Drugs for Amyloid β Protein

- How Were Their Structures Designed? -

Literature Seminar (2013. 2. 2)

Kiyomichi SHINODA

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§1 Introduction

What is Alzheimer's Disease (AD)?

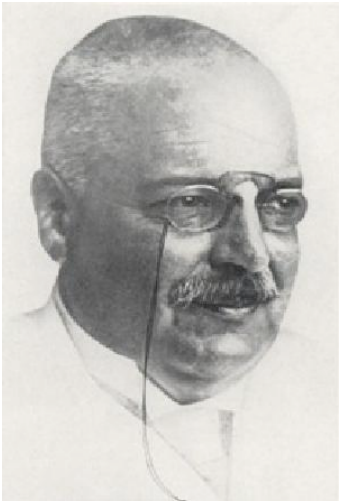
- predominant dementia in Japan (H. Akatsu et al., *J. Neurol. Sci.* **2002**, *196*, 63.)

Discovery who: Dr. Allos Alzheimer (German psychiatrist)

when: 1906

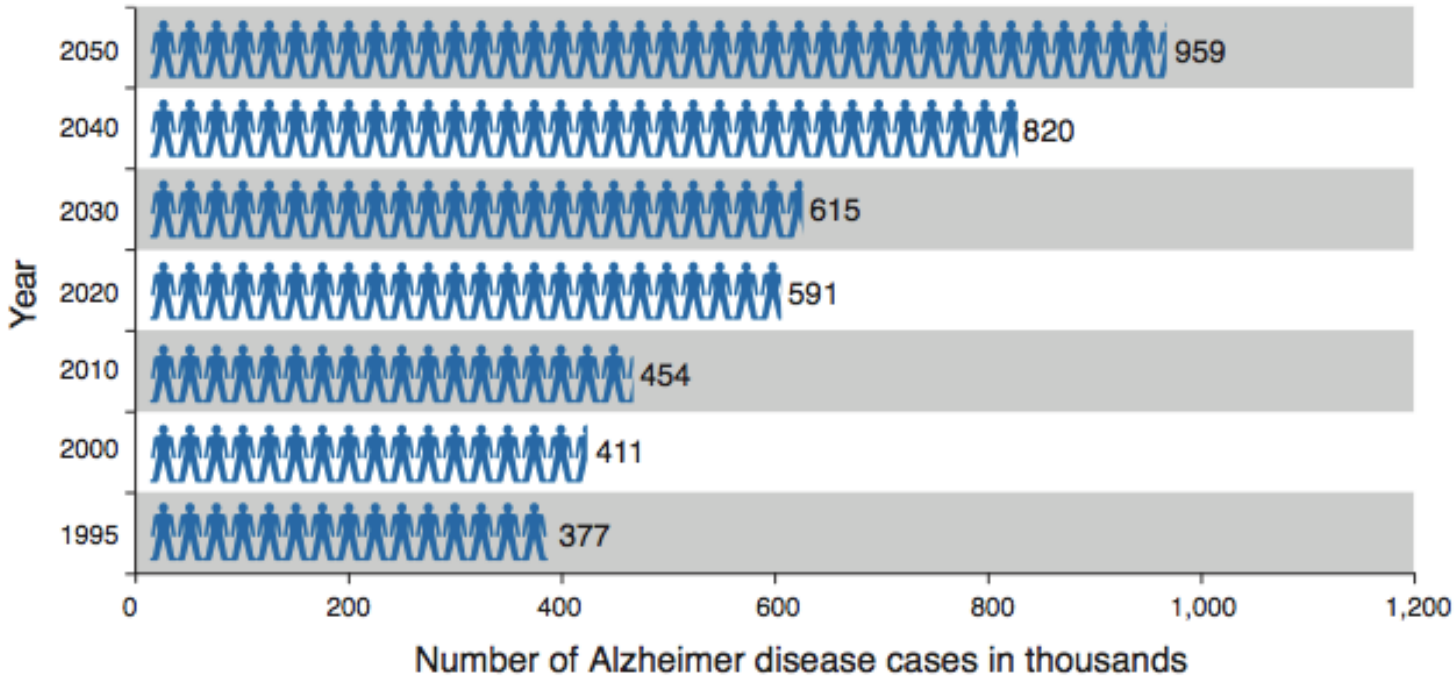
Classification

- Familial AD
- Dementia of Alzheimer type



Dr. Allos Alzheimer
(1864-1915)

Number of Patients in the US

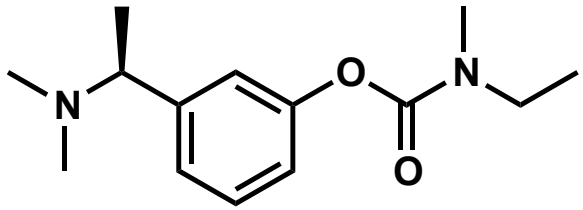


C. Mount and C. Downton, *Nat. Med.* **2006**, *12*, 780.

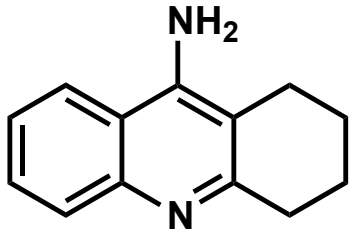
Drug development is a pressing need!

Drugs Placing on the Market Currently

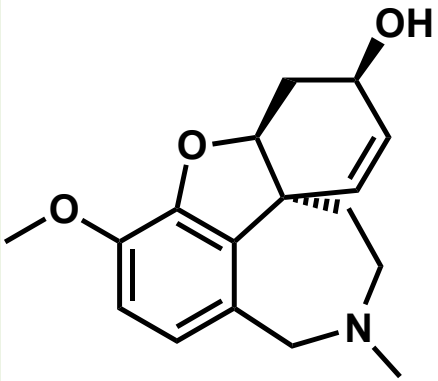
AChE Inhibitors



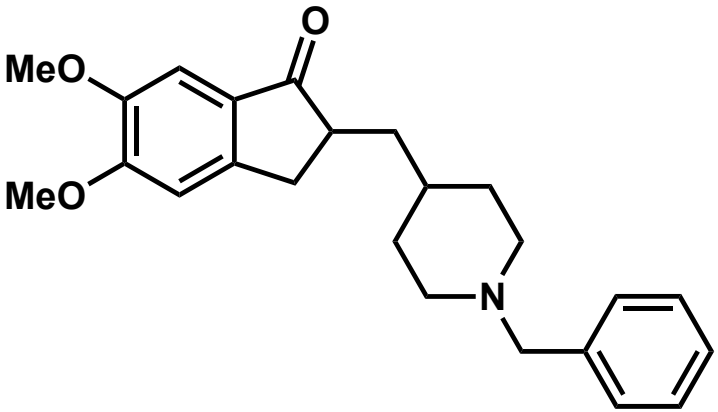
Rivastigmine



Tacrine

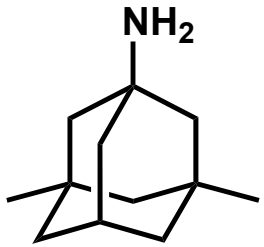


Galantamine



Donepezil

NMDA Antagonist



Memantine

Drugs Placing on the Market Currently

AChE Inhibitors inhibit the decrease in ACh

→ memory improvement

NMDA Antagonist inhibit the too much activation of neurons

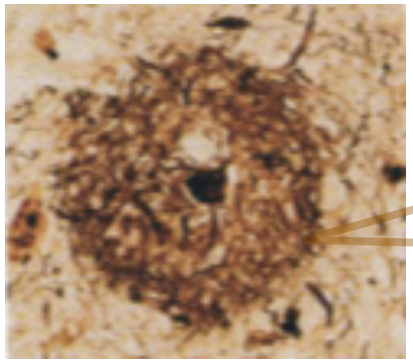
→ prevention of the following cell death

However, they are symptomatic treatments...

Effective and fundamental drugs are needed!

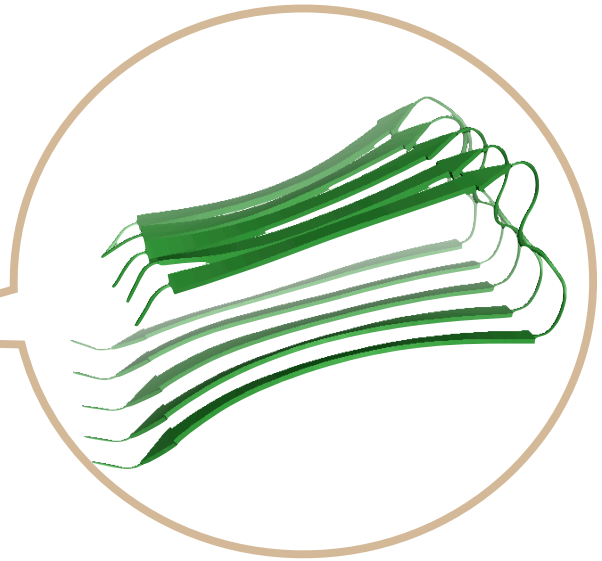
What can be the Target?

Amyloid Cascade Hypothesis



senile plaque

(specific to Alzheimer's Disease)



Amyloid β Protein

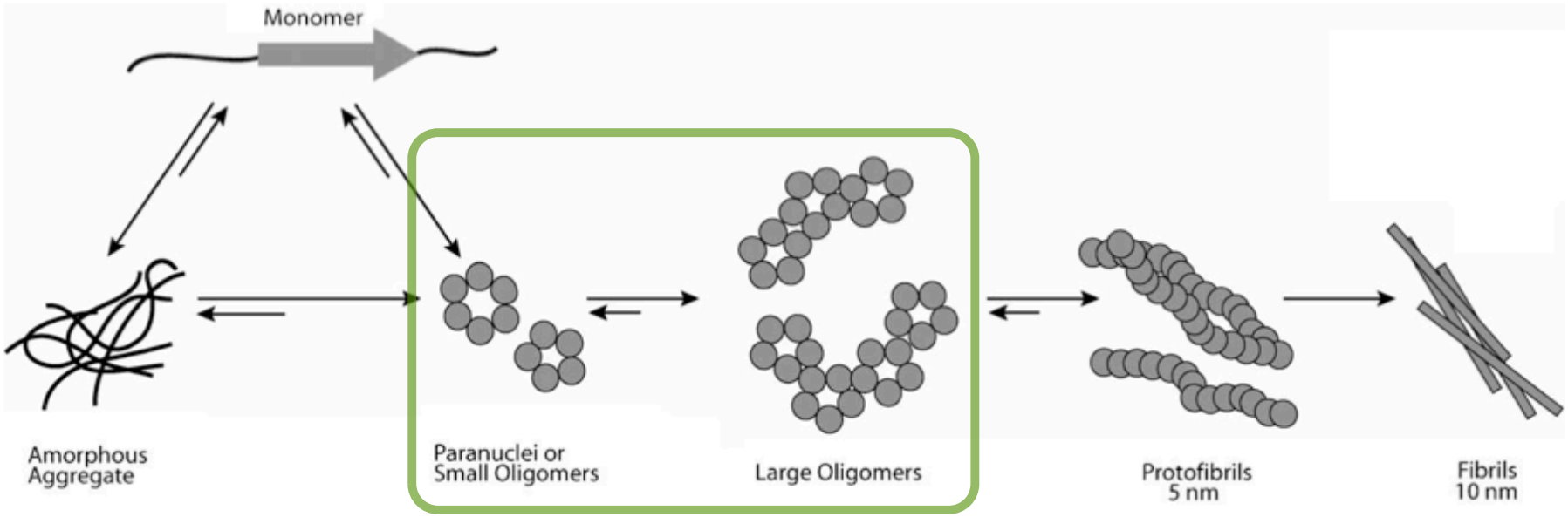
(PBD ID: 2BEG)

Dementia

→ Aβ drugs can treat AD fundamentally!

Note: Sinele plaque figure was taken from "細胞工学 **2012**, *31* (10), 1108."

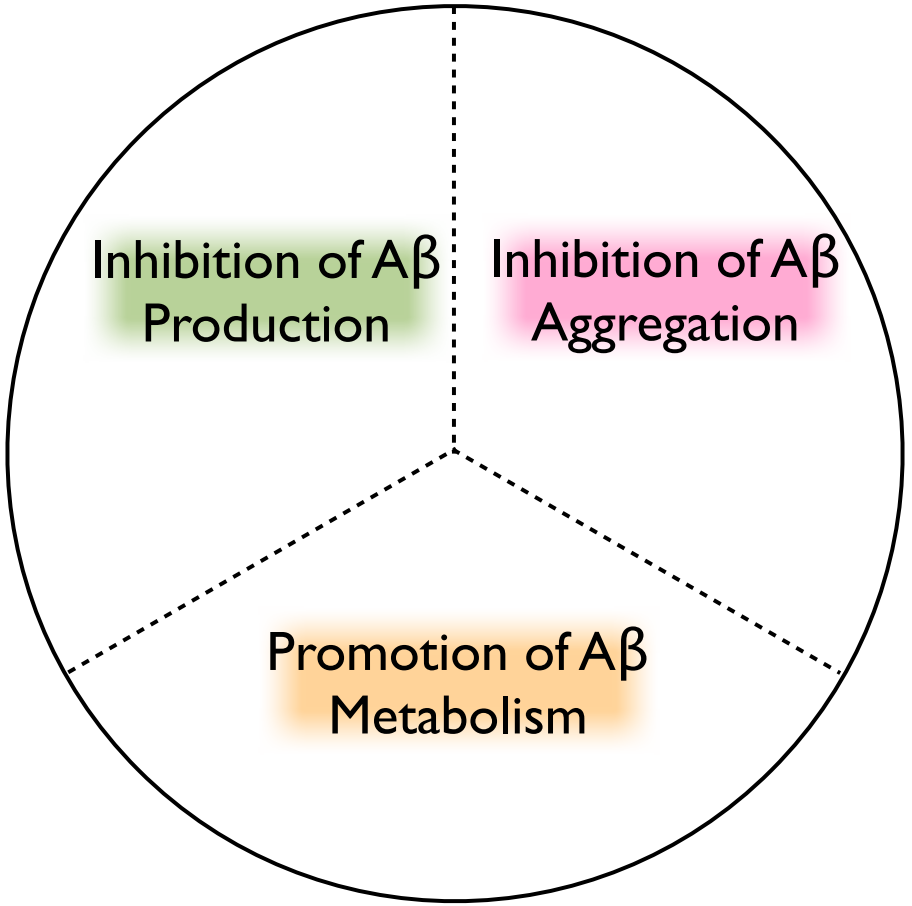
Oligomer Hypothesis



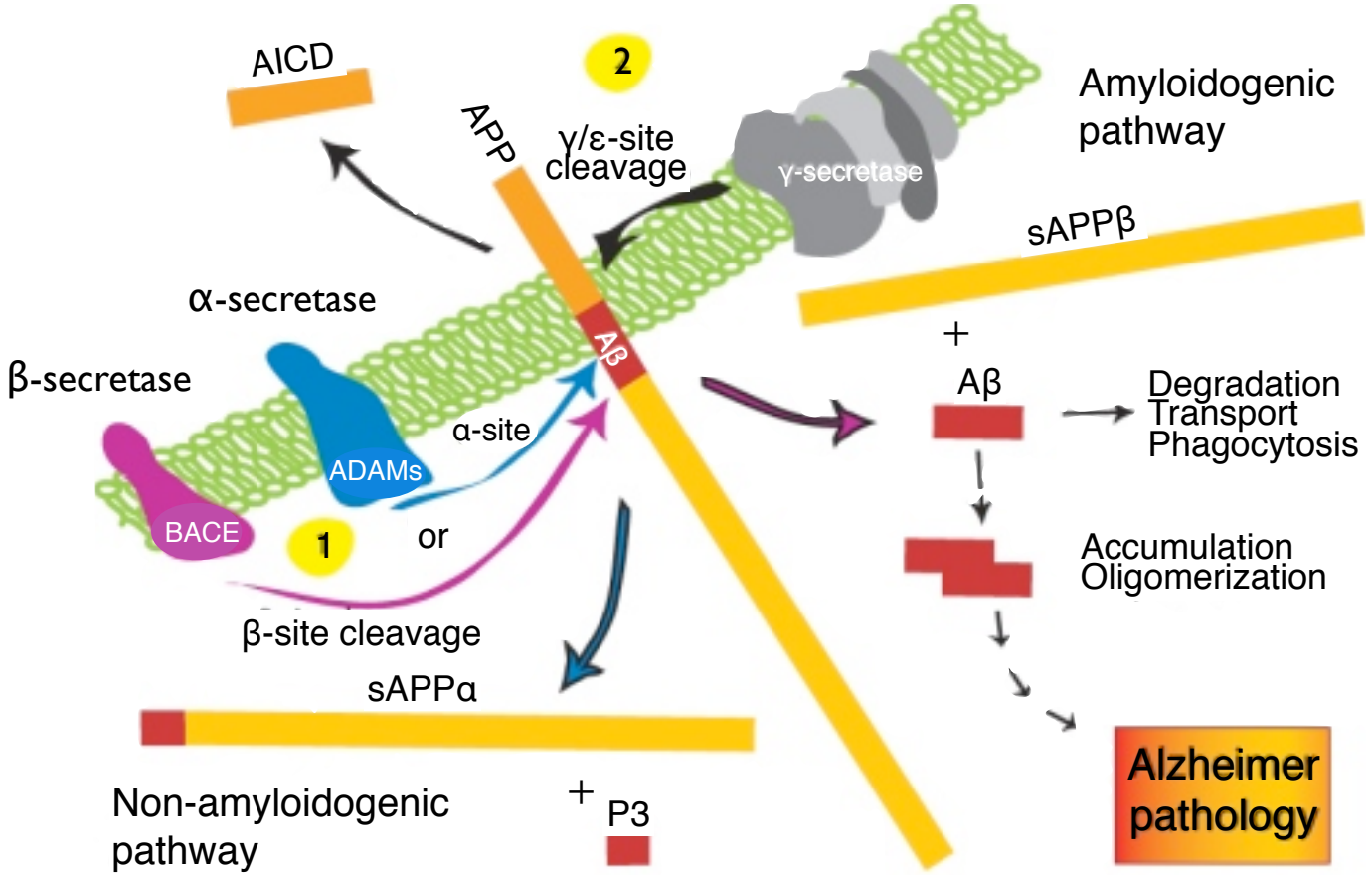
C. I. Stains et al., *ChemMedChem* **2007**, *2*, 1674.

Fibrils are less toxic than oligomers.

Possible Strategies for Aβ Drugs



Aβ Production Pathway



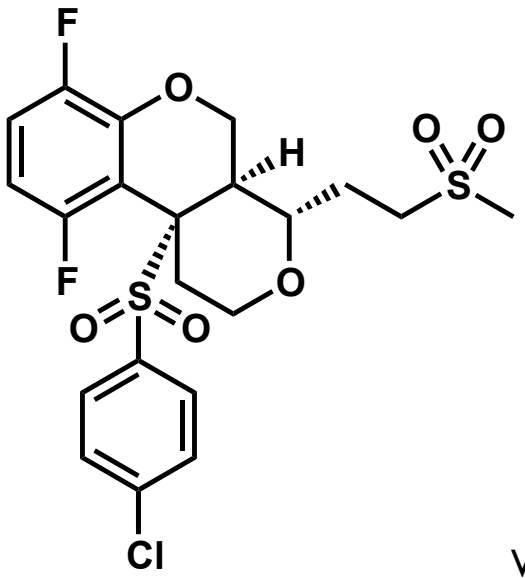
This picture is taken from the following webpage:
Department of Neurochemistry (Stockholm University)
URL: http://www.neurochem.su.se/english/research/2.14201
Note: letters were overwritten for comfortable reading.

How to inhibit Aβ Production?

α-secretase Activator increase non-amyloidogenic pathway

β- (or γ-)secretase Inhibitor inhibit amyloidogenic pathway

Ex.)



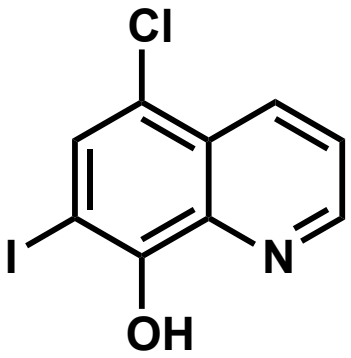
SCH-900229 (Merck)

- γ-secretase Inhibitor
- Aβ40 IC_{50} = 1.3 nM
- Assay was conducted either *in vitro* and *in vivo*.

W.-L. Wu et al., *ACS Med. Chem. Lett.* **2012**, *3*, 892.

How to Inhibit Aβ Aggregation?

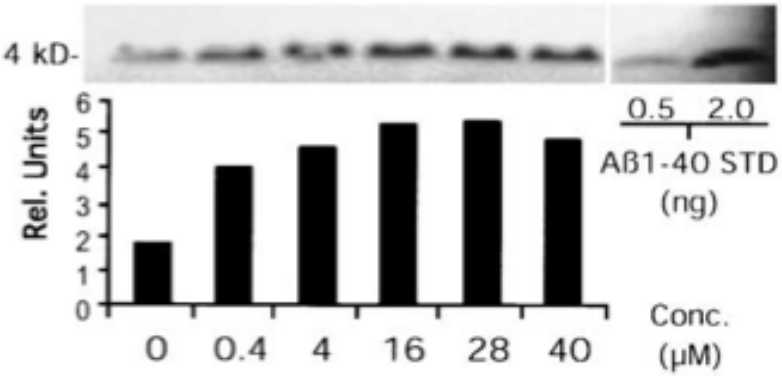
Zn/Cu Chelator Clioquinol (R.A. Cherny et al., *Neuron* **2001**, *30*, 665.)



- prevent toxic interaction between Aβ and metal ion

soluble Aβ

Assay



←western blotting

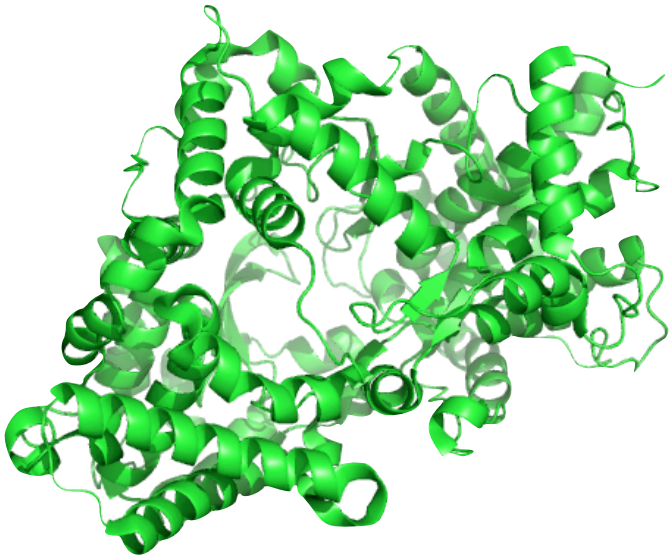
←densitometric quantification

Note: If you need more information, please see Mr. Sonobe's Lit. Seminar (2012.12.1).

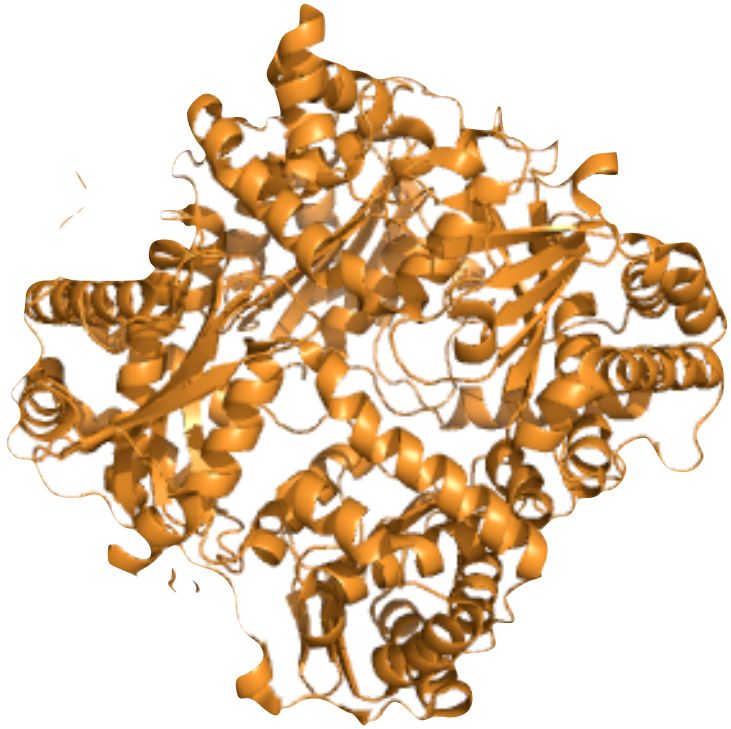
How to Promote Aβ Metabolism?

- major Aβ metabolizing enzymes (E. Mailto et al., *Cell. Mol. Life Sci.* **2008**, *65*, 2574.)

Neprilysin

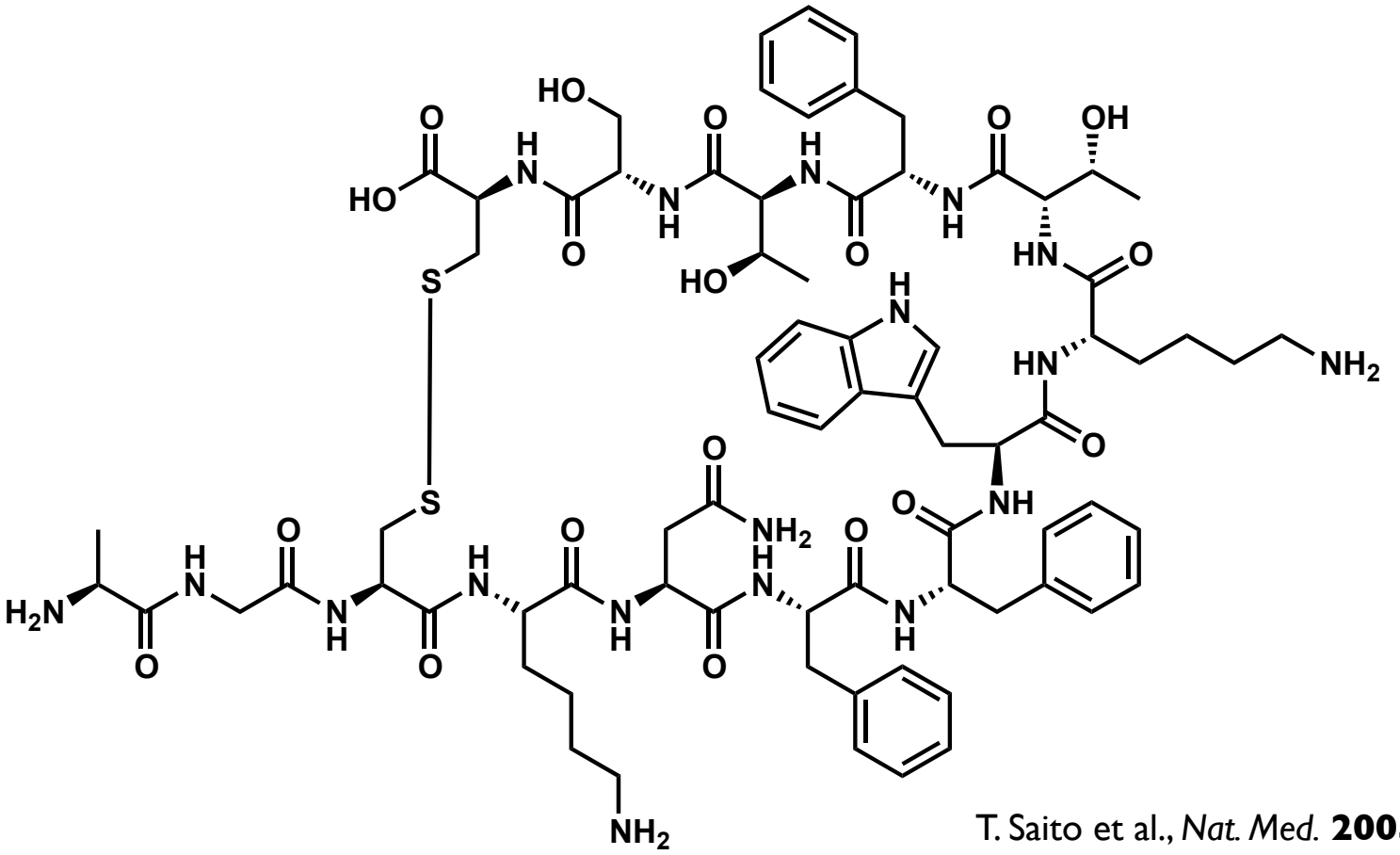


Insulin Degrading Enzyme



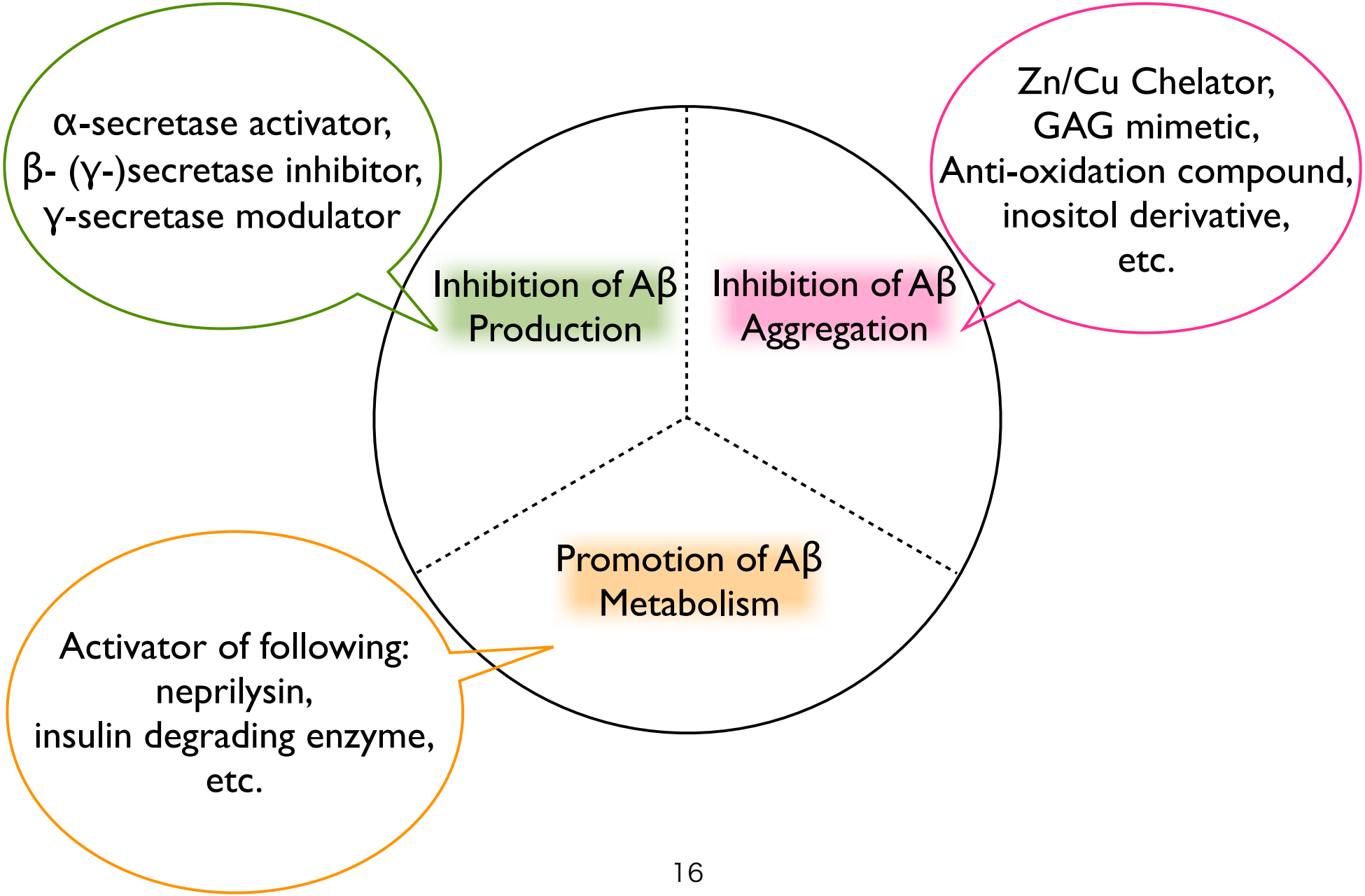
How to Promote Aβ Metabolism?

Neprilysin Activator Somatostatin (peptide hormone)



T. Saito et al., *Nat. Med.* **2005**, *11*, 434.

Summary


α-secretase activator,
β- (γ-)secretase inhibitor,
γ-secretase modulator
Zn/Cu Chelator,
GAG mimetic,
Anti-oxidation compound,
inositol derivative,
etc.
Inhibition of Aβ Production
Inhibition of Aβ Aggregation
Promotion of Aβ Metabolism
Activator of following:
neprilysin,
insulin degrading enzyme,
etc.

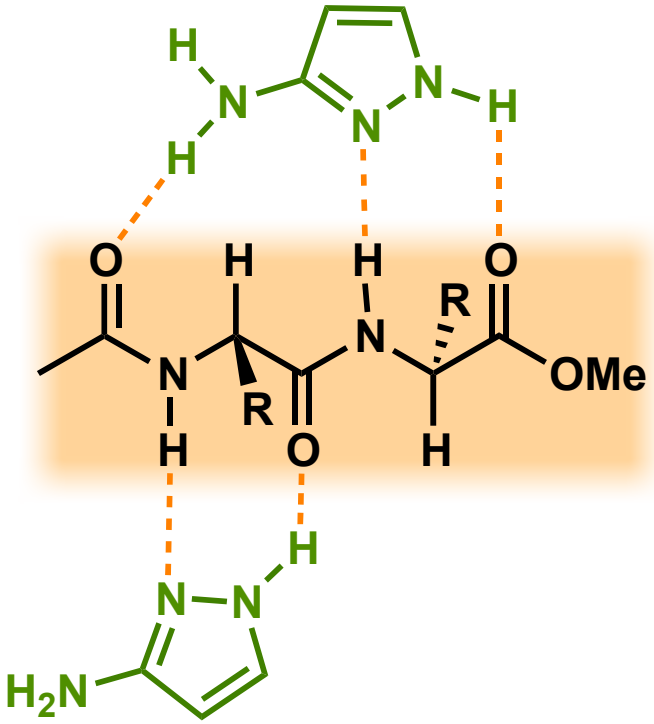
§2 β-Sheet Ligand with Aminopyrazole Moiety

§2.1 Research Background

§2.2 Design of β-Sheet Ligand against Aβ42

β-Sheet Stabilizing Compound

- They presented intermolecular stabilization of β-sheet model.

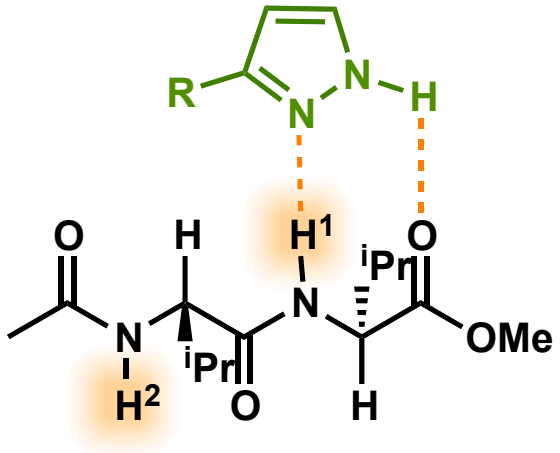


5 hydrogen bonds let dipeptide forming β-sheet structure stably.

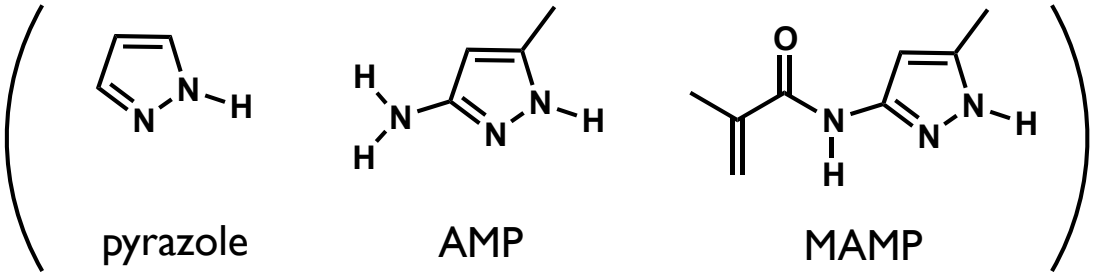
[replica] T. Schrader et al., *Chem. Commun.* **1996**, 2089.; *JACS* **1997**, *119*, 12061.

Favorable Binding Orientation

- ^{1}H-NMR titration analysis (T. Schrader et al., *JACS* **1997**, *119*, 12061.)



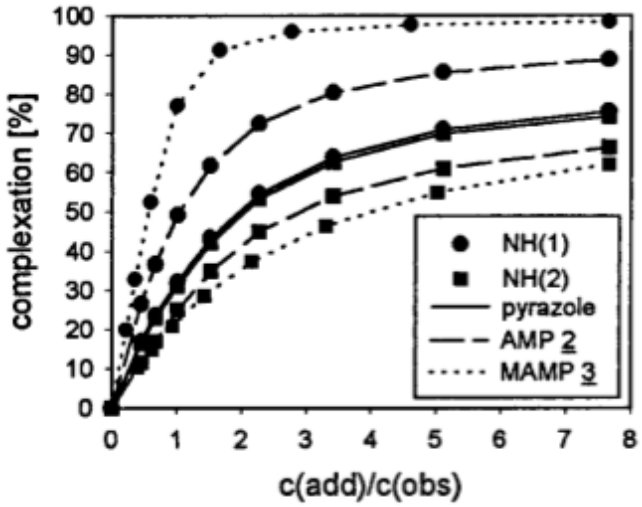
Ac-*L*-Val-*L*-Val-OMe

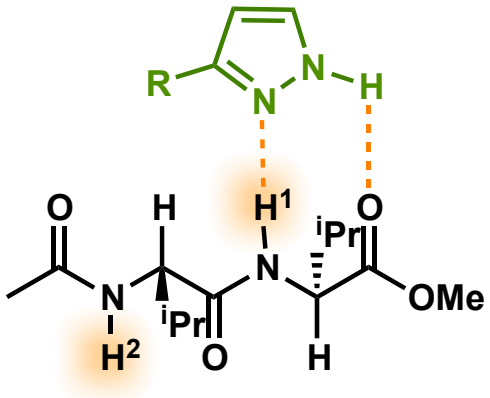


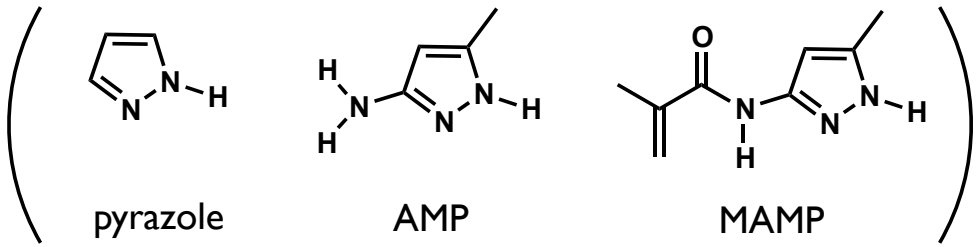
Complexation induced shifts were measured.

Favorable Binding Orientation

- ^{1}H-NMR titration analysis (T. Schrader et al., *JACS* **1997**, *119*, 12061.)

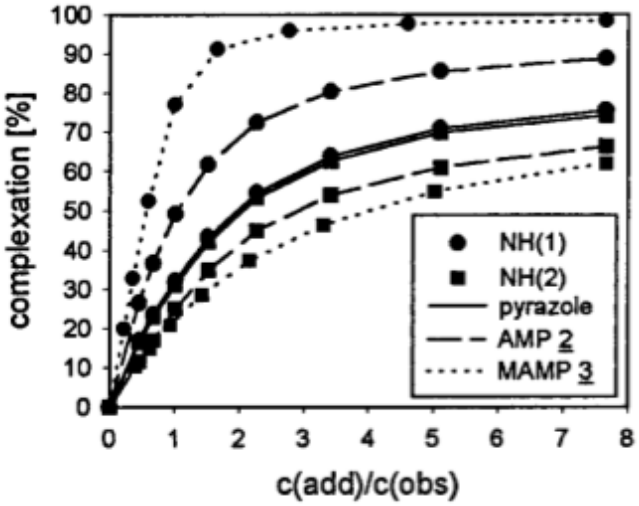


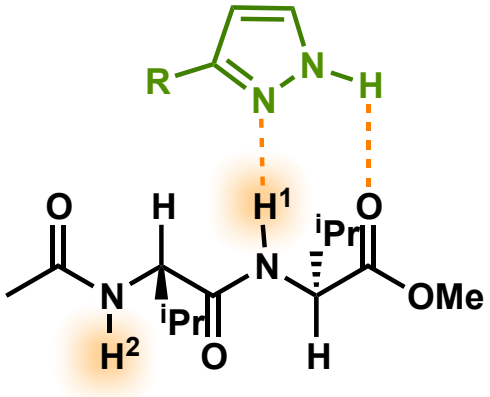




Favorable Binding Orientation

- ^{1}H-NMR titration analysis (T. Schrader et al., *JACS* **1997**, *119*, 12061.)





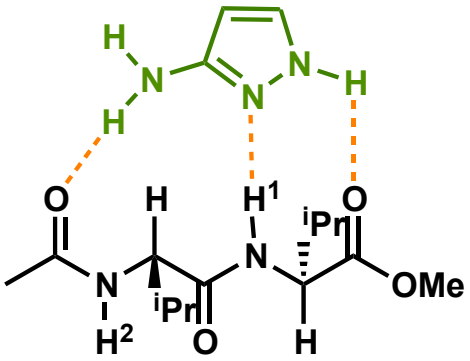
- In pyrazole case, NH(1) and NH(2) draw the same curves, which means these two protons can't be distinguished.
- In AMP and MAMP case, NH(1) and NH(2) draw different curves, which means these two protons can be distinguished.

How can we explain these results?

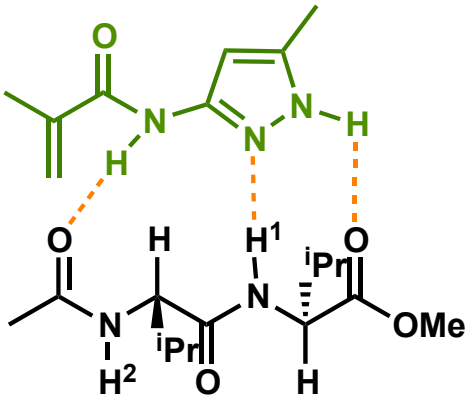
Favorable Binding Orientation

- ^{1}H-NMR titration analysis

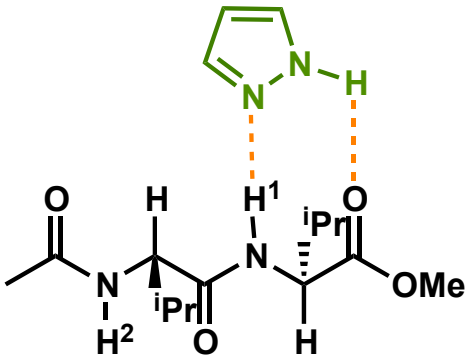
AMP



MAMP



pyrazole

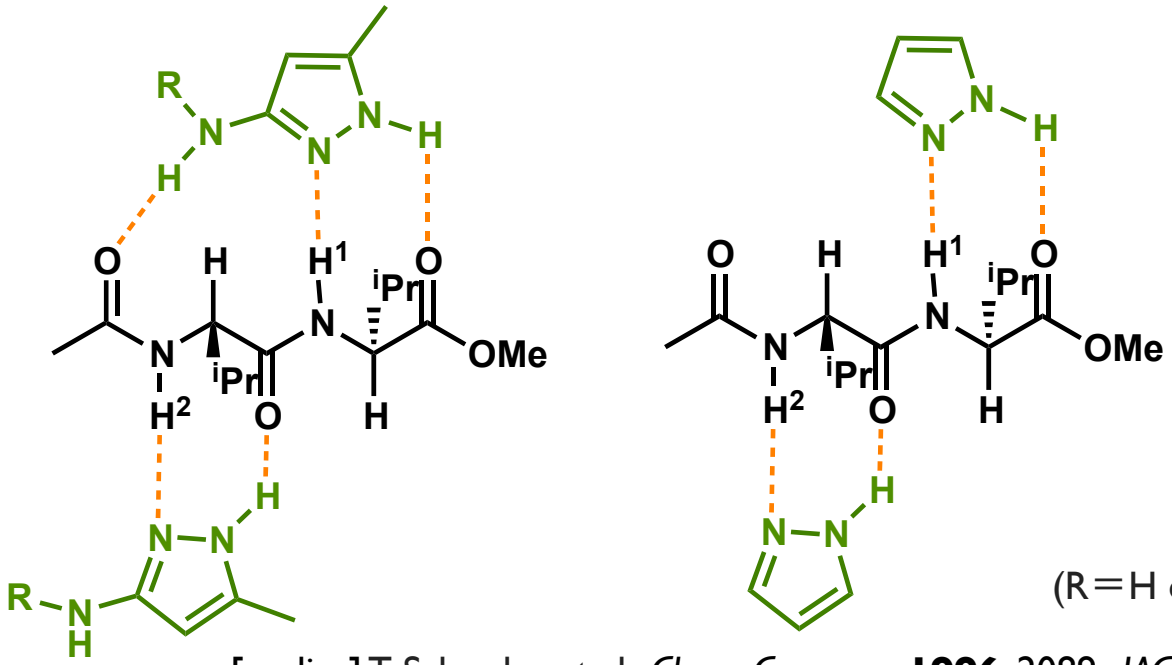


The numbers of hydrogen bonds were different.

T. Schrader et al., *Chem. Commun.* **1996**, 2089.; *JACS* **1997**, *119*, 12061.

Favorable Binding Orientation

- ^{1}H-NMR titration analysis (left: AMP & MAMP, right: pyrazole)



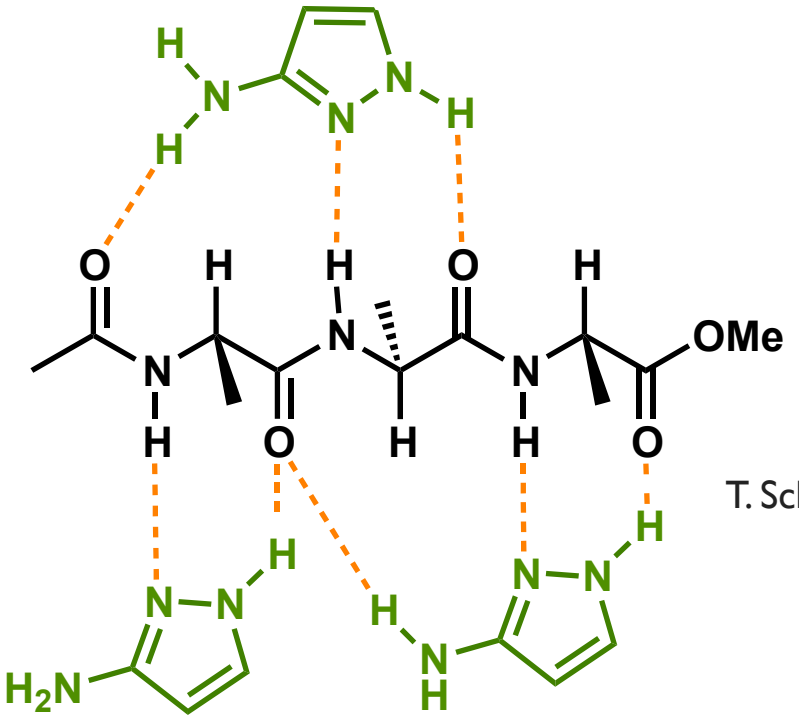
(R = H or methacryloyl)

[replica] T. Schrader et al., *Chem. Commun.* **1996**, 2089.; *JACS* **1997**, *119*, 12061.

AMP & MAMP: top face approach was preferred!
pyrazole: two faces couldn't be distinguished...

Application to Tripeptide

- complexation of AMP with Ac-*L*-Ala-*L*-Ala-*L*-Ala-OMe



[replica]
T. Schrader et al., *JACS* **1997**, *119*, 12061.

(energy minimization
program: CERIUS2
force-field: Dreiding 2.21)

→ Almost same interaction was observed!

§2 β-Sheet Ligand with Aminopyrazole Moiety

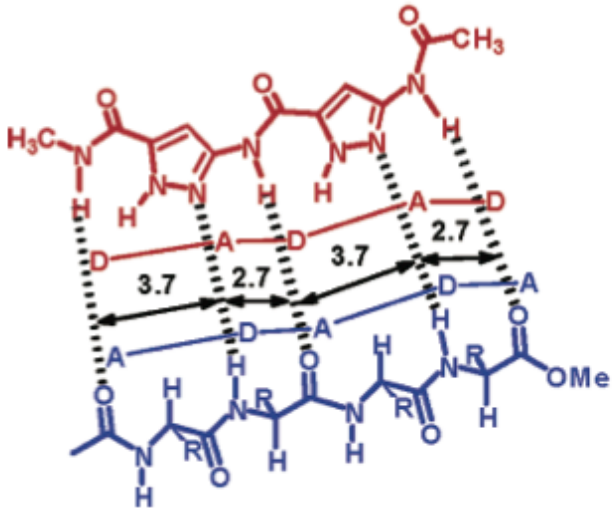
§2.1 Research Background

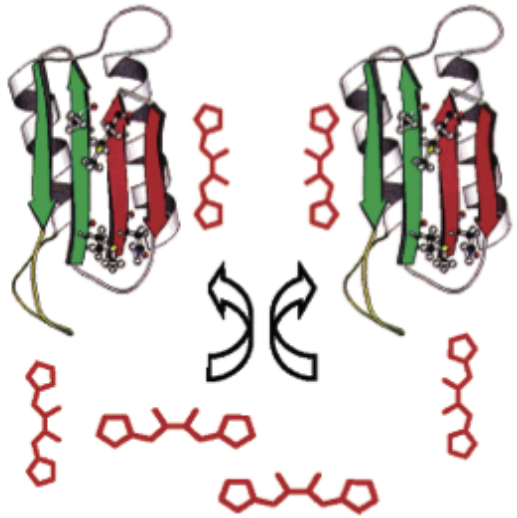
§2.2 Design of β-Sheet Ligand against Aβ42

Application to β-Sheet Ligand

- Aminopyrazole can stabilize β-sheet conformation.

It can also act as a β-sheet ligand!



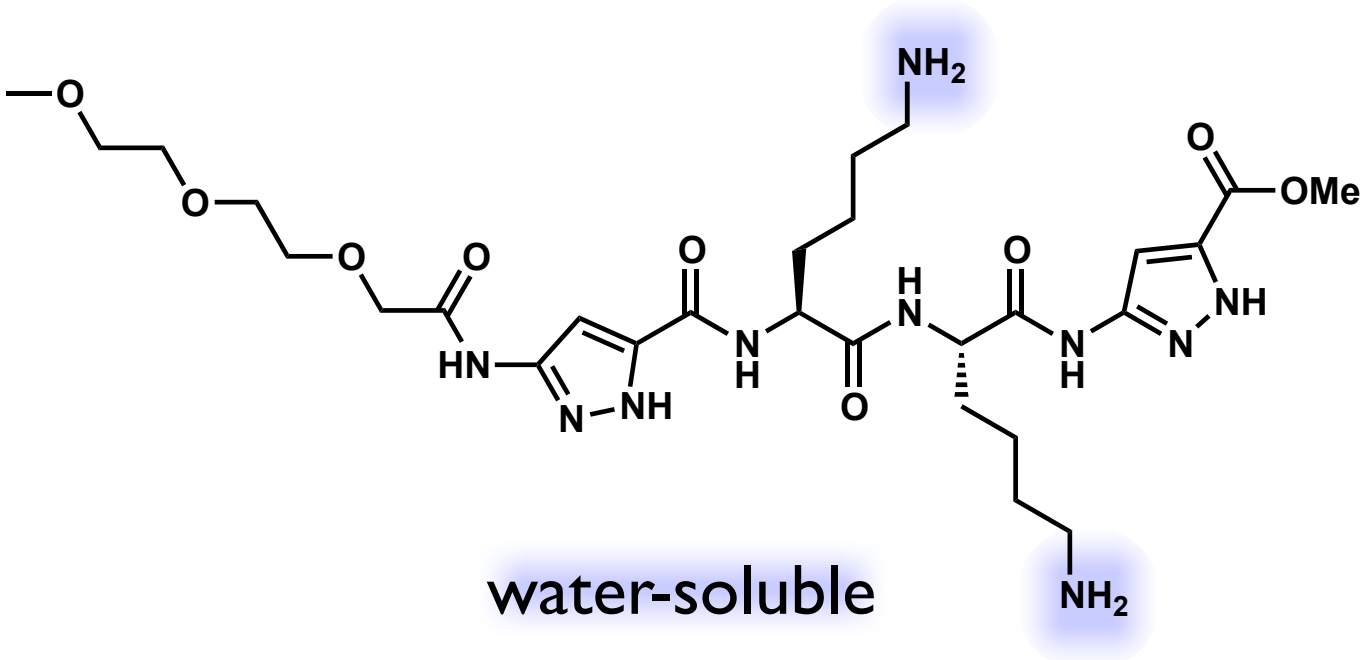


T. Schrader et al., *JOC* **2004**, *69*, 5168.

Water-Soluble β-Sheet Ligand Design

- Structure was optimized by force-field calculations.

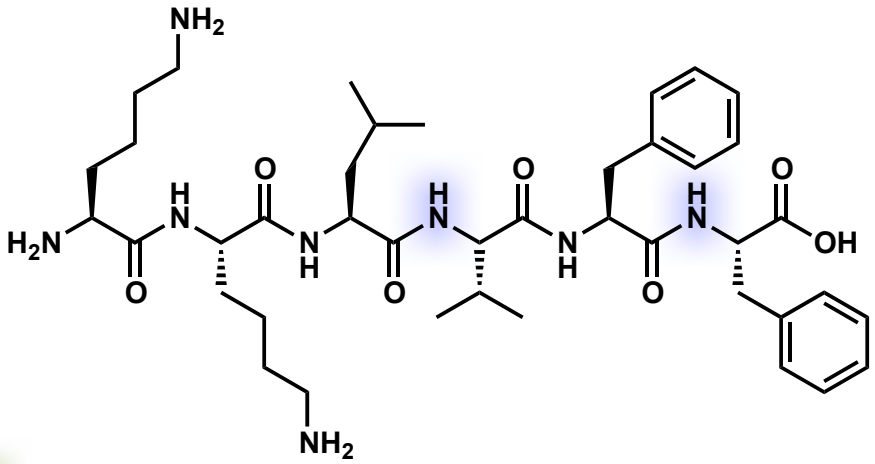
Even number of α-amino acids must be inserted between aminopyrazole moieties.



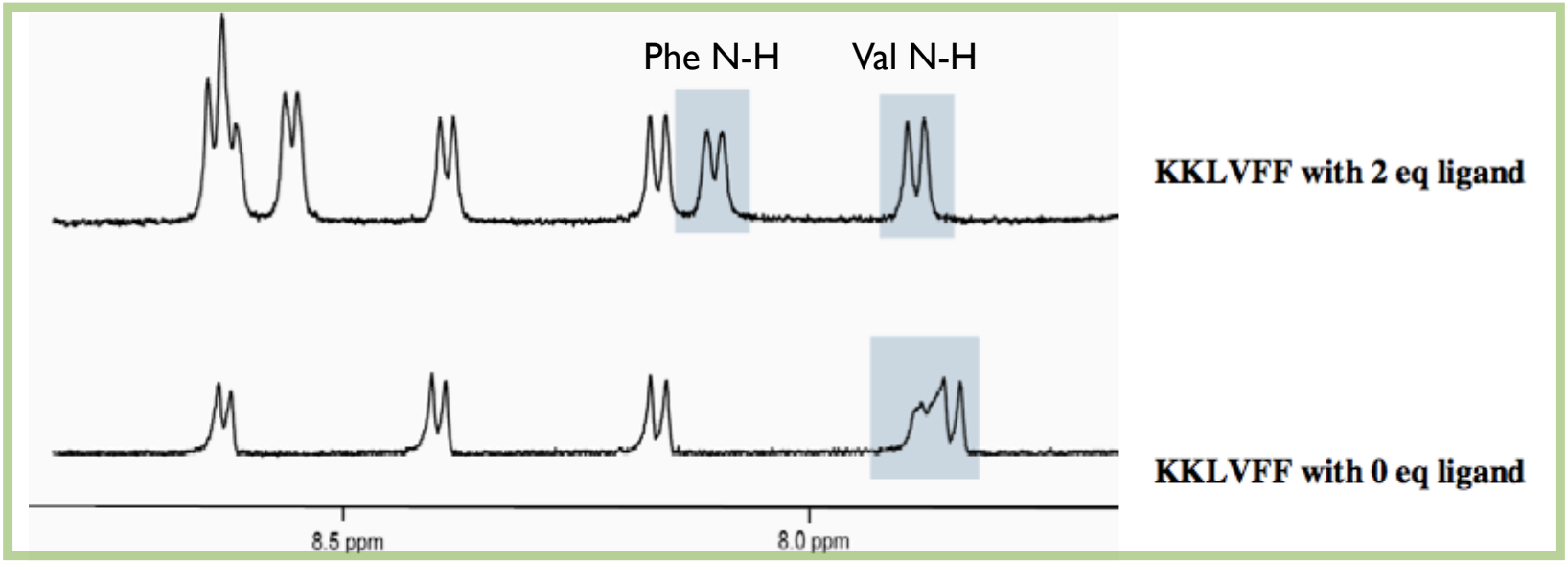
P. Rzepecki and T. Schrader, *JACS* **2005**, *127*, 3016.

Complexation with KKLVFF

KKLVFF ≡



^{1}H-NMR Spectrum

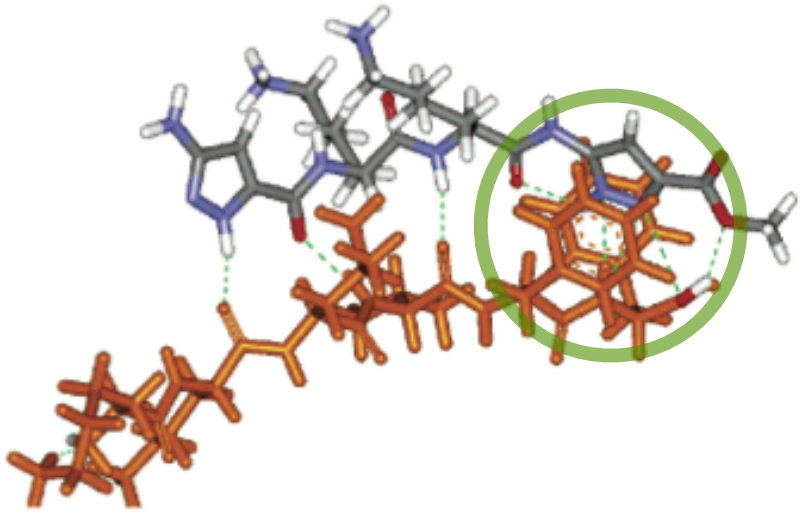


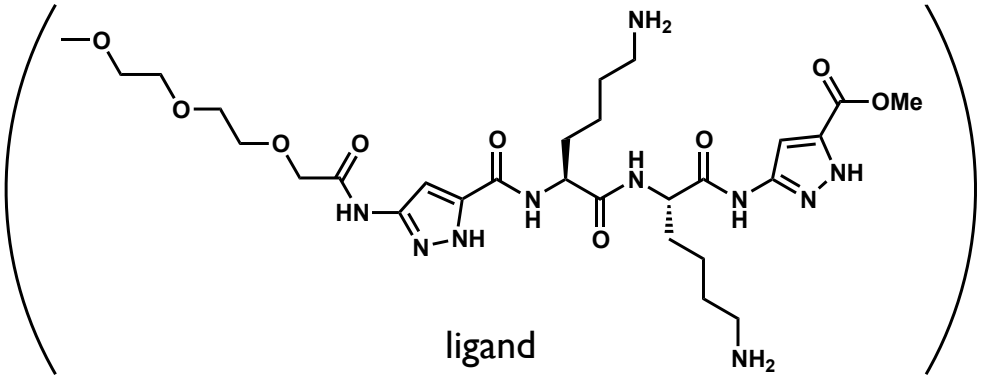
P. Rzepecki and T. Schrader, *JACS* **2005**, *127*, 3016.

Interaction of Ligand and KKLVFF

- Monte Carlo simulation of ligand and KKLVFF

MacroModel 7.2
Amber*
3000steps, water





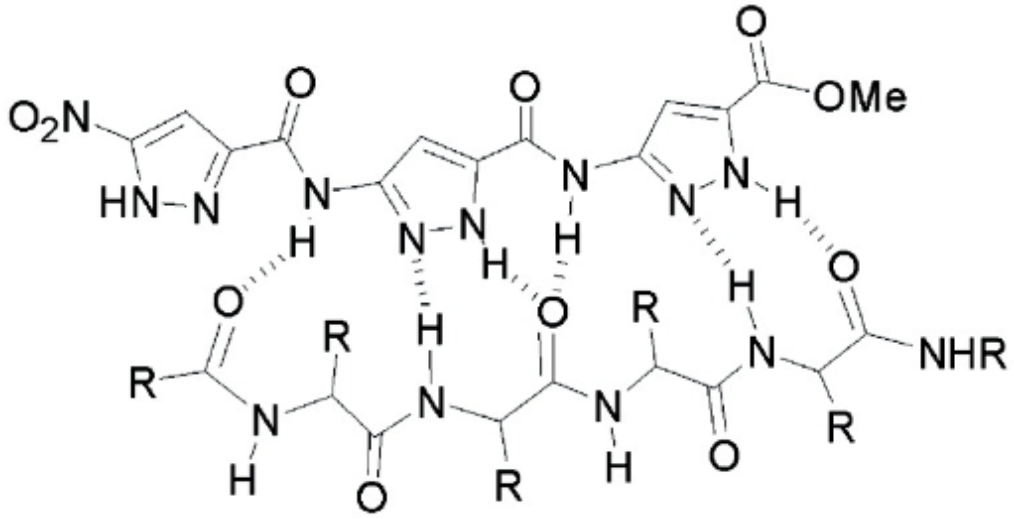
Interaction of pyrazole ring with Phe side chain was observed.

This interaction can be applied to Aβ ligand!

P. Rzepecki and T. Schrader, *JACS* **2005**, *127*, 3016.

Design of β-Sheet Ligand against Aβ42

- They proposed aminopyrazole trimer as the main structure of the ligand.



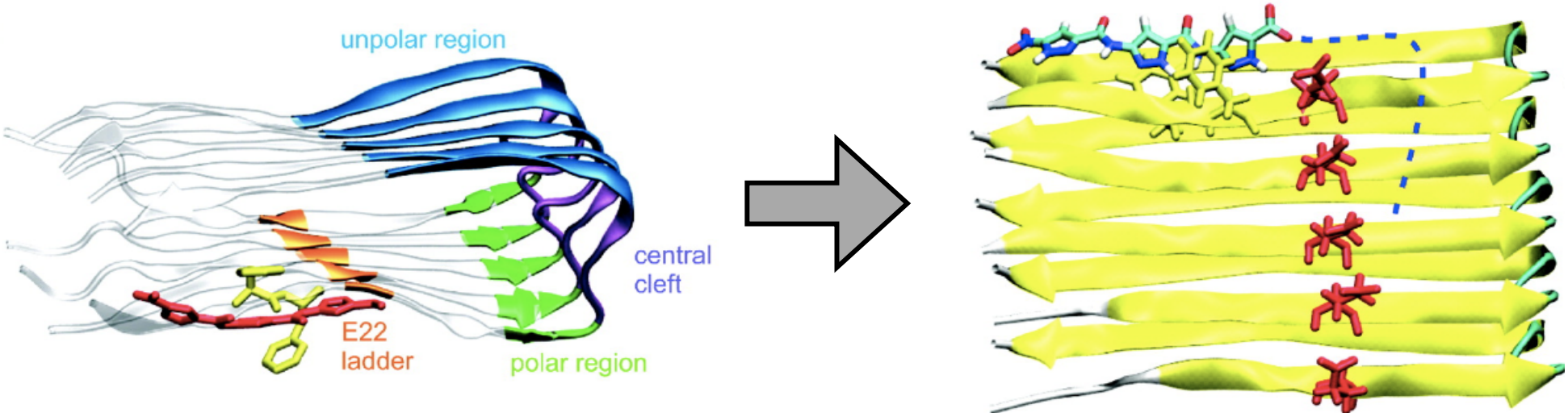
G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

interaction of pyrazole ring and Phe side chain...

This trimer was supposed to interact with residues L17-A21!

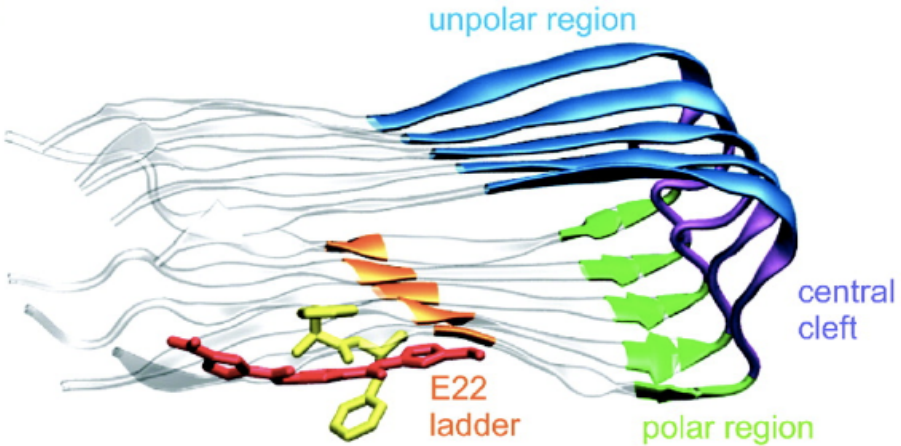
Design of β-Sheet Ligand against Aβ42

- 4 candidate region for interaction (G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.)



Specificity would be increased by attaching C-terminal complementary recognition sites!

Suitable Structure for Each Region



Polar Region (E22-K28) — carboxylate and ammonium groups

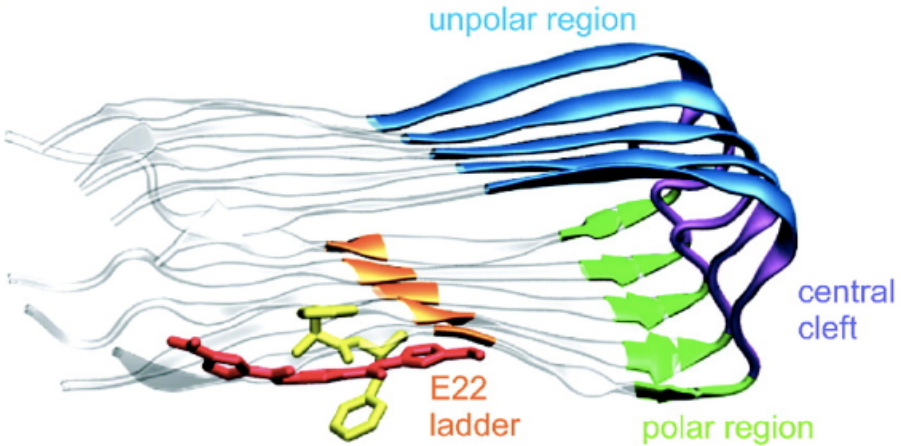
Unpolar Region (I31-V36) — flexible branched hydrocarbons

Central Cleft (G29-A30) — TEG (triethyleneglycol) unit

E22 Ladder — pentacationic appendices

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Which Region can Indeed be Targeted?



Polar Region (E22-K28) ✕

Unpolar Region (I31-V36) ○

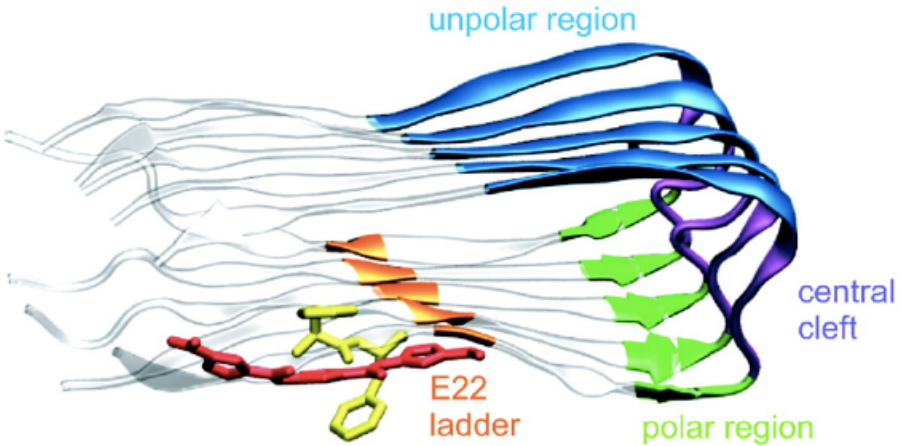
Central Cleft (G29-A30) ○

E22 Ladder ○

D23 and K28 form a stable salt bridge *in silico* and can't access the target region.

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Which Region can Indeed be Targeted?



Polar Region
(E22-K28) ✕

Unpolar Region
(I31-V36) ○

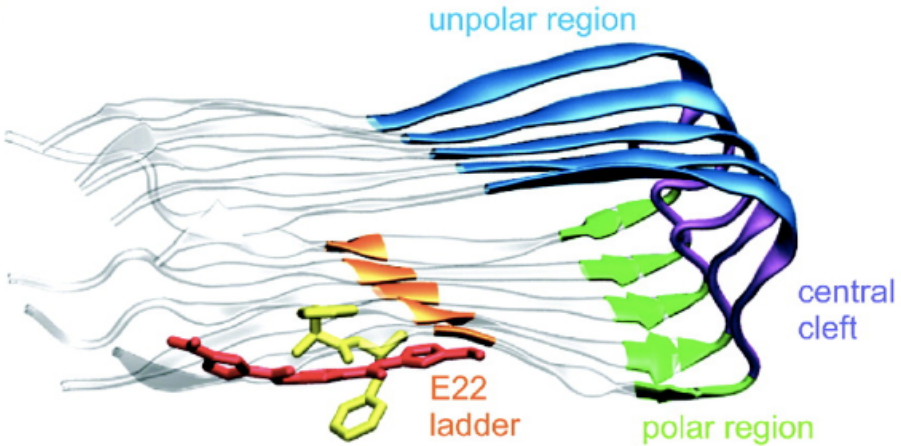
Central Cleft
(G29-A30) ○

E22 Ladder ○

Expected interaction was observed *in silico*.

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Which Region can Indeed be Targeted?



Polar Region (E22-K28) ✕

Unpolar Region (I31-V36) ○

Central Cleft (G29-A30) ○

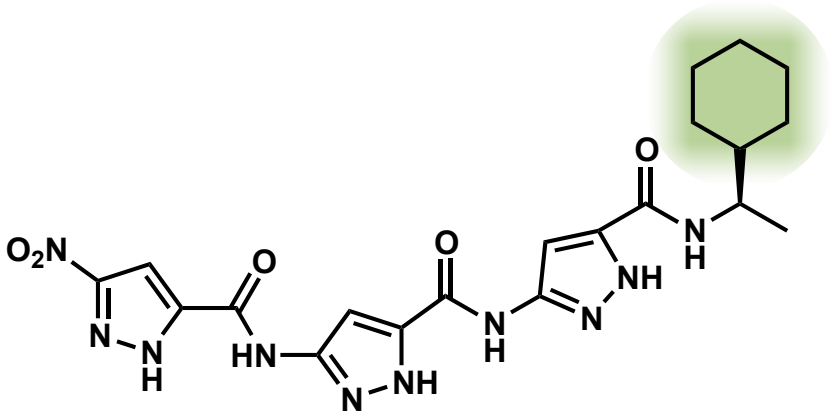
E22 Ladder ○

Long tethers let more than one carboxylate groups interact with penta-Lys.

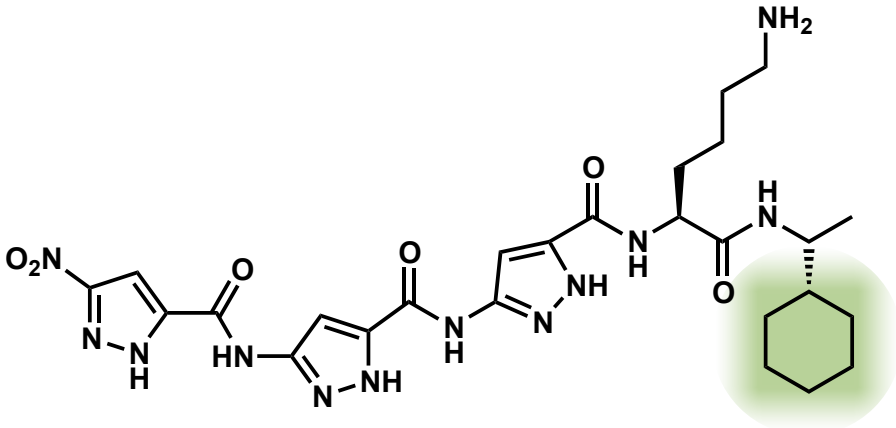
G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to Unpolar Region

- structures and inhibition / disaggregation activity



inhibition: 65%
disaggregation: 62%



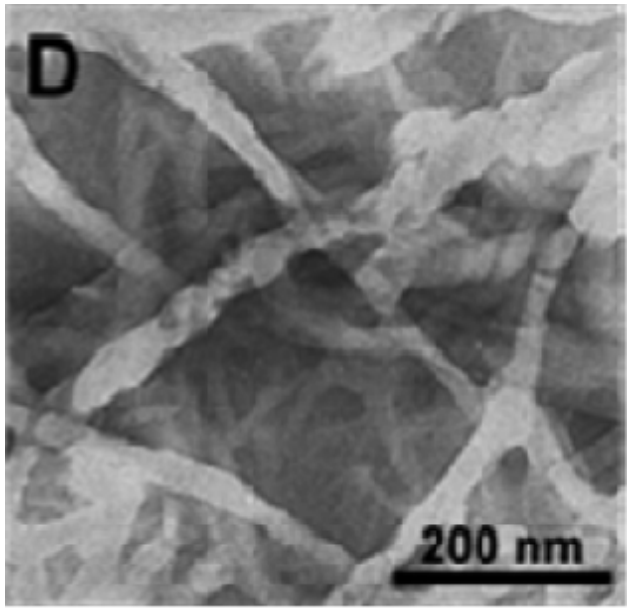
inhibition: 70%
disaggregation: 64%

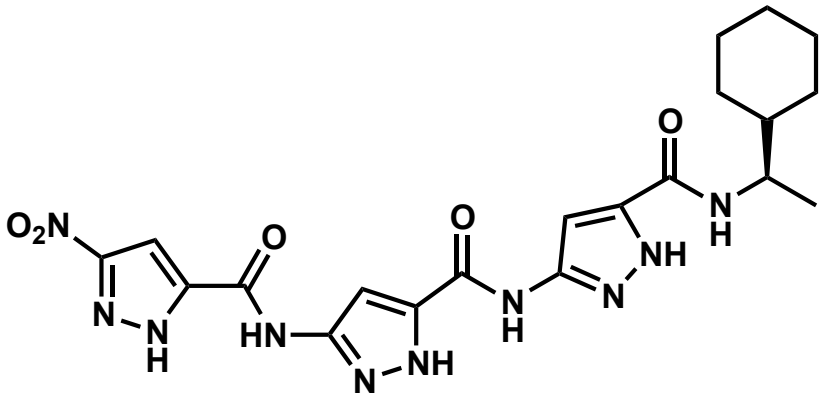
Definition: Aβ alone is 0% for both value

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to Unpolar Region

- Transmission Electron Microscopy (TEM)



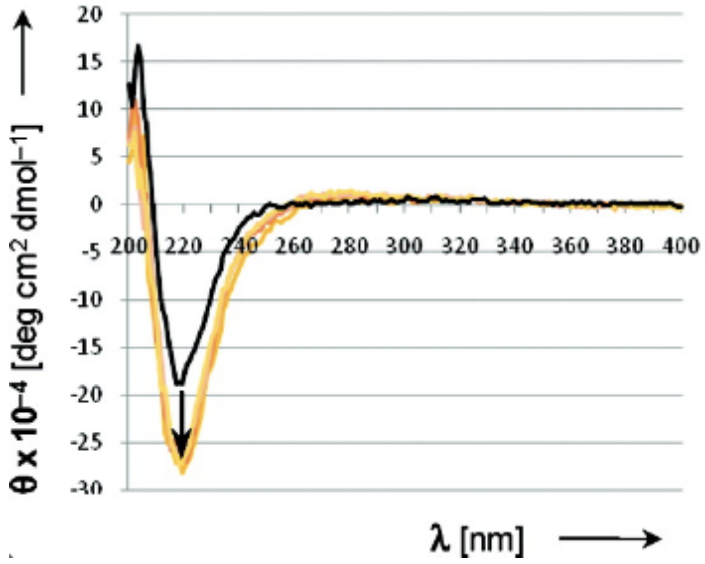


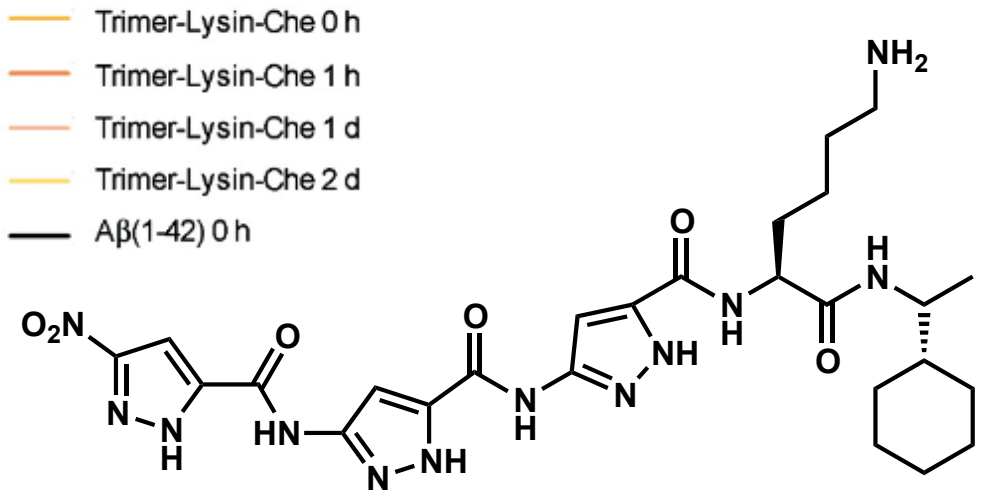
Thick (twisted?) fibril were observed...

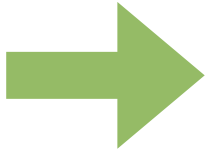
G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to Unpolar Region

- CD spectrometry





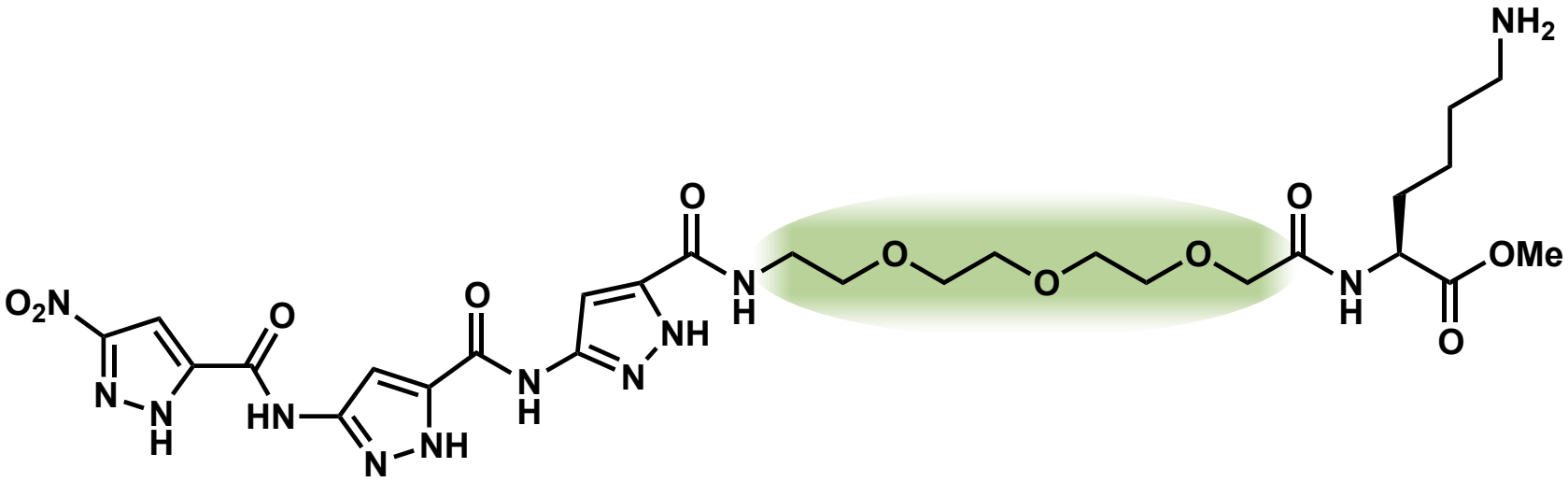


β-sheet band was increased...

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to Central Cleft

- structure and inhibition / disaggregation activity



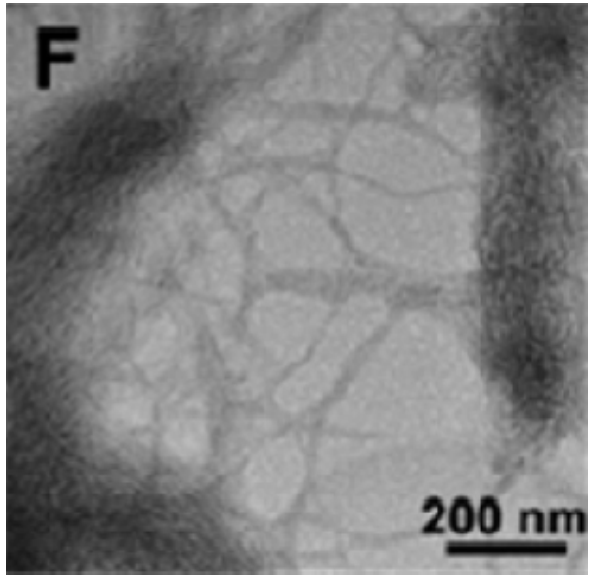
inhibition: 76%
disaggregation: 79%

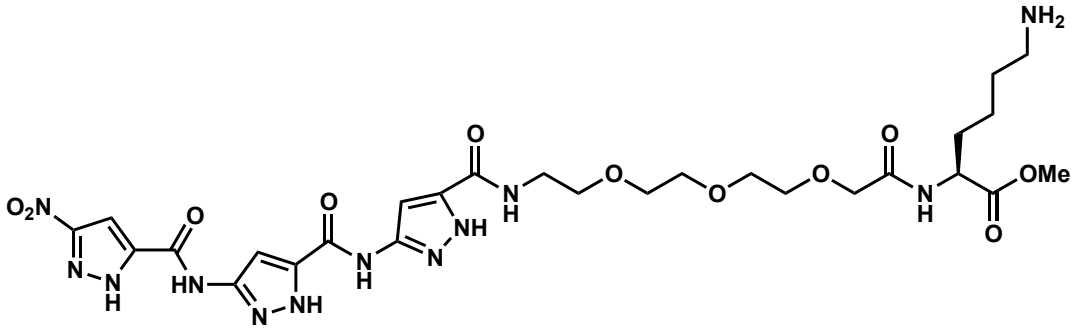
Definition: Aβ alone is 0% for both value

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to Central Cleft

- Transmission Electron Microscopy (TEM)



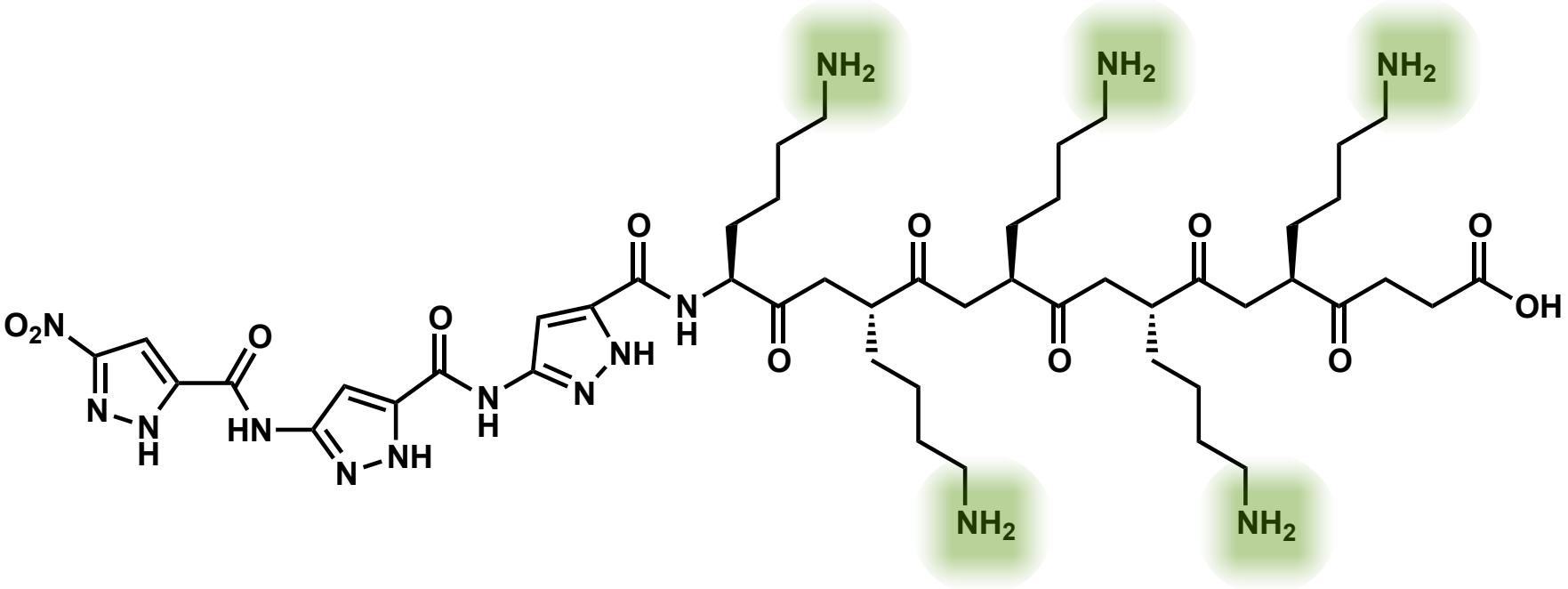


Very thin, delicate structure was observed!

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to E22 Ladder

- structure and inhibition / disaggregation activity



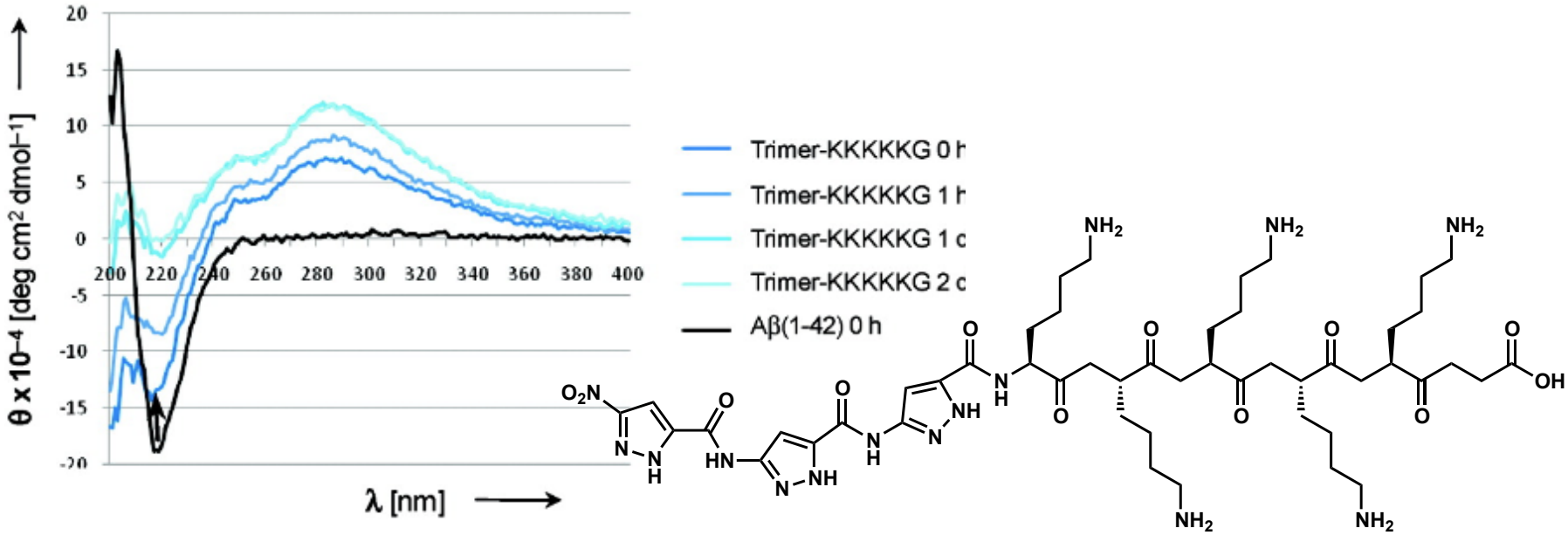
inhibition: 75%
disaggregation: 72%

Definition: Aβ alone is 0% for both value

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Compound Targeting to E22 Ladder

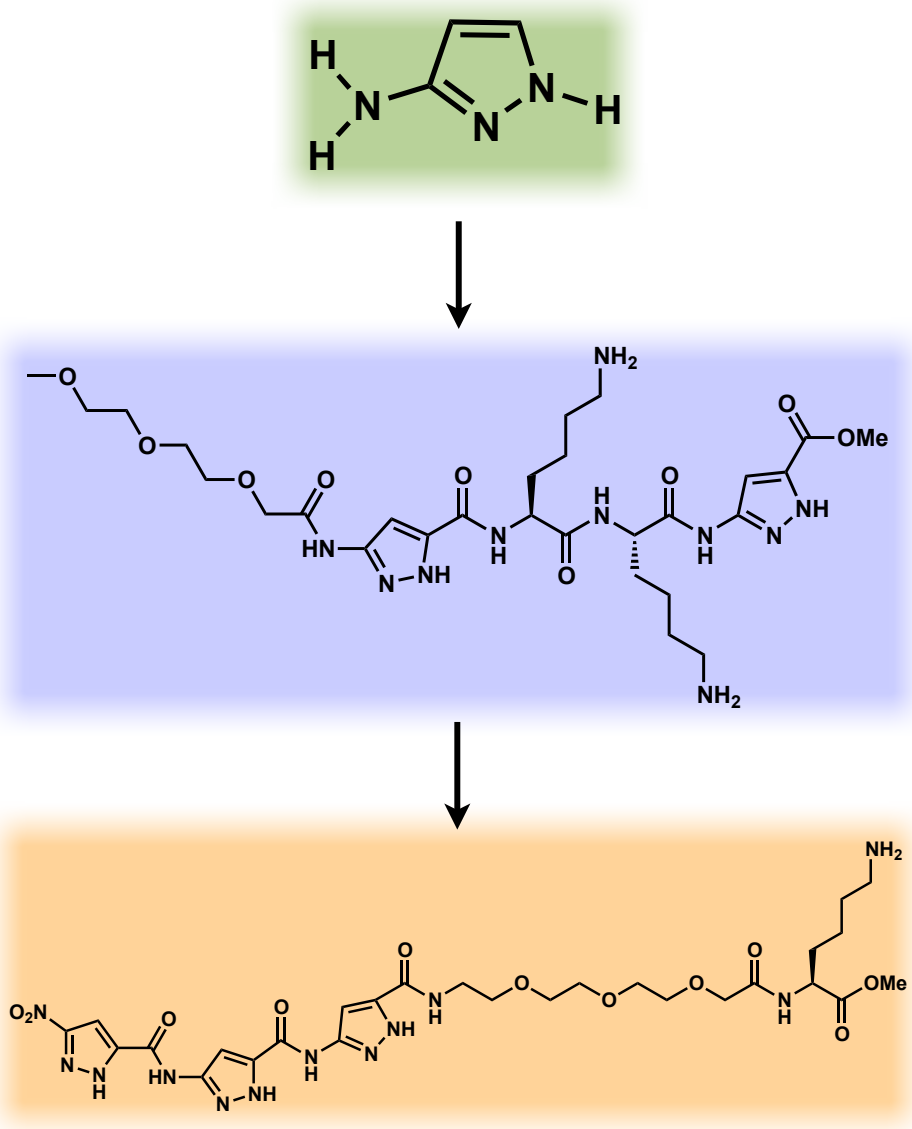
- CD spectrometry



β-sheet was disappeared and random coil was appeared!

G. Bitan, T. Schrader et al., *JACS* **2011**, *133*, 4348.

Summary



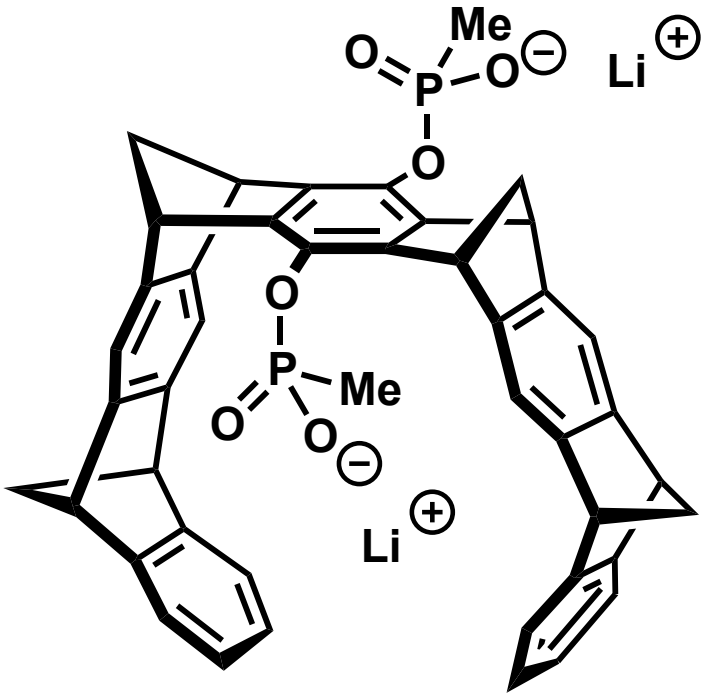
- β-sheet stabilizing molecule
- water-soluble β-sheet ligand
- fibril-structure-based design of β-sheet ligand against Aβ42

§3 Lys-Specific Molecular Tweezers (MTs)

§3.1 Assays of Lys-Specific MTs

§3.2 Research Background

Structure of Molecular Tweezer



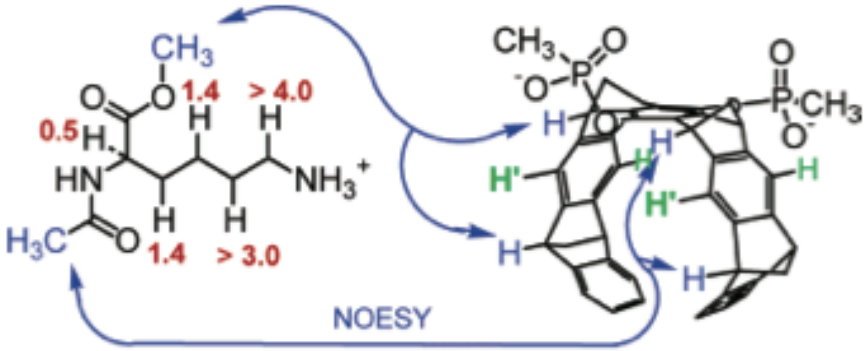
Lys- and Arg-specific molecular tweezer

(T. Schrader et al., *JACS* **2005**, *127*, 14417.)

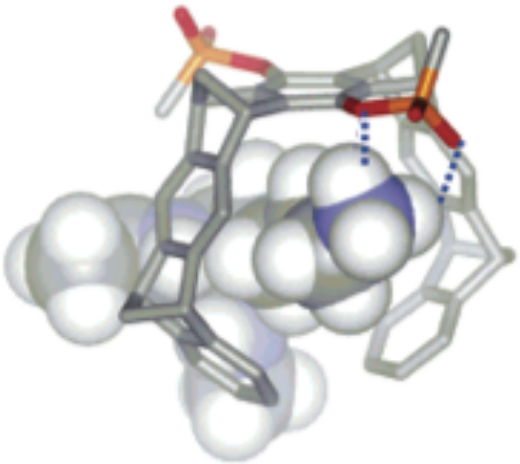
How MT Bind to Lys and Arg?

- binding of MT and Ac-Lys-OMe (T. Schrader et al., *JACS* **2005**, *127*, 14417.)

upfield shift (1H-NMR)
and NOESY cross-peaks

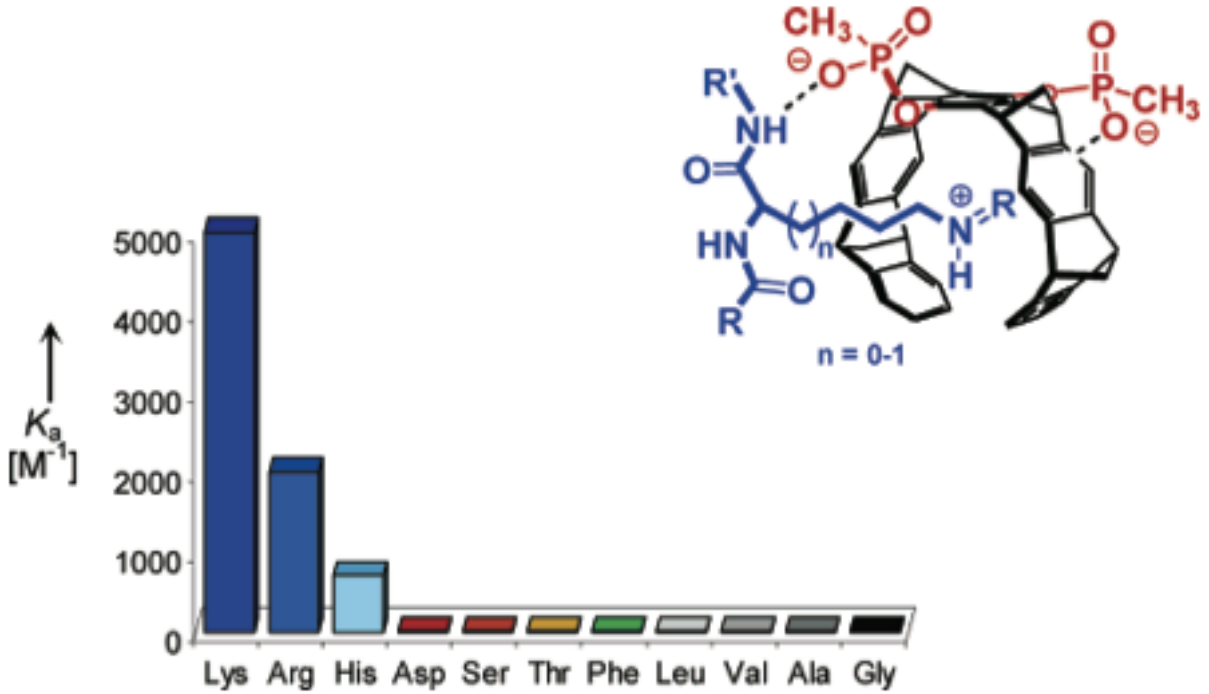


computational simulation



Monte Carlo Simulation
water, 5000steps
MacroModel 7.1, Amber*

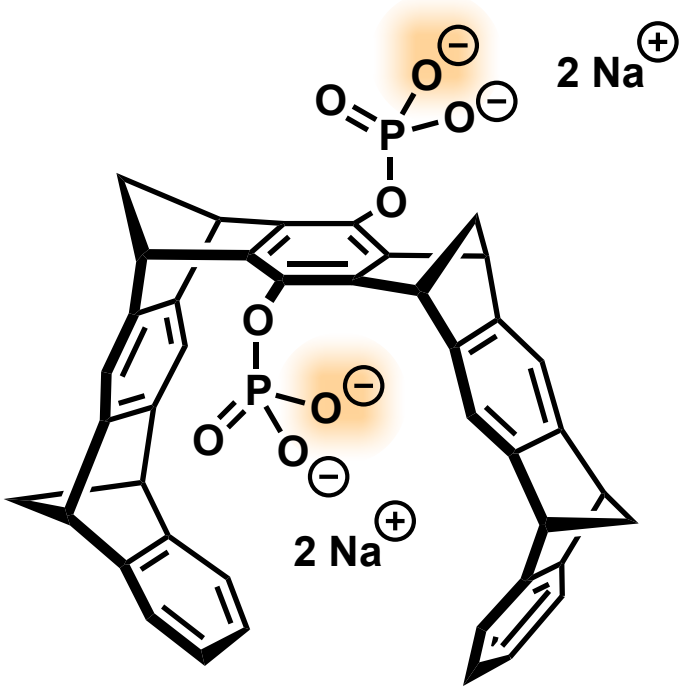
Lys- and Arg-Specificity Evaluation



This MT could bind to Lys and Arg specifically!

T. Schrader et al., *JACS* **2005**, *127*, 14417.

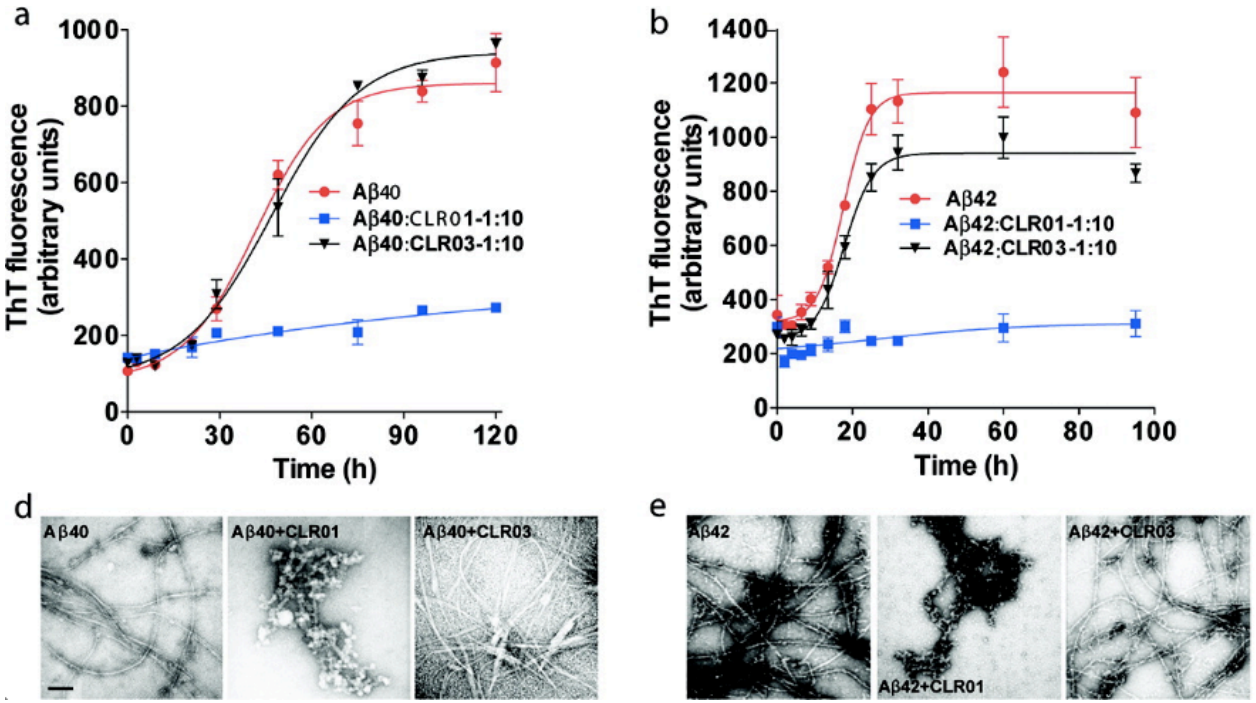
Structure of Lys-Specific MT

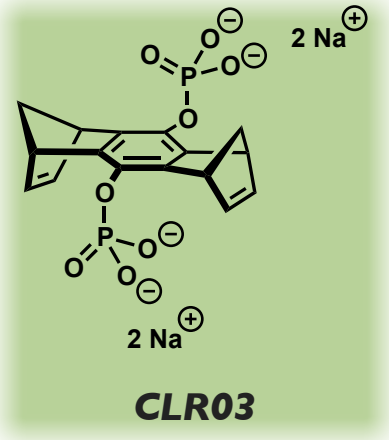


Lys-specific MT "CLR01"
Aβ aggregation inhibitor

(T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.)

Thioflavine T (ThT) Fluorescence Assay



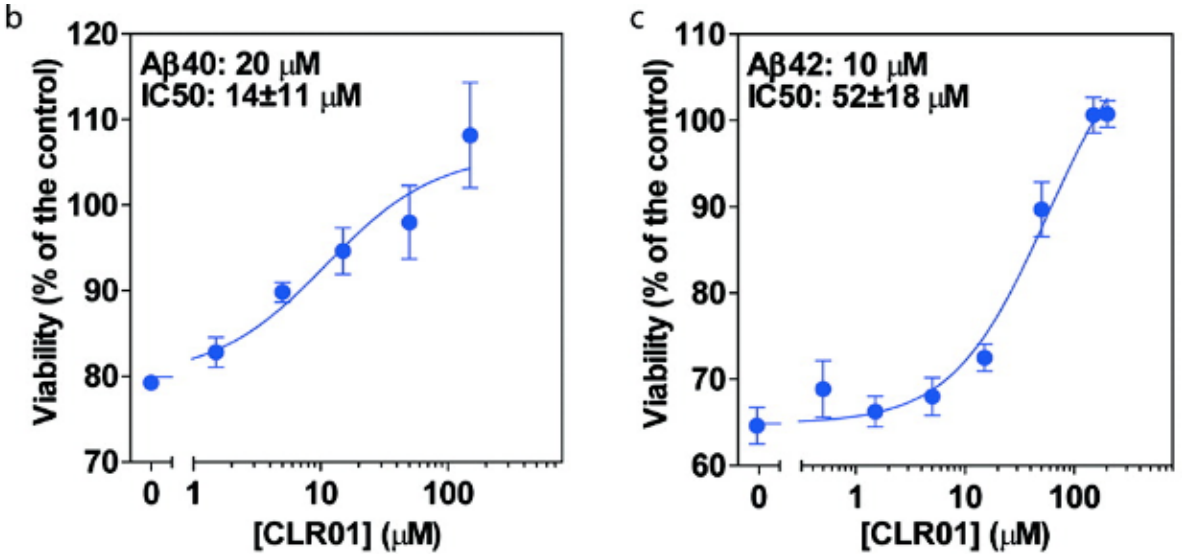


T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

- aggregation → fluorescence increase

→ CLR01 inhibited aggregation of Aβ40 & Aβ42!

Cell Viability Assay

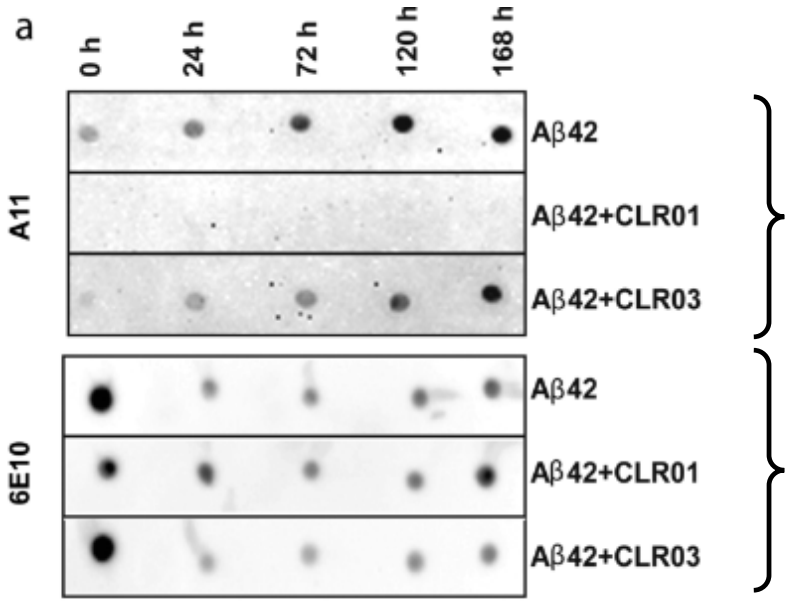


- [CLR01] ≦ 400 μM
- cells: PC-12

T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

Aβ toxicity was inhibited by CLR01!

Immunoreactivity Probing Assay



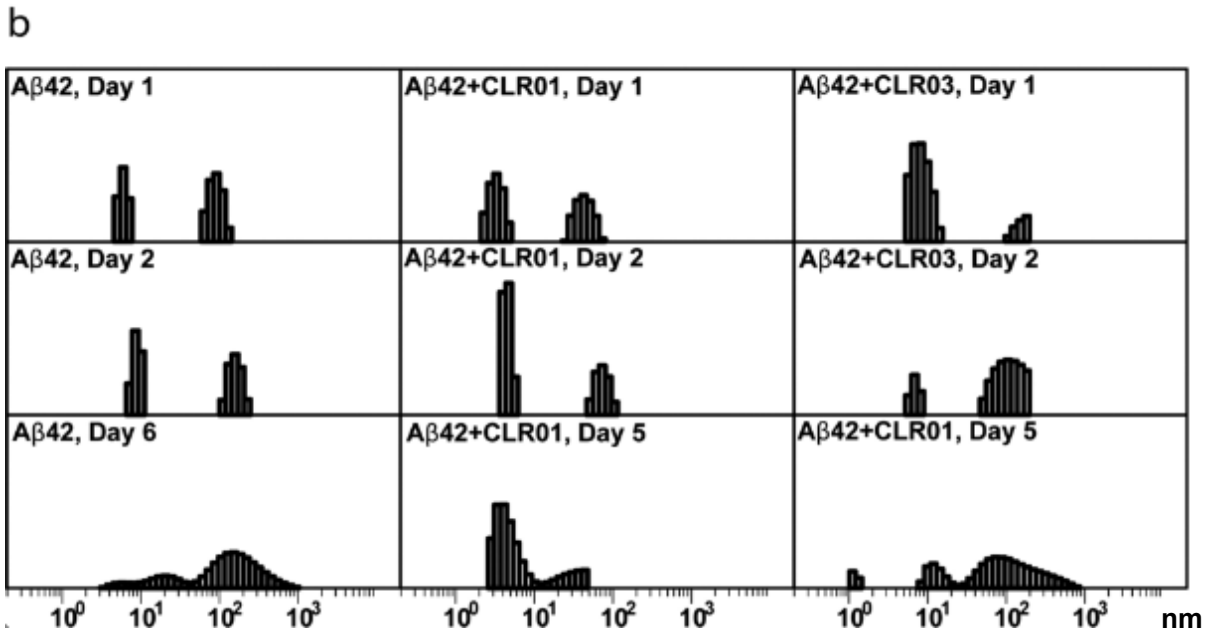
oligomer-specific polyclonal antibody

Aβ-specific monoclonal anitibody

T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

CLR01 inhibited toxic oligomer formation!

Dynamic Light Scattering



T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

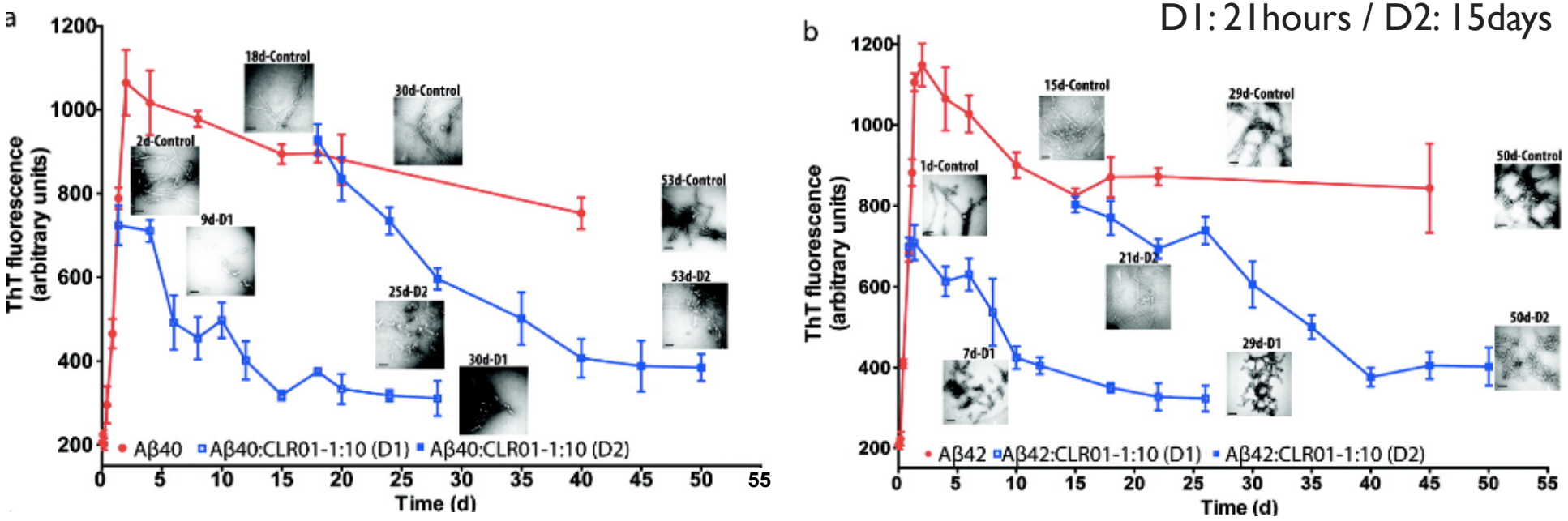
CLR01 inhibited 10^2-10^3 nm size particle formation!

= toxic aggregates

Investigation: nontoxic oligomer was stabilized!

Aβ Fibril Disaggregation Assay

[initiation of aggregation]
D1: 21hours / D2: 15days



T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

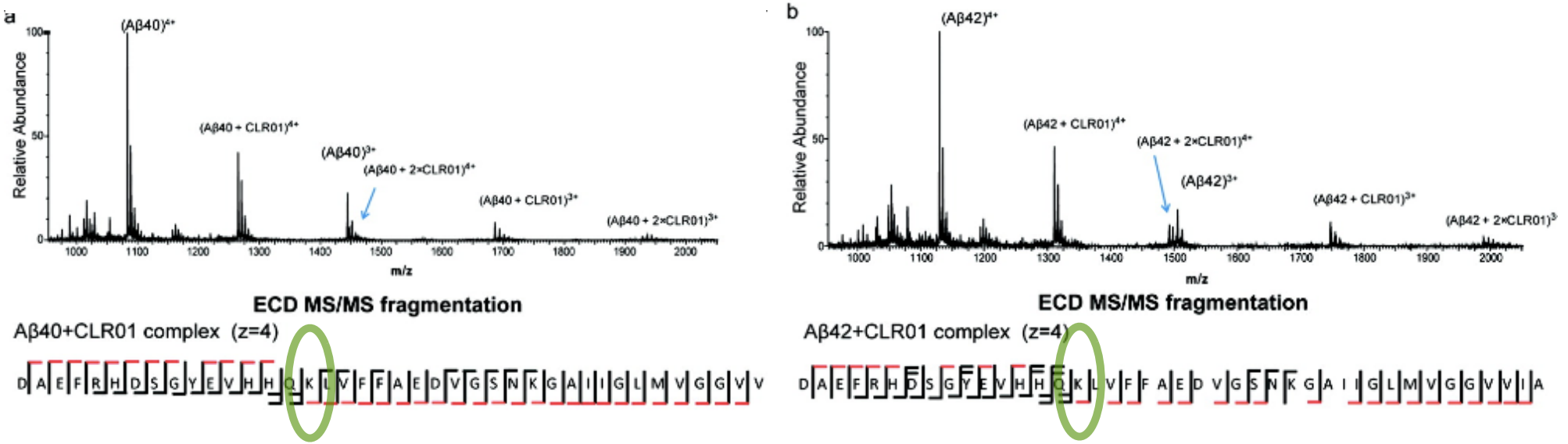
CLR01 could disaggregate Aβ fibrils!

MS & MS/MS Analysis

= fragments with **no** CLR01 attached

= **only** fragments with CLR01 attached are observed

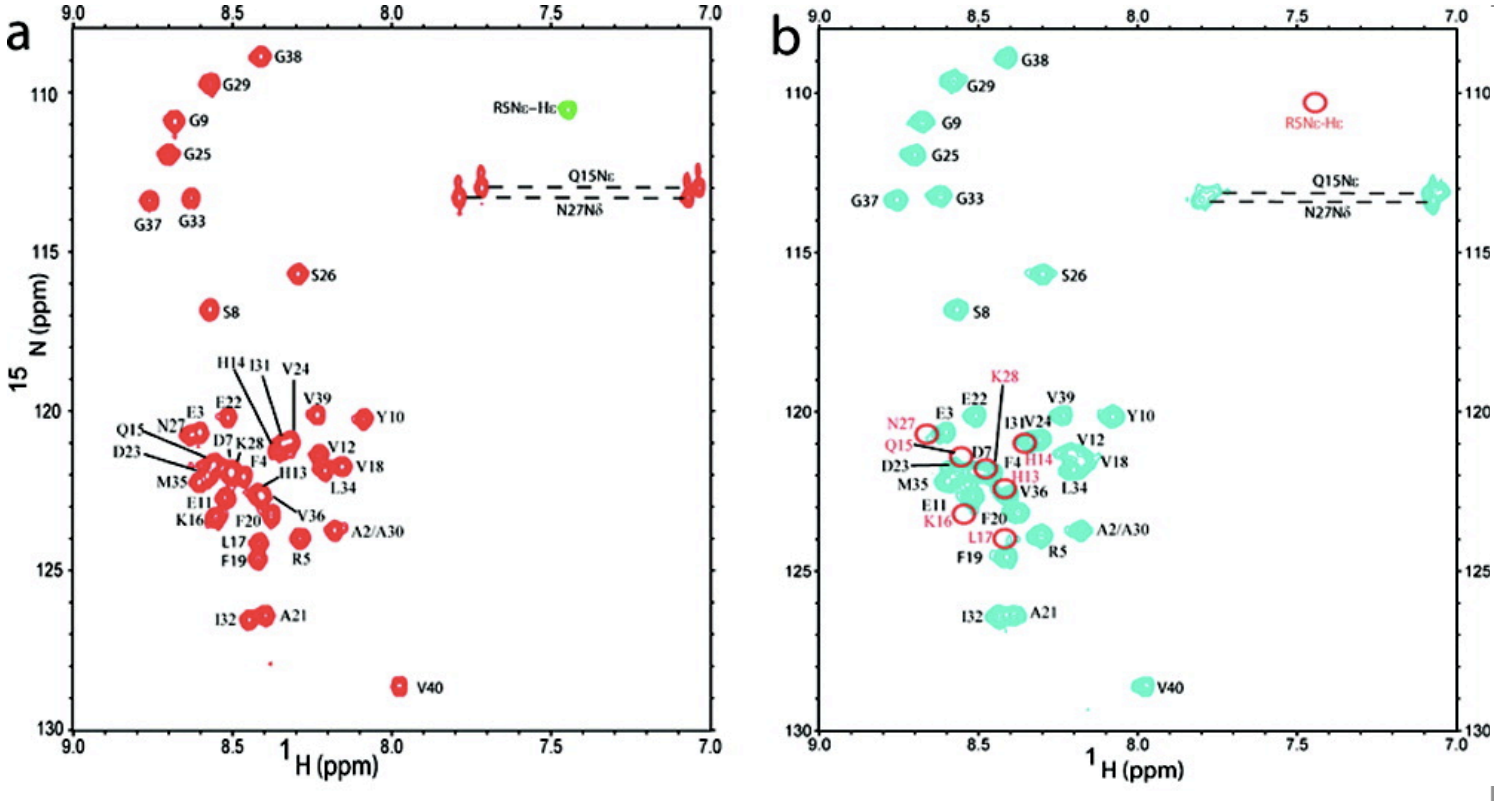
= **both** fragments **with** and **without** CLR01 attached are detected in the same spectrum



T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

CLR01 binding fragments contained Lys!

2D-NMR Analysis (^{15}N-^{1}H HSQC)



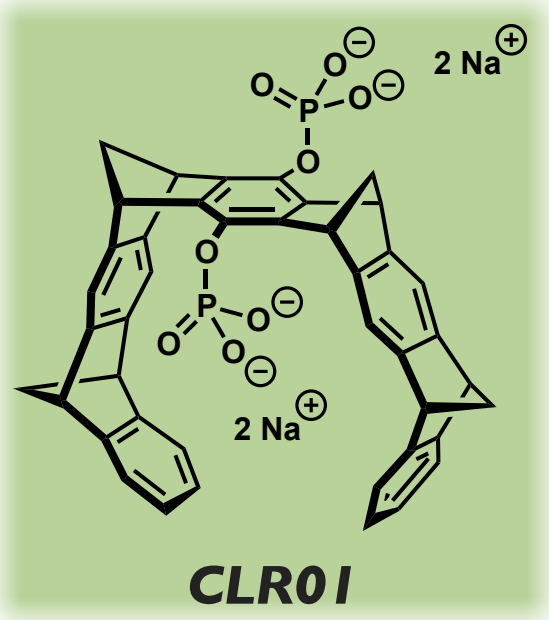
Peaks of Lys, Arg and His disappeared.

Taking care of MS/MS result...

CLR01 bound to Lys specifically!

T. Schrader, G. Bitan et al., *JACS* **2011**, *133*, 16958.

Summary



T. Schrader, G. Bitan et al.,
JACS **2011**, *133*, 16958.

- inhibited aggregation
- inhibited toxicity of oligomer by forming nontoxic oligomer
- disaggregated Aβ fibril
- bound to Lys of Aβ specifically

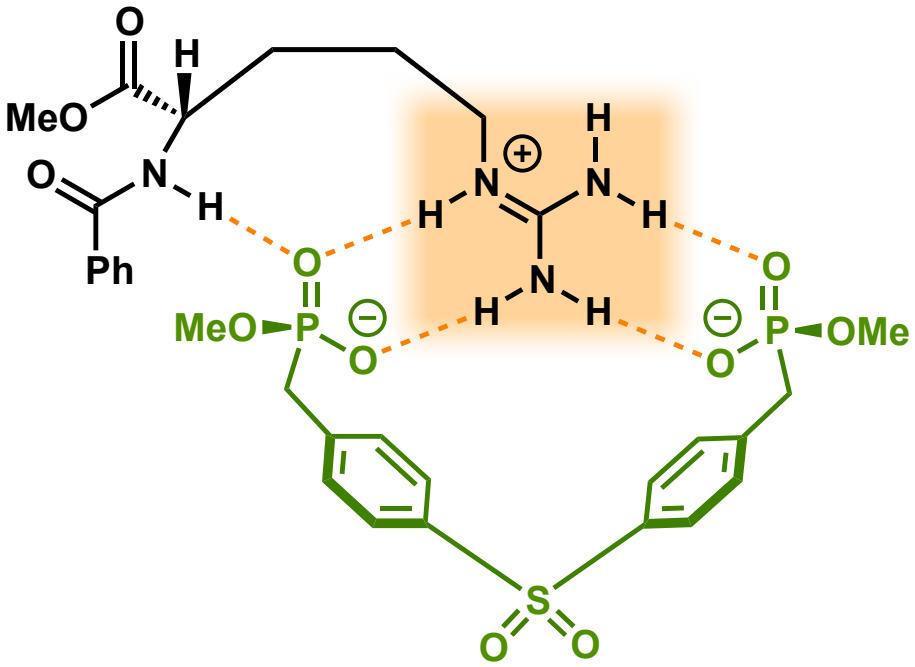
§3 Lys-Specific Molecular Tweezers (MTs)

§3.1 Assays of Lys-Specific MTs

§3.2 Research Background

Alkylguanidium Ions Binding by MTs

- Several cation recognition molecule has been developed.



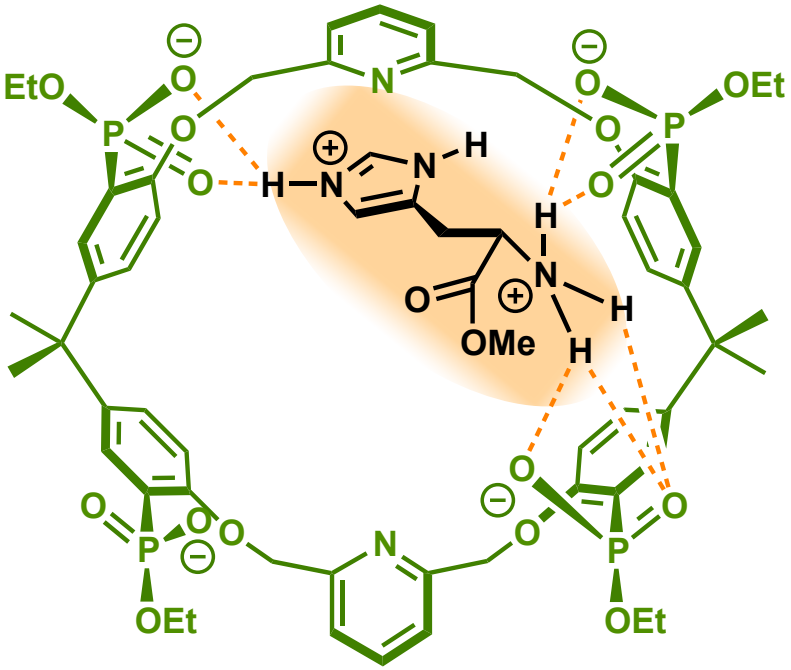
recognition by hydrogen bond

[replica] T. Schrader, *Chem. Eur. J.* **1997**, *3*, 1537.

(force-field calculations
program: CERIUS2
force-field: Dreiding 2.21)

Macrocyclic Receptor for Lys and His

- Only interaction with His methyl ester is shown below.



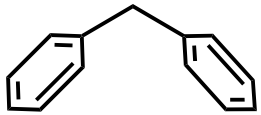
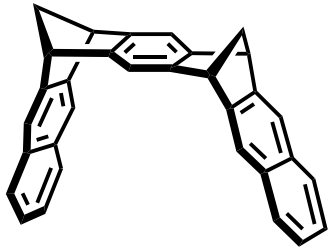
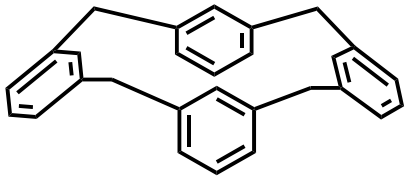
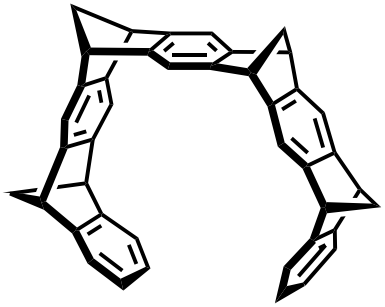
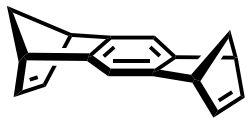
recognition by hydrogen bond and cation-π interaction

This receptor has a room to catch one more His methyl ester.

[replica] T. Schrader et al., *OL* **2001**, *3*, 1597. (energy minimization program: CERIUS2 force-field: Dreiding 2.21)

Molecular Electrostatic Potential

- electron-negativity of non-conjugated arenes' concave face

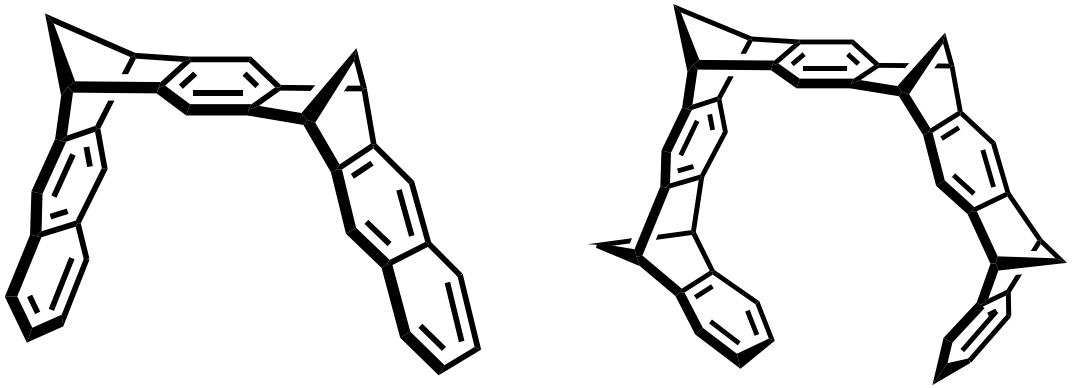
Compound	MEP [kcal/mol]	Compound	MEP [kcal/mol]
	concave: –42.90 convex: –37.50		concave: –49.95 convex: –39.70
	concave: –41.14 convex: –29.30		concave: –54.49 convex: –48.35
	concave: –50.41 convex: –44.50		

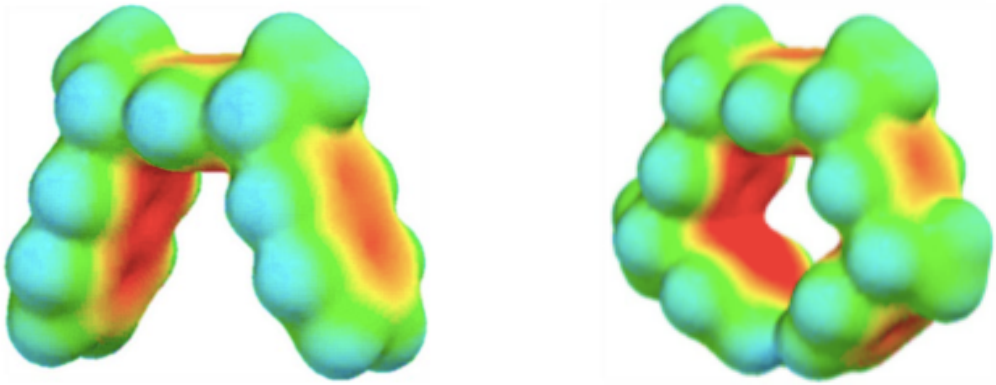
density functional theory calculation (pBP/DN**)

F.-G. Klärner et al., *J. Mol. Model.* **2000**, *6*, 318.

Electro-Static Potential Surface

- non-conjugated arenes' electro-static potential surface



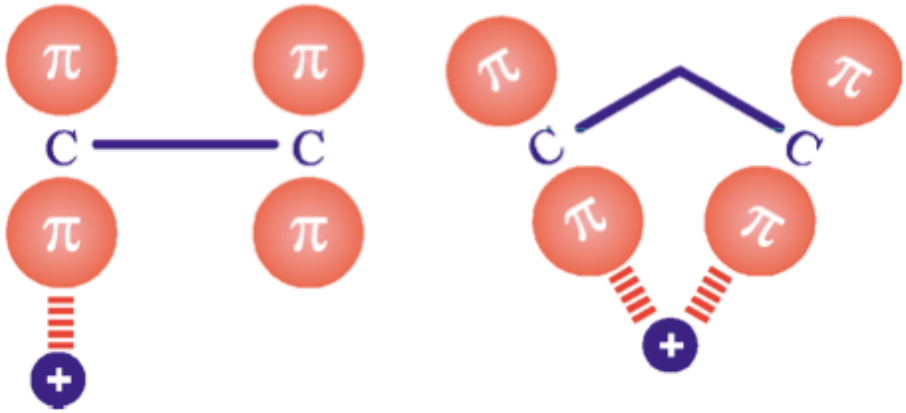


Red color shows electron-negative region.

semiempirical theory calculation (AM1)

F.-G. Klärner et al., *J. Mol. Model.* **2000**, *6*, 318.

Why Concave Face is Electron-Negative?

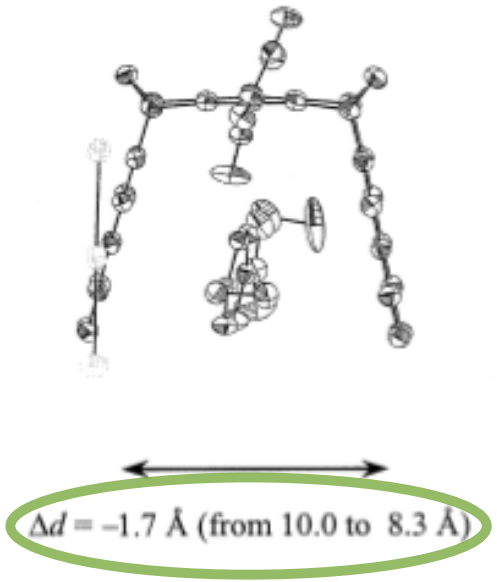


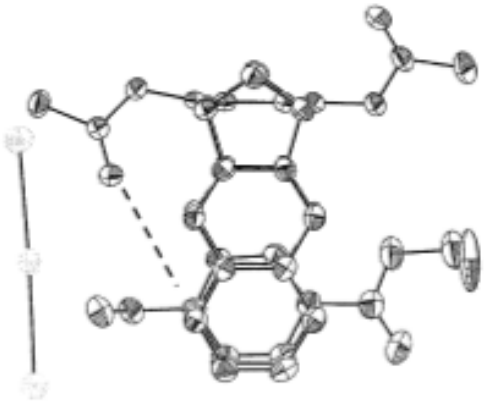
F.-G. Klärner et al., *J. Mol. Model.* **2000**, *6*, 318.

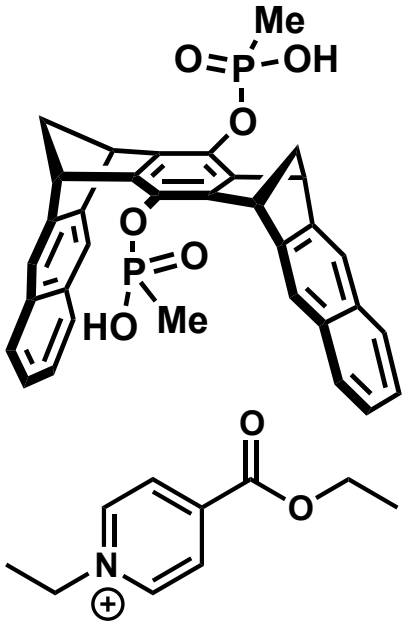
Concave face was more electron negative than isolated one π-electron system.

Strong cation-π interaction was expected!

Fixed Distance Between Aromatic Walls



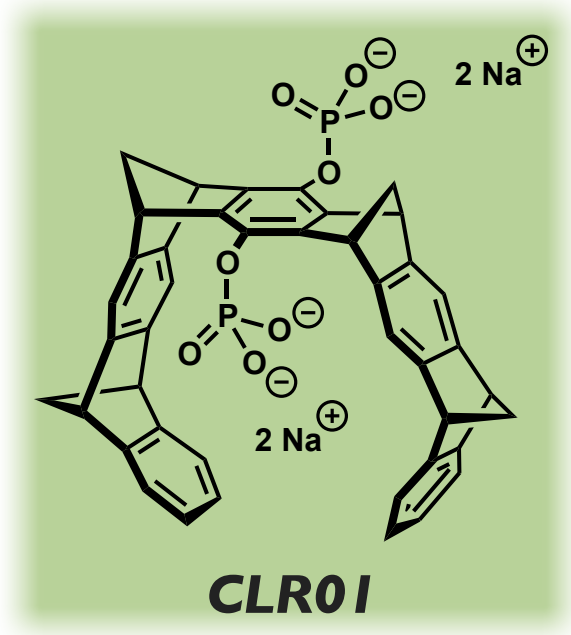




T. Schrader, F.-G. Klärner et al., *ACIE* **2002**, *41*, 1355.

This character could lead to substrate specificity!

Summary



T. Schrader et al., *JACS* **2011**, *133*, 16958.

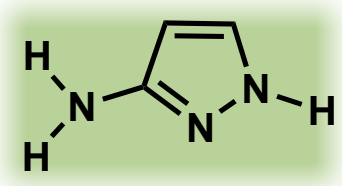
- origin … cation recognition

- Concave face of CLR01 was more electron-negative than an isolated π-electron system.
- Cavity width was fixed.

strong and specific binding to Lys

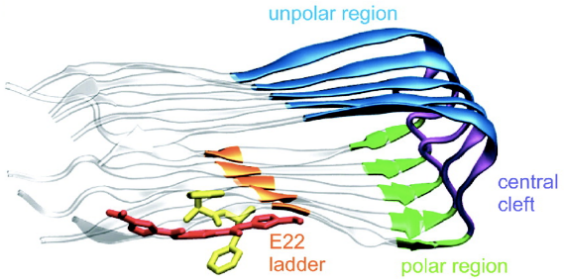
Summary of all Sections

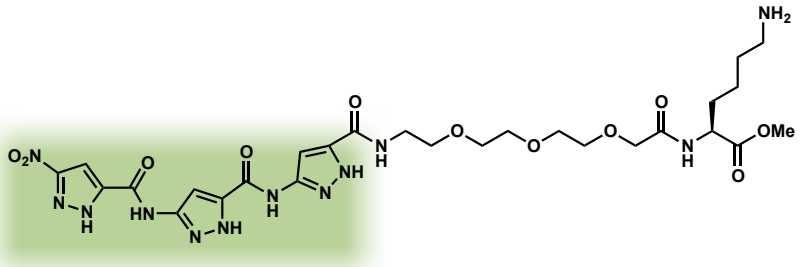
- inhibitor design starting from β-sheet stabilizing



aminopyrazole

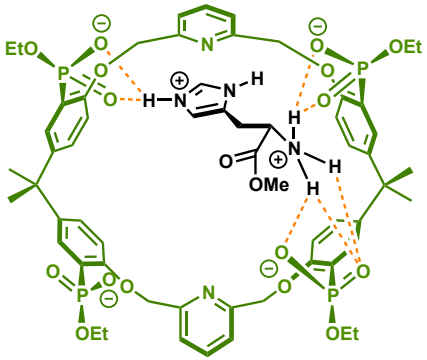
structure-based design





Trimer-TEG-*L*-Lys-OMe

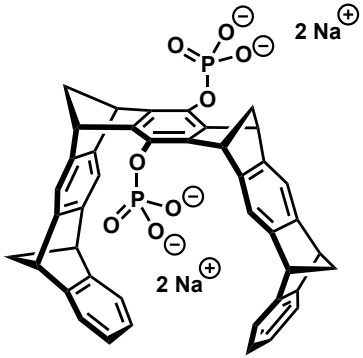
- inhibitor design starting from cation recognition



His, Lys-specific

fixed cavity width

cation-π interaction



Lys-specific