

EUROPEAN INNOVATION DAY 2018
TOKYO (JAPAN), 15 OCTOBER 2018

PERSPECTIVE OF AN EUROPEAN (EX) RESEARCHER: FROM ACADEMIC EUROPE TO ACADEMIC JAPAN

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UNIVERSITÉ
FRANCO
ITALIENNE



JAPAN SOCIETY FOR THE PROMOTION OF SCIENCE
日本学術振興会



Tokyo University of Pharmacy and Life Sciences

Timeline as a researcher



06/1988

- Born in Paris (France)

07/2011

- M.Sc. In Organic Chemistry (France)



07/2015

- Ph.D. in Peptide Science (France & Italy)



UNIVERSITÀ
DEGLI STUDI
FIRENZE



10/2015

- Post-doc in Medicinal Chemistry (Japan)



12/2017

- Joined Biotage (Japan)



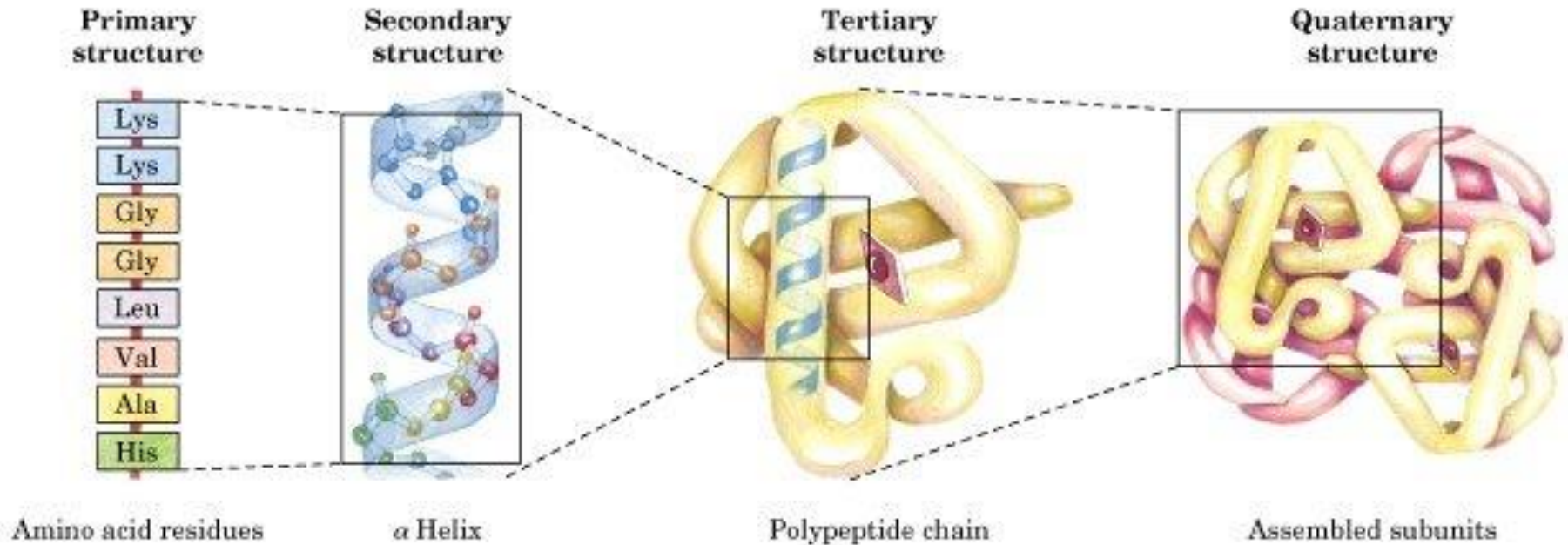
Introduction

Peptides

Ph.D.

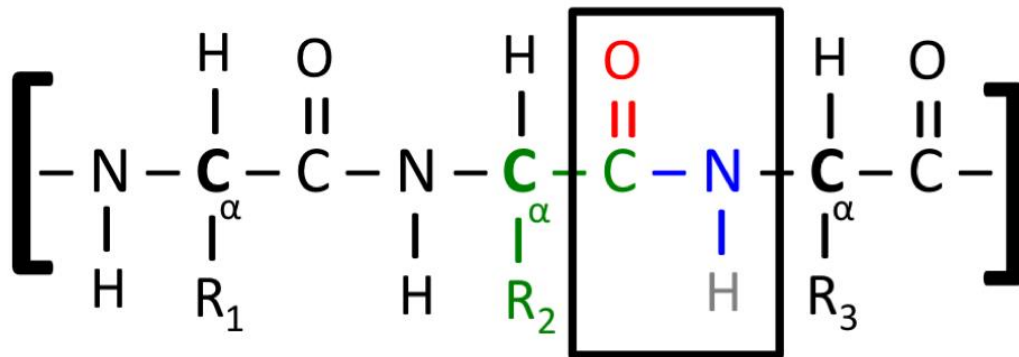
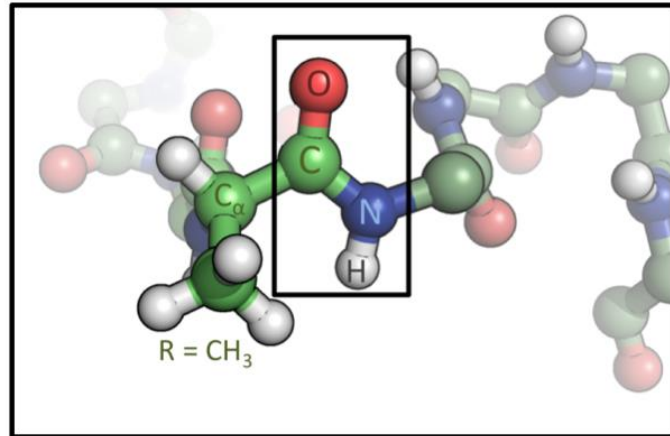
Post-doc

Amino acids, peptides & proteins



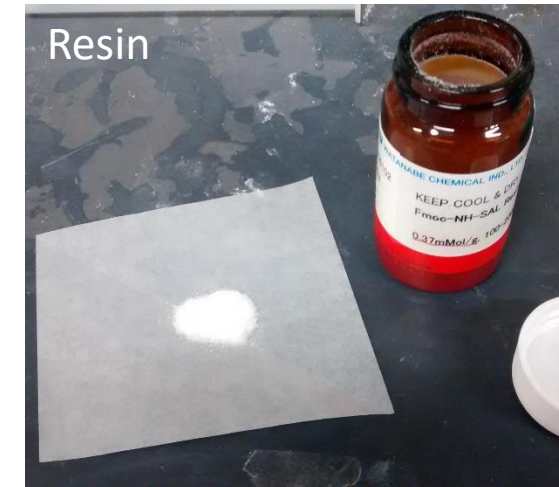
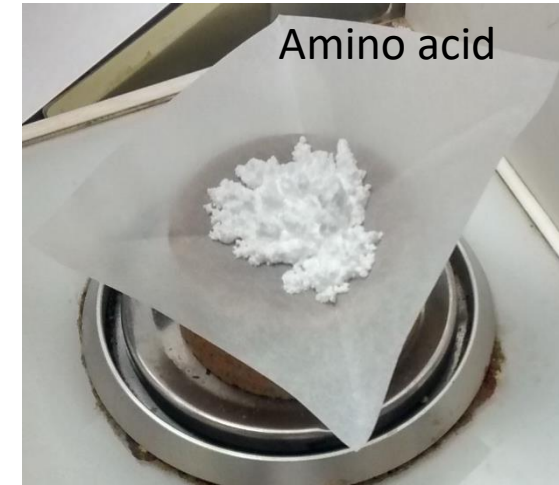
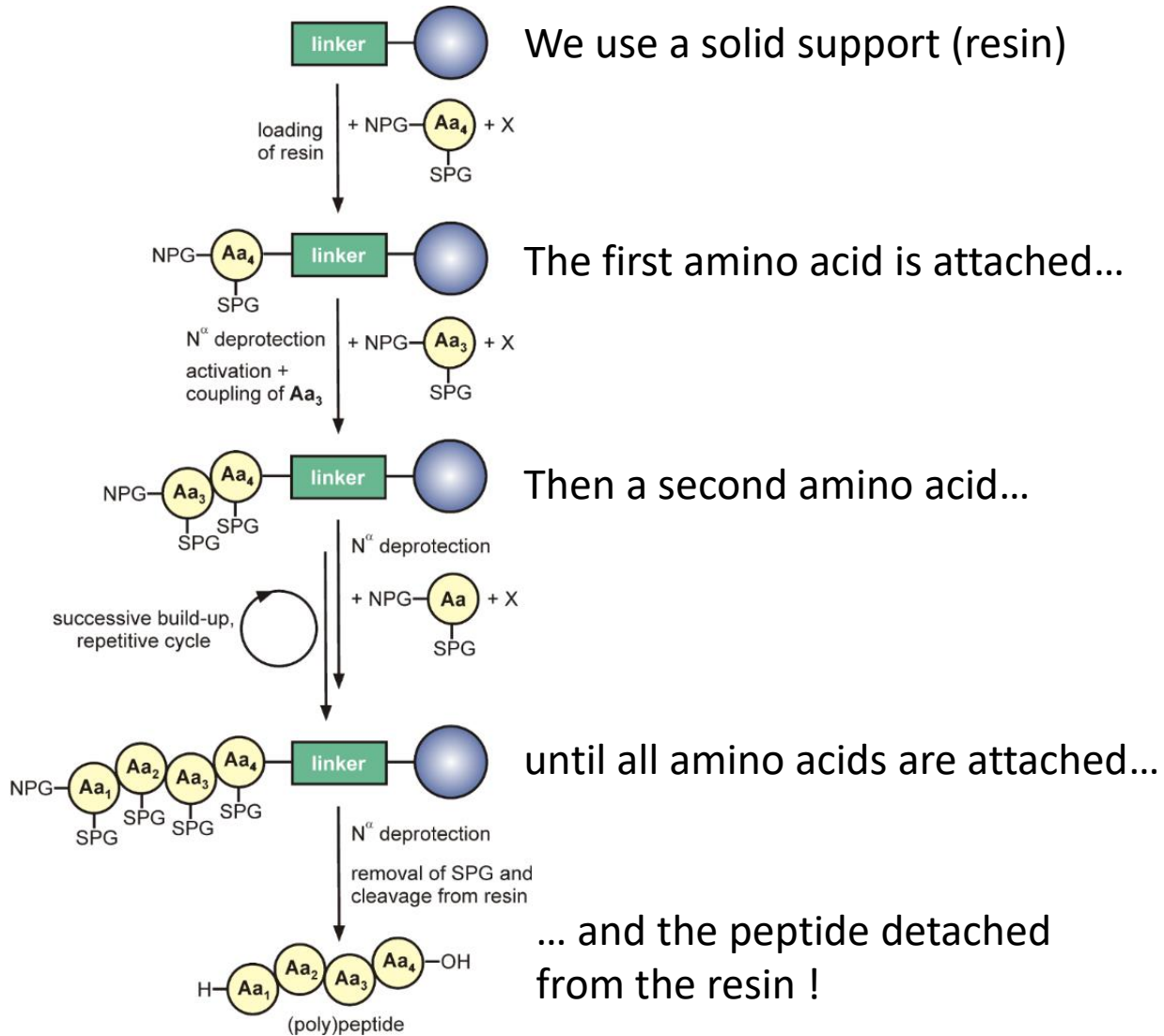
- **Proteins = large molecules made of amino acids**
- **Proteins are essential in living organisms**

Peptides and the amide bond

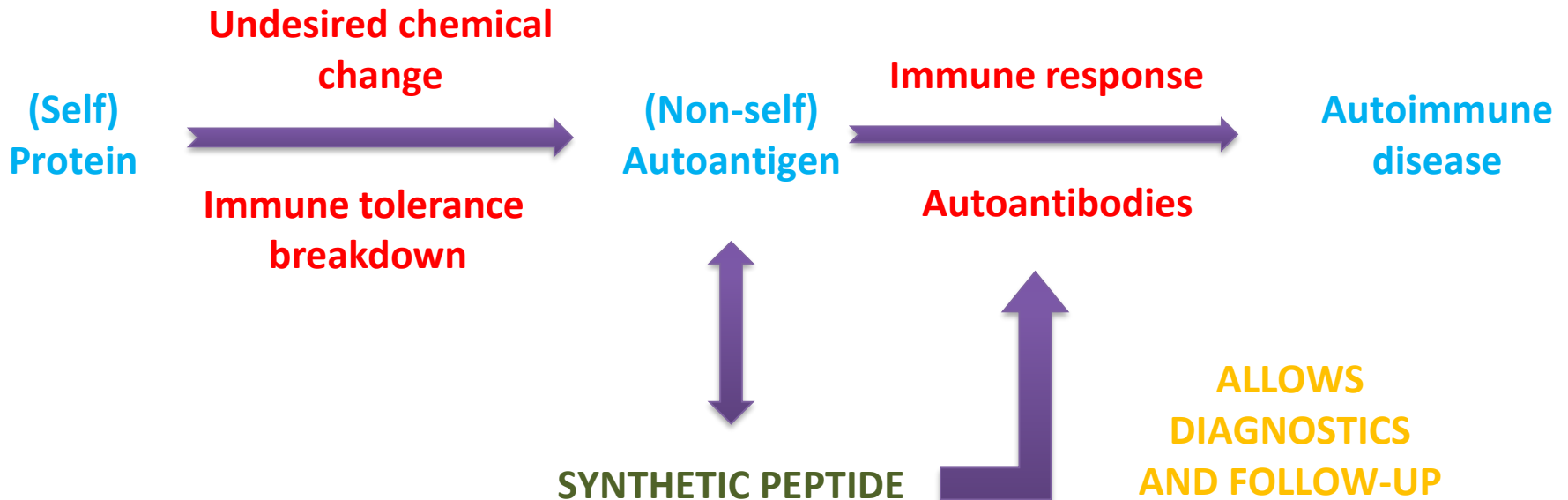


- Peptide = “small” molecule made of amino acids
- Peptide is <100 amino acids (Protein is >100 amino acids)
- Amino acids linked together by amide bonds

How are peptides synthesized?



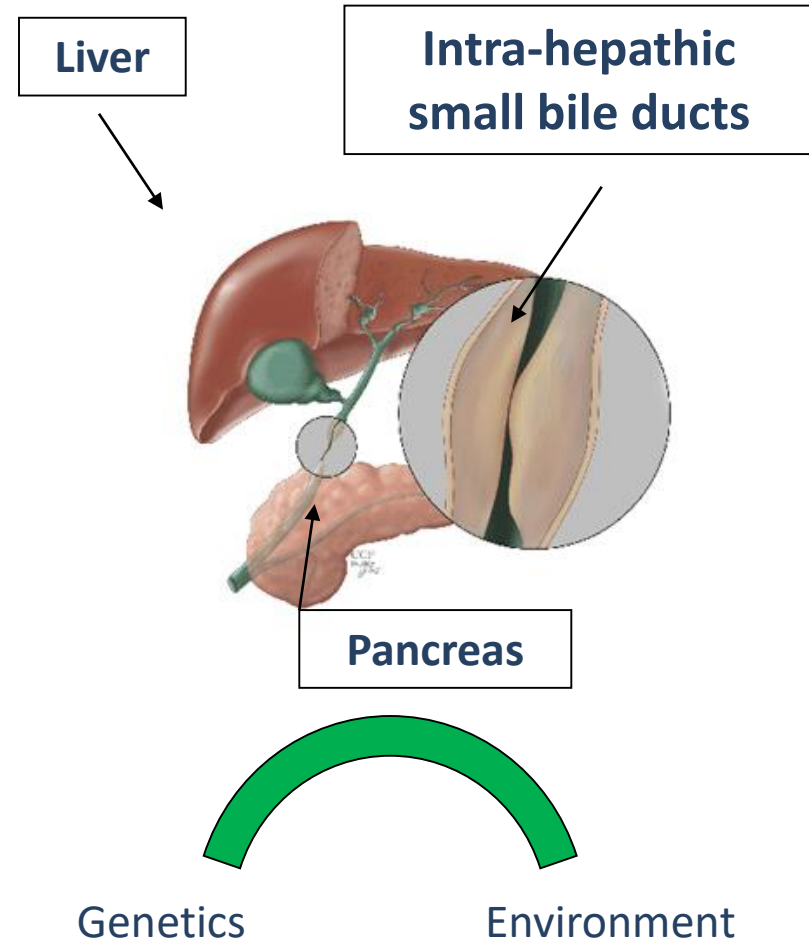
The chemical reverse approach



- **Synthetic peptides as molecular probes for diagnostics !**

Primary Biliary Cirrhosis

- Autoimmune cholestatic disease of the liver
 - Antimitochondrial autoantibodies (AMA) in 90-95% of patients
 - Existing diagnostic methods:
 - detect AMA
 - alkaline phosphatase level
 - liver biopsy
- All of these are not convenient

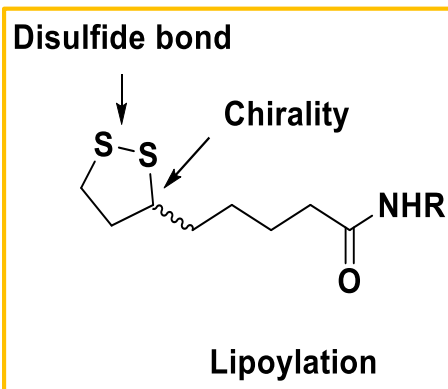
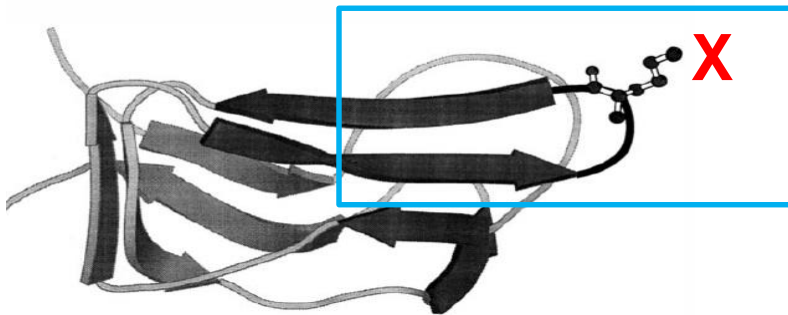


Molecular probes using modified PDC

PDC-E2(167-186):

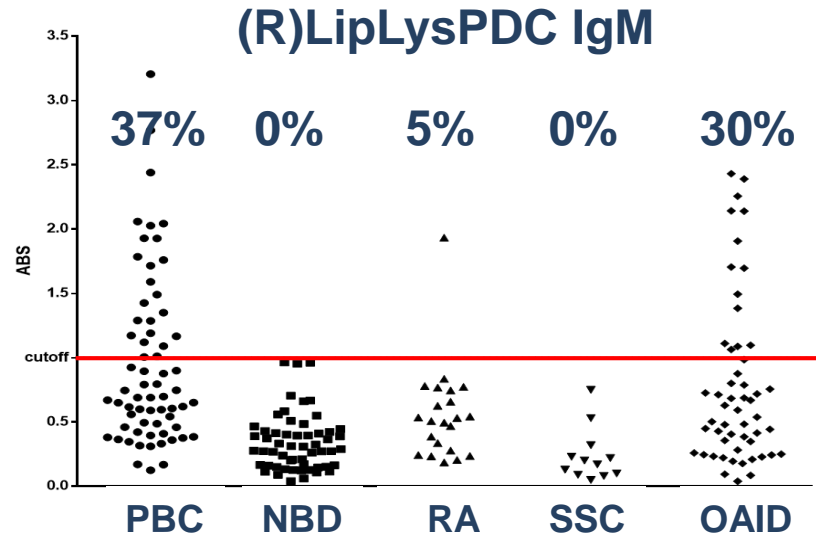
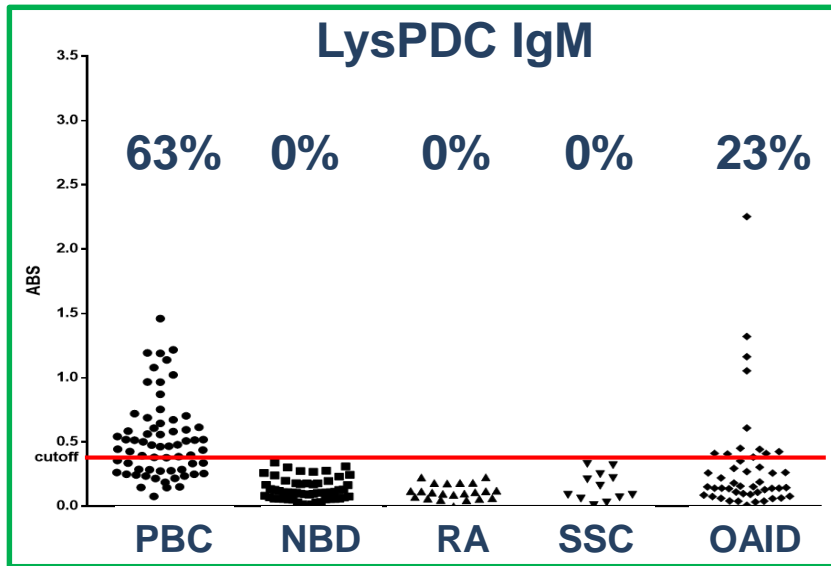
inner lip. domain of PDC-E2
(type I β -turn)

AEIETD-**X**-ATIGFEVQEEGYL-KKKK



- PDC-E2 is the protein targeted by AM antibodies
- The PDC-E2 protein *in vivo* is modified by an exposed **lipoic acid** in **X**
- Undesired chemical modification of PDC triggers this disease?
 - Several analogues with different **X** were synthesized

Molecular probes with patients' sera



- Left: **X** position with no **lipoic acid**
- Right: **X** position with **lipoic acid**

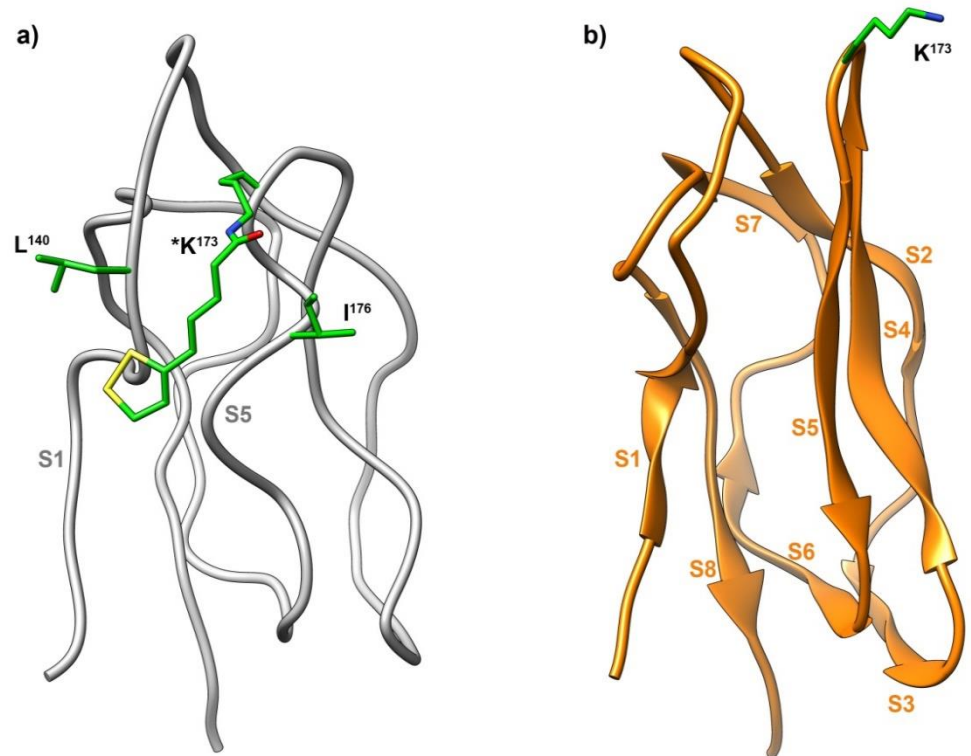
Unlipoylated peptides have higher % positivity towards PBC sera !

- De-lipoylation as an aberrant PTM involved in PBC ?

Molecular dynamics to the rescue

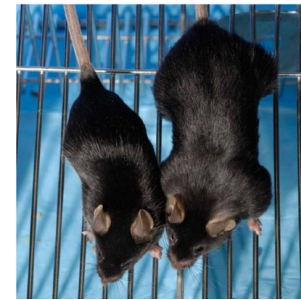
MD simulations with data from existing databases

- Hydrophobic interaction between **lipoic acid** and surrounding amino acids
- Removing the **lipoic acid** changes the tridimensional structure of the protein !
- De-lipoylation changes the protein and triggers an immune response ?



Myostatin & muscular growth regulation

- Myostatin: responsible for negative regulation of muscular growth
 - Natural deficiencies exists (genetics)
 - No myostatin = muscle hypertrophy
- Regulation of myostatin function to force muscle growth ?



Myostatin inhibition



Muscle size
increase?



- Useful strategy for treatment of muscle atrophic diseases, cachexia & aging !

The Peptide A (from previous studies)



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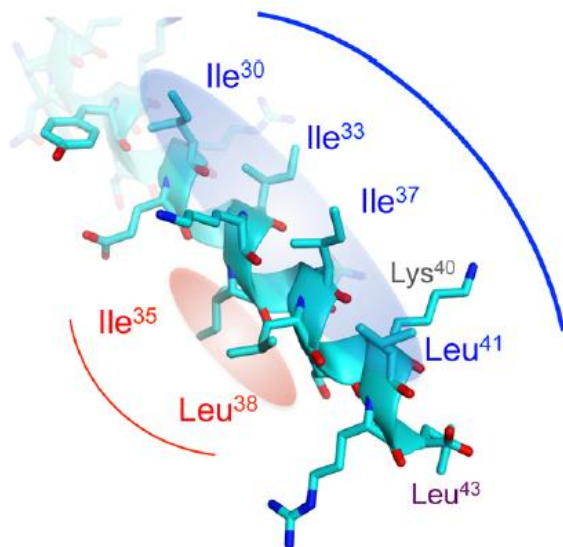
- Peptide A: comes from mouse myostatin prodomain.
- Is able to bind to human myostatin.

Peptide A (wildtype, 23 AA)

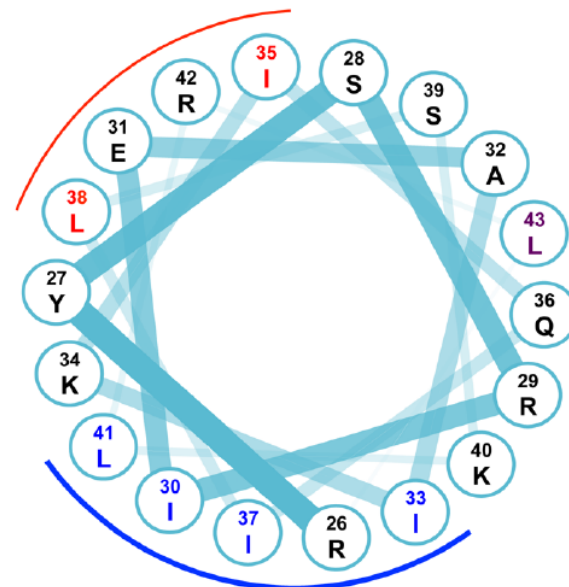
21 43
WRQNTRYSR**IEAIKIQIL**SKLRL-amide

Green : Important AA for the activity

— α -helix



Predicted 3D helical and 2D wheel models show an α -helix with **MAJOR** and **MINOR** hydrophobic faces !



Peptide A: sequence optimization

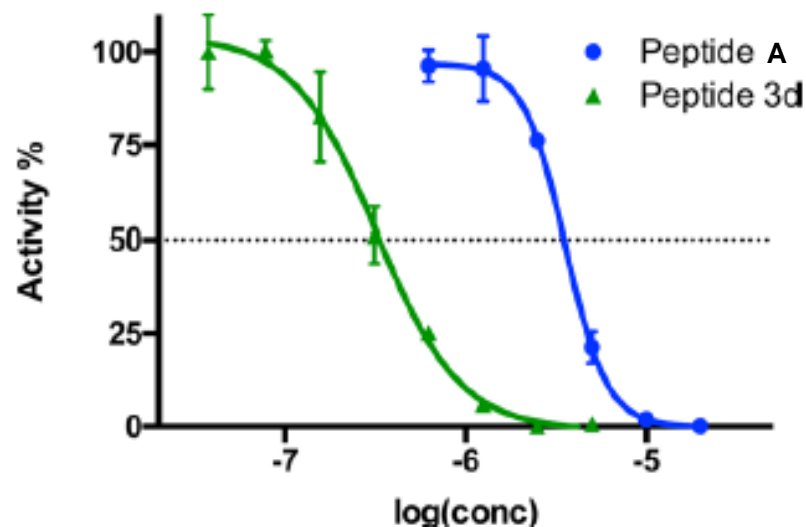
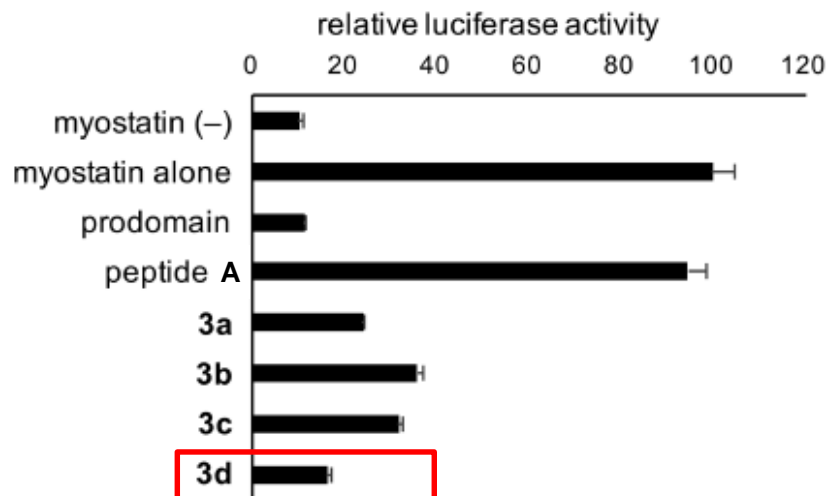


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Myostatin inhibitory peptide **A** (23AA)

21 43
H-WRQNTRYSRIEAIKIQILSKLRL-NH₂

- 3a WRQNTRYSRIEWIKIQIISKLRRL-amide
3b XRQNTRYSRIEAIKIQIISKLRRL-amide
3c XRQNTRYSRIEWIKIQILSKLRRL-amide
3d XRQNTRYSRIEWIKIQIISKLRRL-amide
(X = 2-naphthoxyacetyl group)



Peptide A: $IC_{50} = 3.53 \pm 0.25 \mu M$

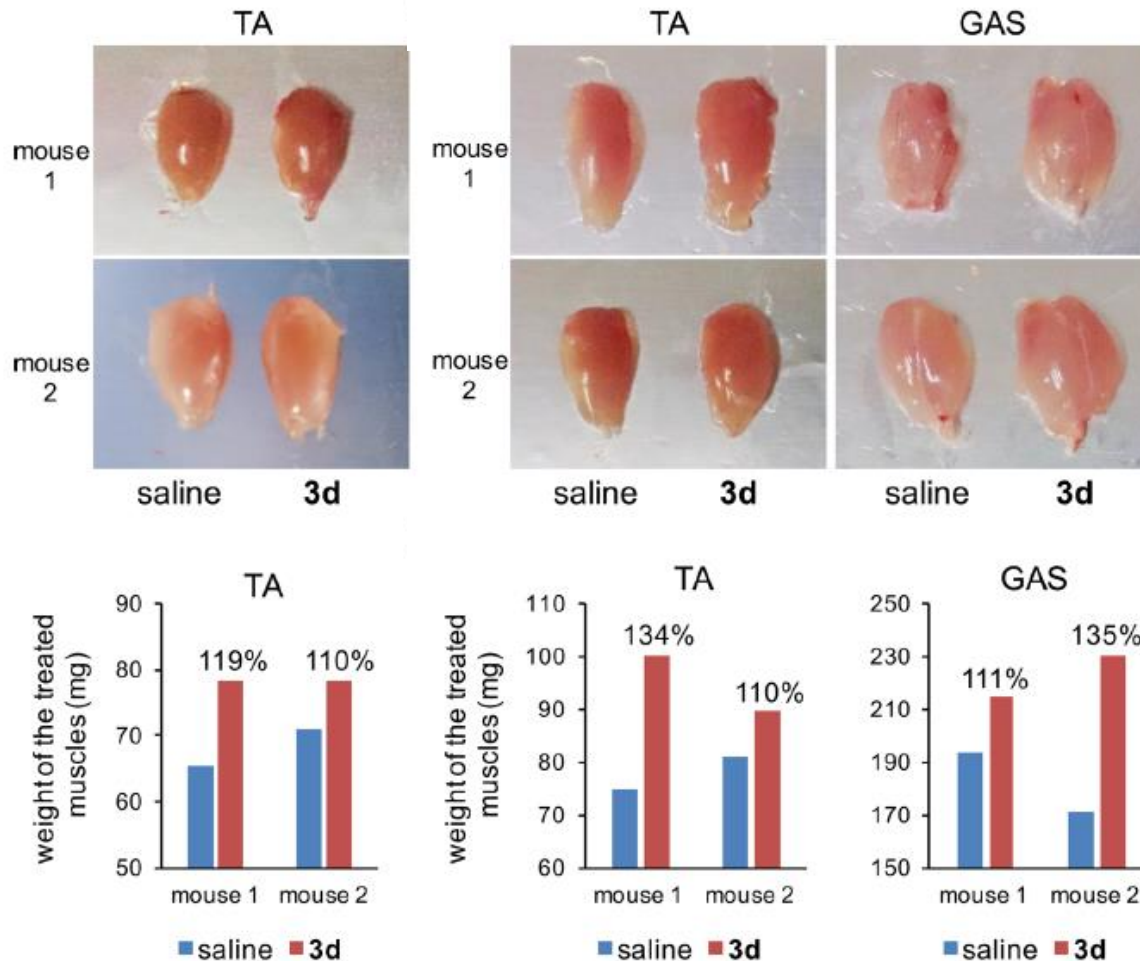
↓ 11x lower !

Peptide 3d: $IC_{50} = 0.32 \pm 0.05 \mu M$

In vivo effects



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- After 6 weeks of treating mice with **Peptide 3d** (intramuscular injections at 0 and 2 weeks)...

➤ ... there is a noticeable increase of muscle fibers size ...

➤ ... and average increase in muscular mass is +20 %!

Peptide A: structure optimization

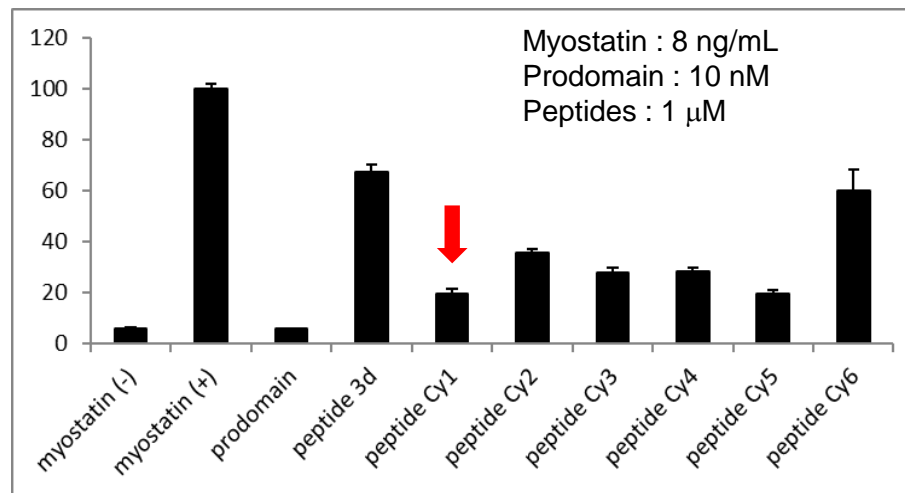
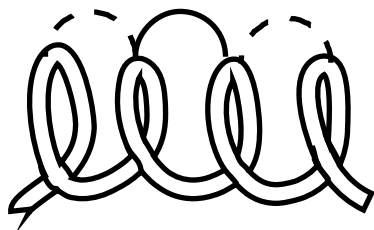


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- Effects of conformational lock on inhibitory potency?
- 2 parts of the peptide bound together to rigidify its structure

3d	XRQNTRYSRIEWIKIQIISKLRRL-NH ₂
Cy1	XRQN C RY C RIEWIKIQIISKLRRL-NH ₂
Cy2	XRQNTRY C RI C WIKIQIISKLRRL-NH ₂
Cy3	XRQNTRYSR C W C IQIISKLRRL-NH ₂
Cy4	XRQNTRYSRIEWIK C II C KLRL-NH ₂
Cy5	XRQNTRYSRIEWIKIQI C KL C L-NH ₂
Cy6	XRQN K RYSD I EWIKIQIISKLRRL-NH ₂

➤ « Cyclization scan »



Life of an EU researcher in Japan

- A valuable experience: **Transferable skills** for future career



- ✓ Group thinking: Interests of **the group** before your own
- ✓ Commitment: Learn to be **dedicated** to an objective
- ✓ Flexibility: Adapt to a **different culture**

Life of an EU researcher in Japan

- ✓ **Working in Japan: an incredible opportunity**
- ✓ **Cultural differences: a lot to learn**
- ✓ **Building a successful international profile**
- **The most important: Enjoy your time in Japan**

Thank you for your kind attention !