Type A & B Niemann-Pick disease and acid sphingomyelinase structure

2023/01/12

B4 Ryota Matsukawa

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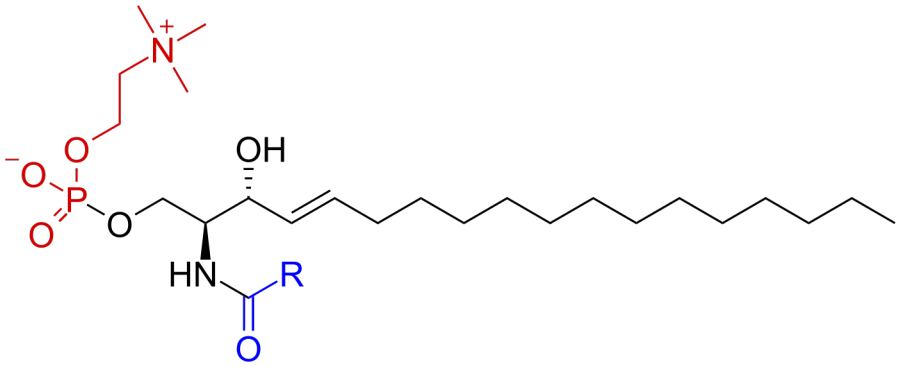
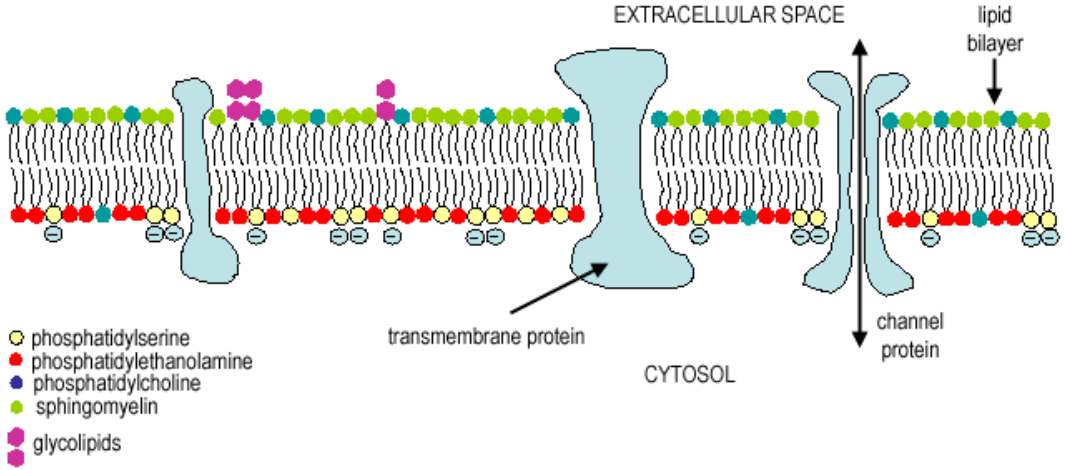
Sphingomyelin and acid sphingomyelinase (ASM)

Sphingomyelin

- Phosphate diester of phosphocholine and ceramide
- Ceramide: sphingosine + fatty acid
- A major component of the outer leaflet of cell membranes

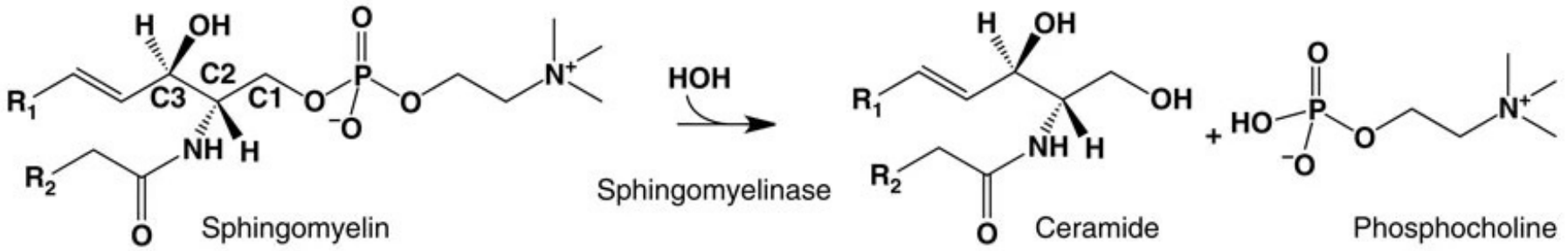


Wikipedia sphingomyelin
https://en.wikipedia.org/wiki/Sphingomyelin



The Cell: The Histology Guide
https://www.histology.leeds.ac.uk/cell/plasma_membrane.php

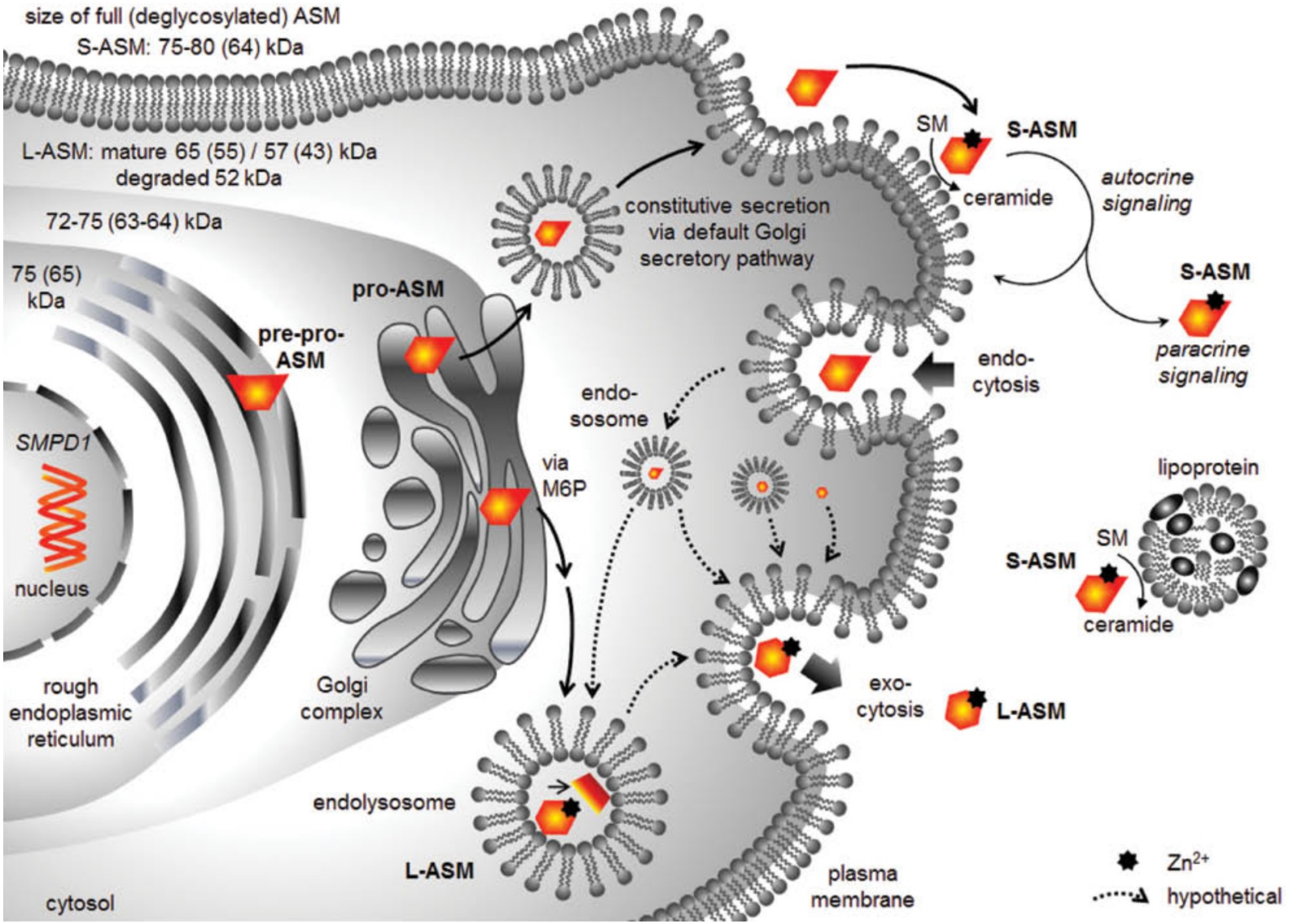
- Sphingomyelin is hydrolyzed by acid sphingomyelinase (ASM)



Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Acid sphingomyelinase (ASM)

Pathway to hydrolysis of sphingomyelin



J. Kornhuber, *et al., Biol. Chem.*, **2015**, *396*, 707.

Niemann pick disease (NPD) type A and B

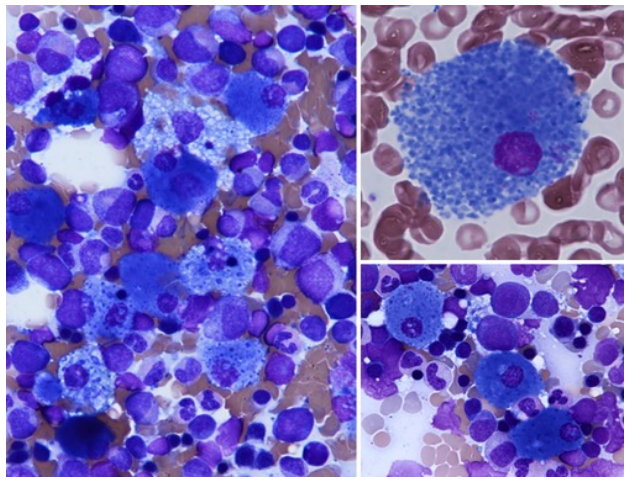
Niemann-Pick disease type A and B

- Acid sphingomyelinase deficiency (ASMD)
  - ・・・ Abnormal accumulation of sphingomyelin in lysosomes or cell membrane in liver, spleen, lung and central nerve system due to mutations in SMPD1 which encoding ASM

- Clinical features and pathology
  - ・・・ Type A: Hepatosplenomegaly (肝脾腫), hypotonia (筋緊張低下), progressive neurological symptoms

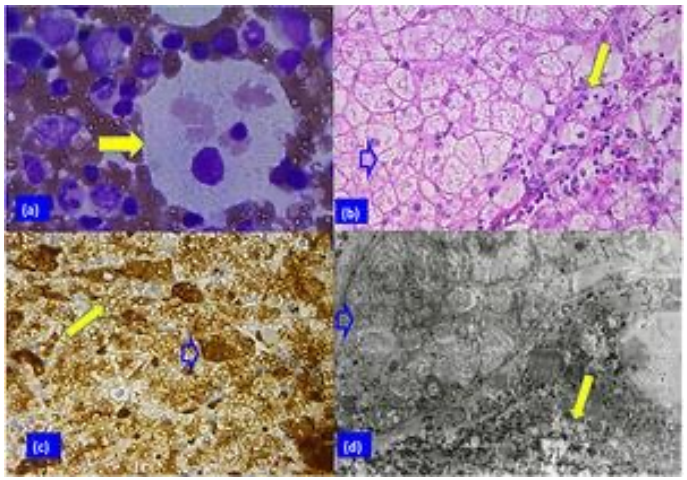
    Type B: Hepatosplenomegaly (肝脾腫), liver failure (肝不全), decreased pulmonary function (肺機能の低下)

Splenomegaly (脾腫)



Jesús, V. *et al., Br. J. Haematol.*, **2016**, 172, 840.

Foam cells (泡沫細胞)



M. Cerón-Rodríguez *et al.*, *Ann. Hepatol.*, **2019**, *18*, 613.

E.H. Schuchman, *et al., Mol. Genet. Metab.*, **2017**, *120*, 27.

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1. Sphingomyelin and Niemann-Pick disease (NPD) type A and B

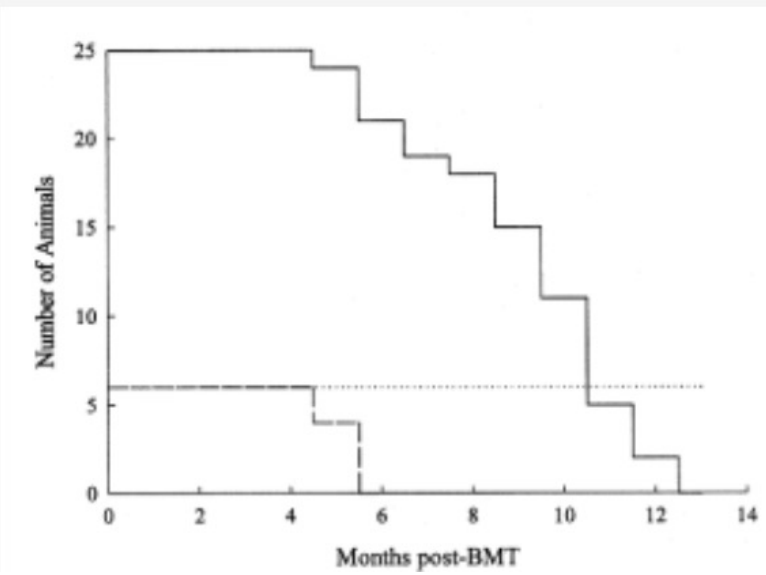
2. Therapy for type A and B NPD
   - Bone marrow transplantation
   - Enzyme replacement therapy
   - Gene therapy

3. Crystal structures of acid sphingomyelinase

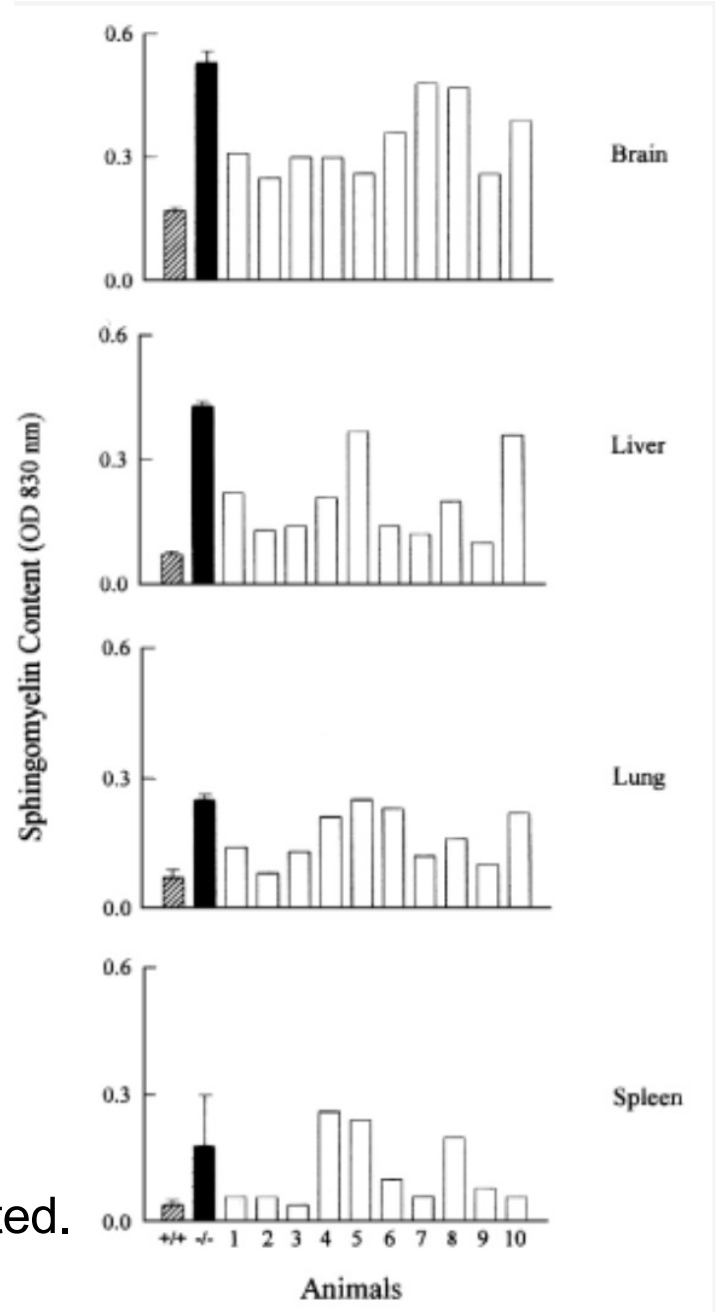
4. Summary

Bone marrow transplantation (BMT)

Bone marrow transplantation for ASM knock-out mouse model



Dotted line: Controls (+/+)
Hatched line: ASMKO controls (-/-)
Solid line: Transplanted ASMKO



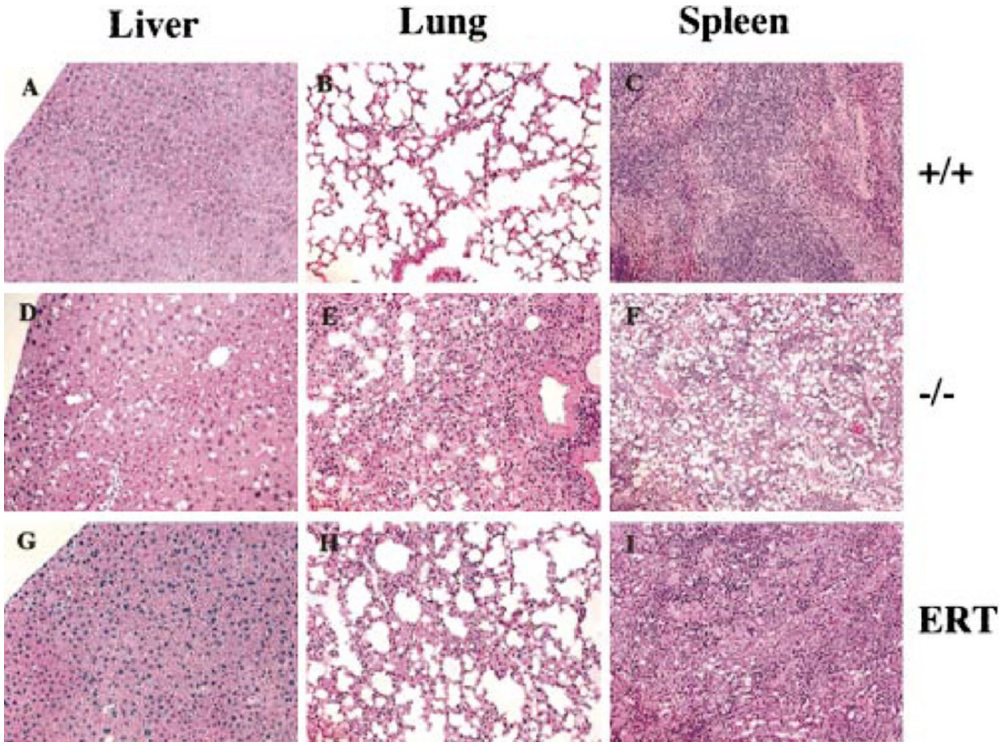
- Sphingomyelin levels in the spleen and liver decreased significantly.
- The effect on the brain was moderate and the progressive neurological disease was not prevented.

S.R. Miranda, *et al., Transplantation*, **1998**, *65*, 884.

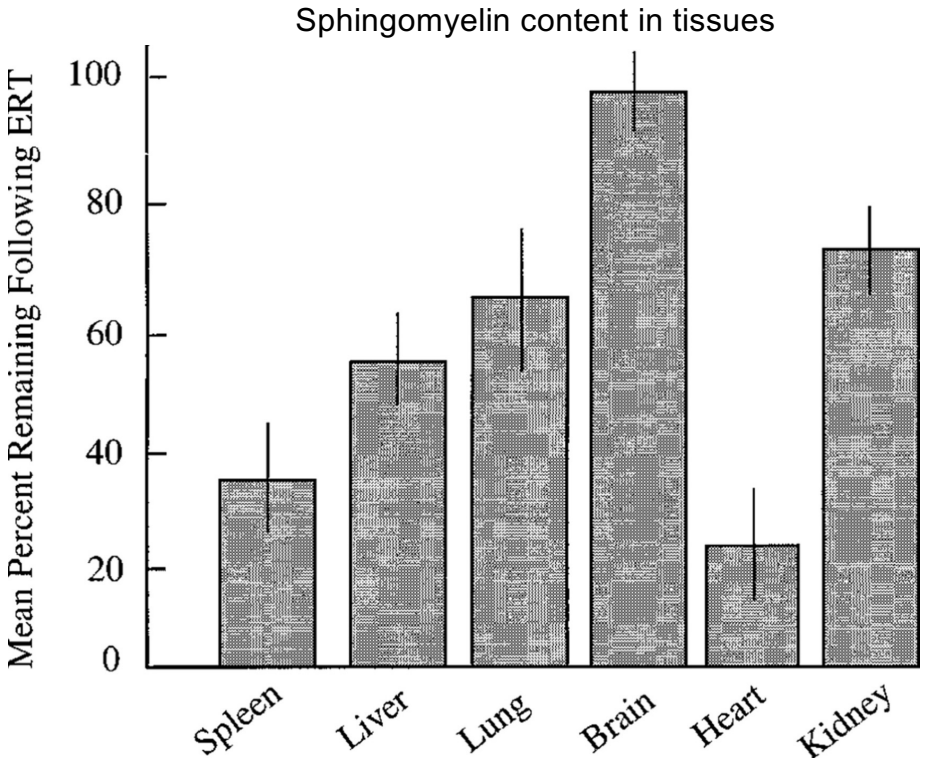
Enzyme replacement therapy (ERT)

Olipudase alfa (Xenpozyme) for ASMKO mouse

Olipudase alfa: Recombinant human ASM



+/+: Normal control, -/-: ASMKO control, ERT: Treated ASMKO



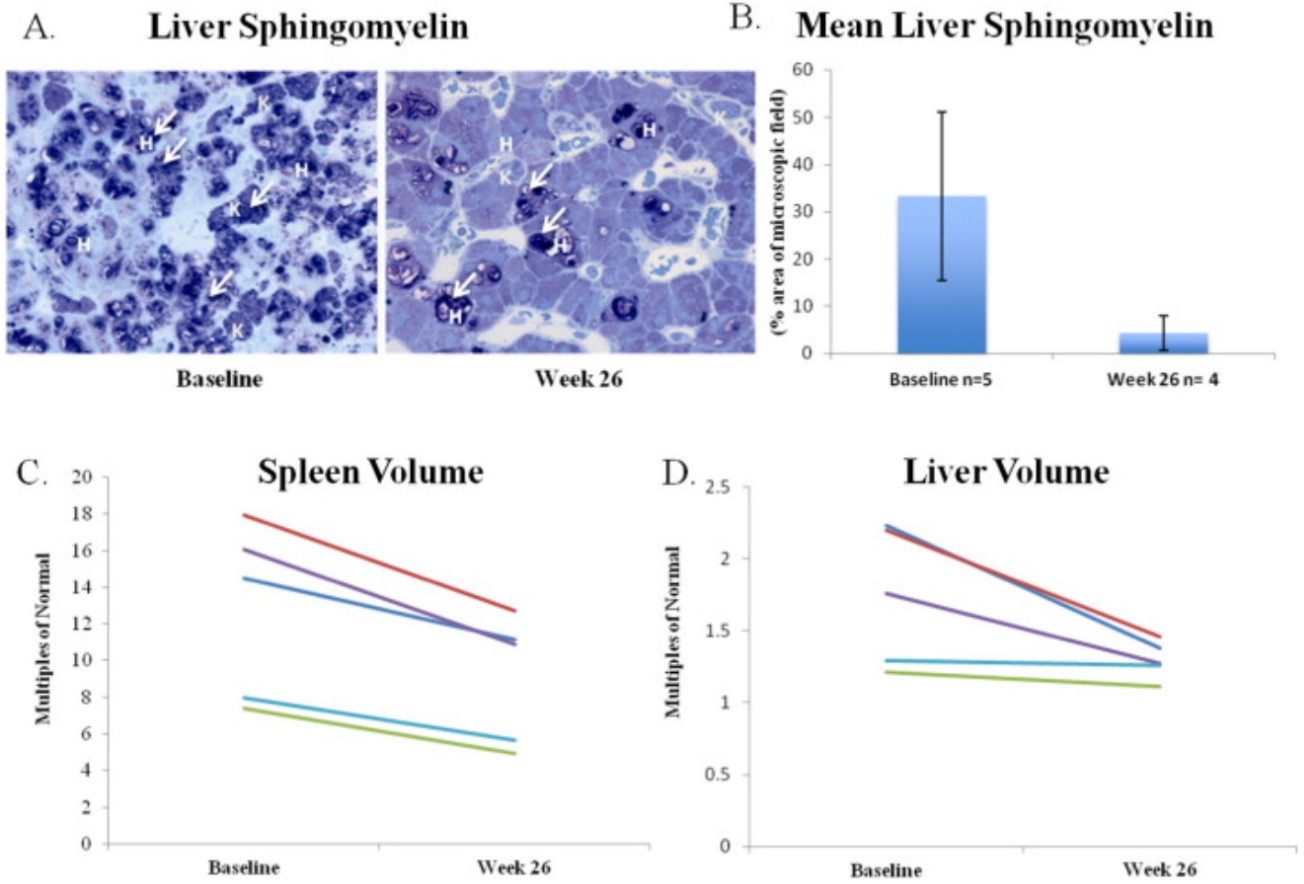
- Histological improvement was observed by infusion of rhASM.

SRP. Miranda, *et al.*, *FASEB J.*, **2000**, *14*, 1988.

Enzyme replacement therapy (ERT)

Olipudase alfa (Xenpozyme) for NPD type B patients

Olipudase alfa: Recombinant human ASM

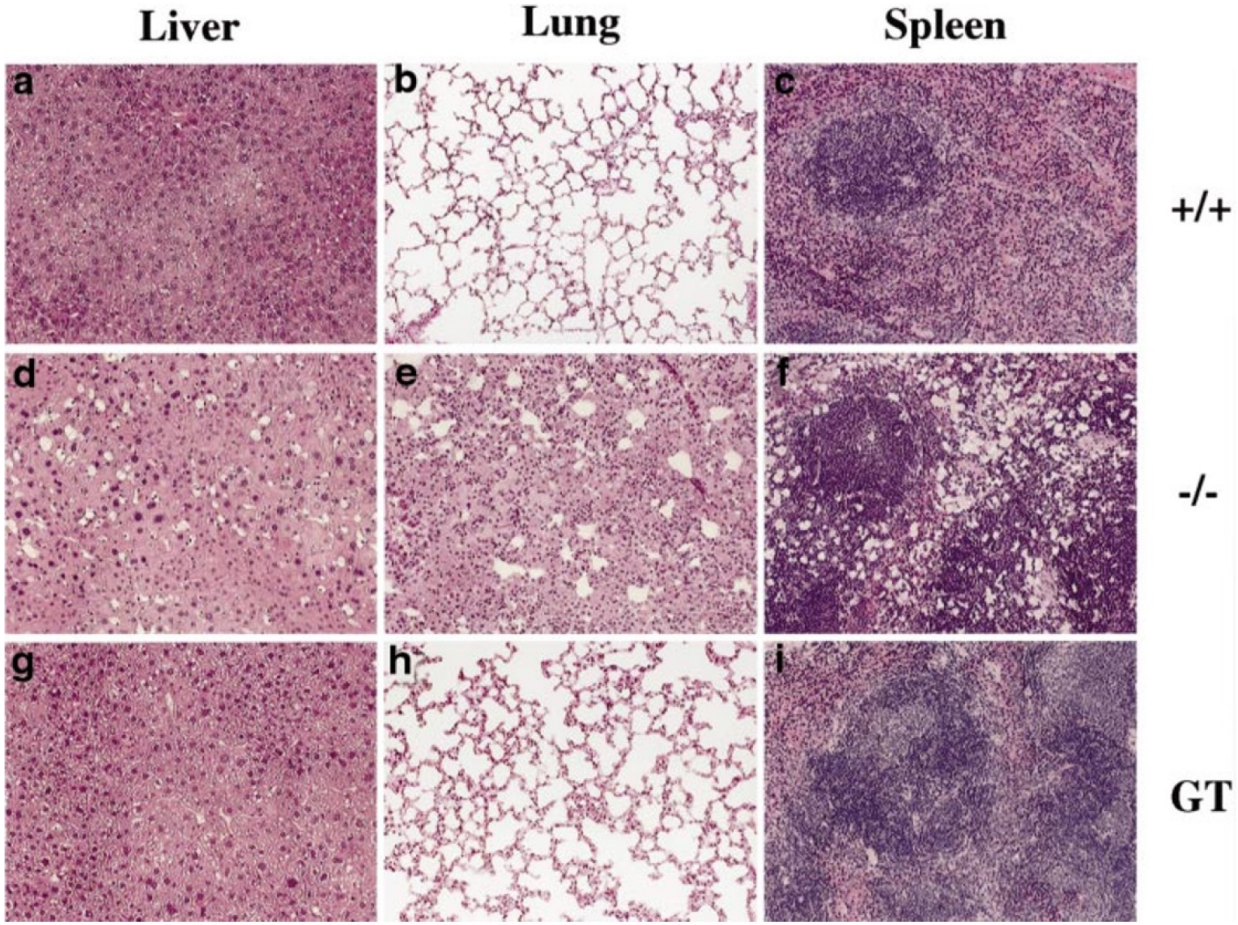


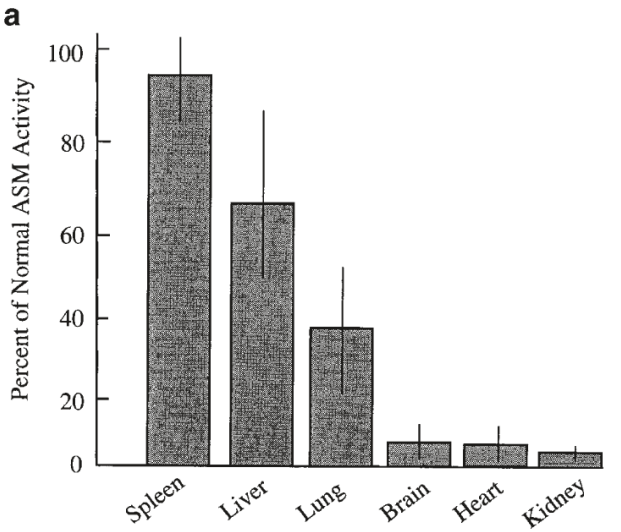
- Accumulated sphingomyelin was debulked and histological improvement was observed by treatment of Olipudase alfa.

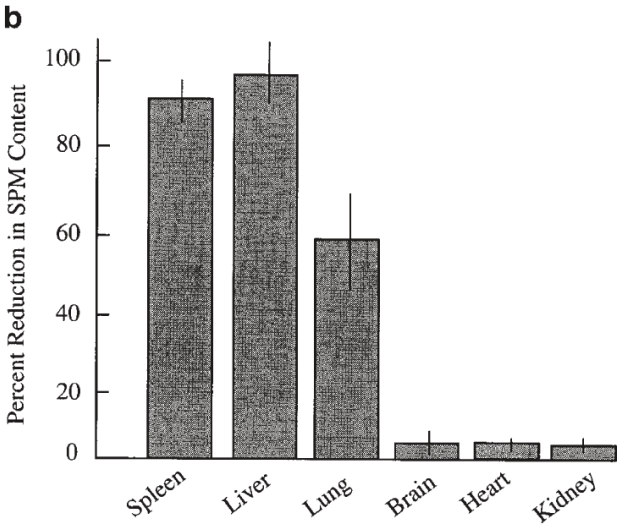
M. P. Wasserstein, *et al.*, *Mol. Genet. Metab.,* **2015**, *116*, 88.

Gene therapy

Hematopoietic stem cell gene therapy







+/+: Normal control, -/-: ASMKO control, GT: Treated ASMKO

- Tissues from the treated mice show markedly less lipid accumulation than ASMKO control.

SRP. Miranda, *et al.*, *Gene Ther.,* **2000**, *7,* 1768.

Gene therapy

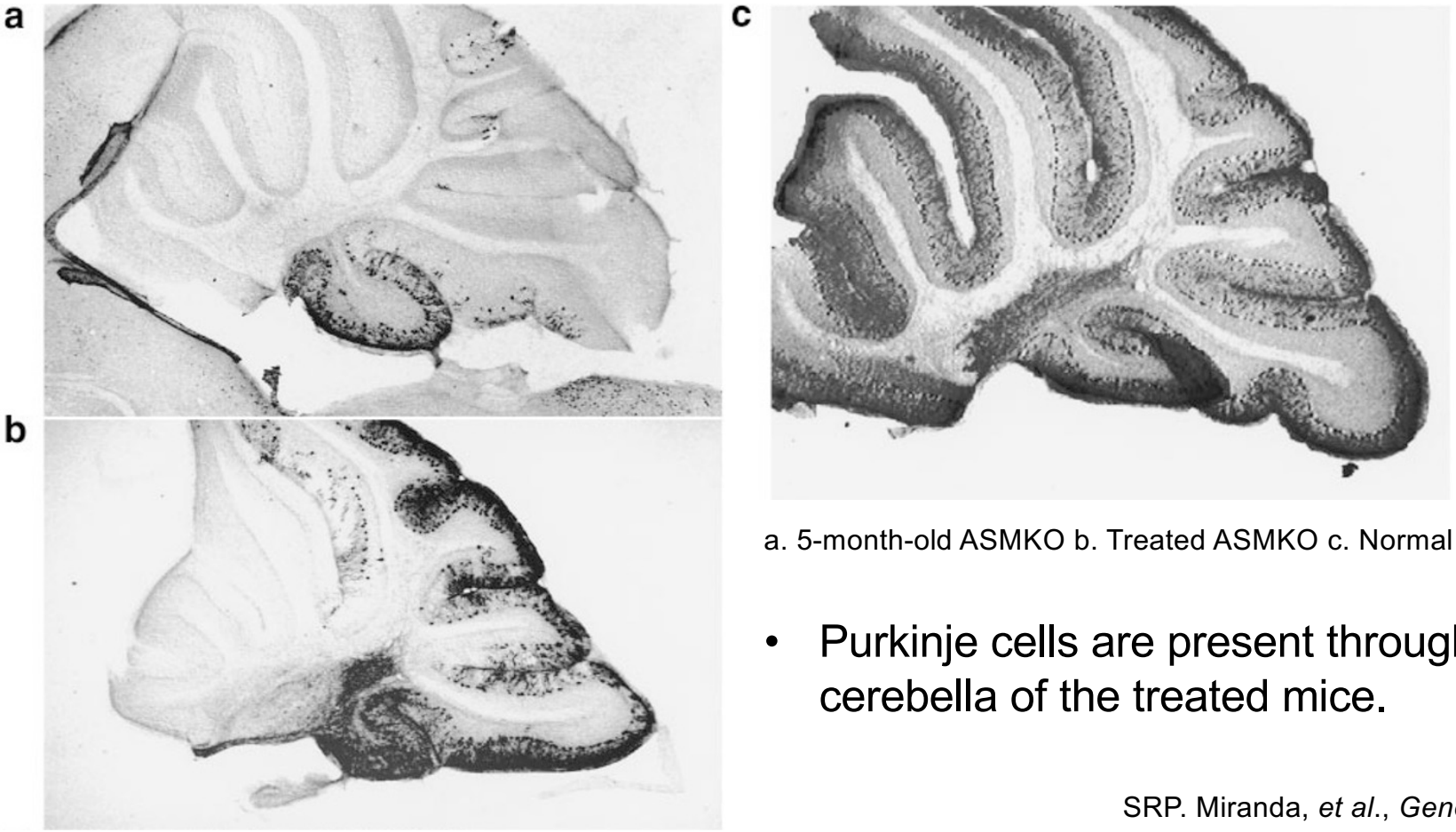
Hematopoietic stem cell gene therapy

- It has been reported that Purkinje cells are reduced in the cerebellum in the ASMKO mouse model.

K. Horinouch, *et al*., *Nat. Genet.,* **1995**, *10,* 288-293.

Gene therapy

Purkinje cell immunohistochemistry



a. 5-month-old ASMKO b. Treated ASMKO c. Normal control

- Purkinje cells are present throughout the cerebella of the treated mice.

SRP. Miranda, *et al*., *Gene Ther.,* **2000**, *7,* 1768.

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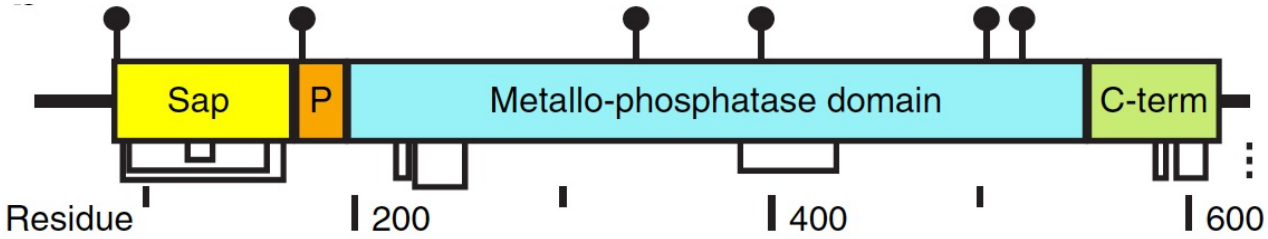
ASM structures

Diagram of ASM domains

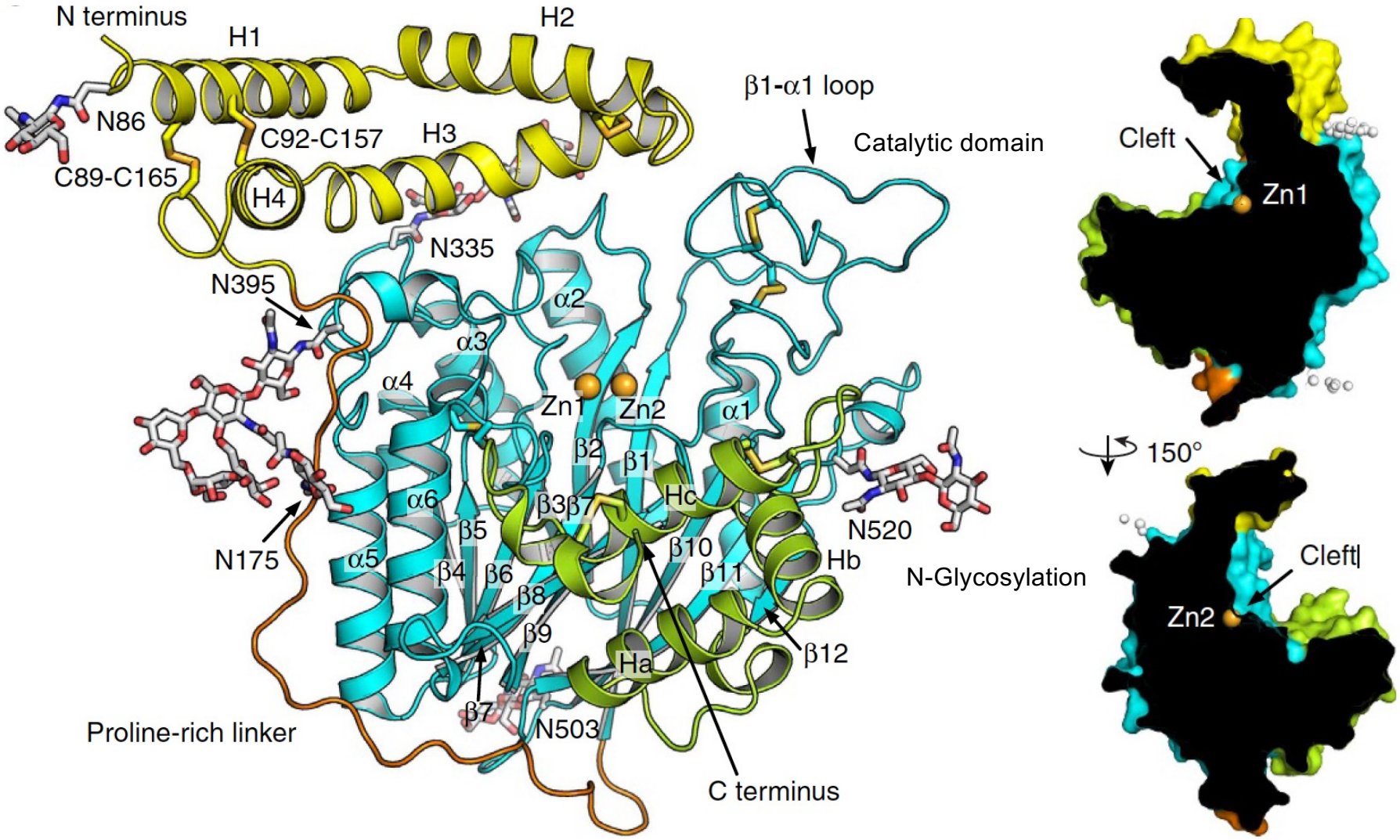


- Yellow: Saposin domain (*N-terminal*)
  Sphingomyelin activator that recognize and locate lipids on the active site
- Orange: Proline-rich linker
  The connection between the saposin and the catalytic domain
- Blue: Metallo-dependent phosphatase catalytic domain
  Active site for hydrolysis of sphingomyelin
- Green: *C-terminal* domain (ill-defined)

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

ASM structures

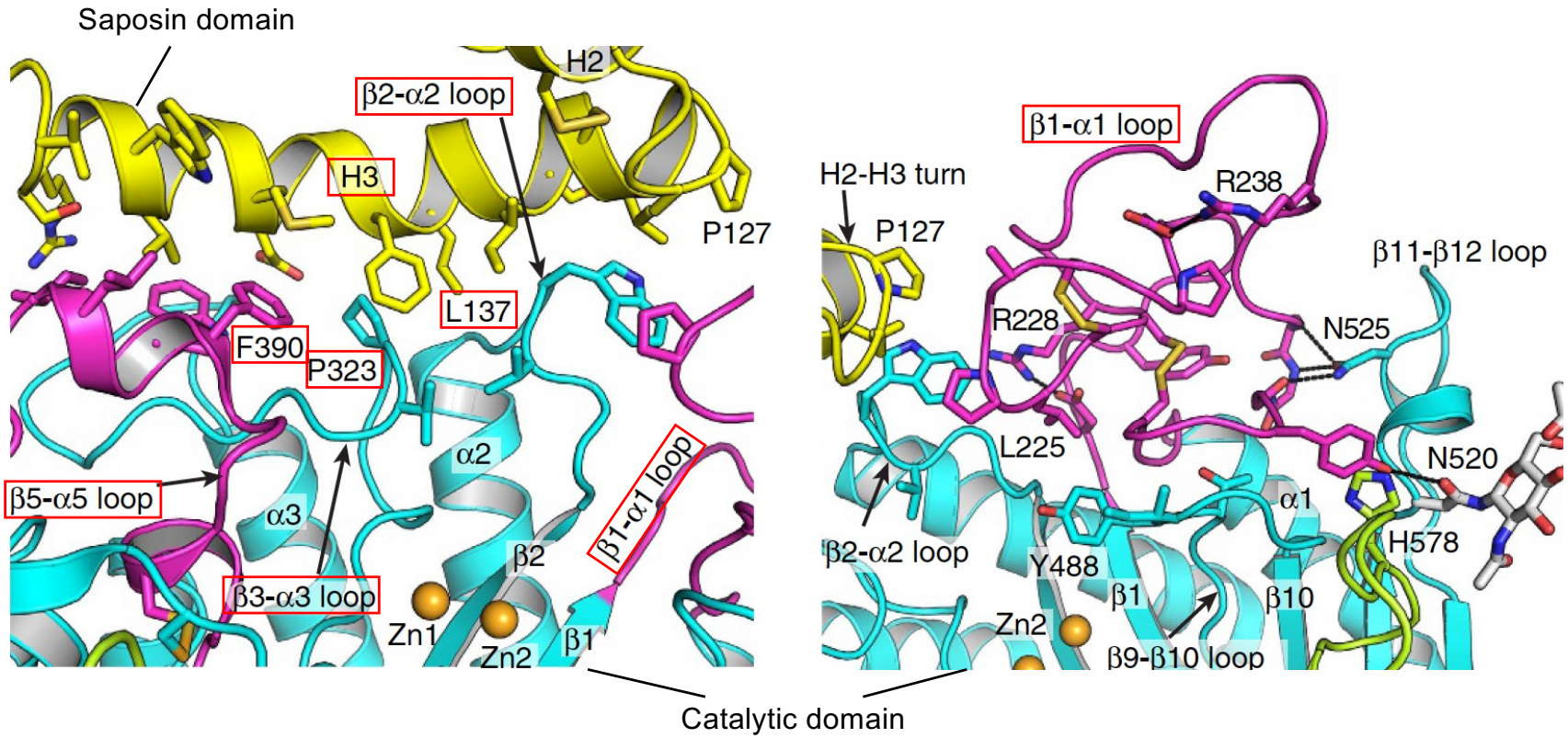
Overall structure of ASM



Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

ASM structure: Domain interactions

Interactions between the saposin domain and catalytic domain

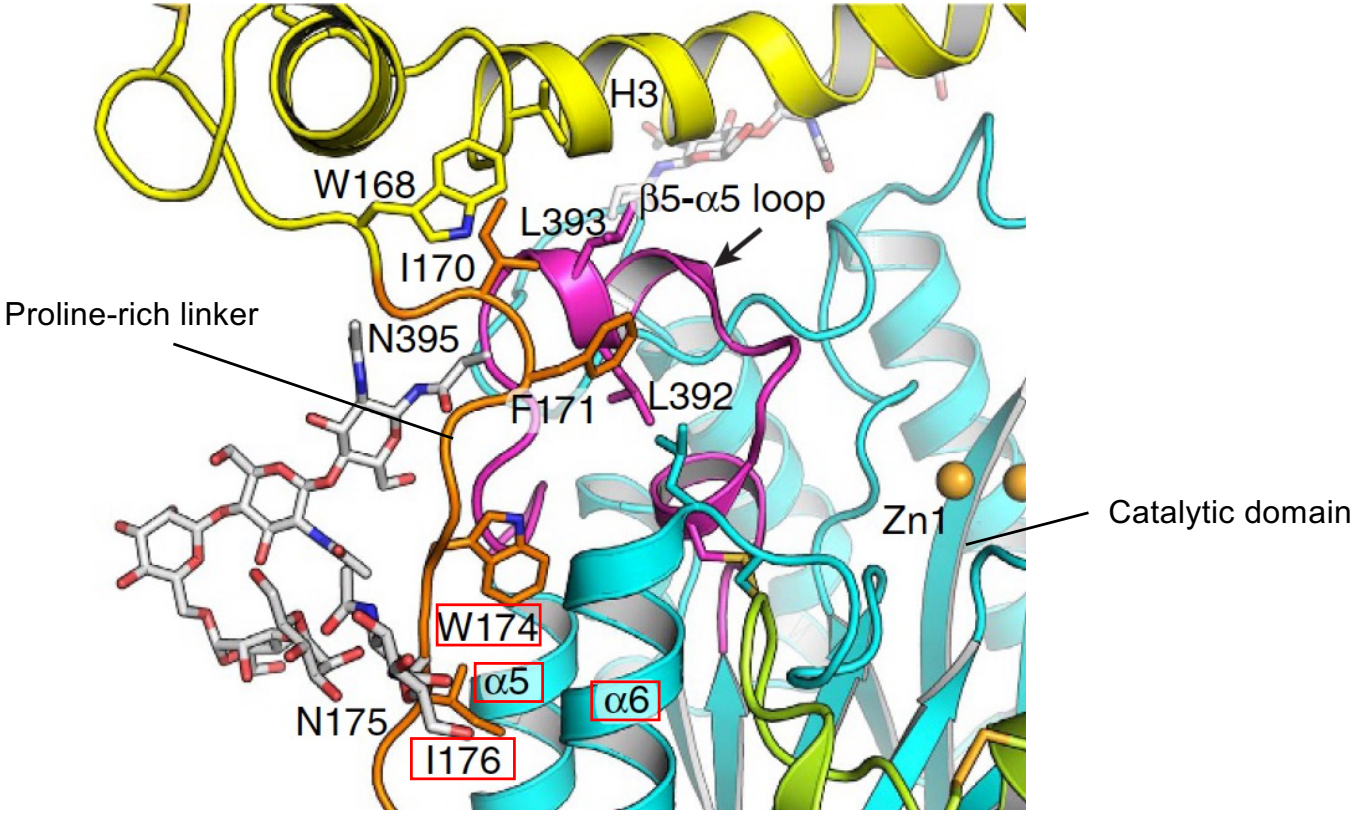


- H3 helix in saposin domain extensively contacts with β1-α1, β2-α2, β3-α3, β5-α5 loop.
- Many mutations occur on the interface residues, such as L137P in H3, P323A on the β3-α3 loop, ΔF390 on the β5-α5 loop.

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

ASM structure: Domain interactions

Interactions between the proline linker and catalytic domain

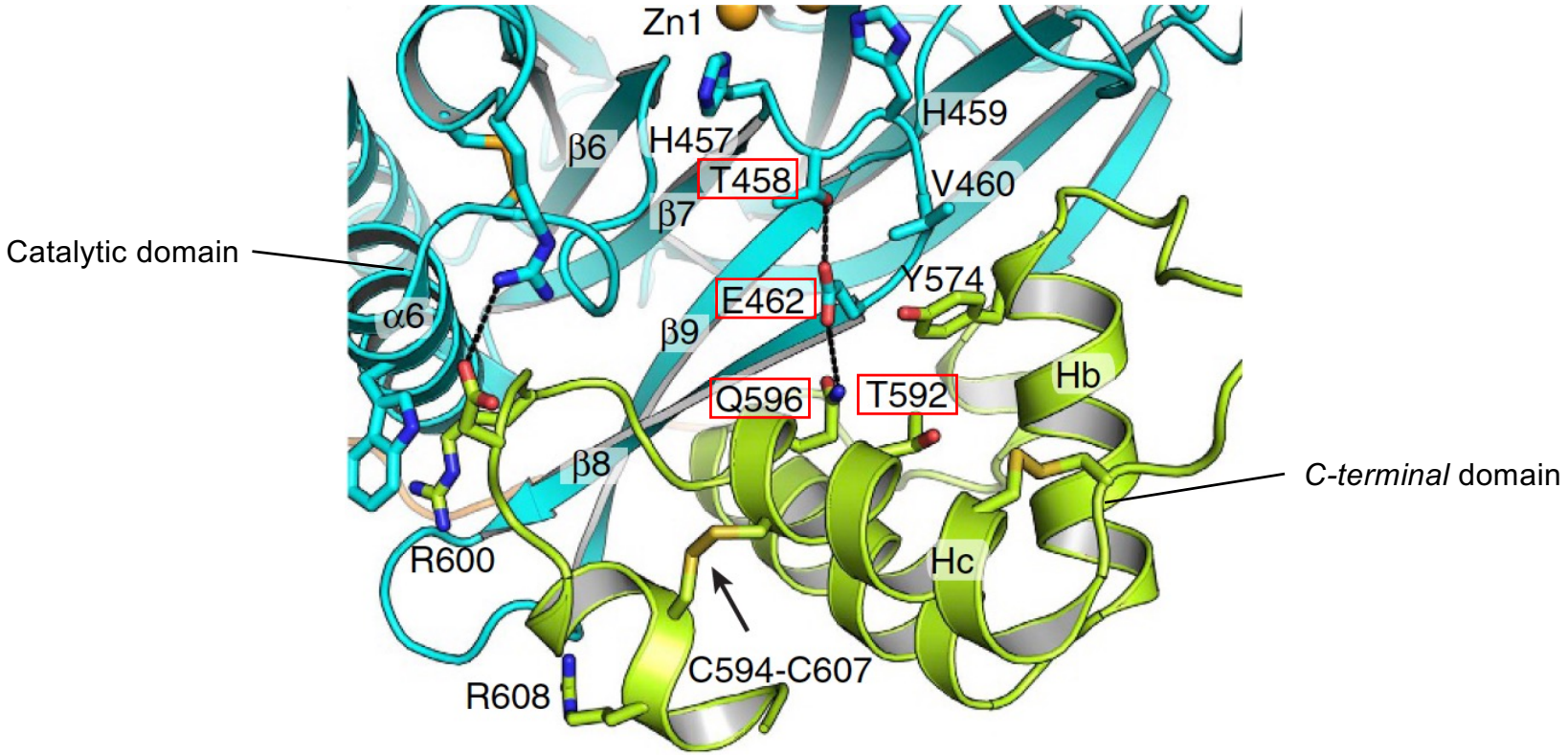


- The proline-rich linker is stabilized by hydrophobic interactions between W174, I176, L178 in the proline-rich linker and α5, α6 helices in the catalytic domain.

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

ASM structure: Domain interactions

Interactions between the proline linker and *C-terminal* domain

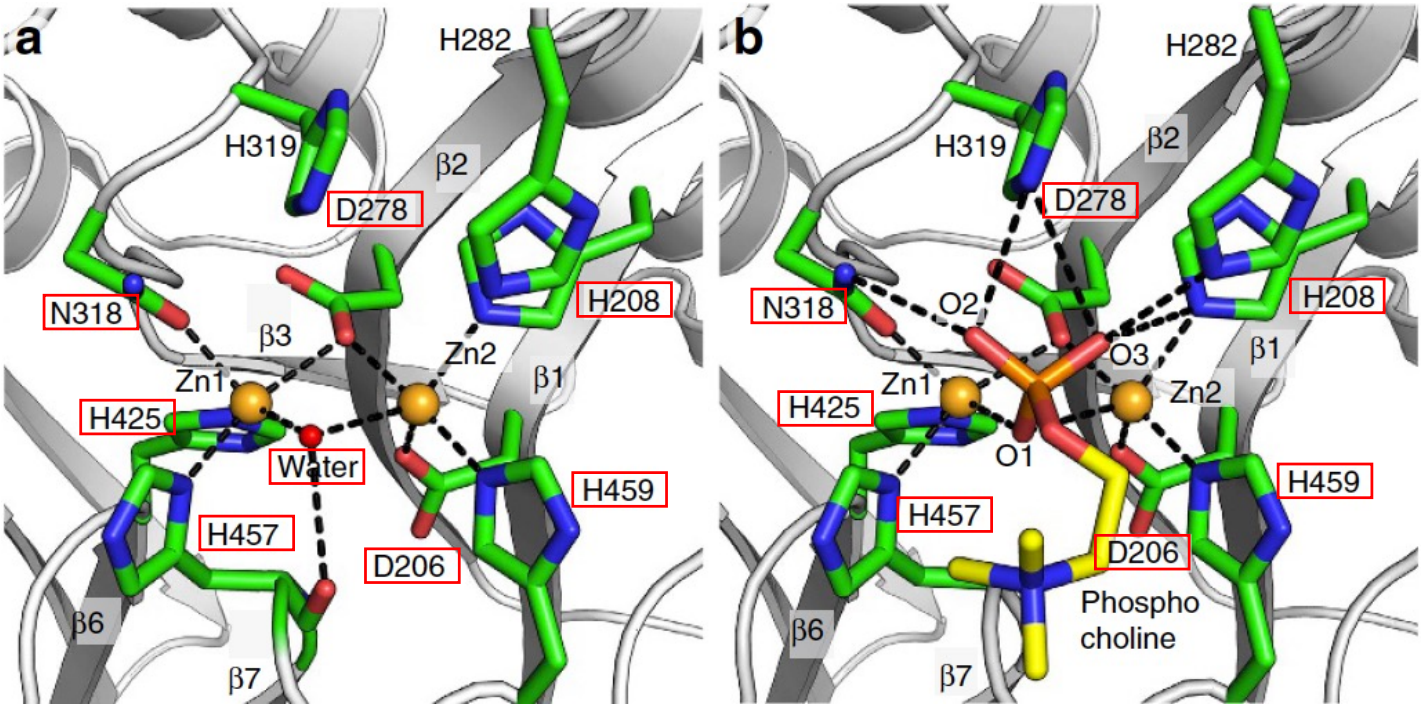


- The *C-terminal* domain contributes to stabilization of the active site, especially β7-β8 loop.
- It has been reported that ΔT592 mutation causes severe ASMD.
  → ΔT592 mutation has a severe impact on ASM folding?

Rodriguez-Pascau, L. *et al.*, *Hum. Mutat.,* **2009**, *30*, 1117.
Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

ASM structure: Zinc and phosphocholine binding in the active site

a. Active site of holo ASM structure
b. Phosphocholine bound structure

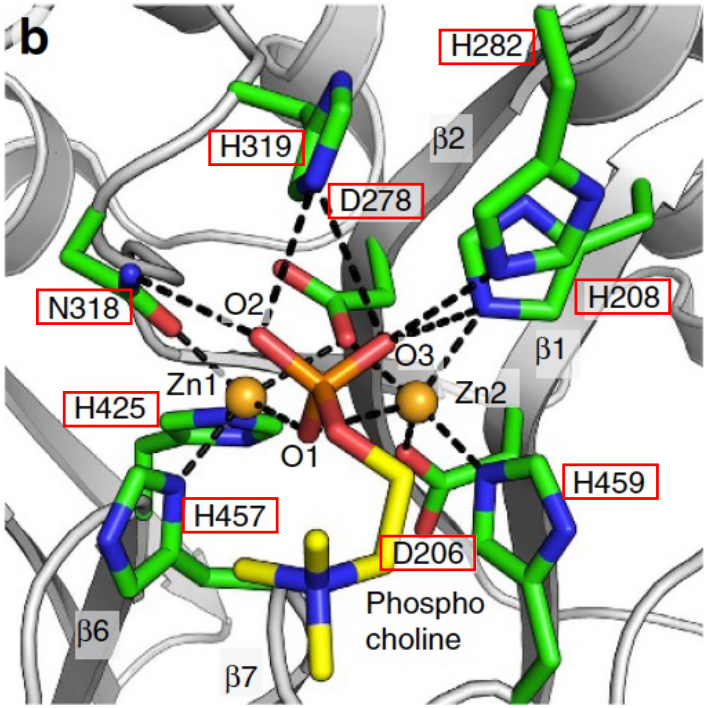


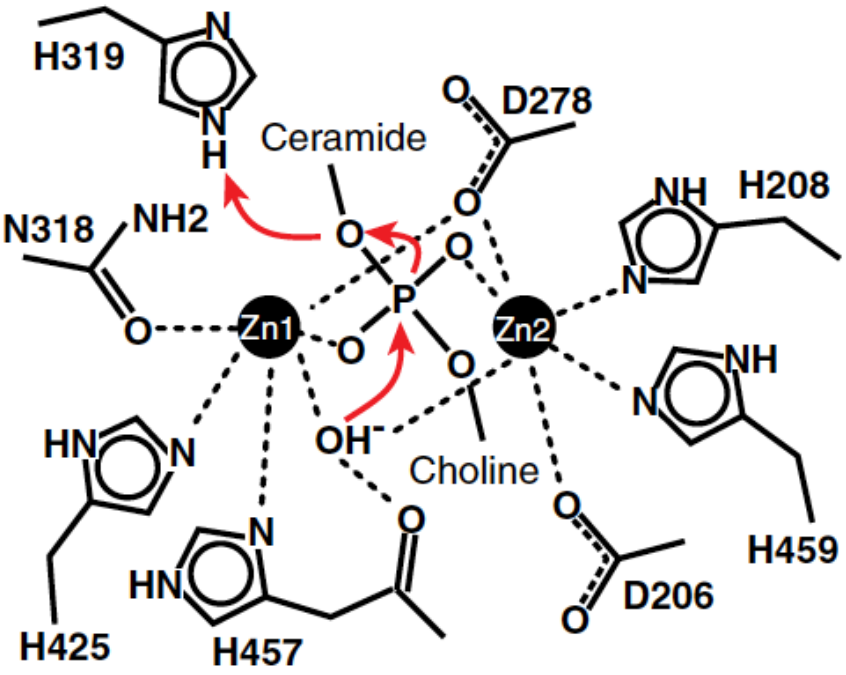
- Both zincs have trigonal bipyramidal geometry.
- A water molecule bridges between Zn1 and Zn2 and is stabilized by hydrogen bonding to the main chain carbonyl of H457.
- Oxygen (O1) in the phosphoryl group replaces the position of the water molecule in the holo ASM structure.

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Hydrolysis of sphingomyelin

Mechanism of hydrolysis of sphingomyelin



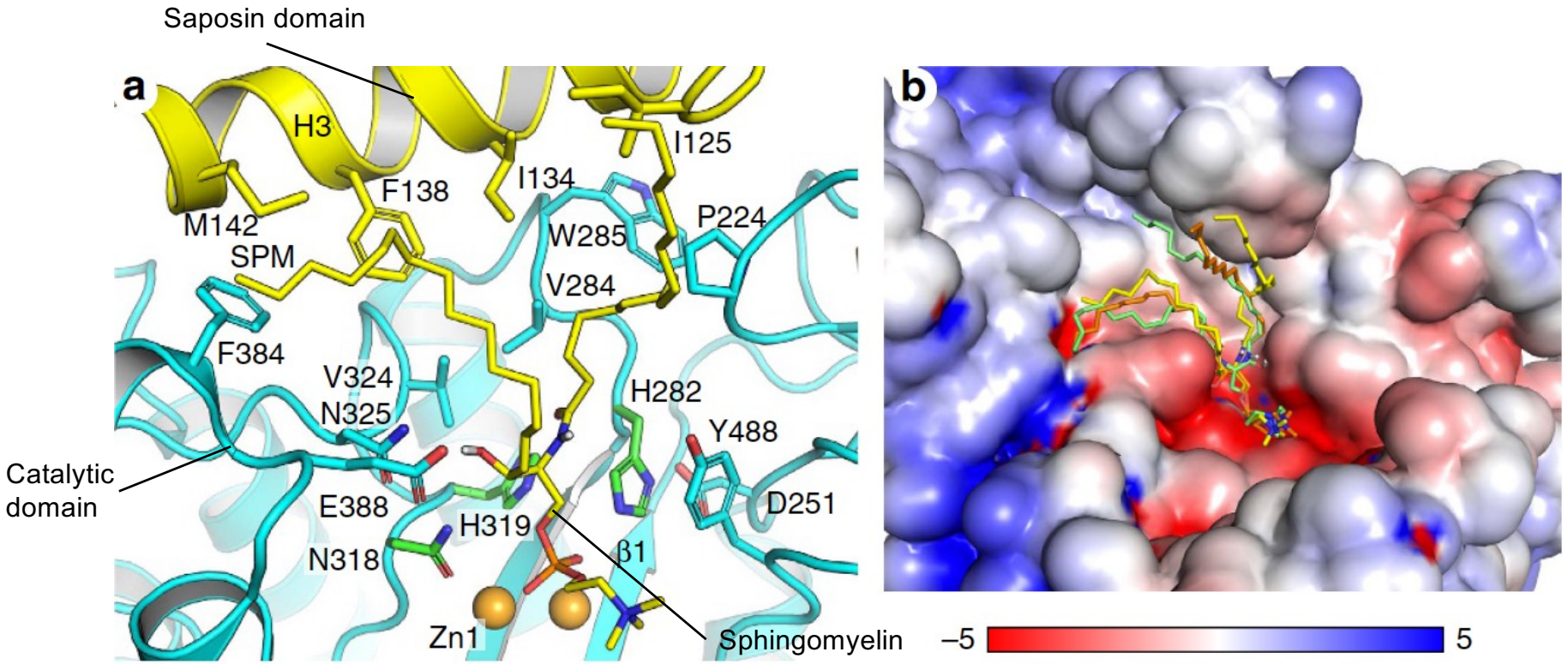


- The coordinated water is deprotonated to hydroxide due to the activation by two zinc ions, and the hydroxide attacks on the phosphorus of sphingomyelin.
- The phosphorus oxygen on the ceramide side extracts a proton from H319.
- Mutation of H319Y causes severe ASMD.

Sikora, J. *et al.*, *Ann. Hum. Genet.*, **2003**, *67*, 63.
Zhou, YF. *et al.*, *Nat. Commun.*, **2016**, *7*, 13082.

Docking model of sphingomyelin on ASM

a. Surface residues around C-16 sphingomyelin
b. Electrostatic potential maps

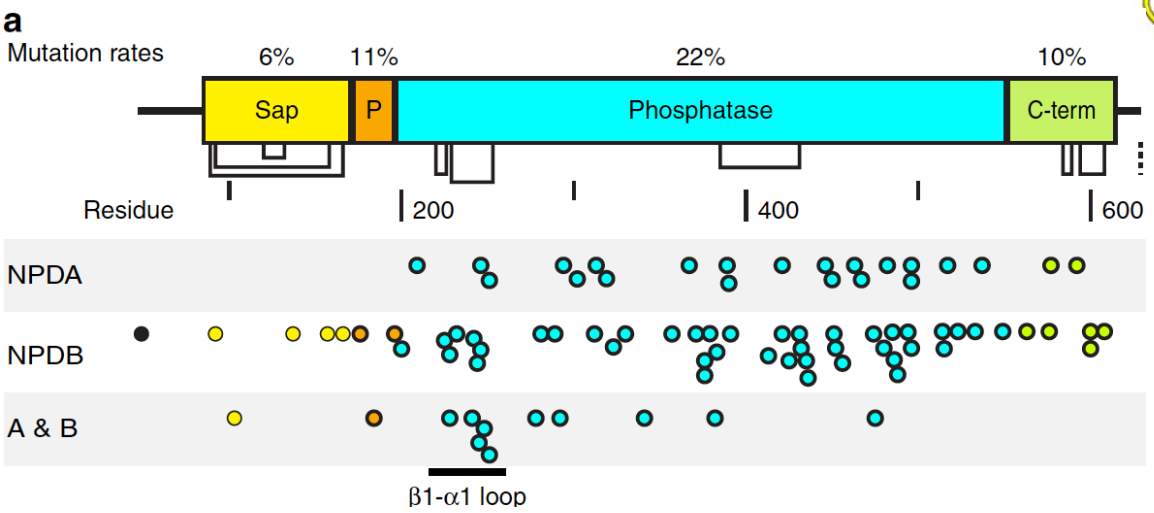


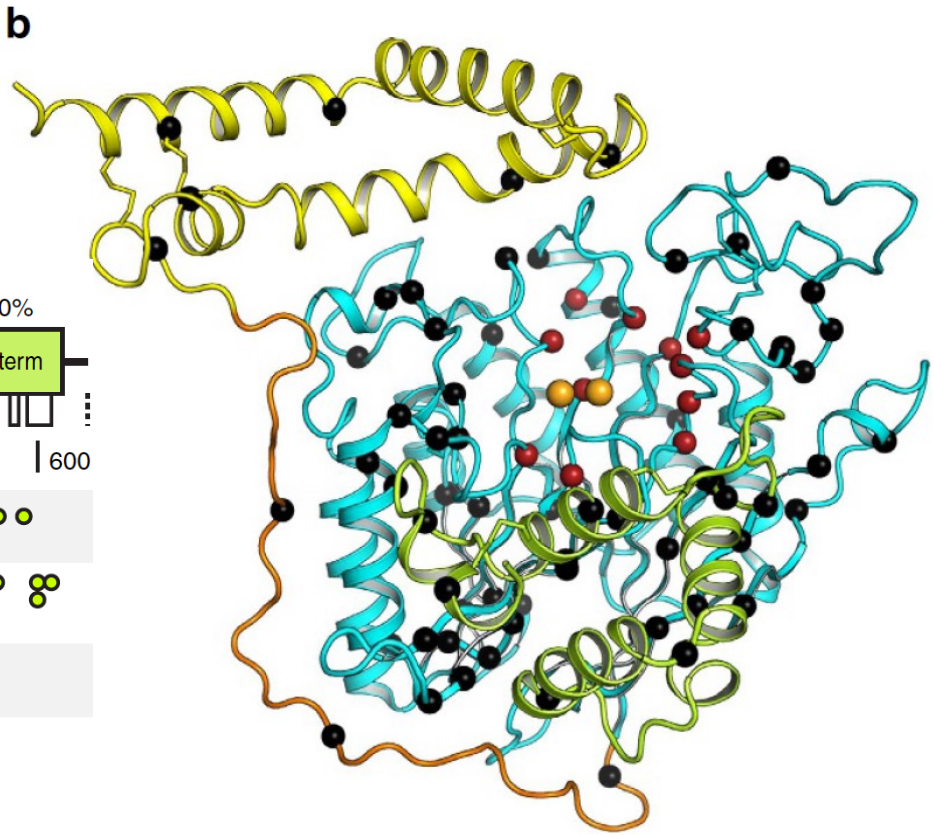
- The hydrophobic ceramide chains in sphingomyelin is accommodated in the saposin concave surface which is rich in hydrophobic residues and not highly charged.

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Mutation in ASM

a. Distribution of mutations on ASM sequence
b. Distribution of mutations on 3D structure



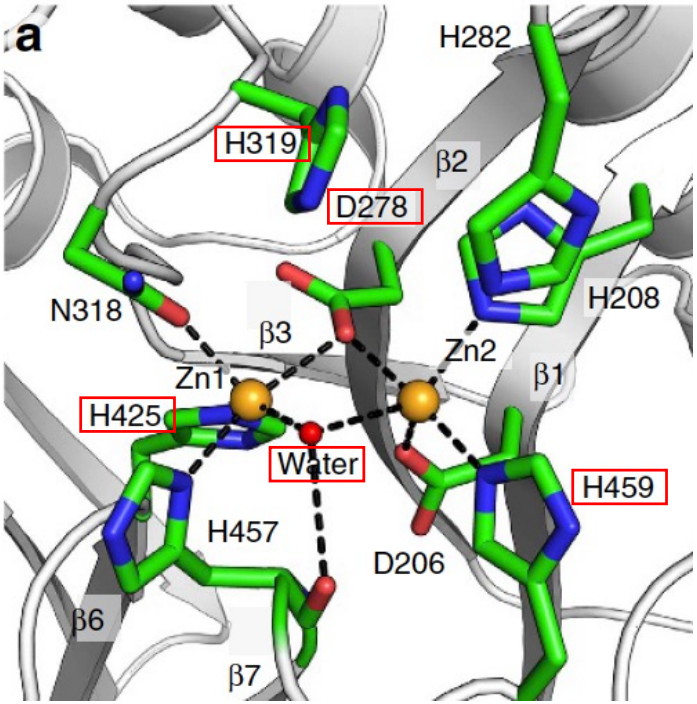


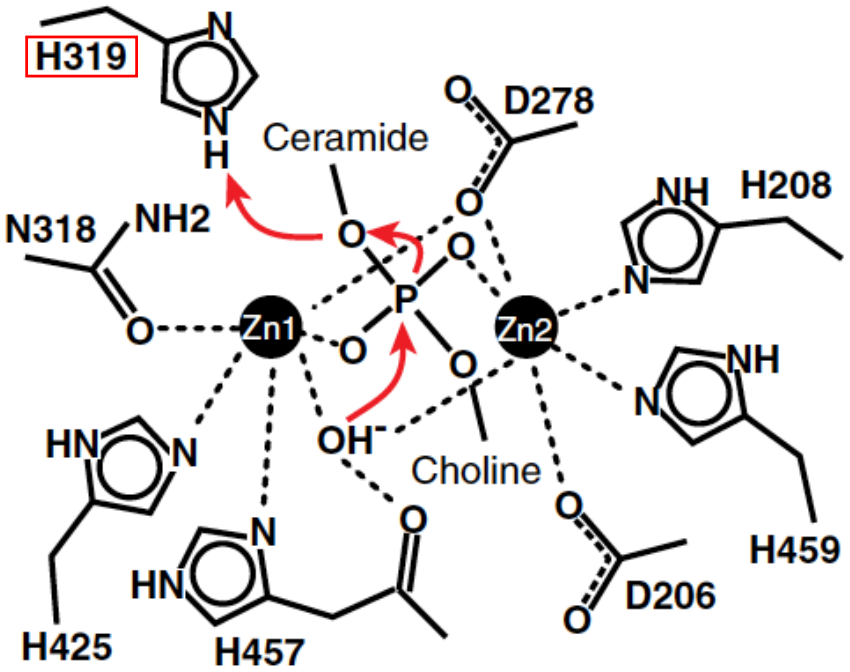
- 82% of mutations are located in the catalytic domain.
- Those mutations are categorized into two groups, according to their disruptive effects on catalytic activity and protein folding.

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Mutation affecting catalytic activity

D278A, H329Y, H425R mutations



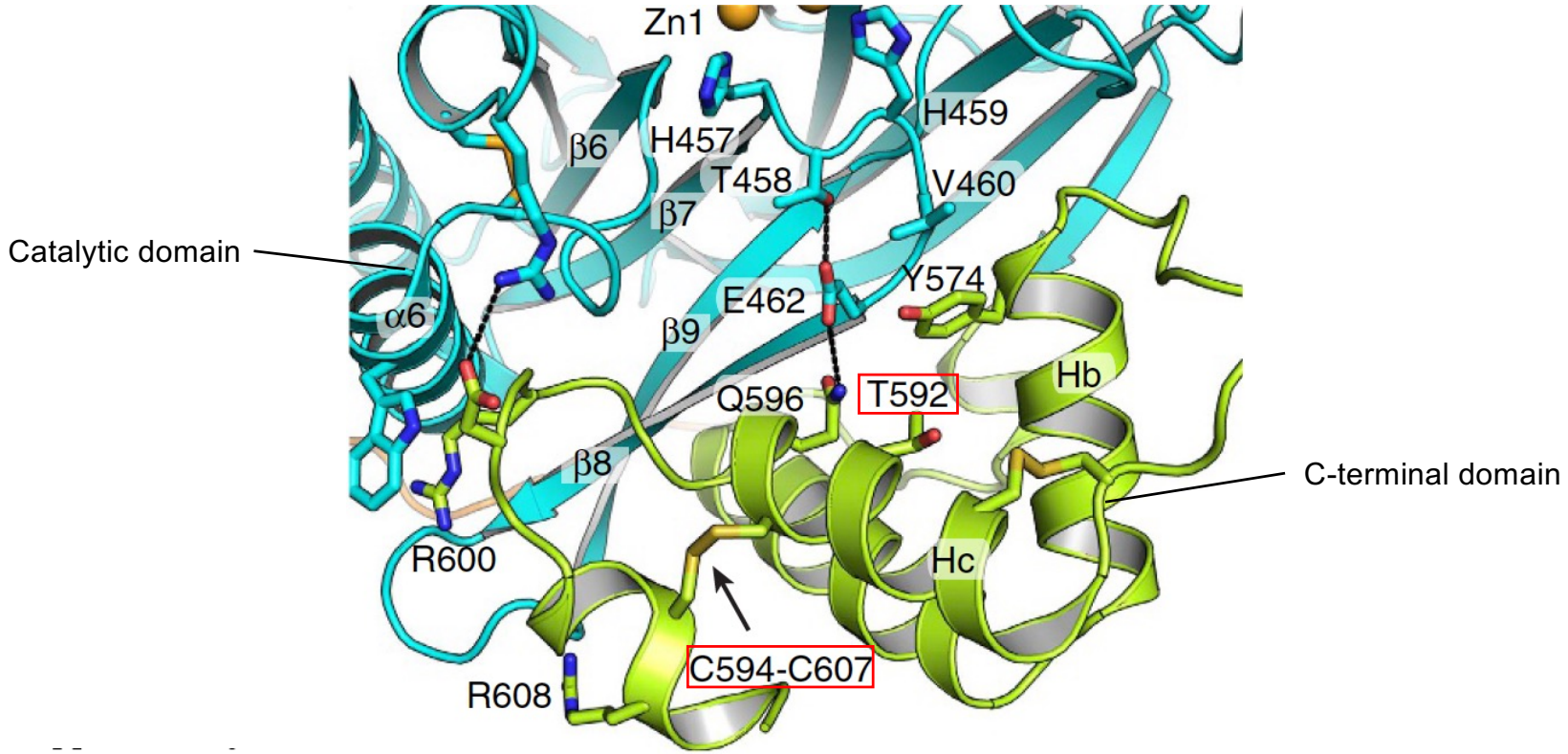


Mutation	NPD[$]	RSA*	Interpretation of mutations based on structure	Category[¶]
D278A	A, B	0.00	Carboxylic acid group coordinate binding of both zinc atoms.	**Active**
H319Y	A	0.09	Essential imidazole ring for catalysis. Long side chain of tyrosine disrupts catalytic reaction.	**Active**
H425R	B	0.00	Direct coordination with zinc. Mutation disrupts zinc binding and catalytic reaction.	**Active**

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Mutation affecting protein folding

ΔT592 mutations



Mutation	NPD[$]	RSA*	Interpretation of mutations based on structure	Category[¶]
delT592	A	0.09	In the middle of Hc helix. A deletion not only breaks the C594-C607 disulfide, but also interferes the interactions between C-terminal domain and catalytic domain. .	Folding

Zhou, YF. *et al., Nat. Commun.*, **2016**, *7*, 13082.

Contents

Summary

- NPD type A and B present abnormal accumulation of sphingomyelin due to deficient activity of ASM.
- NPD type A present progressive neurological symptoms.
- There are no approved therapies for ASMD except for enzyme replacement therapy, which is effective only in NPD type B.
- Crystal structures of ASM revealed the mechanism of hydrolysis of sphingomyelin, in which two zinc ions activate a water molecule for nucleophilic attack of phosphodiester bond.
- Saposin, proline-rich linker, catalytic and *C-terminal* domain together create a cleft specific for sphingomyelin binding.