Glycosylation regulates CD38 assembly on the cell surface

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Miki Hara-Yokoyama

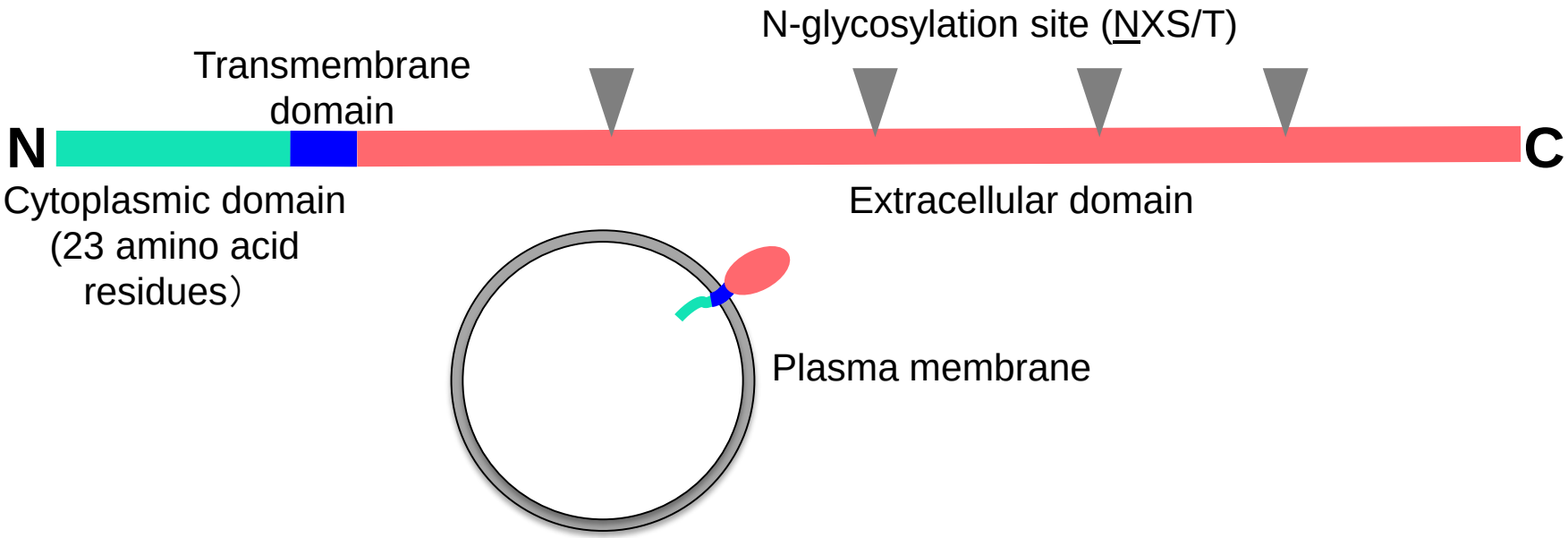
Tokyo Medical and Dental University (TMDU), Japan

Outline

- Introduction of CD38
- Aim of this study
- The structural analysis of assembly
    1) the extracellular domain of CD38 in solution
    2) CD38 on the cell surface
- The functional significance of the assembly
- The regulation by glycosylation

Introduction of CD38

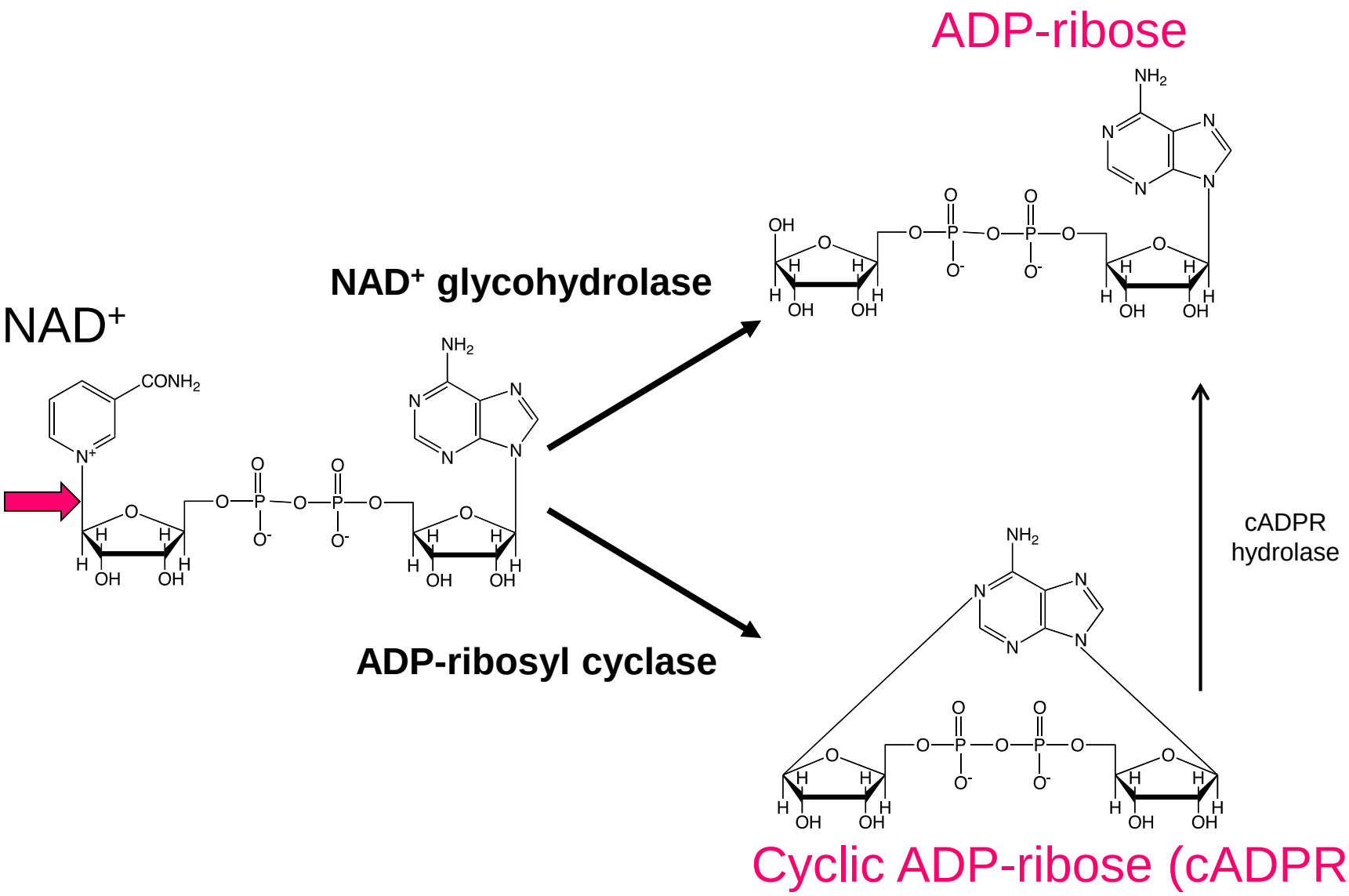
The leukocyte cell-surface antigen CD38 is a multifunctional protein.



Ectoenzyme

Raft-dependent signaling molecule

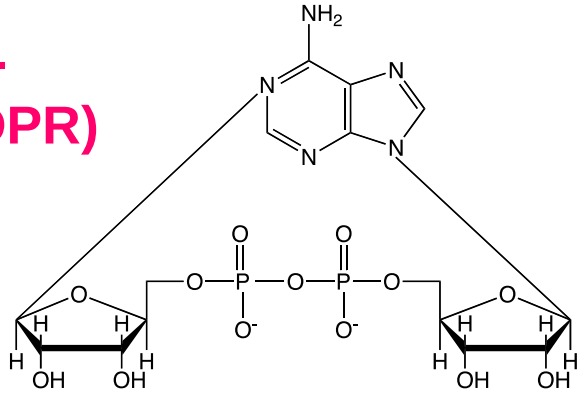
CD38 is the major NAD^+ glycohydrolase in mammals.



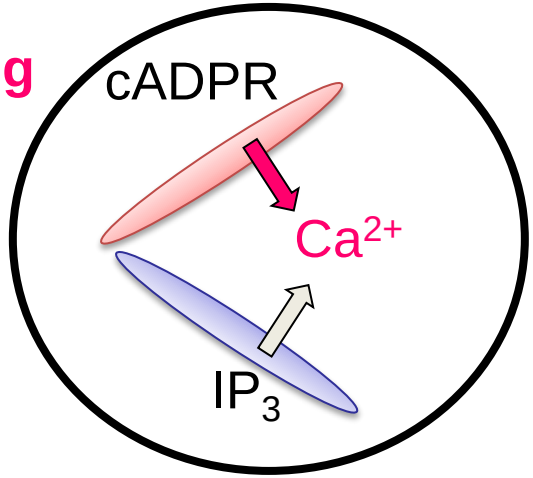
Cyclic ADP-ribose triggers intracellular calcium mobilization in an IP_3-independent manner.

Ectoenzyme	Trafficking of neutrophils and dendritic cells Secretion of insulin and oxytocin

Cyclic ADP-ribose (cADPR)



Ca^{2+} mobilizing messenger



Aplysia

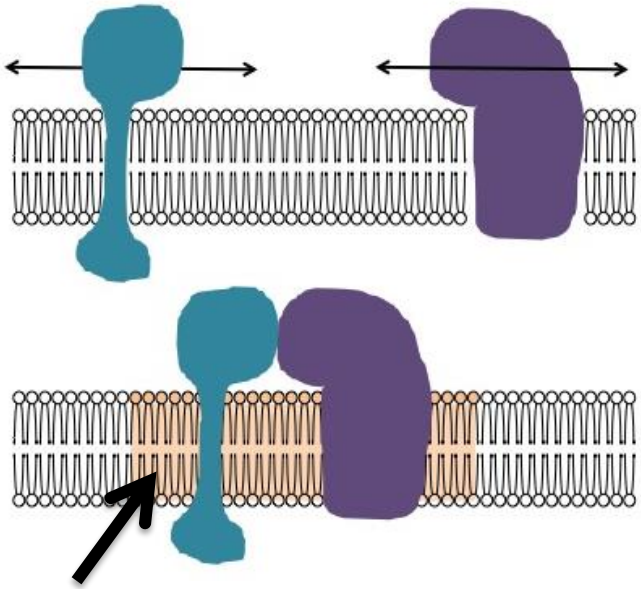
Aplysia ADP-ribosyl cyclase

Human

CD38

Similar structure

CD38 associates with various supramolecular complexes within lipid rafts.

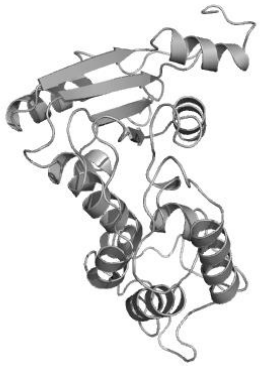


Cholesterol/sphingolipid-enriched membrane domain (lipid raft)

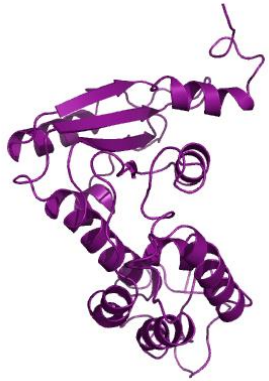
T cells	CD38/CD3/Lck/LAT
B cells	CD38/BCR/CD19/CD81
NK cells	CD38/CD16
Monocytes	CD38/MHC Class II/CD9
Dendritic cells	CD38/CD83/CD11b/CD81

Aim of this study

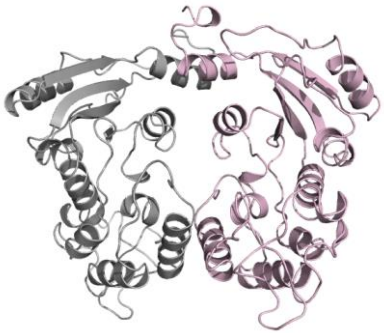
***Aplysia* ADP-ribosyl cyclase (cytosolic protein) 1LBE**



BST1/CD157 (GPI-anchored protein) 1ISF



CD38 (transmembrane protein) 1YH3



Structural basis & functional significance

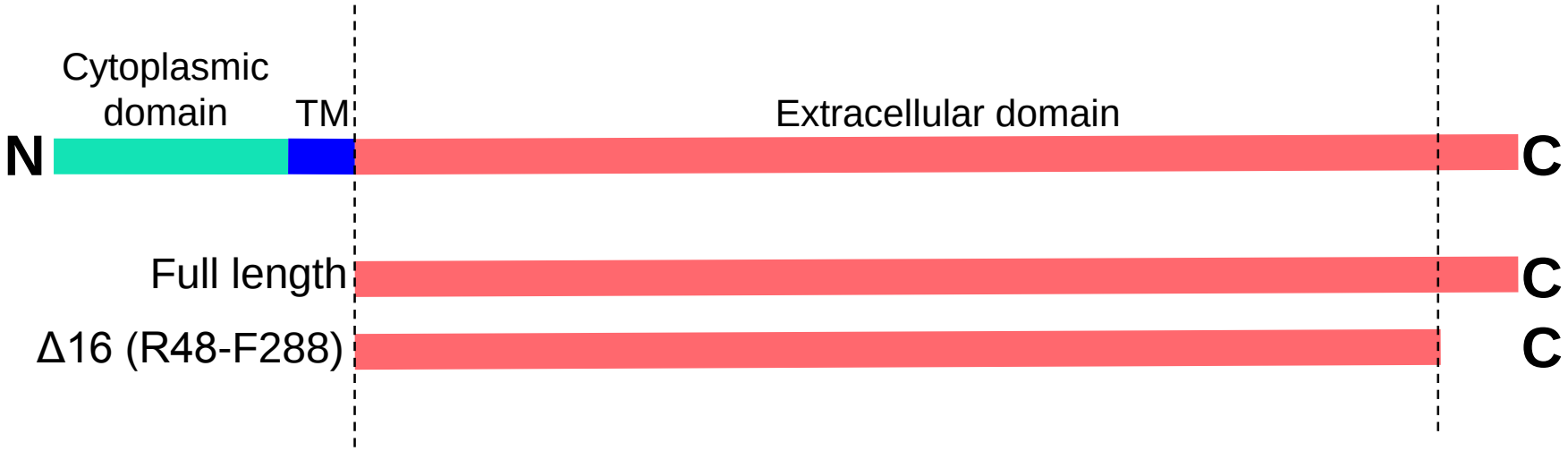
?

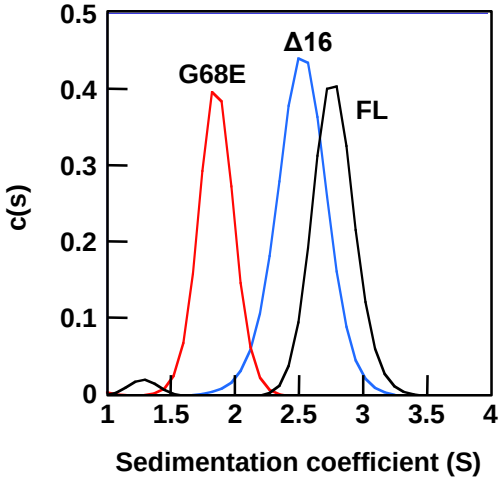
Dimer

Tetrame

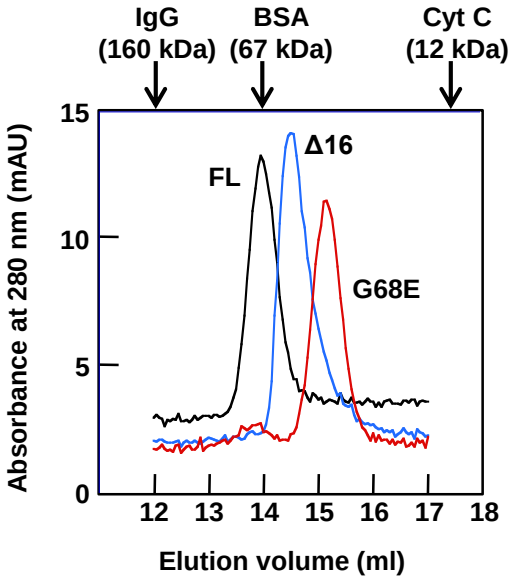
The structural analysis (the extracellular domain of CD38 in solution)

The full-length and the C-terminal-truncated extracellular domains of mouse CD38 exist as homodimers in solution.



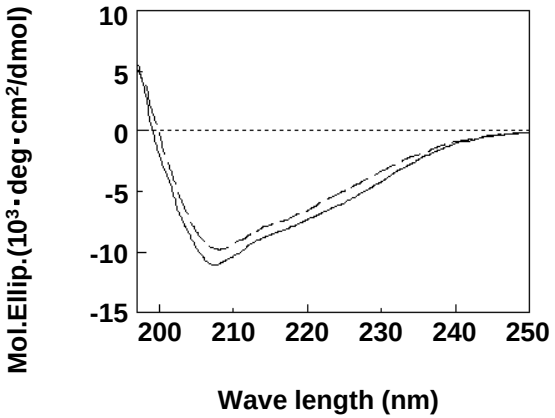


Analytical ultracentrifugation



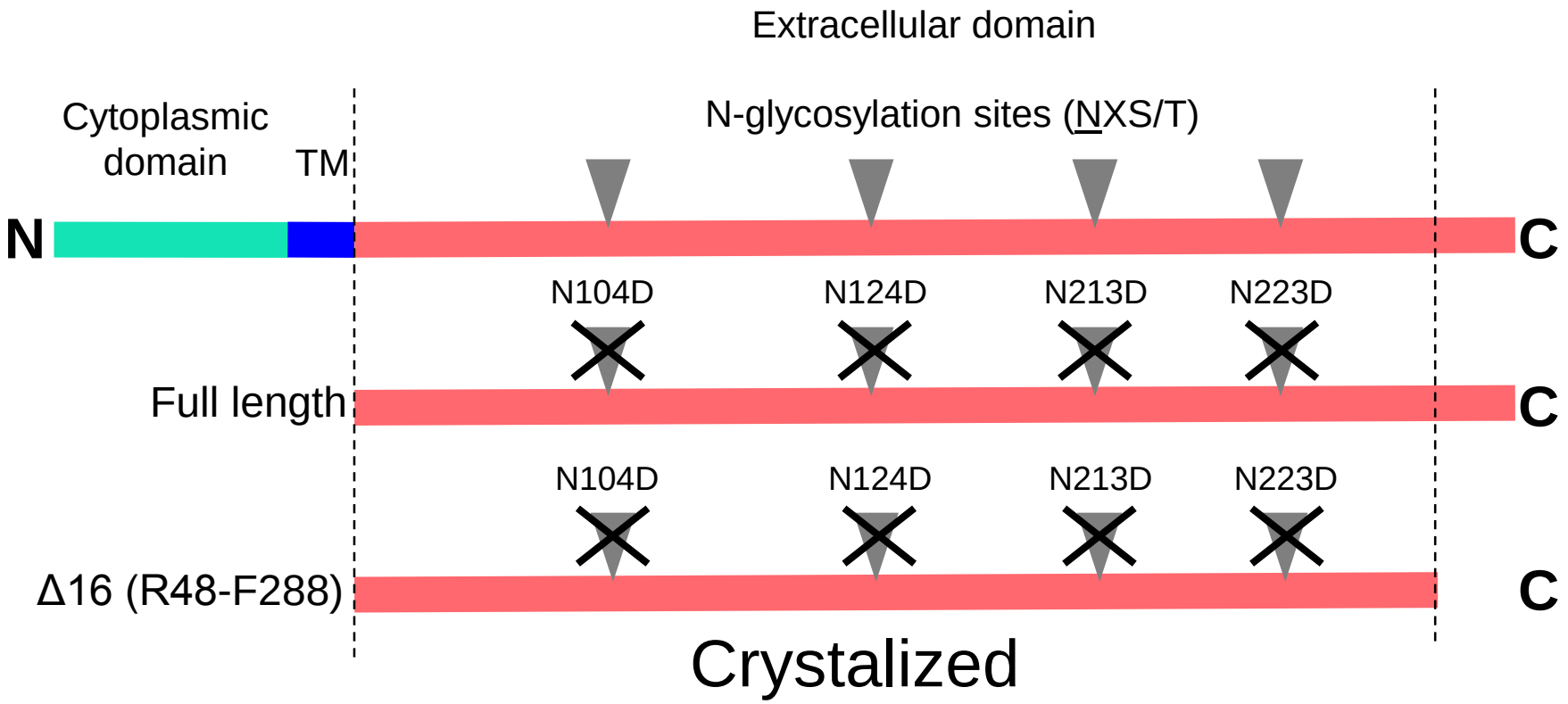
Size exclusion chromatography

The overall structure was not significantly altered by the truncation.



Circular dichroism spectra

Homophilic interfaces were found in the crystal packing of the C-terminal-truncated extracellular domain of CD38 !

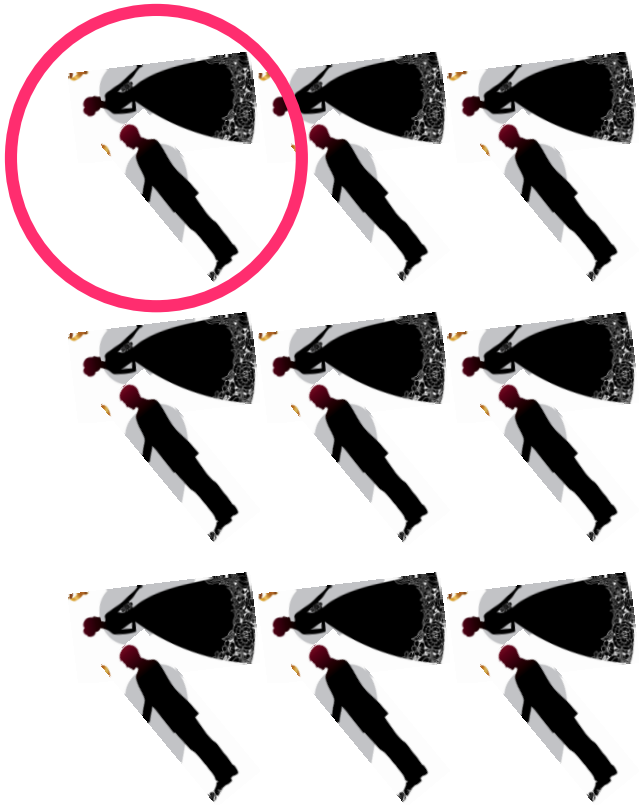


Concept of crystal packing

Information of interfaces

Suggested

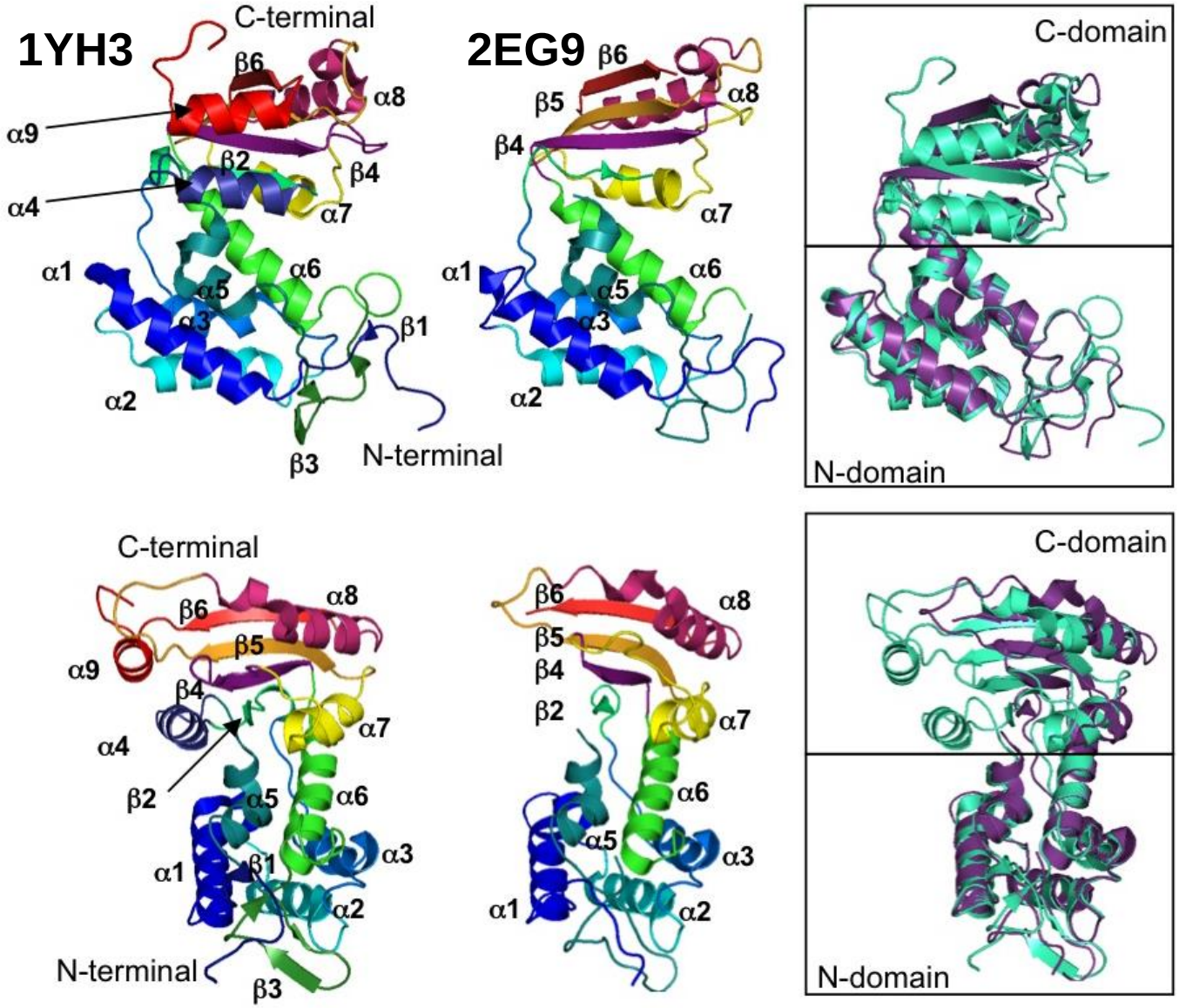
No information



This study (2EG9)

Previous study (1YH3)

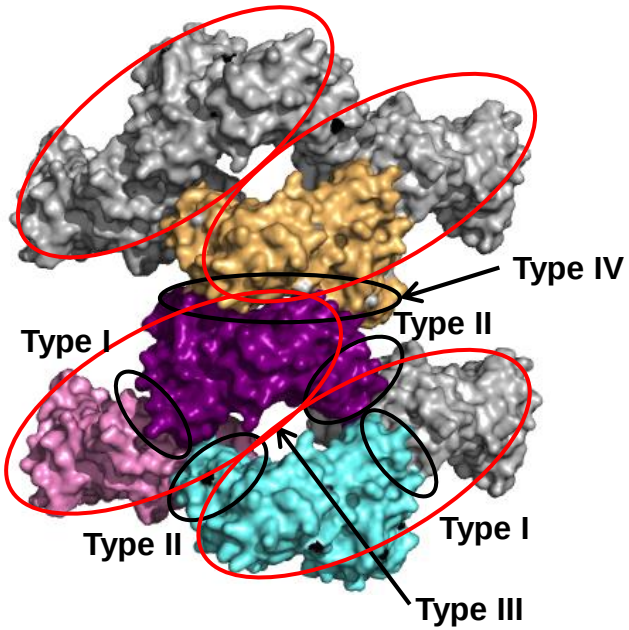
The monomeric structure was not significantly altered by the truncation, except the loss of the α9 helix and the fluctuation of the α4 helix.



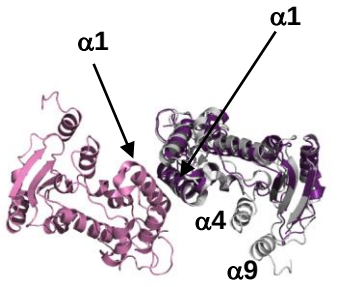
M.Hara-Yokoyama et al, Structure 20: 1585-1595 (2012)

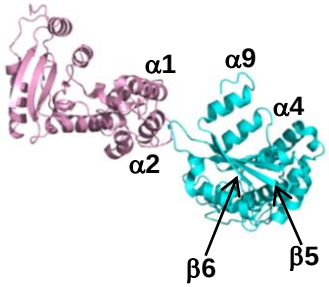
Four types of interfaces (I-IV) were found in the crystal packing of mCD38(R48-F288).

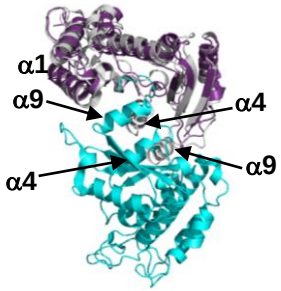
2EG9

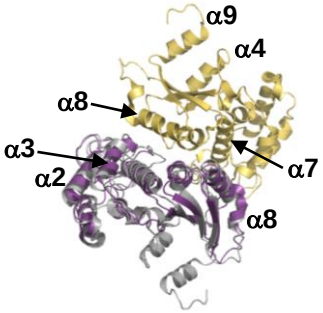


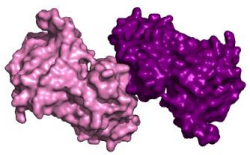
Do the interfaces exist also in solution?

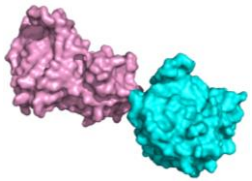


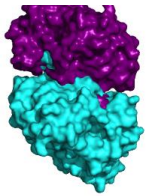


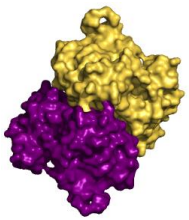










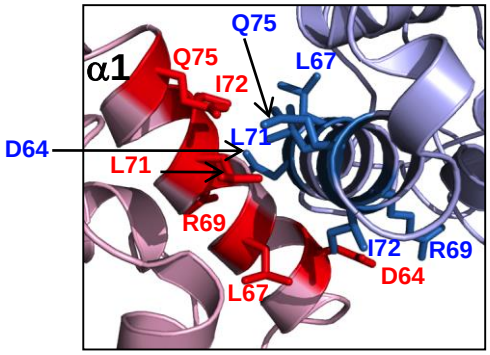


Type I Type II Type III Type IV

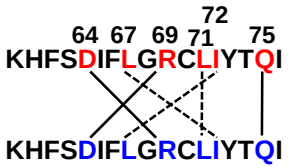
Two molecules of the full-length extracellular domain are oriented according to those in 2EG9.

The results of the G68E mutation support the interaction between the α1 helices of the extracellular domain of CD38 in solution.

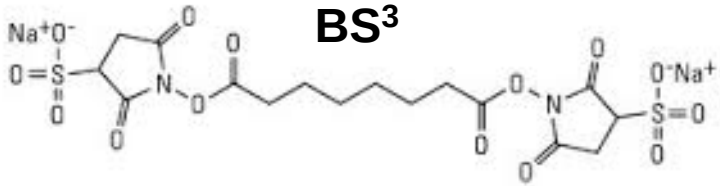
The G68E mutation should disrupt the type I interface.



α1 helix



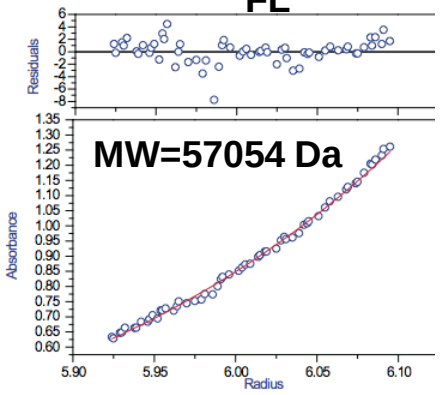
BS³



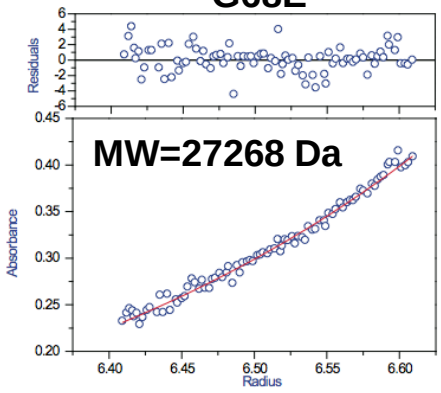
BS3
Bis(sulfosuccinimidyl) suberate
MW 572.43
Spacer Arm 11.4 Å

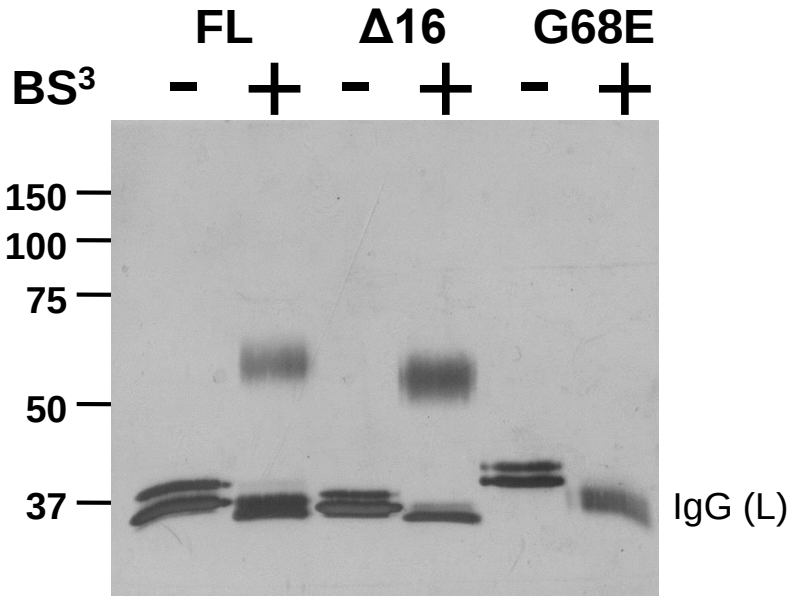
Analytical ultracentrifugation (sedimentation equilibrium)

FL



G68E





The BS^3-dependent crosslinking likely occurs via the type III interaction mode.

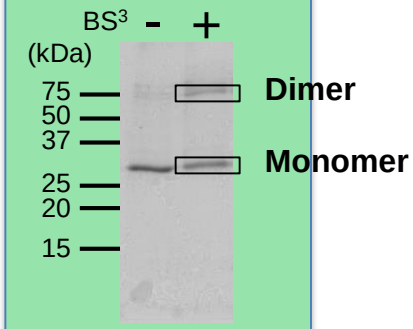
FLAG-CD38

↓

crosslink

↓

SDS-PAGE

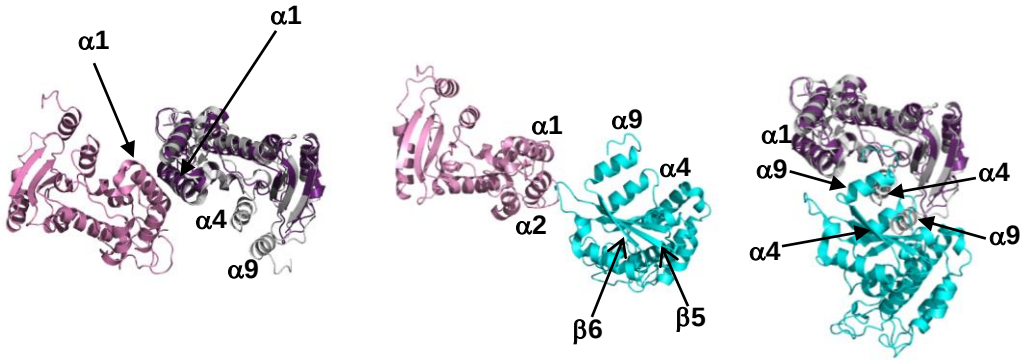


↓

Trypsin digestion

↓

MS analysis



Type I **Type II** **Type III**

Lys145

SKSKHLAHQYTWIQGKMFTLED

m/z = 1481.79 (peak 6)

FLAG-tag

RAMDYKDDDDKLRSLLVWTGEPTTKHFSDIFLGRCLIYTQ

Lys60

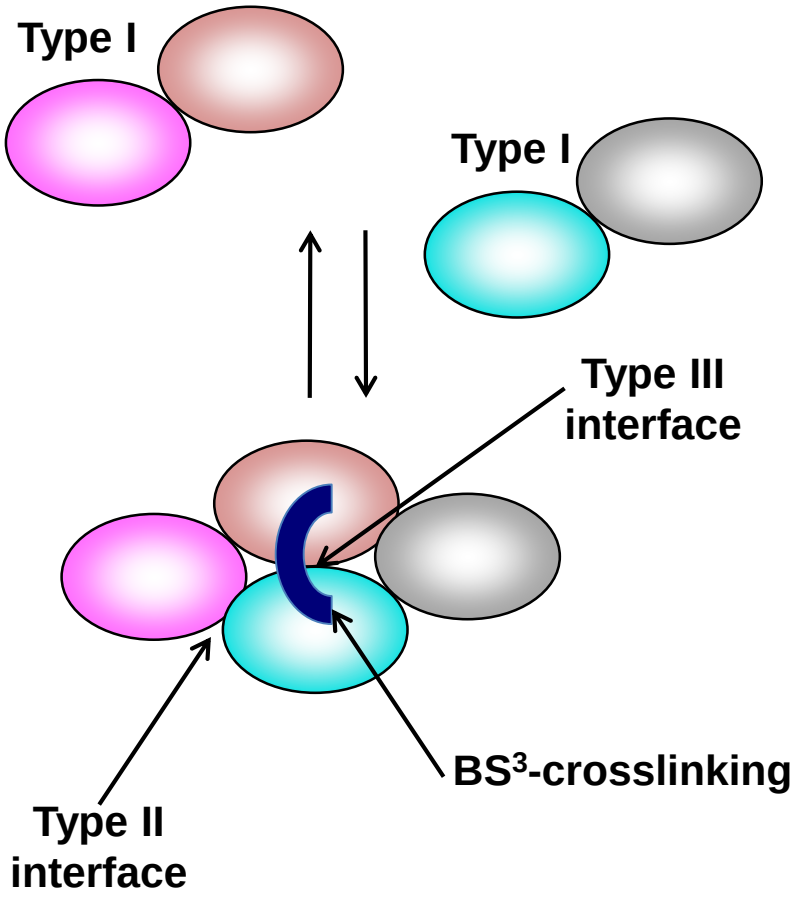
68

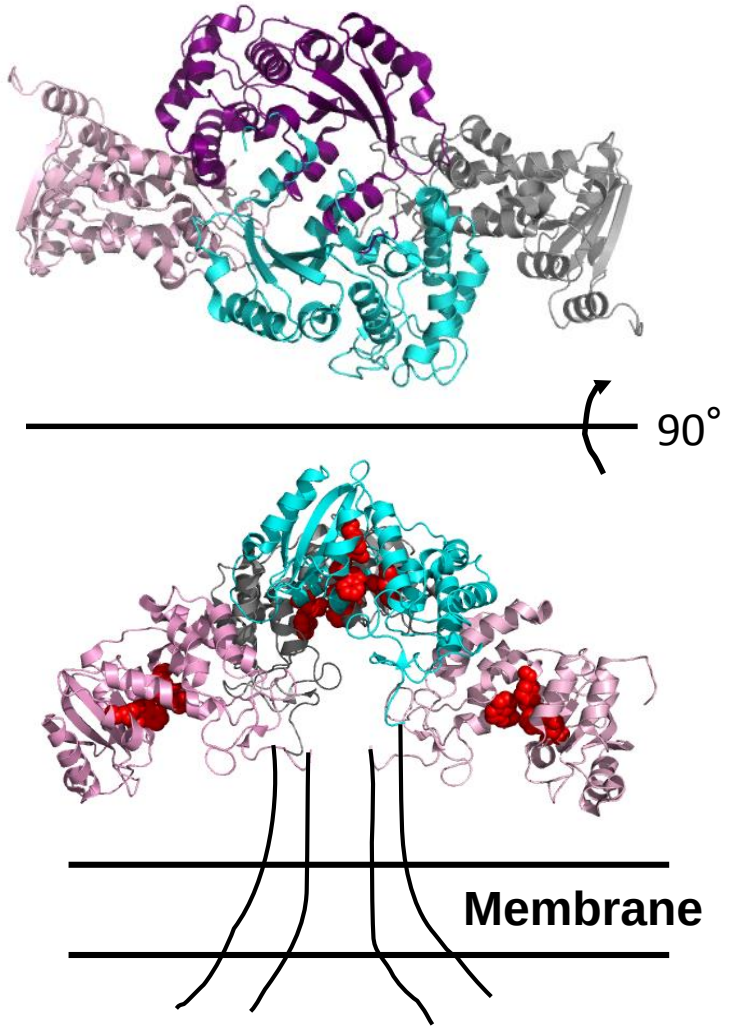
m/z = 1331.75 (peak 1)

m/z = 1091.60 (peak 2)

Monomer	+	+	
Dimer	+	-	

The dimer via the type I interaction mode exist in equilibrium to form a tetramer via the type II/III interaction mode, which is compatible with membrane association.

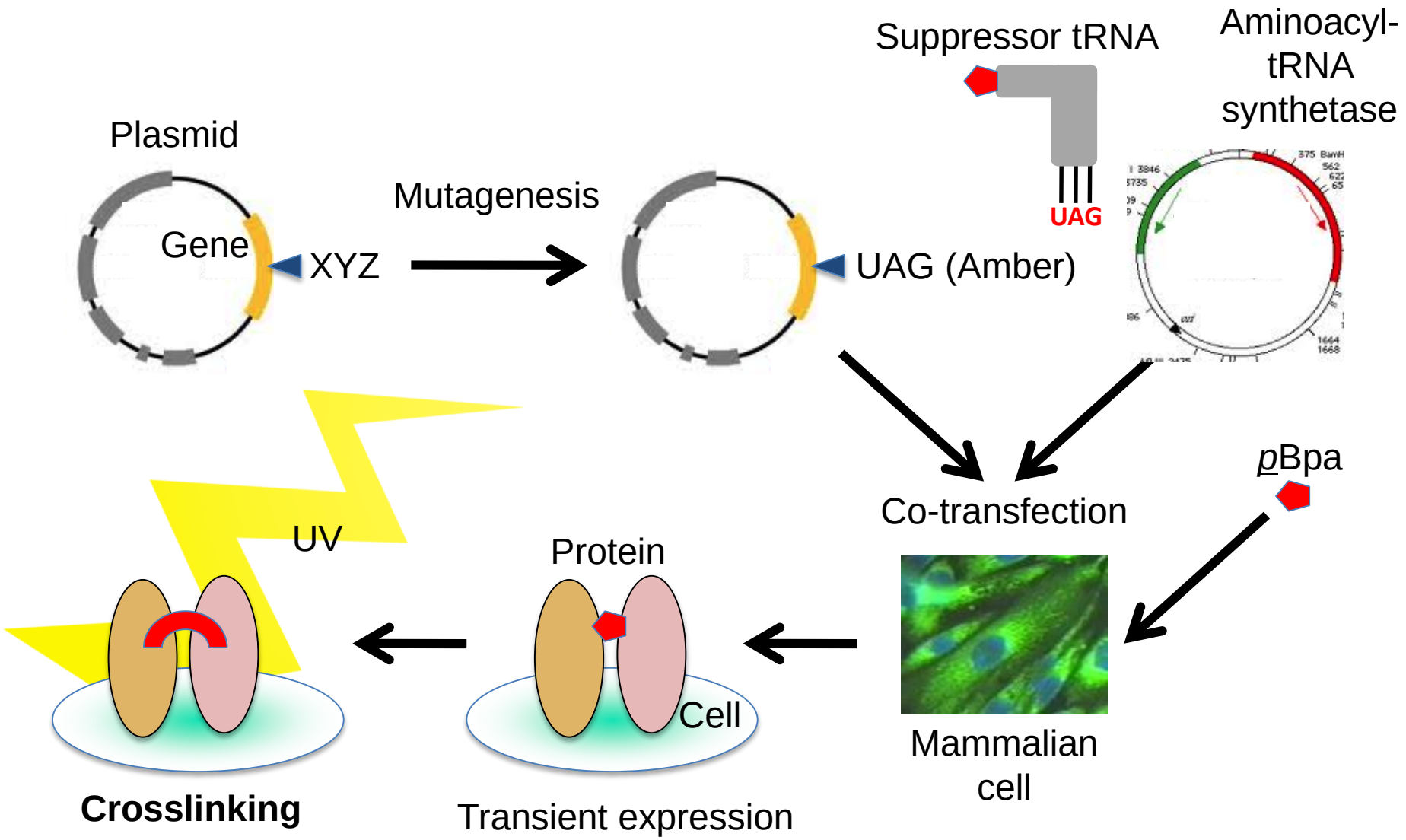




The structural analysis (CD38 on the cell surface)

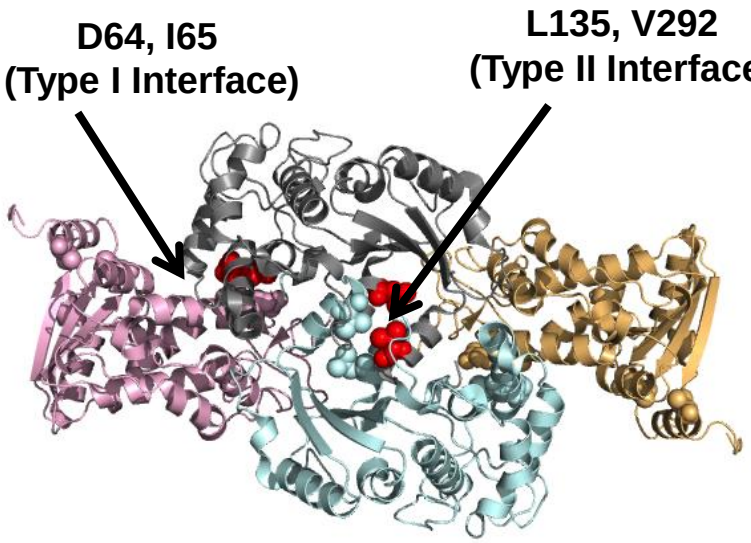
Are the interfaces present in CD38 on the cell surface?

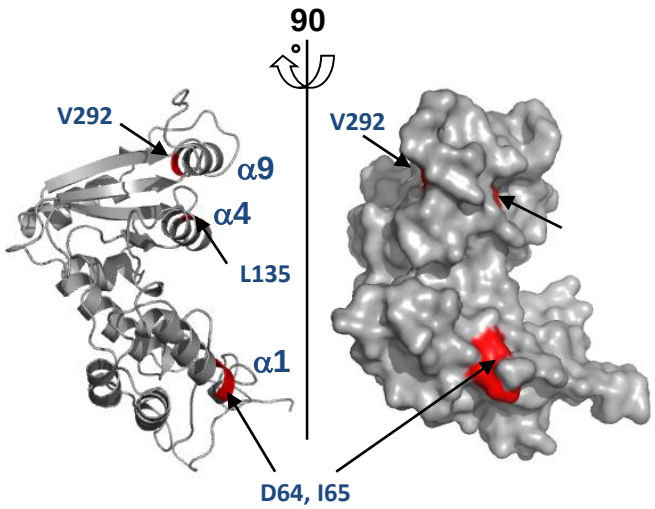
Site-specific crosslinking on the cell surface with an expanded genetic code.



Hino et al, Nature Methods 2:201-206 (2005), Nature Protocol 1: 2957-2962 (2006)

The crosslinking occurs between CD38 molecules and the type I and type II interfaces are involved, suggesting the tetramerization of CD38 on the cell surface.





CHO cells

FLAGx3-CD38/amber

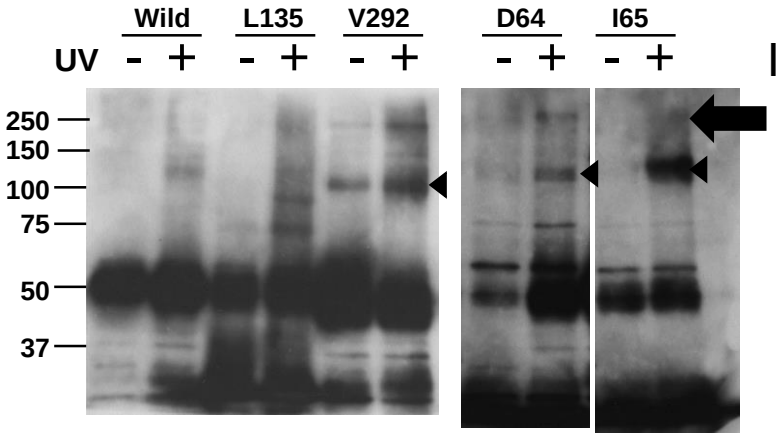
Mycx3-CD38

↓

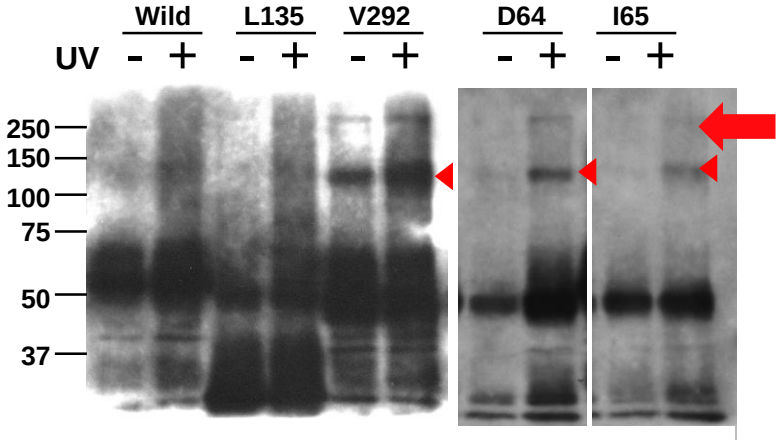
Crosslink

↓

IP: FLAG



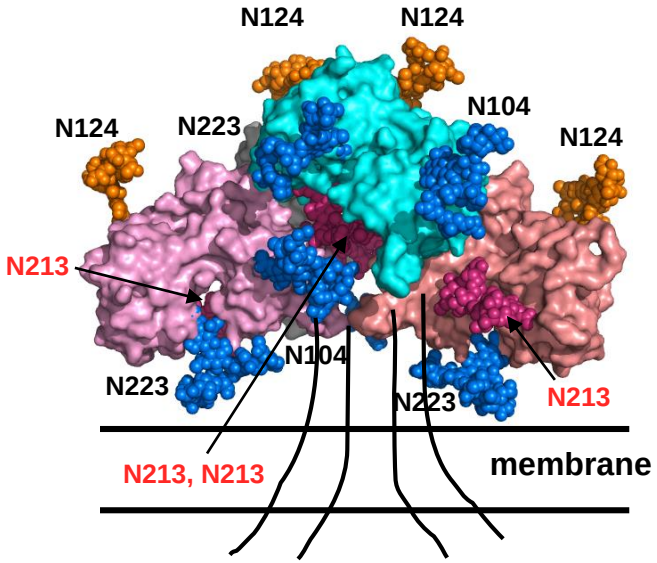
IB: FLAG

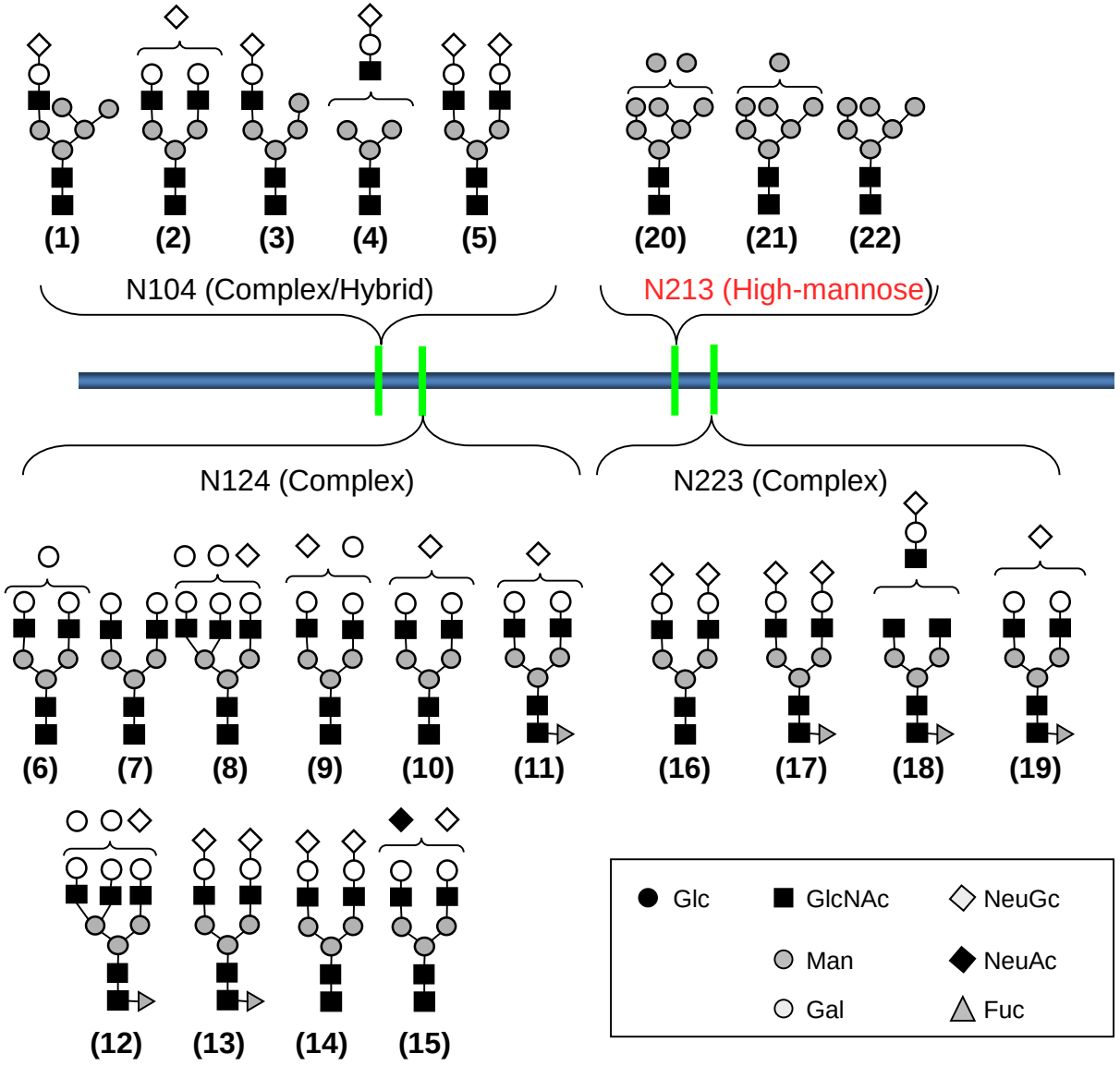


IB: Myc

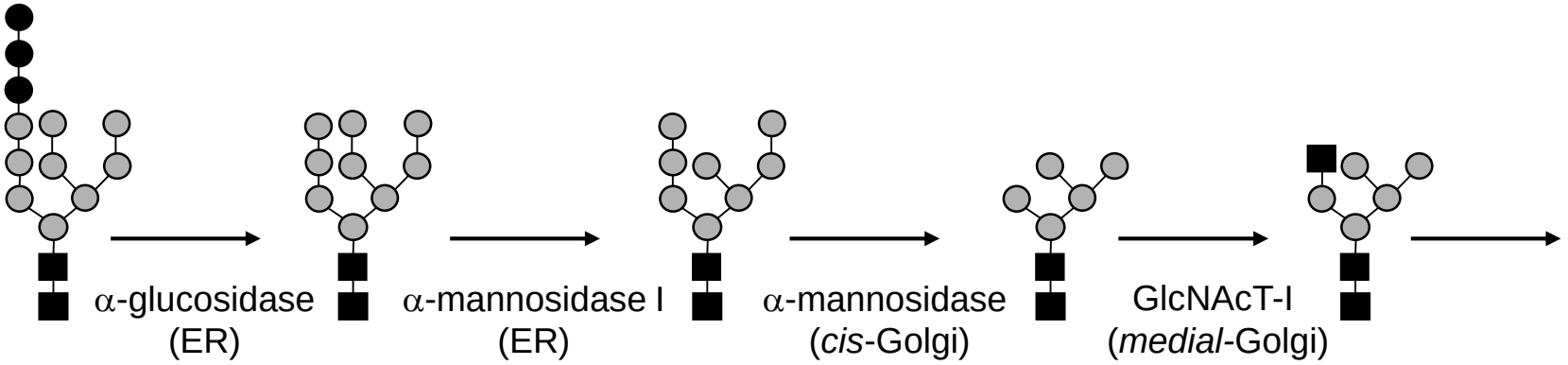
Only the oligosaccharides attached to the N213 residue remained as the high-mannose-type.

A20 cells expressing
FLAGx3-CD38
↓
IP: FLAG
↓
Trypsin digestion
↓
MS analysis

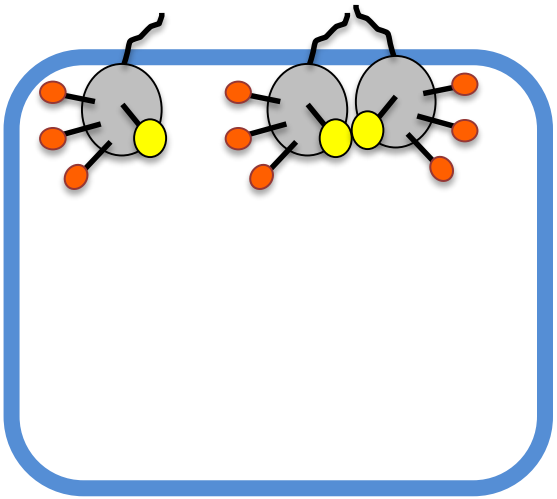




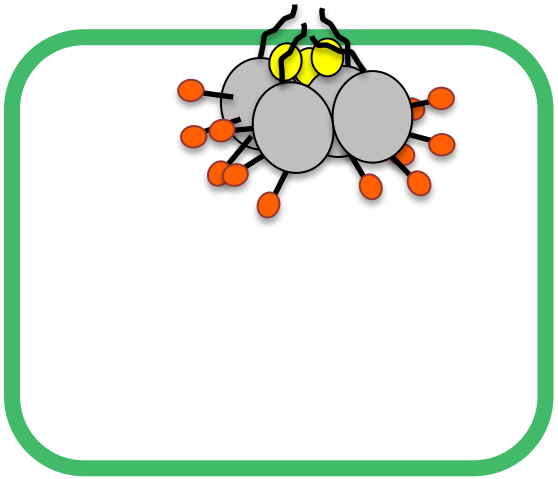
The processing of the N-glycan of CD38 is compatible with tetramerization.



Within the tetramer, the processing enzymes are not accessible to the N213 residues.



ER or *cis*-Golgi



medial-Golgi

The functional significance of tetramerization

Evaluation of the significance of the tetramerization of CD38 on the cell surface.

To impair the type I interface

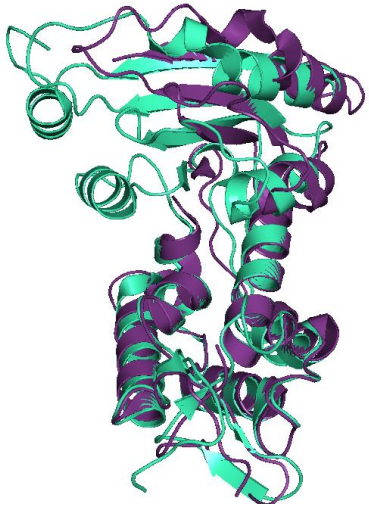
G68E

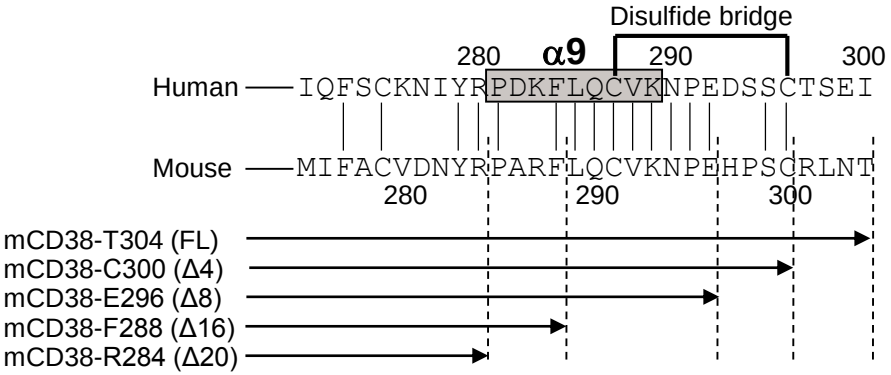
To affect the type III interface

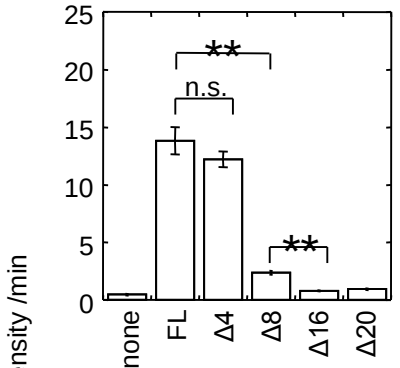
C-terminal deletion
C291A, C300A, C291A/C300A

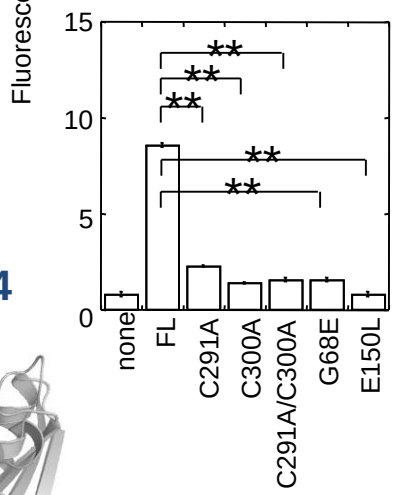
Both the I and type III interfaces are crucial for the tetramerization on the cell surface.

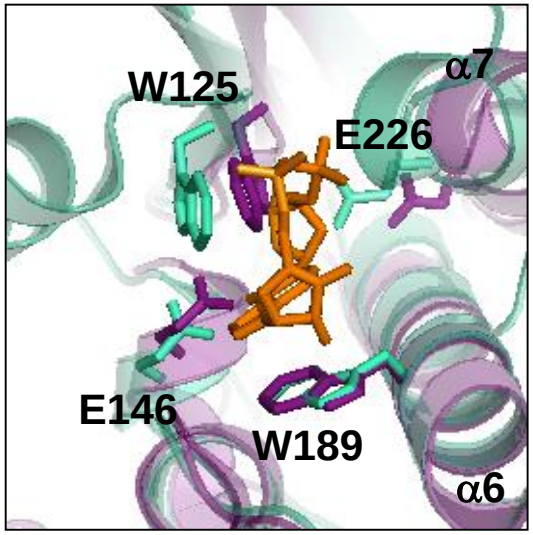
The tetramer structure (both type I and II/III) is required for the catalytic activity of CD38 in A20 cells.

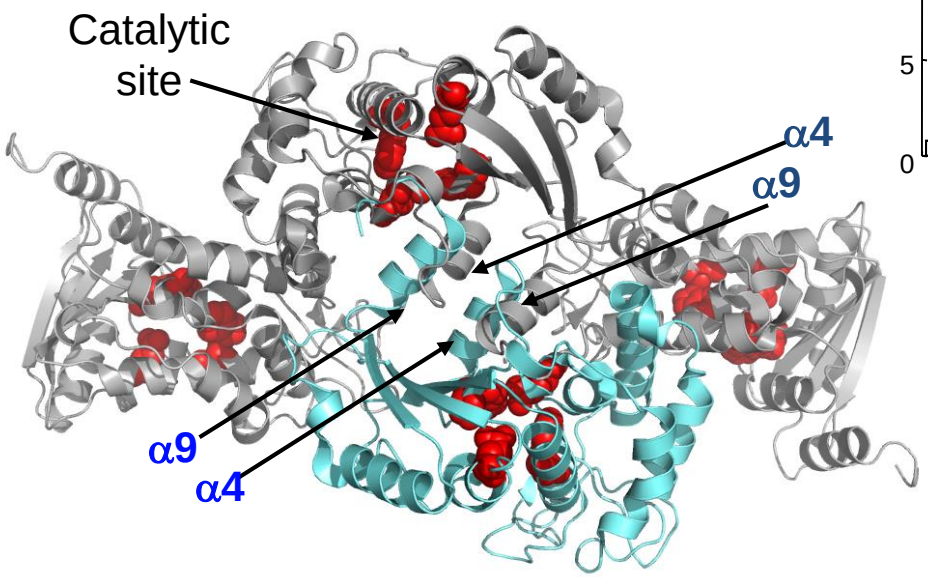












Preparation of detergent-resistant membranes (DRMs)

A20 cells expressing CD38

↓

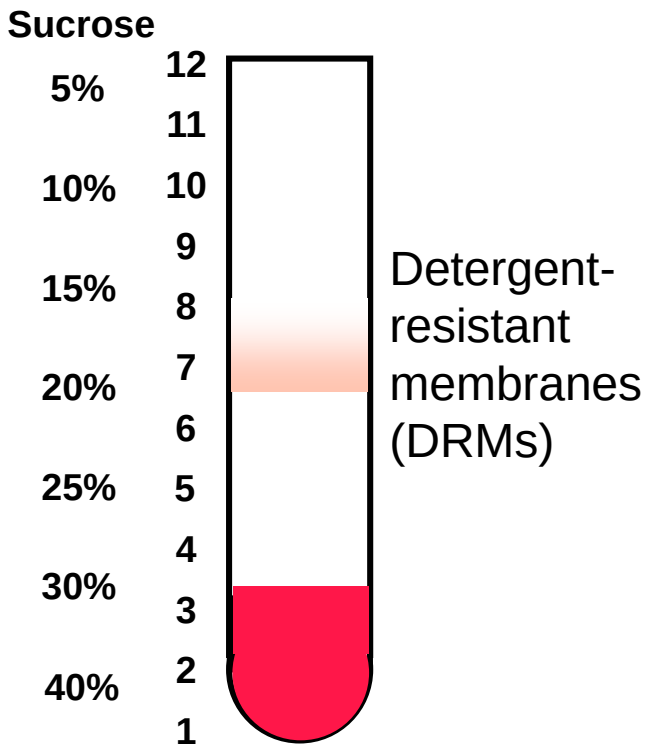
Lysis with Brij-58

↓

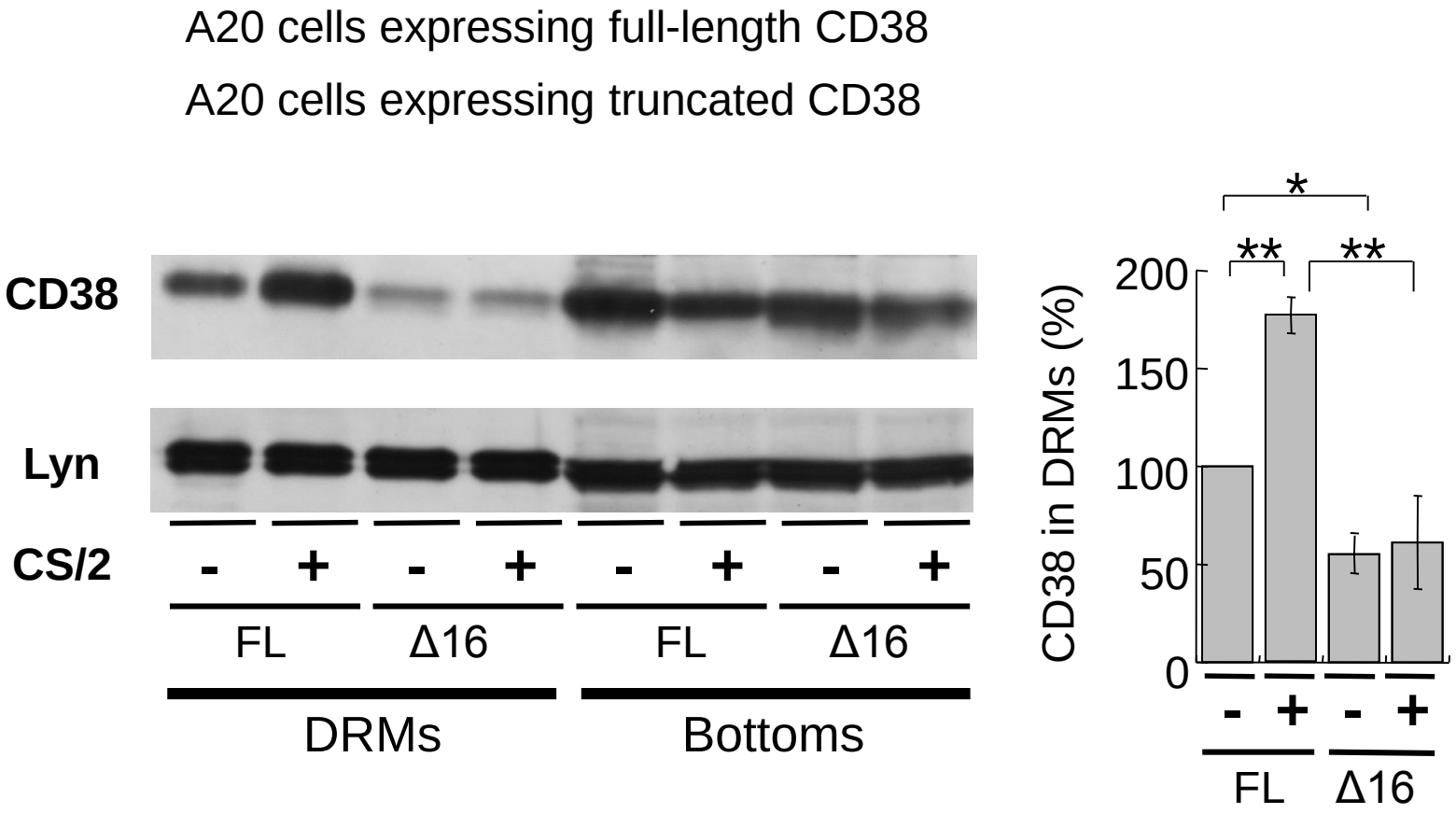
Sucrose density gradient ultracentrifugation

↓

Detergent-resistant membranes (DRMs)

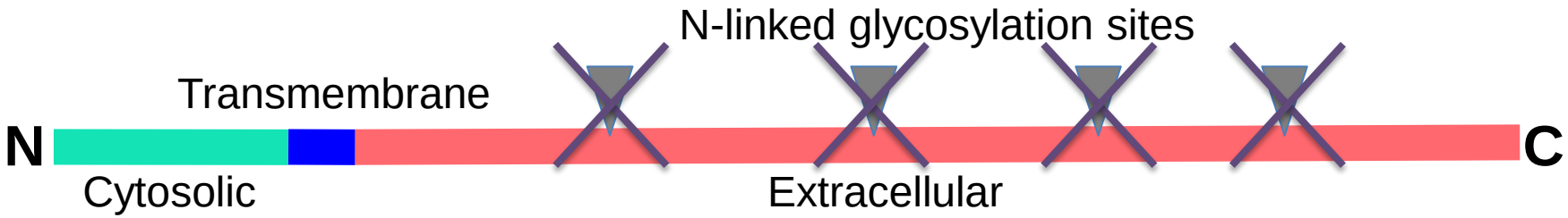


The tetramer structure is required for the association of CD38 with DRMs in A20 cells.



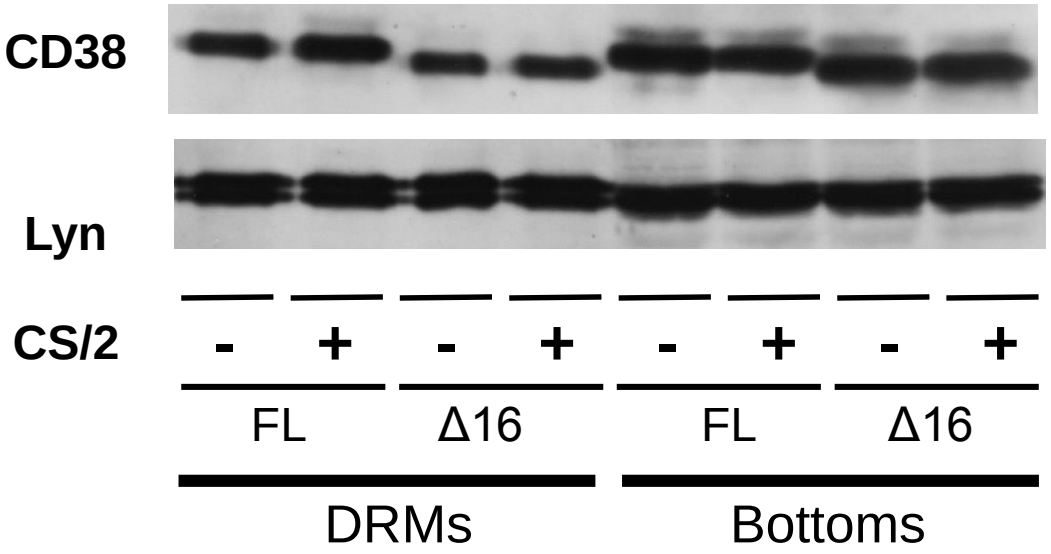
The effect of glycosylation

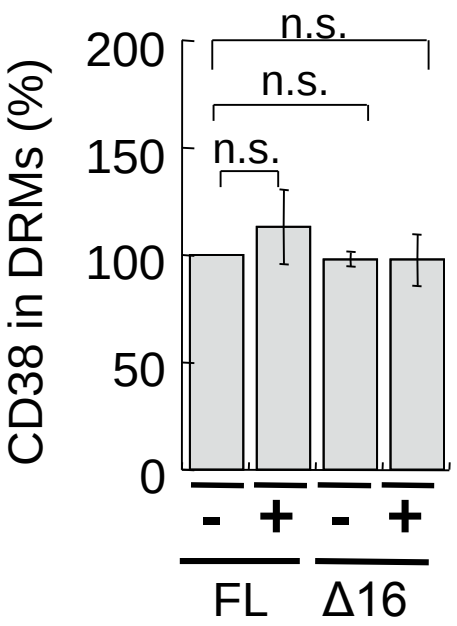
The C-terminal truncation did not alter the amount of nonglycosylated CD38 in DRMs.



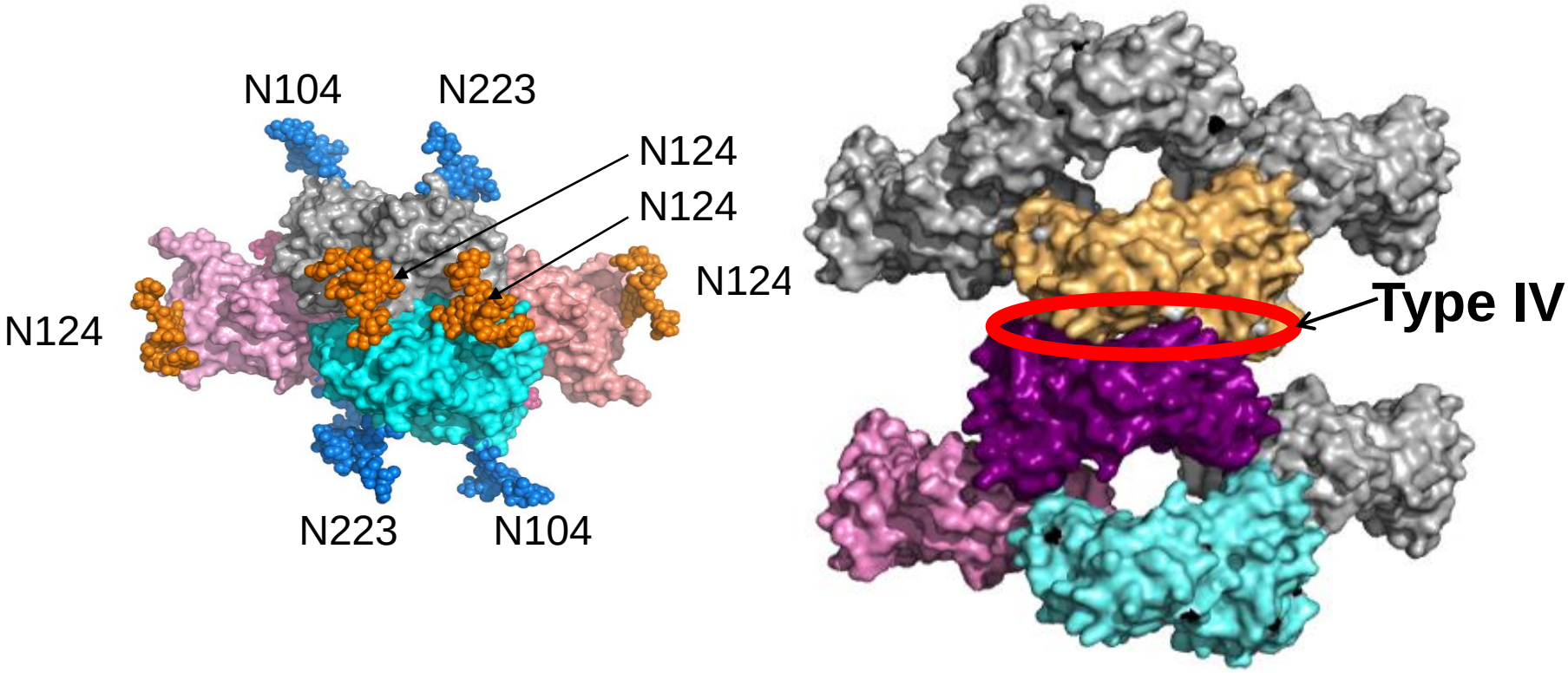
A20 cells expressing full-length deglycosylated CD38

A20 cells expressing truncated deglycosylated CD38

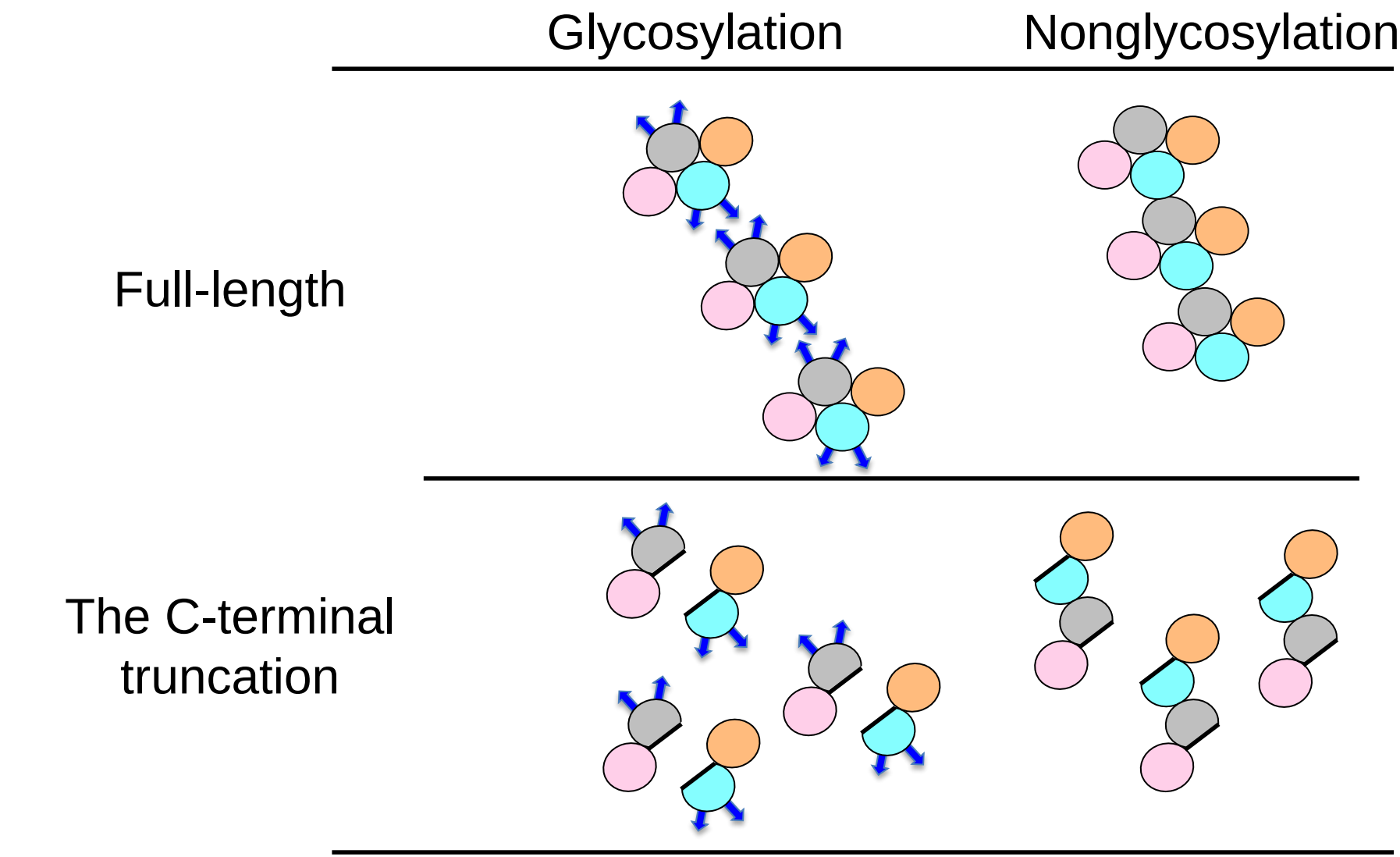




The absence of the N-glycans attached to N104 and N223 enables the formation of the “type IV” interface in the case of cell-surface CD38.

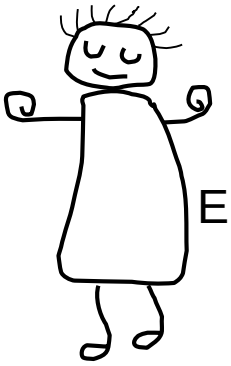


The N-glycans probably regulate the assembly of CD38 on the cell surface by inhibiting the “aggregating” type IV interface.



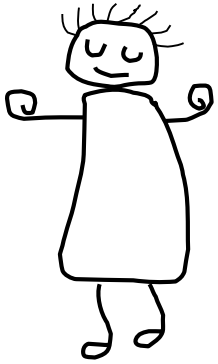
Summary

***Aplysia* ADP-ribosyl cyclase (cytosolic protein) 1LBE**

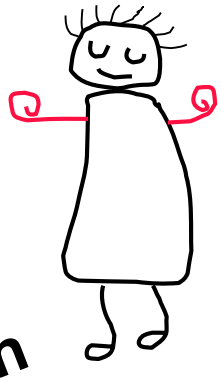


Enzyme

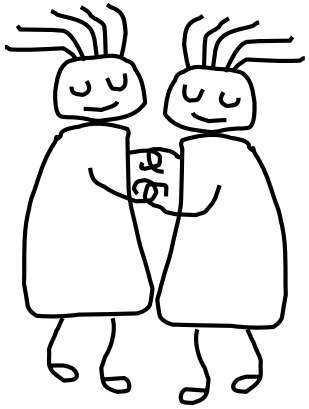
BST1/CD157 (GPI-anchored protein) 1ISF

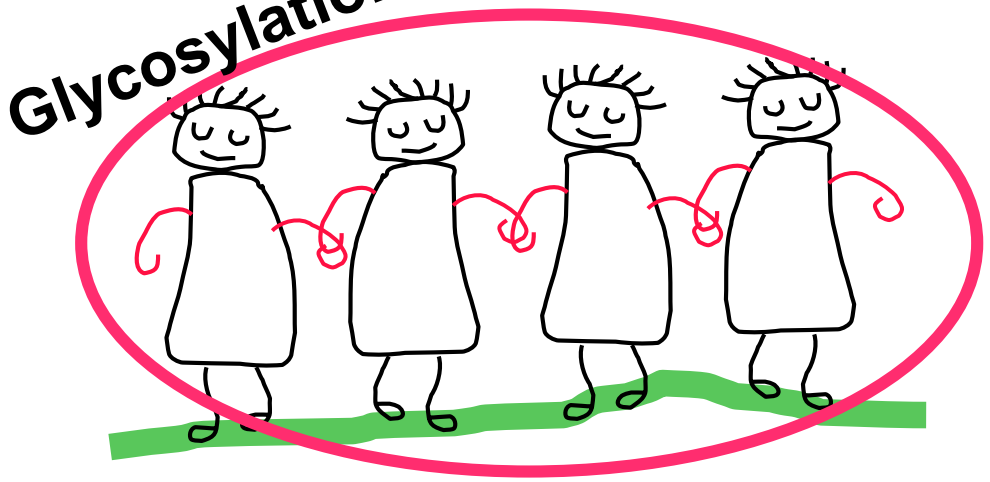


CD38 (transmembrane protein) 1YH3



Enzyme + raft-association





M.Hara-Yokoyama et al, Structure 20: 1585-1595 (2012)

Collaborators

Tokyo Medical and Dental University (TMDU)

- Kazue Terasawa
- Satoru Harumiya
- Katarzyna A. Podyma-Inoue
- Takeshi Kasama
- Hiroshi Takayanagi
- Masaki Yanagishita

RIKEN (SSBC)

- Mutsuko Kukimoto-Niino
- Nobumasa Hino
- Kensaku Sakamoto
- Chiemi Mishima-Tsumagari
- Yoko Kaitsu
- Tomoko Matsumoto
- Motoaki Wakiyama
- Mikako Shirouzu
- Yoshio Hirabayashi
- Shigeyuki Yokoyama

National Institute of Health Sciences

- Satsuki Itoh
- Noritaka Hashimoto
- Yoko Hiruta
- Nana Kawasaki

Musashino University

- Naoko Ustunomiya-Tate

University of Toyama

- Kiyoshi Takatsu

RIKEN (BSI)

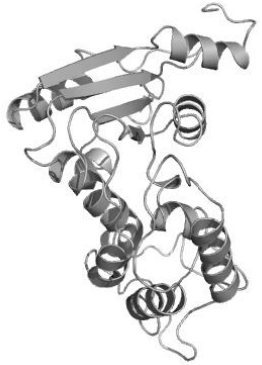
- Yoshio Hirabayashi

University of Tokyo

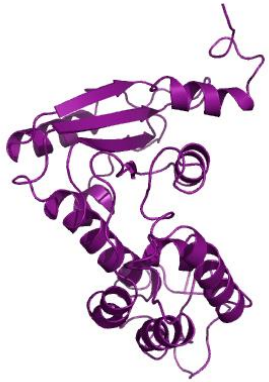
- Toshiaki Katada

We identified the interfaces contributing the tetramer formation.

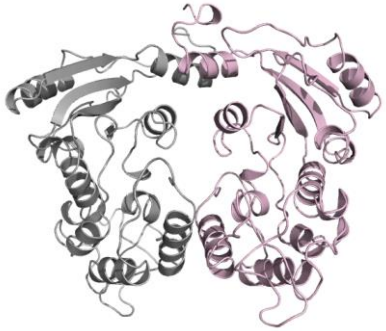
***Aplysia* ADP-ribosyl cyclase (cytosolic protein) 1LBE**

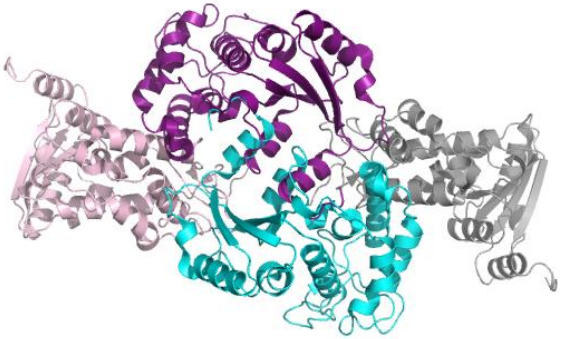


BST1/CD157 (GPI-anchored protein) 1ISF



CD38 (transmembrane protein) 1YH3

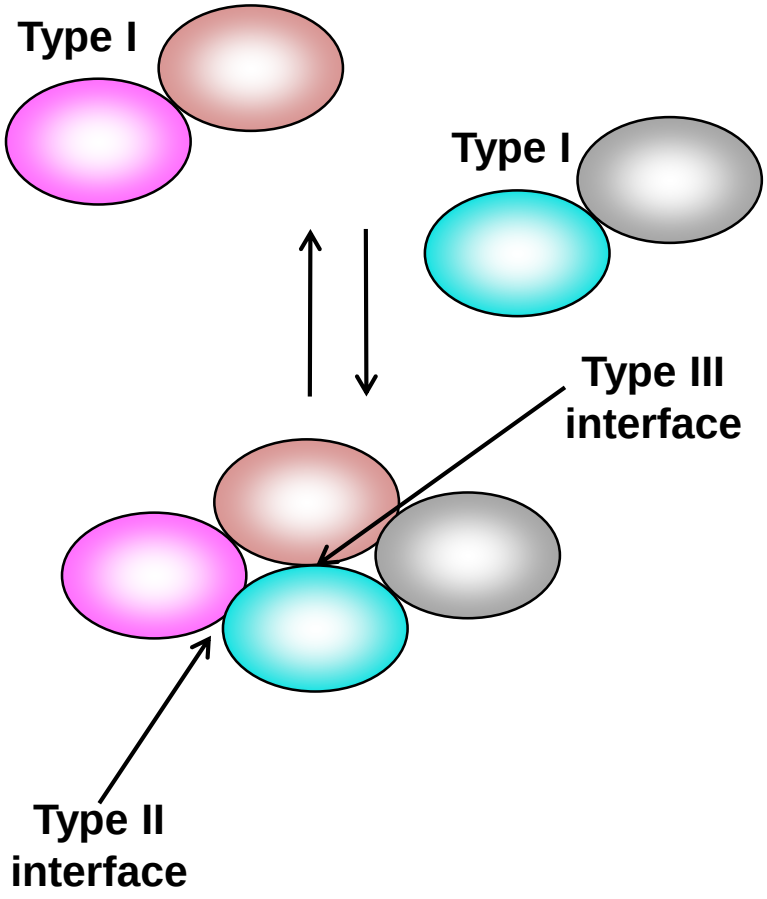


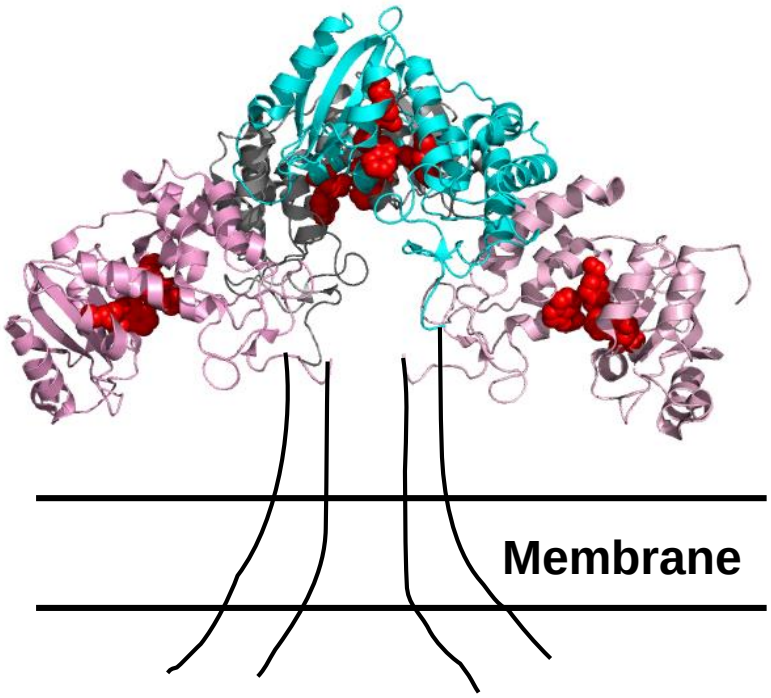


2EG9

M.Hara-Yokoyama et al,
Structure 20: 1585-1595 (2012)

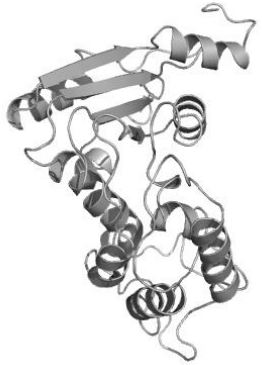
The dimerization of core dimers provides a structural basis for the previously reported tetramerization of CD38 on the cell-surface.



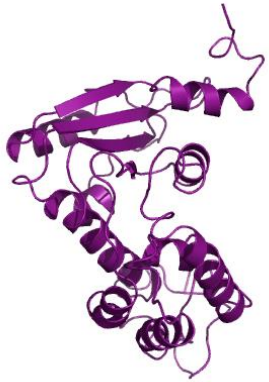


CD38の細胞膜上での四量体構造は機能と密接に関与する

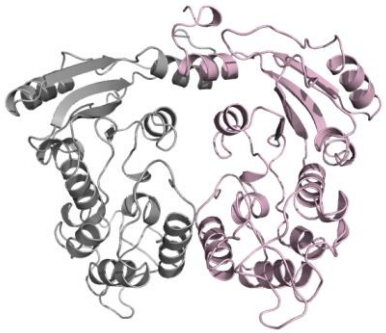
***Aplysia* ADP-ribosyl cyclase (cytosolic protein) 1LBE**

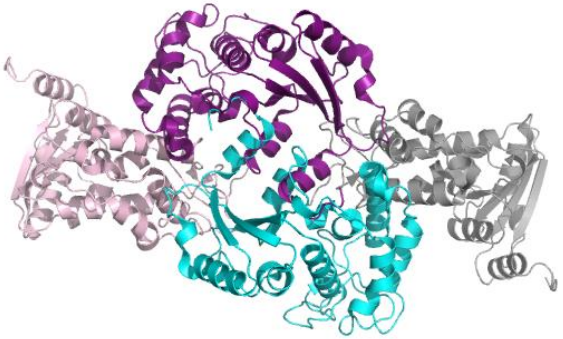


BST1/CD157 (GPI-anchored protein) 1ISF



CD38 (transmembrane protein) 1YH3

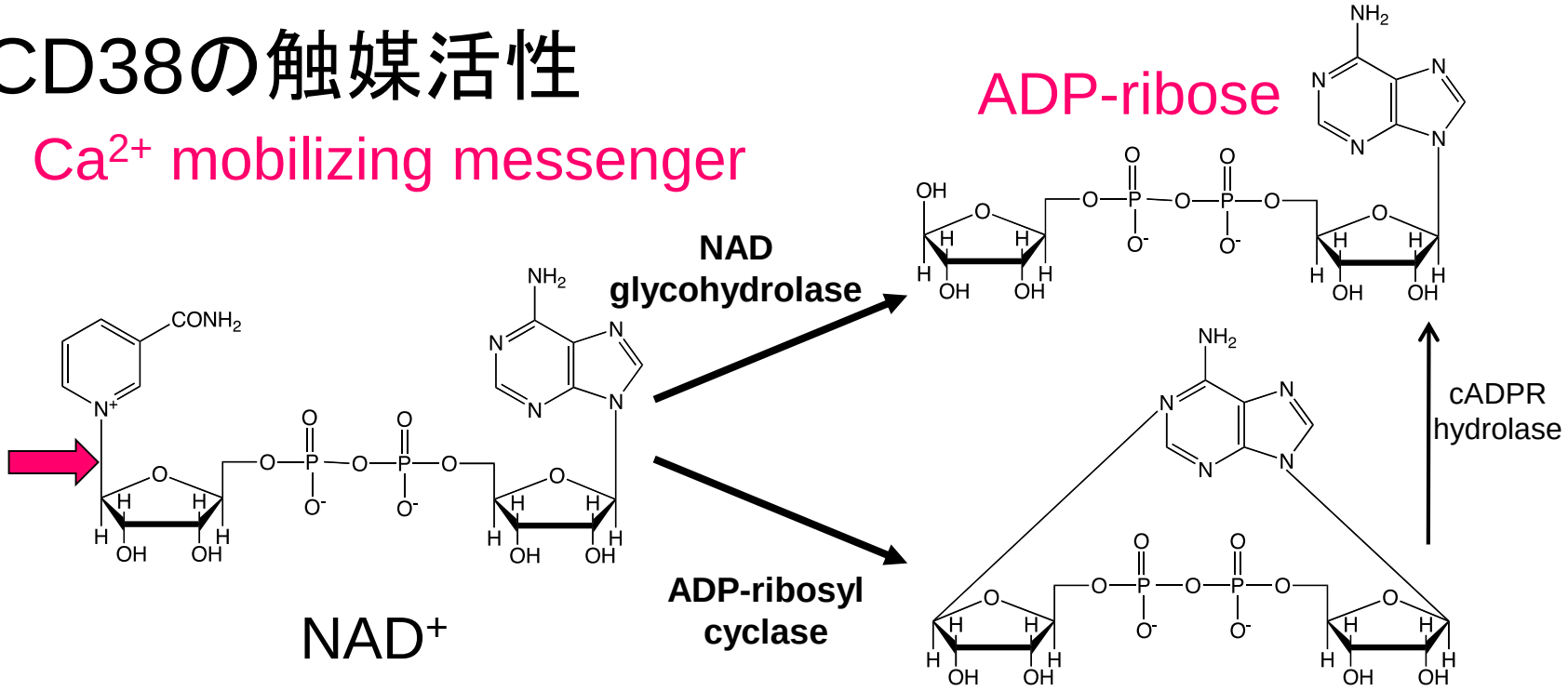


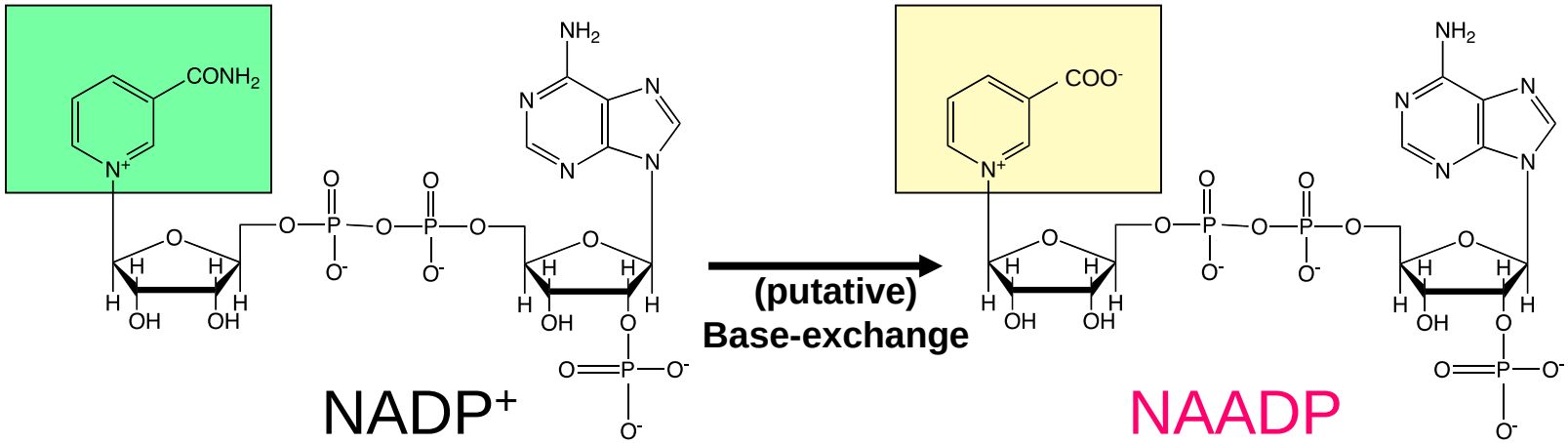


M.Hara-Yokoyama et al,
Structure 20: 1585-1595 (2012)

CD38の触媒活性

Ca^{2+} mobilizing messenger



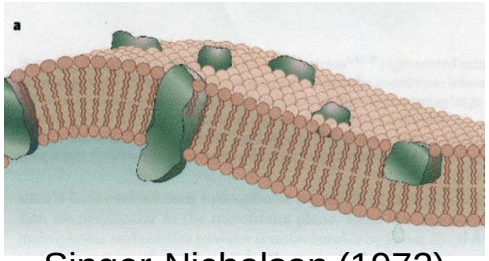


細胞内カルシウム動員活性をもつ

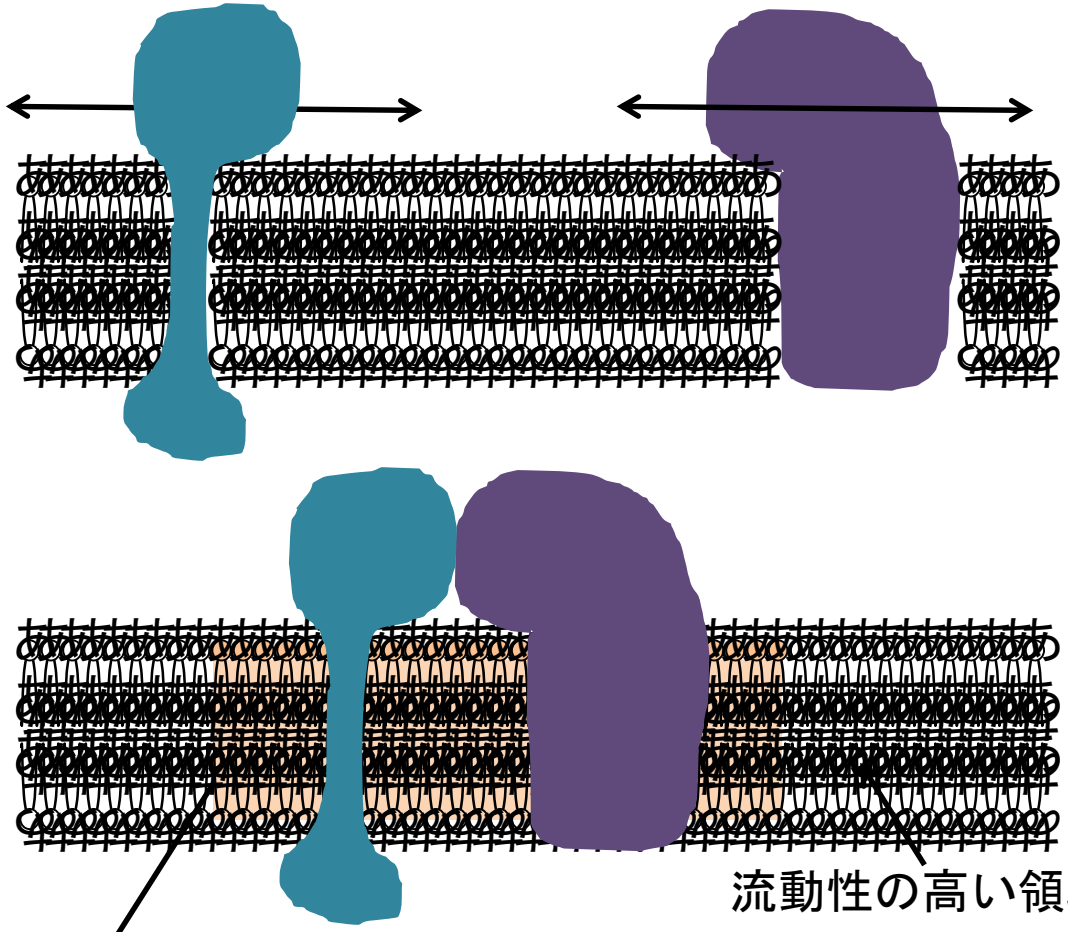
CD38は膜ドメインに存在する

膜ドメインとは

脂質二重層



Singer-Nicholson (1972)



生体膜には不均一性がある

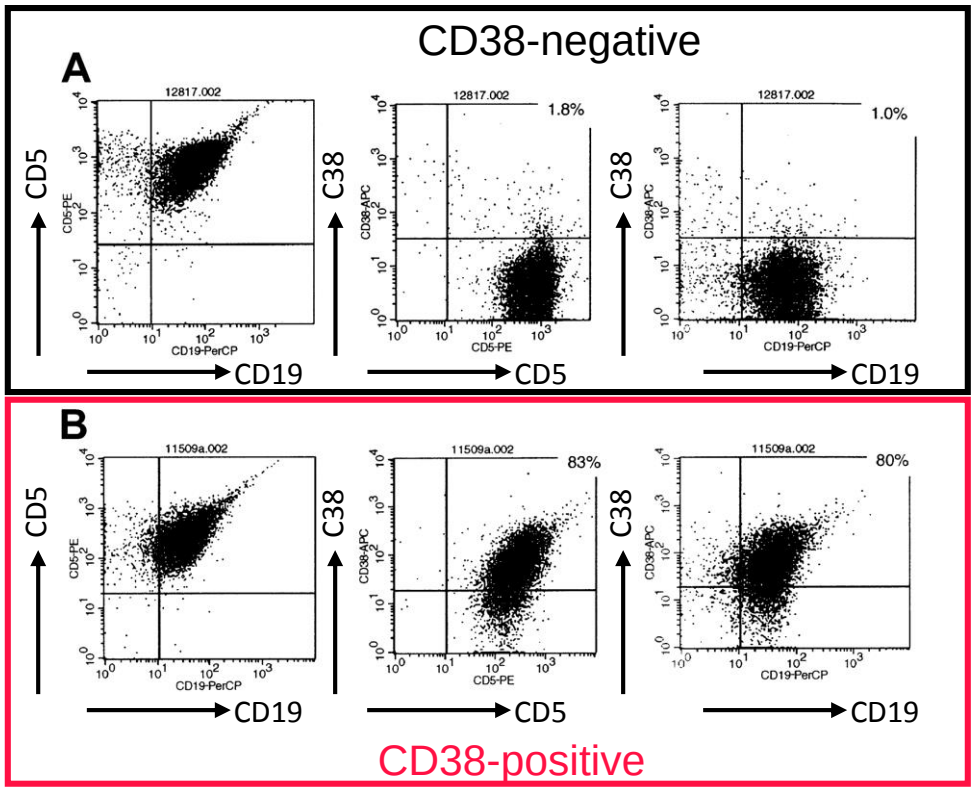
CD38 is recognized as a negative prognostic indicator in B-CLL patients.

Peripheral blood

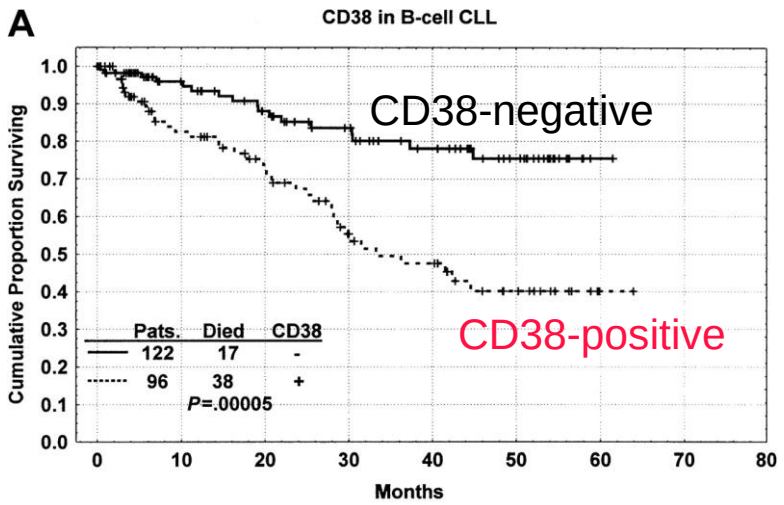
↓

Flow cytometry (CD5/CD19/CD38)

B-CLL cells

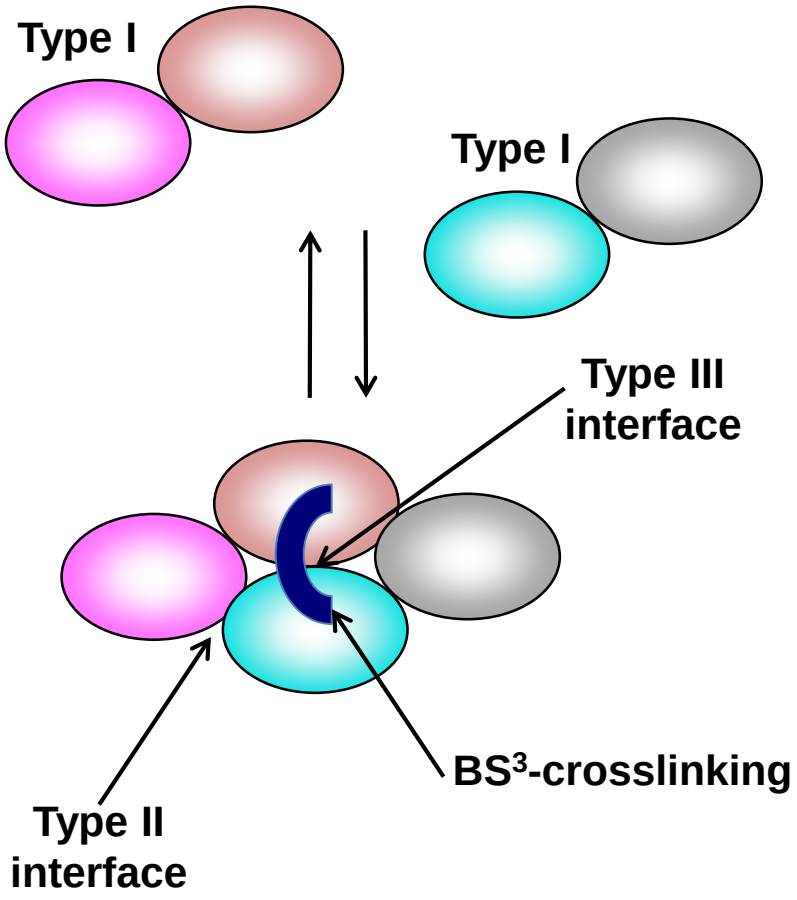


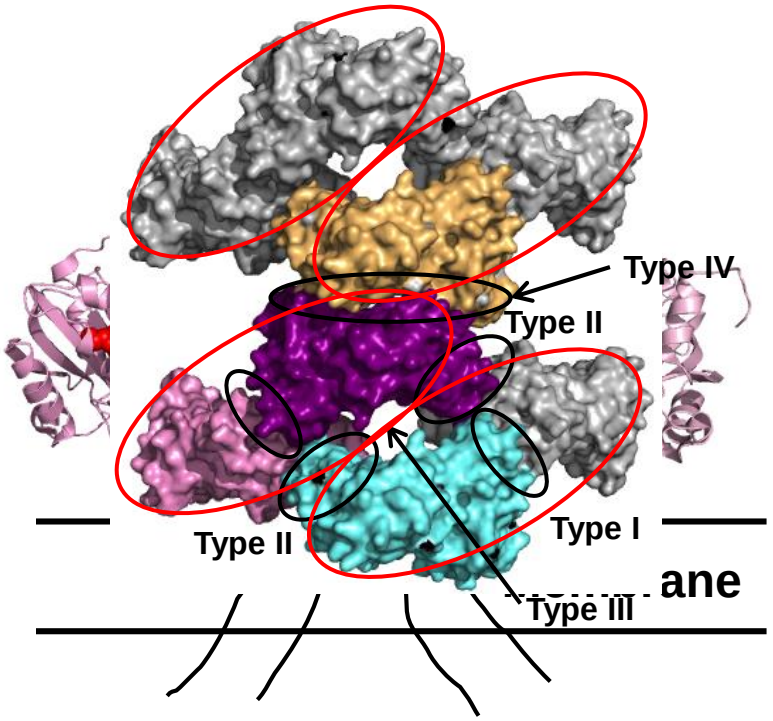
Kaplan-Meler survival curve



BLOOD 98:181-186 (2001)

The dimer via the type I interaction mode is further considered to exist in equilibrium to form a tetramer via the type II/III interaction mode, which is compatible with membrane association.





Daratumumab (anti-CD38 mAb)

Updated results of a key Phase 1/2 trial testing the potential new myeloma therapy daratumumab were released.

> “Daratumumab continues to show substantial promise as potential new treatment for multiple myeloma (ASCO2015)”
> Publihsed: May 30, 2015