A carboxylated Zn-phthalocyanine inhibits the fibril formation of Alzheimer's amyloid β peptide

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Background and Purpose

Alzheimer's Disease

Alzheimer's disease (AD) is a common dementia disease of the elderly

Histopathological features of AD

1. **Degenerative and dystrophic neurons**
2. **Reactive glial cells**
3. **Extracellular Aβ peptide deposition**
4. **Intracellular neurofibrillary tangles**

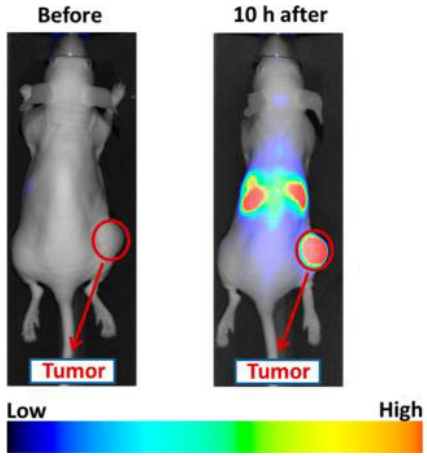
Genetic and animal studies demonstrated the vital role of Aβ peptide in AD pathology

Current Management of Alzheimer's Disease

- **Diagnosis**
  - **Mainly depends on clinical assessment**
  - **Neuro-imaging is valuable, but either expensive or nonspecific**
  - **Definitive diagnosis can be done by autopsy examination of patient's brain.**
- **Treatment**
  - **Currently no disease modifying therapy is available**

Near-infrared Imaging

- **Diagnostic tools**
  - **Near-infrared imaging is a technique to detect target molecules with near-infrared probe.**
- **Example**
  - **NIR fluorophore conjugated folic acid is used as a probe to visualize folate receptor**
  - **Phthalocyanine-LHRH is used to visualize tumor *in vivo***



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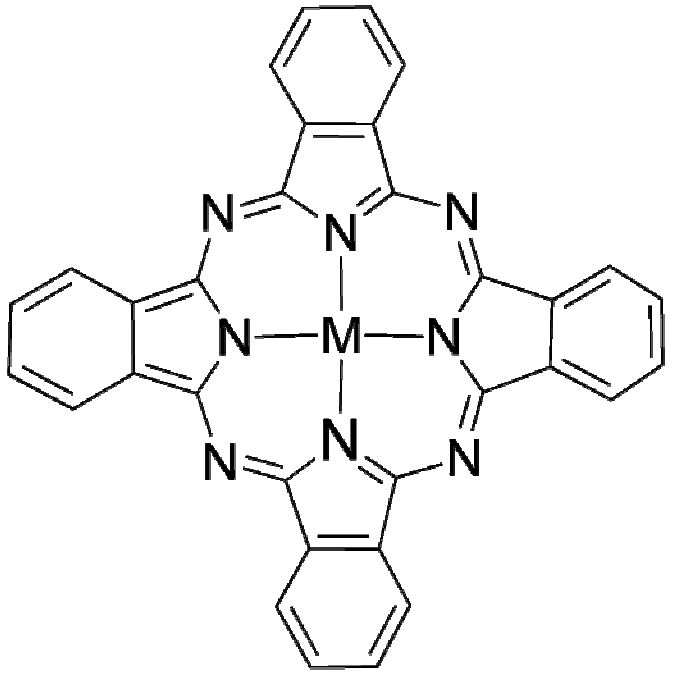
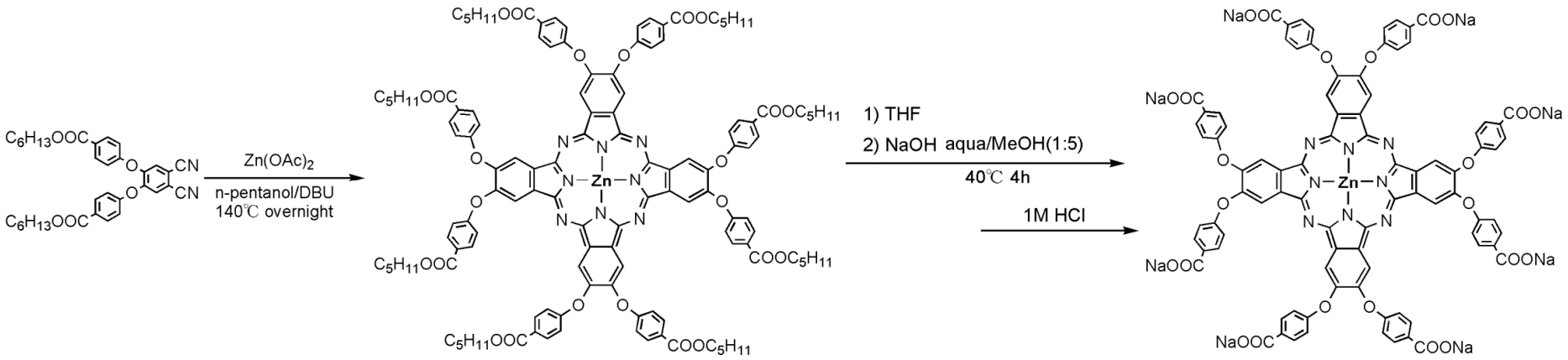
Phthalocyanine

- **Phthalocyanine is a large macrocyclic compound having optical properties in near-infrared region**

- **Water insoluble or tends to aggregate in water, resulting quenching of fluorescence properties**
- **Water soluble phthalocyanine substituted with sodium carboxylate group was prepared.**

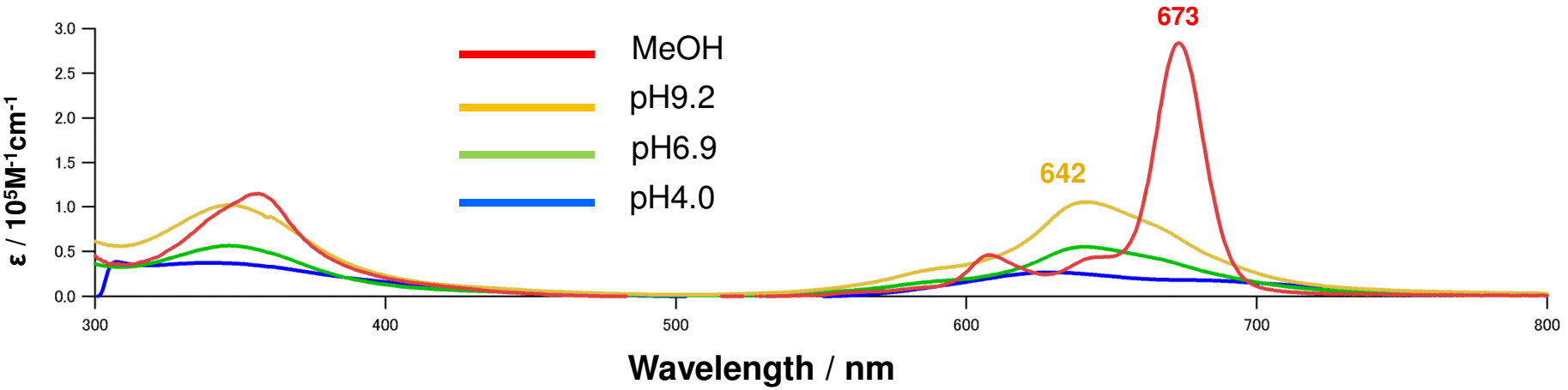


Syntheses and Properties of ZnPc Complex



yield 46%

UV-vis Spectra of $ZnPc(COONa)_8$ in Buffer Solutions and MeOH at R.T.



Perspective

- **Digagnostic strategy**
  - **Can phthalocyanine bind to Aβ be detected with NIRS?**

- **Therapeutic strategy**
  - **Potential of phthalocyanine for inhibition of amyloidogenesis**

Aim of this study

To investigate the interaction of phthalocyanine with Aβ peptide during fibril formation process

Methods and Results

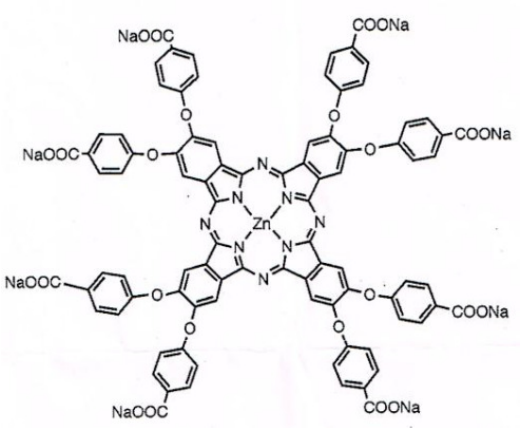
Phthalocyanines:

1. Inhibition of Aβ fibril formation
2. Destabilization of Aβ fibril
3. Binding to Aβ
4. Secondary structure and hydrophobicity
5. Inhibition of oligomer formation
6. Cell toxicity

Phthalocyanines used in the study

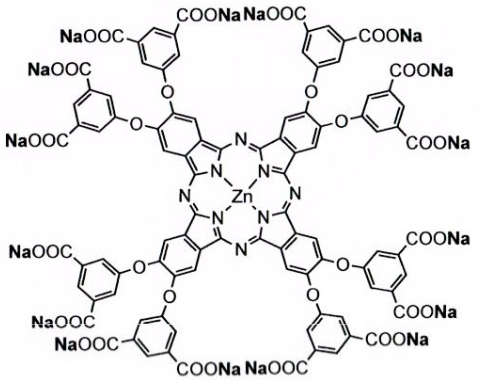
(A)

$ZnPc(COONa)_8$
(water soluble)



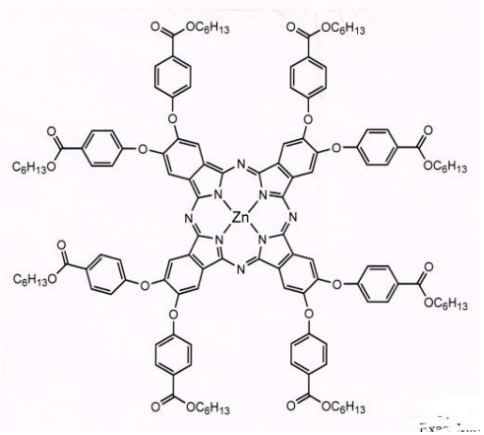
(B)

$ZnPc(COONa)_{16}$
(water soluble)



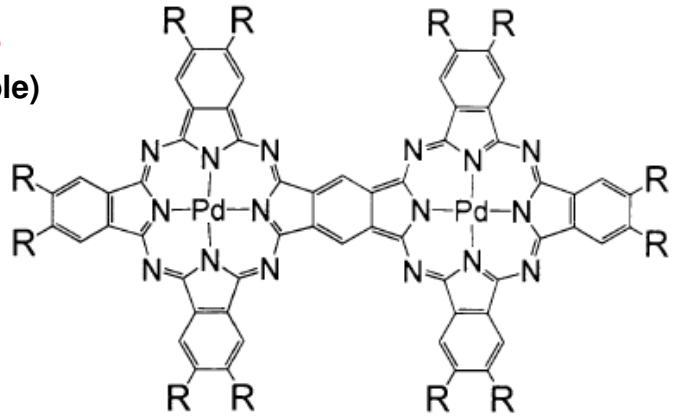
(C)

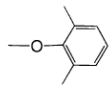
$ZnPc(OOC_5H_{11})_8$
(water insoluble)



(D)

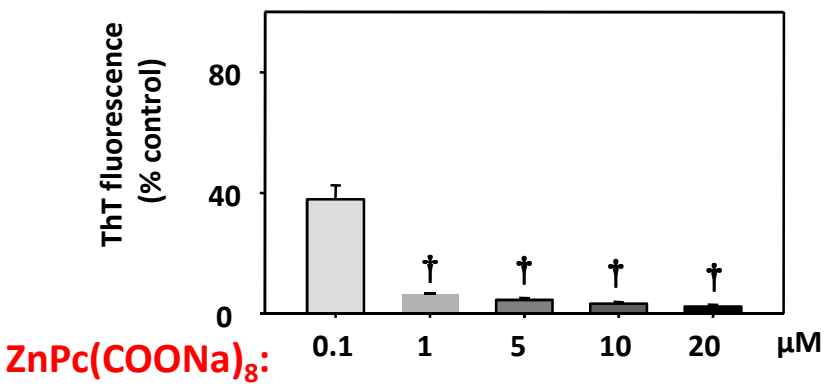
PdPc dimer
(water insoluble)



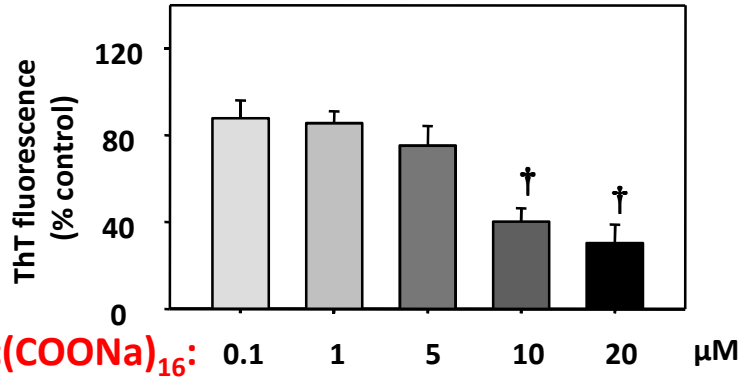
R = 

Effects of phthalocyanines on $A\beta_{1-40}$ fibril formation

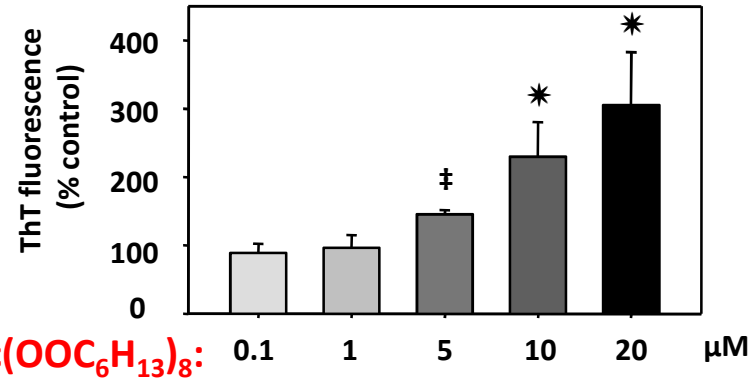
$A\beta_{1-40}$ (50 μM)



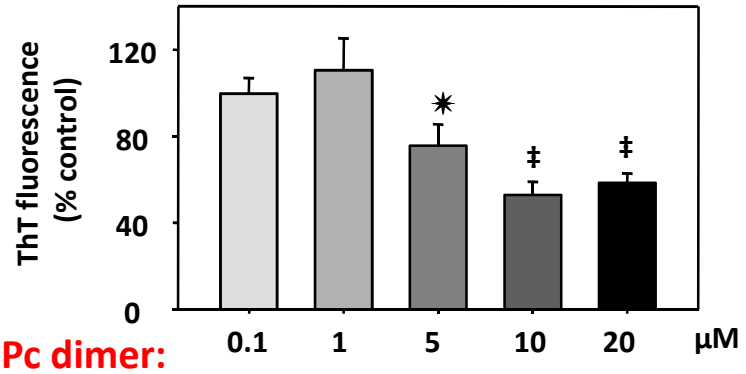
$ZnPc(COONa)_8$:



$ZnPc(COONa)_{16}$:



$ZnPc(OOC_6H_{13})_8$:

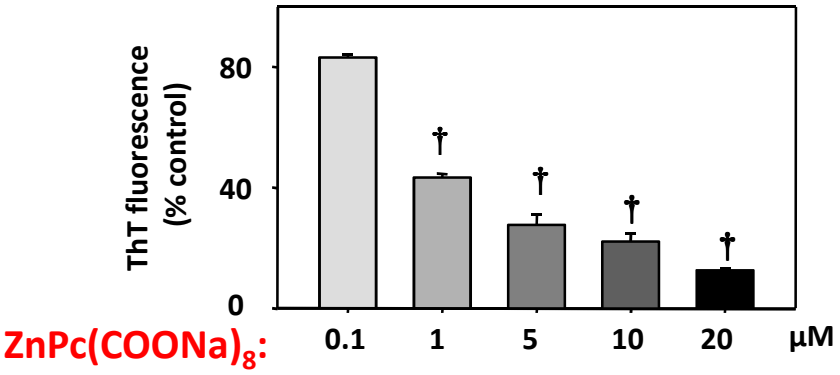


PdPc dimer:

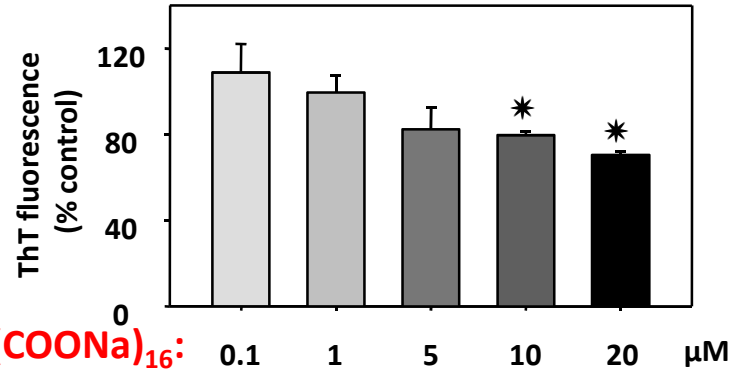
*48 h incubation

Effects of phthalocyanines on $A\beta_{1-42}$ fibril formation

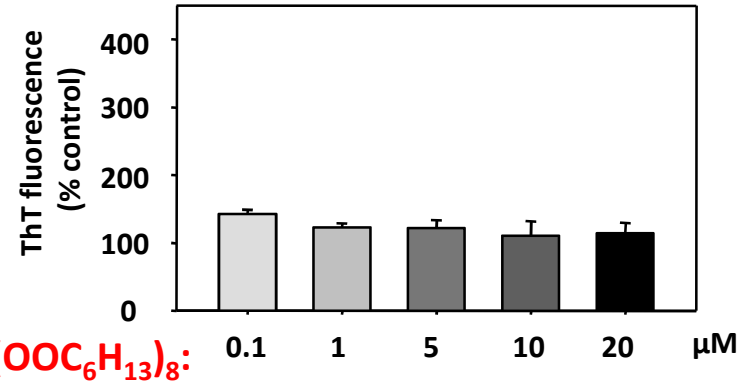
$A\beta_{1-42}$ (12.5 μM)



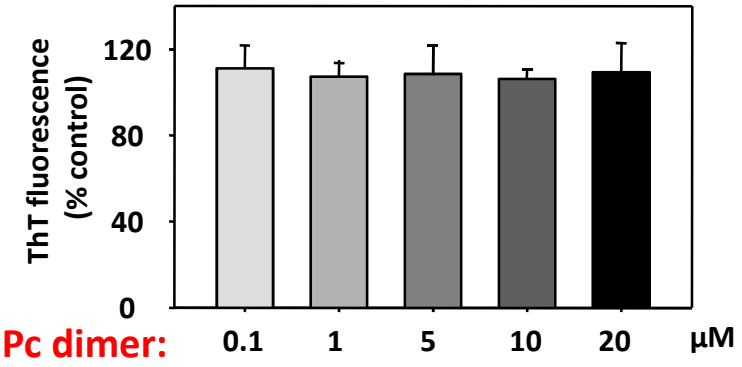
$ZnPc(COONa)_8$:



$ZnPc(COONa)_{16}$:



$ZnPc(OOC_6H_{13})_8$:

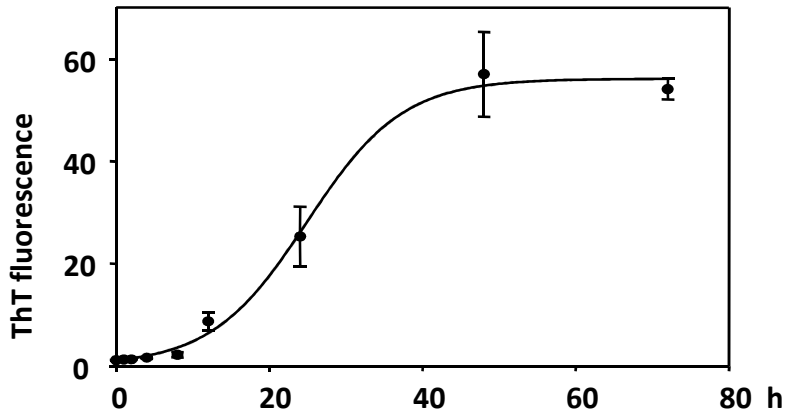


PdPc dimer:

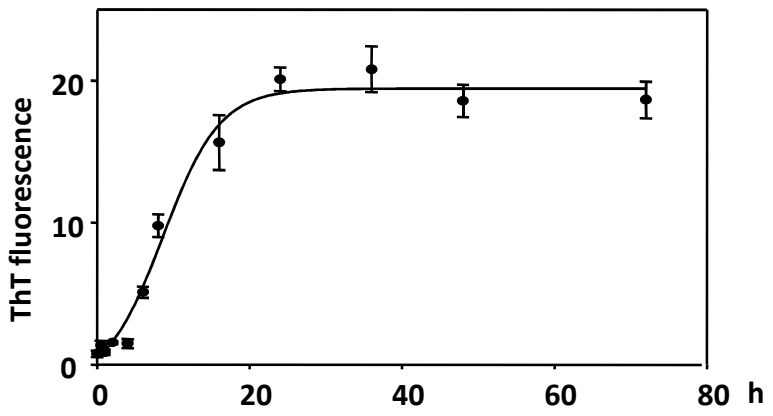
*24 h incubation

Aβ fibril formation kinetics

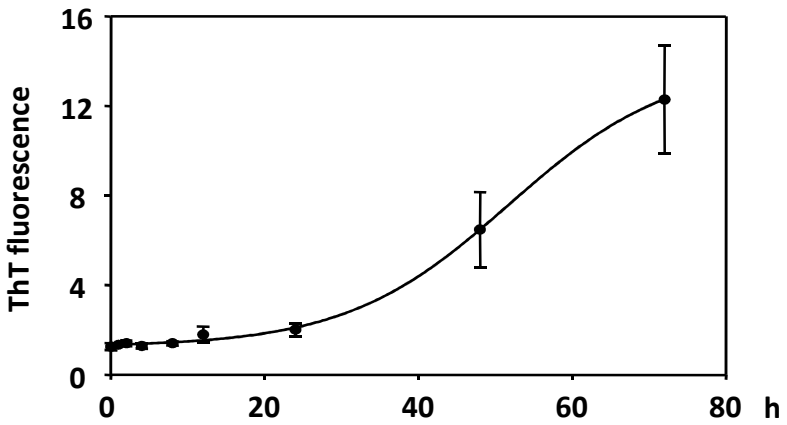
$A\beta_{1-40}$ (50 μM)



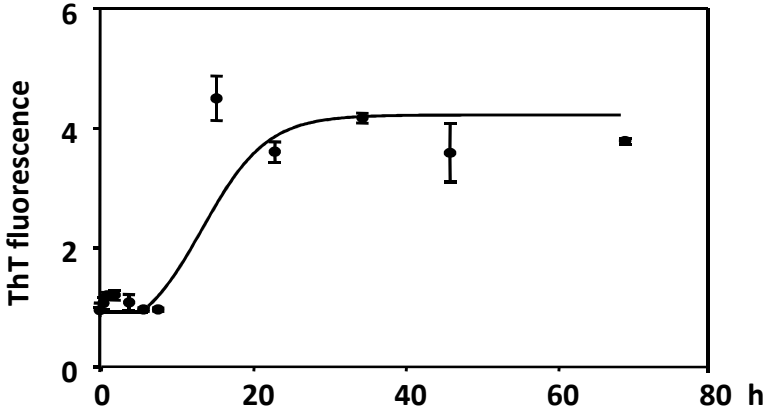
$A\beta_{1-42}$ (12.5 μM)



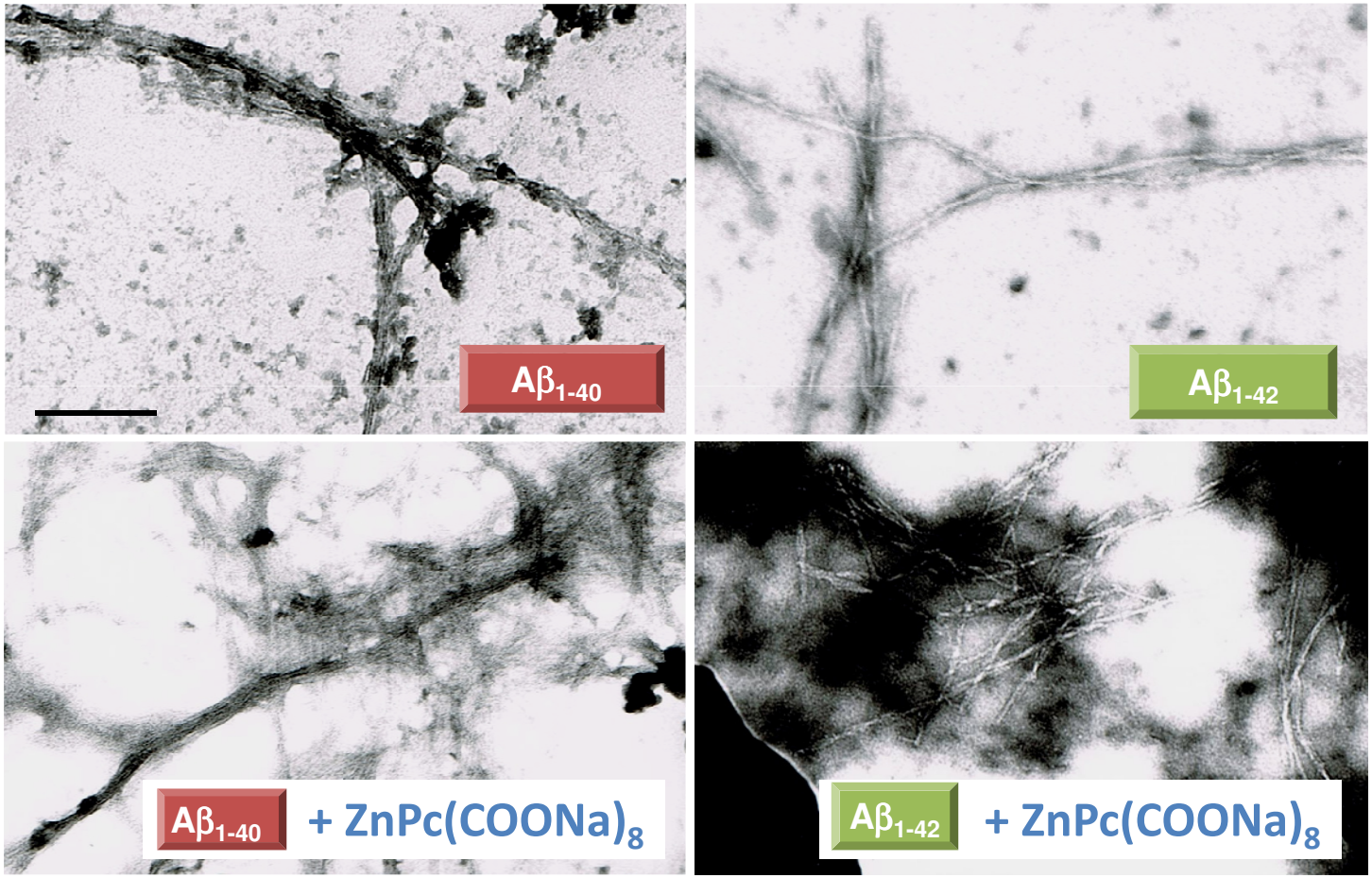
$A\beta_{1-40}$ + $ZnPc(COONa)_8$



$A\beta_{1-42}$ + $ZnPc(COONa)_8$



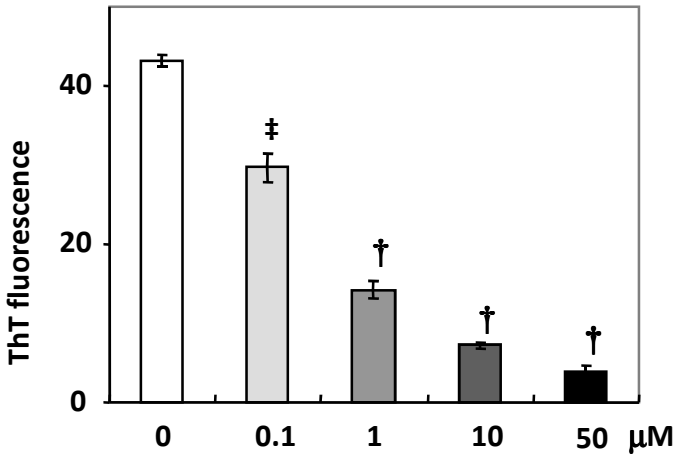
Aβ fibril morphology

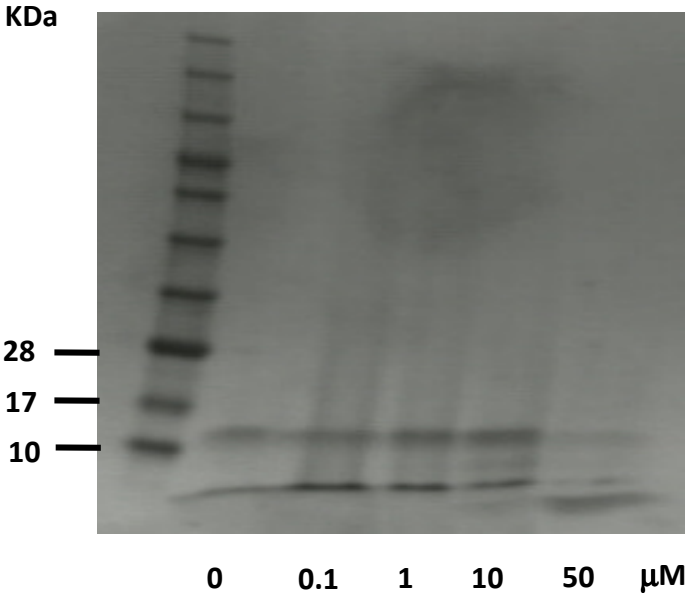


Bar=200 nm

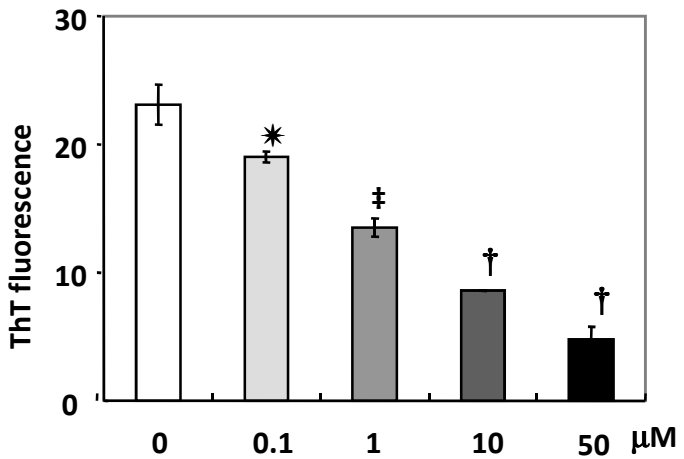
Aβ fibril destabilization

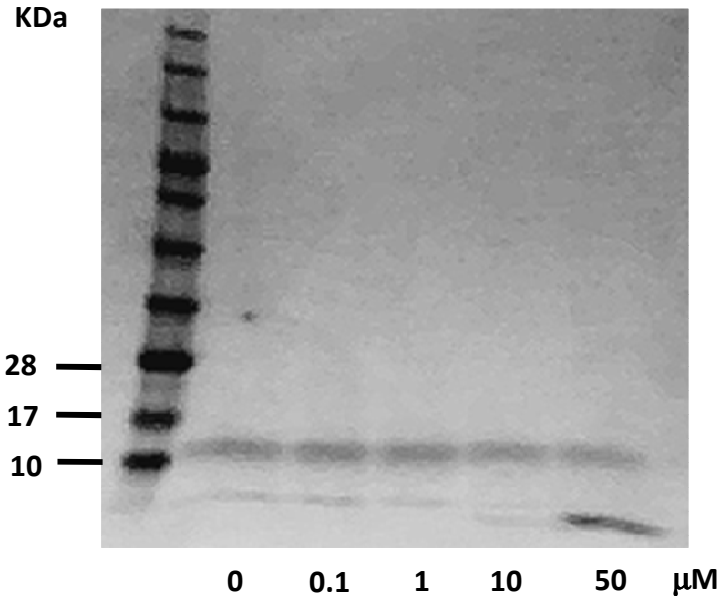
$A\beta_{1\text{-}40}$ fibril (50 μM) + $ZnPc(COONa)_8$





$A\beta_{1\text{-}42}$ fibril (25 μM) + $ZnPc(COONa)_8$





Binding of phthalocyanine to $A\beta_{1-42}$ peptide or fibril

buffer

$A\beta_{1-42}$

$A\beta_{1-42}$ fibril

$A\beta_{1-42}$

+

$ZnPc(COONa)_8$

Incubation (24 h)

→

Immunoprecipitation with Aβ Ab

NIR scan

$ZnPc(COONa)_8$ (μM)

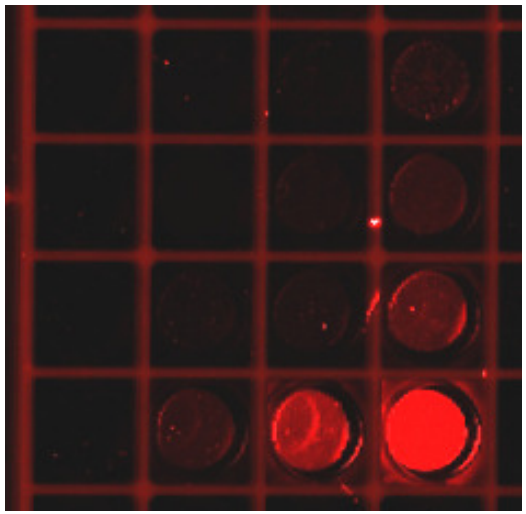
0 1 5 10

buffer

$A\beta_{1-42}$

$A\beta_{1-42}$ fibril

$A\beta_{1-42}$



Aβ antibody

+

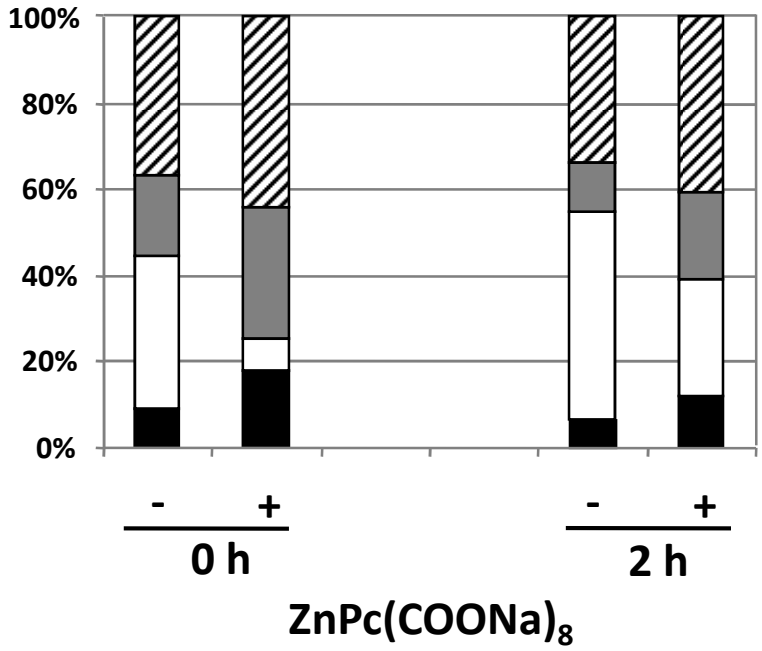
-

+

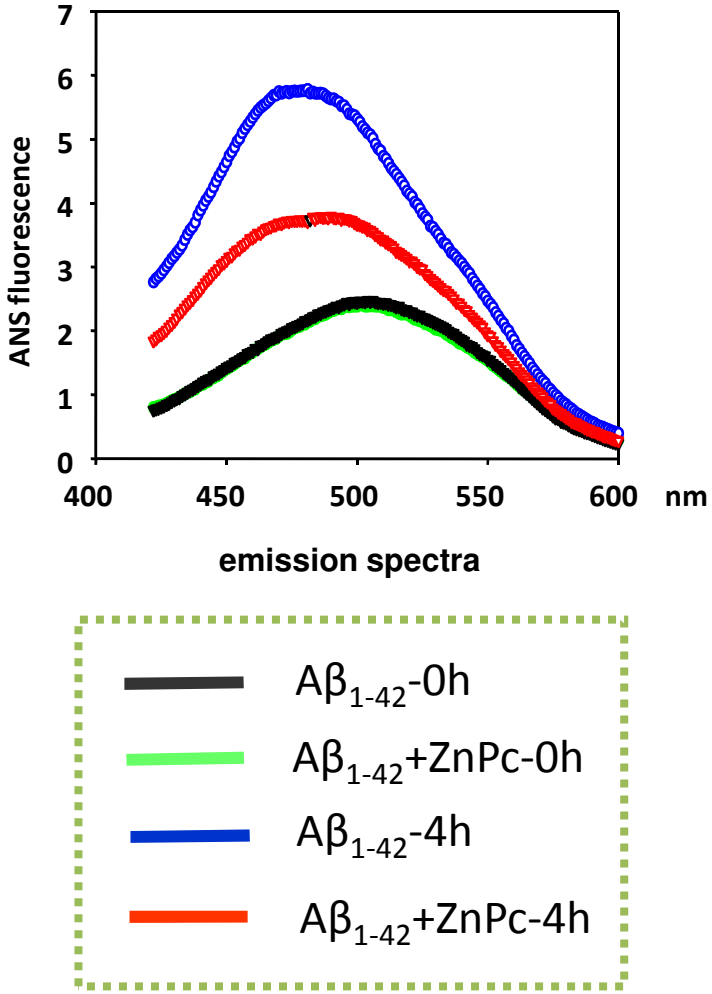
+

Secondary structure and hydrophobicity of Aβ peptide or fibril

$A\beta_{1-40}$ CD spectroscopy



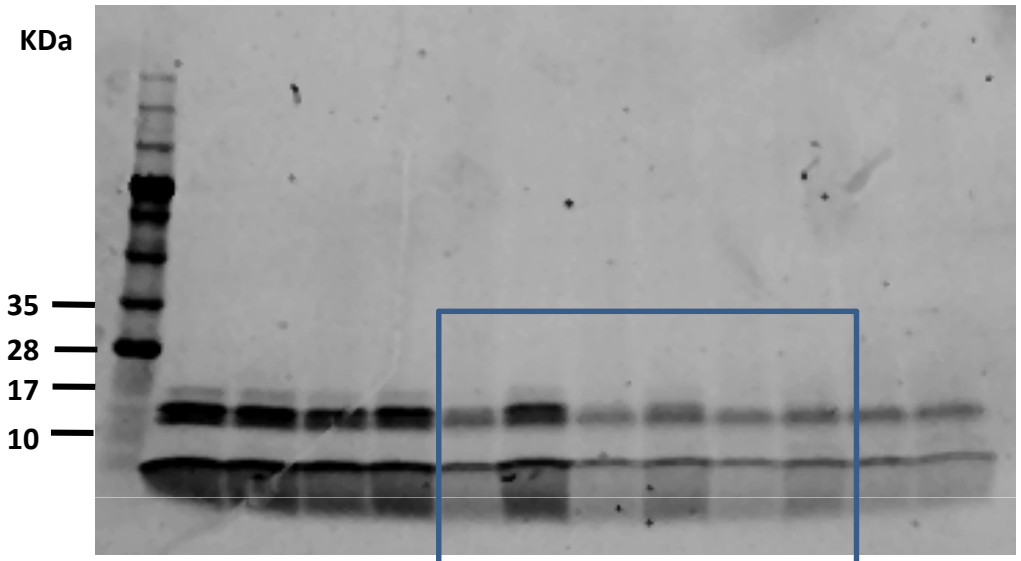
$A\beta_{1-42}$ ANS fluorescence assay



Molecular species of $A\beta_{1-42}$ during fibril formation

Coomassie blue staining (SDS PAGE)

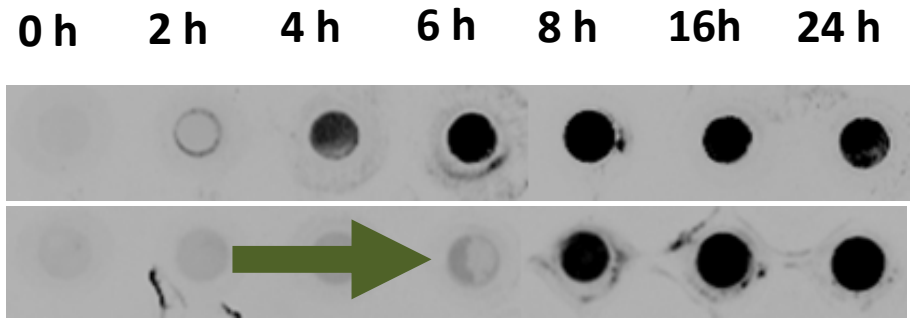
$A\beta_{1-42}$ (50 µM)



Dot blot immunoassay with oligomer specific Ab

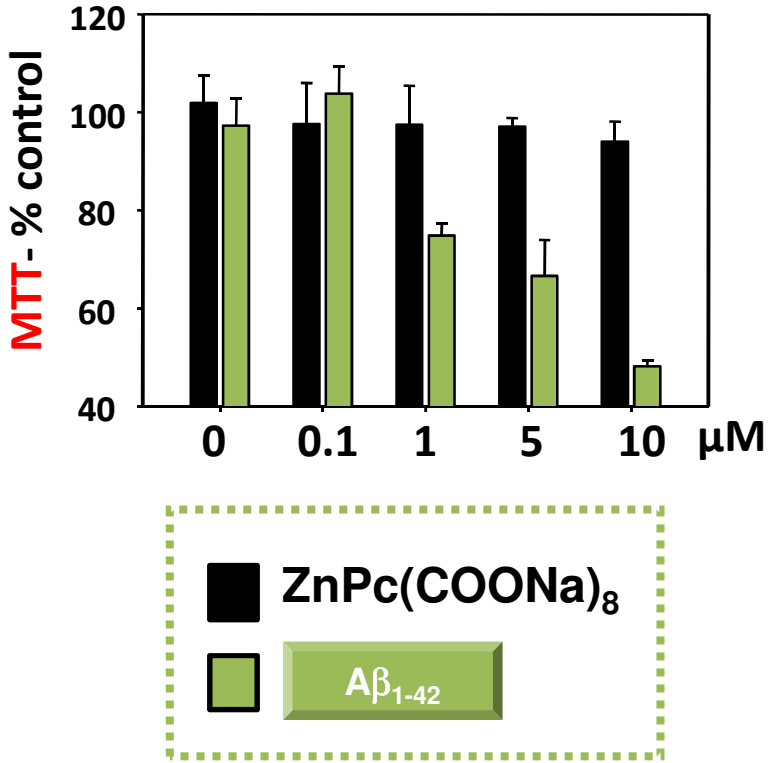
$A\beta_{1-42}$

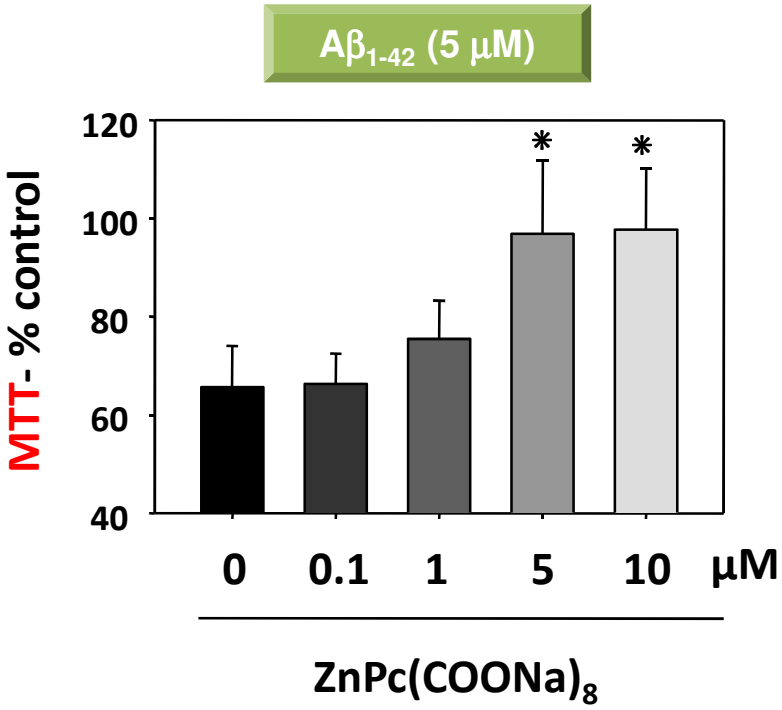
$A\beta_{1-42}$ + $ZnPc(COONa)_8$



delayed oligomer formation

Cell viability assay of neuronal cell





ZnPc(COONa)$_8$ protects A1 neuronal cells from Aβ-induced toxicity

Summary of the results

- **Water soluble ZnPc(COONa)$_8$**
  - **has near-infrared properties**
  - **binds to Aβ peptide**
  - **inhibits its oligomer and subsequent fibril formation process**
  - **destabilizes preformed Aβ fibrils**
  - **decreases β-sheet conformation and hydrophobicity**
  - **possesses neuroprotective feature against Aβ peptide**

Conclusion

Phthalocyanine could be used as a safe diagnostic probe as well as a therapeutic tool for AD.

Acknowledgements

- **Shimane University**
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    - **Dept. Laboratory Medicine**
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Laboratory Medicine

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