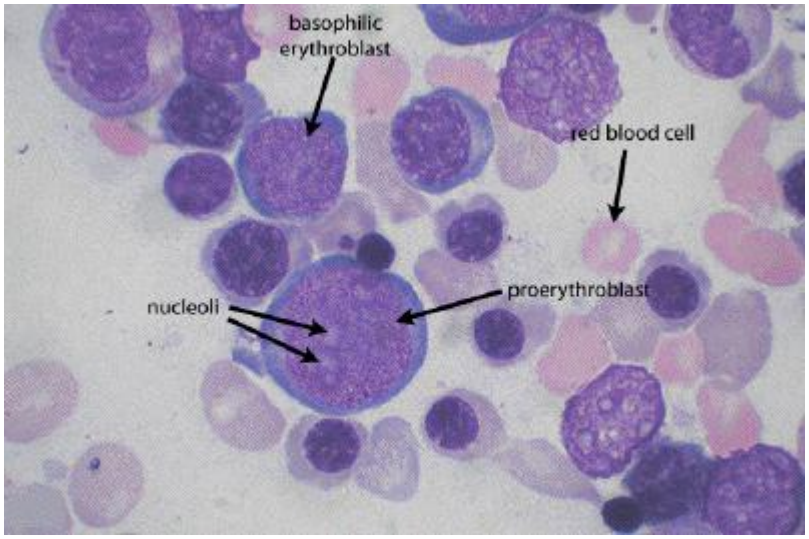
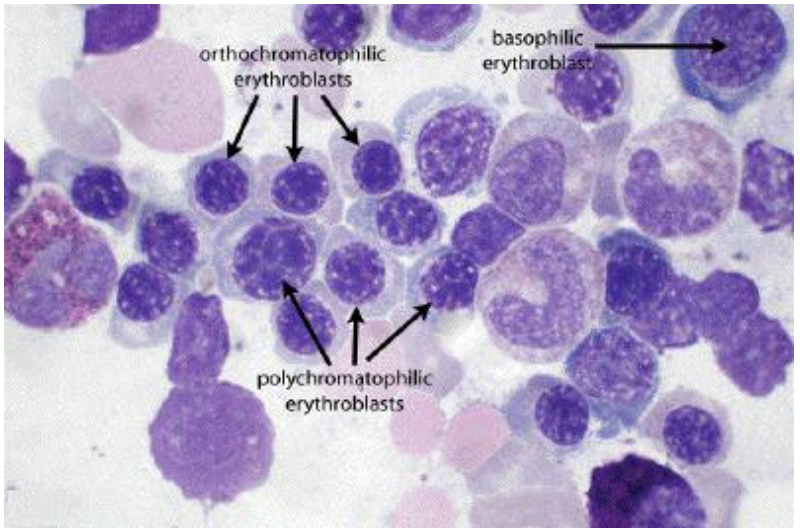
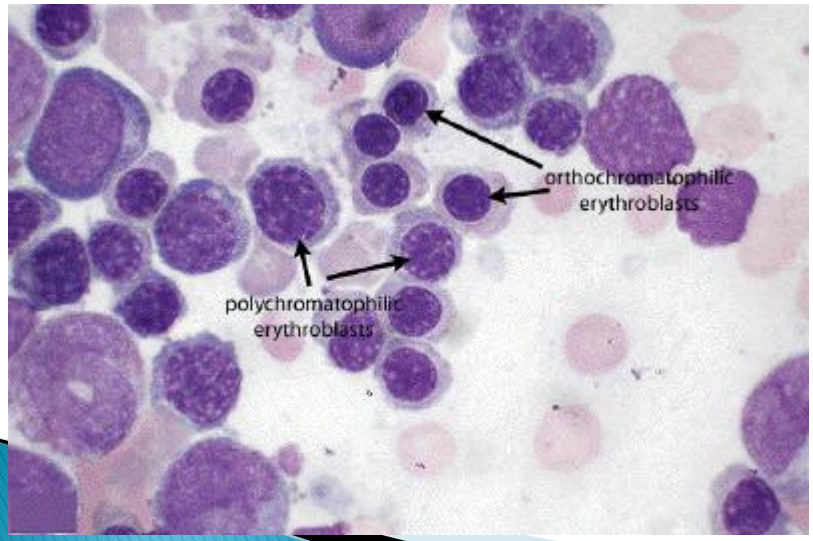
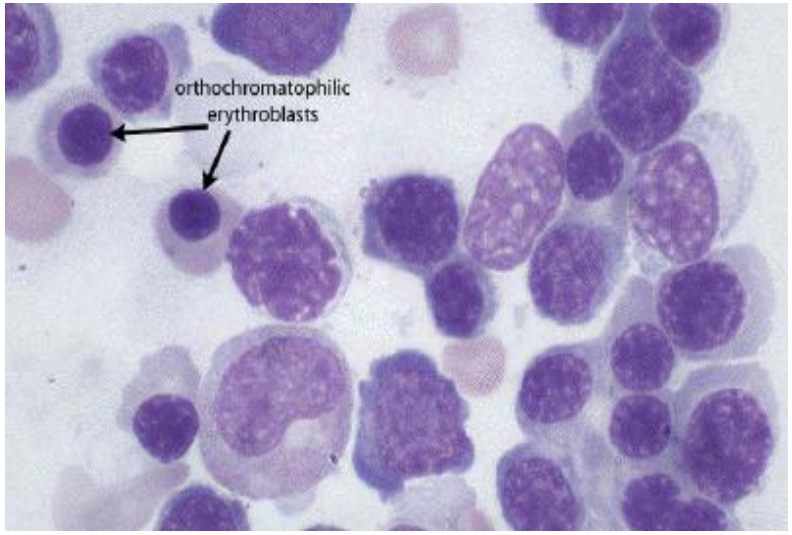


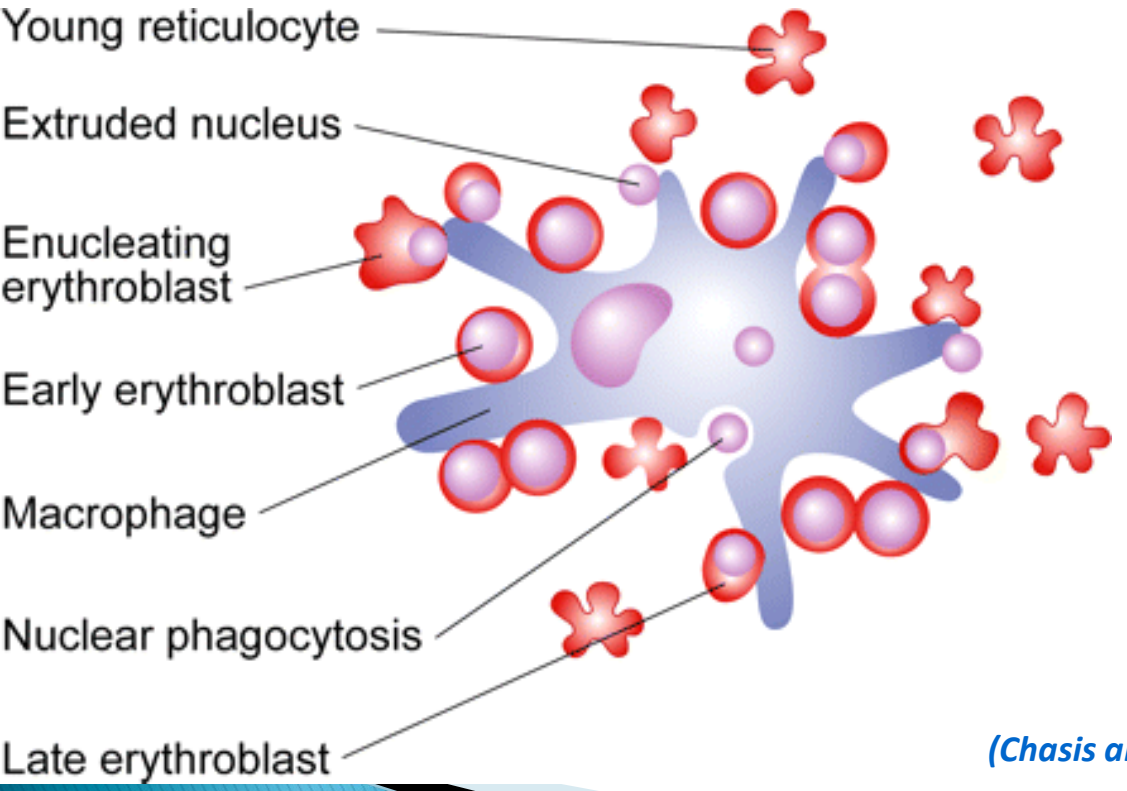






ERYTHROPOIESIS

Proliferation and differentiation processes occurring within the **erythroid niche**



Early-stage erythroblasts are larger cells with centrally located nuclei; **more differentiated** erythroblasts are smaller cells containing nuclei located adjacent to plasma membranes.

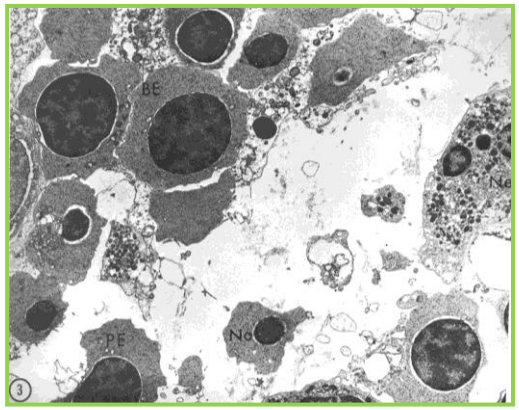
Expelled nuclei undergo phagocytosis by central macrophage.

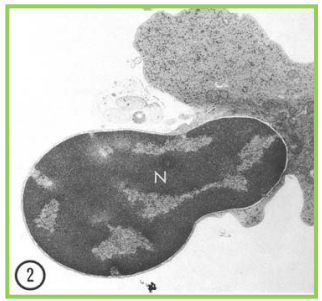
Young multilobulated reticulocytes are initially attached to the macrophage surface and later detach

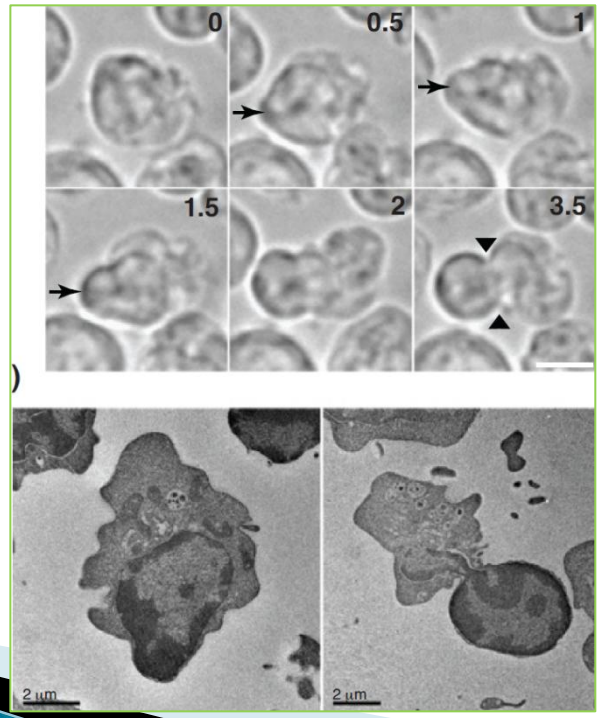
(Chasis and Mohandas, BLOOD 112(3); 2008)

(Ji et al., Trends in Cell Biology; 2011, Vol. 21)

ENUCLEATION

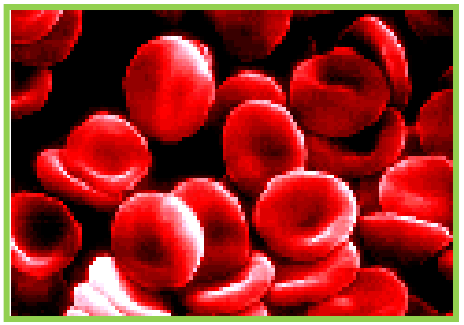


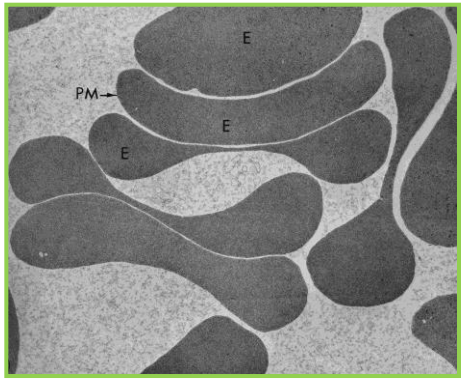


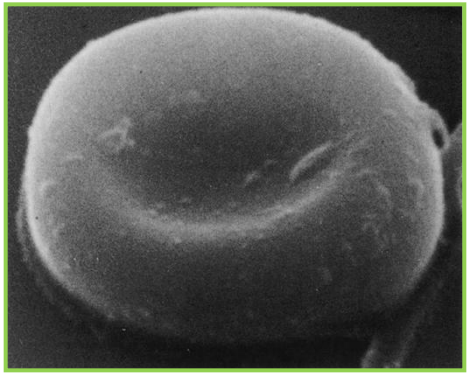


min

Time-lapse live-cell imaging







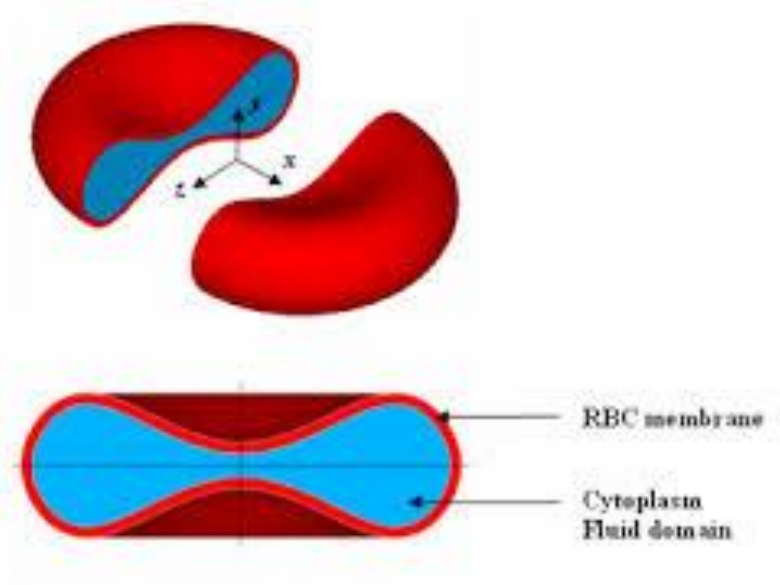
Αμφίκοιλο δισκοειδές

Mammalian red blood cells have no nuclei, no internal organelles, no major biosynthetic repair mechanisms

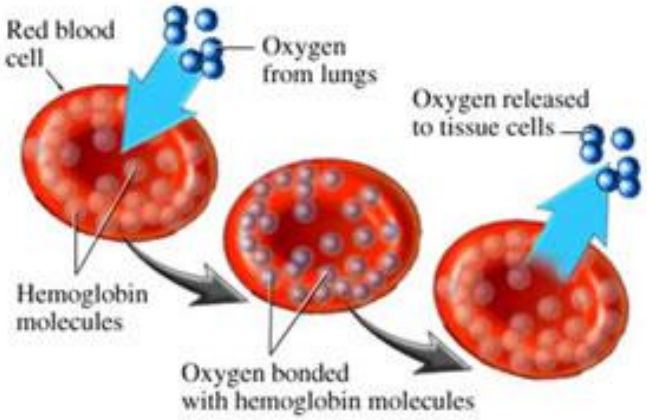
Despite this, they survive in circulation for about 120 days

This implies that their membranes have some adaptation that allows them to survive for this length of time

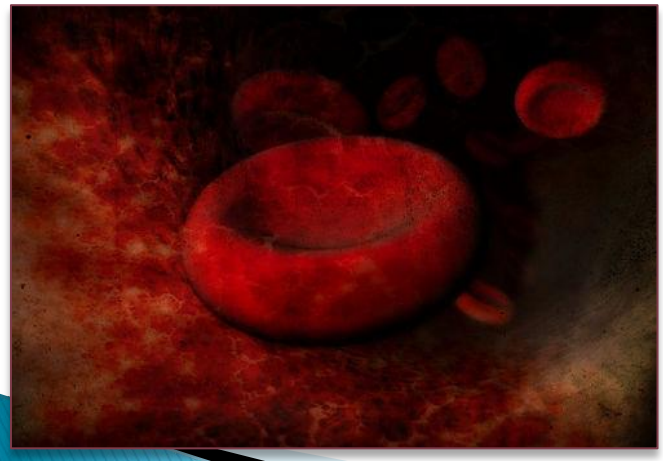
An unsupported lipid bilayer would be unable to endure the rigours of circulation

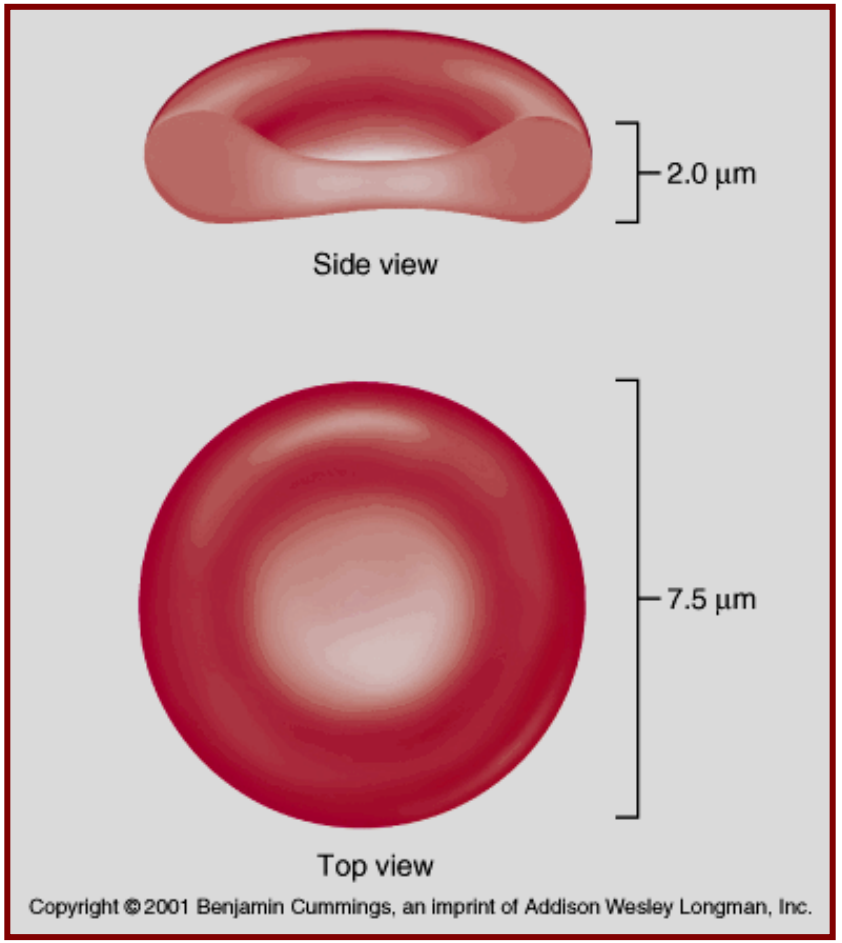


The unique discoid shape of the RBC plasma membrane provides the biological and mechanical properties necessary to perform its primary role in Hb-mediated oxygen transport throughout the body



ΕΡΥΘΡΟΚΥΤΤΑΡΟ





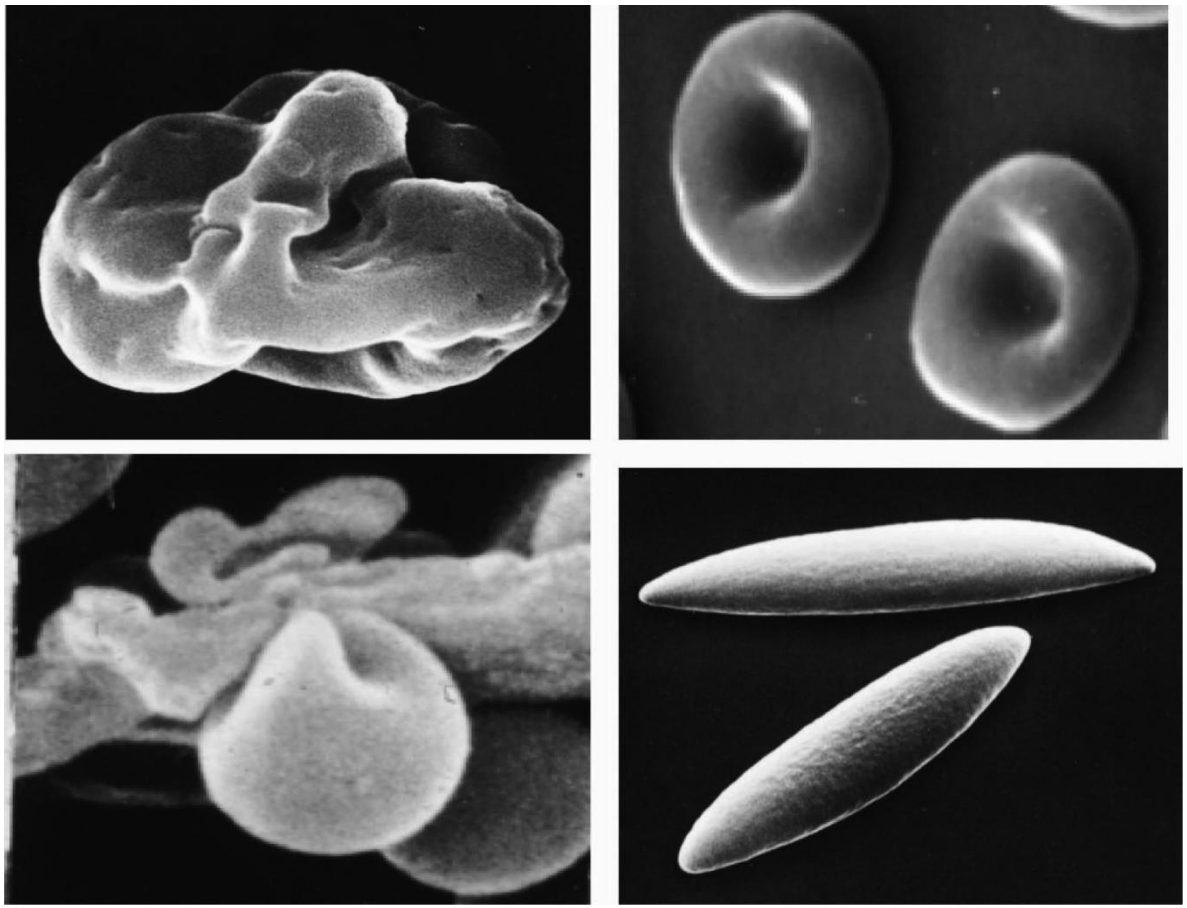
$90\mu m^3$ -$140\mu m^2$ ($98\mu m^2$)

Only structural component: antigenic, transport, mechanical

48 h

Multilobular reticulocyte

mature discoid red cell



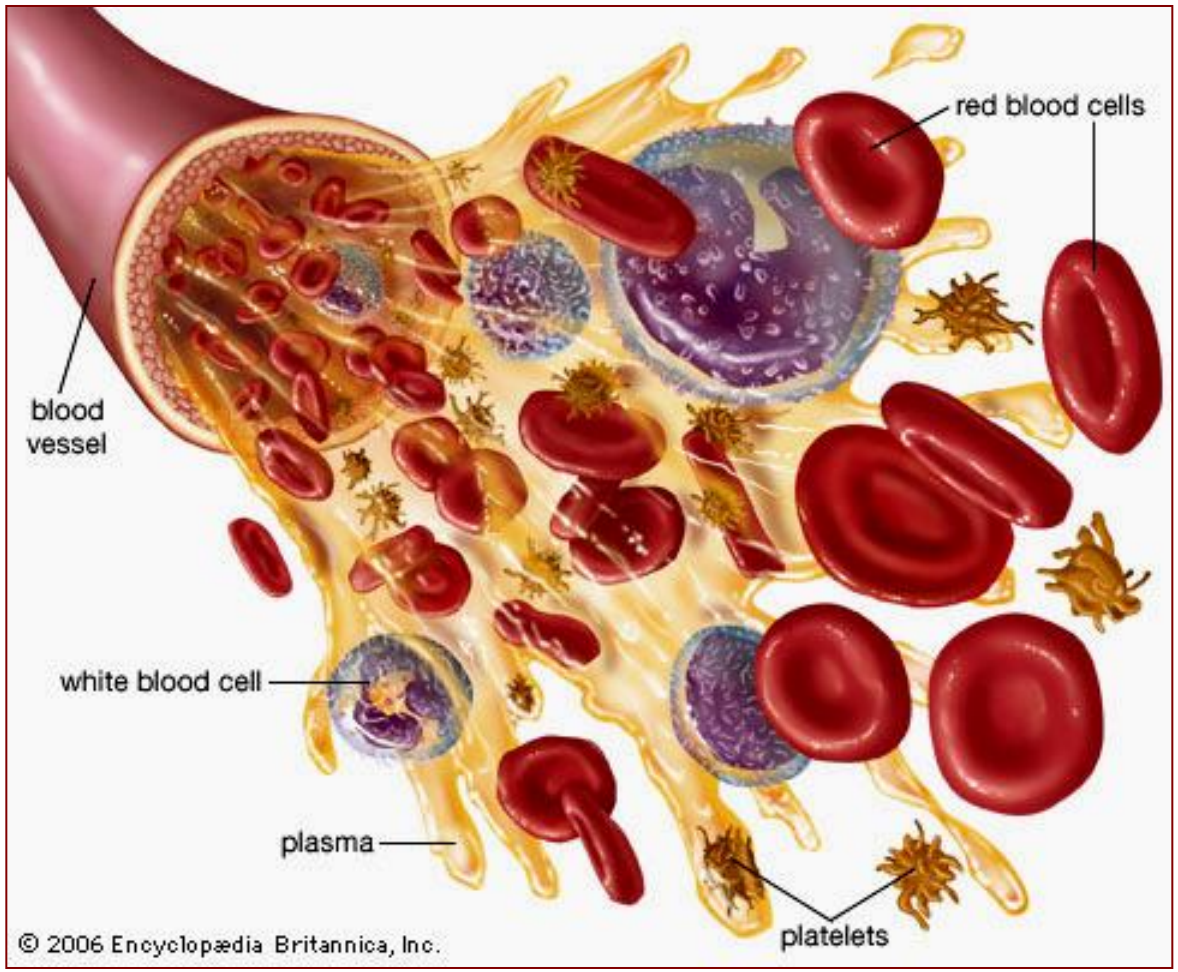
deformation

red cell traversing from the splenic cord to splenic sinus

Ellipsoidal cells

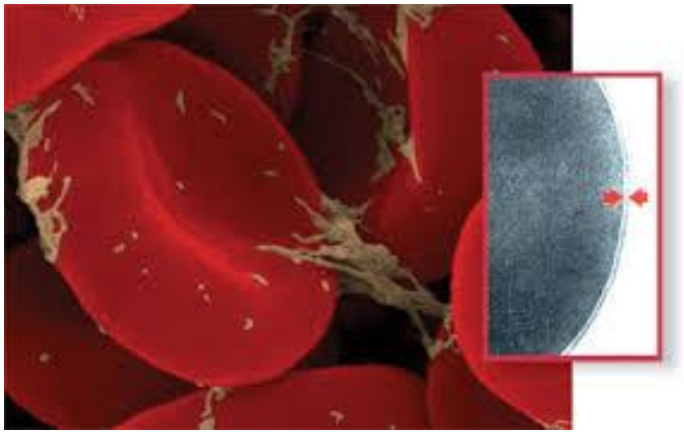
(Mohandas and Gallagher, BLOOD 112(10):2008)

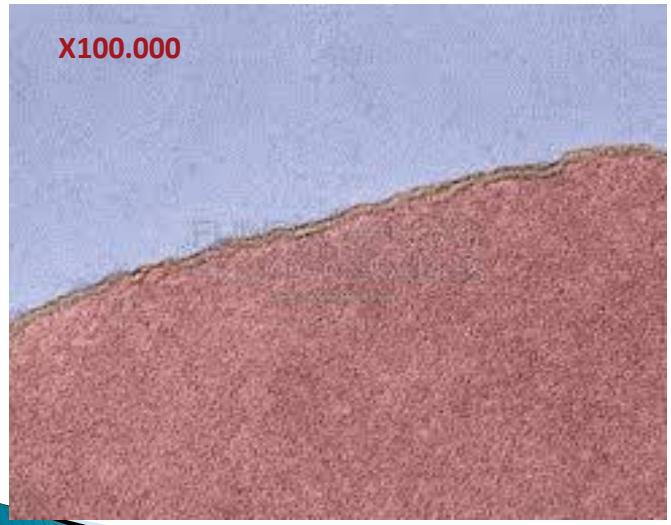
•ελαστική/εκτατή/συμπιέσιμη-ανθεκτική (structural resistance)



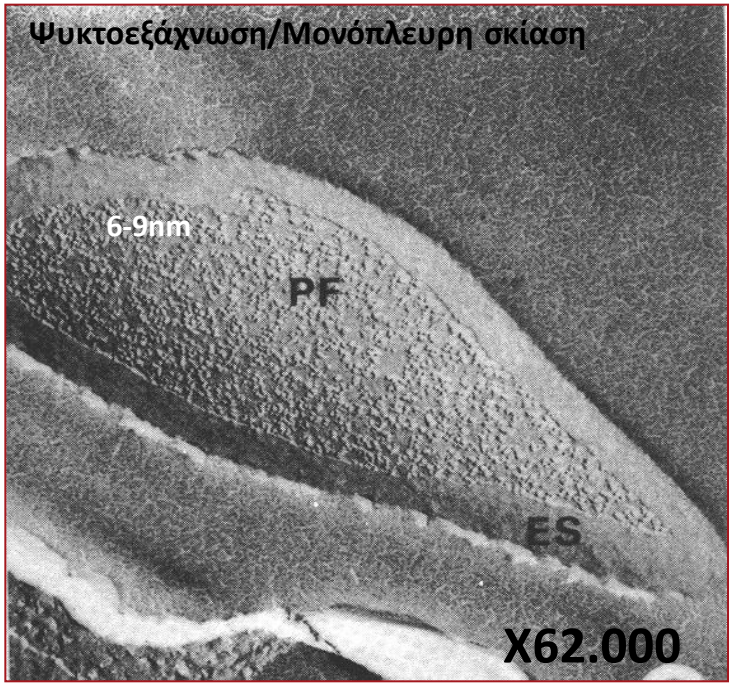
[Membrane Effects in a Red Blood Cell - YouTube]

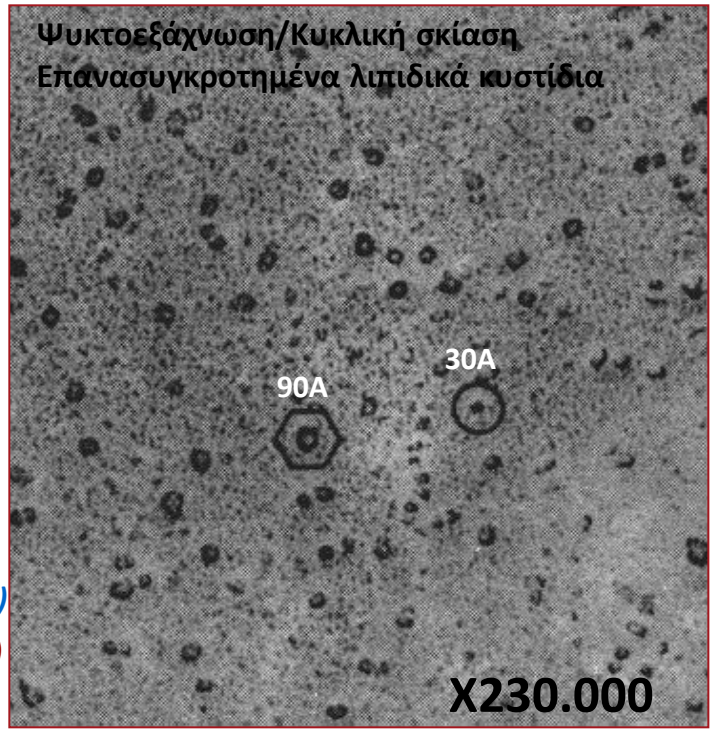
ΕΡΥΘΡΟΚΥΤΤΑΡΙΚΗ ΜΕΜΒΡΑΝΗ





1960-70
Daniel Branton
Hans Moore





(Margaritis et al., 1977; J.Cell Biology, 72:47)

(Μαργαρίτης & Παπασιδέρη, 1979; Materia Medica Greca, 1:47)

ΛΕΙΤΟΥΡΓΙΚΟΙ ΡΟΛΟΙ

- **Τυπικοί και «ιδιαίτεροι»**

- **Μεταφορά υλικών και πληροφορίας, ηλεκτροχημικό δυναμικό, επικοινωνία, ανοσολογική αναγνώριση, μεταγωγή σήματος κλπ**

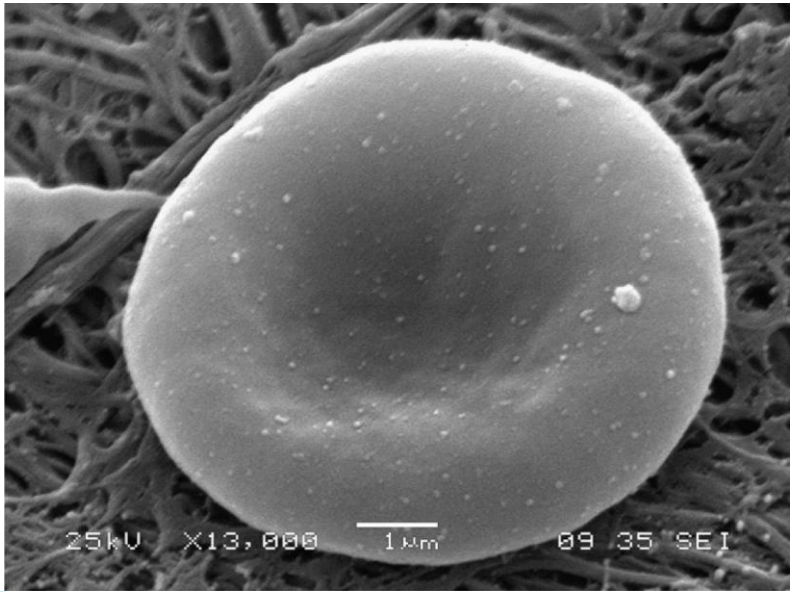
- **Ικανότητα ελαστικής παραμόρφωσης RBC (cellular deformability)**

Κανένα κύτταρο δεν έχει την ιδιότητα αυτή στο συγκεκριμένο βαθμό (θηλαστικά)

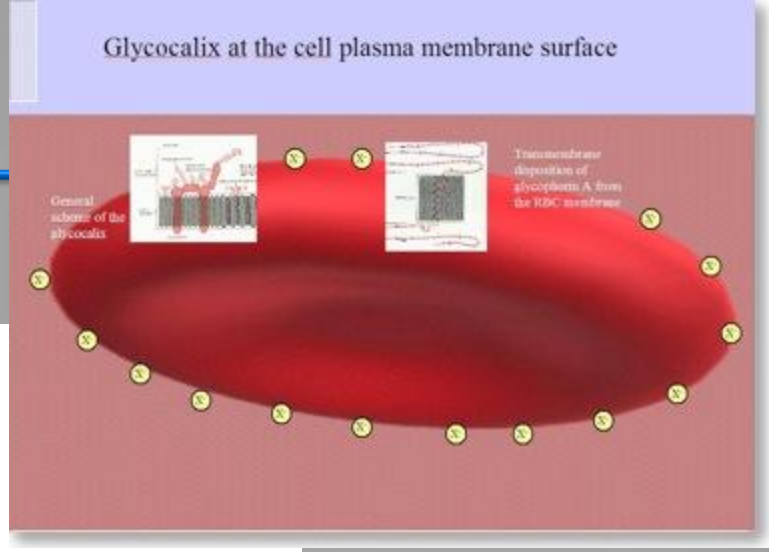
- ❑ **κυτταρική *γεωμετρία (S/V)***
- ❑ ***ιξώδες κυτταροπλάσματος [Hb]***
- ❑ ***μηχανικές ιδιότητες μεμβράνης***

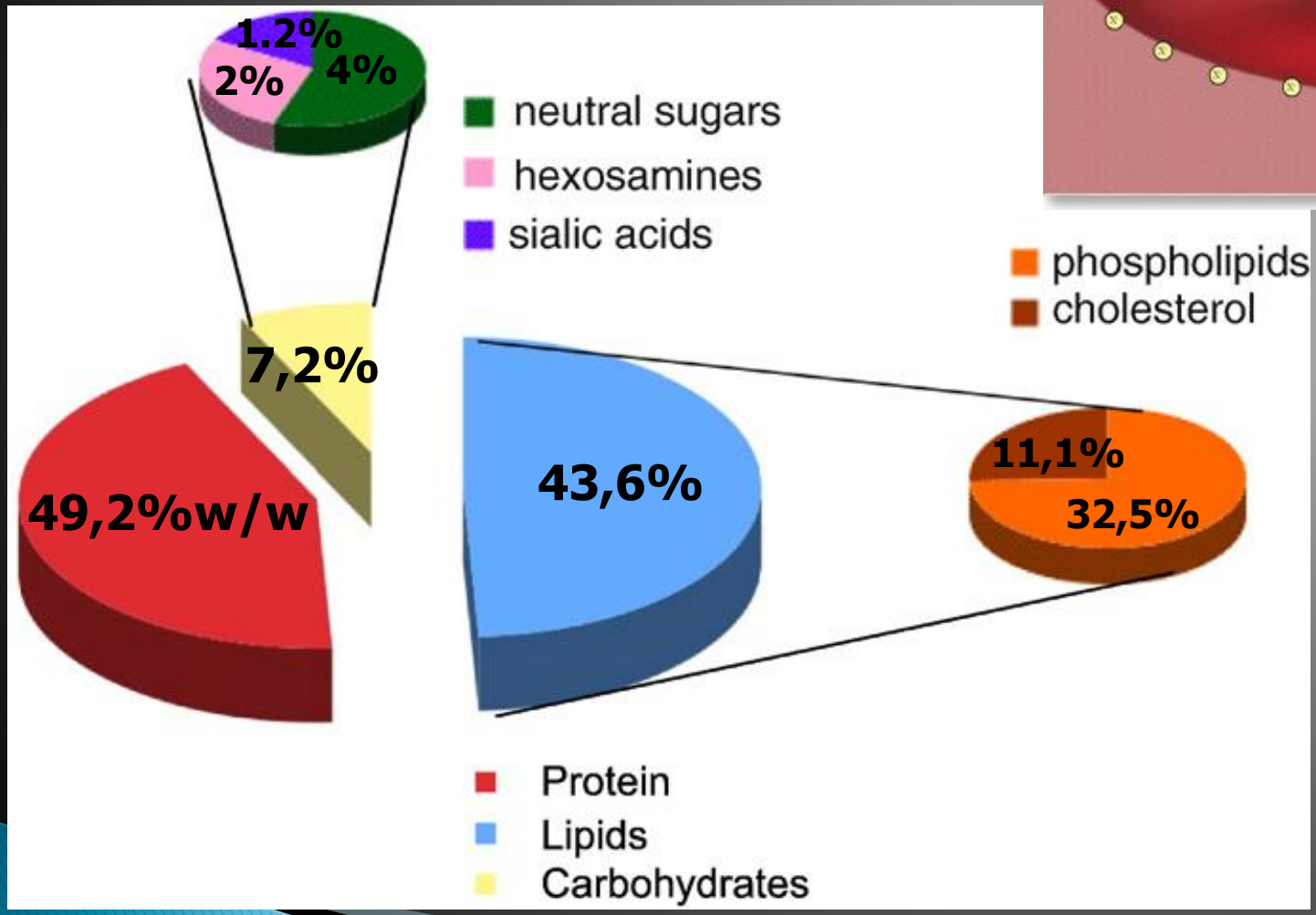
- ✓ **membrane deformability**
- ✓ **membrane stability**

(Whelihan and Mann, THROMBOSIS RESEARCH 2013)



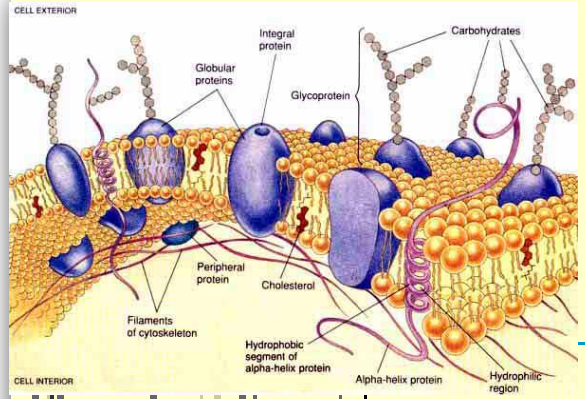
ΣΥΣΤΑΣΗ ΜΕΜΒΡΑΝΗ





90%Hb
10% H2O

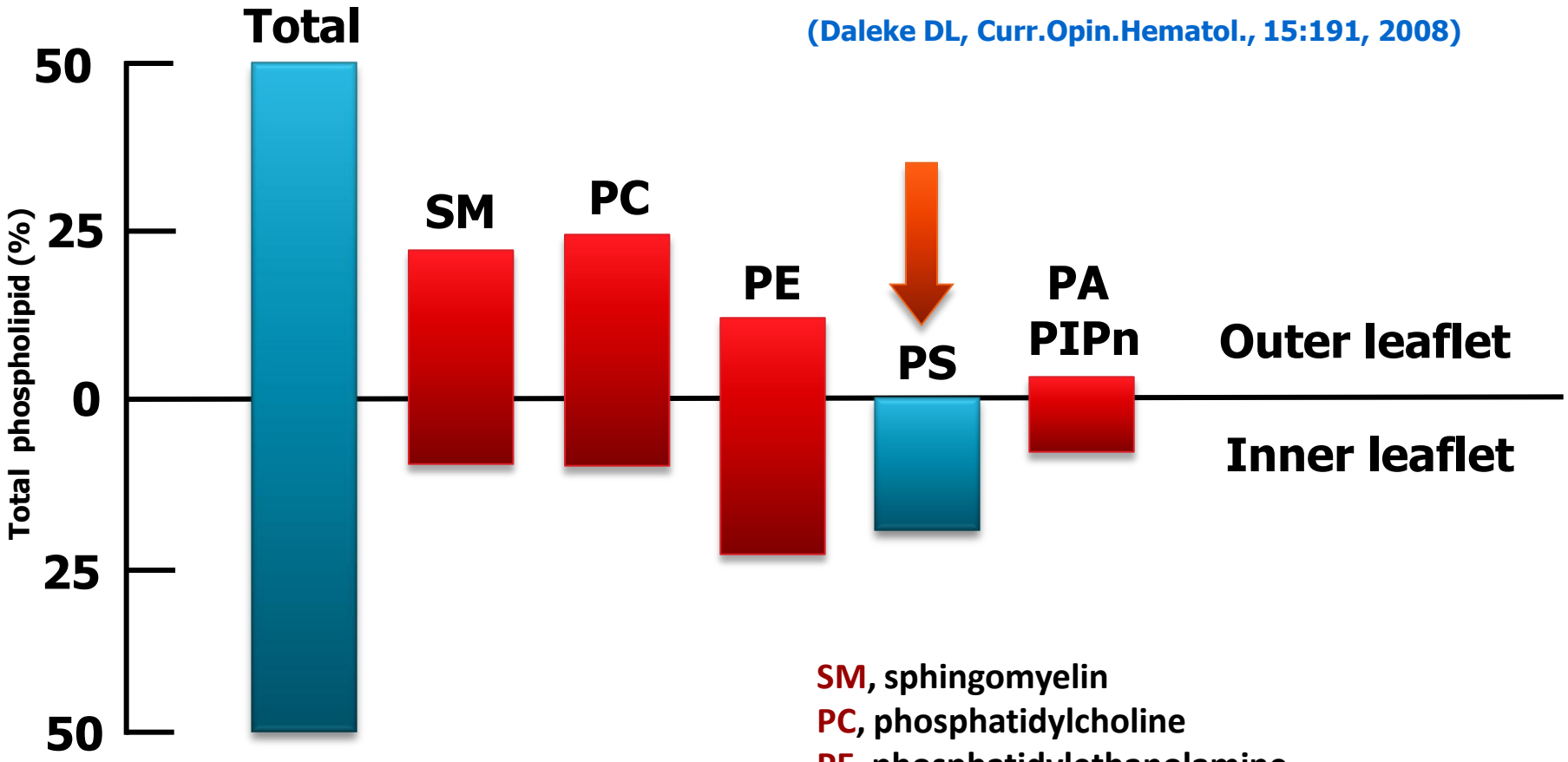
(Pasini et al., Journal of Proteomics 2010)



	Percentage of Total Composition in			
	Human Erythrocyte Plasma Membrane	Human Myelin	Beef Heart Mitochondria	*E. coli* Cell Membrane
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidylethanolamine	18	20	27	65
Phosphatidylglycerol	0	0	0	18
Phosphatidylinositol	1	1	7	0
Phosphatidylserine	8.0	8.0	0.5	0
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0
Others	0	0	23.5	17

Source: Data from C. Tanford, *The Hydrophobic Effect* (New York: Wiley, 1973).

ΛΙΠΙΔΙΑΚΗ ΚΑΤΑΝΟΜΗ



(Daleke DL, Curr.Opin.Hematol., 15:191, 2008)

SM, sphingomyelin
PC, phosphatidylcholine
PE, phosphatidylethanolamine
PS, phosphatidylserine
PA, phosphatidic acid
PIPn, phosphatidylinositol-(n)-phosphate

ΛΙΠΙΔΙΑΚΗ ΚΑΤΑΝΟΜΗ

Energy-dependent -independent

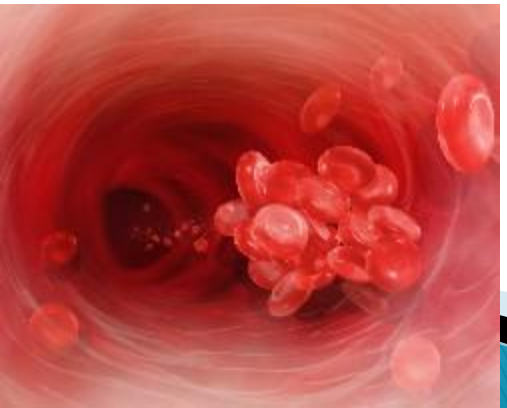
Phospholipid transport proteins: flippases, flopases, scramblases

PS externalization: λειτουργικές επιπτώσεις

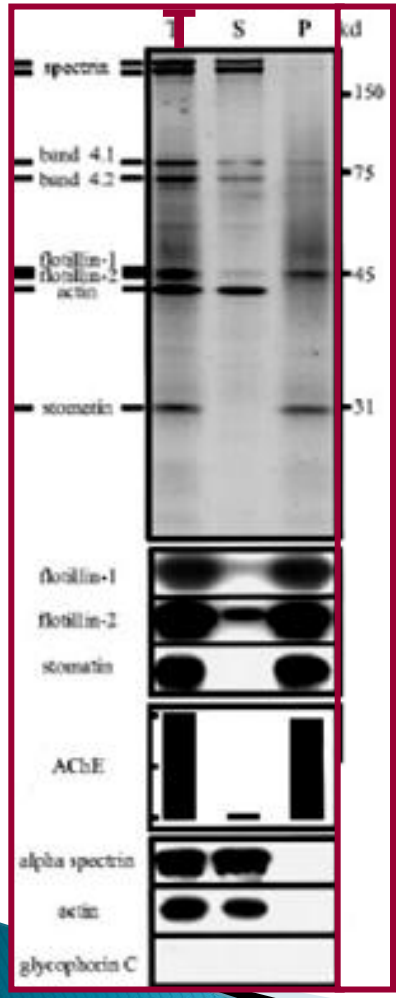
Αναγνώριση από μακροφάγα

Premature destruction of thalassemic, sickle RBCs

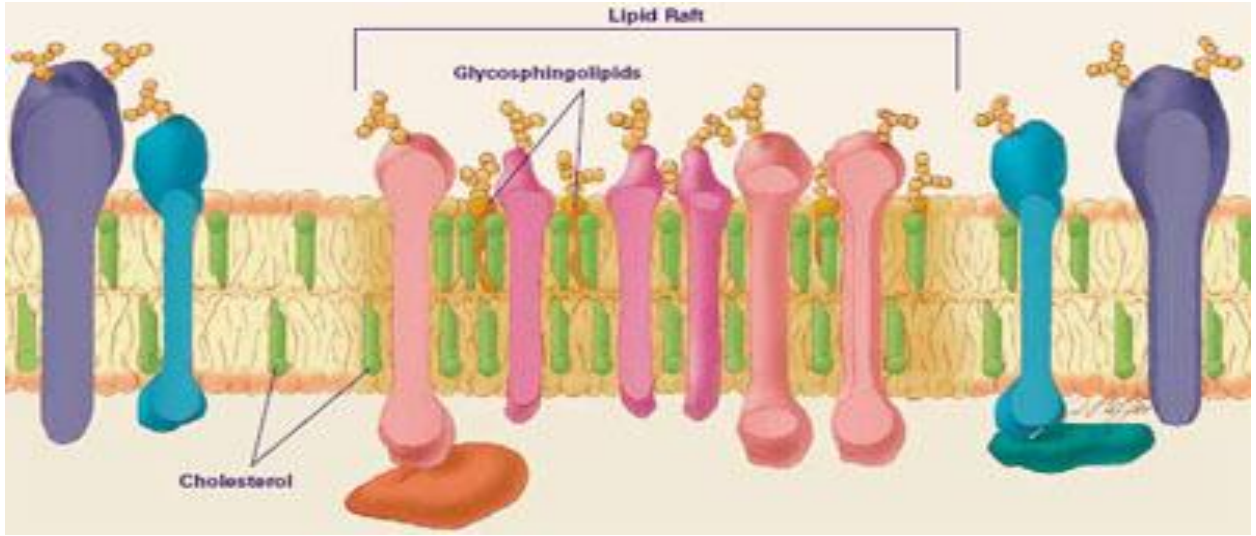
Adhesion to vascular endothelial cells



LIPID RAFTS



Lipid rafts EM: πλούσια σε σφιγγολιπίδια, χοληστερόλη και πρωτεΐνες (0.1%-3% of total EM proteins) όπως GPI-anchored proteins (AChE), stomatin (Δ, 7.2b), flotillins (Δ), B3, peroxiredoxin-2, Hb, synexin, sorcin



Μερική συσχέτιση ορισμένων κατηγοριών RBC-LR με το σκελετό μέσω ηλεκτροστατικών αλληλεπιδράσεων

(Salzer and Prohaska, 2001; *Blood, 97:1141*)

LIPID RAFTS

Protein	Molecular weight, kDa	Membrane association*	Remarks
α-globin	15	Cytoplasmic	Hemoglobin complex
β-globin	16	Cytoplasmic	Hemoglobin complex
GAPDH	36	Cytoplasmic	Conversion of G3P to 1,3-BPG
Peroxiredoxin-2	21.9	Cytoplasmic	Eliminates peroxides; signaling?
S-100β	10.5	Cytoplasmic	Dimer with α chain; binds p53, tubulin, and Ca^{2+}; (dis)assembly of microtubules and intermediate filaments
CA-I	28.8	Cytoplasmic	Reversible hydration of CO_2
Flotillin-1	47	Endofacing hairpin loop	Organization of caveolae and/or lipid rafts
Flotillin-2	45	Endofacing hairpin loop	High-order flotillin oligomers; raft scaffolding component
Stomatin	31	Endofacing hairpin loop	Associates with Glut1; cation transport
Gαs	44	Endofacing	GPCR activation of adenylate cyclase
CD55	55-70	GPI-linked	Decay accelerating factor
CD58	64-73	GPI-linked	Unknown function in erythrocytes
CD59	20-40	GPI-linked	Membrane inhibitor of reactive complement lysis
Glut1	54	Multipass (12)	May bind stomatin; passive glucose transport
Band 3	101	Multipass (14)	Binds protein 4.2 and ankyrin; "Cl shift"
AQP1	28	Multipass (6)	Water channel protein for erythrocytes and renal PCT
β_2-AR	65	Multipass (7)	Gαs-coupled receptor
Duffy	35-43	Multipass (7)	Chemokine and *P vivax* receptor; GPCR-like
Scramblase	35	Single-pass	Movement of membrane phospholipids

(Murphy et al, 2004; *Blood, 103:1920*)

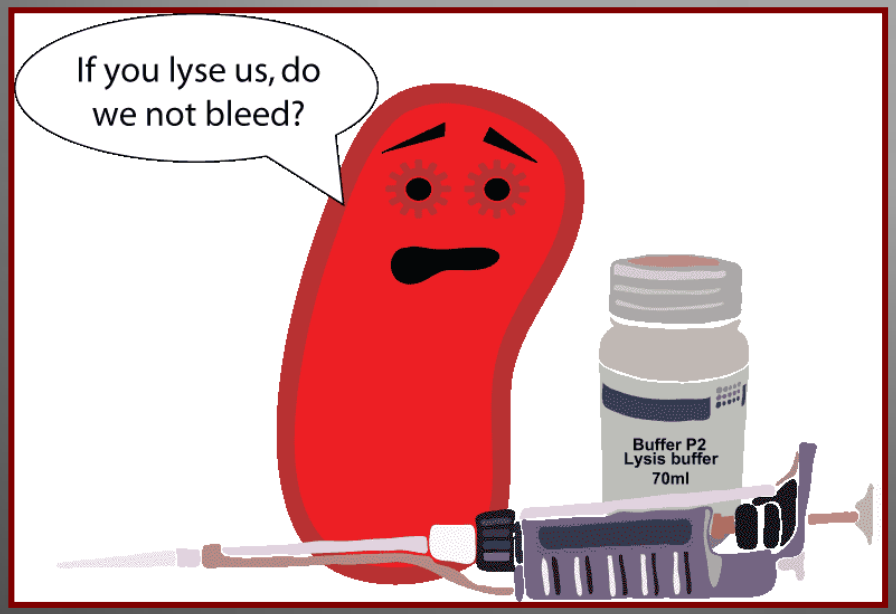
5 κύριες και πολλές δευτερεύουσες πρωτεΐνες

Πολλαπλοί πληθυσμοί Lipid Rafts (stomatin/flotillin/synexin)

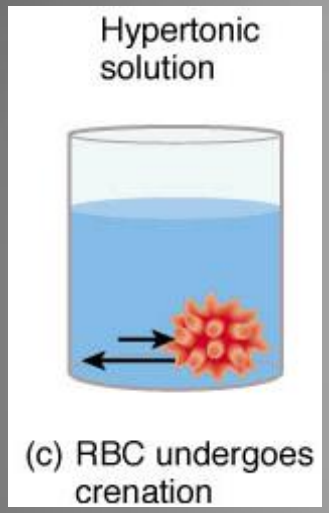
ΑΠΟΜΟΝΩΣΗ

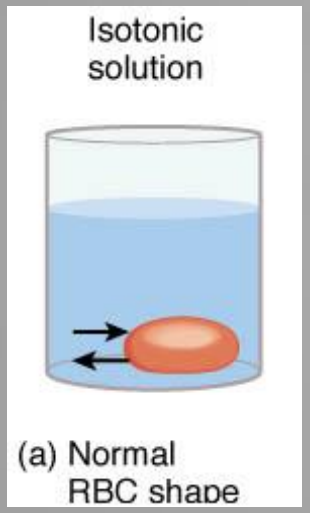
- Απομόνωση: Υποτονική αιμόλυση (20mOsm)
- Dodge et al., 1963; *Arch. Biochem. Biophys., 100:119*
- Steck et al., 1971; *Biochemistry 10:2617*
- *St*eck T.L., 1974; *J. Cell Biol., 62:1*

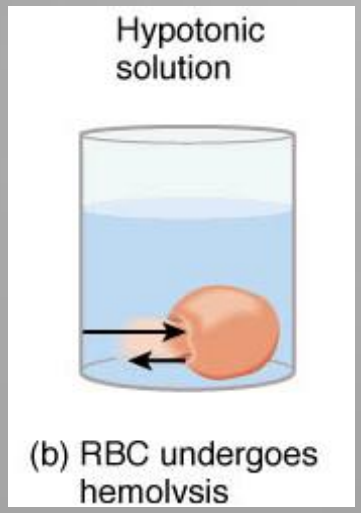
· "White ghosts"

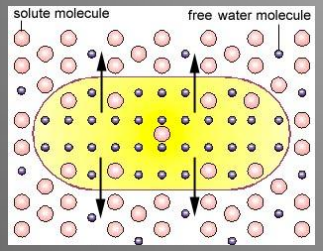


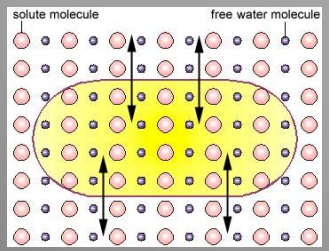
ΟΣΜΩΤΙΚΗ ΣΥΜΠΕΡΙΦΟΡΑ

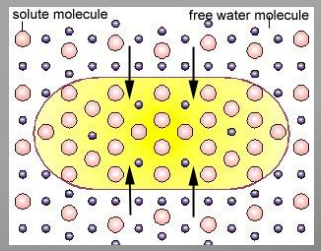


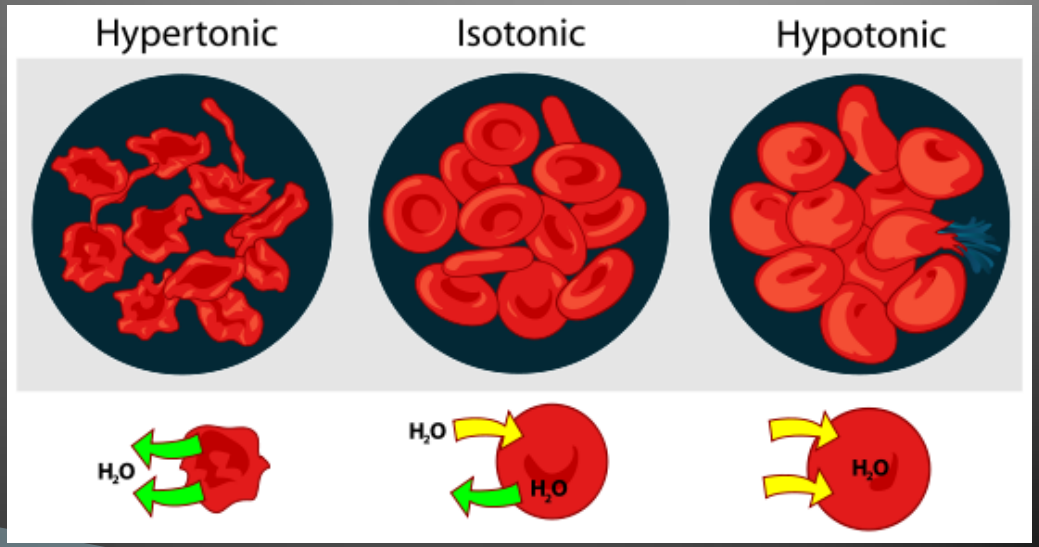








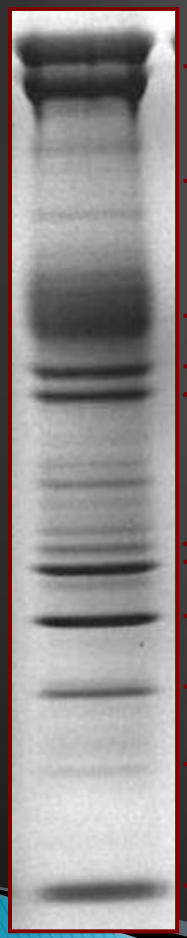




https://www.youtube.com/watch?v=OYoaLzobQmk

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

Οι πρωτεΐνες της ερυθροκυτταρικής μεμβράνης διαχωρίζονται με SDS ηλεκτροφόρηση (κατά το σύστημα Fairbanks/Laemmli) και βάφονται με τη χρωστική μπλε του Coomassie



σπεκτρίνες (1, 2) (280kd)

αγκυρίνες (2.1, 2.2, 2.3 κλπ)

Ζώνη 3 (AE1) (90-100kd)

4.1R

4.2 (παλλιδίνη) (72kd)

4.9 (δεματίνη)

5 (β-ακτίνη) (45kd)

6 (G3PD)

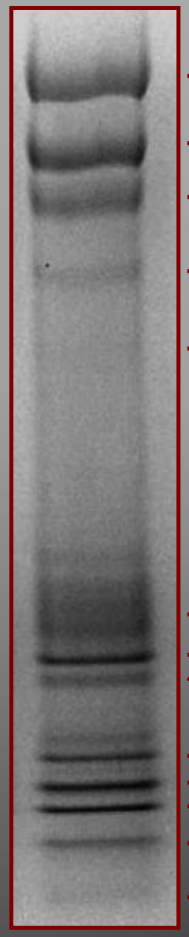
7 (7.2b στοματίνη) (32kd)

8 (?) (21kd)

αιμοσφαιρίνη (12-16kd)

ghosts

(Laemmli U.K., 1970)



αSp

βSp

ank 2.1

2.2

2.3

3

4.1

4.2

4.9

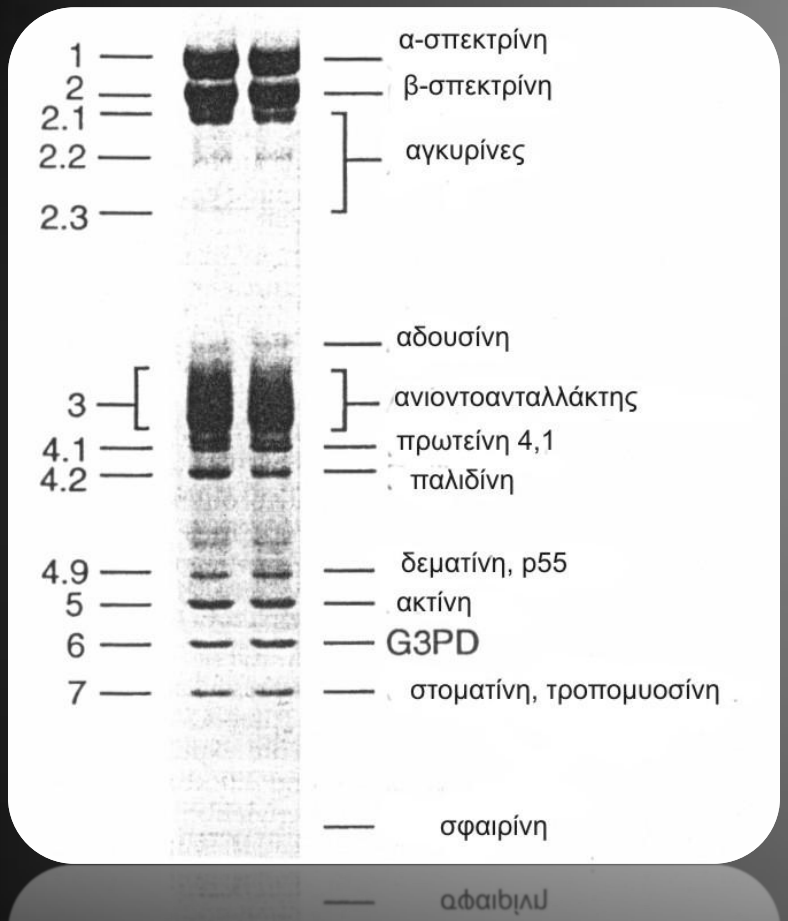
5

7

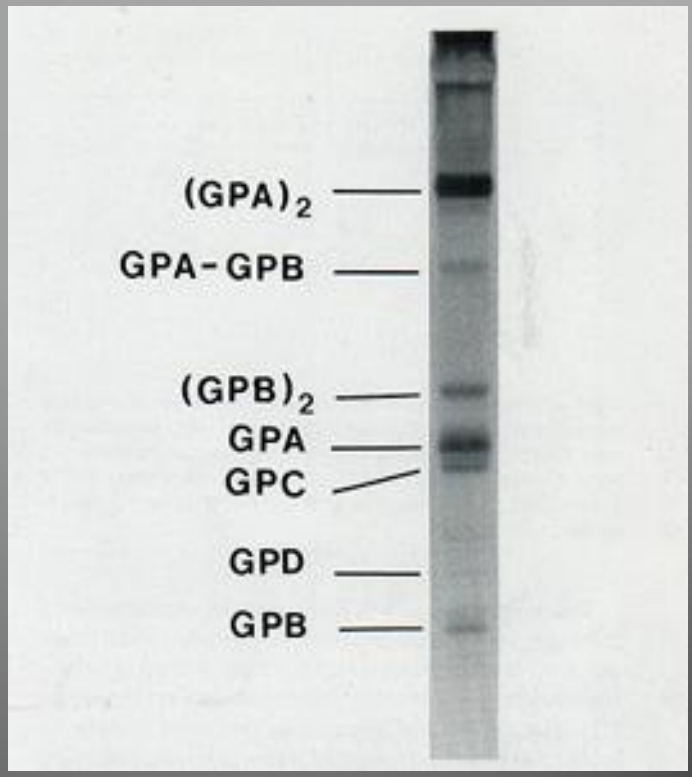
8

(Fairbanks et al., 1971)

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

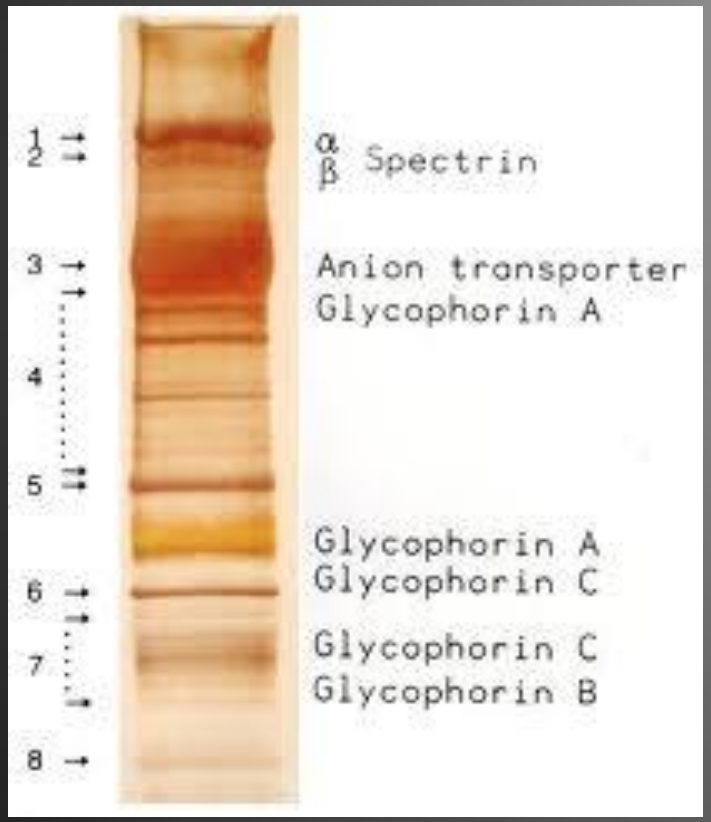


γλυκοφορίνες



Οι **γλυκοφορίνες** και οι **πρωτεΐνες Rh** προσδένουν ελάχιστα αυτή τη χρωστική και δεν γίνονται ορατές (*Costa et al., 1990*).

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ



Χρώση αργύρου

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

Band No.[a]	Protein Name	Subunit Molecular Weight	Probable State of Assembly	Number of Copies per Cell	Role
Peripheral Proteins					
1	α-Spectrin	260,000	$\alpha_2\beta_2$ tetramers	10^5 tetramers	Membrane skeleton
2	β-Spectrin	225,000			
2.1	Ankyrin	215,000	Monomer	10^5	Links skeleton to band 3
*	Adducin	105,000 / 100,000	Heterodimer	3×10^4	
4.1	—	78,000	Monomer	2×10^5	Involved in spectrin junctions
4.2	Palladin	72,000	?	2×10^5	?
4.9	Demantin	48,000	Trimer?	5×10^4	Involved in spectrin–actin interaction
5	Actin	43,000	Oligomers of 12–17 units	5×10^5	Involved in spectrin junctions
*	Tropomyosin binding protein	43,000	Monomer	3×10^4	Binds tropomyosin
6	Glyceraldehyde-3-phosphate dehydrogenase	35,000	Tetramer	5×10^5	Glycolytic enzyme
*	Tropomyosin	29,000 / 27,000	Heterodimer	7×10^4	Binds to actin
7	—	29,000	?	5×10^5	?
8	—	23,000	?	10^5	?
Integral Proteins					
3	—	89,000	Dimer + tetramers	10^6 dimers	Anion channel
4.5		55,000	?	1.5×10^6	Glucose transport
	Glycophorin A	31,000	Dimer	4×10^5	Cell recognition
	Glycophorin B	23,000	?	$\sim 10^5$	Cell recognition
	Glycophorin C	29,000	?	$\sim 10^5$	Linkage to 4.1?

ource: Most of the data are from V. Bennett, *Annu. Rev. Biochem.* (1985) 54:273–304.

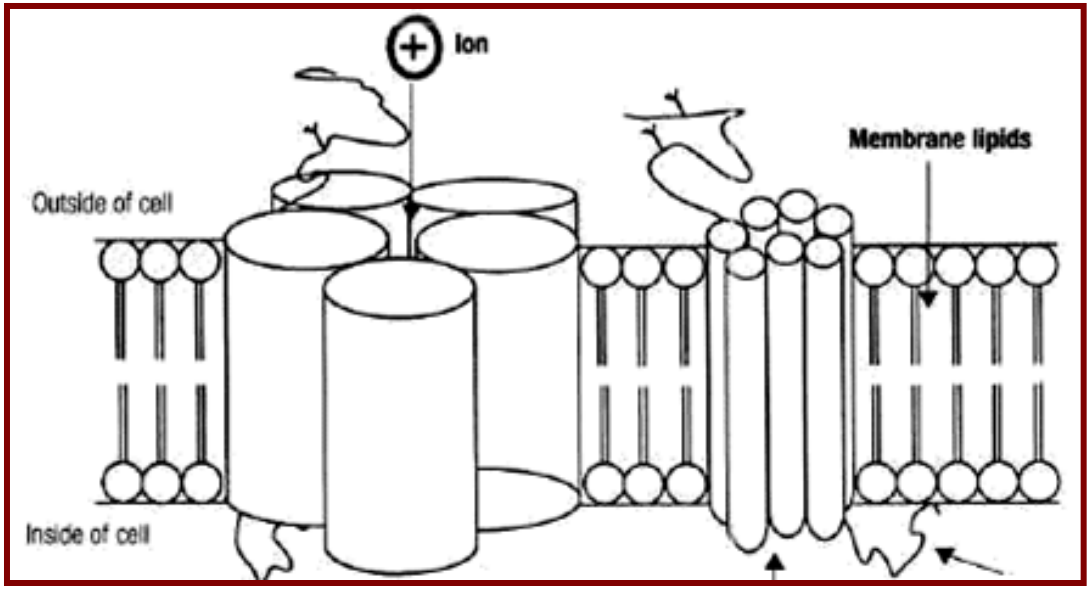
Band numbers correspond to those in Figure 10.17. The glycophorins do not stain well with protein ains but can be detected by carbohydrate-specific stains.

Components that do not constitute major bands on gels but have demonstrable roles in membranes.

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

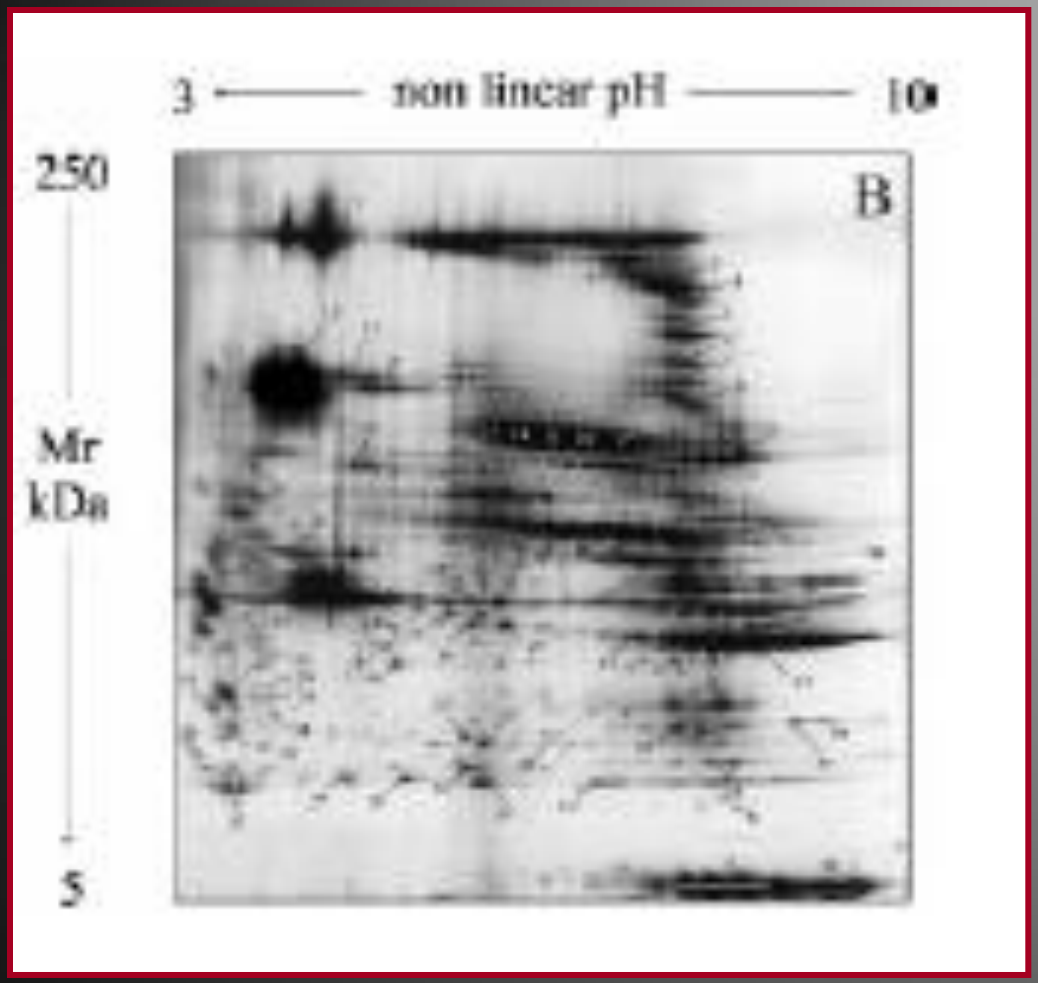
Πρωτεΐνες	Αριθμός αμινοξέων	MB σε KD	Μονομερή ανά RBC	Γονίδια και χρωμοσωμική θέση	Μέγεθος γονιδίων	exons	Μέγεθος mRNAs kb
Σπεκτρίνη α-αλυσίδα	2.429	281	242.000	SPTA1, 1q21	80	52	8.0
Σπεκτρίνη β-αλυσίδα	2.137	246	242.000	SPTB, 14q23-q24.1	>100	36	7.0
Αγκυρίνη	1.880	206	124.000	ANK1, 8p11.2	>120	42	6.8, 7.2
Ζώνη-3	911	102	1.200.000	SLC4A1, 17q21-q22	17	20	4.7
Πρωτεΐνη 4.1	588	66	200.000	EPB41, 1p36.2-p34	>250	>23	5.6
Πρωτεΐνη 4.2	691	77	200.000	EPB42, 15q15	20	13	2.4

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ



Οι πρωτεΐνες της ερυθροκυτταρικής μεμβράνης έχουν μεγάλο βαθμό συγκρότησης/ολιγομερισμού

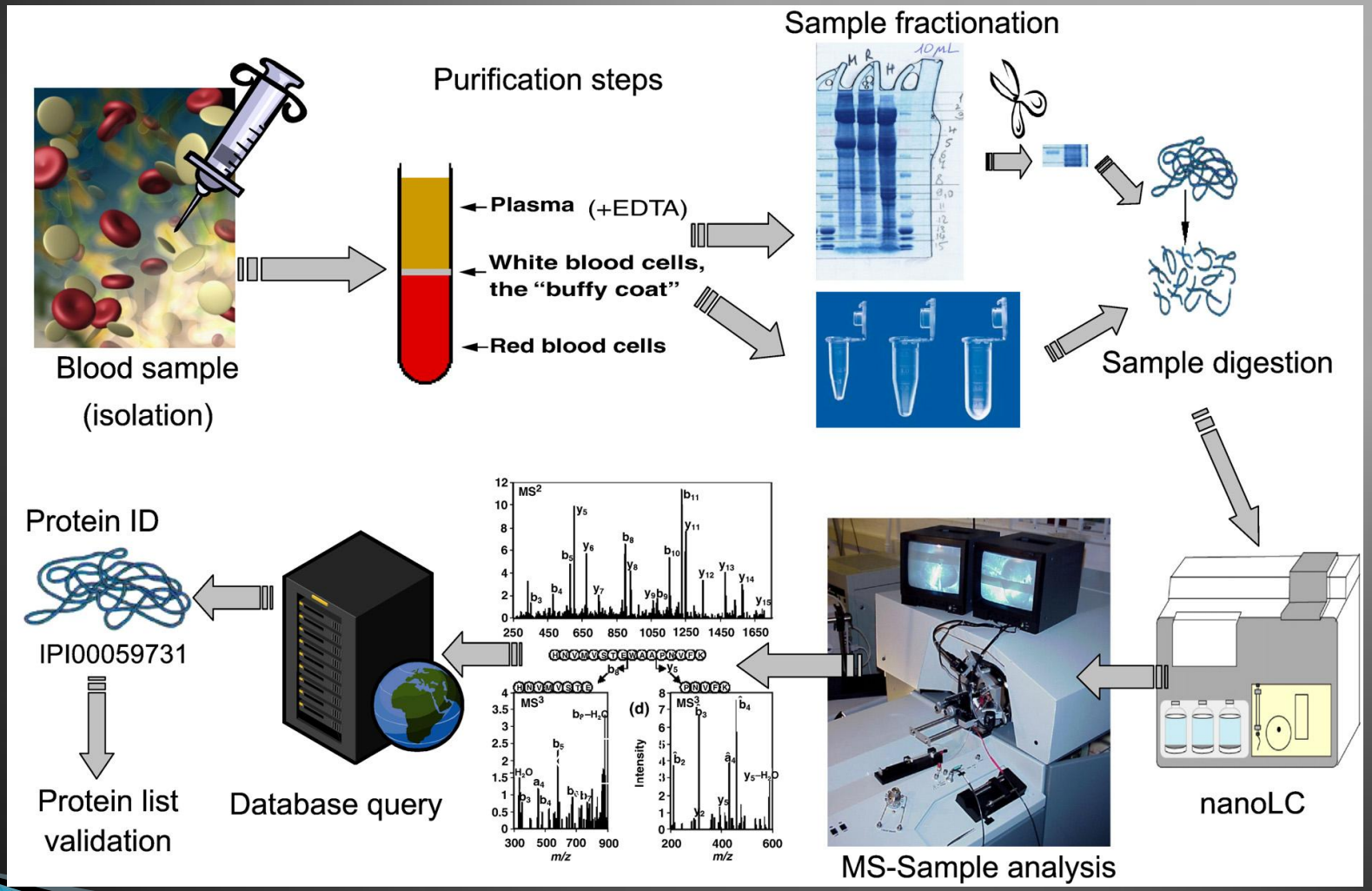
ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ



> 500 spots

(Bruschi et al., 2005; *J Proteome Res., 4:1304*)

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ



(Pasini et al., Journal of Proteomics 2010)

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

Proteins identified in RBC membrane fractions

No.	Protein description	Molecular mass (Da)	Gi Number	Sequence coverage (%)	No. of identified peptides
1	Spectrin α chain, erythrocyte	279,916.5	1174412	48.0	77*
2	Spectrin β chain, erythrocyte	246,468.1	17476989	48.0	76*
3	Ankyrin 1, splice form 2	206,067.9	105337	45.0	55
4	Ankyrin 1, isoform 4, erythrocytic	203,416.6	10947036	45.0	50
5	Ankyrin 1, isoform 2, erythrocytic	189,011.2	10947042	46.0	48
6	Similar to ankyrin 1	206,264.8	13645508	51.0	46
7	Protein band 4.2, erythrocytic	79,946.5	107446	33.0	21
8	Protein band 4.1 (elliptocytosis 1, RH-linked)	66,398.5	4758274	45.0	17
9	Protein band 3, erythrocytic	101,792.3	4507021	28.0	17
10	Protein band 4.1, erythrocytic	97,016.9	14916944	32.0	16
11	Actin β chain	41,812.8	481515	47.0	12*
12	Flotillin 1, erythrocytic	47,355.3	5031699	47.0	12
13	Membrane protein p55, erythrocytic, (palmitoylated)	52,296.5	4505237	35.0	11
14	Flotillin 2	47,142.3	18587629	29.0	11
15	Protein band 4.9 (dematin), erythrocytic	45,514.4	13623437	40.0	10
16	Protein band 7.2b, stomatin	32,598.5	1103842	47.0	10
17	Glyceraldehyde-3-phosphate dehydrogenase	36,054.2	31645	51.0	10
18	Tropomyosin 3, cytoskeletal	29,032.7	136096	55.0	10
19	Solute carrier family 2 (facilitated glucose transporter), member 1	54,117.8	5730051	13.0	6
20	Similar to flotillin 2	42,565.9	13277550	15.0	6
21	Tropomyosin isoform	28,420.1	1082876	36.0	6
22	Glucose transporter glycoprotein	37,879.6	3387905	17.0	5
23	Tropomyosin α chain (smooth muscle)	26,576.7	136100	37.0	5
24	Actin α 2, aortic smooth muscle	42,108.1	1070613	20.0	5
25	Adducin α subunit, erythrocyte	80,955.1	12644231	10.0	5*
26	Rabphilin-3 A-integrating protein	80,858.2	1082757	8.0	5*
27	C-1-tetrahydrofolate synthase, cytoplasmic	101,559.2	115206	6.0	4
28	Translation initiation factor 2C, 2	66,252.2	18570004	10.0	4
29	Aldolase A	39,288.8	229674	17.0	4
30	Tropomodulin	40,569.2	4507553	16.0	3*
31	RAP2B, member of RAS oncogene family	20,504.4	11433346	43.0	3
32	Arginase type 1 erythroid variant	35,664.1	18535612	12.0	3
33	Arginase type 1	34,734.9	10947139	12.0	3
34	Creatine kinase, muscle	43,101.1	14763181	21.0	3
35	B-CAM protein	63,566.7	2134798	8.0	3
36	ATP-binding cassette half-transporter	99,712.3	11245444	5.0	2
37	RAP1A, member of RAS oncogene family or RAP1B	20,987.1 20,824.7	4506413 7661678	14.1 14.1	2 2
38	Calcium transporting ATPase 4	137,920.2	14286105	2.5	2
39	Rh blood D group antigen polypeptide	45,136.5	10800054	4.0	2
40	Channel-like integral membrane protein	16,239.5	1314306	15.0	2
41	Glycophorin A precursor	16,429.6	1070639	21.0	2
42	Solute carrier family 29 (nucleoside transporter), member 1	50,219.4	4826716	3.5	2
43	Glycophorin A	14,784.8	106140	23.0	2
44	Glutathione transferase	27,053.4	809436	19.0	2
45	Glycophorin C, isoform 1	13,810.6	4504229	20.0	1
46	Aquaporin 1	28,526.0	4502177	7.0	1
47	Erythroblast membrane-associated protein	52,604.8	17489129	3.0	1
48	Similar to glycophorin A	16,371.6	13529077	20.0	1
49	Cell surface glycoprotein CD44	39,433.8	7512338	4.0	1
50	Vesicle-associated membrane protein 2 (synaptobrewin 2)	12,648.7	7657675	15.0	1
51	Similar to adhesive plaque matrix protein precursor	106,879.1	17481669	1.9	1
52	Poly (A)-specific ribonuclease	73,451.0	4505611	3.0	1
53	Similar to RAS-related protein RAL-A	23,566.8	14740792	7.0	1
54	Presenilin-associated protein	39,862.4	6409316	6.0	1
55	Duodenal cytochrome *b*	31,611.2	13376257	3.5	1
56	bA421H8.2 (novel protein)	16,743.7	17402228	9.0	1
57	Similar to RAS-related protein RAB-15	23,517.9	18596861	5.0	1
58	CD59antigenp18–20	17,067.4	17473237	5.0	1
59	Rhesus D category VI type III protein	45,247.7	2765839	1.9	1
60	RAB 35, RAS oncogene family	23,025.2	5803135	6.0	1
61	Ral A binding protein	76,063.4	5803145	2.1	1
62	Hypothetical protein XP_100510	8,049.3	18577723	16.0	1
63	ATP-binding cassette, subfamily C, member 6	164,904.4	6715561	0.9	1
64	Phosphoribosyl pyrophosphate synthetase	34,834.2	4506127	7.0	1
65	Unknown protein	46,884.2	18089137	3.6	1
66	Similar to Lutheran blood group	59,287.7	18589892	3.1	1
67	Phosphatidylinositol-4-phosphate 5 kinase, type III	46,078.6	1730569	4.4	1
68	Hypothetical protein XP_100665	35,877.6	18604339	7.0	1
69	Hypothetical protein XP_100619	18,567.6	18604359	15.0	1
70	Block of proliferation 1	83,629.5	23830903	1.5	1
71	Similar to tropomyosin	10,804.3	18590249	13.0	1
72	Hypothetical protein XP_061743 or XP_089854	48,719.0 31,487.9	17472555 18577194	2.5 4.0	1 1
73	Hypothetical protein XP_106269	12,703.8	18558481	22.0	1
74	Hypothetical protein XP_100925	22,863.4	18601384	8.0	1
75	Zona pellucida binding protein	40,169.8	5902116	3.4	1
76	2′,3′-cyclic-nucleotide 3′-phosphodiesterase	4,242.9	7435185	60.0	1
77	Lyn B protein	56,033.3	2117805	4.3	1
78	KIAA0340	117,819.0	2224621	2.1	1
79	Hypothetical protein XP_091724	144,900.8	18588504	1.1	1
80	Hypothetical protein XP_091430	27,641.2	18586430	6.0	1*
81	Similar to tropomyosin 4	18,426.8	14729747	6.0	1*
82	HGTD-P	17,342.4	9295192	10.0	1*
83	Hypothetical protein XP_095819	291,206.1	18572484	0.6	1*
84	Far upstream element binding protein	67,534.4	1082624	2.5	1*
85	Hypothetical protein XP_103707	13,374.6	18551195	12.0	1*
86	Hypothetical protein XP_092517	41,409.4	18552304	2.6	1*
87	Enhancer protein	41,289.8	1345400	4.6	1*
88	Hypothetical protein	15,770.3	18551736	12.0	1*
89	KIAA1741 protein	123,305.7	12698027	1.7	1*
90	Ig heavy chain V-V region	10,995.4	87863	16.0	1*
91	DC 38	31,691.4	12005984	4.7	1*

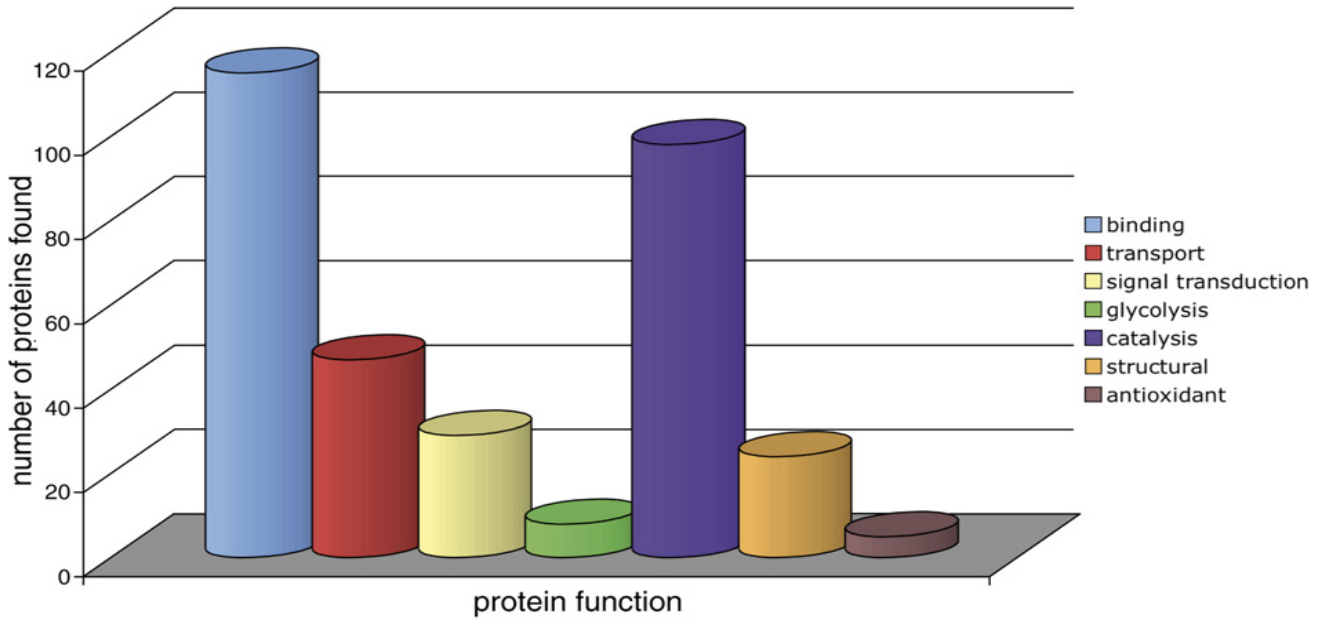
*, The proteins found primarily in the low-ionic-strength spectrin extract from RBC membranes.

(Kakhniashvili et al., 2004; *Mol Cell Proteomics, 3:501*)

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ

(Pasini et al., Blood 2006): 340 membrane proteins and 252 soluble proteins

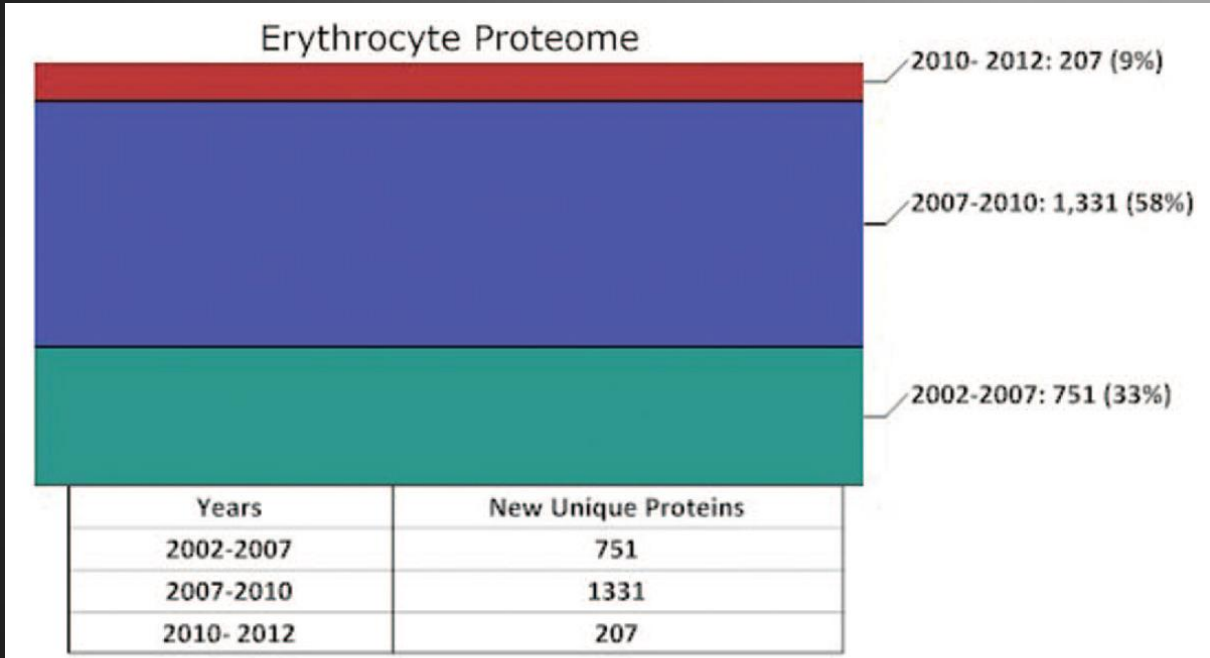
(Goodman et al., Exp.Biol.Med 232:1391, 2007): 751 RBC membrane proteins)



integral membrane
membrane-associated
GPI-anchored
Extracellular
cytoskeletal proteins

Binding (115), catalytic activity (98), transporters (47), signal transducer (29), structural activity (24) (small GTPases the most numerous intracellular signaling proteins in RBCs)

ΠΡΩΤΕΪΝΙΚΗ ΣΥΣΤΑΣΗ



Years	New Unique Proteins
2002-2007	751
2007-2010	1331
2010- 2012	207

Figure 1 Growth of our knowledge of the erythrocyte proteome from 2002 to 2012

the number of unique proteins has grown from 751 in 2007 to 2289 as of 2013

Goodman et al., Exp Biol and Med 238:509; 2013

5 ΔΕΚΑΕΤΙΕΣ ΜΕΛΕΤΗΣ

αφθονία, προσβασιμότητα

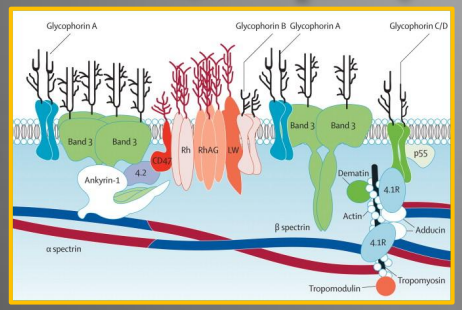
νέες πρωτεϊνες, συμπλέγματα, δομές

καθαρότητα

μη-ερυθροειδή κύτταρα

κληρονομικές μεμβρανοπάθειες

μη-αιματολογικοί κλινικοί φαινότυποι



κοινές αιματολογικές (και μη-) νόσοι

ωρίμανση, γήρανση, κυτταρική απόσυρση

εξωκυττάρια κυστιδιοποίηση

αποτελεσματικότητα μεταγγίσεων

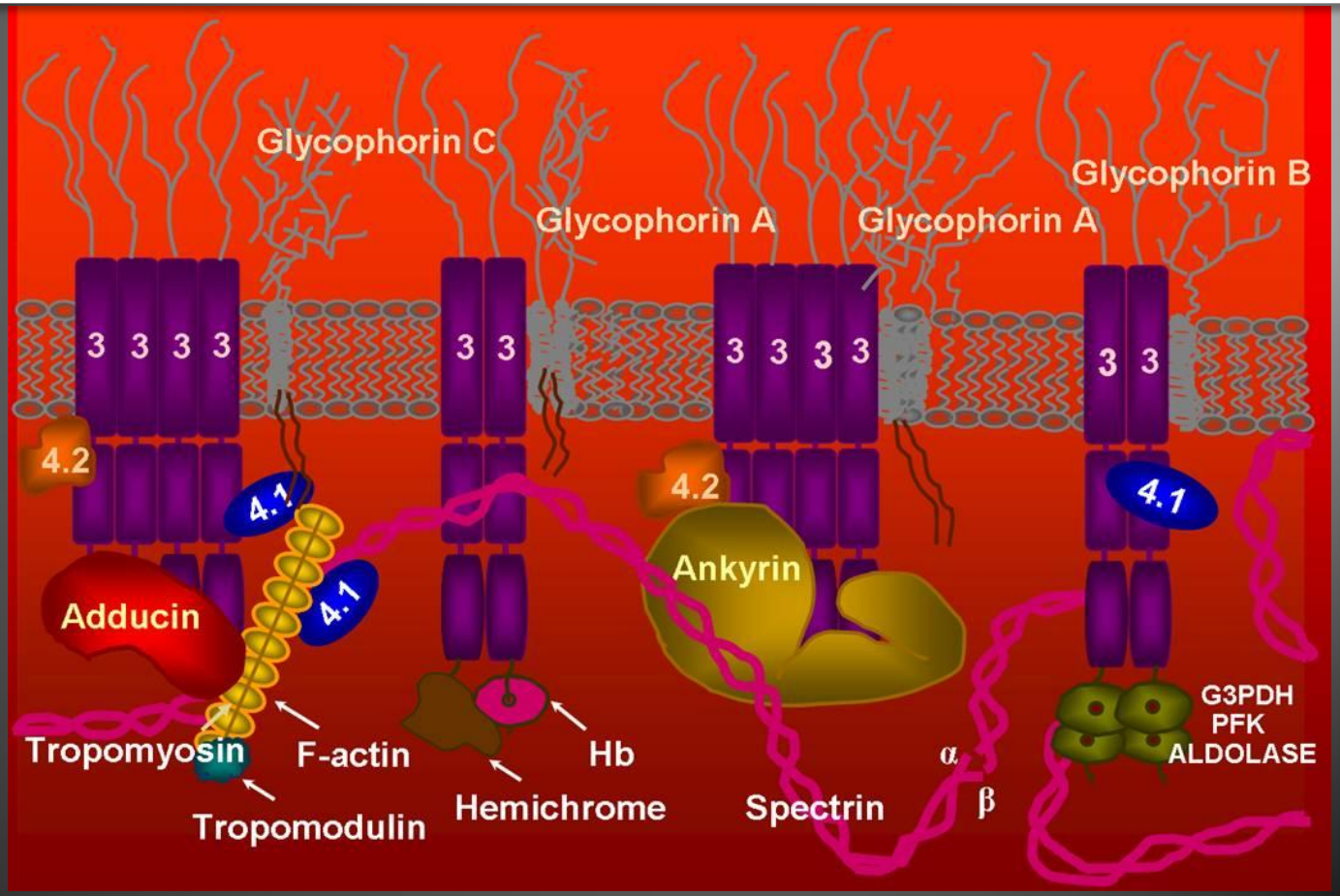
Lipid rafts

αρχετυπικό σύστημα μελέτης

ΠΚΘ, απουσία πυρήνα, μιτοχονδρίων

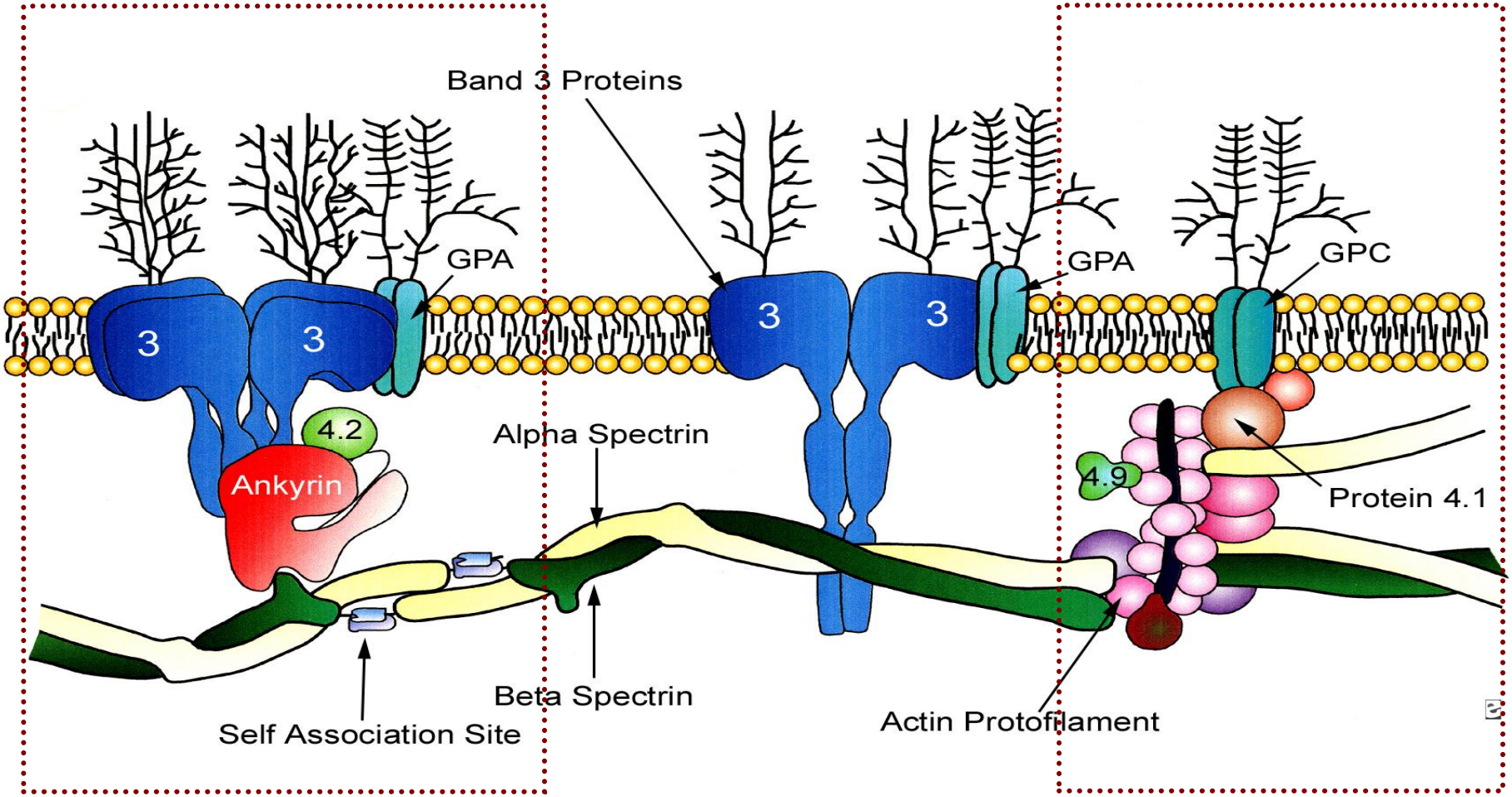
ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

The RBC bilayer is crowded with the transmembrane proteins needed for its performance, e.g., for sustaining its osmotic balance and for exchanging anions. Most of these proteins or their complexes are linked with the skeleton

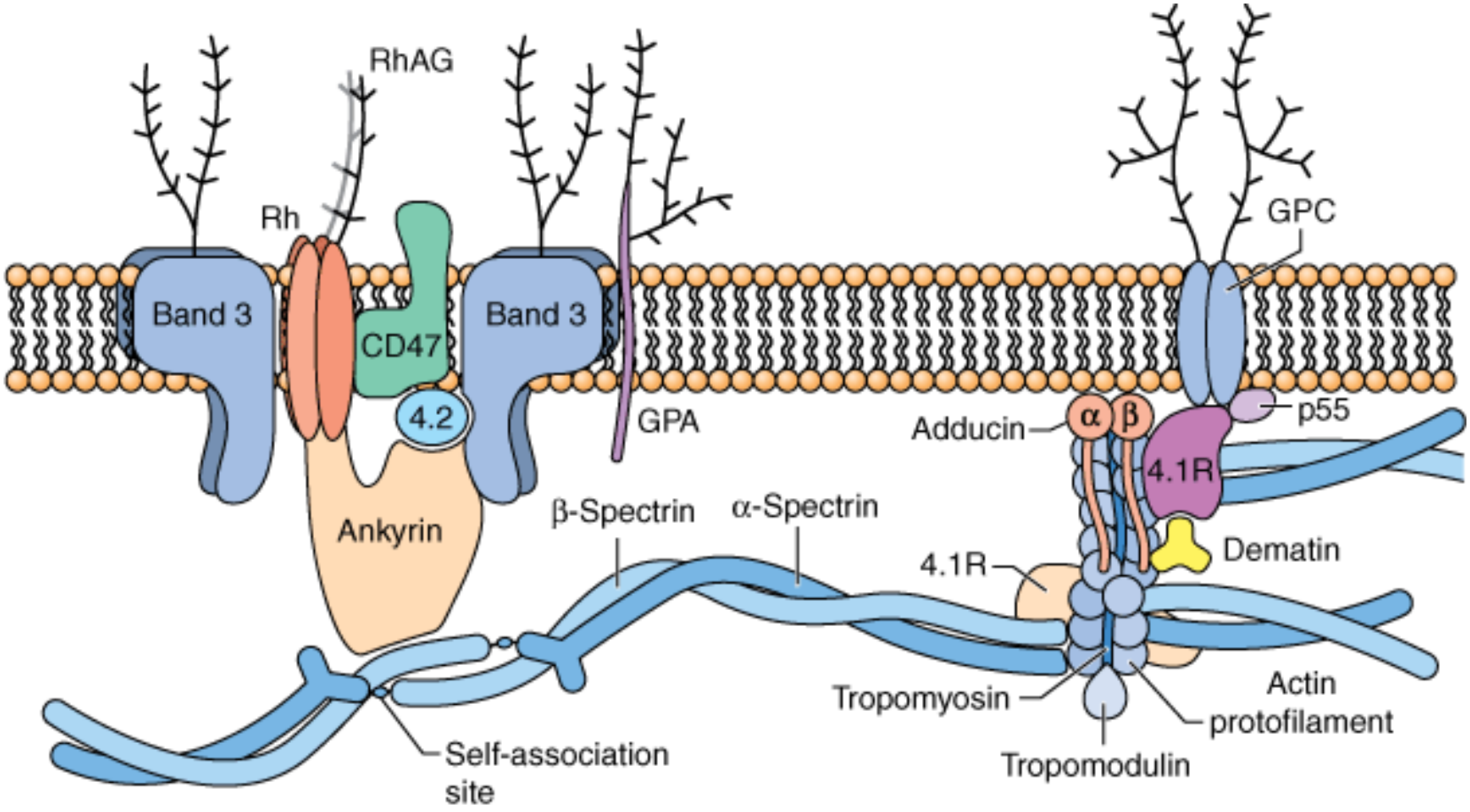


Συνεκτικότητα διπλοστοιβάδας-σκελετού, **ακεραιότητα** μεμβράνης

(απώλεια επιφάνειας, κυστιδιοποίηση, fragmentation, HS)

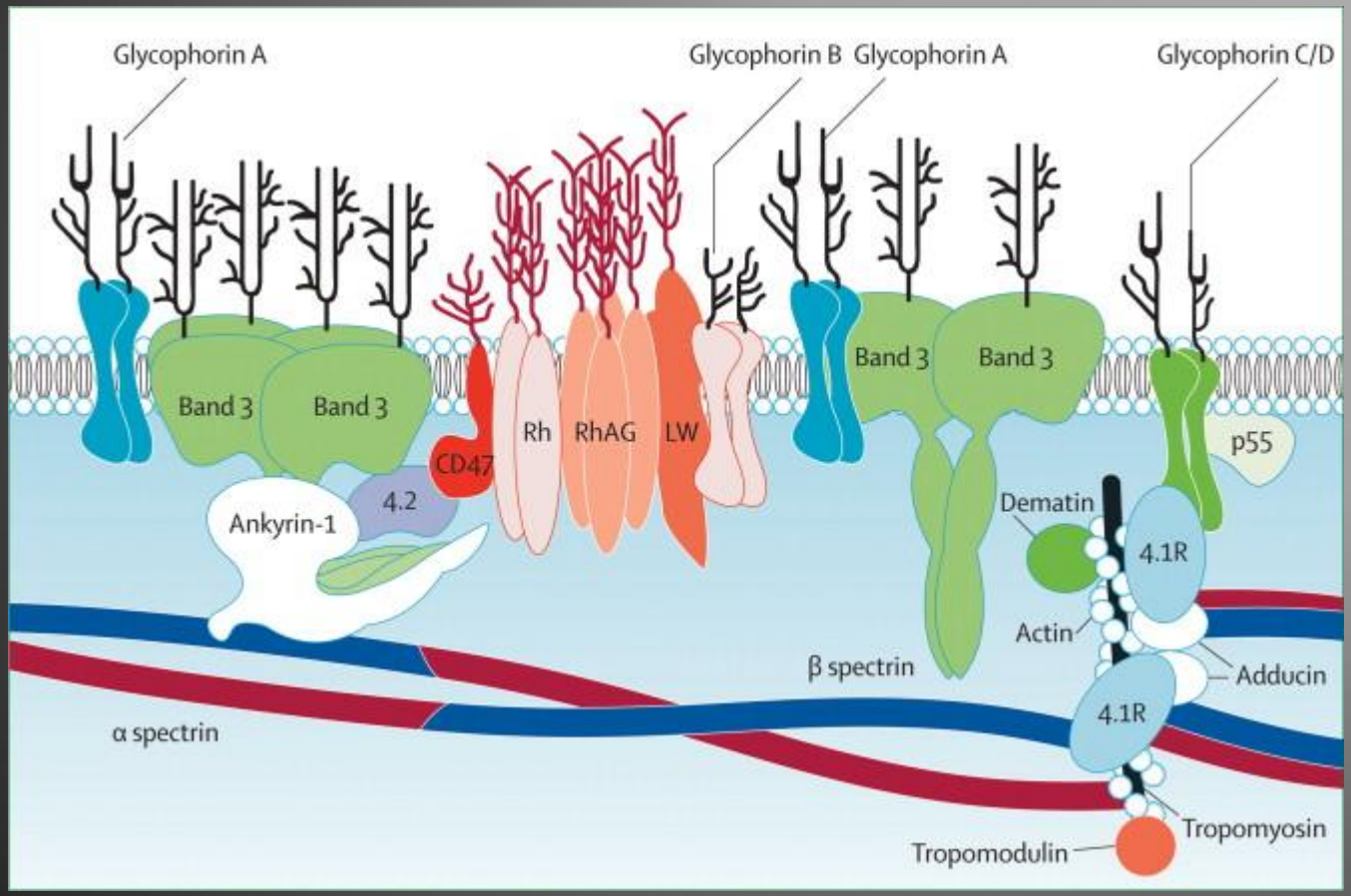


Μηχανική σταθερότητα μεμβράνης (GpC-/- RBCs)



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

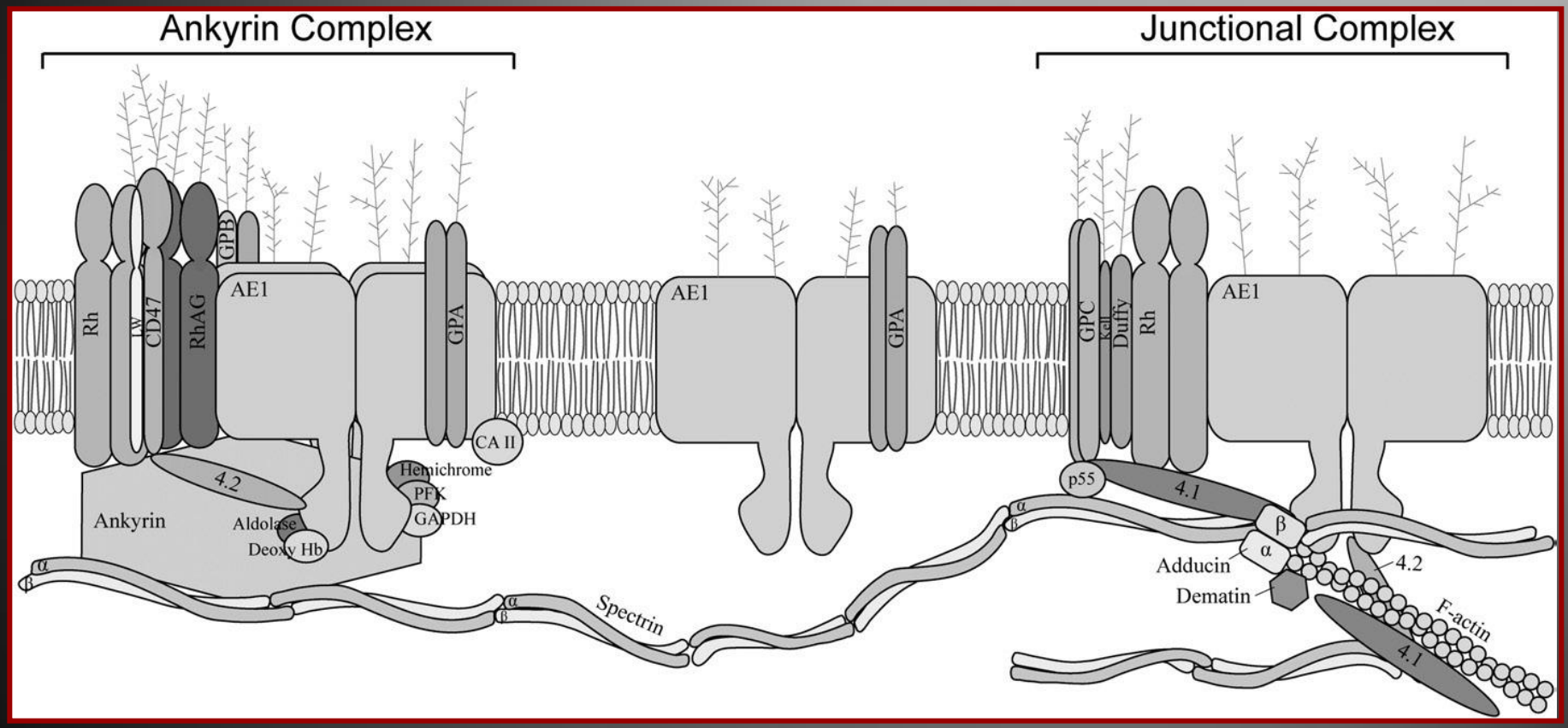
ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ



Lateral and vertical interactions

The 4.1R complex: band 3 dimers, blood group antigens (Rh, Duffy, Kell, XK) and glycophorin C (GPC)

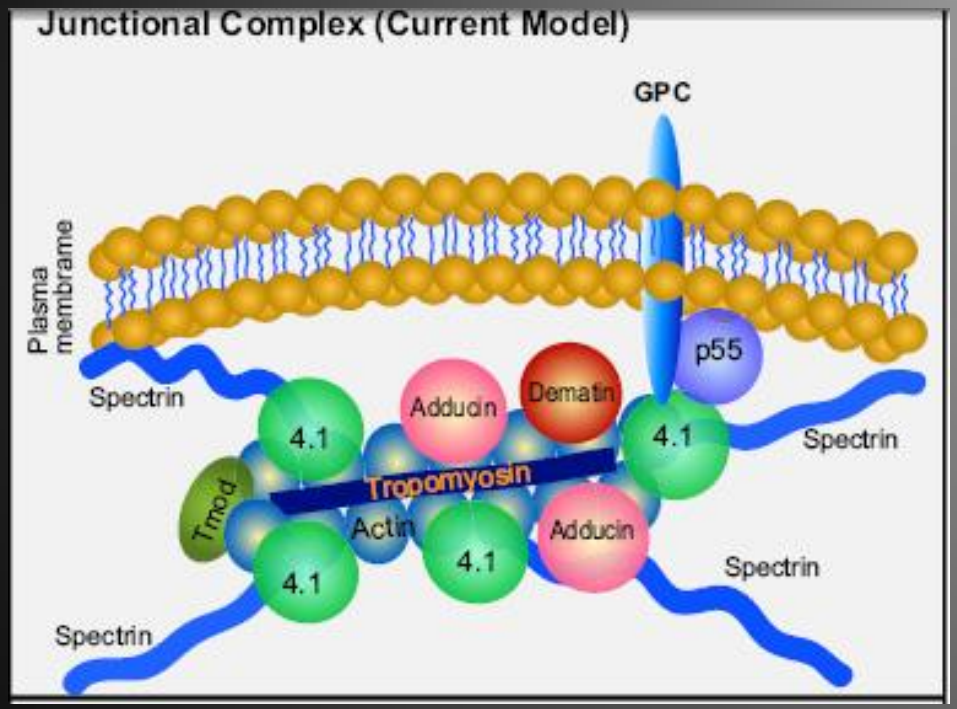
The GPC, Rh, Duffy, and XK interact directly with 4.1R while band 3 binds to adducin

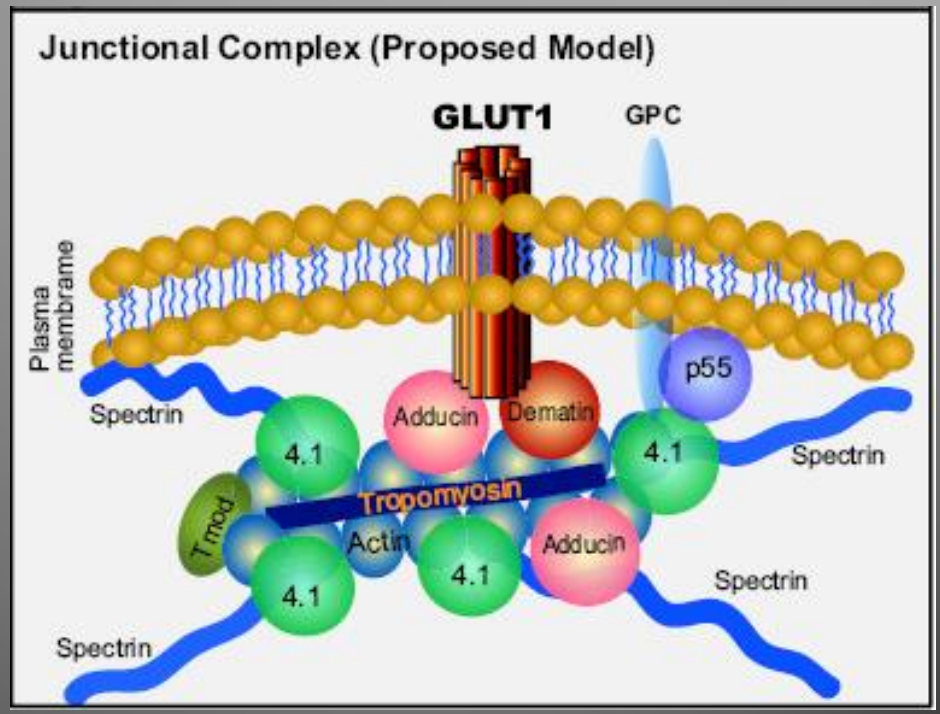


Band 3 multiprotein interactions in the human erythrocyte membrane
(Salomao et al. PNAS USA 105:8026, 2008)

ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

The Dematin–Adducin –GluT1 bridge





The unusually high abundance of GLUT1 in human RBCs, and its rapid glucose transport kinetics that far exceeds the physiological sugar requirement of the RBC, has raised the possibility that GLUT1 and other related transporters may in fact perform a structural rather than the transport function in adult RBC

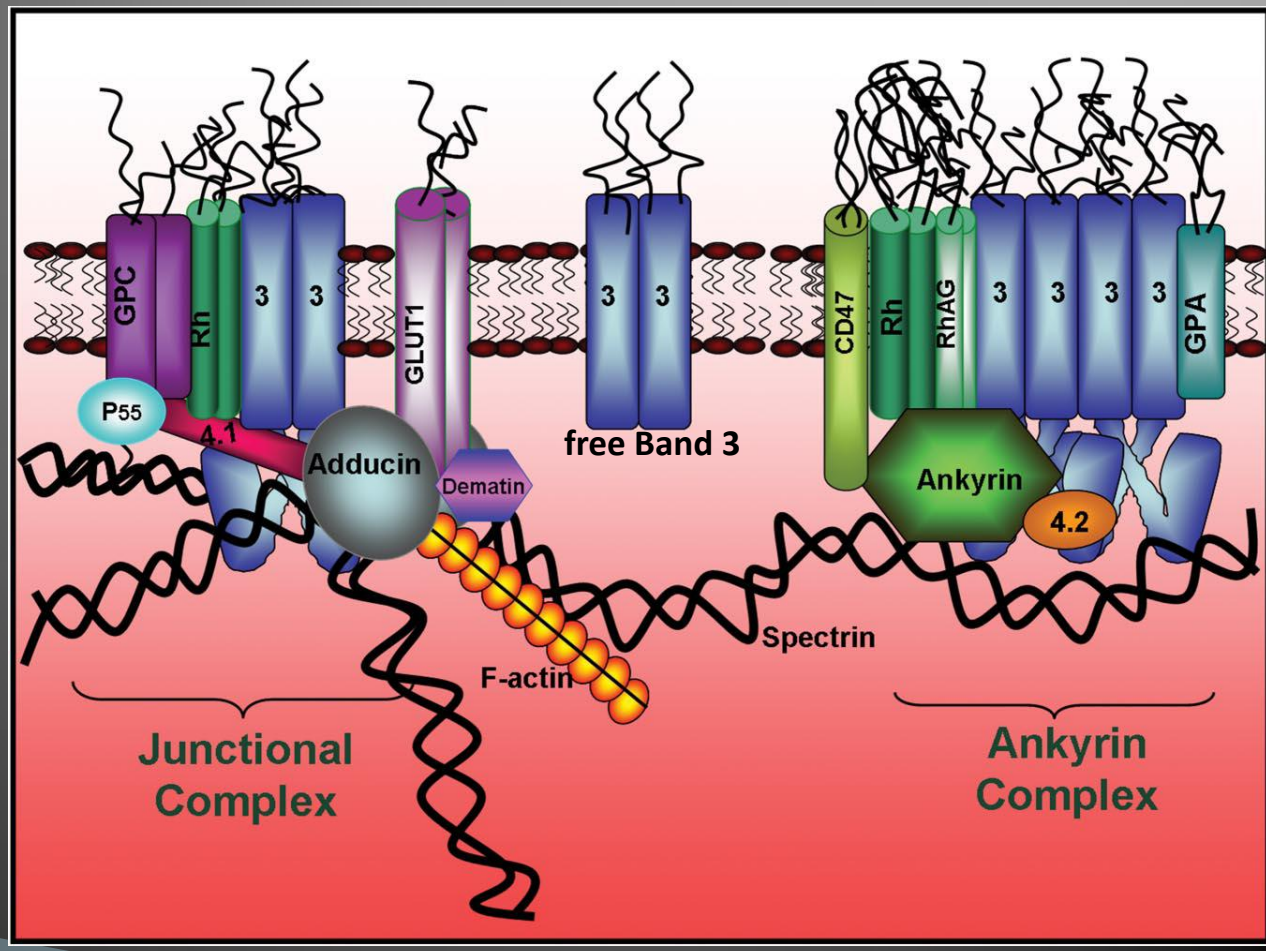
(Khan et al., 2008 J Biol Chem 283:14600)

ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

The Band 3- Adducin -Spectrin bridge

Adducin
(actin capping protein)

**(binds Sp,
facilitates Sp-actin
directly binds to B3 ⇒
Attaches JC to membrane)**



(Anong et al., 2009 BLOOD 114(9):1904)

ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

Blood Groups Ags

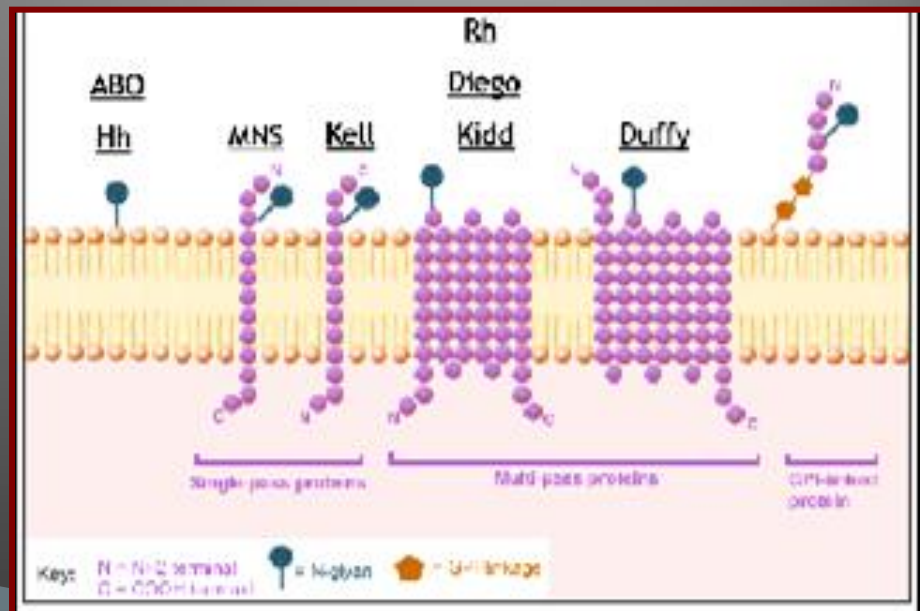
30 blood group systems recognized on human RBCs

5 systems Ags: carbohydrate structures (on glycoproteins and glycolipids, ABO-H Bombay phenotype (AgH-/anti-H)

Ags of two systems (LE, CH／RG) are not intrinsic to the RBCs but acquired from plasma.

Ags of 23 systems: defined by the protein sequence of RBCs membrane proteins.

Band 3 / AE1: **Diego system**
GpA,B: **MNS system**. GpC/D: GE system
Rh polypeptides (C,c,D,E,e)
RhAG (Rh-associated glycoprotein)
AQP1: **CO system** Ags C0(a) and Co(b)



ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

Rh blood group system

49 antigens
(D- RHD gene/RhDprotein)
C, c, E, and e (RHCE gene/RhCE protein)

Rh positive or negative refer to the D antigen only (RhD gene)- Το Ag-D είναι περισσότερο **ανοσογόνο** από όλα τα non-ABO antigens

Rhesus complex: RhD, RhCE, RhAG proteins

In contrast to the ABO blood group (IgM φυσικά)- the Rh Abs are IgG antibodies which are acquired through exposure to Rh-positive blood (ασύμβατη κύηση ή μετάγγιση-immunization against Rh only occur under first Ag-contact)

Rh null phenotype: ΚΛΙΝΙΚΗ ΚΑΤΑΣΤΑΣΗ-no Rh Ags (Rh or RhAG)-structural abnormalities in RBCs (stomatocytosis?), αποσταθεροποίηση μεμβράνης-hemolytic anemia

ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

Rh complex

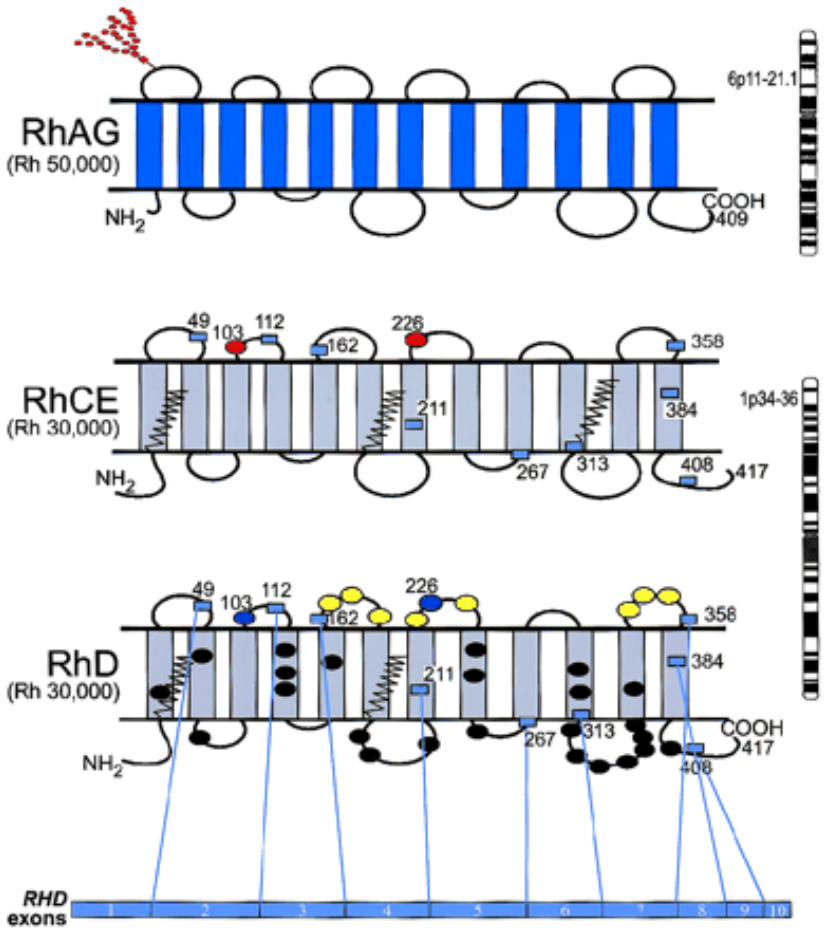
- **RhAG (channel for neutral gases NH3 and CO2)**
  (Overhydrated hereditary stomatocytosis)

- **tetramer of Rh polypeptides (D and CE)** (no transport proteins, but facilitate the correct assembly of the other transport proteins in the membrane)
- **CD47**
- **LW**
- **glycophorin B**

Genes/proteins: RhAG, RhCE, and RhD

RhAG: 409 aa, MW 50kDa, RHAG gene, 6p11-p21.1

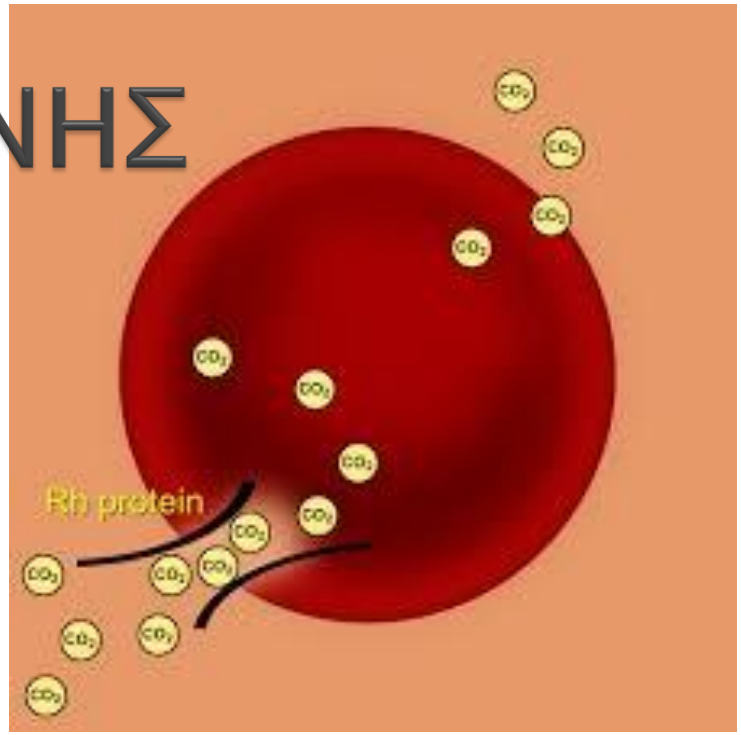
RhCE and RhD: MW 30 kDa, RHCE and RHD adjacent genes, 1p34-p36



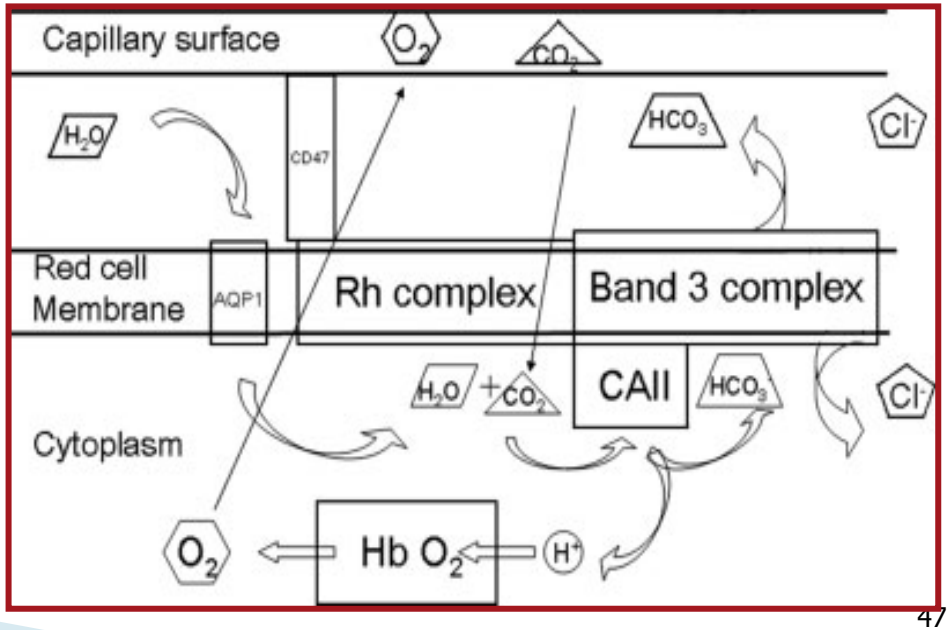
(Avent and Reid, Blood 95(2):375; 2000)

ΔΟΜΗ ΤΗΣ ΜΕΜΒΡΑΝΗΣ

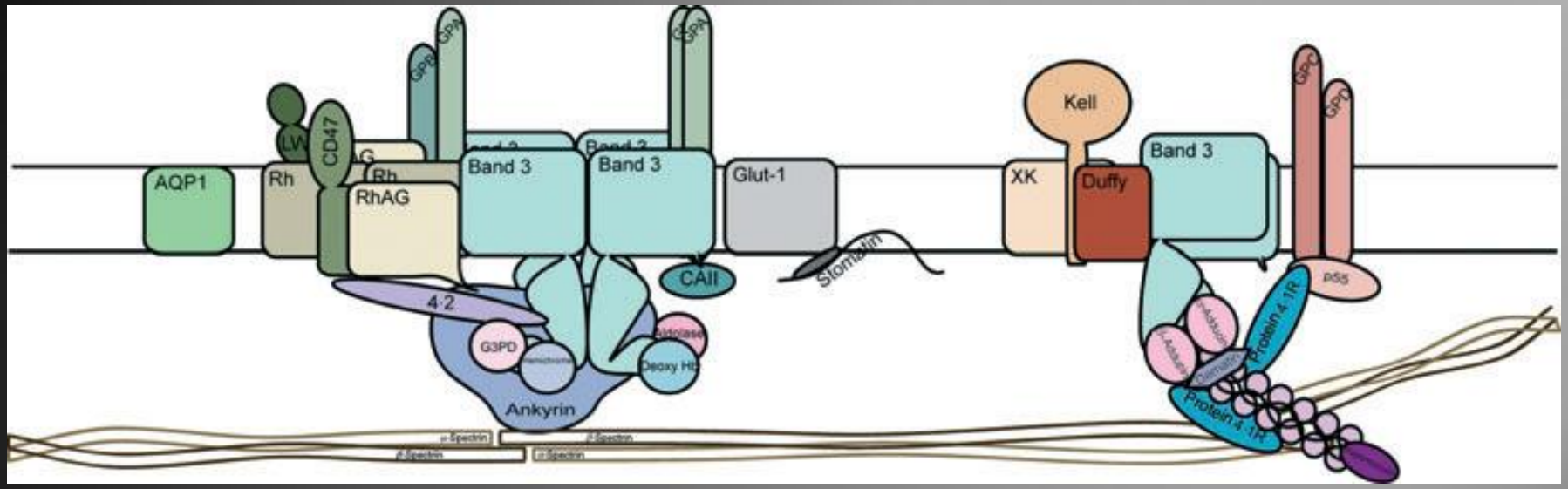
Rh complex



Rh proteins function as relatively **nonspecific channels** for **neutral small molecules** and might act as **gas channels** for oxygen and carbon dioxide, ammonium etc

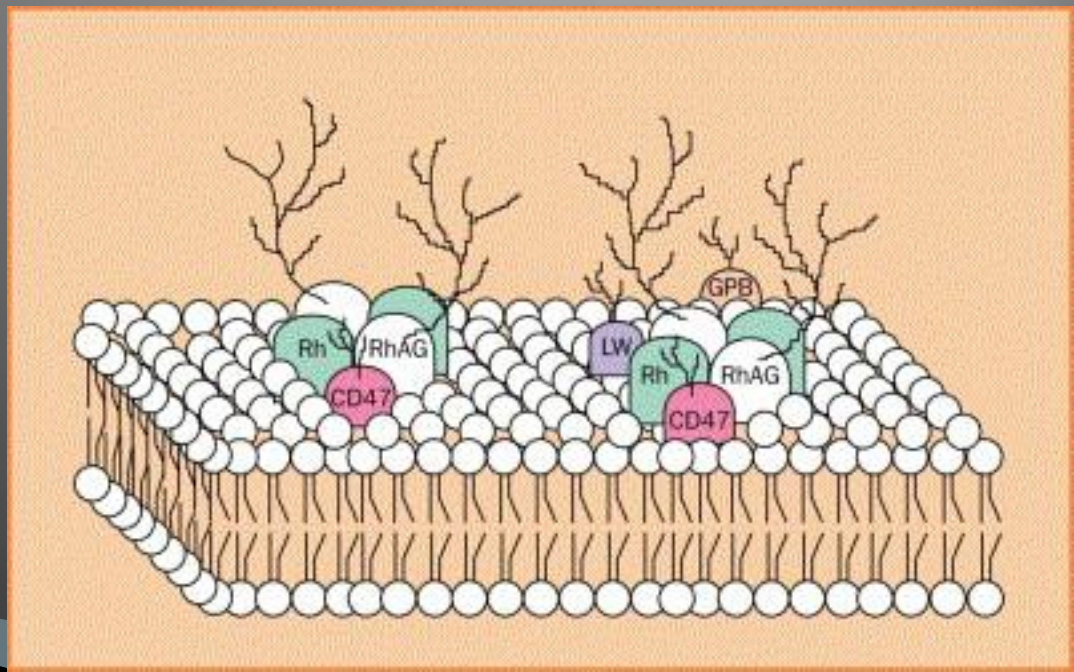


B3-Rhesus macrocomplex

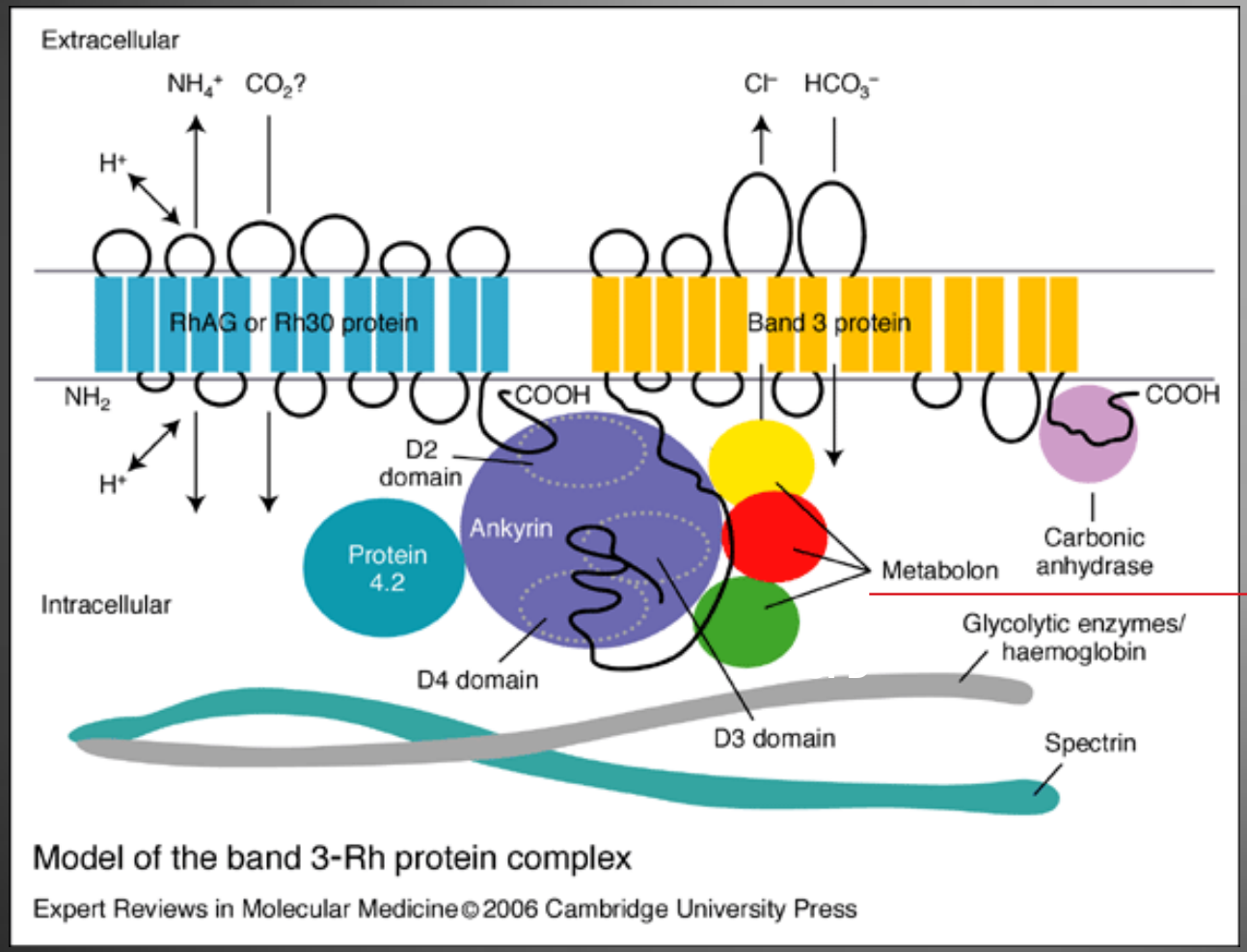


4.2↓→ ↓CD47, RhAG glycozyl.

B3-mut→ ↓4.2, ↓CD47, Rh, RhAG



B3-Rhesus macrocomplex



-ATP

RBC metabolism, ion & gas transport function

ΜΕΜΒΡΑΝΙΚΕΣ ΠΡΩΤΕΪΝΕΣ

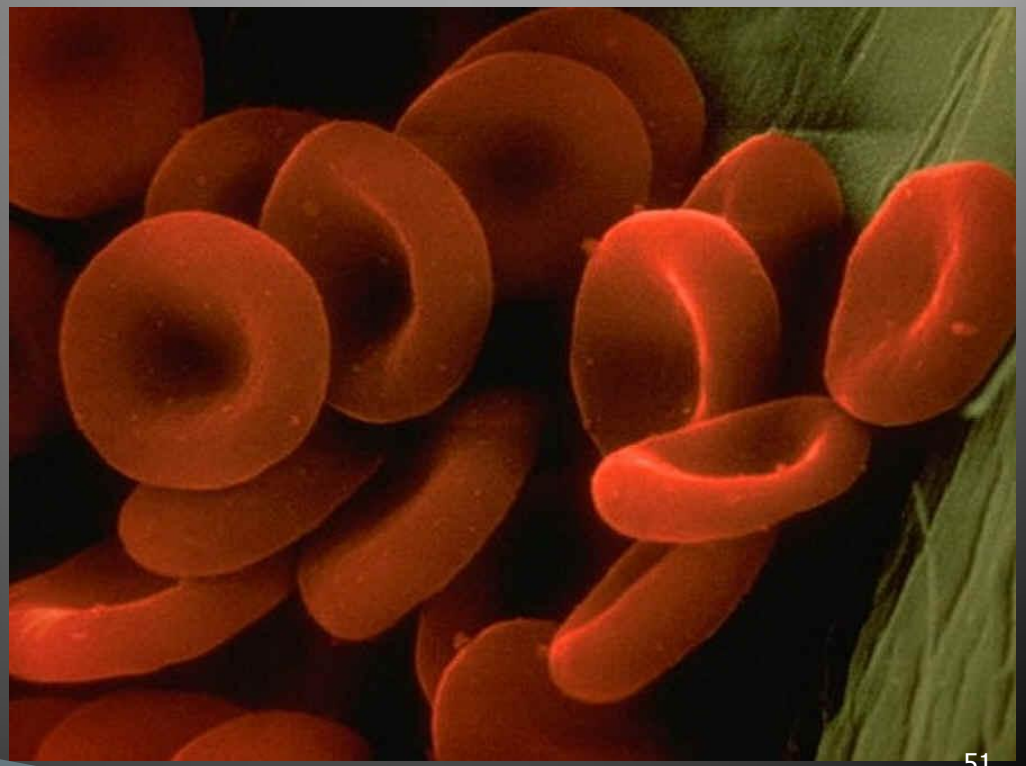
50 διαμεμβρανικές πρωτεΐνες (100-1.000.000 αντίγραφα/RBC)

25 = blood group antigens

- Transport proteins
- Adhesion proteins (ICAM-4, Lu)
- Signaling receptors
- Structural integrity

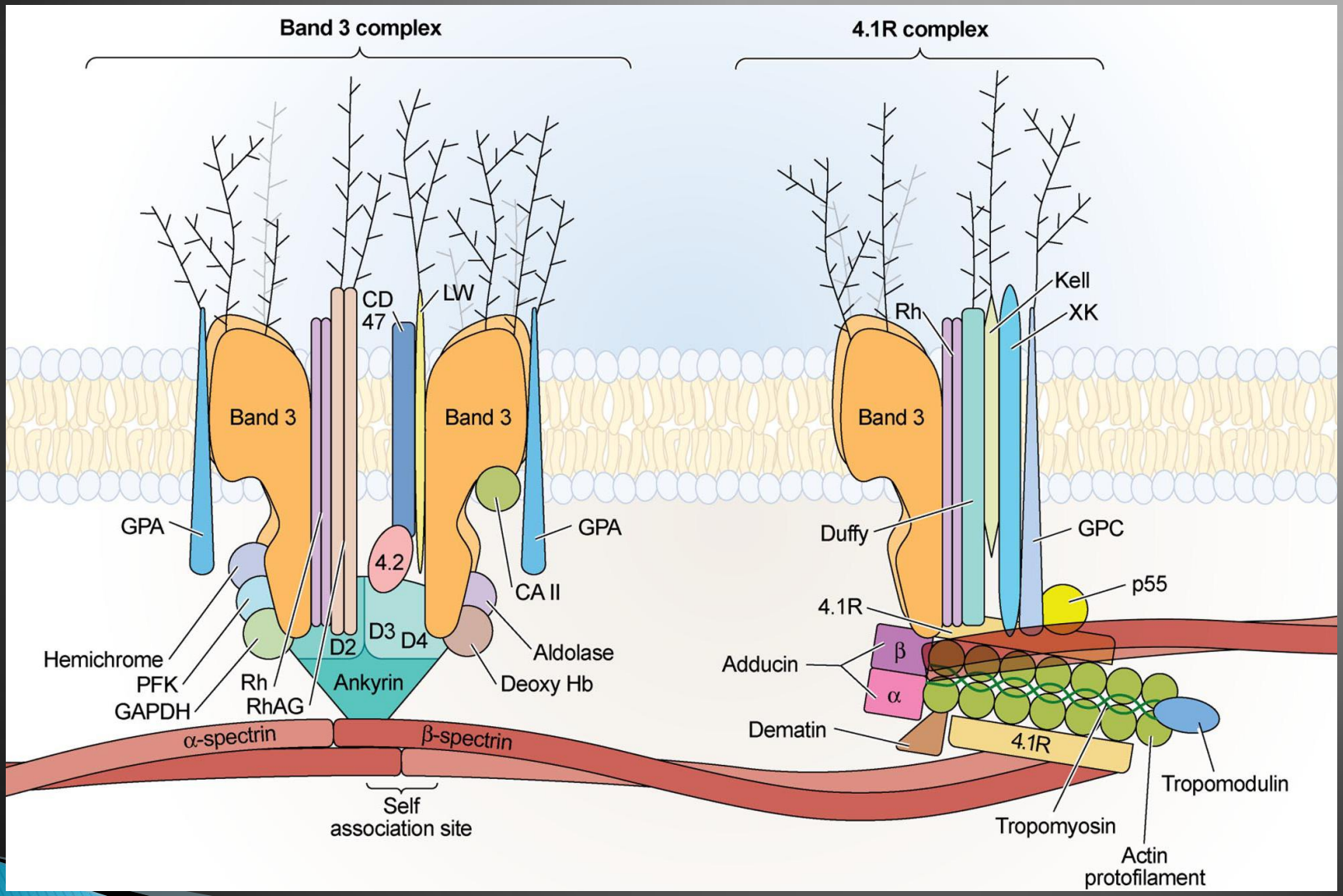
RBC DEFORMABILITY

1. Surface area loss
2. Change in cell volume



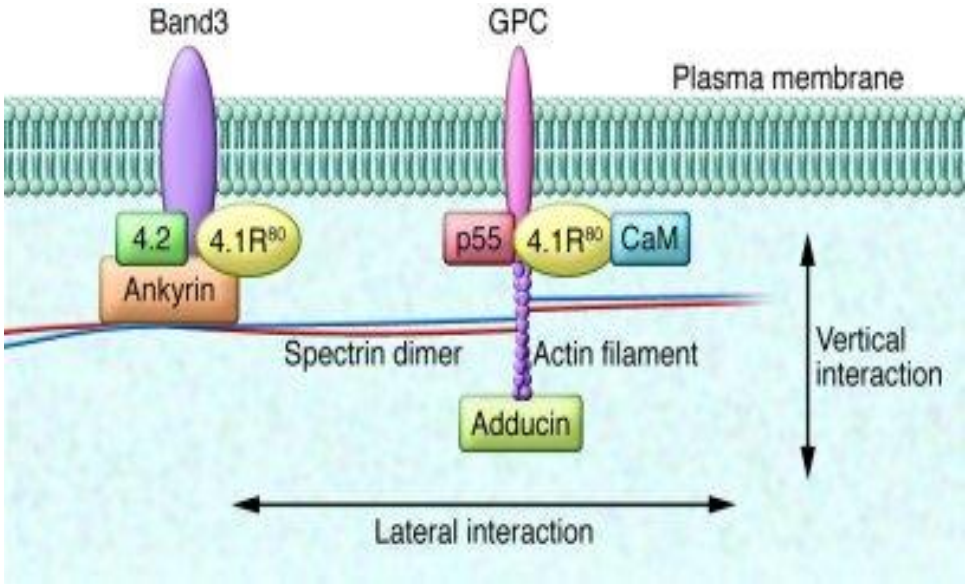
ΔΙΑΤΗΡΗΣΗ ΕΠΙΦΑΝΕΙΑΣ

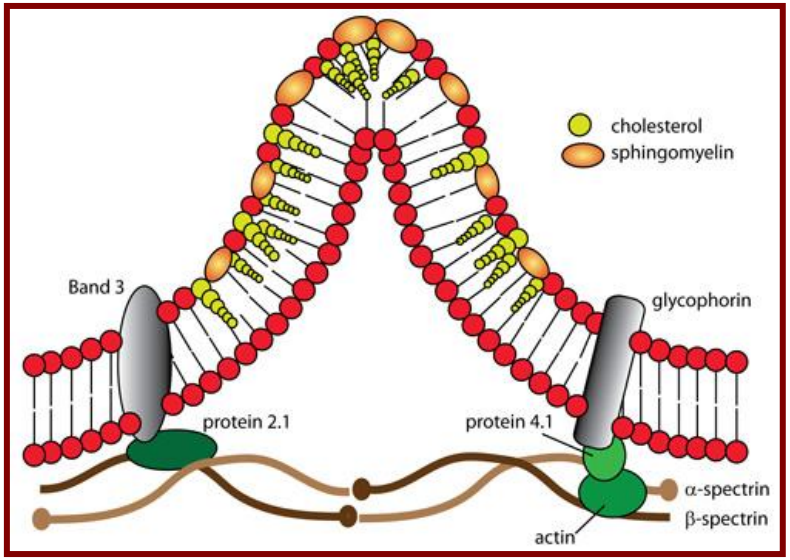
(Salomao et al., 2008; PNAS, 105: 8026)



STRUCTURAL INTEGRITY PROTEINS

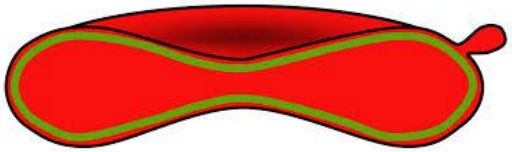
ΔΙΑΤΗΡΗΣΗ ΕΠΙΦΑΝΕΙΑΣ





Edward J. Benz Jr. J Clin Invest. 2010; 120(12):4204–4206

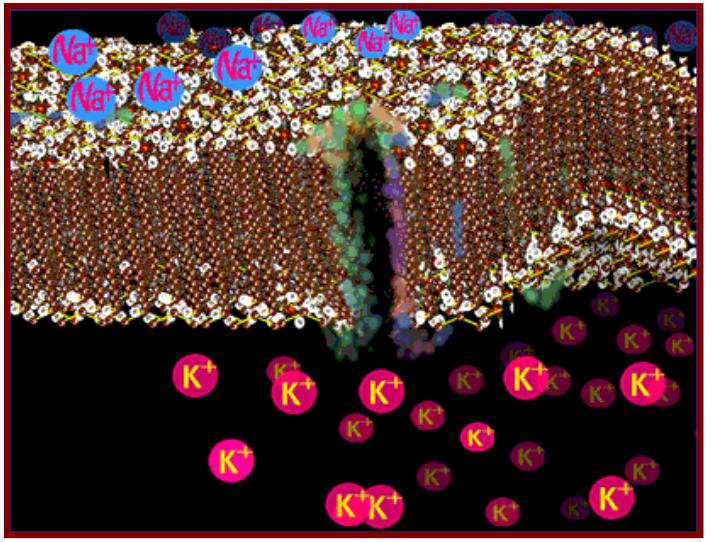
https://www.youtube.com/watch?v=0jra_ZgqCx4



1. **Membrane protein linkages with skeletal proteins: regulating cohesion between lipid bilayer and MSk (Preventiion of membrane vesiculation**

2. **Mechanical stability of MSk (Prevention of membrane breakup)**

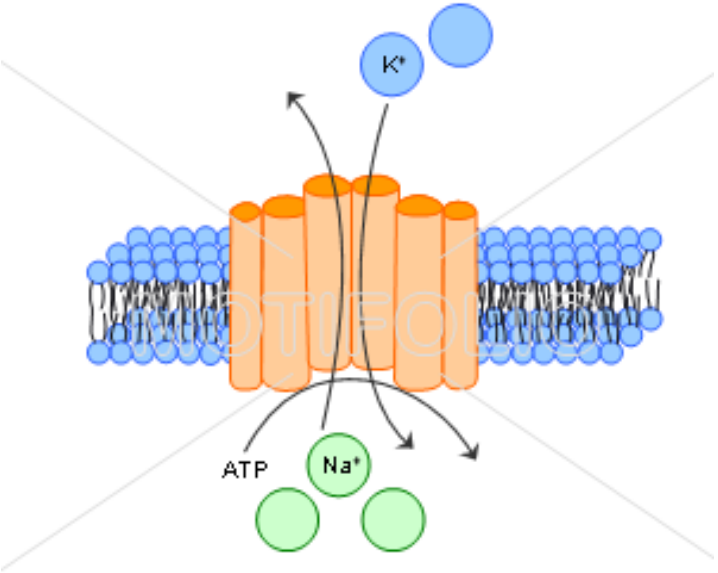
ΔΙΑΤΗΡΗΣΗ ΟΓΚΟΥ



RBC volume is regulated through:
(1) active membrane transport, e.g. pumps
(2) gradient-driven passive transport
(3) a number of channels

While the RBC membrane is permeable to some extent to water and anions, it is extremely impermeable to cations, requiring specific transport systems for them.

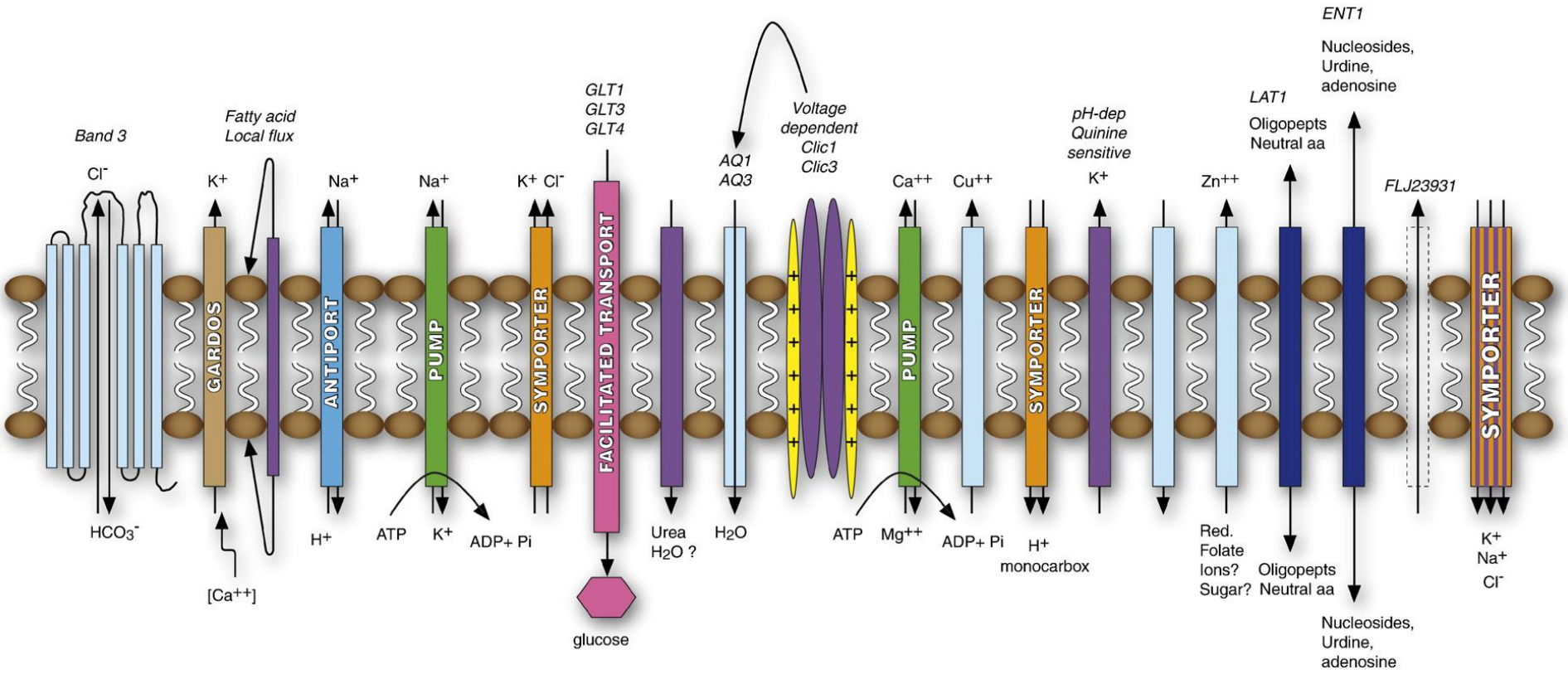
The Na/K-ATPase – an electrogenic pump



To maintain an intracellular cation concentration opposite to that of the plasma (low potassium, high sodium and calcium**) the RBC relies on two ATP-dependent cation pumps:**

- **Na+–K+-ATPase**
- **calmodulin activated Mg2+-dependent Ca2+-ATPase**

ΠΡΩΤΕΪΝΕΣ ΜΕΤΑΦΟΡΕΙΣ



(Pasini et al., J Proteomics 2010)

Violet: low expressed transporters. The voltage dependent channels (Clic 1, Clic 3) are believed to regulate the Aquaporin channels. Symporters: in orange. Antiporters: in blue. Facilitated transport: rosa. Pumps: in green. Bi-directional transport: dark blue. Channels activated in consequence of an internal stimulus: in brown. All others: pale blue. Hypothetical transporters: just sketched.

ΠΡΩΤΕΪΝΕΣ ΜΕΤΑΦΟΡΕΙΣ

Ζώνη-3 (anion transporter)

Aquaporin (water transporter)

Glut-1 (glucose transporter)

Kidd antigen protein (urea transporter)

RhAG (gas transporter, CO2)

Na+ K+ ATPase

Ca++ ATPase

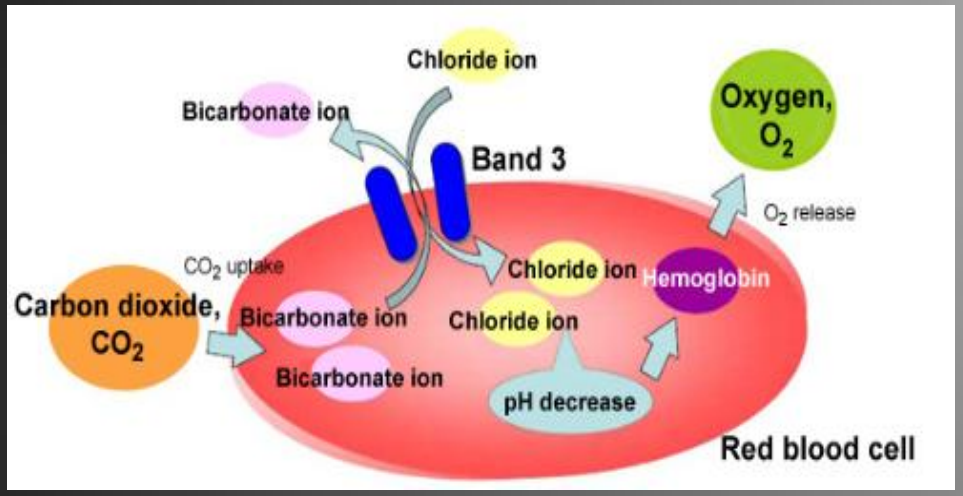
Na+ Cl- cotransporter

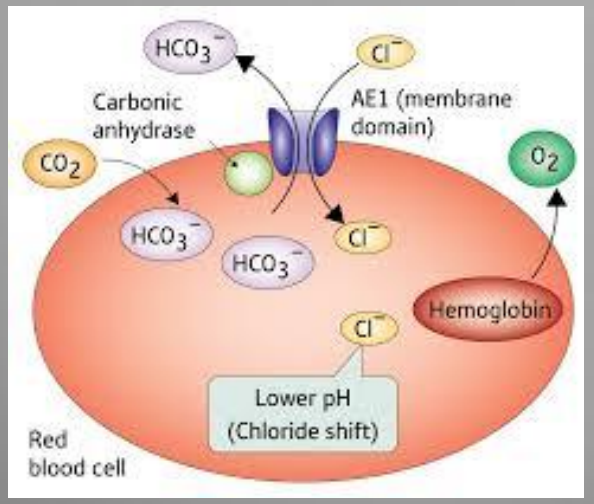
Na+ K+ cotransporter

Gardos channel

ΑΝΙΟΝΤΟΑΝΤΑΛΛΑΚΤΗΣ

Membrane transport protein "Band3" uses ion exchange transport mechanism to promote the release of oxygen from hemoglobin.





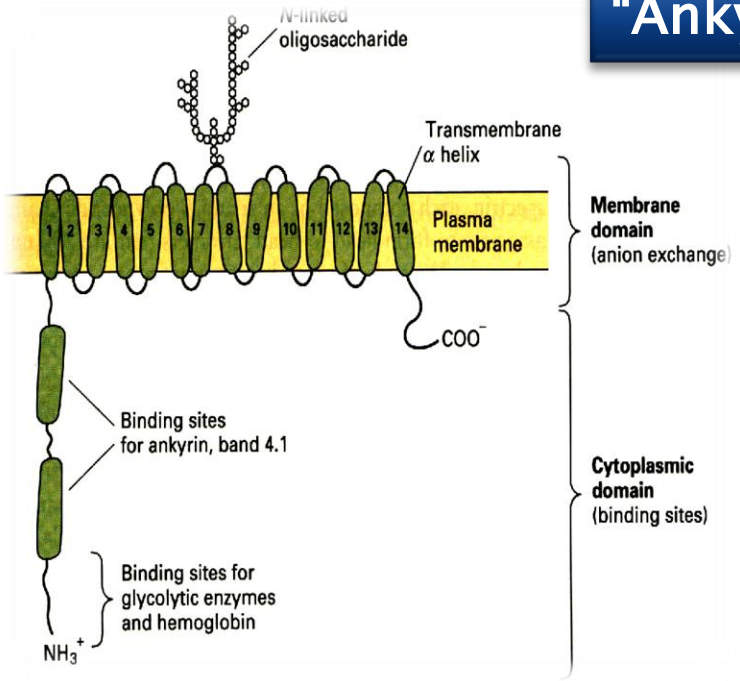
Within RBCs carbonic anhydrase: catalyses the reaction CO_2+H2O to form carbonic acid (which rapidly dissociates into a proton and bicarbonate ion). The proton binds to Hb

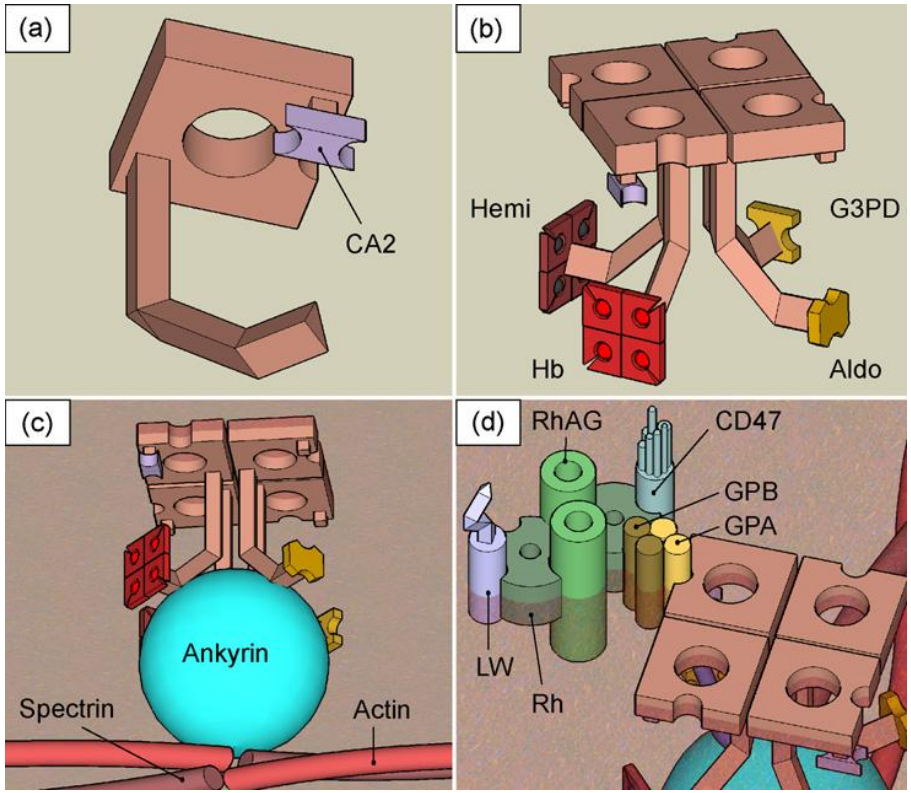
Bicarbonate is transported by band 3 out of the red cell in exchange for chloride, thus allowing CO2 to be transported to the lungs predominantly as bicarbonate (approximately 5 billion bicarbonate ions are transported out of the RBC in a span of about 50 ms!!!)
In the pulmonary capillaries, bicarbonate is again transported by band 3 across the red cell membrane into the cell and chloride is expelled.

As hemoglobin binds oxygen and releases protons, carbonic anhydrase goes to work again, catalyzing the conversion of bicarbonate and a proton to CO2 and water. The CO2 diffuses out of the red cell and is expired.

ΑΝΙΟΝΤΟΑΝΤΑΛΛΑΚΤΗΣ

"Ankyrin-receptor"

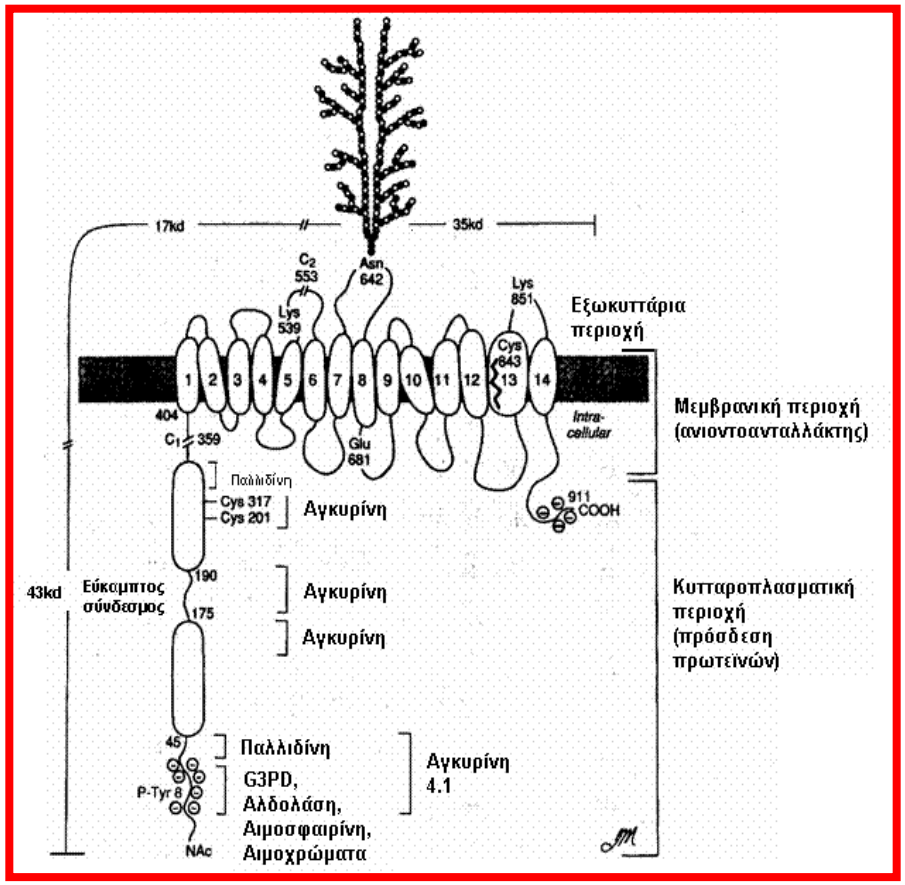




ank, CA, 4.2, 4.1R, G3PD, aldolase, Hb…

(Walsh and Stewart, RA 2010)

ΑΝΙΟΝΤΟΑΝΤΑΛΛΑΚΤΗΣ



Δύο δομικά και λειτουργικά διακριτές περιοχές:

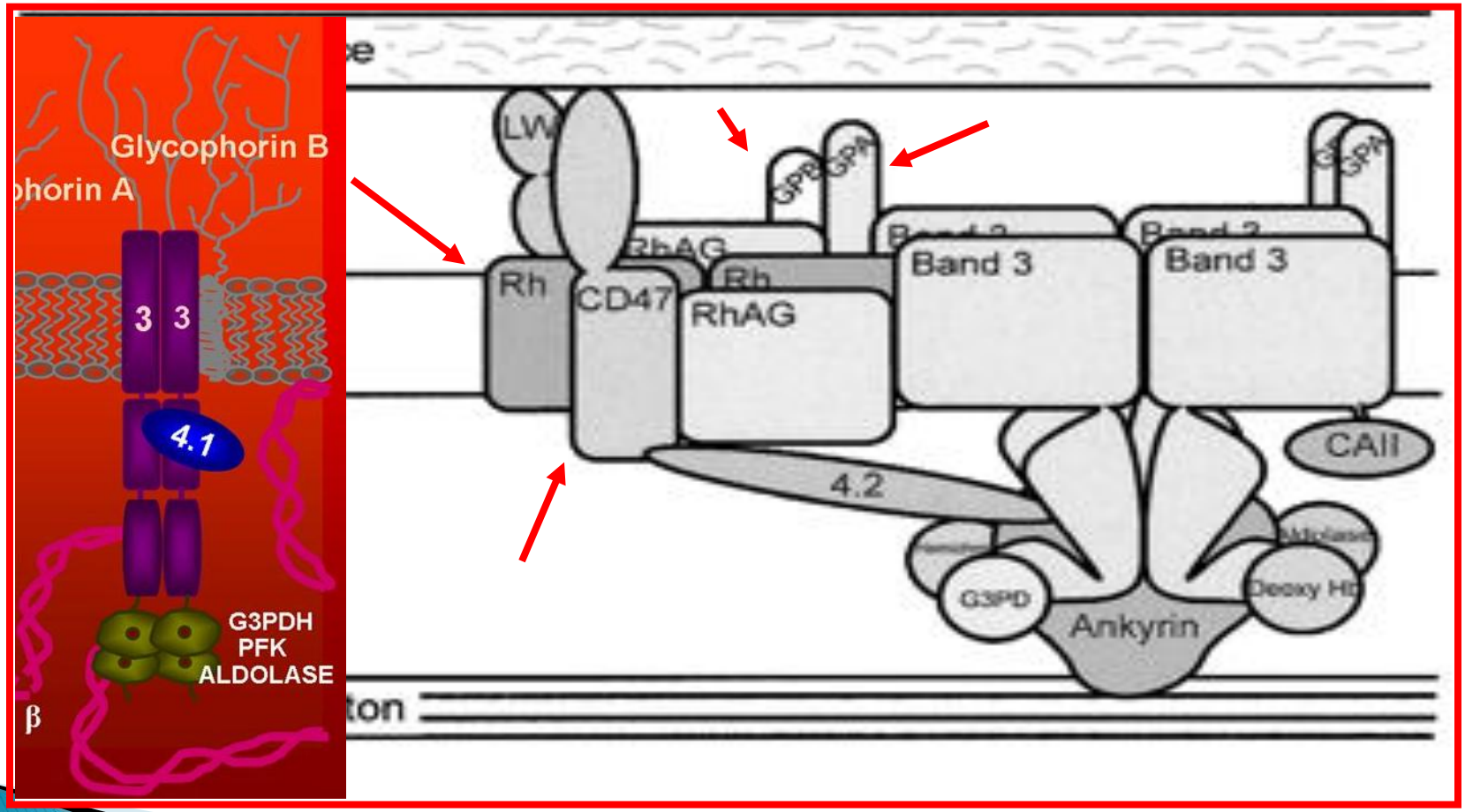
(1) Cyt 43-kd: (aa 1-359)-Με θέσεις δέσμευσης για αρκετές πρωτεΐνες

(2) Membrane domain 52-Kd (17kd+35kd) (aa 360-911), η οποία σχηματίζει το δίαυλο ανταλλαγής ανιόντων (*Becker & Lux, 1993*).

Gene: SLC4A1 at 17q21.

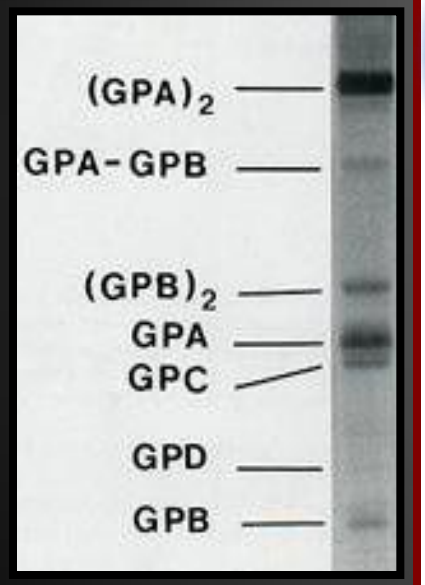
ΑΝΙΟΝΤΟΑΝΤΑΛΛΑΚΤΗΣ

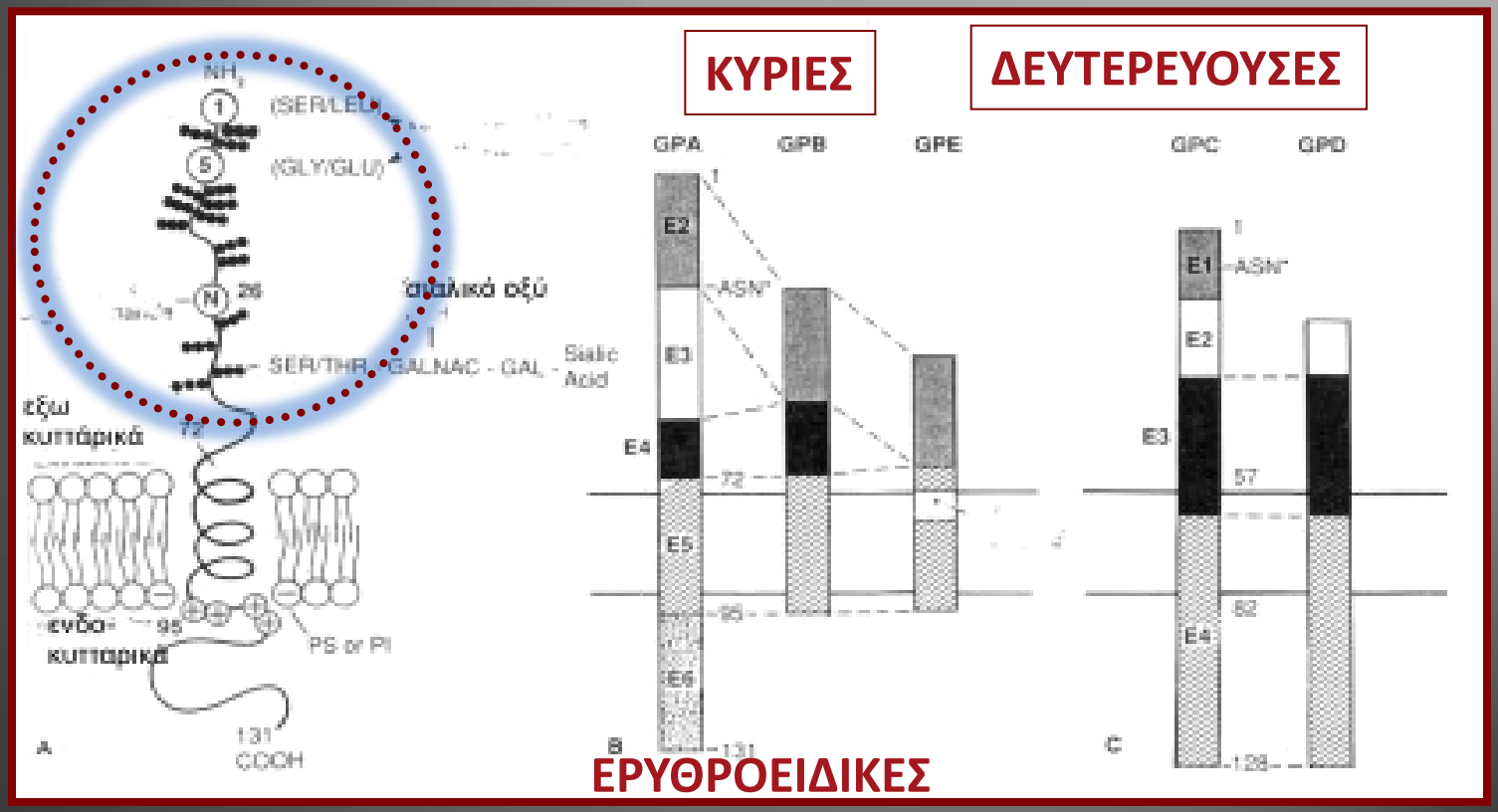
Η ζώνη-3 είναι ο κύριος συνδέτης της μεμβράνης με το σκελετό



- γήρανση/οξείδωση/μόλυνση
- HS, οξέωση νεφρικών σωληναρίων κλπ

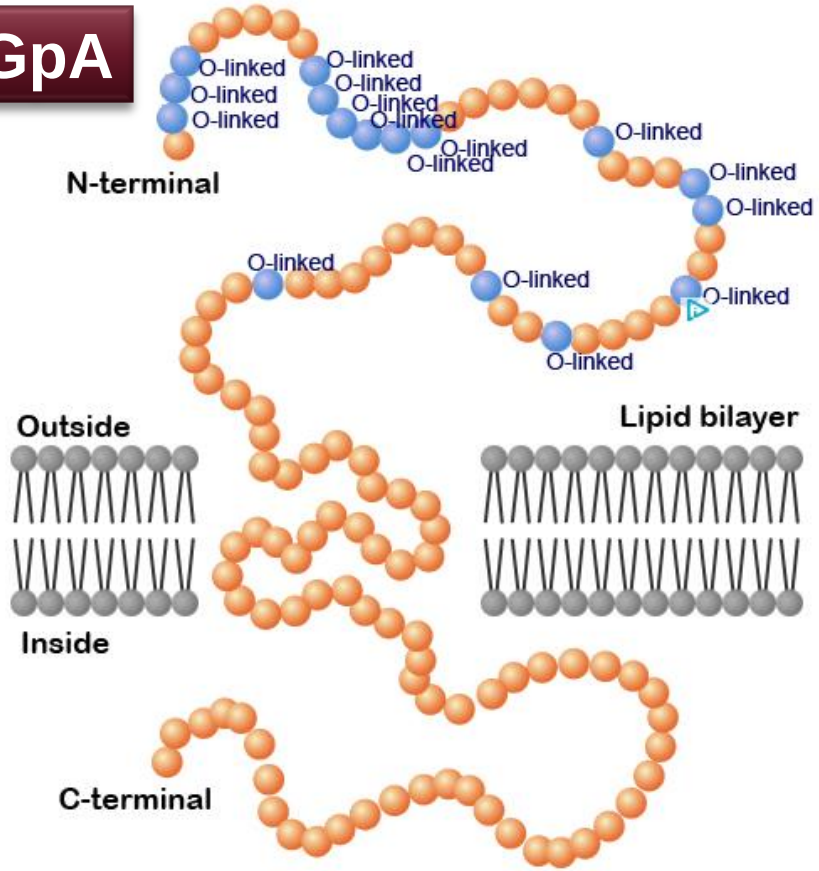
ΓΛΥΚΟΦΟΡΙΝΕΣ





ΓΛΥΚΟΦΟΡΙΝΕΣ

GpA



RBC-specific

- ✓**Αντιγόνα ομάδων αίματος MN, Wright**
- ✓**Cell recognition/erythrophagocytosis**
- ✓**Λειτουργεί αντισταθμιστικά με band 3**

ΓΛΥΚΟΦΟΡΙΝΕΣ

RBC-specific

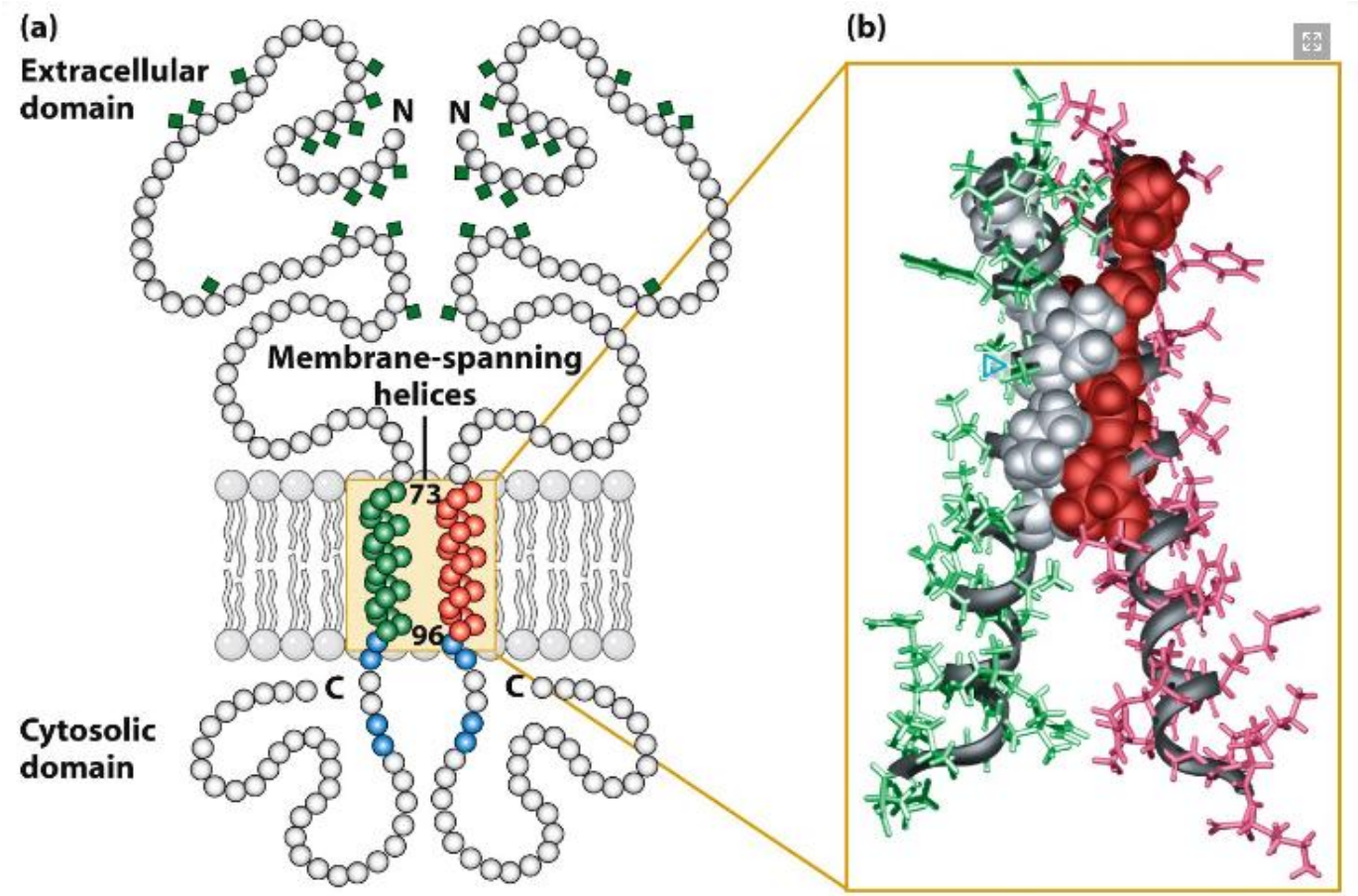
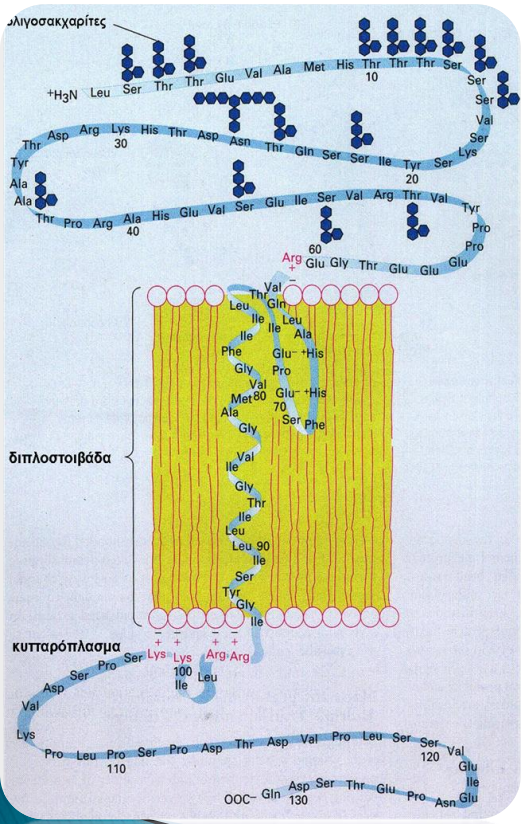


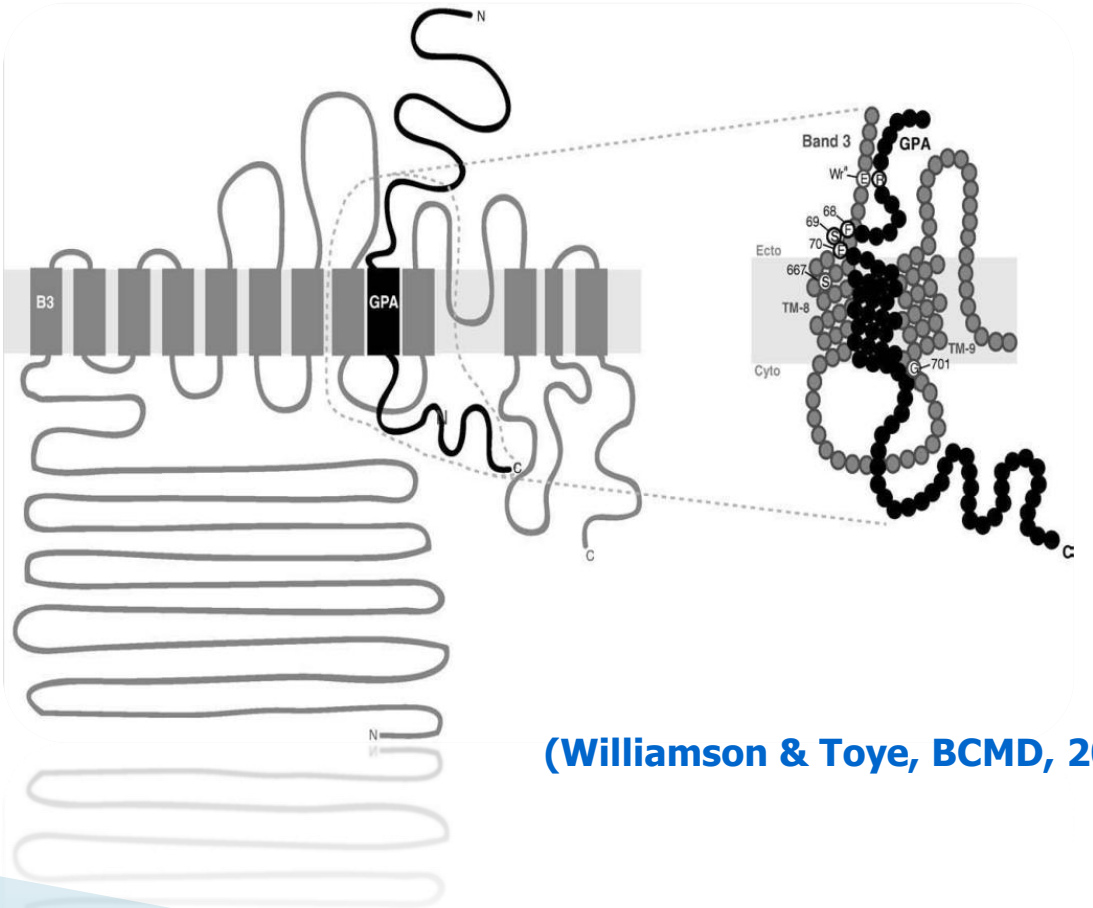
Figure 10-15
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

ΓΛΥΚΟΦΟΡΙΝΕΣ

RBC-specific

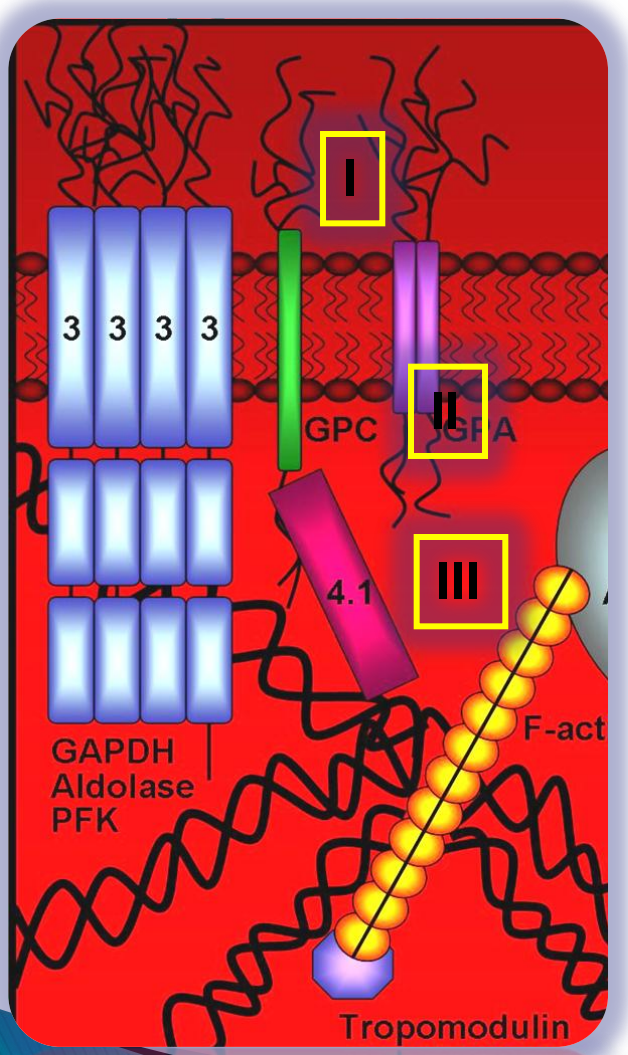
Η GpA είναι πρωτεΐνη-συνοδός της Β3 κατά τη βιοσύνθεση/trafficking





(Williamson & Toye, BCMD, 2008)

ΓΛΥΚΟΦΟΡΙΝΕΣ

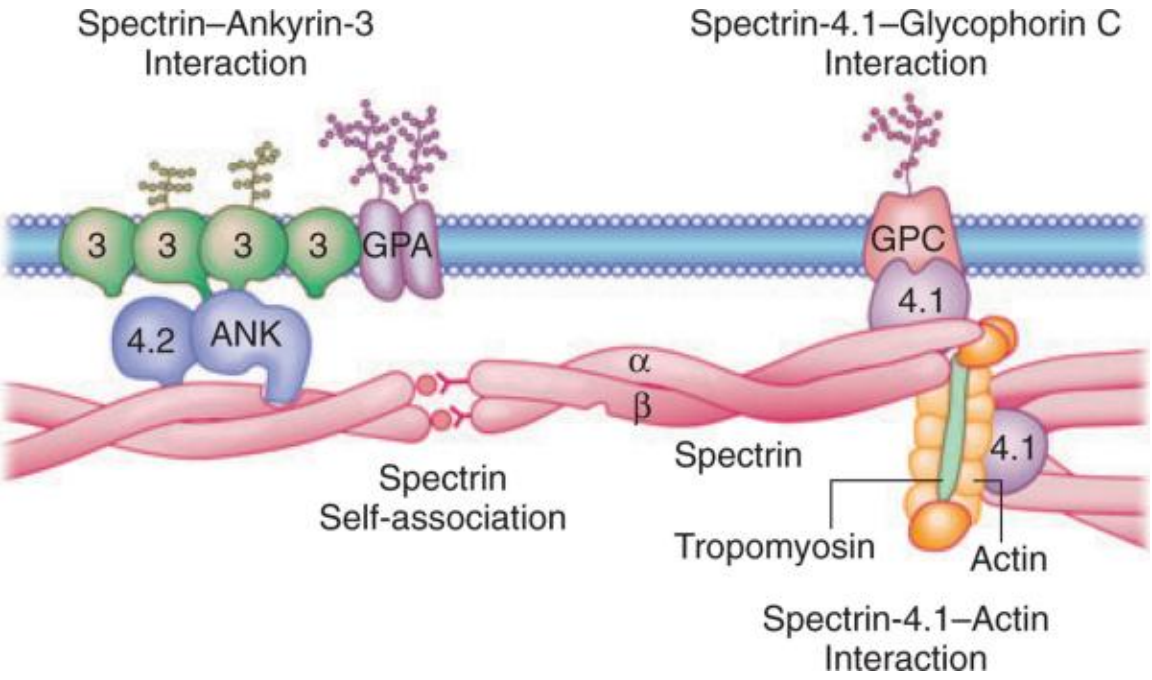


Γλυκοφορίνη C

- Μη-ερυθροειδικές (πνεύμονες, ήπαρ, νεφροί)
- Gerbich (Ge), Lsa, Webb, Dha
- 3 domains

ΓΛΥΚΟΦΟΡΙΝΕΣ

Γλυκοφορίνη C



Ρύθμιση κυτταρικού σχήματος
Ρύθμιση μηχανικών ιδιοτήτων

GpC-----4.1R*-----p55

Hereditary elliptocysosis

CD47

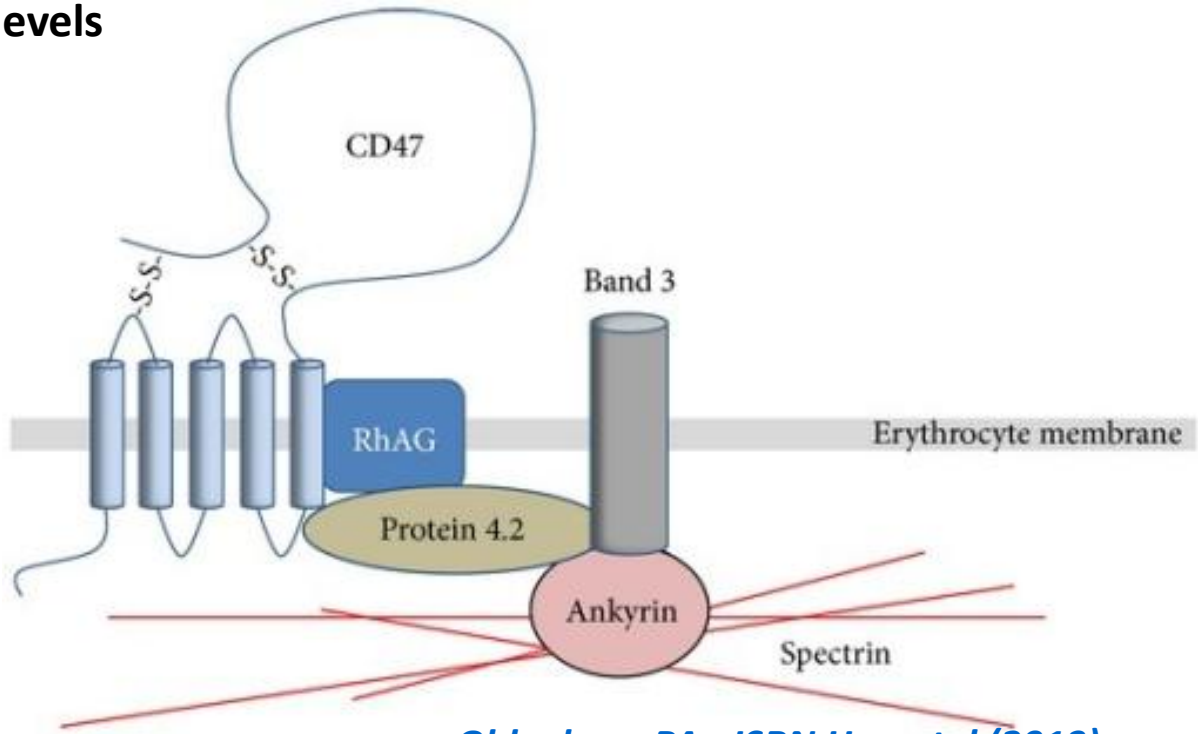
IAP, Integrin–associated protein

5x10⁴ copies/RBC (do not express integrins)

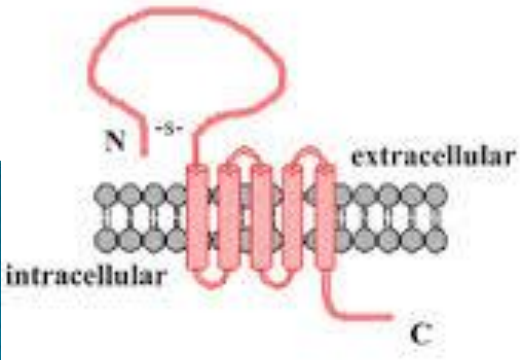
Surface glycoprotein, 50kd, high levels

Mechanosensor (signalling)

CD47 interacts with the Rh/RhAG complex and also associates with protein 4.2, which links CD47 to the band 3/ankyrin complex and the spectrin cytoskeleton.
(*) not all CD47 appears to be associated with this multiprotein complex in RBC membranes



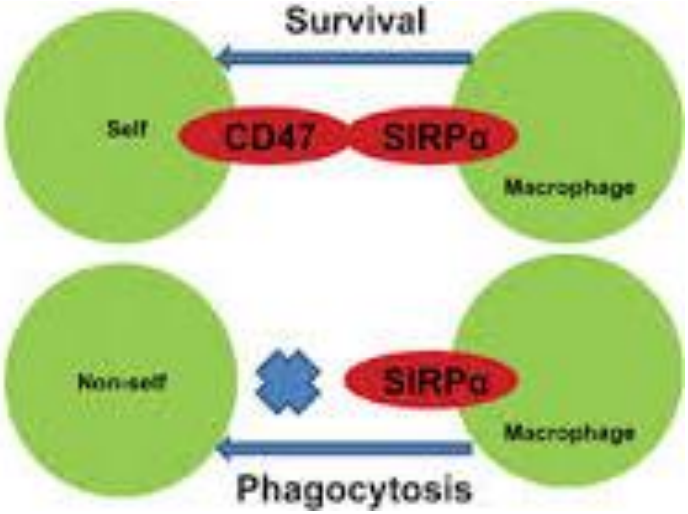
Oldenborg PA - ISRN Hematol (2013)

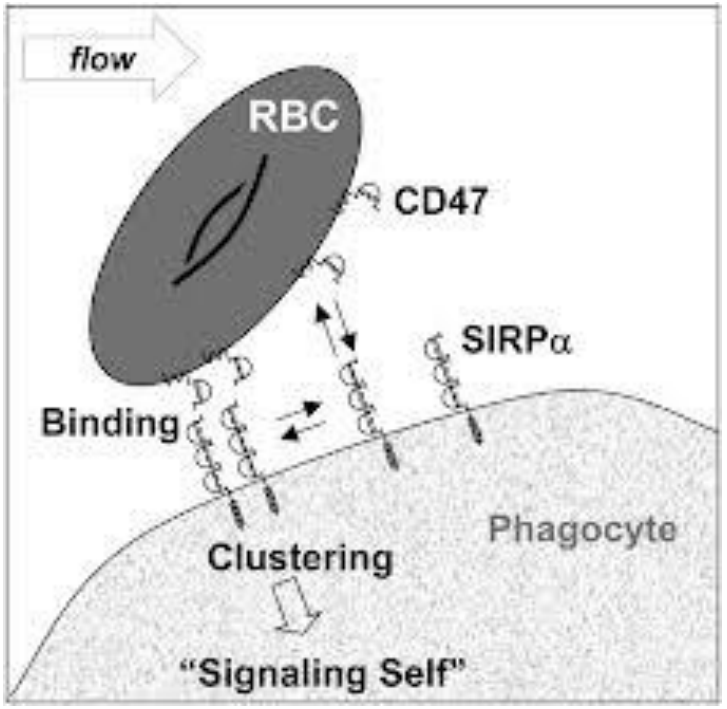


CD47

Marker of self for RBCs

Η CD47 προσδένεται στη ρυθμιστική πρωτεΐνη α (SIRPa) των μακροφάγων, οδηγώντας σε παρεμπόδιση της ενεργοποίησής τους & της ερυθροφαγοκυττάρωσης (CD47-SIRPa signalling)





Zheleznyak et al., Molecular Imaging 12(8):1536; 2013

CD47

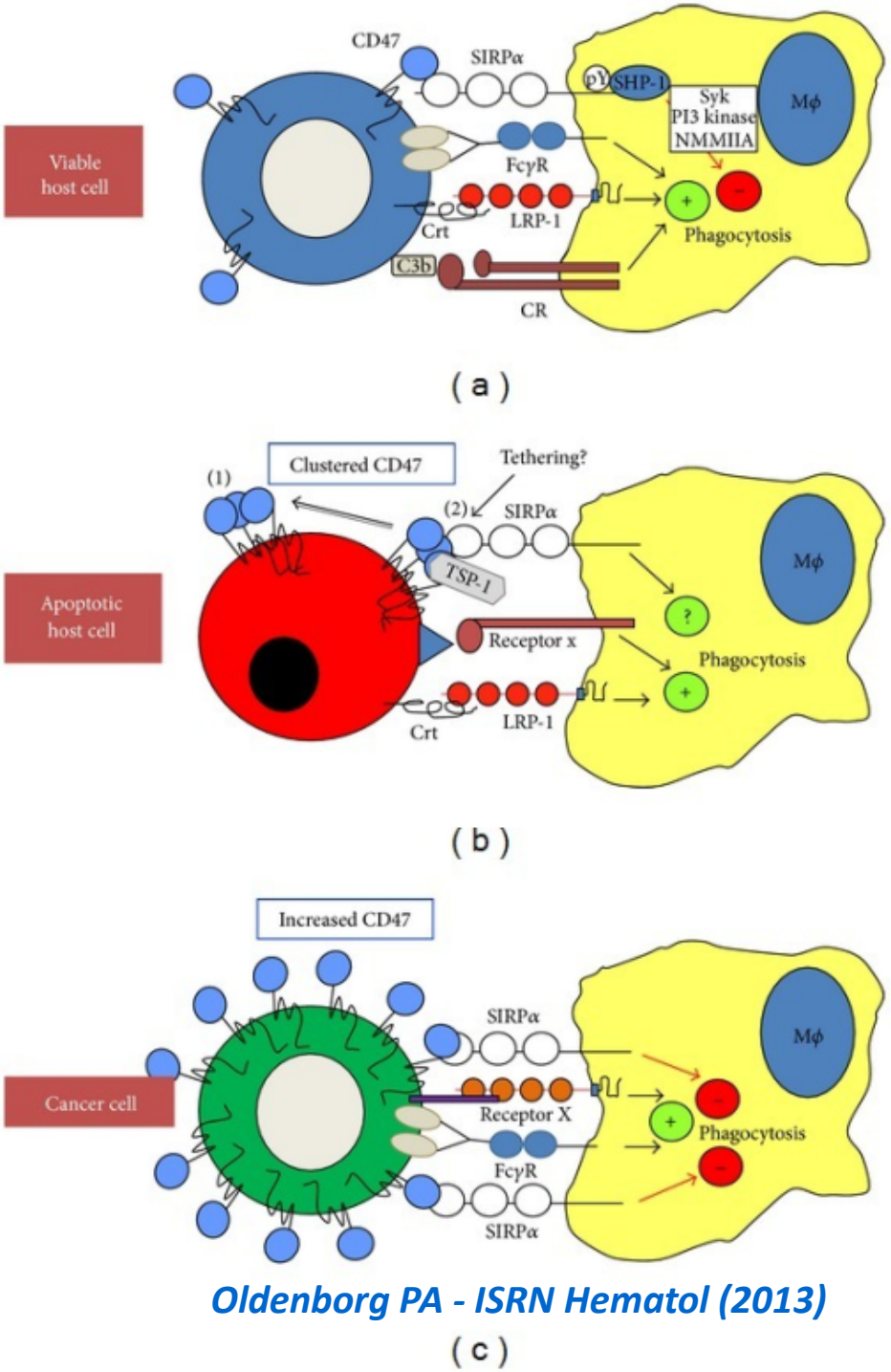
Marker of self

CD47 has been shown to be overexpressed by various tumor types as a means of escaping the antitumor immune response

a) CD47 on **viable normal host cells** can bind to **SIRPα** on a phagocytic cell (e.g., a macrophage (Mφ)), which induces **Tyr phosphorylation** of SIRPα and recruitment of **SHP-1**. This can inhibit prophagocytic signaling through **Fcγ receptors**, **complement receptors (CR)**, or LRP-1. Phagocytosis inhibition may involve signaling through Syk and PI3 kinase

b) On **apoptotic cells**, CD47 becomes clustered in the PM. Clustered CD47 may also **bind to SIRPα** without inducing inhibition of phagocytosis, but may rather **promote tethering** of the apoptotic cell to the phagocyte. This function may also involve **TSP-1** and so far uncharacterized mechanisms that can also promote phagocytosis.

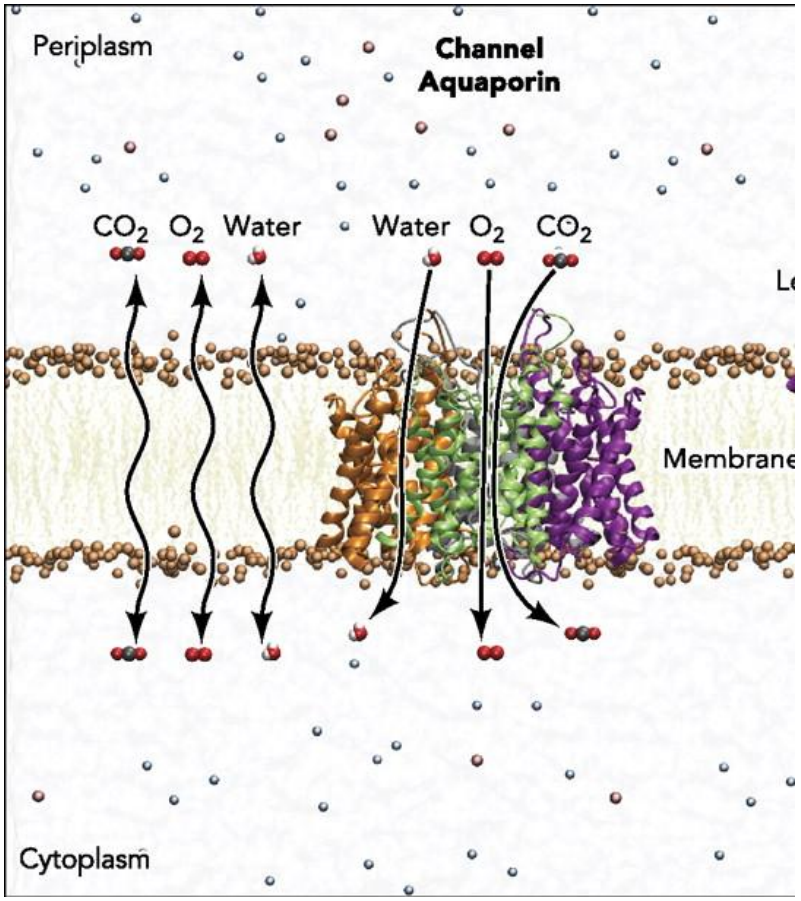
c) **Cancer cells** may **increase their expression of CD47** to **strengthen the inhibitory signals** through SIRPα and to more potently inhibit phagocytosis ediated by Fcγ receptors and other prophagocytic receptors (receptor x).



Oldenborg PA - ISRN Hematol (2013)

ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)

- **Channel-forming integral protein (CHIP28), Water Channel Proteins (269aa)**
- **AQP1 = Αρχετυπικό μέλος υδατοπορινών (RBC 1980's) -** NH2, COOH in cytoplasm
- Nobel Prize in Chemistry, 2003
- Άφθονη σε νεφρούς, επιθήλια



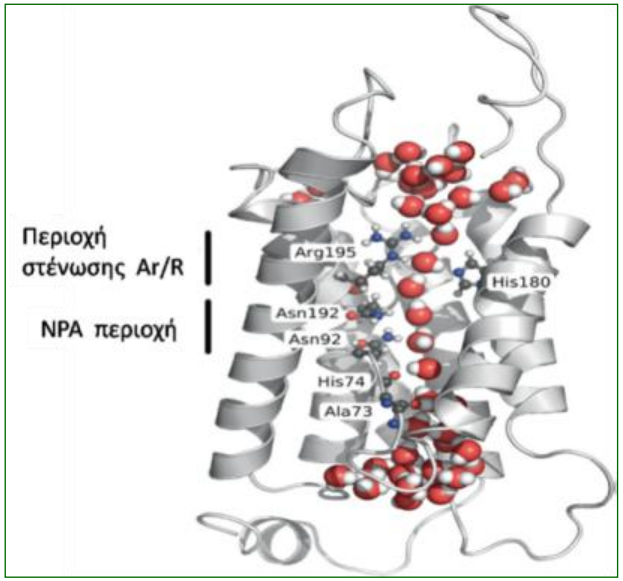
- **όχι αλληλεπιδράσεις με άλλες πρωτεΐνες (?)**
- **συμμετοχή σε λιπιδιακές σχεδίες**

➢Κύριος πρωτεϊνικός δίαυλος νερού - Οσμωτικός-υδατοεπιλεκτικός πόρος - **Ρύθμιση ταχείας και αμφίδρομης μετακίνησης νερού**

➢promoting the **rehydration** of the RBC after their shrinkage in the hypertonic environment of the renal medulla

ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)

$3x10^9$ μόρια/μονομερές/δευτερόλεπτο



Μεταφορά μορίων νερού

(Hub et al., Handb. Exp. Pharmacol.., 190:60, 2009)

https://www.youtube.com/watch?v=1Uw6u0fzNsE

At least 11 aquaporin proteins have been identified in mammals with 10 known in humans

ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)

Monomer: six membrane-spanning regions-α-helical domains

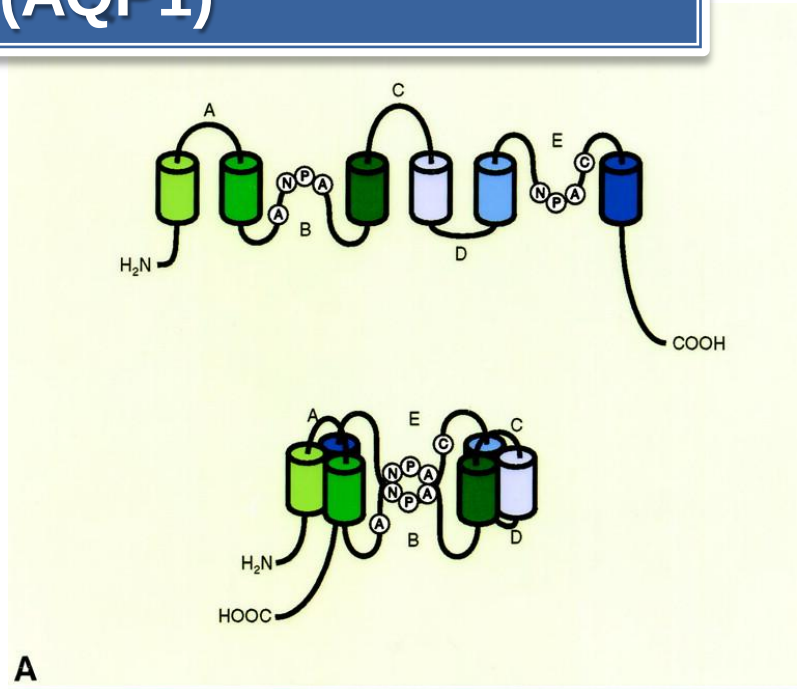
both intracellular NH_2 and COOH termini

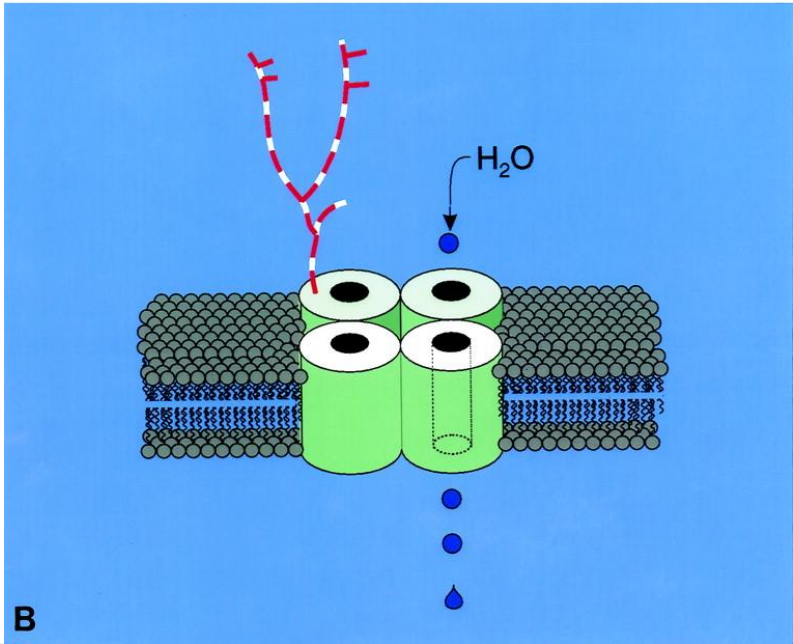
Ομοτετραμερές (4 πόροι)

Γλυκοζυλίωση (a large glycan attached to only one monomer)

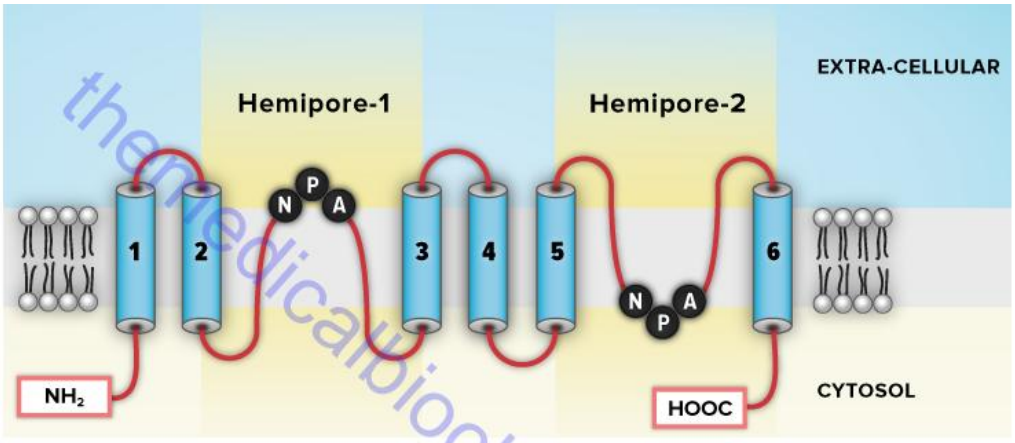
Internal tandem repeat structure with 2 NPA sequences (asparagine-proline-alanine) – Form tight turn structures that interact in the membrane to form the pathway for translocation of water across the membrane

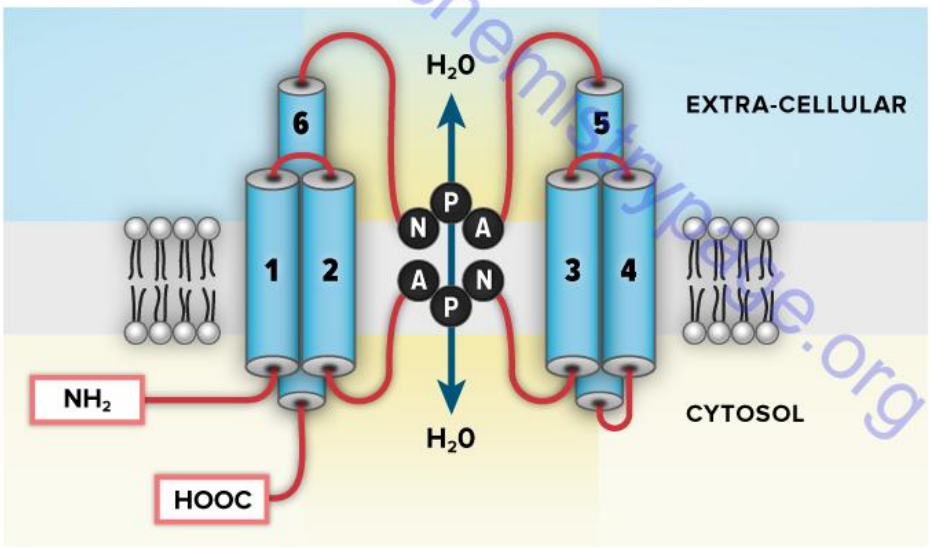
(*Nielsen et al., Physiological Reviews 82(205):2002*)





ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)



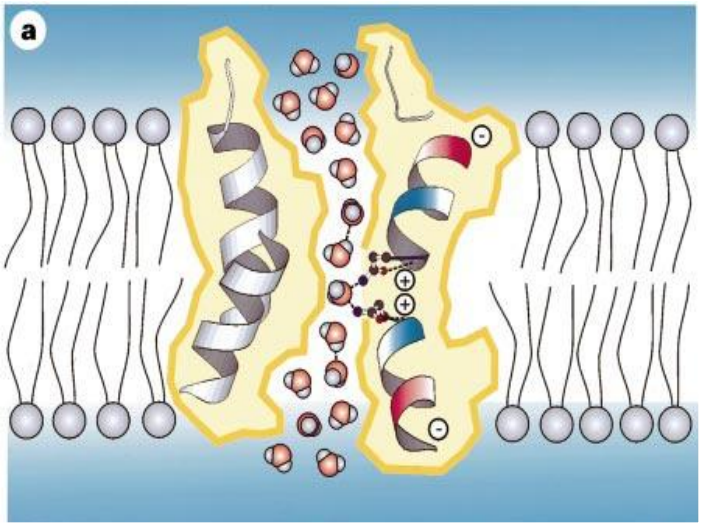


linear array of the protein indicating the two regions of helical domains that interact to form the three dimensional orientation of the protein. The pore that forms in the aquaporins is composed of two halves referred to as hemipores. Amino acids of the pore that are critical for water transport are the asparagine (N), proline (P) and alanine (A) residues indicated in each hemipore.

how the two hemipores interact to form the functional aquaporin

ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)

Μεταφορά του νερού μέσω της υδατοπορίνης-1
(Murata et.al, Nature 407:604, 2000)



Λειτουργία της AQP1

- **Στα ερυθροκύτταρα**

Ταχεία και ρυθμιζόμενη μεταφορά μορίων H_2O

Ώσμωση (~85%)

Διάχυση (~64%)

προσαρμογή στο υπέρτονο περιβάλλον του νεφρού/ επιδείνωση της συρρίκνωσης των RBCs στο νεφρό

- **Μεταφορά CO_2 (αντικρουόμενα αποτελέσματα)**

Μελέτες σε εμπύρηνα κύτταρα/ ωοκύτταρα Xenopus/ μελέτες προσομοίωσης

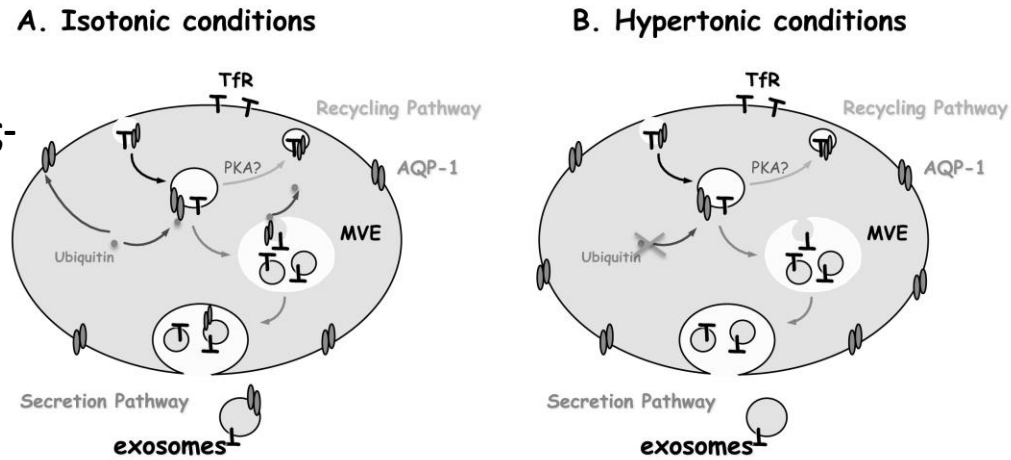
- **Μεταφορά NO και O_2**
- **Διαπερατότητα σε ιόντα**
- **Διαπερατότητα σε NH_3**

ΥΔΑΤΟΠΟΡΙΝΗ-1 (AQP1)

Ερυθροκύτταρα με έλλειψη της Aqp-1

- **φυσιολογική μορφολογία**
- **ήπια αιμόλυση**
- **αιματολογικές παράμετροι σε φυσιολογικά επίπεδα**
- **μείωση στη διαπερατότητα μορίων νερού λόγω διάχυσης ~64%**
- **μείωση στη διαπερατότητα μορίων νερού λόγω όσμωσης ~85%**

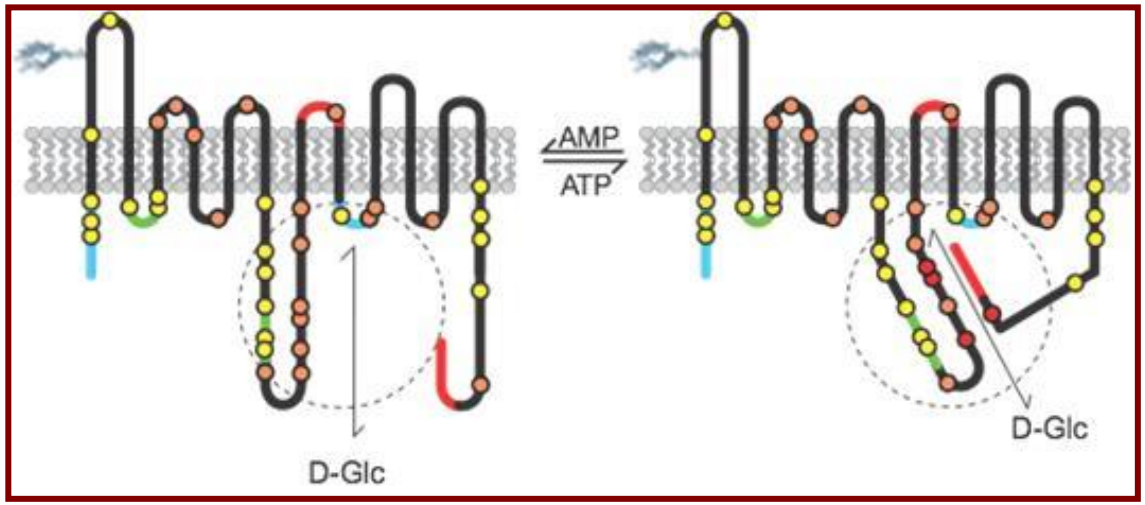
✓ **Ωρίμανση ΔΕΚ in vitro: απομάκρυνση μορίων υδατοπορίνης-1 μέσω εξωσωμάτων, με ουμπικουιτινιλίωση- ρύθμιση από την οσμωτικότητα του εξωκυττάριου περιβάλλοντος- προσαρμογή στις συνθήκες οσμωτικότητας της περιφερικής κυκλοφορίας**



Προτεινόμενο μοντέλο διαλογής της Aqp-1 στα εξωσώματα κατά την ωρίμανση των ΔΕΚ (Blanc et.al, Blood, 2009)

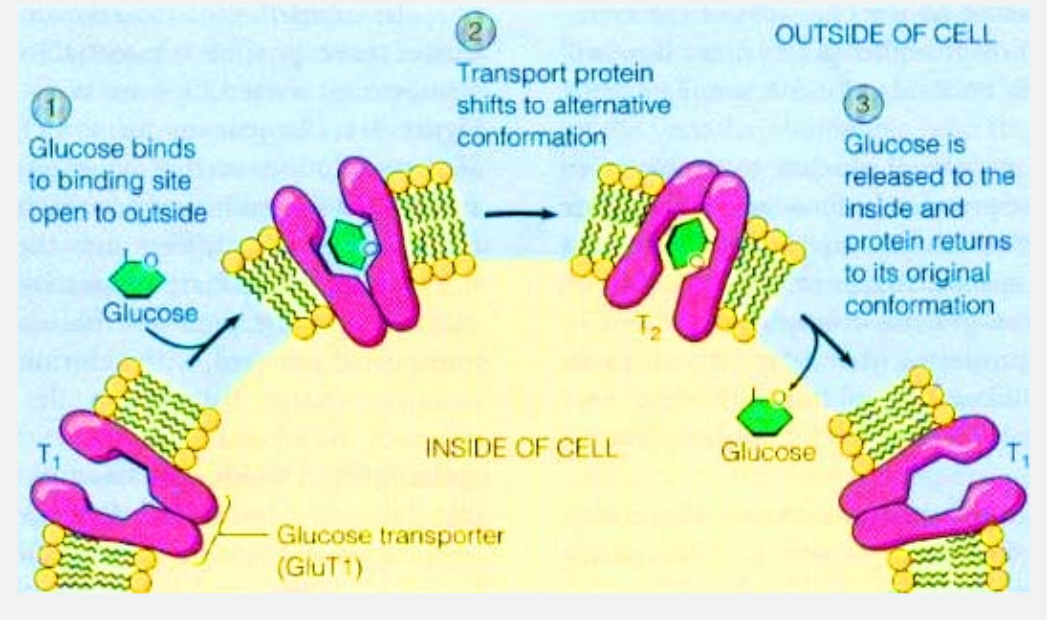
ΜΕΤΑΦΟΡΕΑΣ ΓΛΥΚΟΖΗΣ

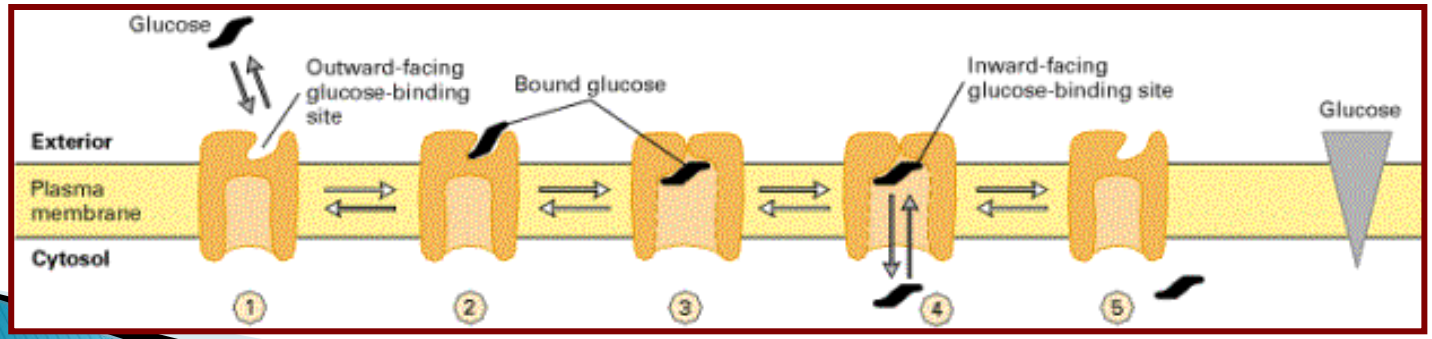
- **GLUT1**
- Μη-ερυθροειδική
- 55 kDa, **200.000 copies/cell**
- **10%** of the total membrane protein
- 12TM, NH2, COOH
- Part of **4.5b**
- Ετερογενώς **γλυκοζυλιωμένη**
- ↓ σακχ.καταλοίπων ⇒ ενεργότητα μεταφορέα
- Dimers/tetramers



ΜΕΤΑΦΟΡΕΑΣ ΓΛΥΚΟΖΗΣ

GLUT1 alter conformation to facilitate the transport of glucose



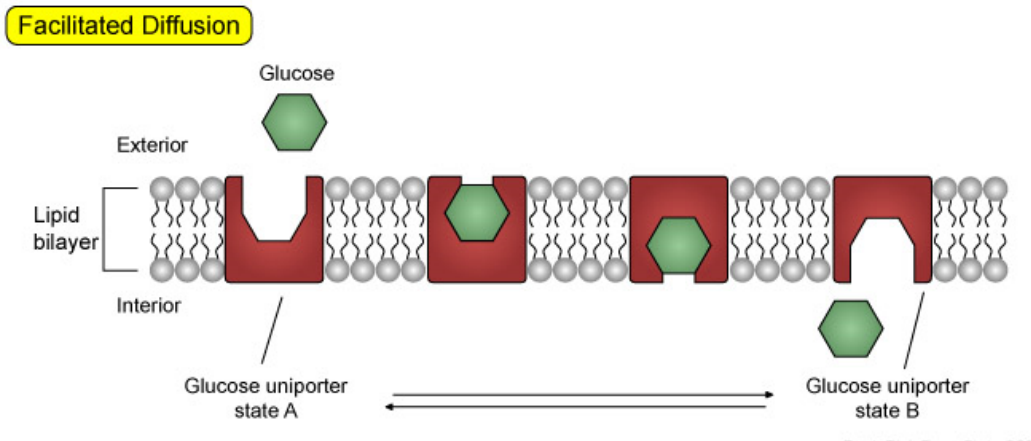


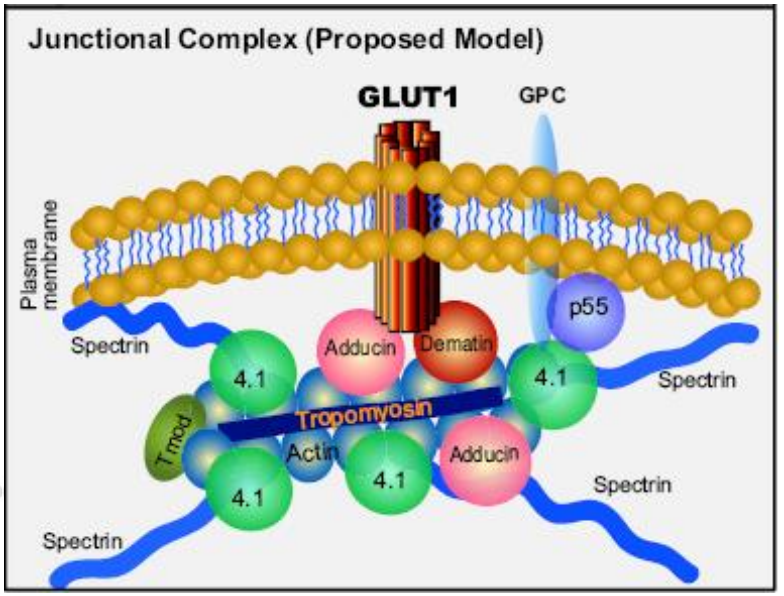
ΜΕΤΑΦΟΡΕΑΣ ΓΛΥΚΟΖΗΣ

principal functions of GLUT1:

(1) to facilitate transport of glucose
(2) Structural role: interaction of both dematin and adducin
Macrocomplex containing the three proteins

GLUT1 exists in extraordinarily high abundance in erythrocytes for 2 reasons unrelated to glucose uptake…





(Khan et al., 2008 J Biol Chem 283:14600)

ΜΕΤΑΦΟΡΕΑΣ ΓΛΥΚΟΖΗΣ

Vitamin C (ascorbate/ascorbic acid) is a water-soluble molecule that is essential for life- antioxidant that prevents cellular damage from oxygen radicals, vitamin E of plasma lipoproteins as well as the RBC membrane components from oxidative defects, a mechanism that is especially important in atherosclerosis

Most vertebrates satisfy their need for ascorbate by synthesizing it de novo from glucose (RBCs GLUT1 or GLUT4) , but some mammals including humans have lost this ability and must obtain vitamin C from their diets

ascorbate and its oxidized form dehydroascorbate (DHA) are taken up from the diet and carried in the plasma to cells throughout the body

Cells then readily reduce DHA to ascorbate. Many cells have high-affinity transporters for vitamin C, but mature RBCs do not have these transporters and cannot take up vitamin C from plasma

Montel-Hagen et al. (2008) demonstrate that the Glut1 of mature human RBCs enables efficient uptake of DHA by these cells. There is a switch in Glut1 substrate specificity from glucose to DHA that is regulated by stomatin, a membrane protein that binds to Glut1. The switch in substrate affinity of Glut1 is a unique feature of those mammals that are incapable of de novo synthesis of vitamin C

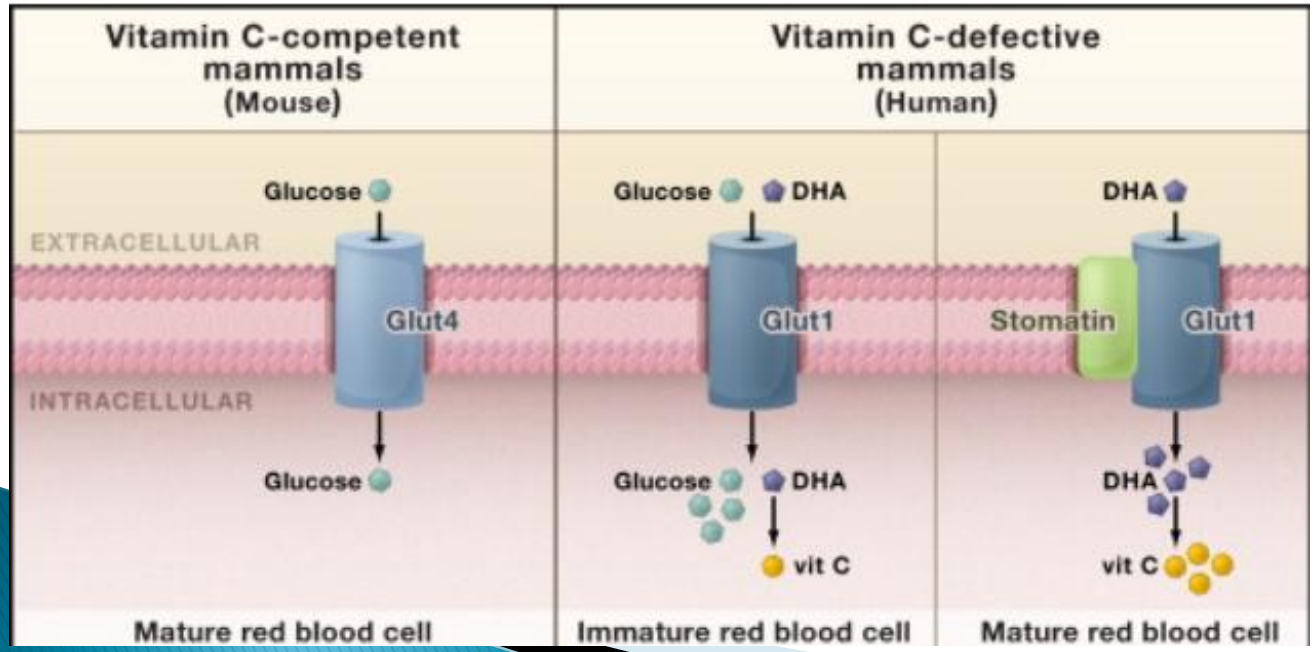
ΜΕΤΑΦΟΡΕΑΣ ΓΛΥΚΟΖΗΣ

(3) to facilitate transport of L-dehydroascorbic acid (DHA) across the membrane

As an effective ROS scavenger, plasma ascorbic acid is oxidized to dehydroascorbic (DHA) that enters RBCs through GLUT1

Inside RBCs, DHA is quickly reduced to ascorbic and then it slowly diffuses back to plasma thus increasing the plasma concentration of vitamin C.

the physical association of GLUT1 with stomatin favors DHA transport at the expense of glucose transport activity



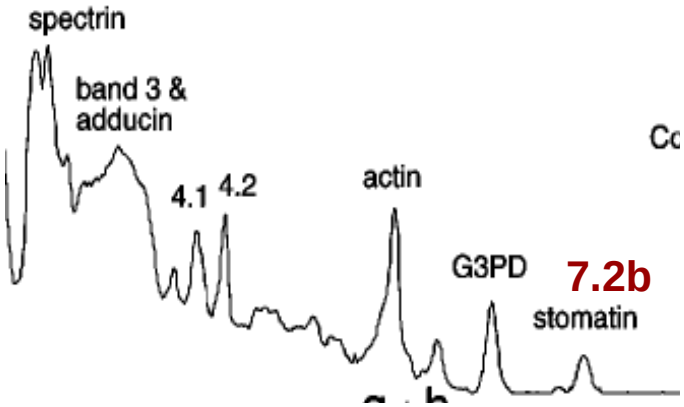
Immature human RBCs express Glut1 and primarily take up glucose in preference to DHA.

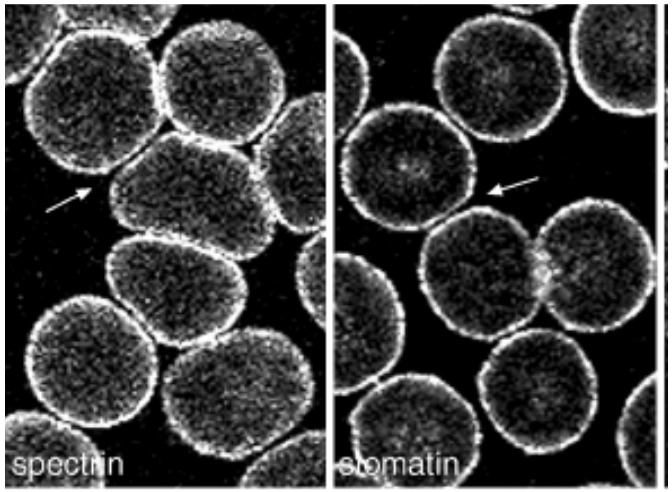
During RBC differentiation, expression of stomatin increases. Stomatin binds to Glut1 and changes its substrate preference from glucose to DHA

Troadec and Kaplan, Cell 132(6):921, 2008

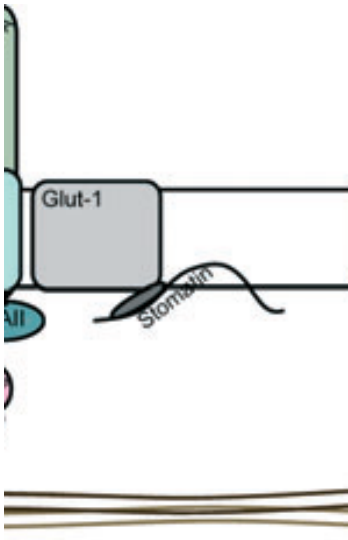
(Anstee, Vox Sanguinis, 100:140, 2011)

ΣΤΟΜΑΤΙΝΗ





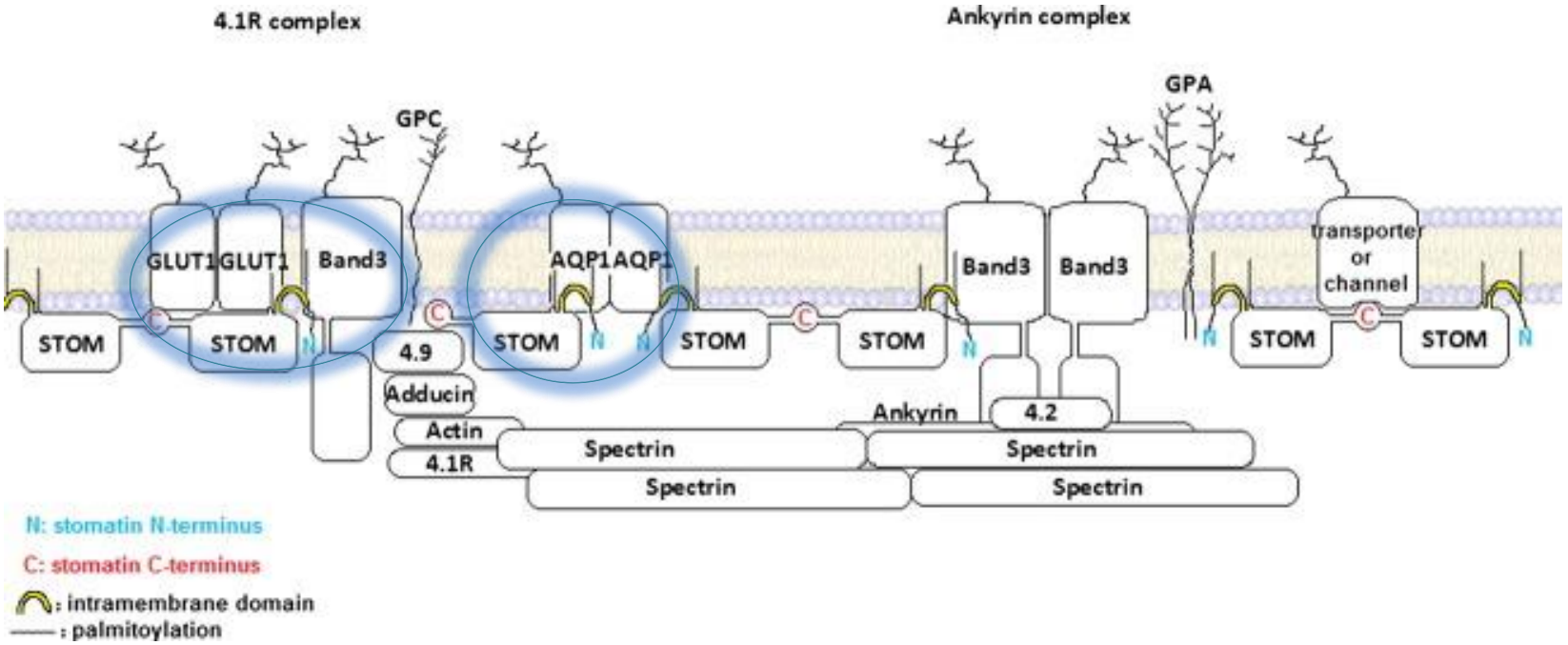
(Wang &Morrow, 2000)



- Major protein of RBC membrane
- 31kDa, 7.2b
- Εκφράζεται σε διαφορετικούς ιστούς
- Membrane-associated
- Homo-oligomeric (9-12 monomers)
- Κύριο συστατικό των Lipid Rafts
- Enriched in membrane folds and protrusions
- Membrane organization, cholesterol-dependent regulatory processes
- **Interacts with and modulates various ion channels and transporters**

ΣΤΟΜΑΤΙΝΗ

•**Αλληλεπιδρά με GLUT1, Ζώνη-3, aquaporin-1, CD47, flotillins, calcium pump (*Rungaldier et al., BBA 1828:956; 2013*)**



Overhydrated Hereditary Stomatocytosis (OHS)

Sharp reduction or absence of stomatin- mutations in the Rh-associated glycoprotein gene (**RhAG**).

stomatin acts as **a molecular switch** that **binds to Glut1 and changes its substrate preference from glucose to DHA (**partly converts the **Glut1** into a transporter for **L-DHA).**

In the **absence of stomatin**, the DHA transport by Glut1 undergoes a 2-fold decrease while glucose uptake is significantly increased.

ATP-άση ΙΟΝΤΩΝ Ca^{++}

- 140 kDa
- Εξαρτώμενη από ATP **αποβολή Ca^{++}**
- **Ανταλλαγή Ca^{++}/H^{+}**
- Ενεργοποιείται από το σύμπλεγμα calmodulin/Ca^{++}

Levels of calcium must be carefully controlled in RBCs

Increase in calcium ions up-regulates a number of enzymes (e.g. calpains) and processes (e.g. proteolysis) that lead to RBC dehydration and protein degradation.