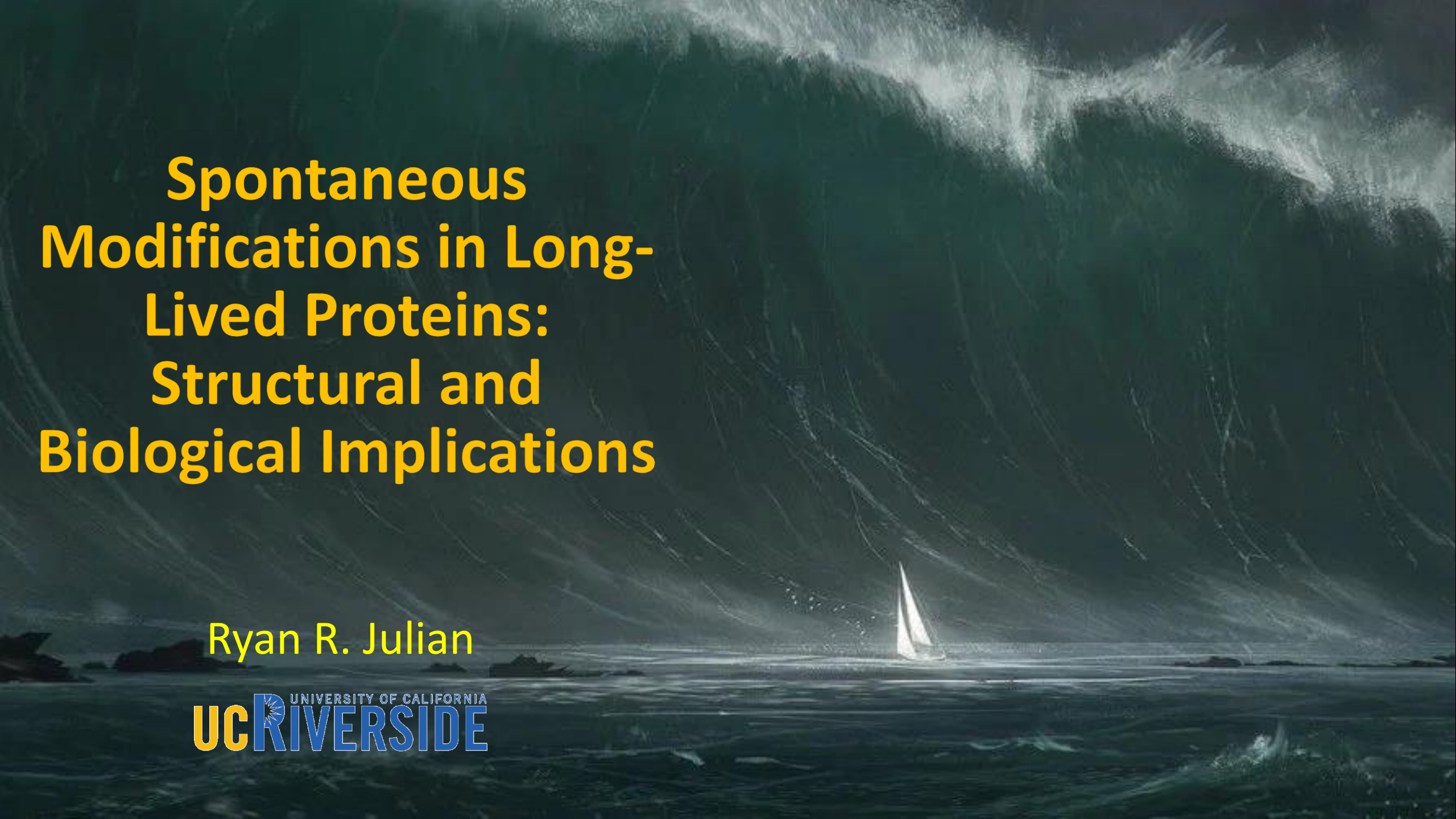
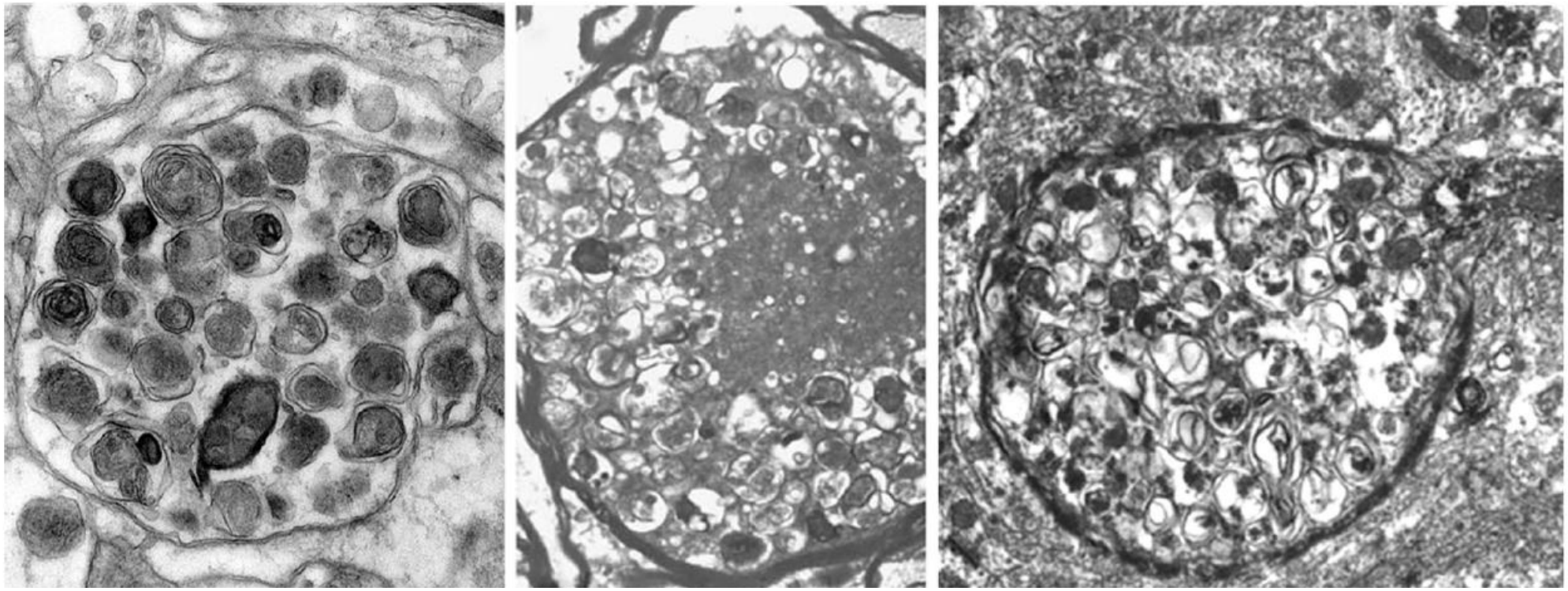


Spontaneous Modifications in Long-Lived Proteins: Structural and Biological Implications

Ryan R. Julian

UC RIVERSIDE UNIVERSITY OF CALIFORNIA

Lysosomal storage

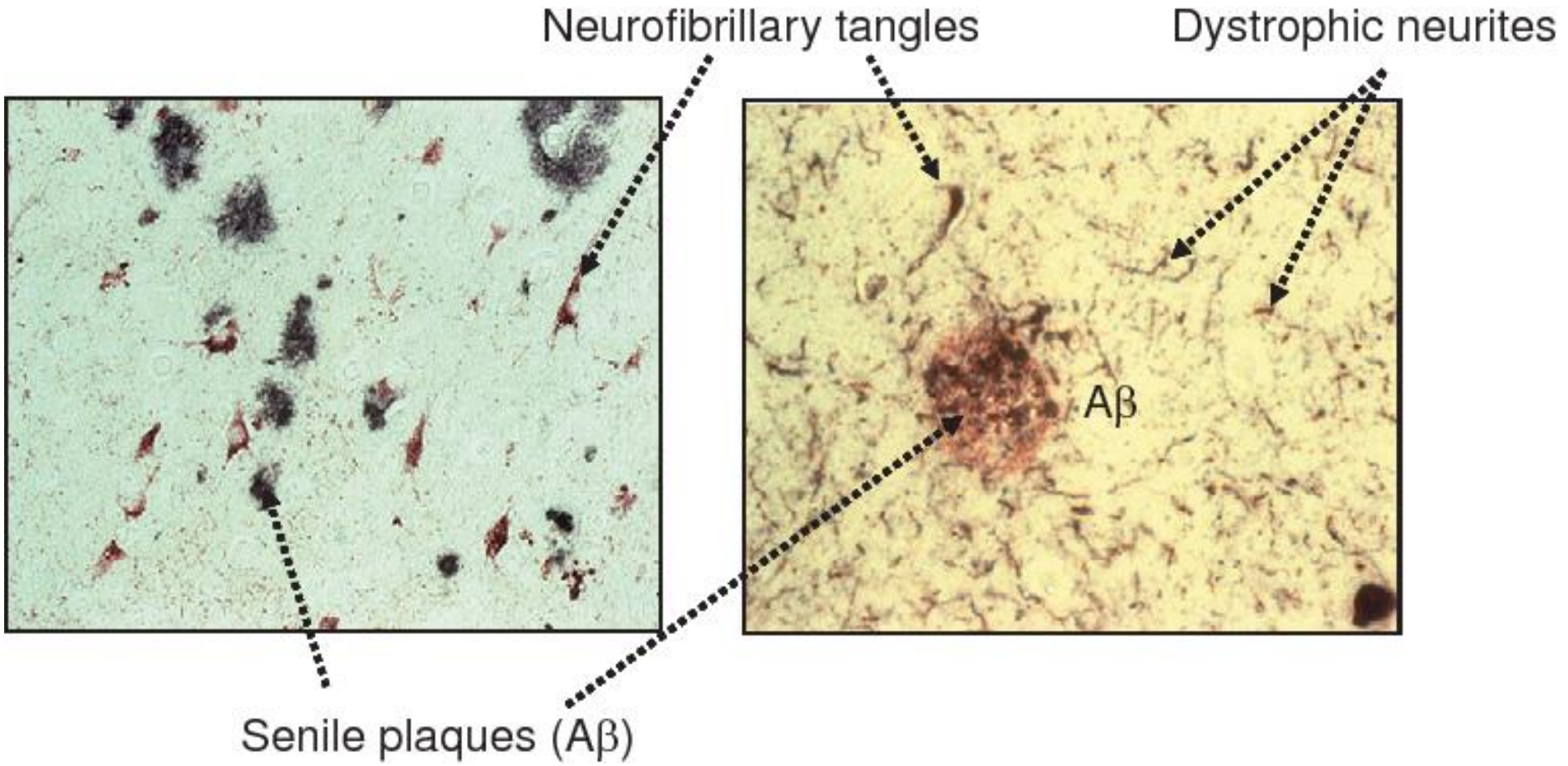


Alzheimer's Disease | Niemann Pick C | Alpha-mannosidosis

Dystrophic neurite cross-sections showing accumulation of lysosomal intermediates that actually occur prior to observation of amyloid deposits. This storage is ubiquitous in AD and lysosomal storage disorders (LSDs).

Nixon and coworkers, Chpt 10 in *Autophagy of the Nervous System*

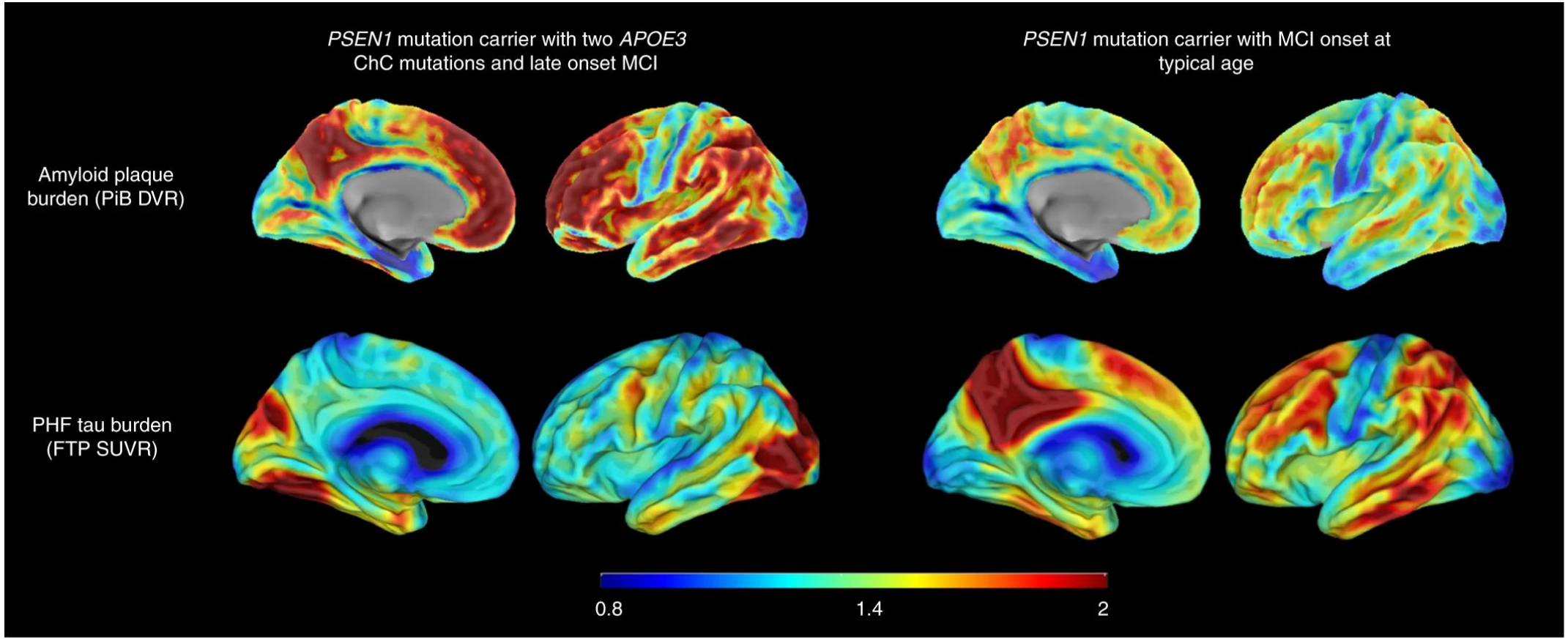
AD Basic Observations



- AD brains contain plaques that consist of abeta and neurofibrillary tangles of tau
- The amyloid hypothesis posits that Aβ aggregation is cause of AD, now updated to be small, soluble oligomers

Skaper, S. D. International Review of Neurobiology; 2012; pp 277–316.

Is plaque really the cause of AD?



- Dozens of failed clinical trials
- Poor correlation between plaque and dementia in many cases

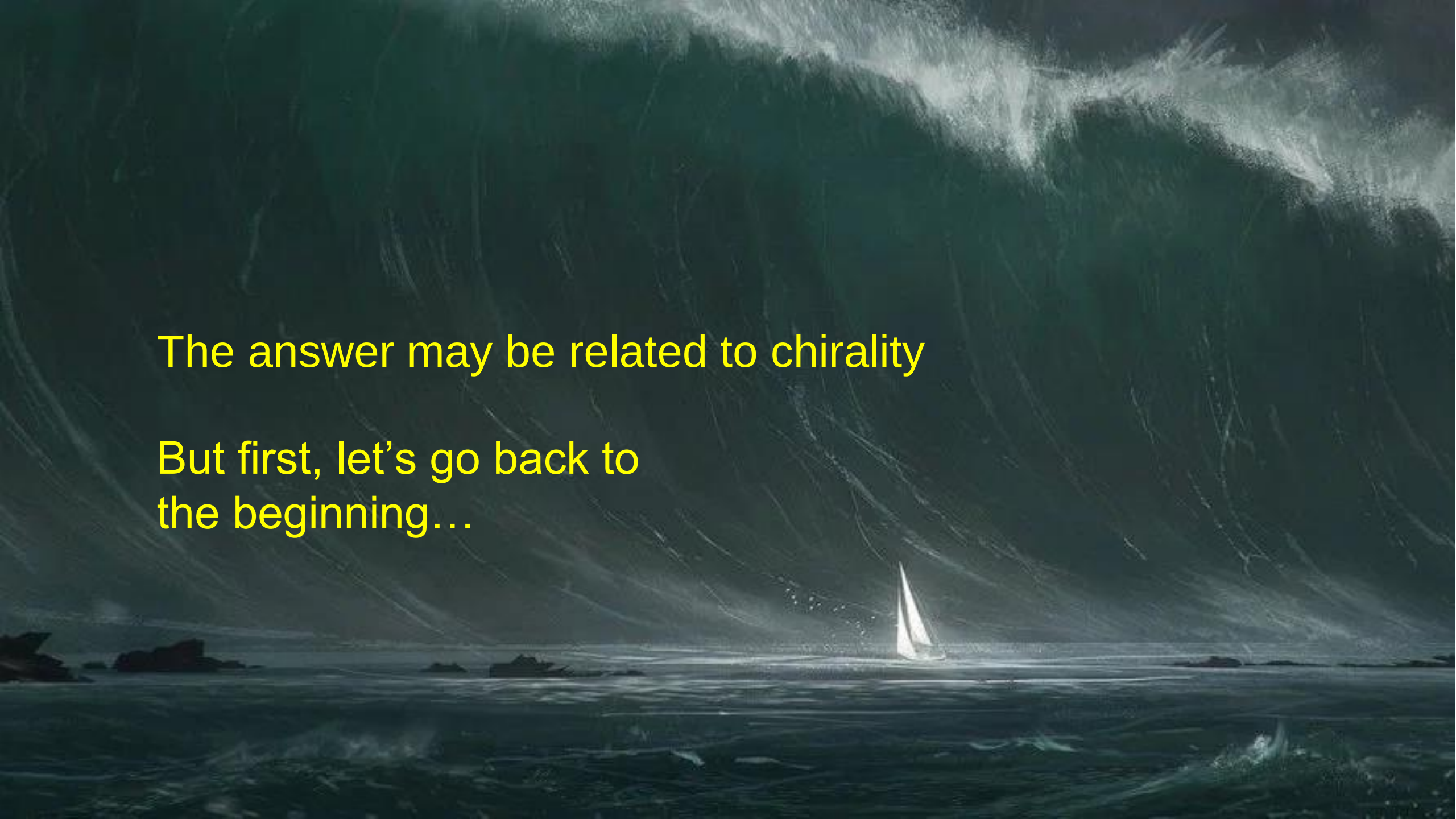
Arboleda-Velasquez, J. F; et al. *Nat. Med.* **2019**.

AD and LSDs are somehow connected, but how?

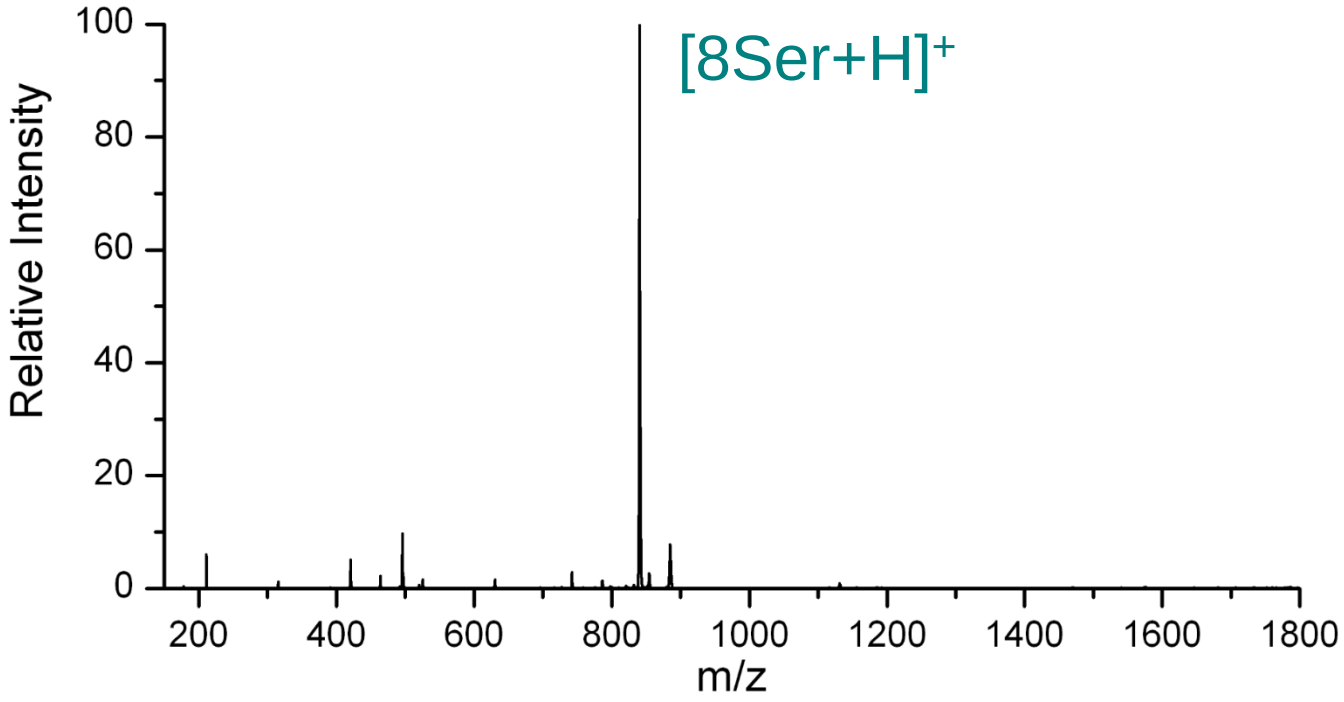
CHAPTER 10

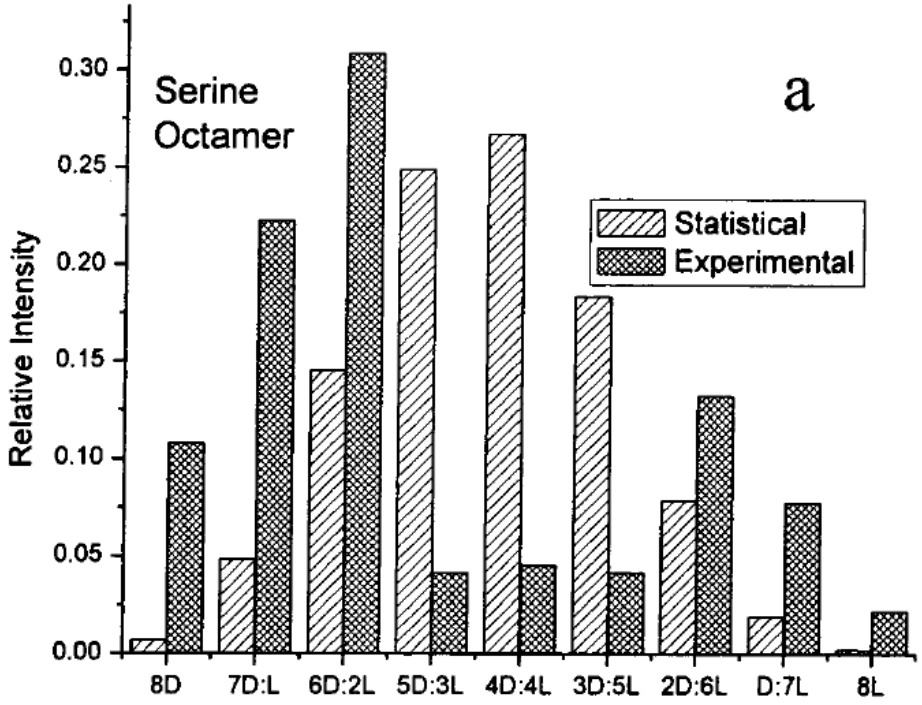
AUTOPHAGY FAILURE IN ALZHEIMER'S DISEASE AND LYSOSOMAL STORAGE DISORDERS: A COMMON PATHWAY TO NEURODEGENERATION?

Devin M. Wolfe and Ralph A. Nixon


The answer may be related to chirality
But first, let’s go back to
the beginning…

The Infamous Serine Octamer



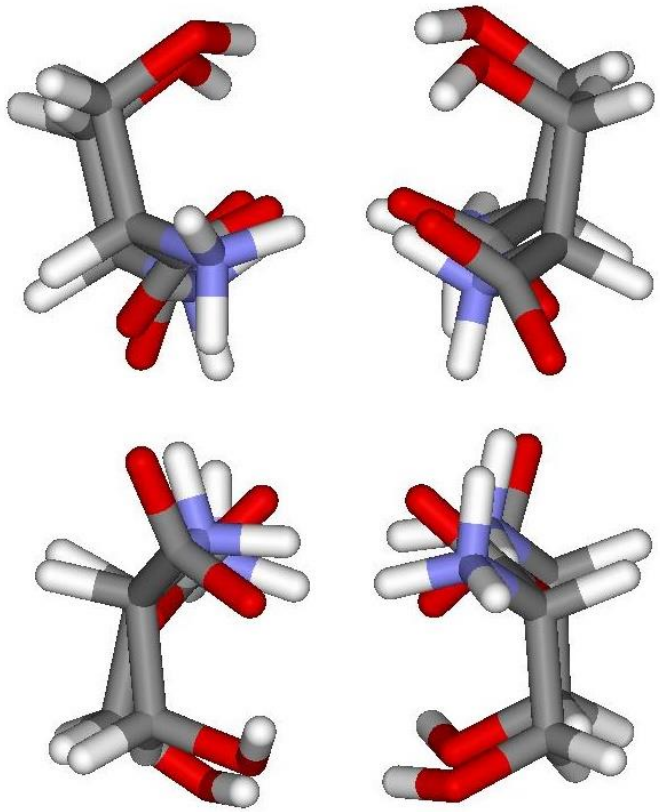


Julian, R. R. et al JPC **2002**, 1219

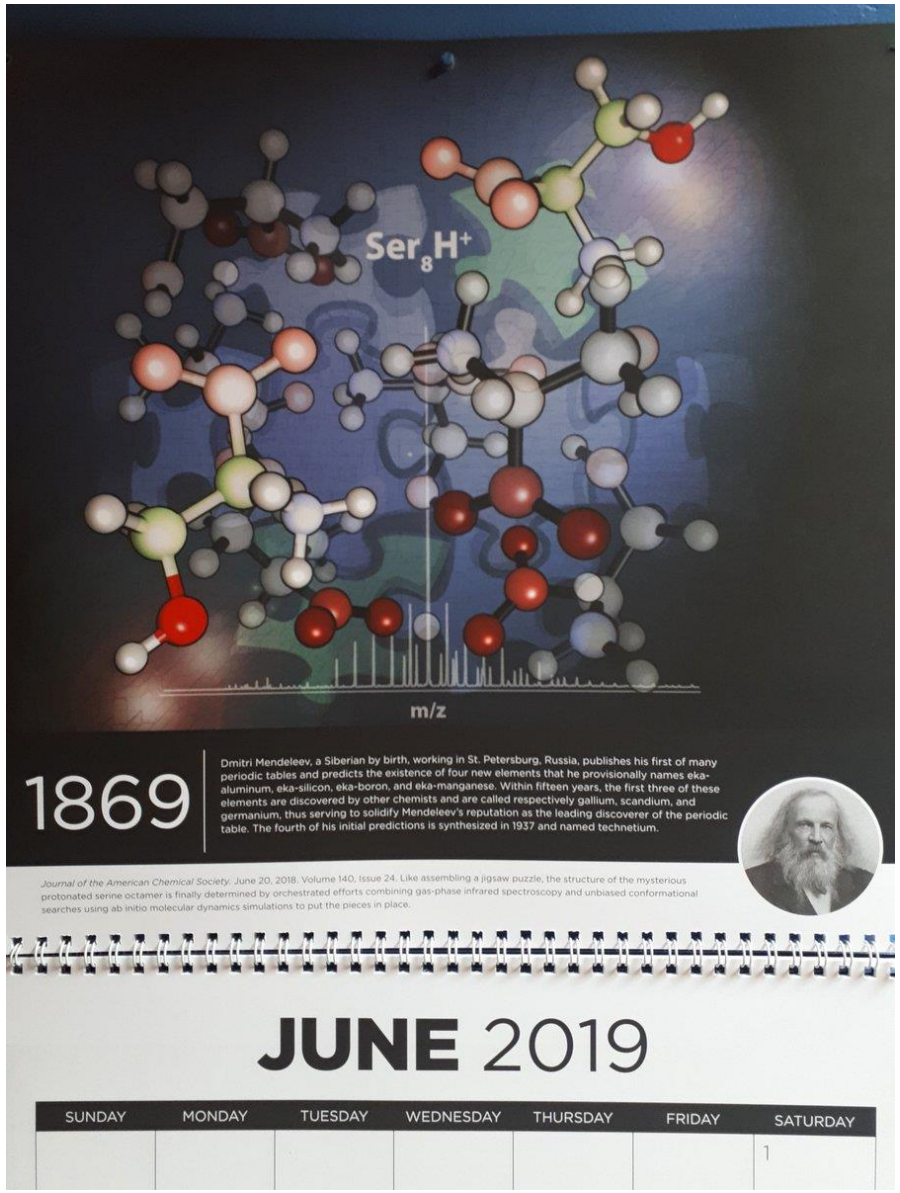
Serine Octamers: Cluster Formation, Reactions, and Implications for Biomolecule Homochirality**

*Sergio C. Nanita and R. Graham Cooks**

The Infamous Serine Octamer



20 years and 65 papers later, the mystery has been solved… You can find it in the June illustration of your 2019 ACS calendar.



Homochirality and D-residues in Nature

D-amino acids are commonly found in the venom of spiders, snakes and snails where they have been intentionally incorporated by a racemase enzyme.
–why?

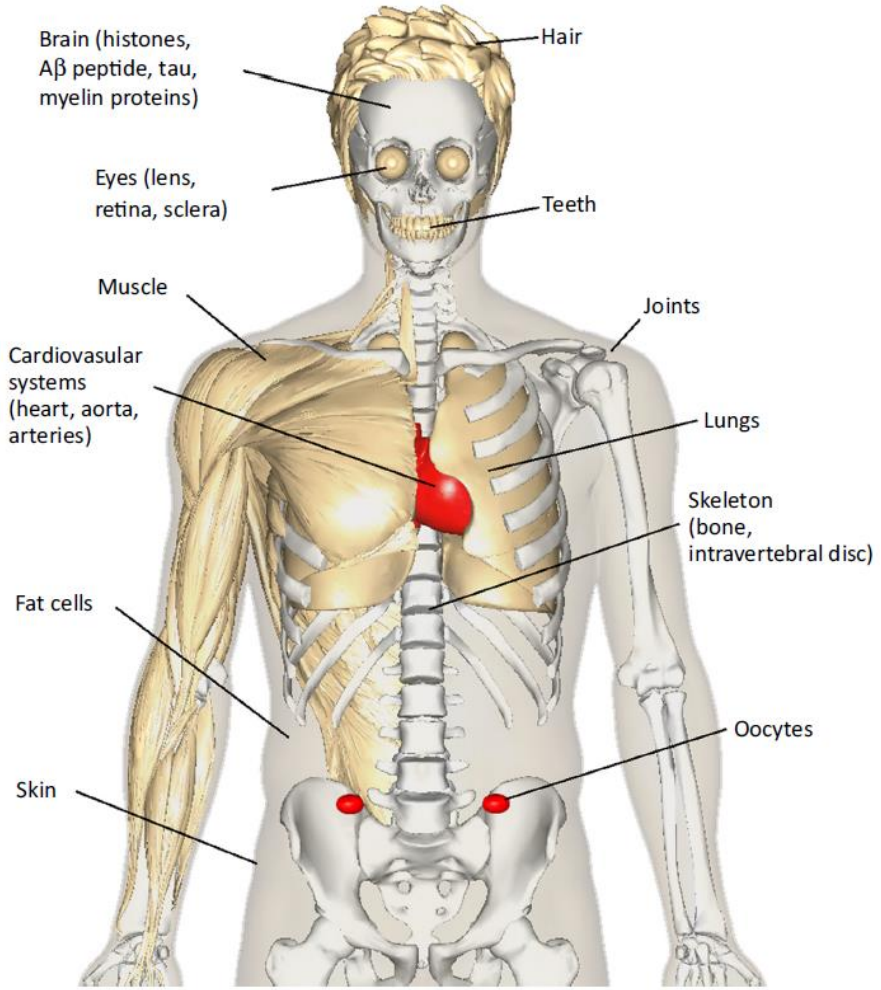
D-residues in people

Opinion

Old Proteins in Man: A Field in its Infancy

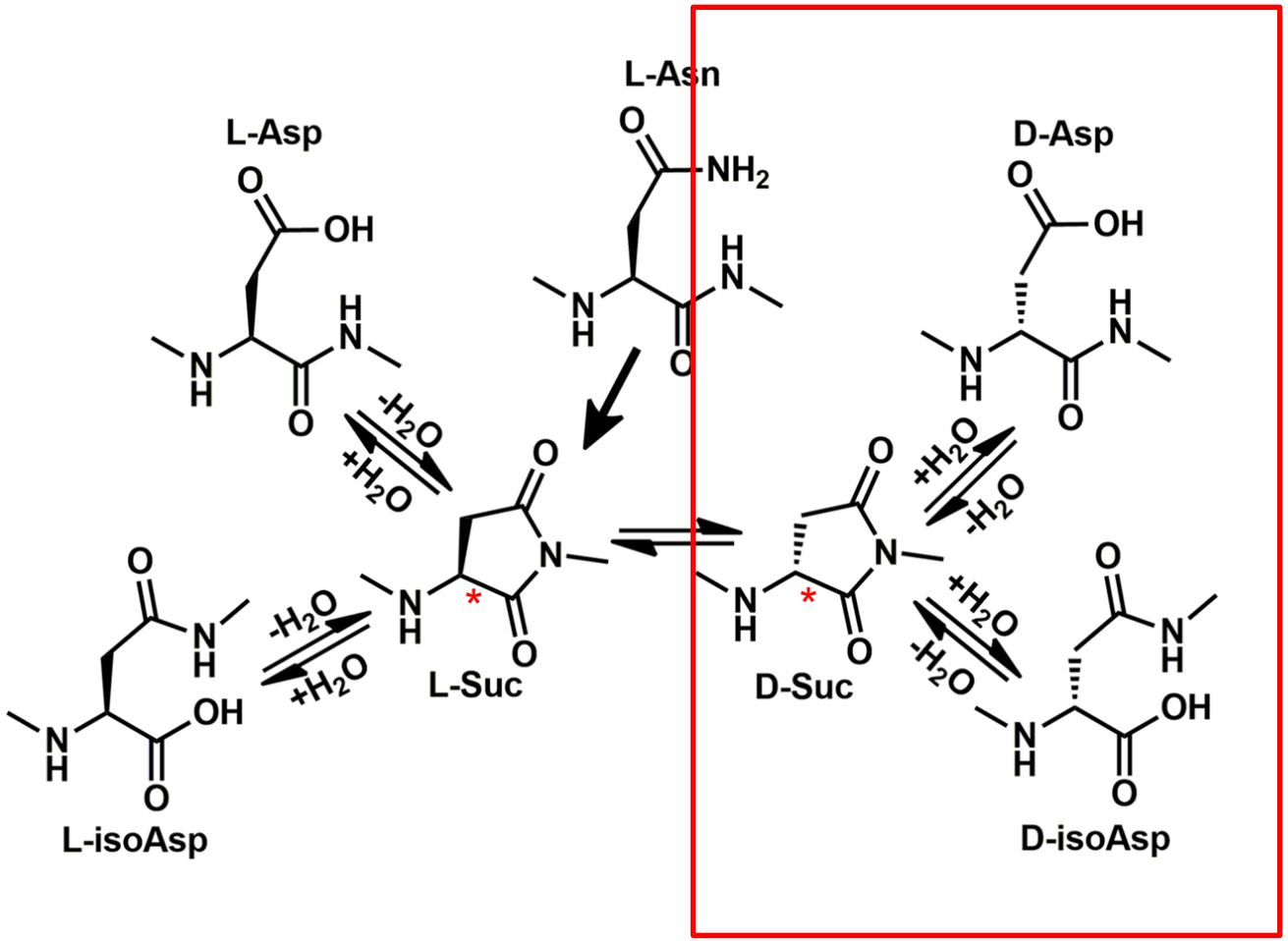
Roger J.W. Truscott,[1,*] Kevin L. Schey,[2] and Michael G. Friedrich[1]

- Over time, spontaneous chemical modifications (i.e. not enzymatically created) can accumulate in long-lived proteins, including the formation of D-residues or other isomers.

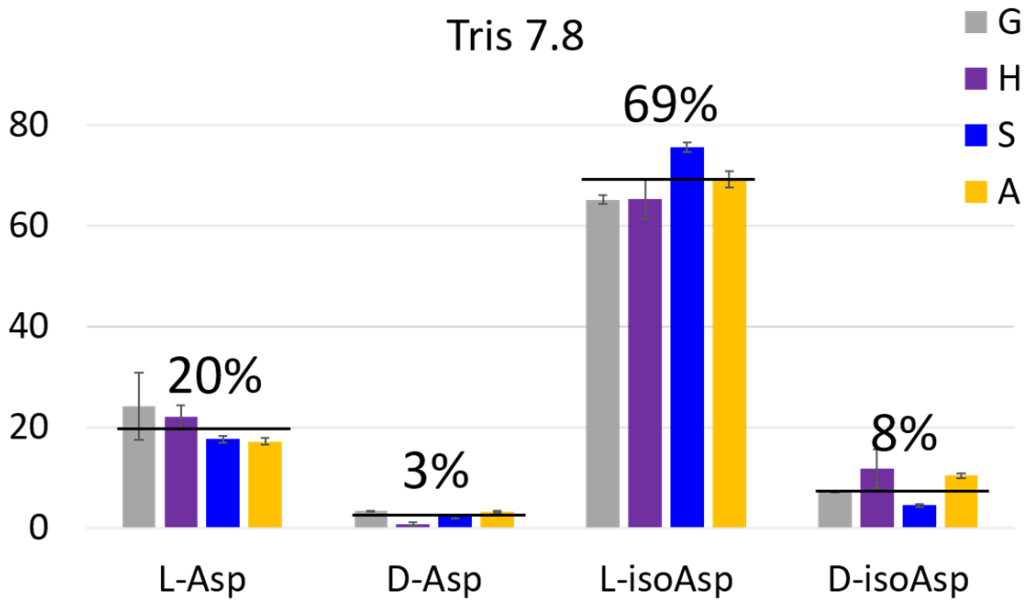


Truscott et al. *Trends Biochem. Sci.* **2016**, *41* (8), 654–664.

Asp isomerization is most common

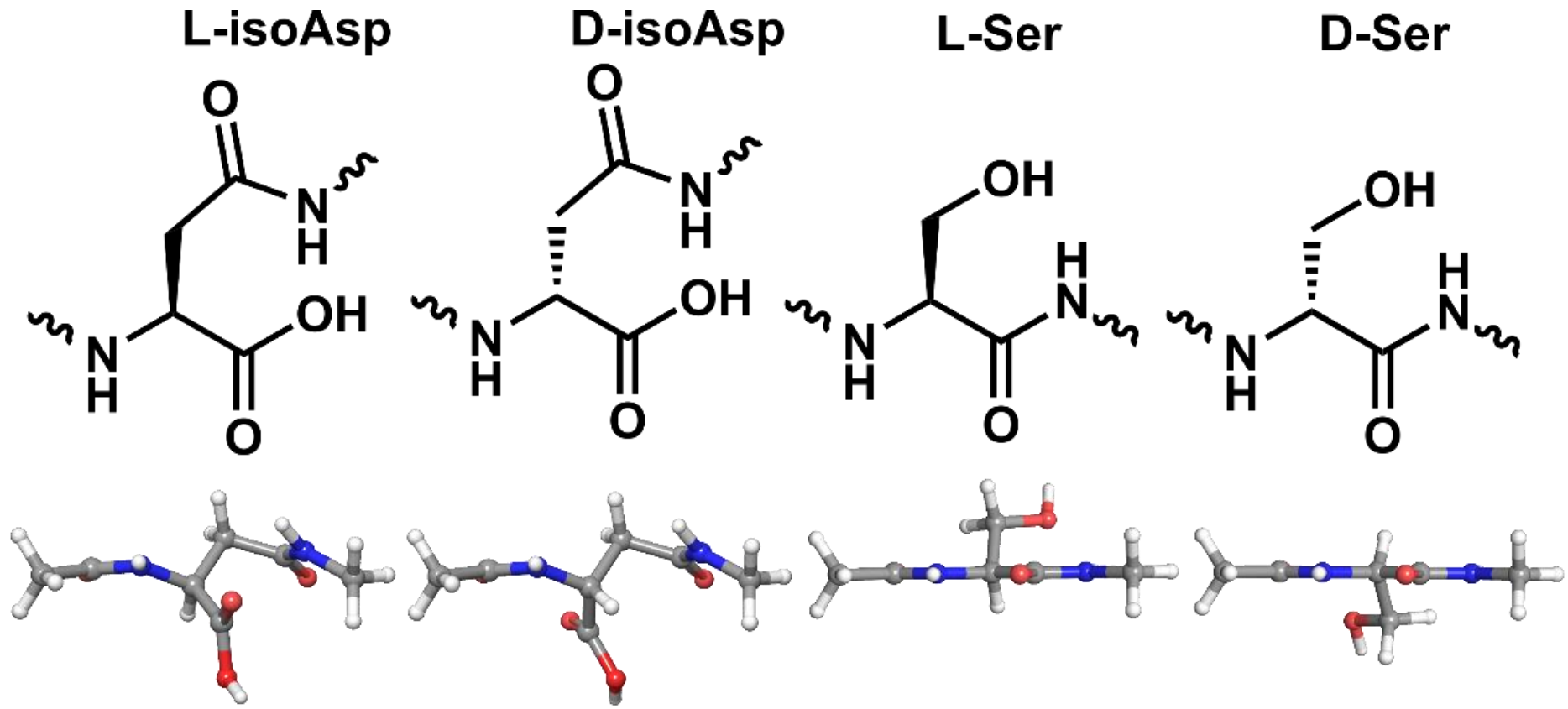


Formation of D-isomers is much slower than L-isoAsp



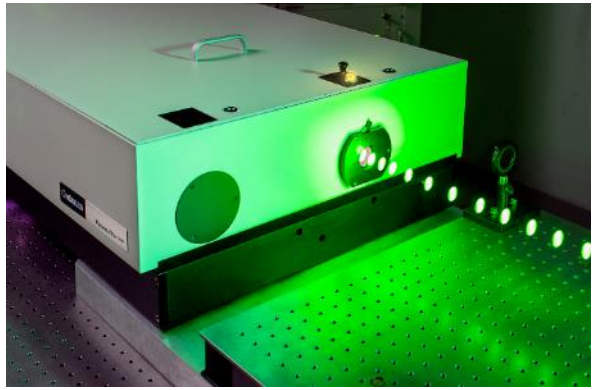
Julian et al, *ACS Chem. Biol*. **2017**, 12, 2875.

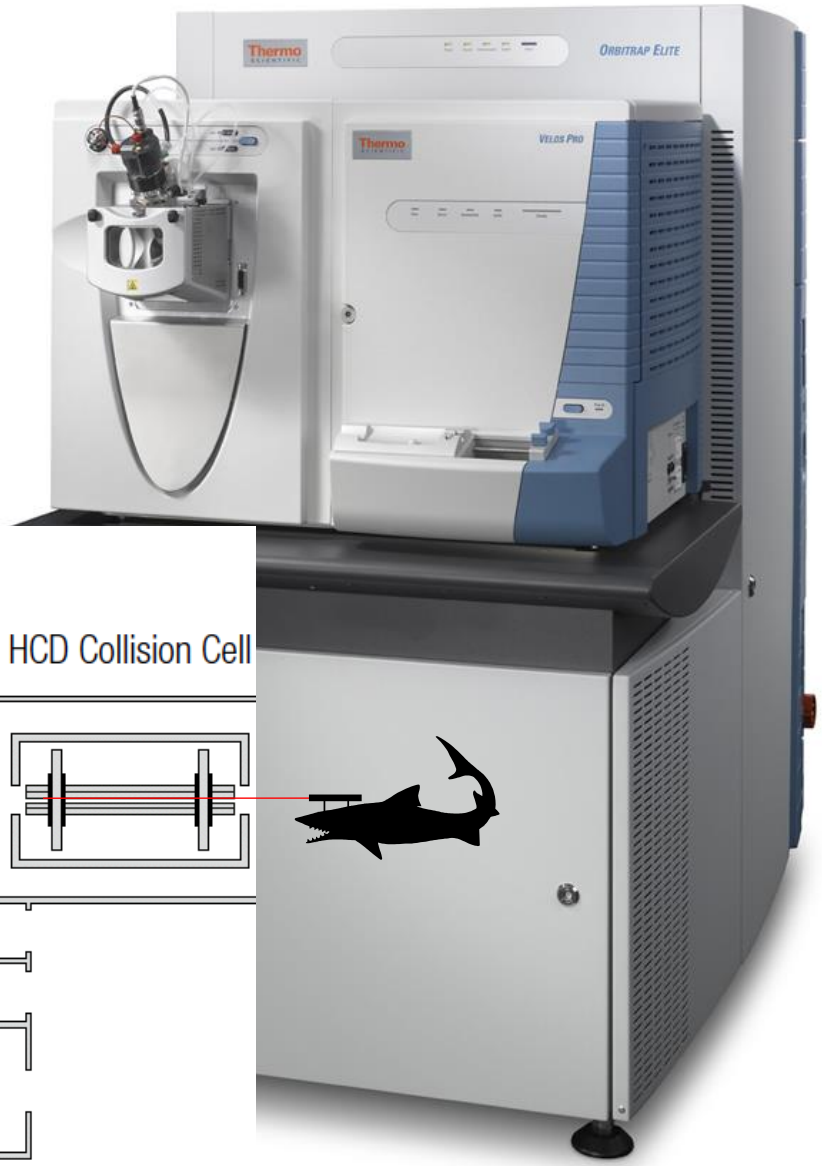
Isomerization/epimerization structural effects

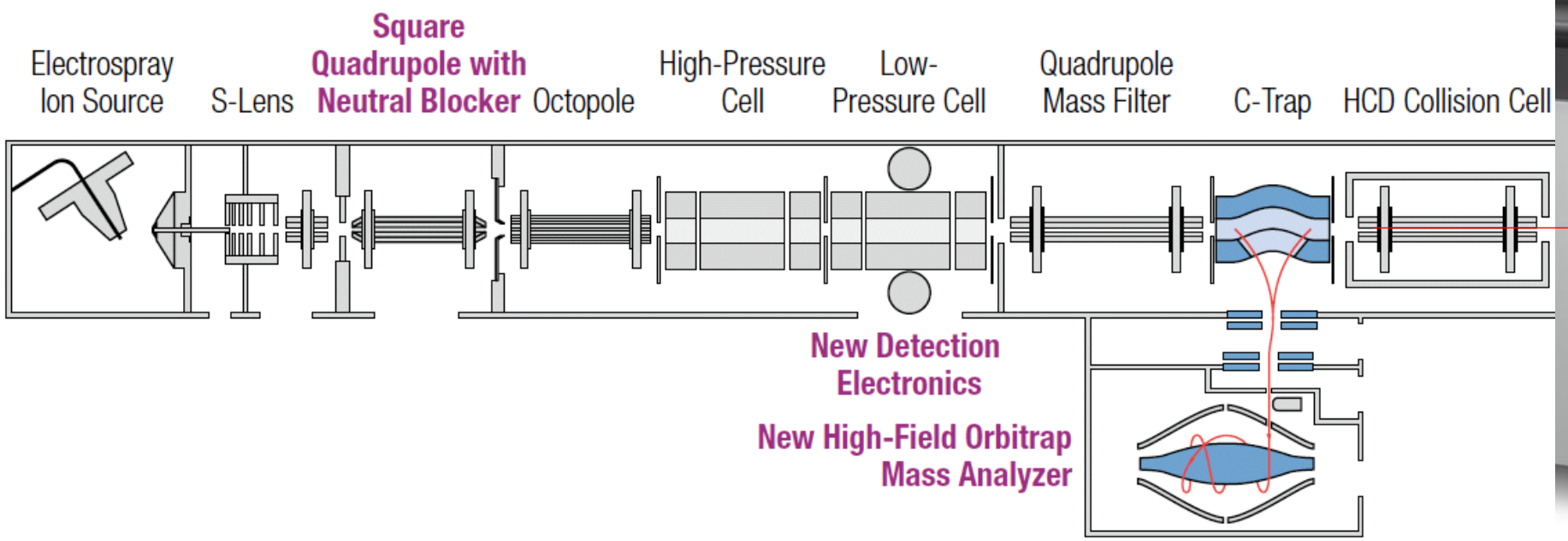


Although these changes are subtle in some ways, (don't change mass, functional groups are retained), they drastically alter structure at the residue level.

Modified Mass Spectrometry



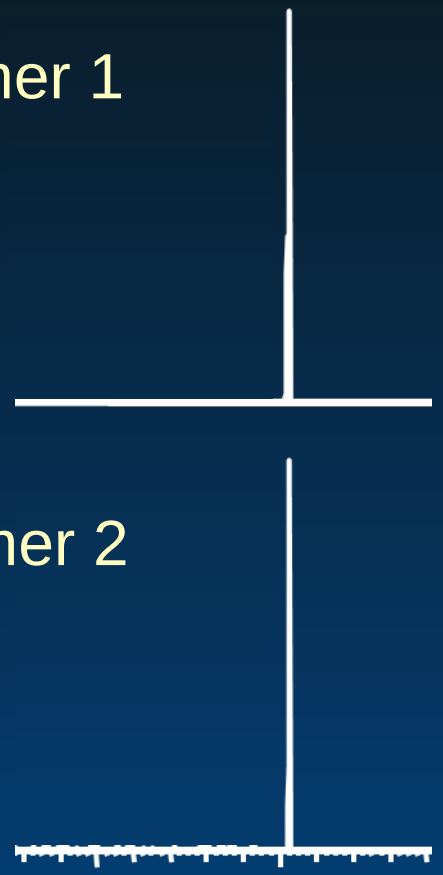




The Challenge: Isomerization doesn’t change mass!

Full MS analysis cannot identify isomers or epimers, even if they are already separated.

Identification of this type of spontaneous chemical modification is therefore much more challenging than traditional PTM identification.



The Answer: MS/MS can identify Isomers

- Spectra for both isomers needed
- Intensity of select fragments differs

$$R_{isomer} = \frac{R_1}{R_2}$$

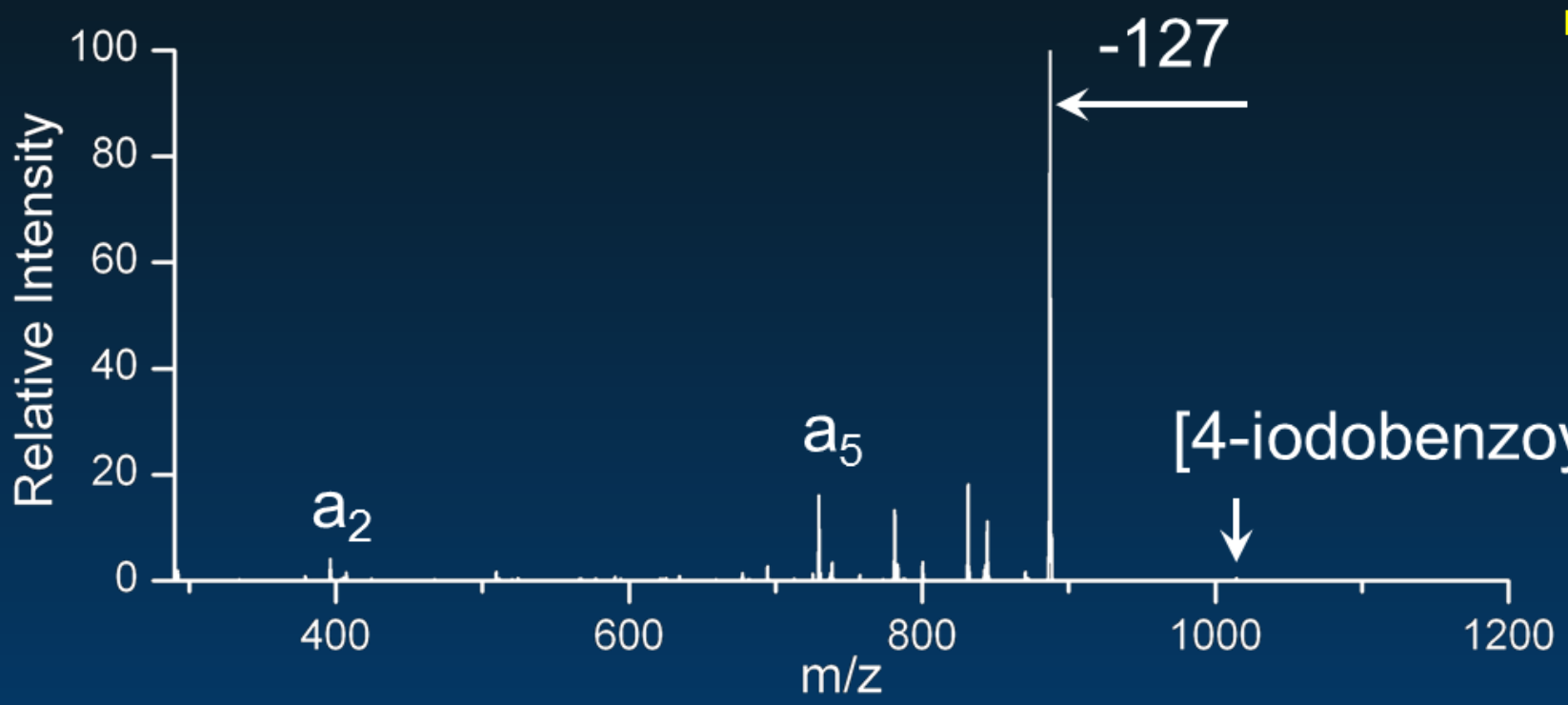
$$R_1 = b_1/a_1$$

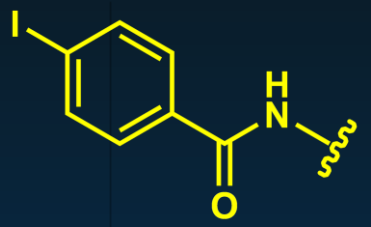
$$R_2 = b_2/a_2$$

R_{isomer} = 1, no isomer discrimination.
R_{isomer} > 1, a larger number reflects higher degree of isomer recognition.

Cooks *J. Am. Chem. Soc.* **2000,** *122*, 10598.

Improving MS/MS with Radical Directed Dissociation (RDD)

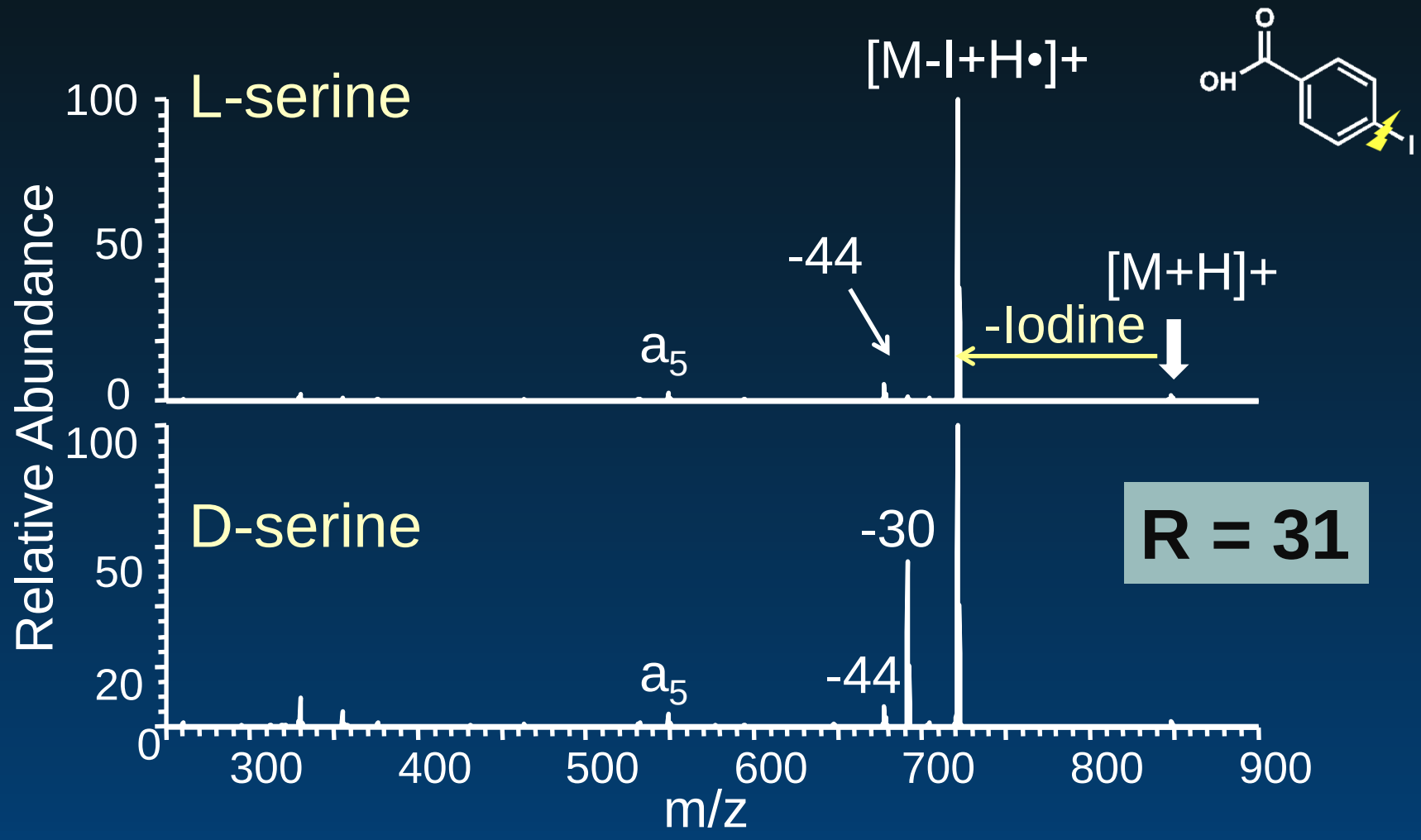




yield: 99.8%

Radicals can be created site-specifically via photodissociation of carbon-iodine bonds. Radical yield is quantitative.

RDD yields high R values

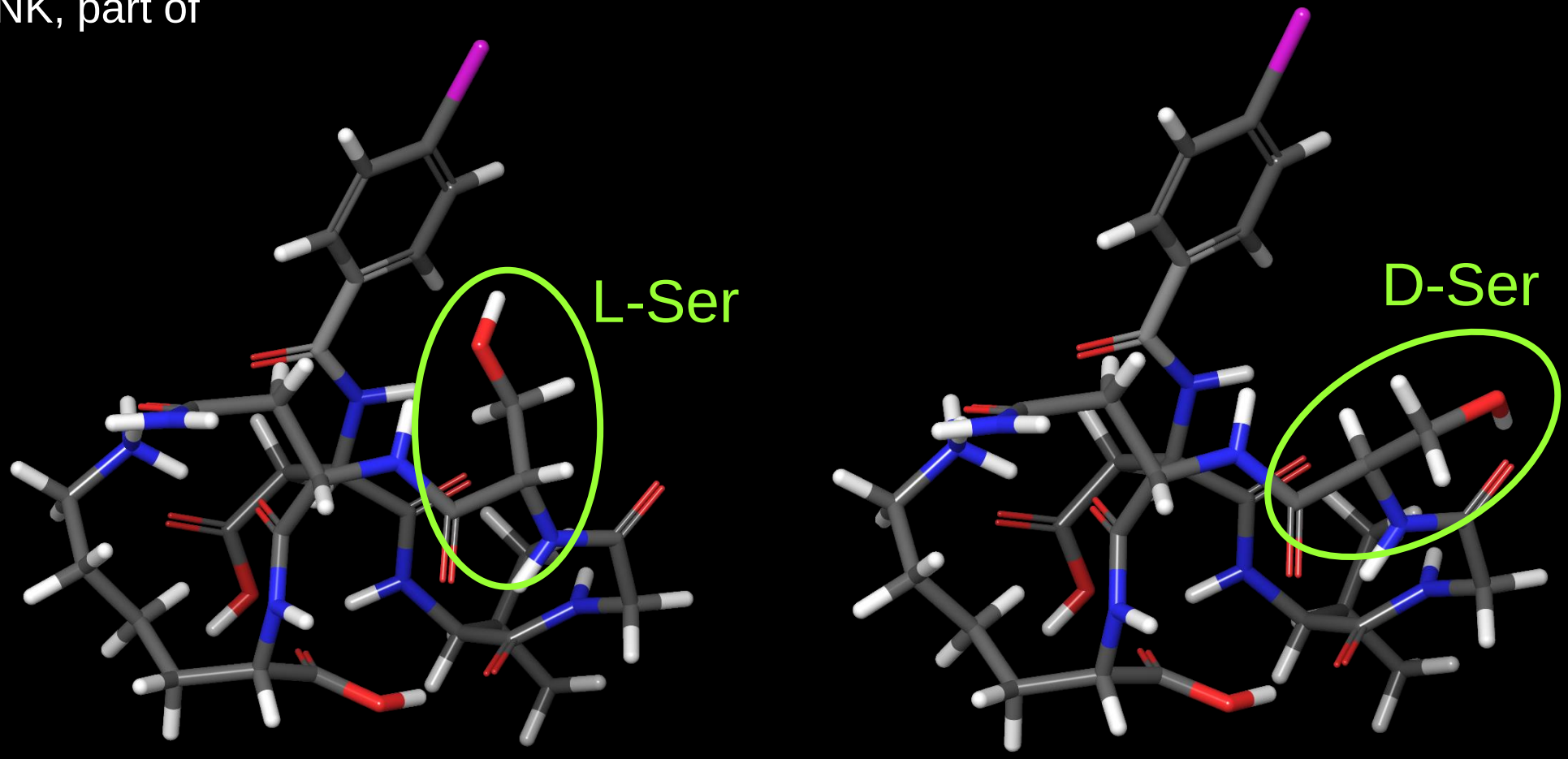


IB-DVGSNK, part of Aβ-40, epimerization is observed in plaques.

Tao, Y et al *Anal. Chem.* **2012**, *84*, 6814.

RDD is sensitive to 3D structure

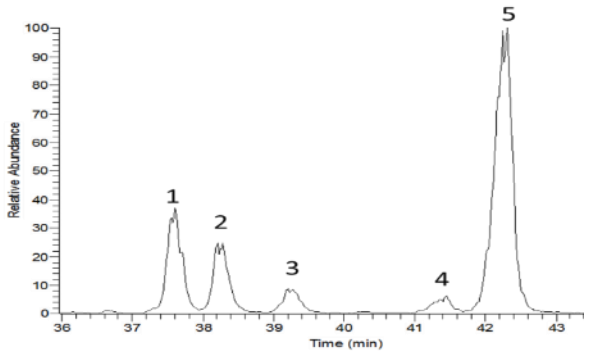
IB-DVGSNK, part of Aβ1-40

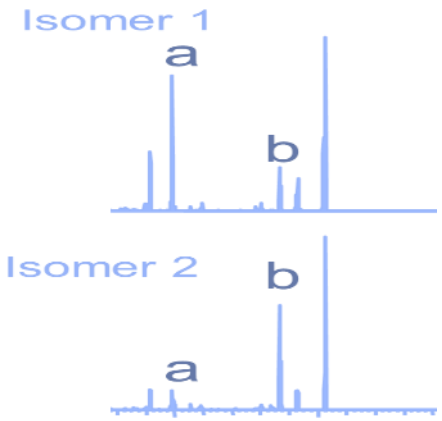


RDD is sensitive to minor changes in structure, including side chain chirality.

Tao, Y et al *Anal. Chem.* **2012**, *84*, 6814.

Isomer/Epimer ID Workflow





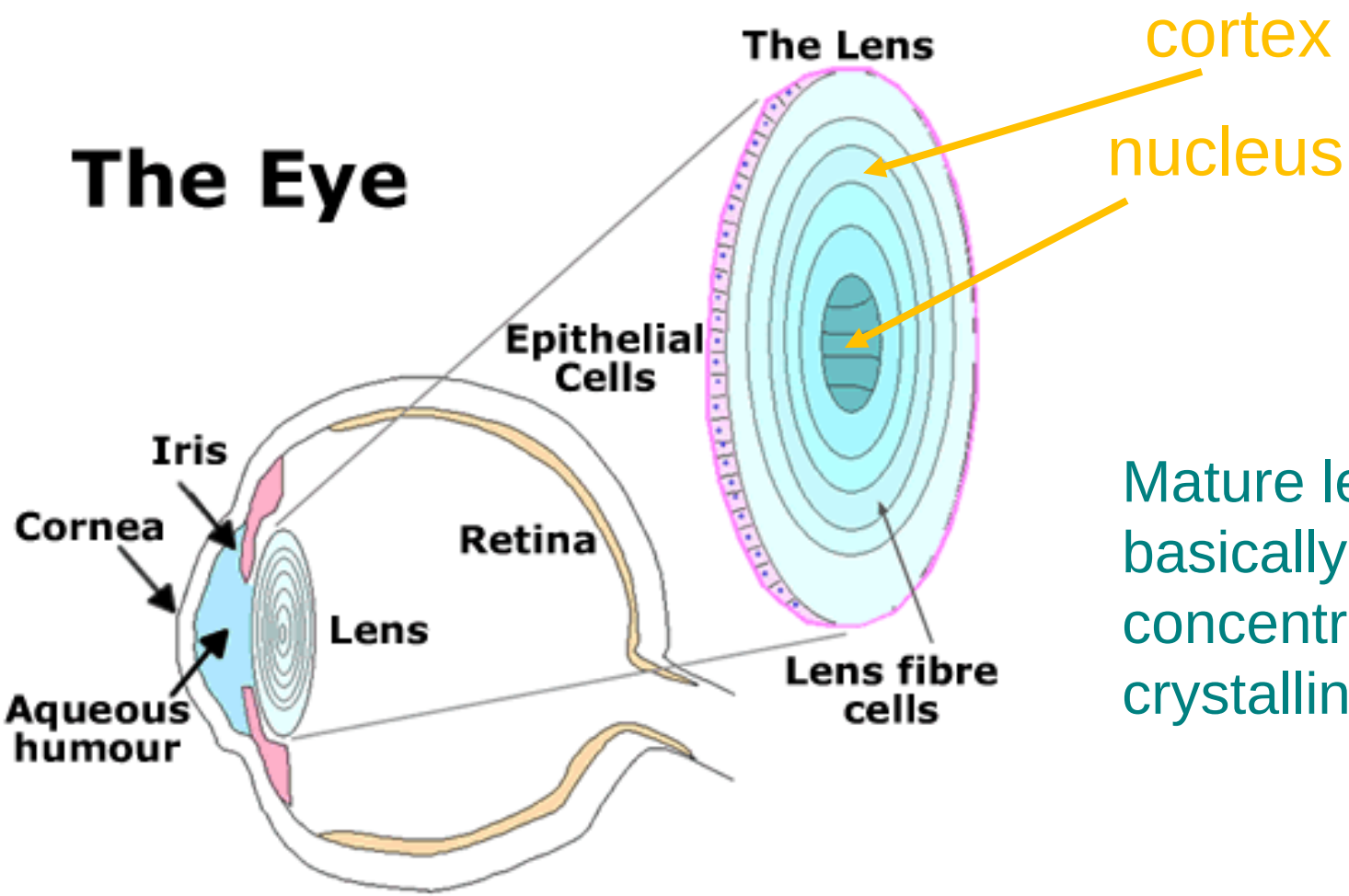
Isolate peptides/proteins from biological sample → Traditional LC-MS for identification

Repeated analysis of hit list to identify potential isomers → Isomers present same m/z in multiple LC peaks

MS/MS for confirmation and additional identification → Different MS/MS spectra for identical precursor m/z indicates isomer/epimer

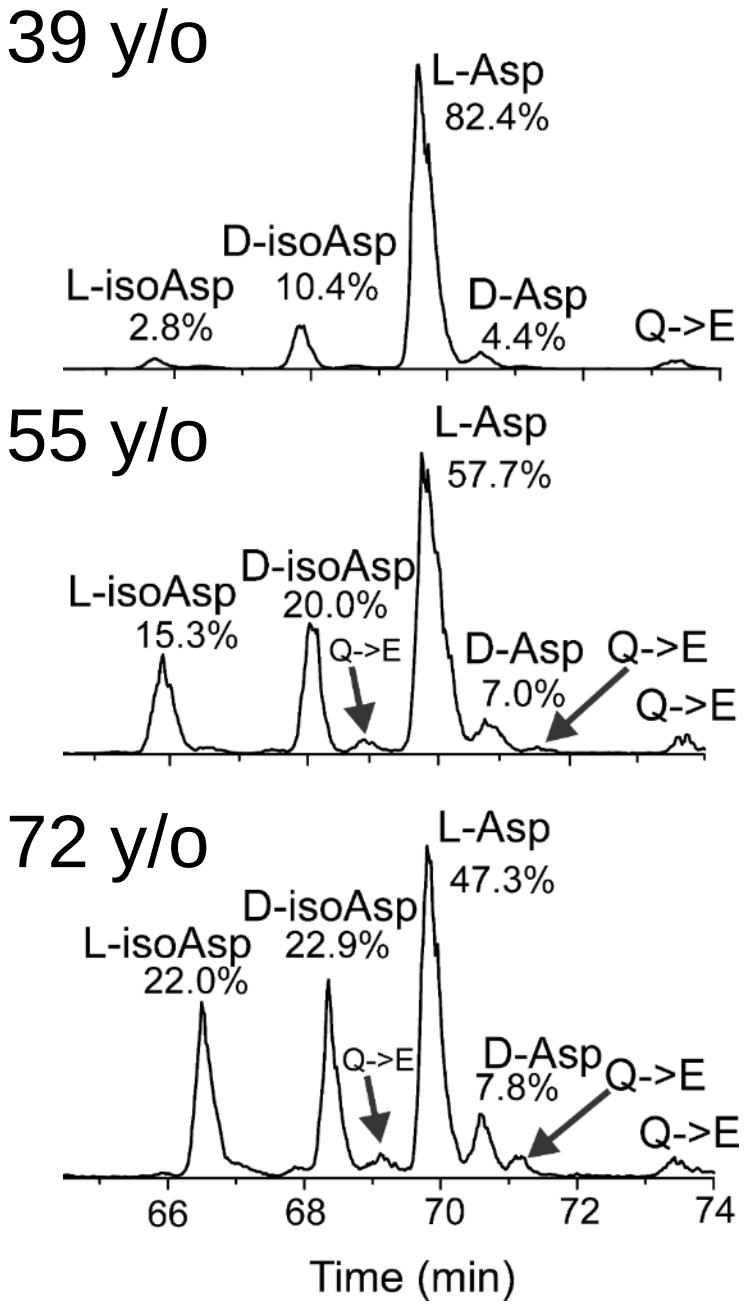
No synthetic standards are needed.

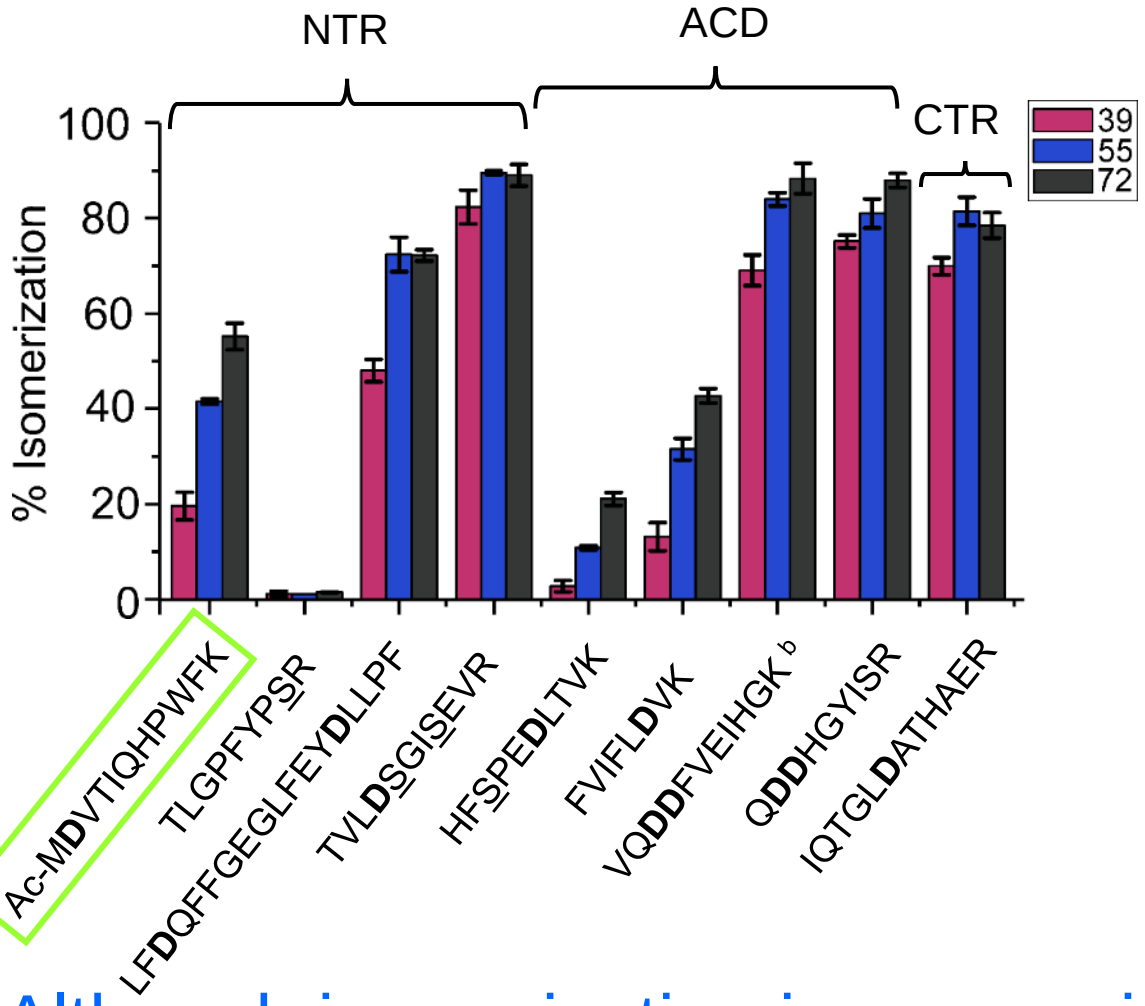
Crystallin Proteins in Lens Never Turnover



Mature lens fiber cells are basically bags of highly concentrated protein, mostly crystallins.

Isomerization vs Age in Human Lenses

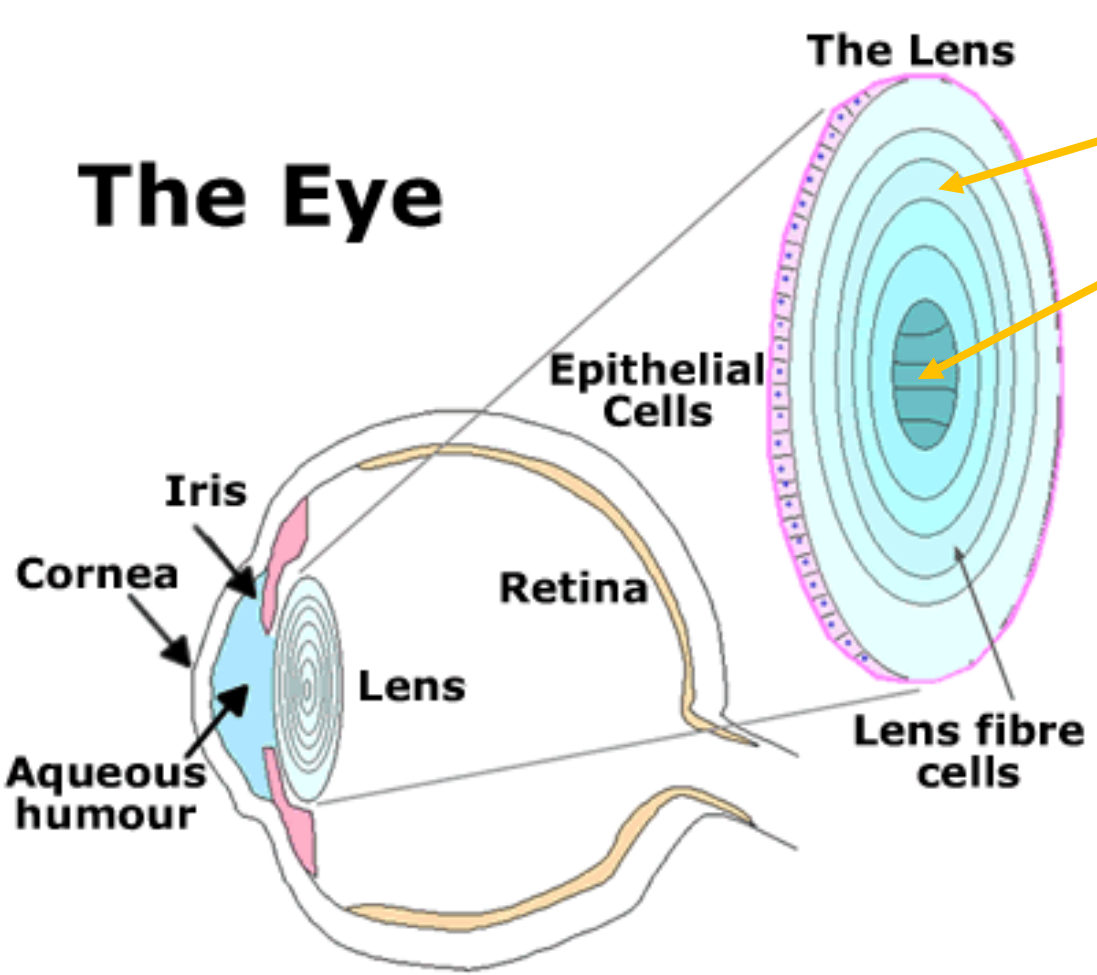




- Although isomerization increases in general with age, the slope varies considerably (data from Alpha-A)

Julian, *Exp Eye Res*. **2018**, 131

The lens continues to grow throughout life

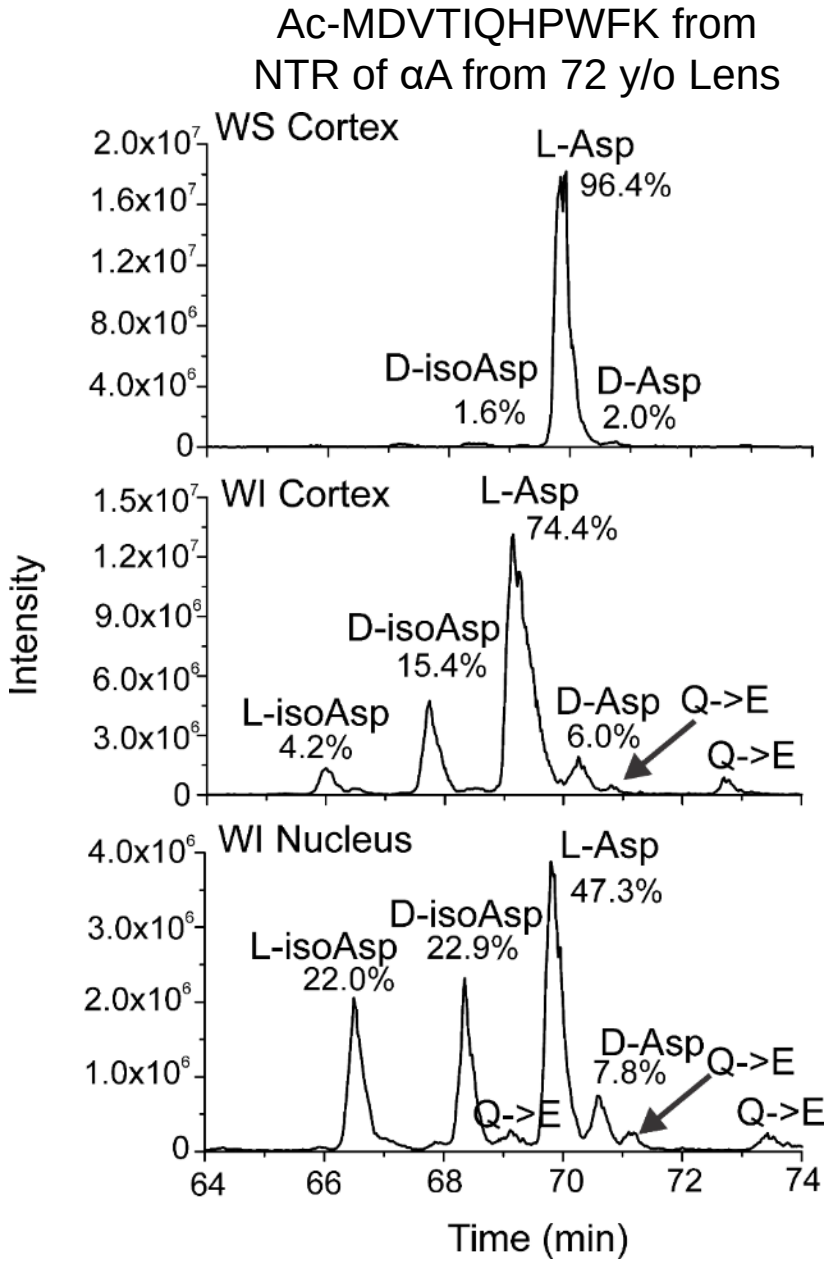


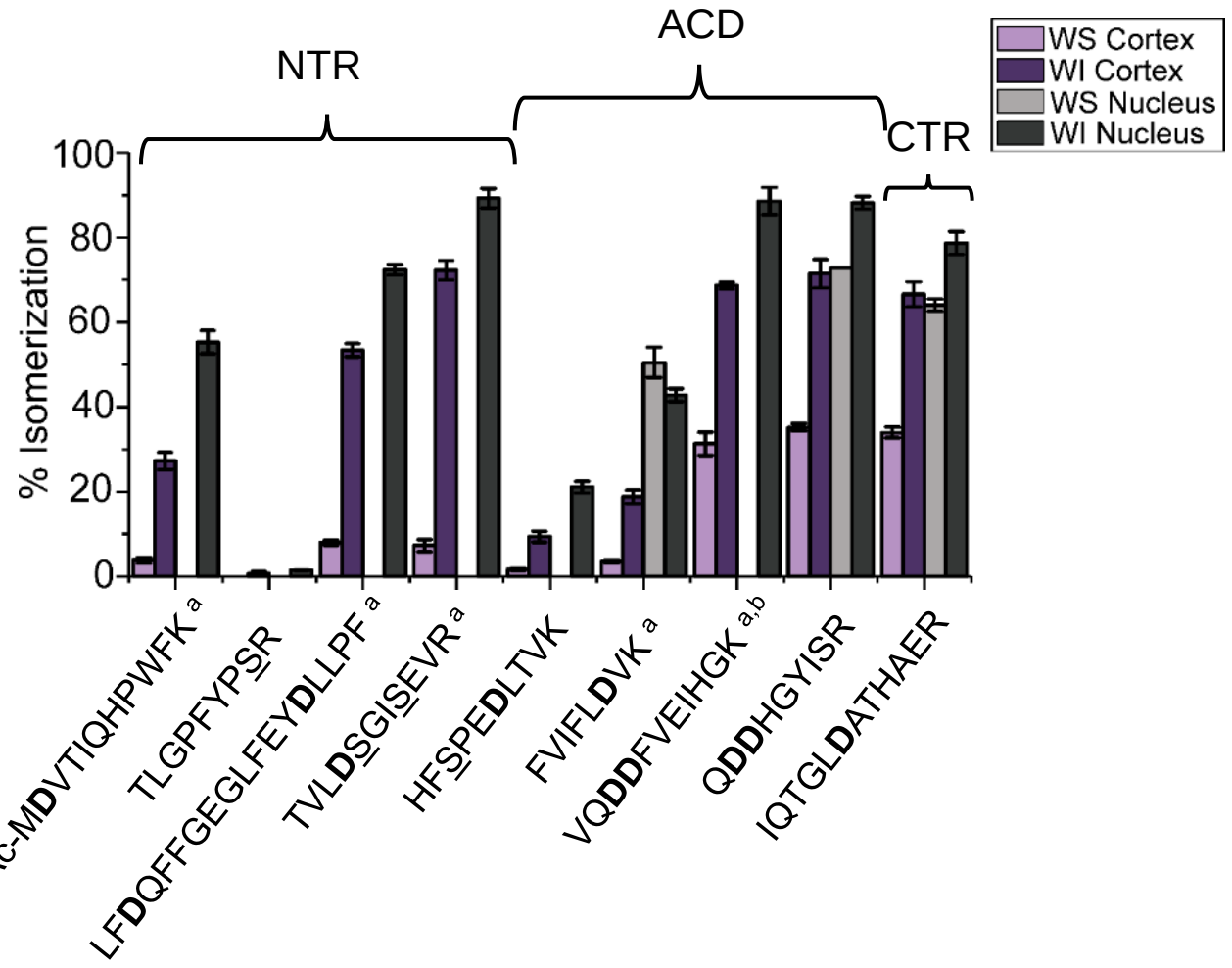
cortex

nucleus

A history of the protein modification is stored in the layers of the lens.

Isomerization vs Location

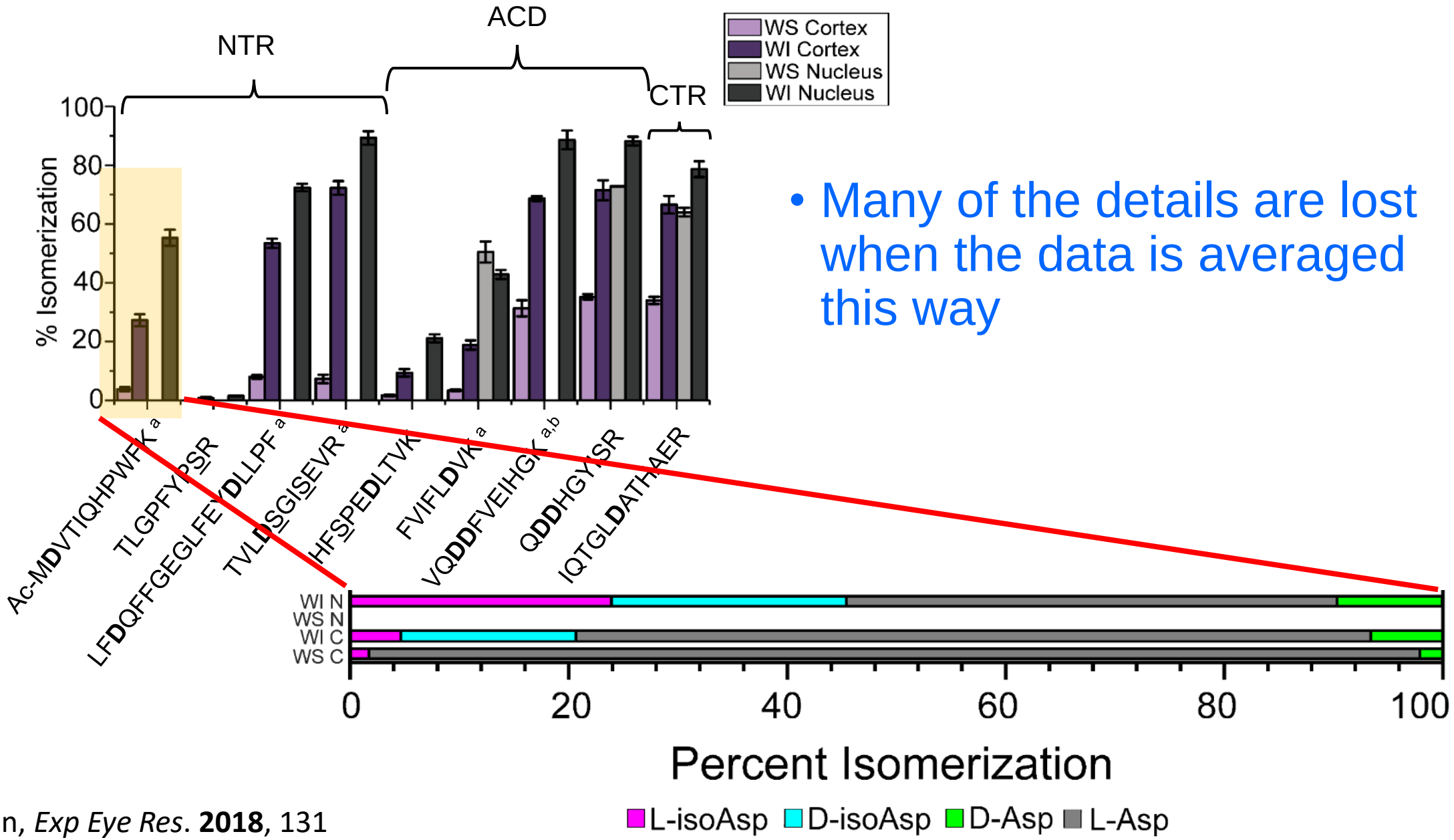




- A more dramatic and consistent trend is observed for isomerization versus location/fraction

Julian, *Exp Eye Res*. **2018**, 131

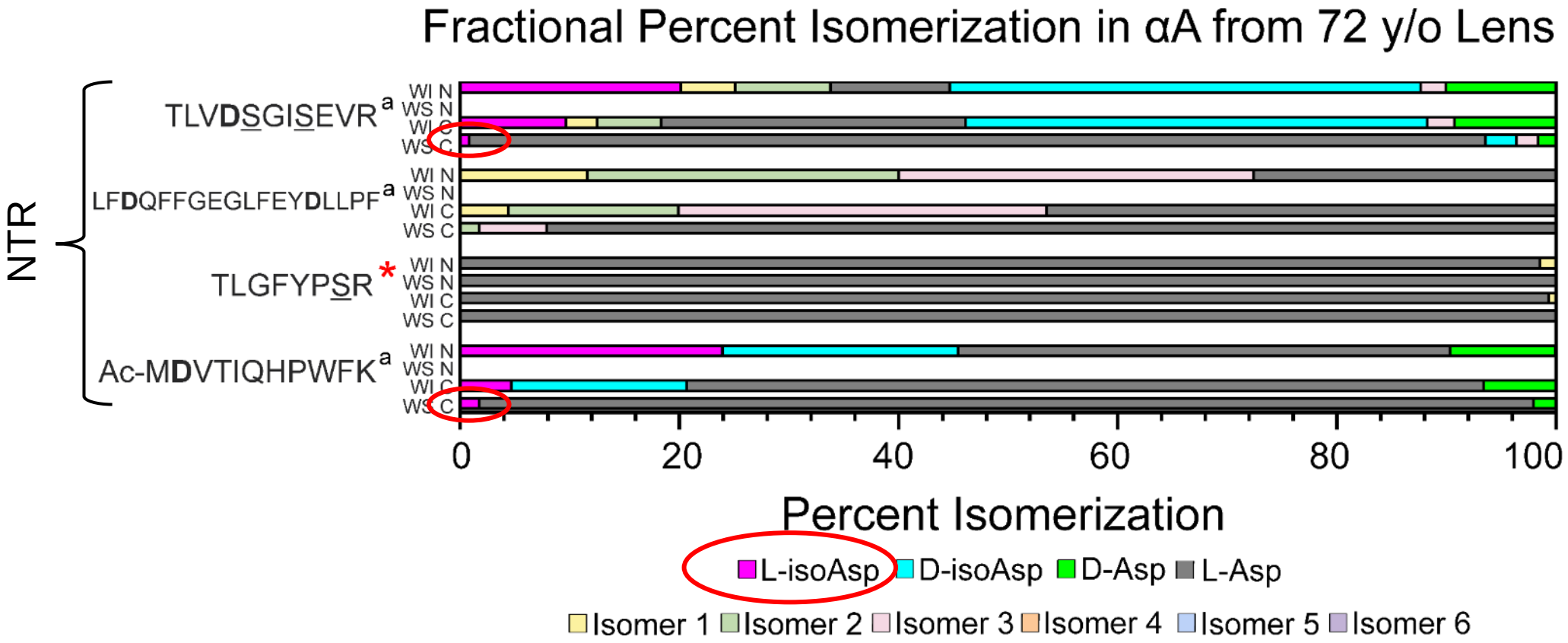
Drilling further into the Data



- Many of the details are lost when the data is averaged this way

Julian, *Exp Eye Res*. **2018**, 131

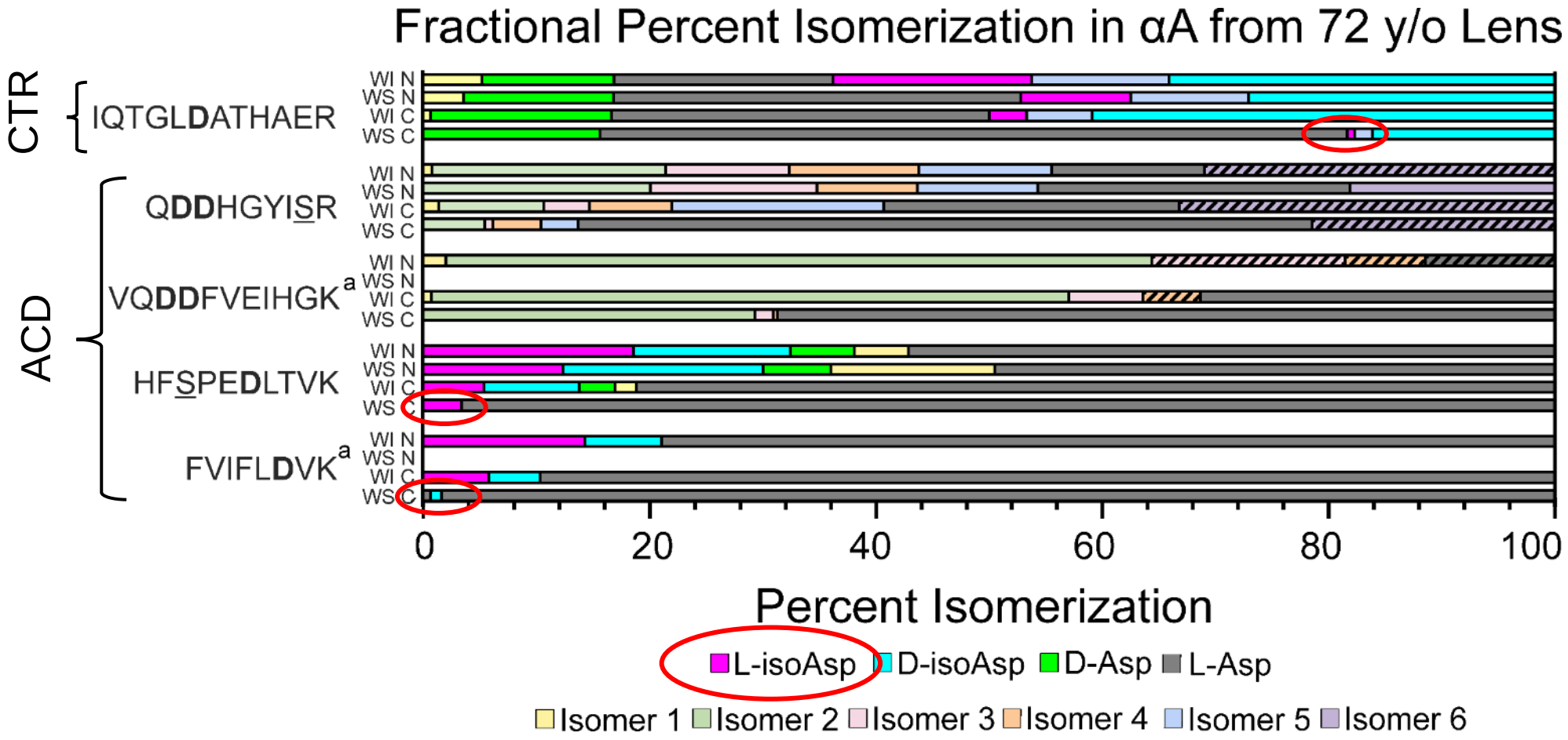
Drilling further into the Data



- Not all isomers are identified, but note lack of L-isoAsp in WS cortex

Julian, *Exp Eye Res*. **2018**, 131

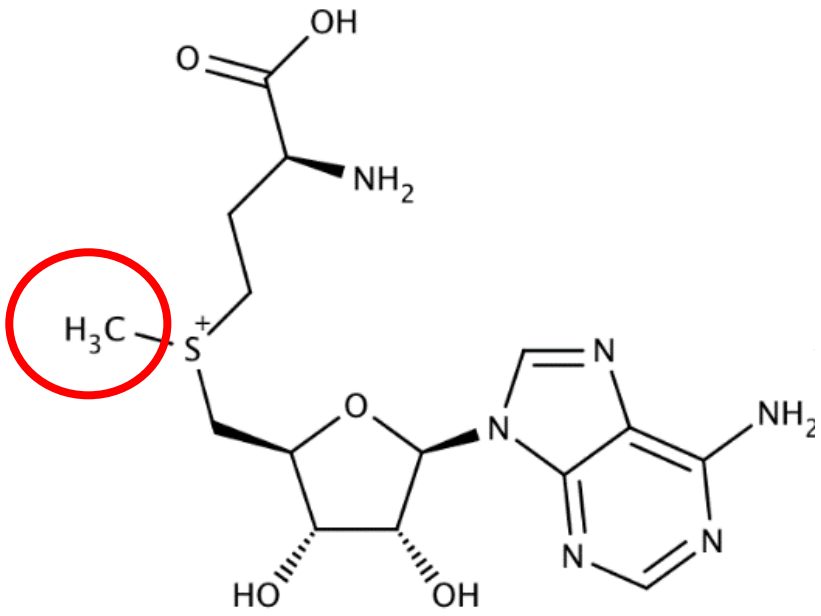
Drilling further into the Data



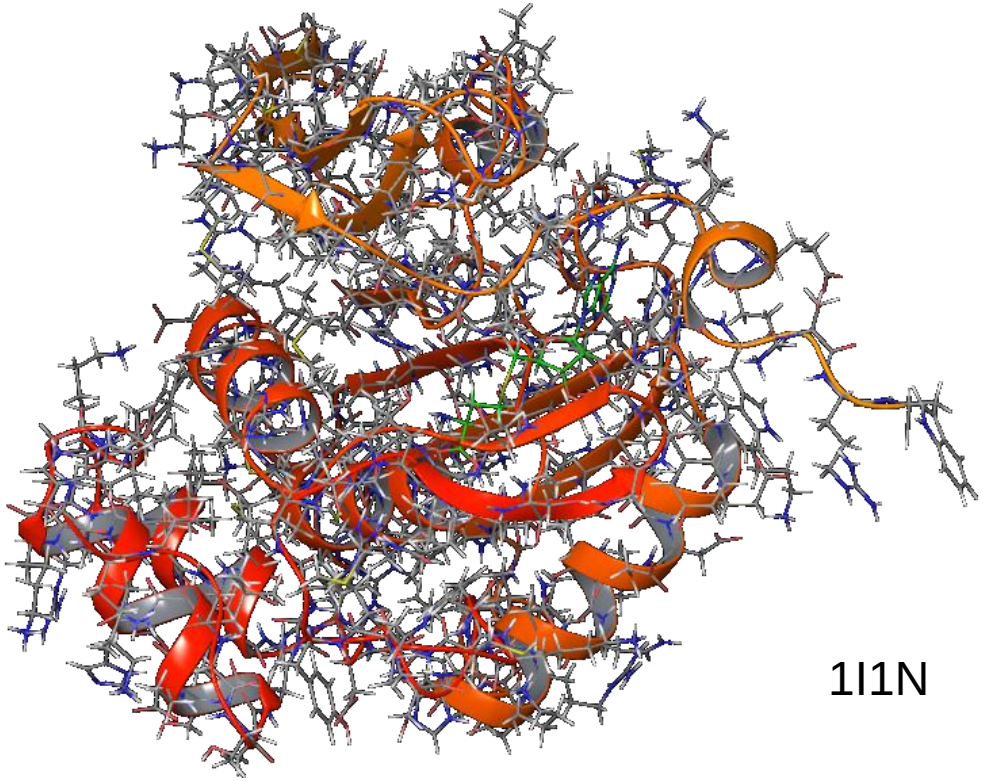
- Again L-isoAsp minimal in WS cortex, probably due to PIMT

Julian, *Exp Eye Res*. **2018**, 131

PIMT is a crucial protective enzyme



S-adenosyl-L-methionine

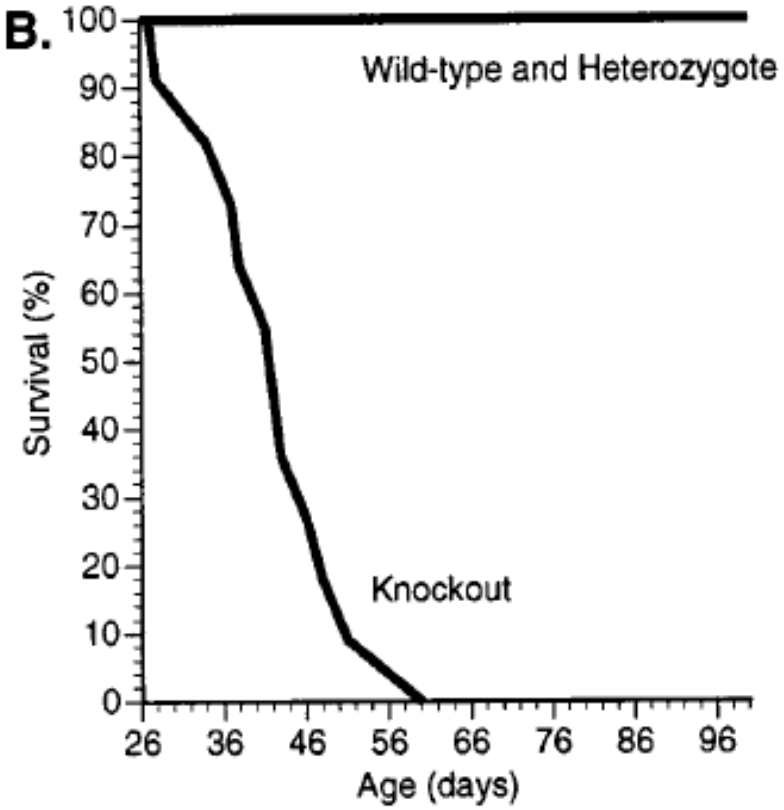


1I1N

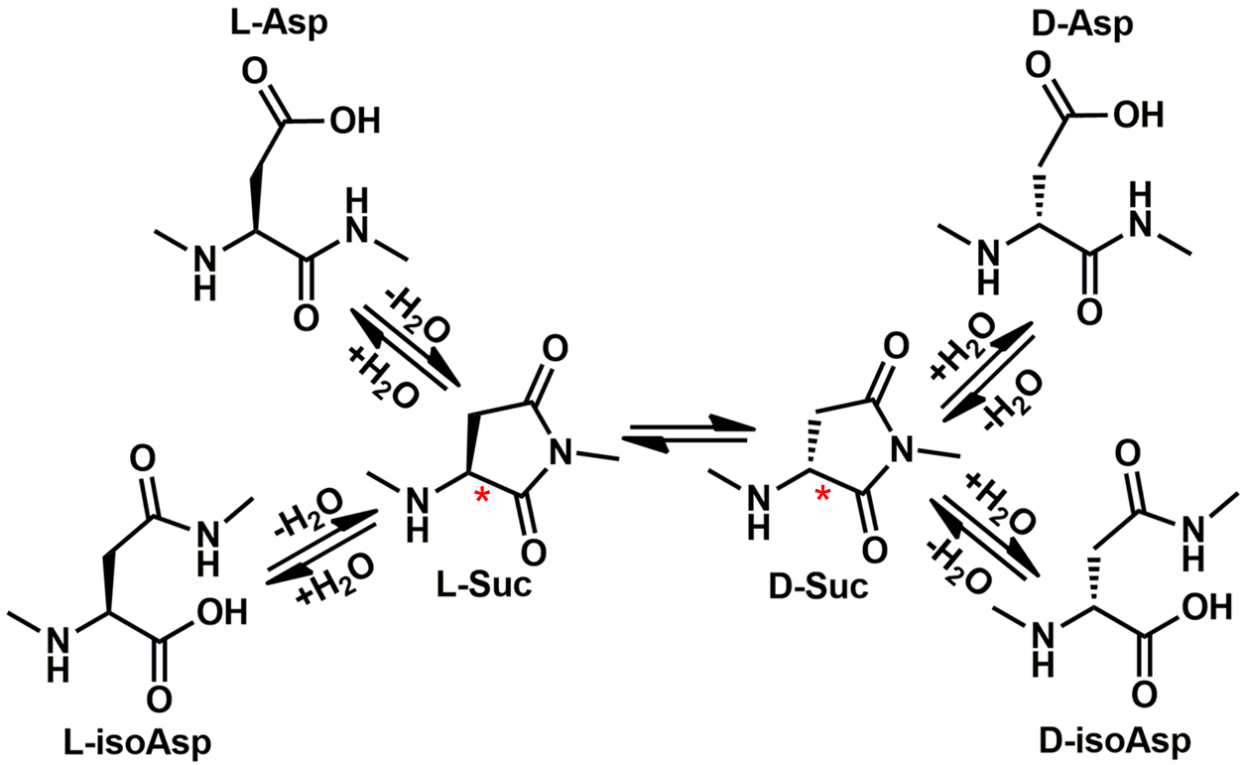
- PIMT is a repair enzyme that methylates L-isoAsp > D-Asp
  - No activity toward D-isoAsp

PIMT is a crucial protective enzyme

The loss of PIMT is rapidly fatal in knockout mice.



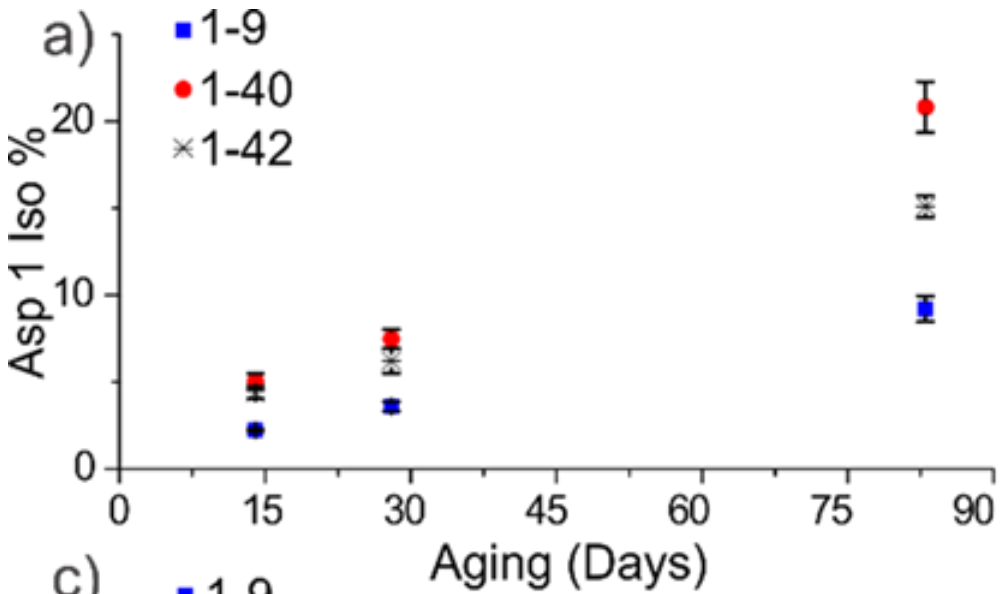
Kim, et al. *PNAS* **1997**, *94*, 6132

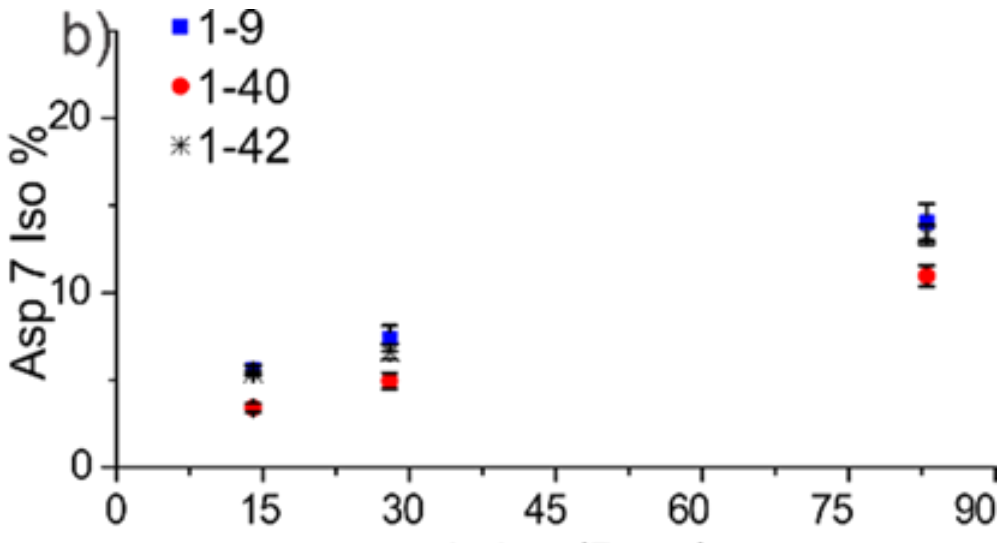


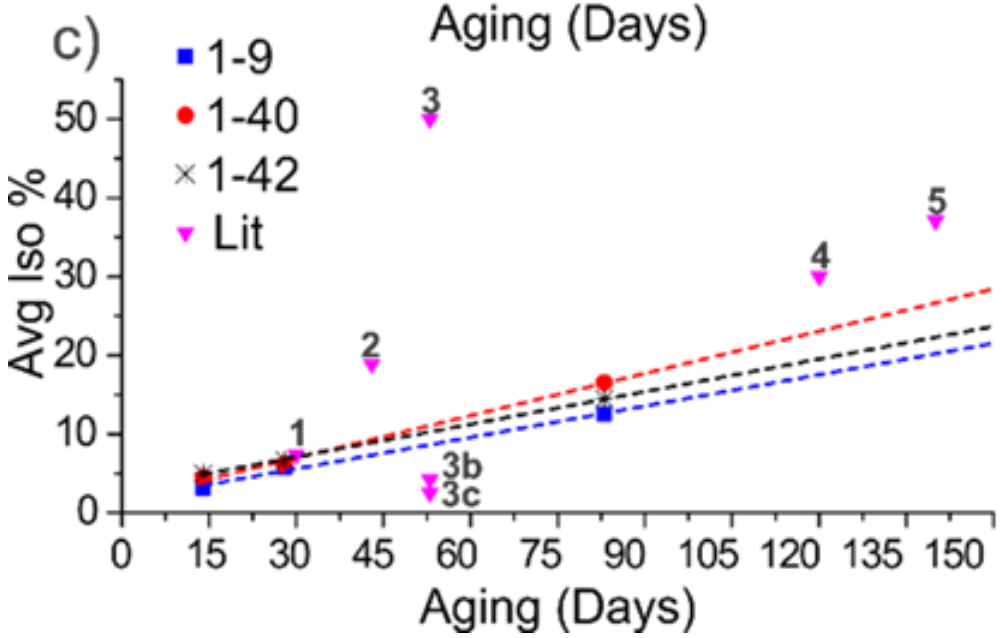
The mechanism of action is rather inefficient

Lowenson et. al. *J. Biol. Chem.* **1991**, *266*, 19396.

Isomerization rates are pretty fast

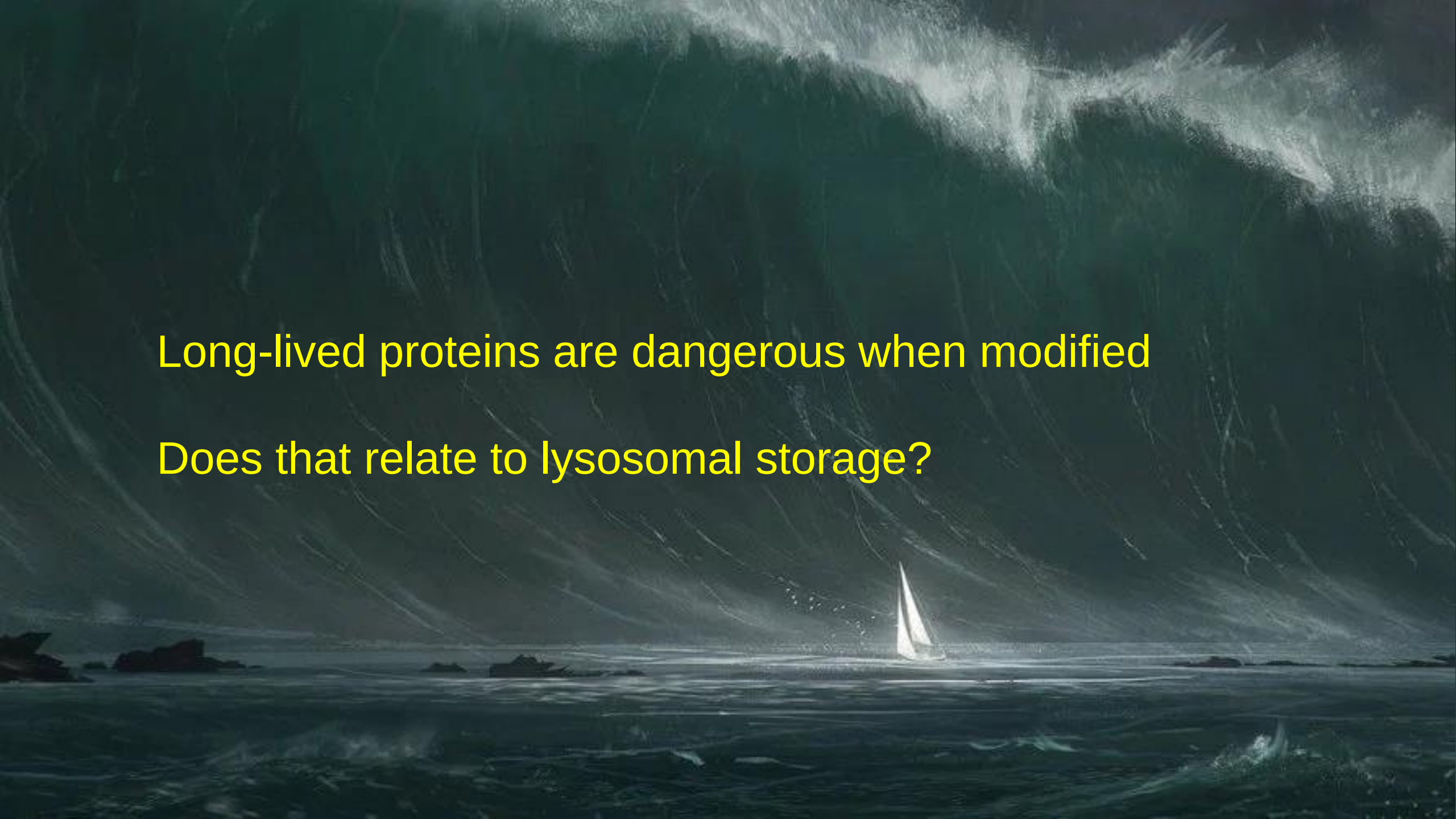






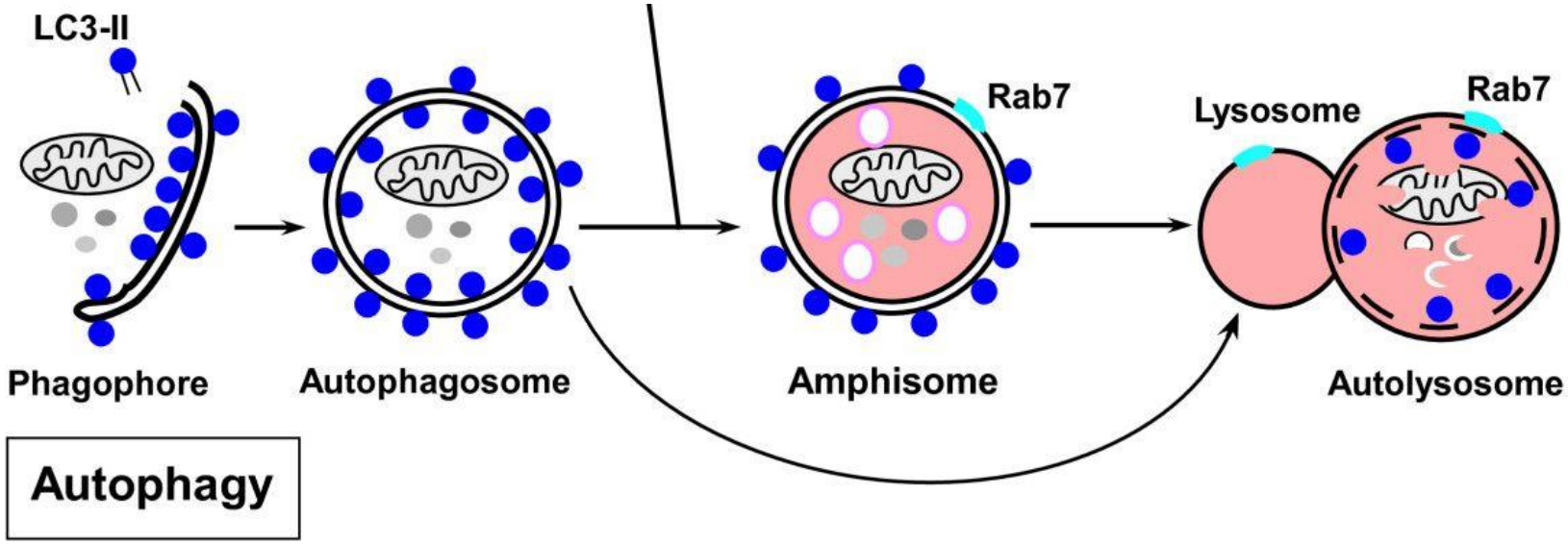
Asp isomerization rates have not been extensively studied, but all known values suggest accumulation within a timeframe of weeks.

Julian, *ACS Cent. Sci.* **2019**, 1387


Long-lived proteins are dangerous when modified
Does that relate to lysosomal storage?

Autophagy and Lysosomes

To eat one's self



Hansen et al *BMC Biol.* **2011**, 9, 39

Autophagy and Lysosomes

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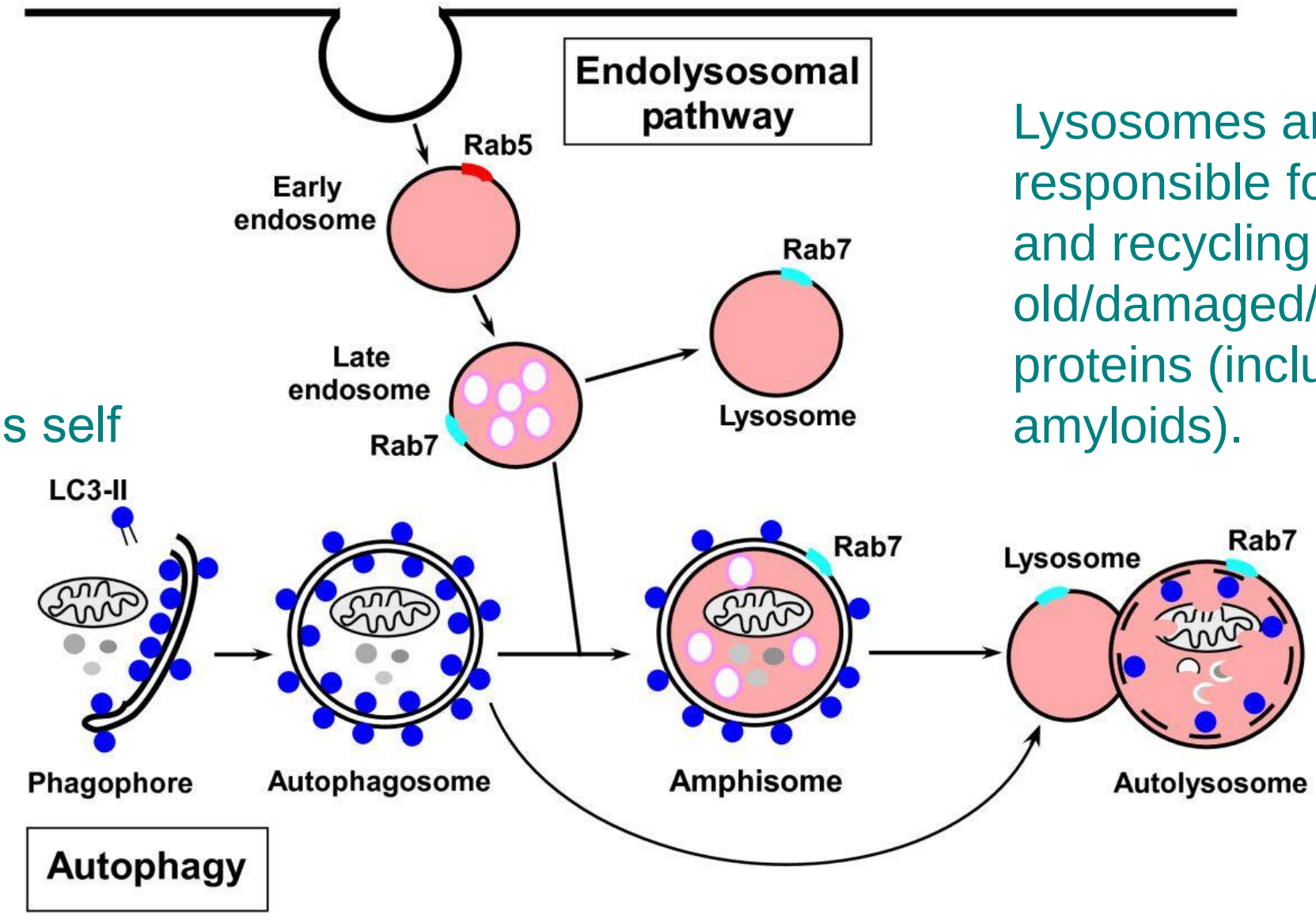
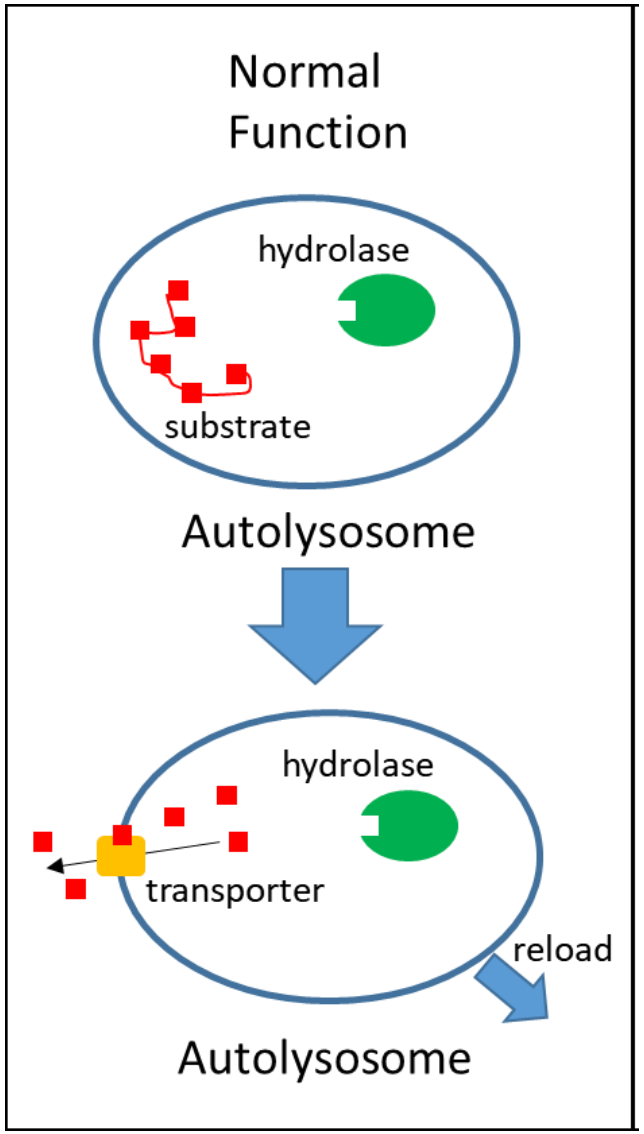


Lysosomes are responsible for digesting and recycling old/damaged/misfolded proteins (including amyloids).

Hansen et al *BMC Biol.* **2011**, 9, 39

Lysosomal storage disorders (LSDs)

Lysosomes normally breakdown proteins into amino acids that are then transported out for making new proteins.



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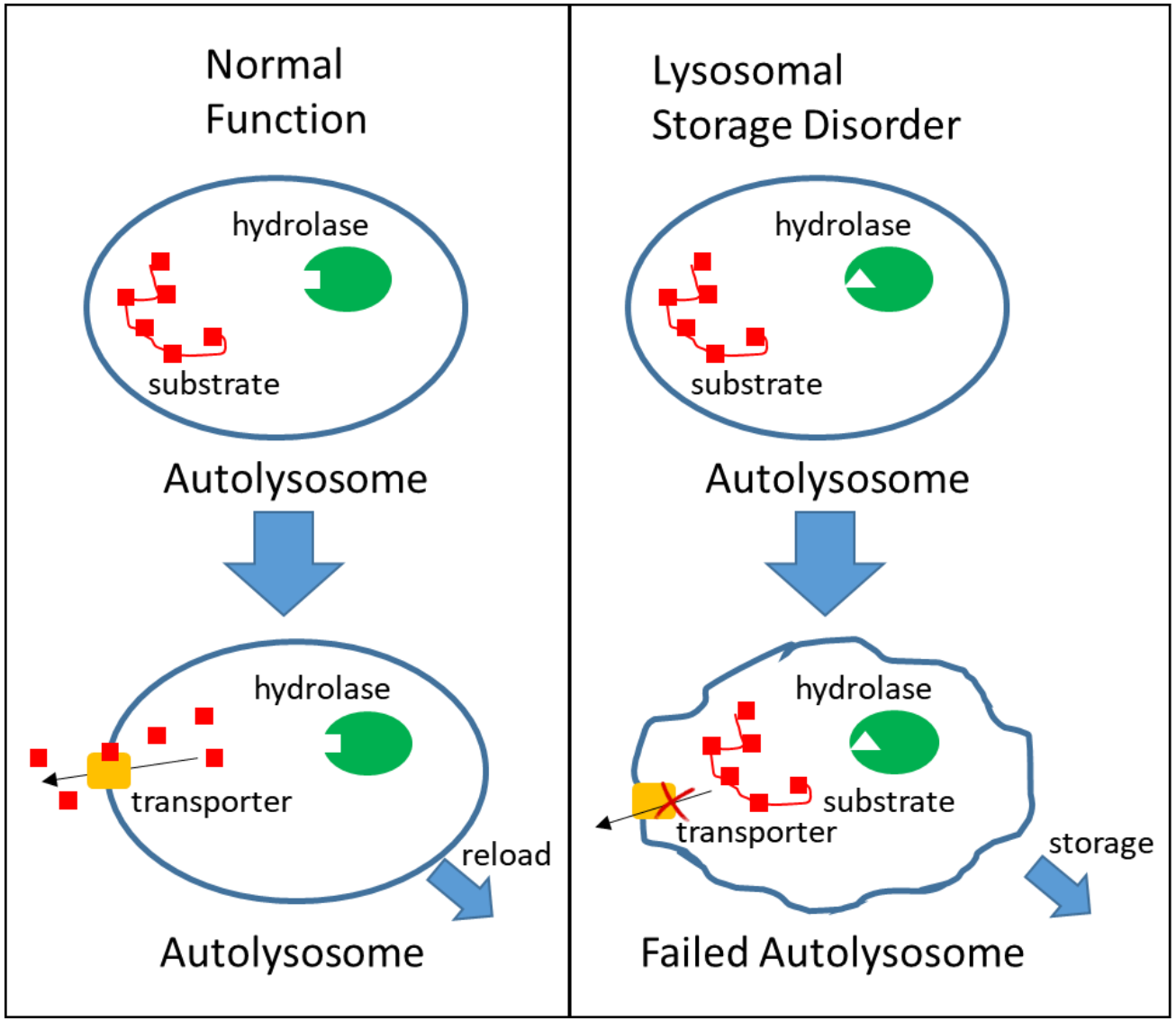
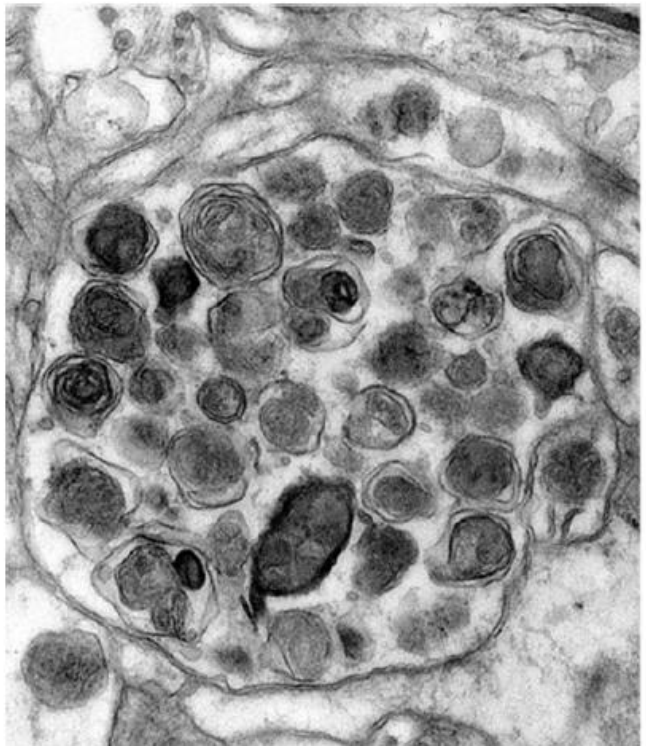
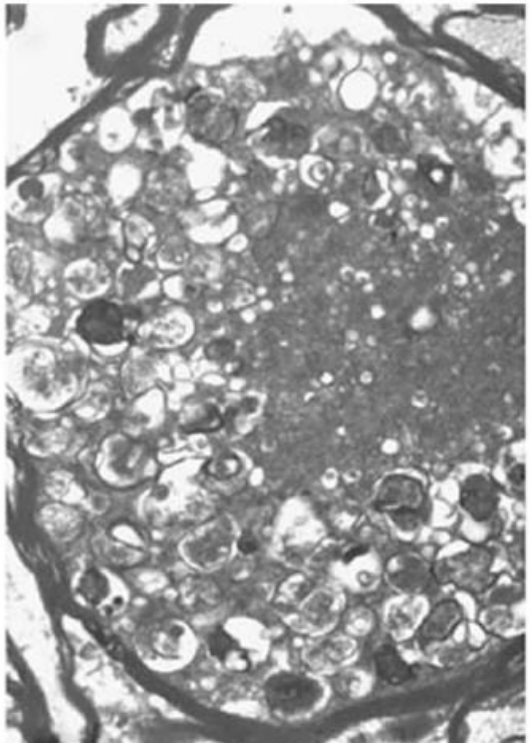


In LSD, genetic mutation incapacitates a hydrolase or transporter, preventing processing of the substrate or export. Eventually the failed autolysosome is ‘stored’.

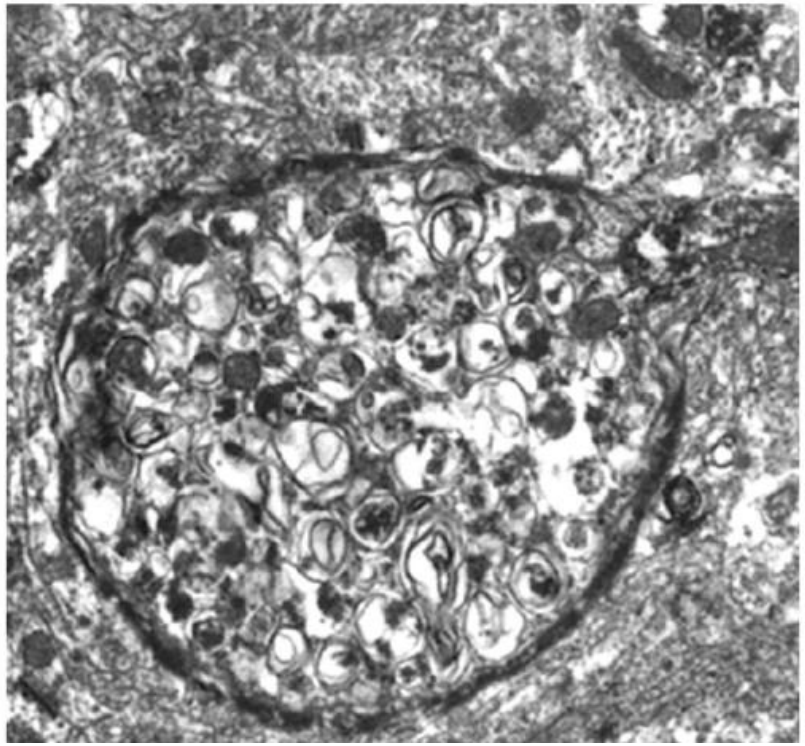
Lysosomal storage



Alzheimer's Disease



Niemann Pick C

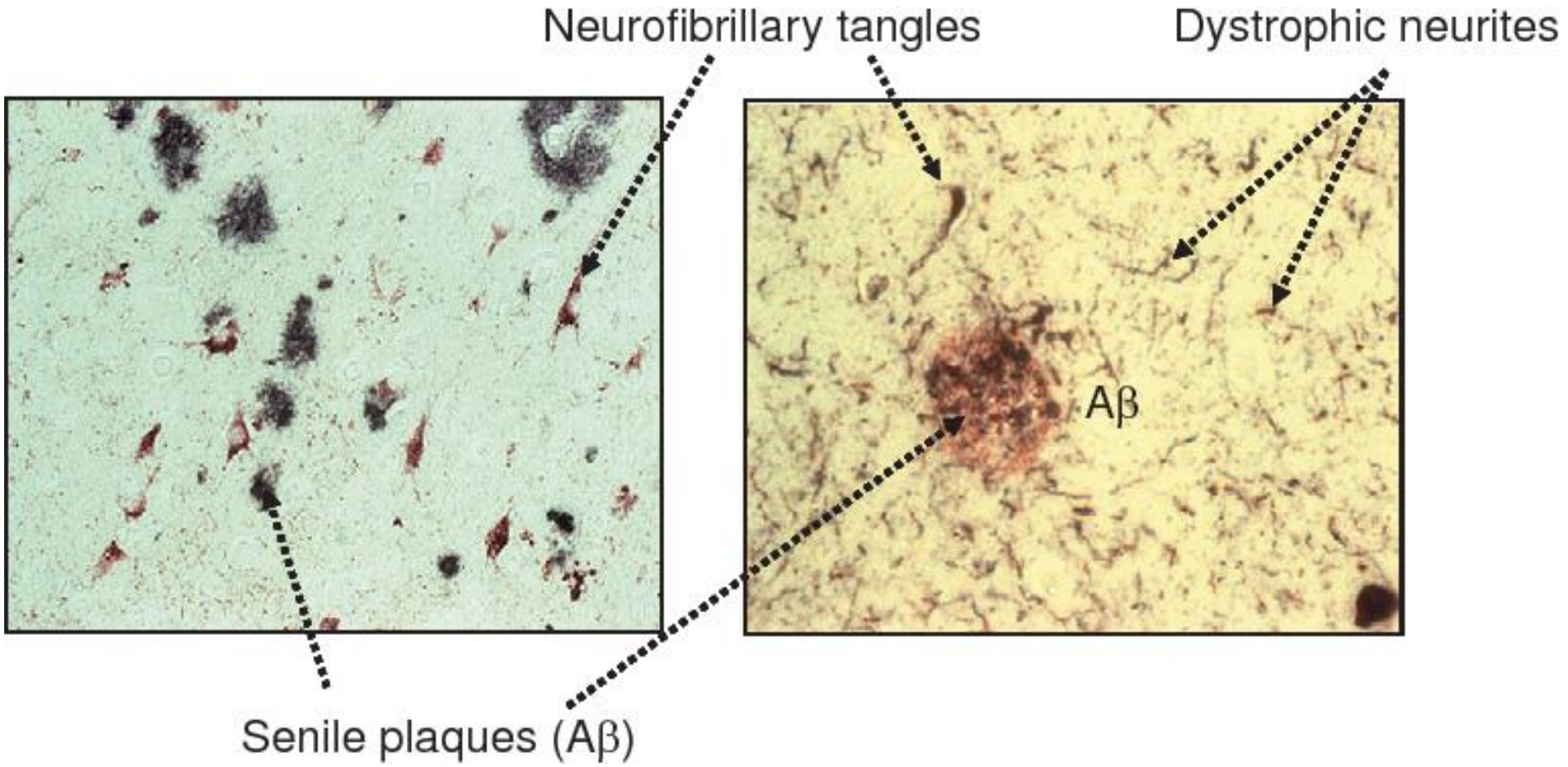


Alpha-mannosidosis

Dystrophic neurite cross-sections showing accumulation of lysosomal intermediates that actually occur prior to observation of amyloid deposits. This storage is ubiquitous in AD and lysosomal storage disorders (LSDs).

Nixon and coworkers, Chpt 10 in *Autophagy of the Nervous System*

AD Basic Observations



- We know that tau and Aβ are strongly associated with AD

Skaper, S. D. International Review of Neurobiology; 2012; pp 277–316.

What is the frequency of iso/epi sites in LLP's?

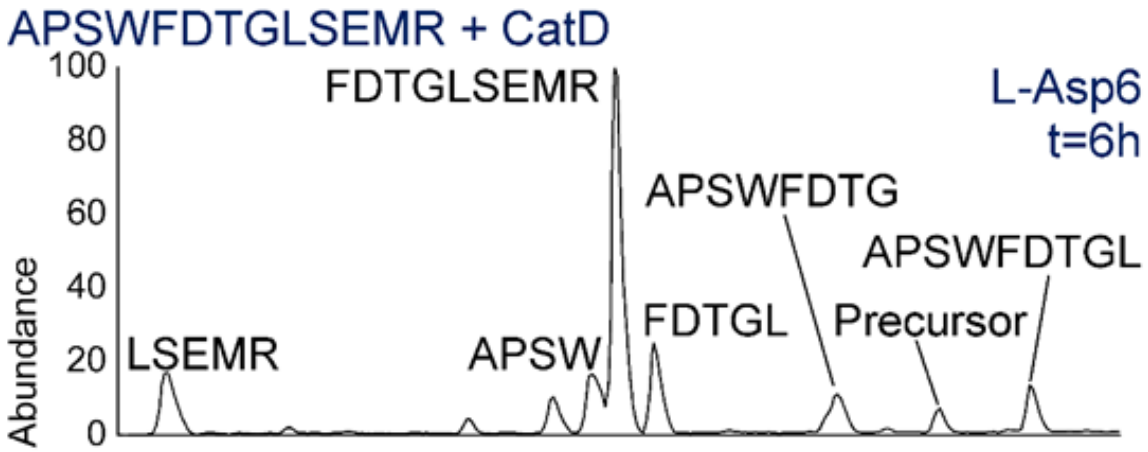
Human Aβ

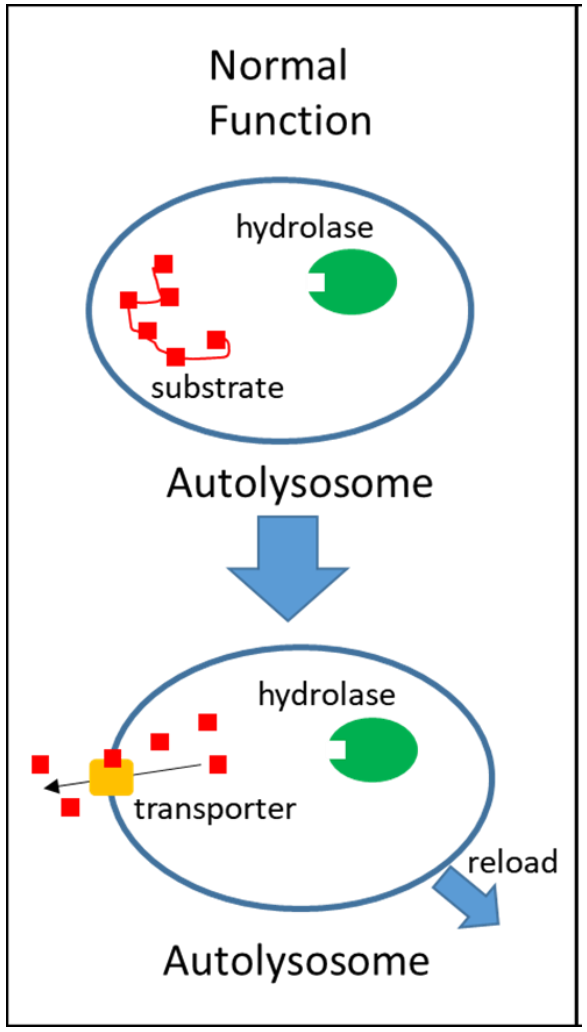
DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA

Human Tau

MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
HVTQEPESGK VVQEGFLREP GPPGLSHQLM SGMPGAPLLP EGPREATRQP SGTGPEDTEG
GRHAPELLKH QLLGDLHQEG PPLKGAGGKE RPGSKEEVDE DRDVDESSPQ DSPPSKASPA
QDGRPPQTAA REATSIPGFP AEGAIPLPVD FLSKVSTEIP ASEPDGPSVG RAKGQDAPLE
FTFHVEITPN VQKEQAHSEE HLGRAAFPGA PGEGPEARGP SLGEDTKEAD LPEPSEKQPA
AAPRGKPVSR VPQLKARMVS KSKDGTGSDD KKAKTSTRSS AKTLKNRPCL SPKHPTPGSS
DPLIQPSSPA VCPEPPSSPK YVSSVTSRTG SSGAKEMKLK GADGKTKIAT PRGAAPPGQK
GQANATRIPA KTPPAPKTPP SSGEPPKSGD RSGYSSPGSP GTPGSRSRTP SLPTPPTREP
KKVAVVRTPP KSPSSAKSRL QTAPVPMPDL KNVKSKIGST ENLKHQPGGG KVQIINKKLD
LSNVQSKCGS KDNIKHVPGG GSVQIVYKPV DLSKVTSKCG SLGNIHHKPG GGQVEVKSEK
LDFKDRVQSK IGSLDNITHV PGGGNKKIET HKLTFRENAK AKTDHGAEIV YKSPVVSGDT
SPRHLSNVSS TGSIDMVDSP QLATLADEVS ASLAKQGL

How do lysosomal proteases handle iso/epi mods?

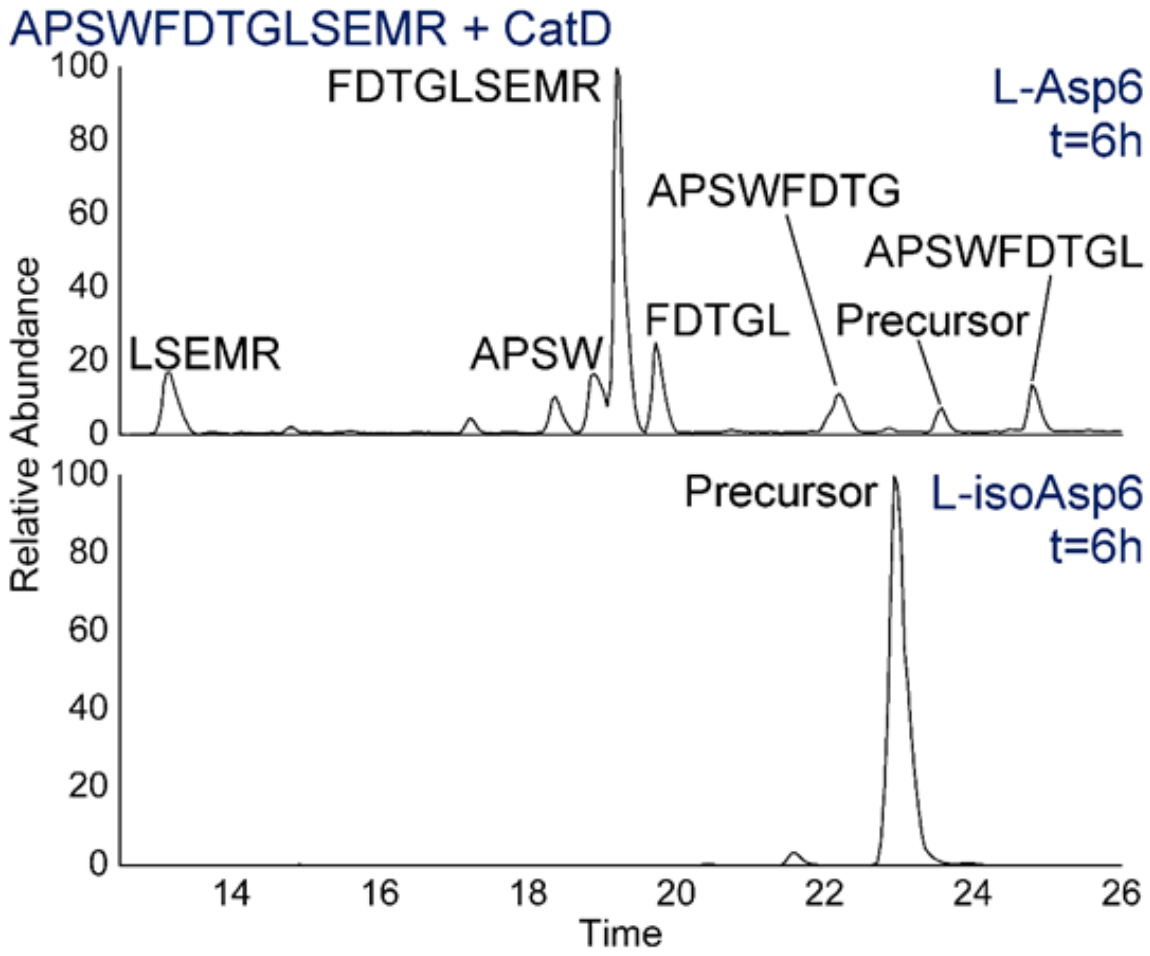


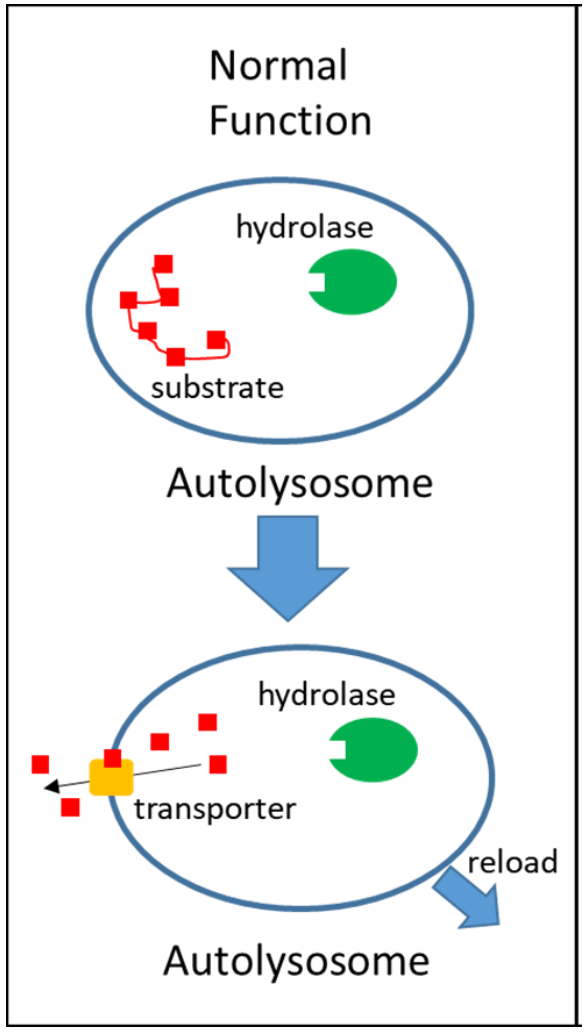


Cathepsin D is the most abundant lysosomal protease. It is an aspartic endopeptidase with cleavage preference at hydrophobic residues.

Julian, *ACS Cent. Sci*. **2019**, 1387

How do lysosomal proteases handle iso/epi mods?





Cathepsin D is the most abundant lysosomal protease. It is an aspartic endopeptidase with cleavage preference at hydrophobic residues.

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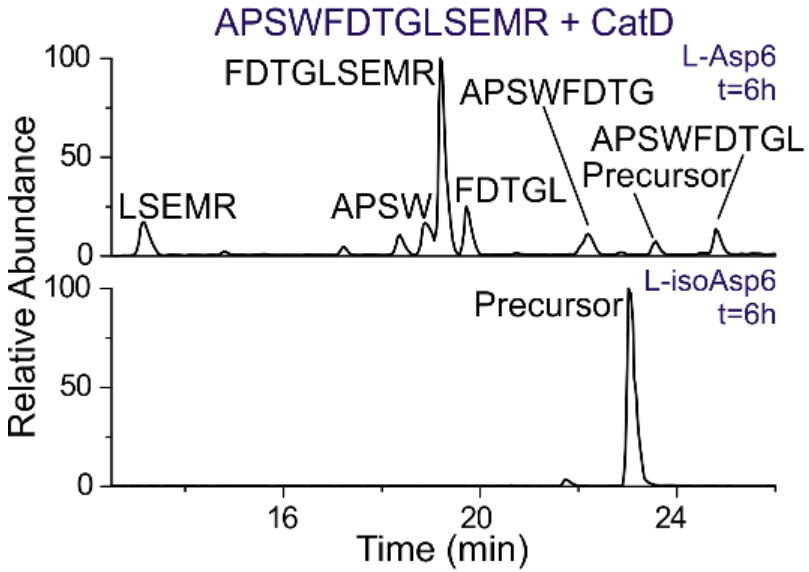
Cathepsins cannot digest near iso/epi mods



CatD Digestion Sites

L-Form	APSWFDTGLSEMR
L-isoAsp	APSWFDTGLSEMR
D-Asp	APSWFDTGLSEMR
D-isoAsp	APSWFDTGLSEMR
D-Ser3	APSWFDTGLSEMR
D-Ser10	APSWFDTGLSEMR

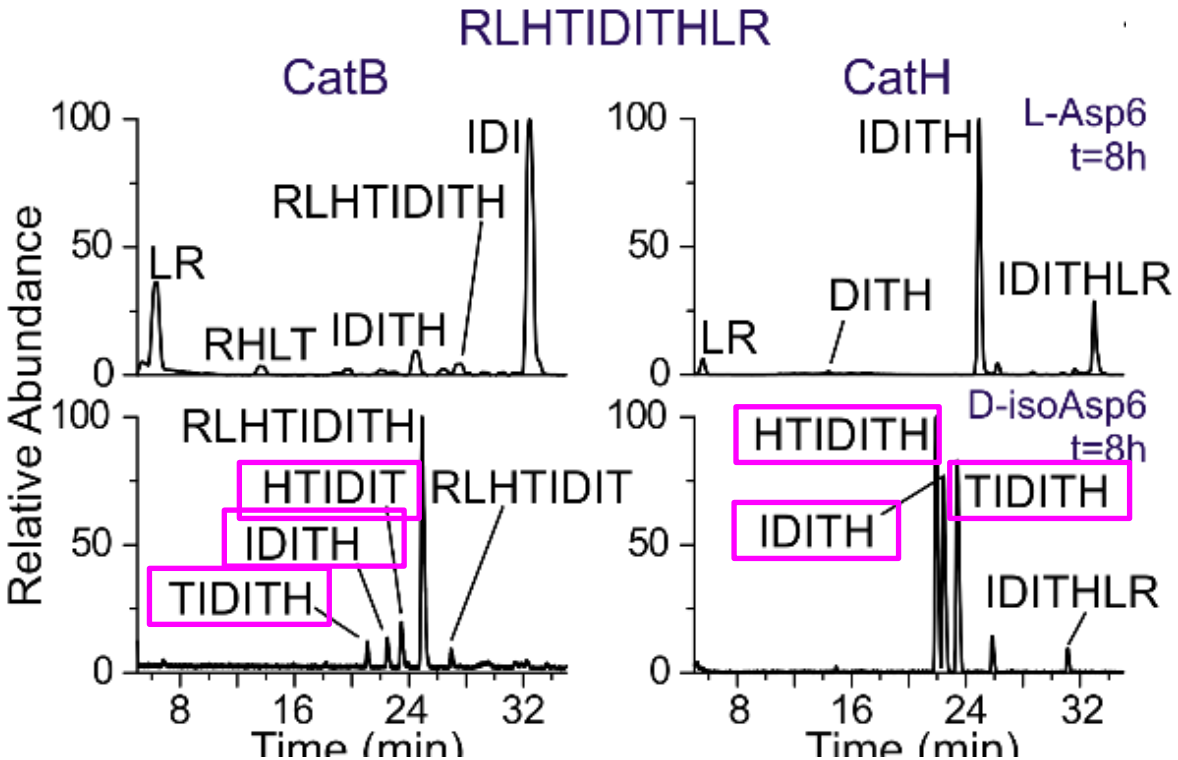
CatL Digestion Sites

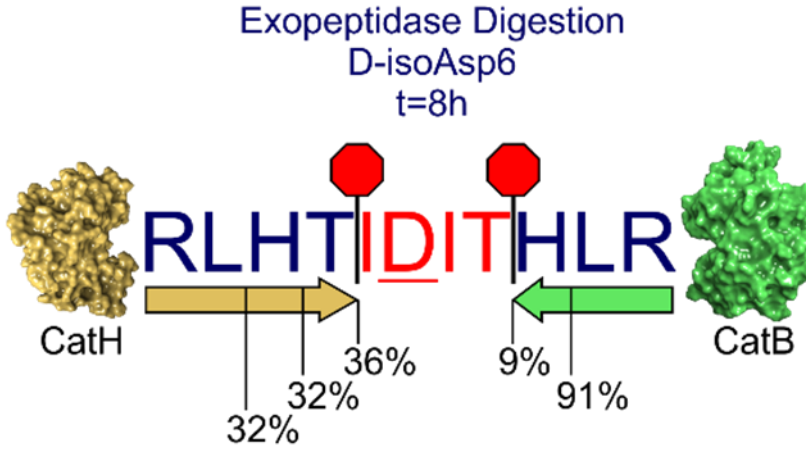
L-Form	APSWFDTGLSEMR
L-isoAsp	APSWFDTGLSEMR
D-Asp	APSWFDTGLSEMR
D-isoAsp	APSWFDTGLSEMR
D-Ser3	APSWFDTGLSEMR
D-Ser10	APSWFDTGLSEMR

Digestion by cathepsins D and L is severely impacted by iso/epi modifications at either Asp or Ser residues.

Julian ACS Cent. Sci. **2019**, 1387

What is the effect on exopeptidases?

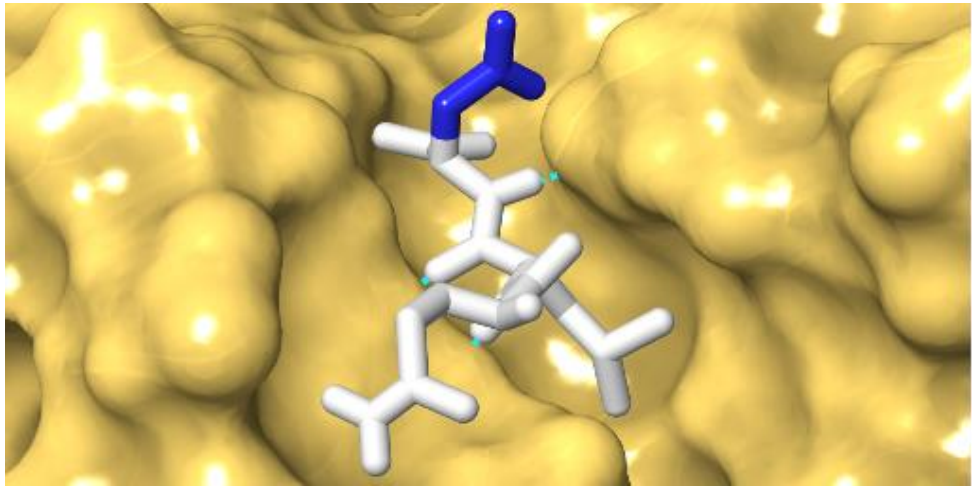


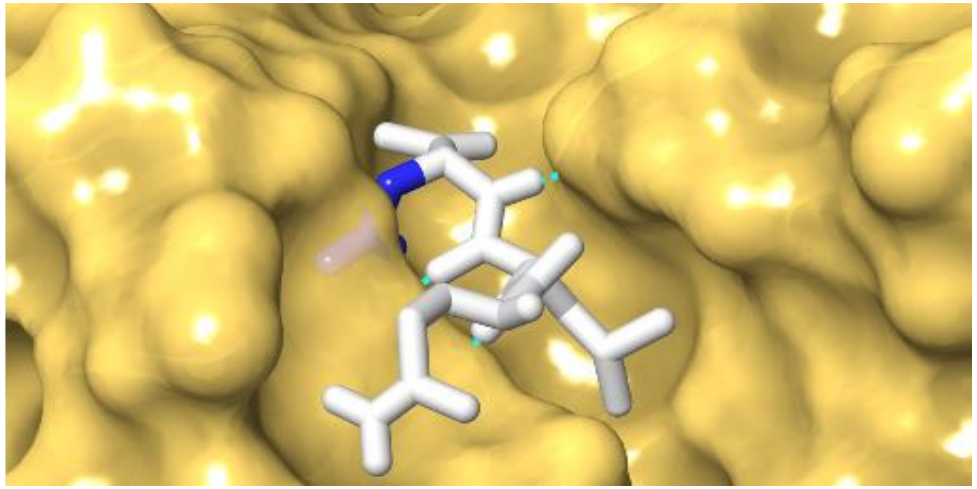


Exopeptidases digest from the termini, but are also unable to penetrate near iso/epi modified sites.

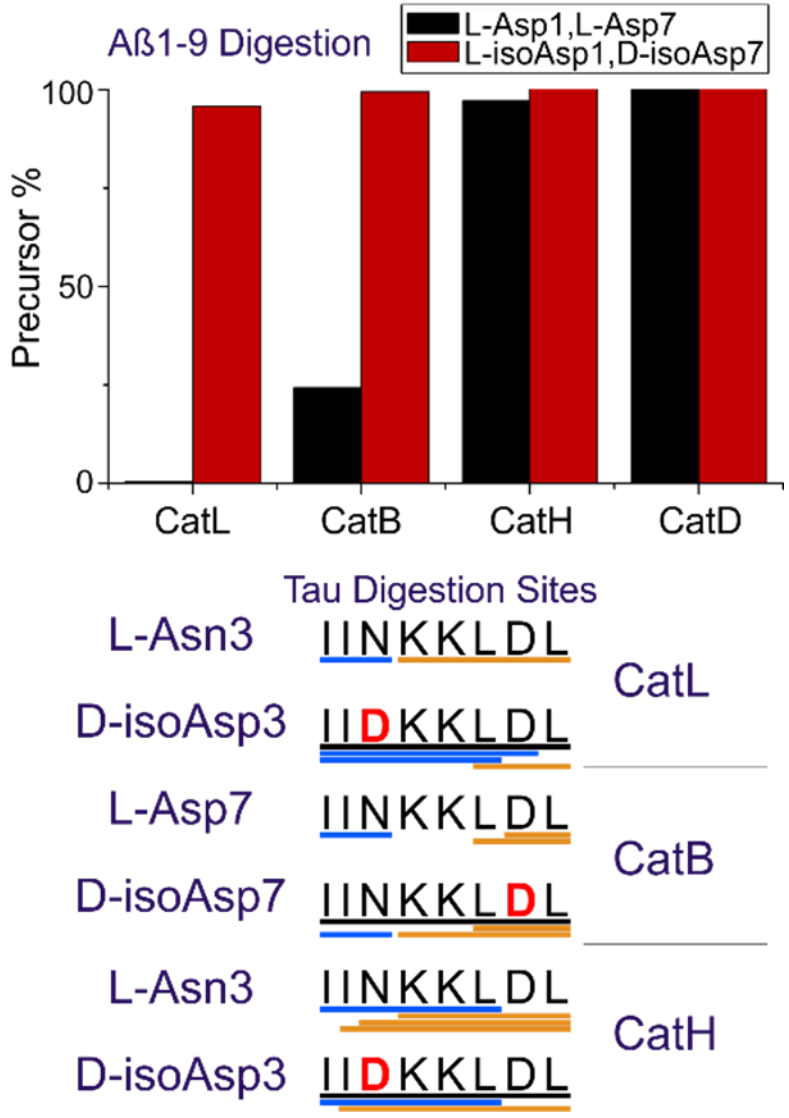
Julian, *ACS Cent. Sci*. **2019**, 1387

Similar results are found in brain proteins





These results are easily explained by examination of protease active sites (CatL shown above).



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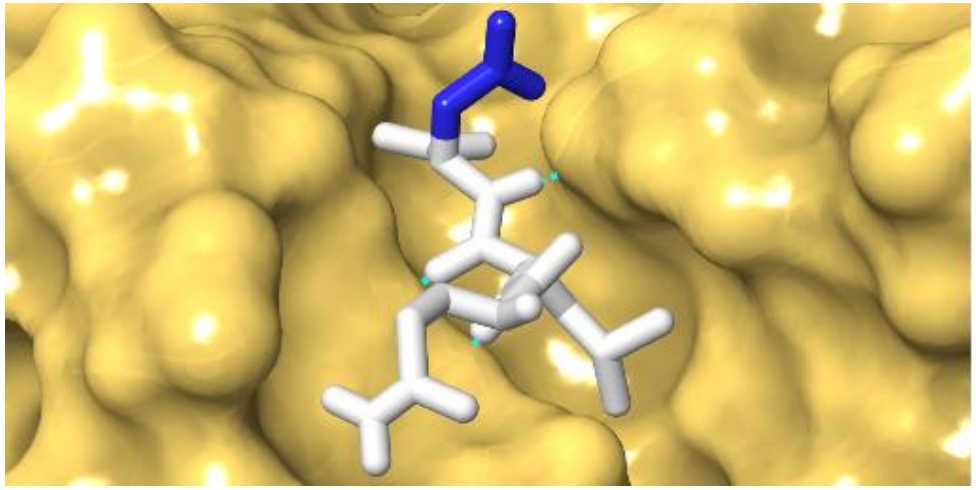

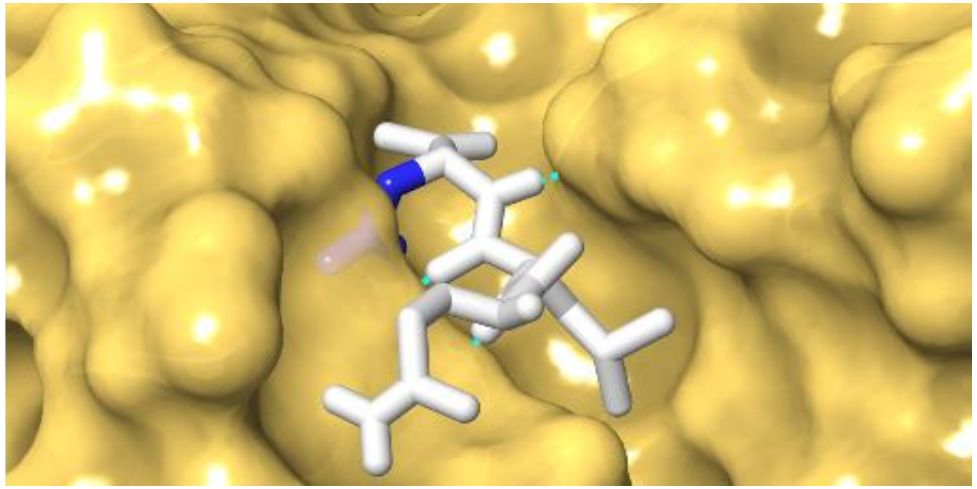

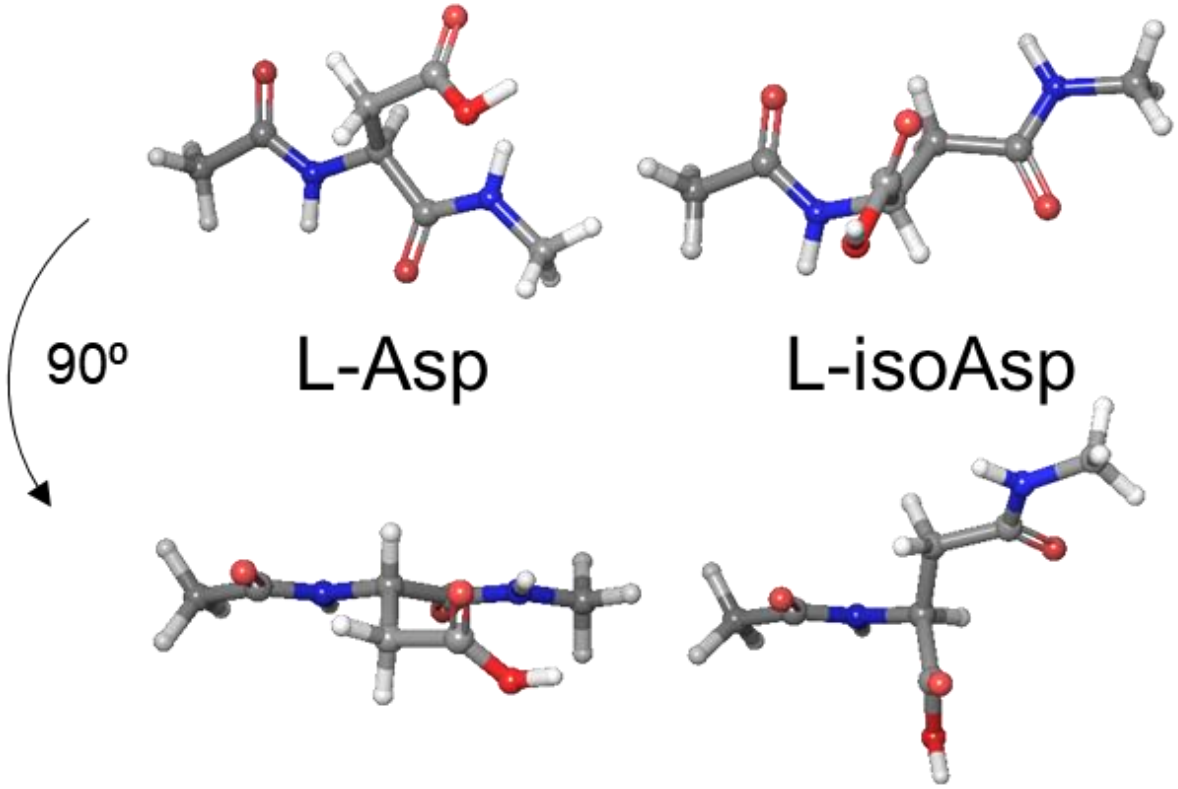

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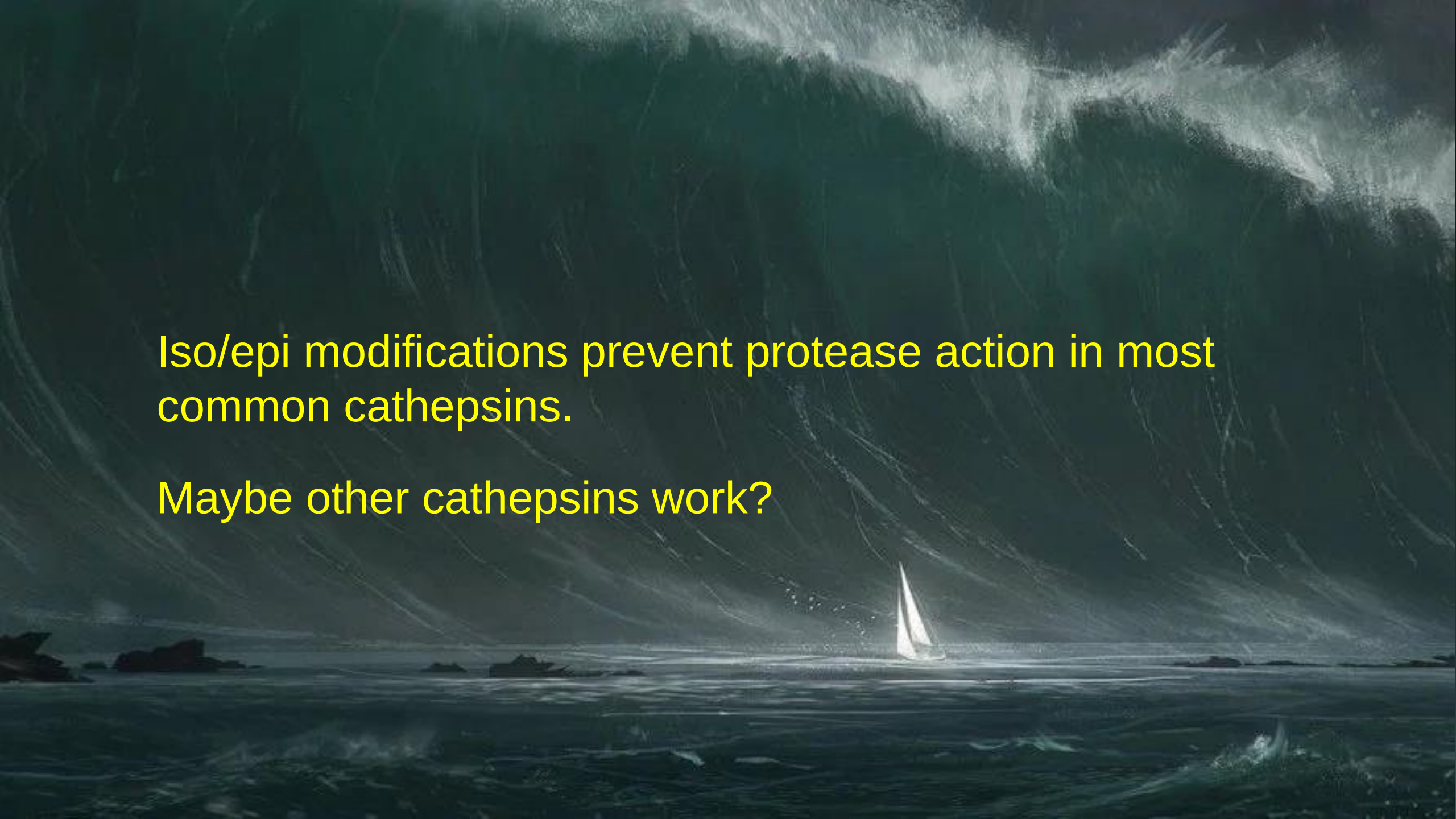
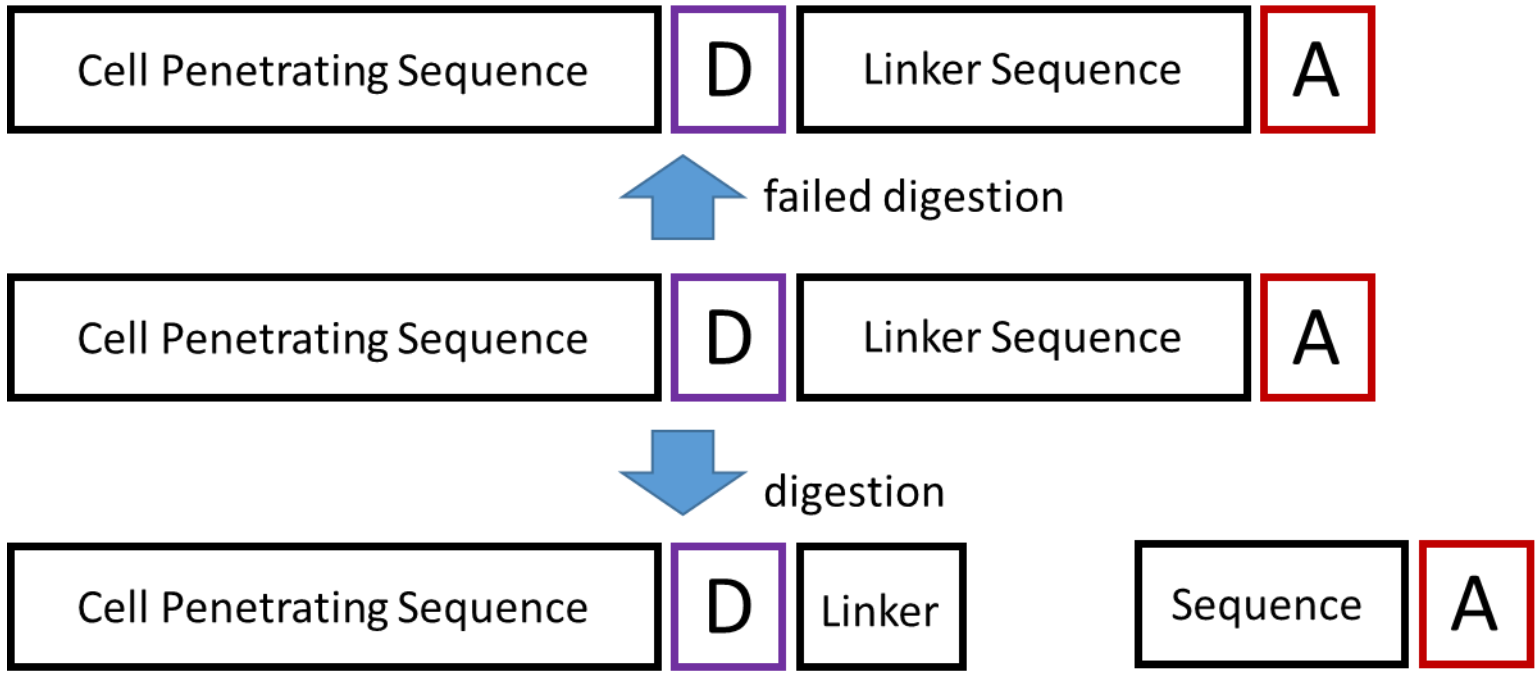

Iso/epi modifications prevent protease action in most common cathepsins.
Maybe other cathepsins work?

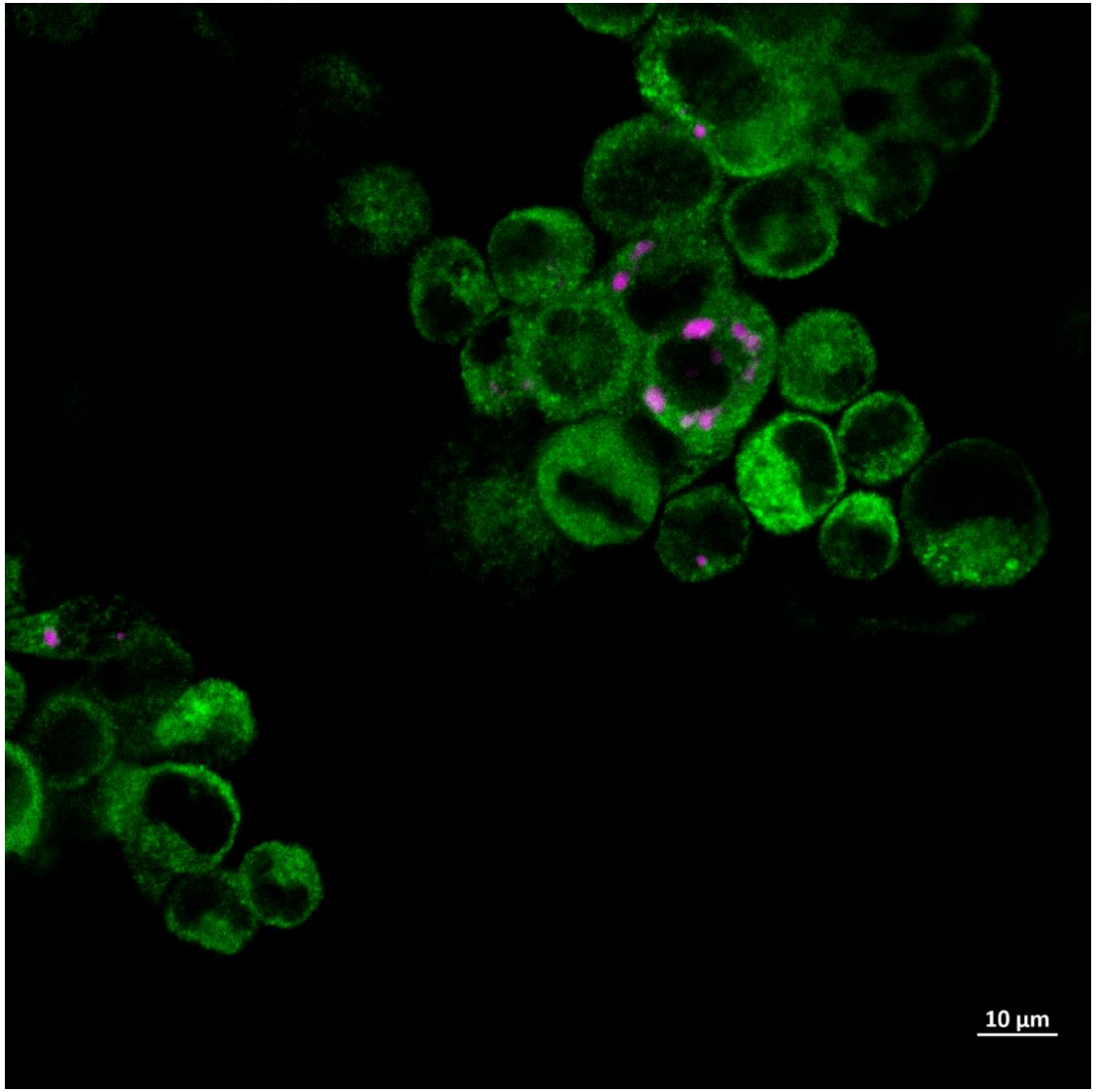
Experiments with living cells



We designed a cell-penetrating peptide that will fluoresce when a linker sequence is cleaved.

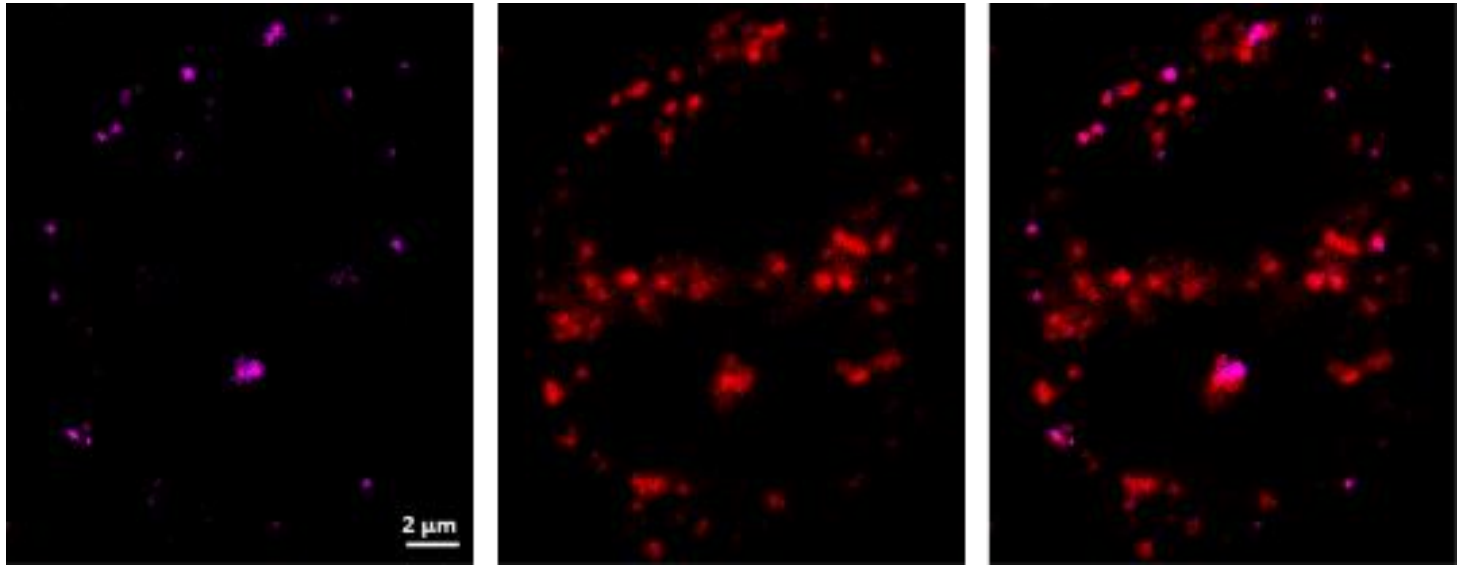
Experiments with living cells

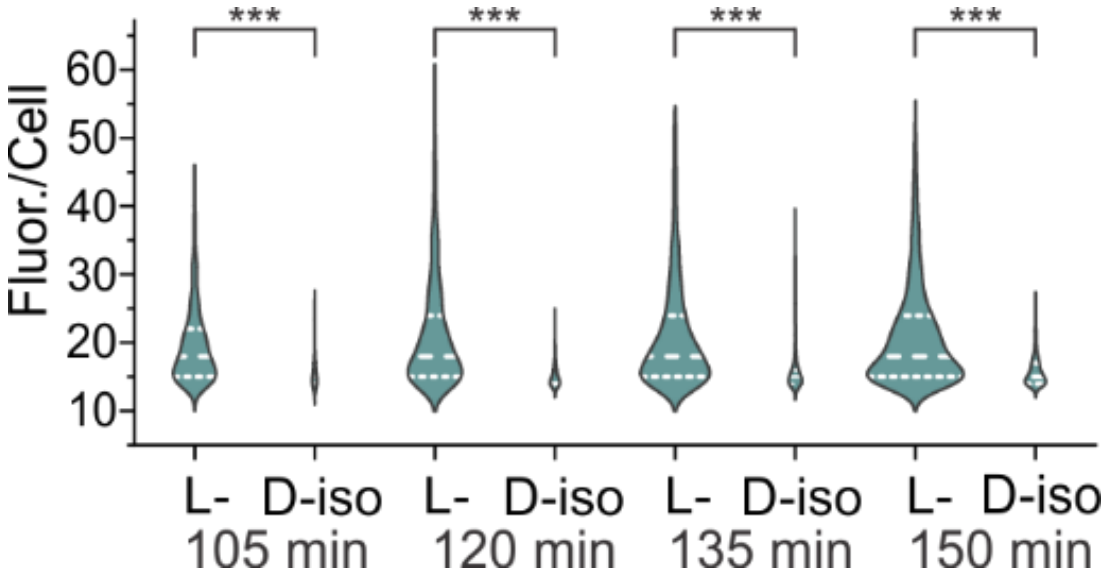
SH-SY5Y microglial cells with punctate fluorescence, consistent with delivery into endo-lysosomal pathway.



Experiments with living cells

Overlap with lysotracker confirms delivery to lysosomes.





Violin plot of relative digestion of modified vs canonical peptide.

Julian, *ACS Cent. Sci*. **2019**, 1387

UDC 576.5

Isomerization of Asp7 Increases the Toxic Effects of Amyloid β and Its Phosphorylated Form in SH-SY5Y Neuroblastoma Cells

E. P. Barykin, I. Yu. Petrushanko, K. M. Burnysheva, A. A. Makarov, and V. A. Mitkevich*

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 119991 Russia

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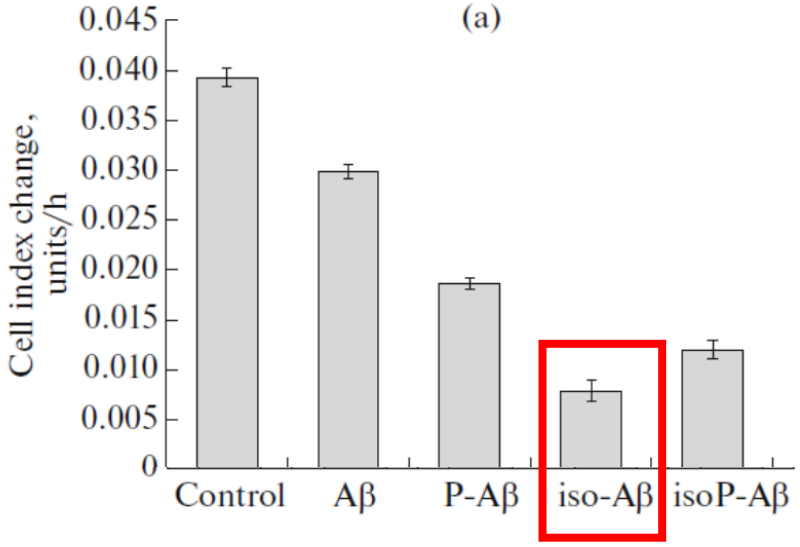
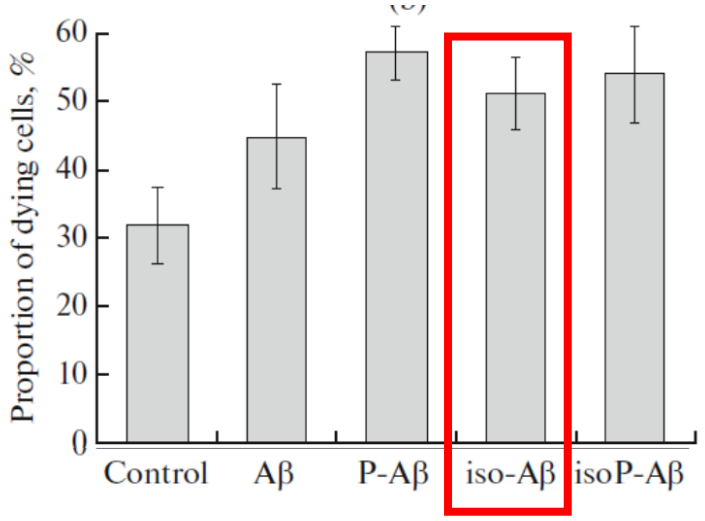
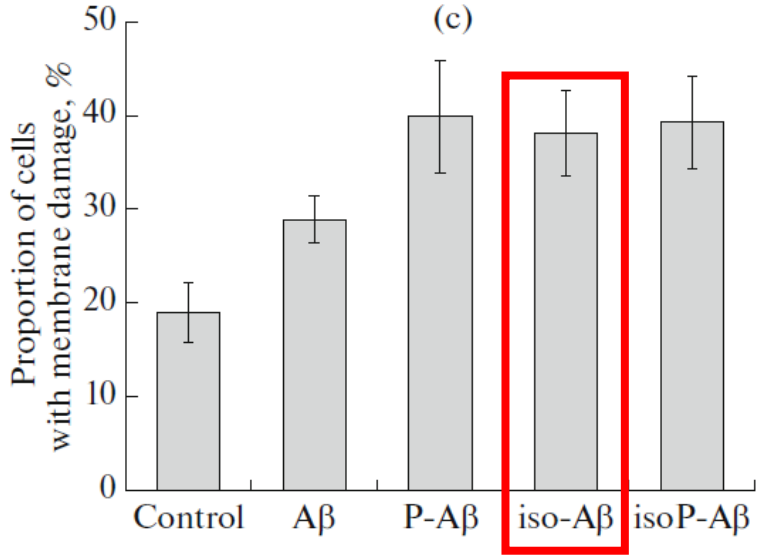
Received February 16, 2016; in final form, February 20, 2016



Cell growth.



Cell death



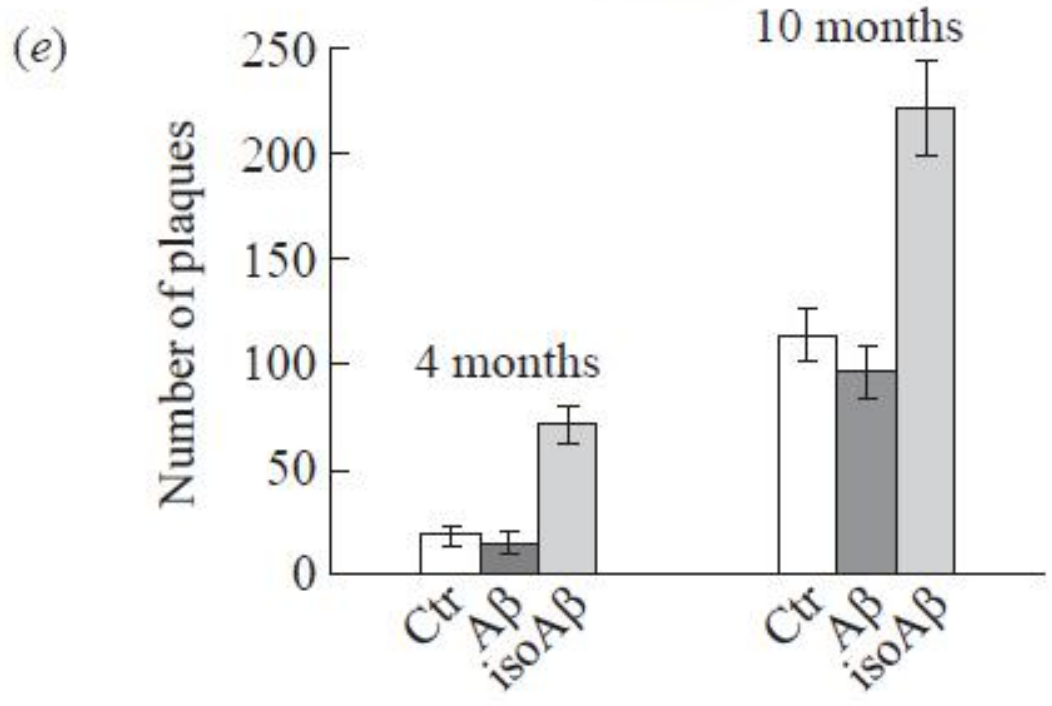
Membrane damage

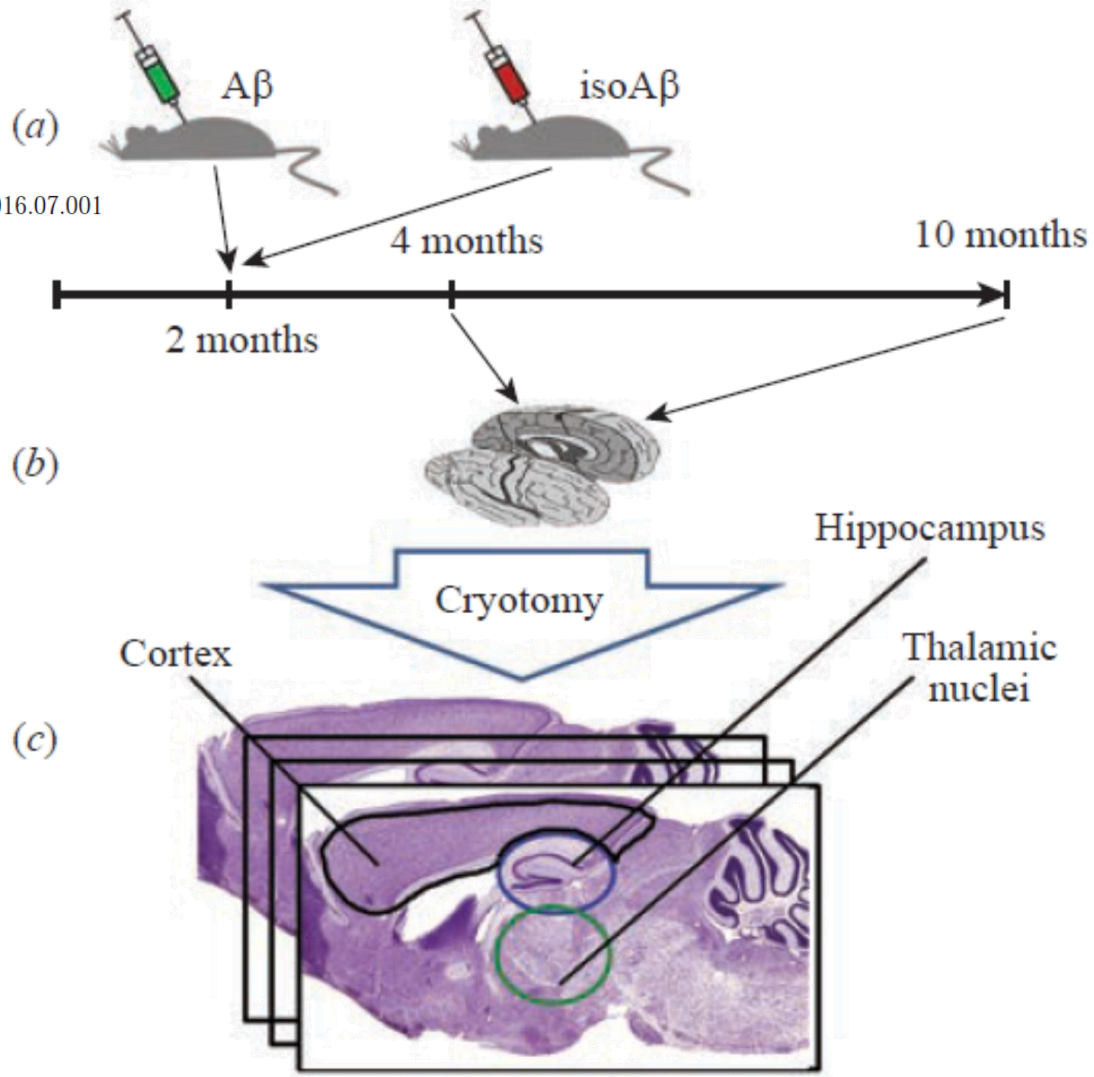
Amyloid-β containing isoaspartate 7 as potential biomarker and drug target in Alzheimer's disease

Sergey A. Kozin, Vladimir A. Mitkevich and Alexander A. Makarov*

V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 1405; e-mail: aamakarov@eimb.ru

DOI: 10.1016/j.mencom.2016.07.001





Full-length $A\beta_{1-42}$

Amyloid-β containing isoaspartate 7 as potential biomarker and drug target in Alzheimer's disease

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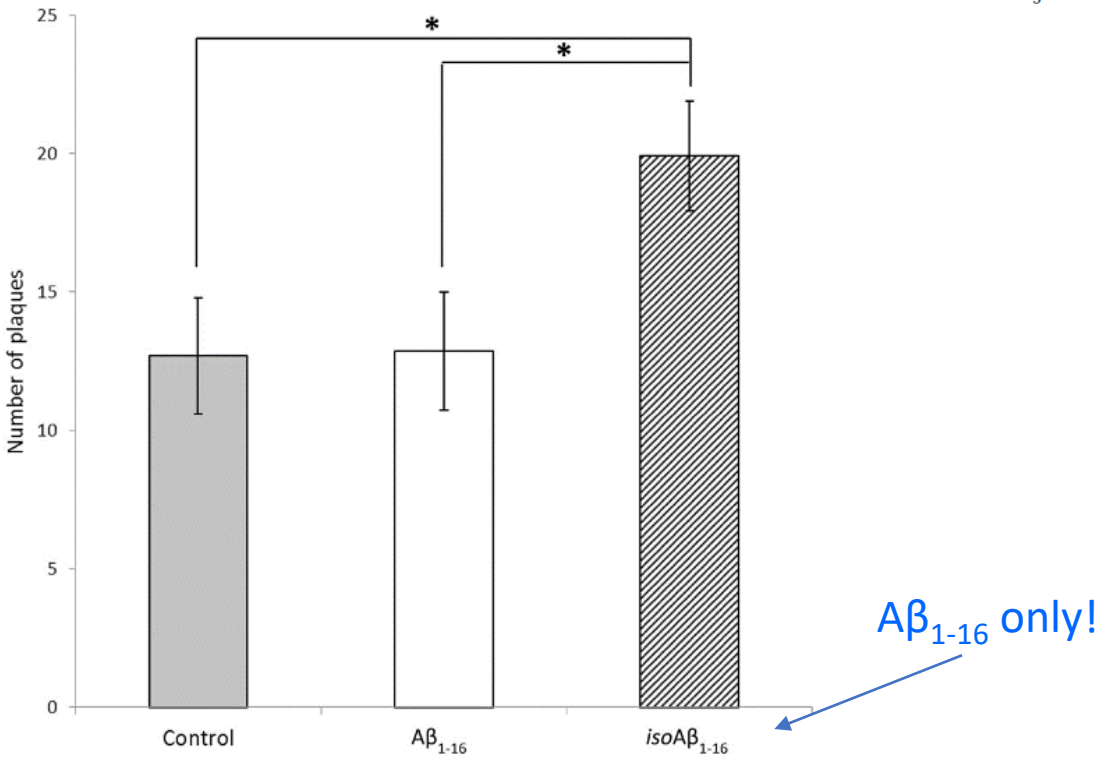
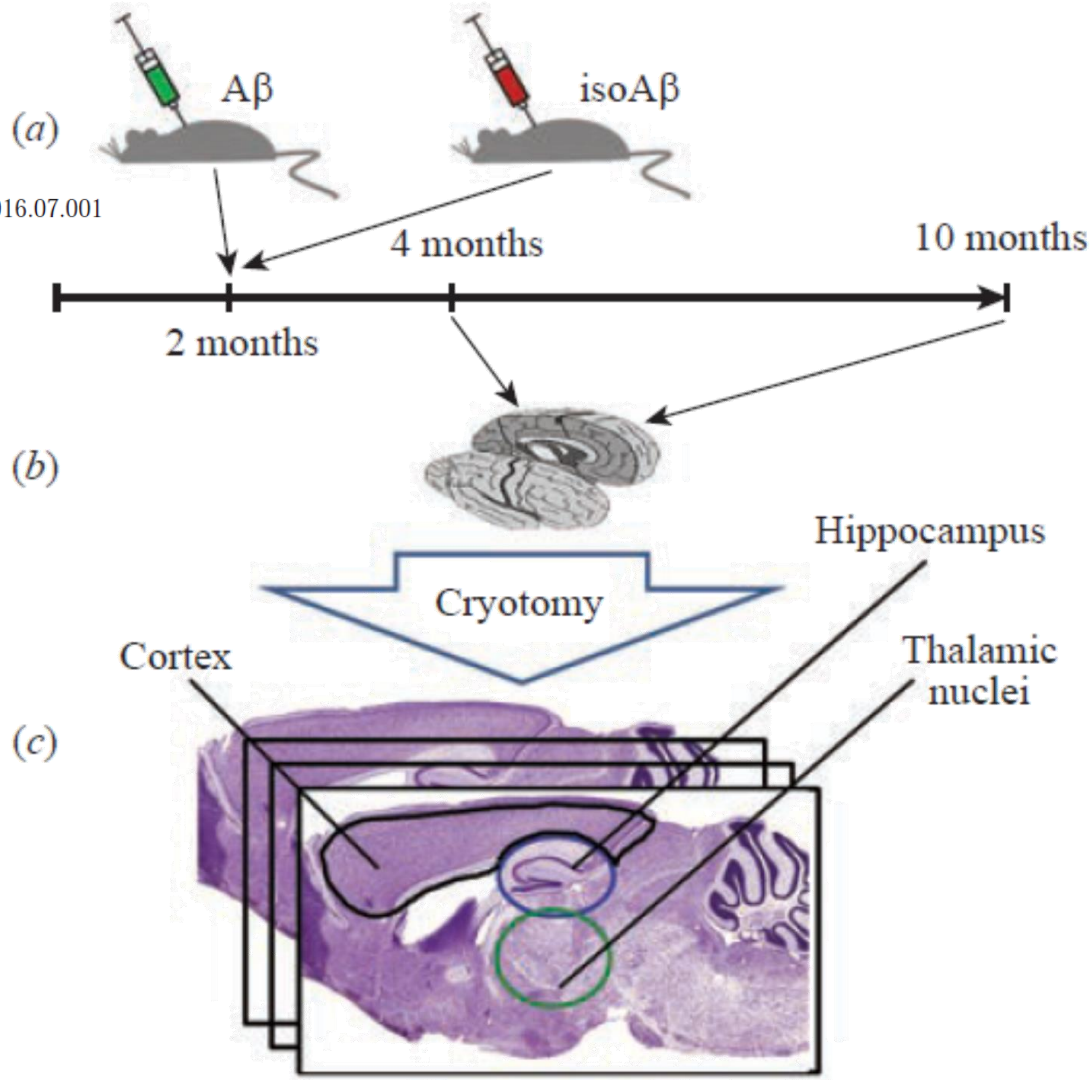
V. A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 499 135 1405; e-mail: aamakarov@eimb.ru

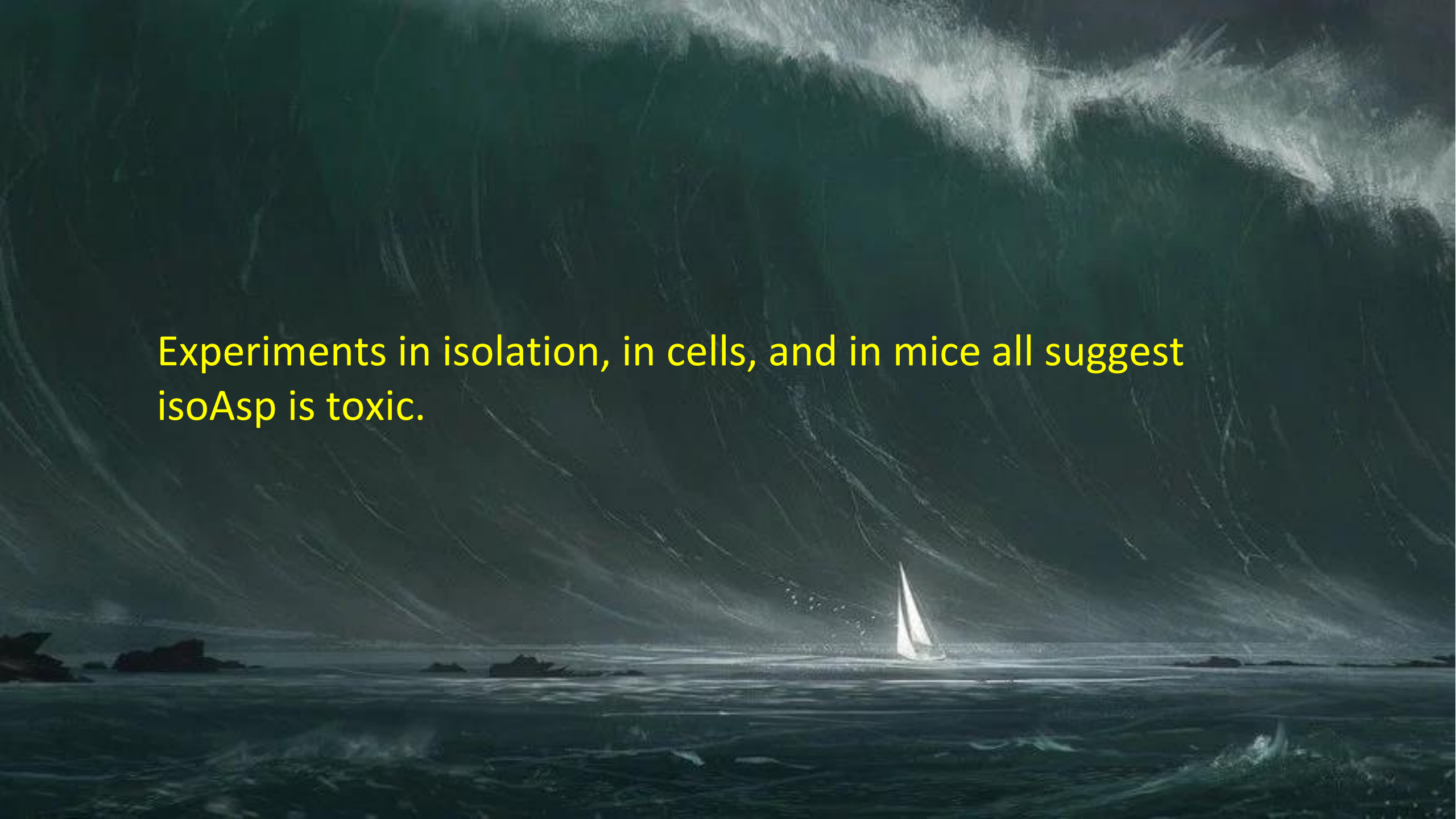
DOI: 10.1016/j.mencom.2016.07.001



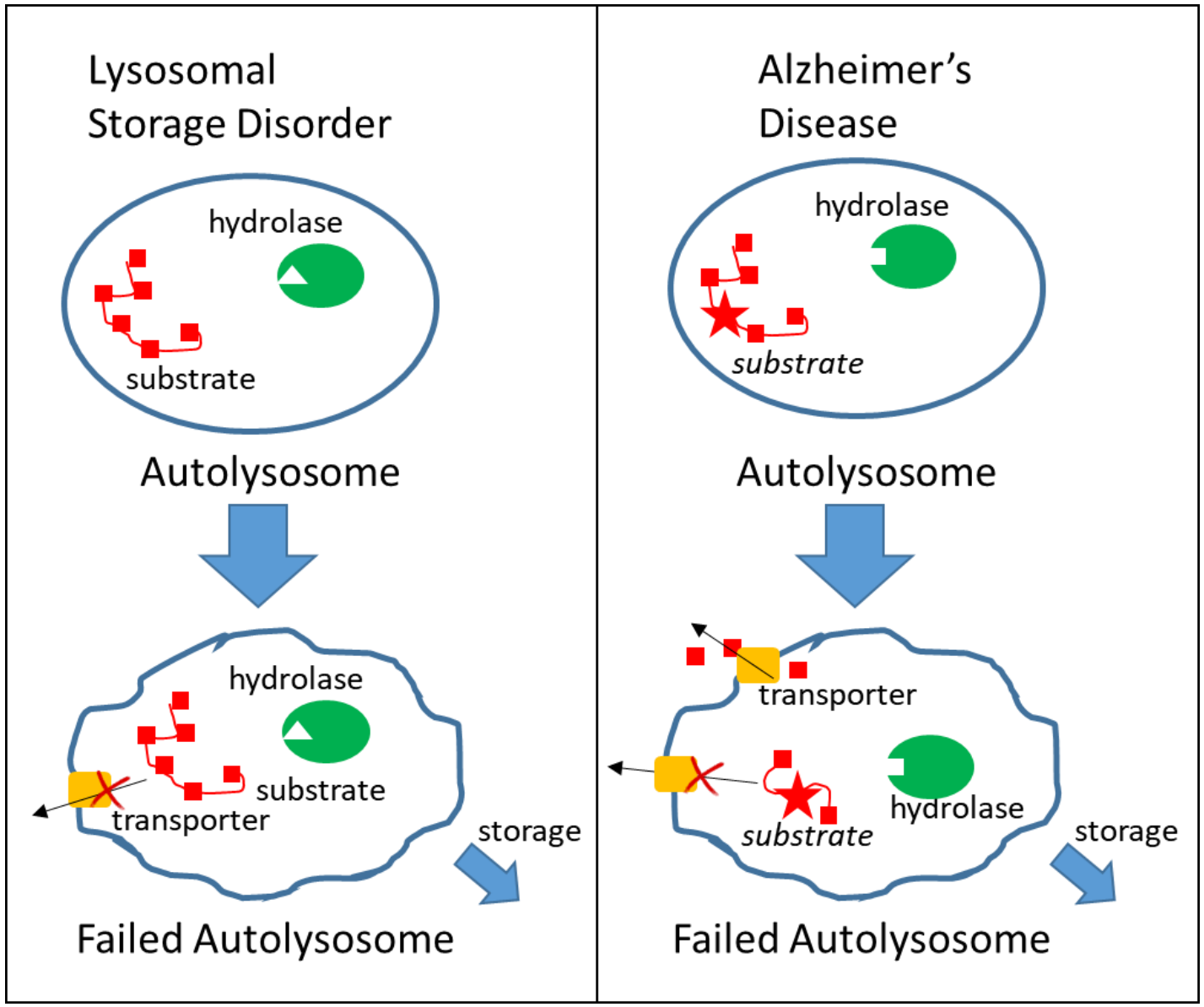
Aβ$_{1-16}$ only!

Fig. 2 Histological quantification of amyloid plaques found in CA1, CA2, and CA3 regions and the dentate gyrus of hippocampus of 4-month-old 5XFAD transgenic mice. *Bar charts* show (as mean ± SEM) number of plaques per section in the PBS-injected




Experiments in isolation, in cells, and in mice all suggest isoAsp is toxic.

What causes lysosomal failure in AD?



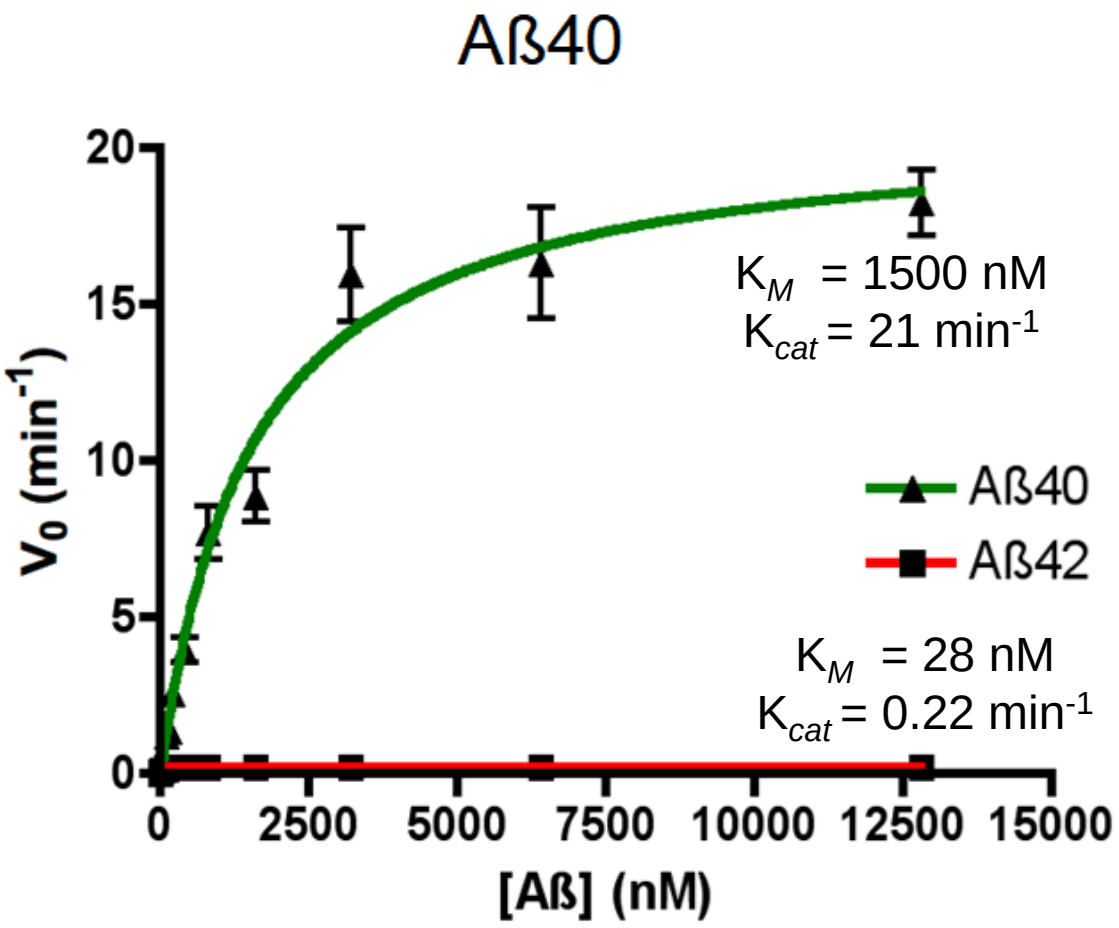
Idea: In AD, it is a failure to process a modified substrate that leads to lysosomal storage.

-modified substrate should accumulate over time

-modified substrate should evade normal digestion

-modified substrate should not be recognized by transporters

What about Aβ 40 vs 42?

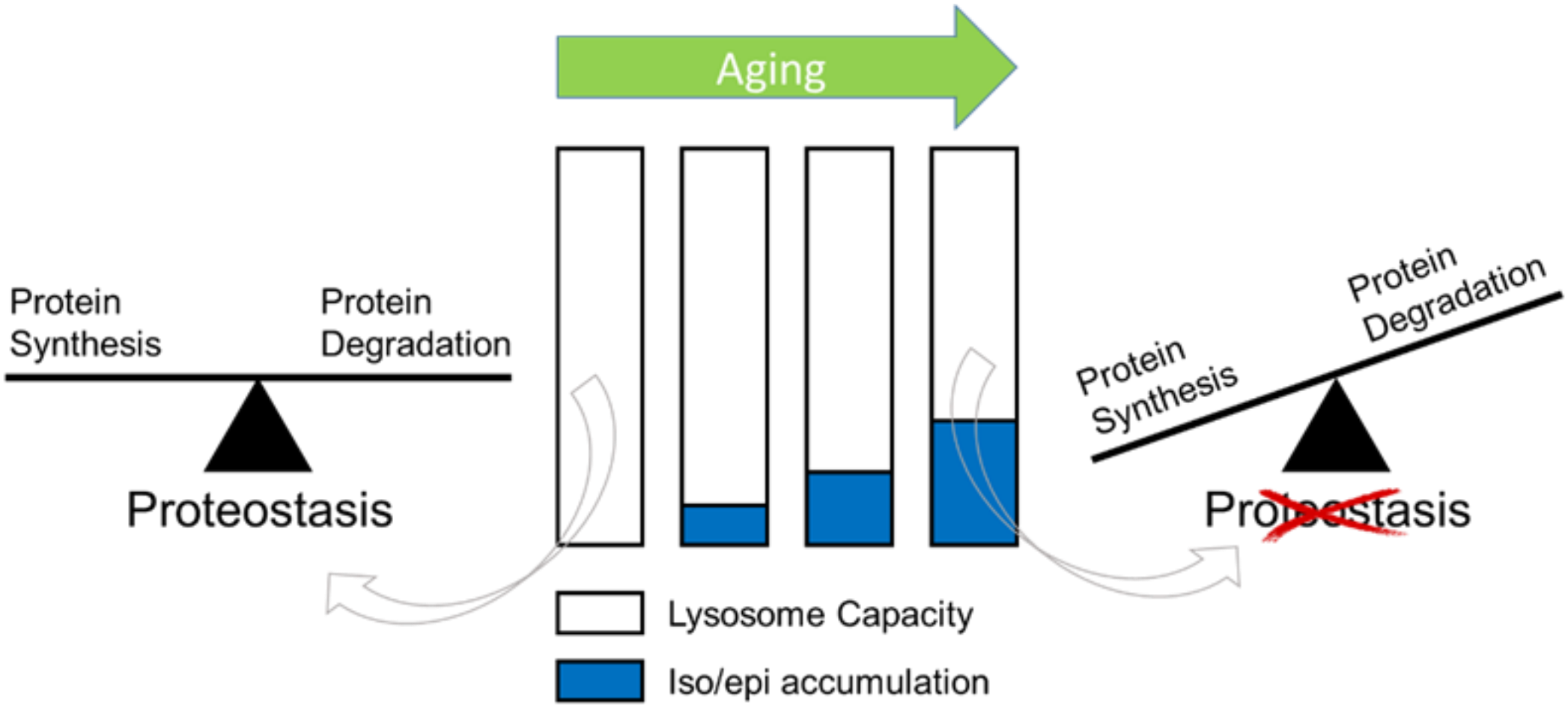


Aβ42 is more aggregation prone and increased production is associated with higher risk of AD.

Aβ42 is also problematic for the lysosome. It binds so tightly to CatD that it behaves as an inhibitor.

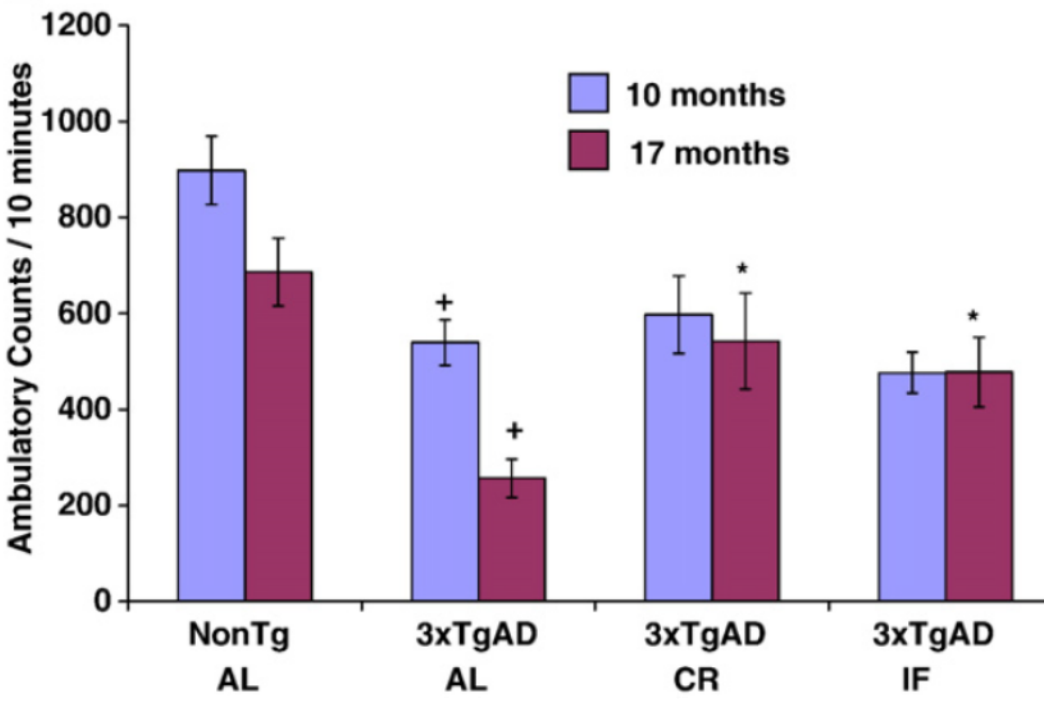
Results courtesy of: Malcolm A. Leissring, Ph.D. UCI MIND

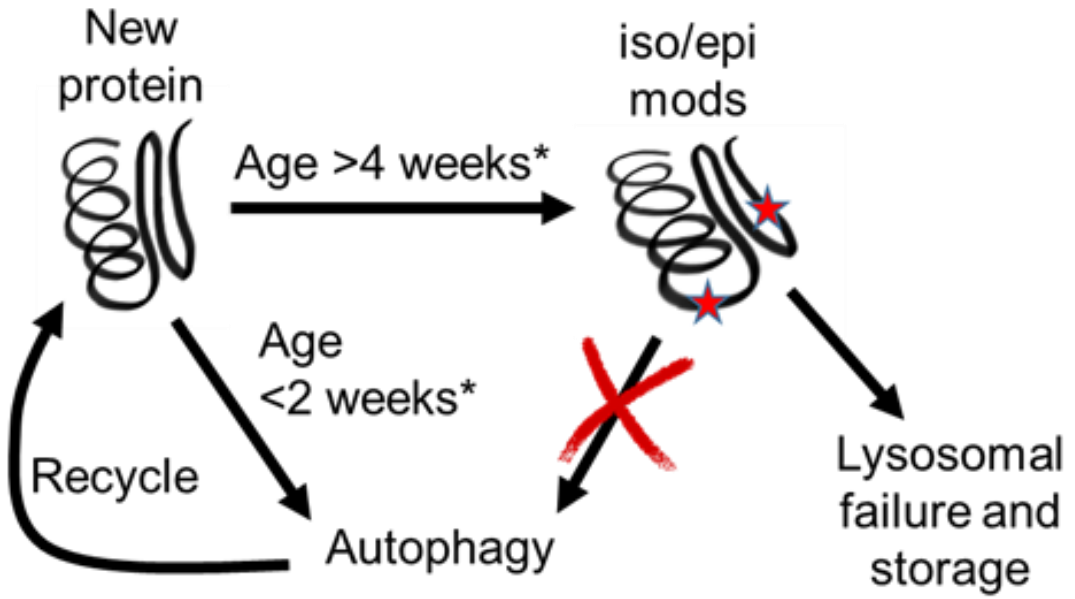
Proteostasis requires sufficient lysosome capacity



Over time, lysosome capacity is reduced by accumulation of iso/epi peptides that cannot be degraded.

How do we combat lysosomal storage?





Frequent autophagy induced by fasting or calorie restriction is protective.

Halagappa, *Neurobiol. Dis*. **2007**, 26, 212.

Summary

- Long-lived proteins may be an important key to understanding aging—not due to loss of function, but due to persistence
- Isomerization and epimerization of long-lived proteins is a likely cause of lysosomal storage associated with Alzheimer’s Disease
- Increasing the frequency of autophagy should be protective

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Jin Tang

Brielle Van Orman

Evan Hubbard

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