*Pure Appl. Chem.*, Vol. 81, No. 2, pp. 255–262, 2009.
doi:10.1351/PAC-CON-08-07-02


# Artificial proteases toward catalytic drugs for amyloid diseases*

Tae Yeon Lee and Junghun Suh‡

*Department of Chemistry, Seoul National University, Seoul 151-747, Korea*

*Abstract*: We have proposed catalytic drugs based on artificial proteases as a new paradigm in drug design. Catalytic cleavage of the backbone of a protein related to a disease may effect a cure. Catalytic drugs can be designed even for proteins lacking active sites. Soluble oligomers of amyloid β-42 peptide ($A\beta_{42}$) are implicated as the primary toxic species in amyloid diseases such as Alzheimer's disease (AD). Cleavage of $A\beta_{42}$ included in an oligomer may provide a novel method for reduction of $A\beta_{42}$ oligomers, offering a new therapeutic option. The Co(III) complex of cyclen was used as the catalytic center for peptide hydrolysis. Binding sites of the catalysts that recognize the target were searched by using various chemical libraries. Four compounds were selected as cleavage agents for the oligomers of $A\beta_{42}$. After reaction with the cleavage agents for 36 h at 37 °C and pH 7.50, up to 30 mol % of $A\beta_{42}$ (4.0 μM) was cleaved, although the target oligomers existed as transient species. Considerable activity was manifested at the concentrations of the agents as low as 100 nM.

*Keywords*: artificial proteases; catalytic drugs; amyloid diseases; Alzheimer's disease; amyloid.

## INTRODUCTION

In the conventional approaches to designing drugs that regulate the activities of disease-related proteins such as enzymes or receptors, attempts are made to discover small molecules that bind to the active sites of the disease-related proteins with high selectivity and affinity. As illustrated schematically in Fig. 1, the interaction between the protein and the drug molecule is stoichiometric.

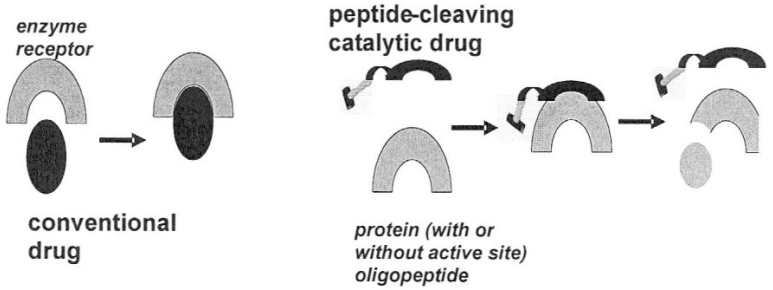



**Fig. 1** Comparison of peptide-cleaving catalytic drugs with conventional drugs.

*Paper based on a presentation at the 17th International Conference on Organic Synthesis (ICOS 17), 22–27 June 2008, Daejeon, Korea. Other presentations are published in this issue, pp. 169–298.

‡Corresponding author

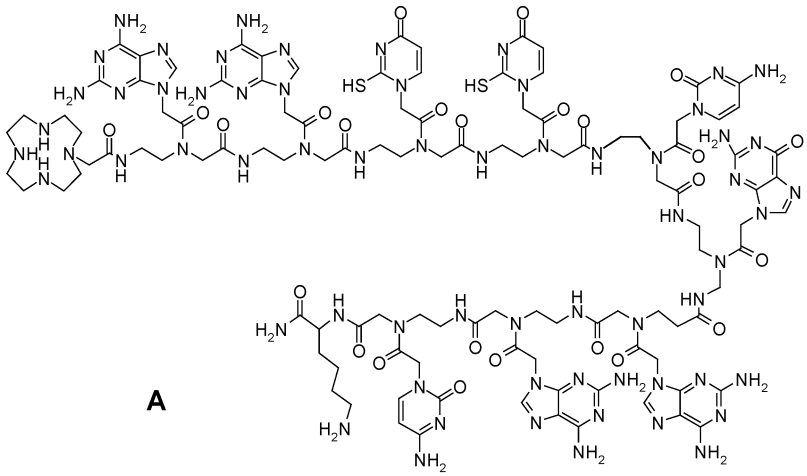
Target-selective artificial proteases are synthetic catalysts that catalyze the hydrolysis of peptide bonds of the target oligopeptides or proteins selectively. When the substrate of a target-selective artificial protease is a disease-related oligopeptide or protein, the artificial protease can abolish the activity of the substrate by acting as a peptide-cleaving catalytic drug as illustrated in Fig. 1 [1,2].

Peptide-cleaving catalytic drugs have several advantages in comparison with conventional drugs. First, the catalytic nature of peptide cleavage allows decreases in the amounts of the drug dosage and the subsequent reduction of the side effects for the peptide-cleaving catalysts. Second, strong binding to the target is not required if the peptide cleavage is fast enough for the peptide-cleaving catalysts. Third, the peptide-cleaving catalysts can be obtained even for proteins or oligopeptides that do not have active sites since the catalyst may recognize any part of the target molecule.

The idea of peptide-cleaving catalytic drugs has been proposed recently by this laboratory [1,2]. So far, successful target-selective artificial proteases have been designed only in this laboratory. Herein, how the concept of peptide-cleaving catalytic drugs has evolved is briefly described together with its potential applications especially toward amyloid diseases such as Alzheimer's disease (AD).

## TARGET-SELECTIVE PEPTIDE-CLEAVING CATALYSTS REPORTED PREVIOUSLY

The first protein-cleaving catalyst selective for a target protein was synthesized by using myoglobin (Mb) as the target [3,4]. The catalysts such as Cu(II) or Co(III) complex of **A** were selected by using a combinatorial library of cyclen-containing peptide nucleic acid oligomers.

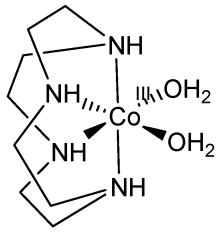


**A**

Kinetic data showed that up to 2.5 or 6 molecules of Mb were cleaved by each molecule of Cu(II)**A** or Co(III)**A**, respectively, indicating the catalytic nature of the action of the cleavage agents. The pH dependence of $k_{cat}$ for Co(III)**A** manifested optimum activity at pH 7.5. The cleavage sites were identified by matrix-assisted laser desorption/ionization with time-of-flight (MALDI-TOF) mass spectra of reaction products.

It is noteworthy that Co(III) complexes may be more suitable for medical uses compared with Cu(II) complexes since metal transfer to metal-abstracting materials in a living body should be substantially slower for Co(III) complexes due to the exchange-inertness of Co(III). To date, the Co(III) complex of cyclen [Co(III)cyclen] has been mainly used as the catalytic center for artificial metalloproteases that are aimed at design of catalytic drugs. Kinetic data for the cleavage of Mb, γ-globulin, and albumin by Co(III)cyclen revealed that proteins would resist cleavage in the presence of 5 μM

Co(III)cyclen for at least several weeks at 37 °C [5]. Thus, side effects due to attack of Co(III)cyclen-containing peptide-cleaving catalytic drugs at non-target proteins would be negligible.

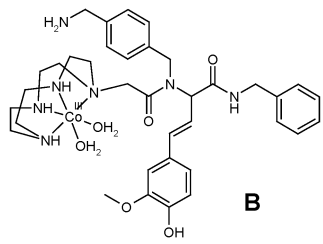



**Co(III)cyclen**

Although the Mb-cleaving catalysts were the first target-selective artificial proteases, their molecular weights (ca. 3000) were too large to use the catalysts as drugs and to analyze the mechanism of the catalytic action. In addition, Mb is not related to a disease. In the next step to establishment of drug discovery exploiting peptide-cleaving catalysts, design of artificial proteases possessing considerably smaller molecular weights as well as high selectivity for proteins directly related to diseases was undertaken. The first peptide-cleaving catalyst meeting those criteria has been designed by using peptide deformylase (PDF) as the target [6].

Inhibitors of PDF are searched as candidates for new antibiotic drugs [7]. The active PDF has Fe(II) ion in the active site, which reacts readily with oxygen. To obtain a stable variant, the Fe(II) ion is often substituted with Zn(II), although Zn(II)-PDF has reduced activity by 2–3 orders of magnitude. *E. coli* Zn(II)-PDF was used as the target enzyme in this study.

The catalyst (**B**) for cleavage of PDF was obtained by using a library of catalyst candidates synthesized by the Ugi [8] condensation. MALDI-TOF mass spectrum of a reaction mixture obtained by incubation of PDF with **B** disclosed that the cleavage site was Gln(152)-Arg(153). The optimum pH for the action of **B** was 7.5. The lower limit of $k_{cat}$ was estimated as 0.05 $h^{-1}$. Evidence for the hydrolytic nature of cleavage of proteins such as Mb and PDF came from *N*-terminal and *C*-terminal sequencing of the cleavage products [2].




**B**

To gain insights into the mechanism of the cleavage of PDF by **B**, docking simulations were performed for the complex formed between PDF and **B**. Figure 2 shows that the catalytic head and the central acyclic chain of the catalyst interact with the *C*-terminal α-helix, while the three aromatic tails make contact with the helical and the loop structures residing above the active site. An expanded view disclosed several modes of interactions between the catalyst and the side chains of PDF. The multiple number of the interactions between **B** and PDF suggested by the simulation experiments accounts for the high selectivity manifested by **B** and PDF.

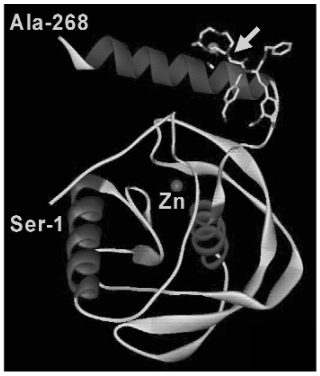



**Fig. 2** The lowest-energy conformation of **B**-PDF complex predicted by the docking simulations: Arrow indicates the cleavage site.

Cleavage of various kinds of monomeric oligopeptides were tested by using chemical libraries of catalyst candidates. Catalysts (**C**, **D**) for cleavage of angiotensin I, an octapeptide, and angiotensin II, a decapeptide, were obtained [9]. Angiotensin II is an important target for designing drugs for hypertension [10]. The cleavage products were obtained by oxidative decarboxylation of *N*-terminal aspartate to produce pyruvate (Fig. 3) instead of hydrolysis of the peptide backbone. The oxidation catalysts do not belong to artificial proteases since peptide hydrolysis is not involved, but they may be also exploited as catalytic drugs.

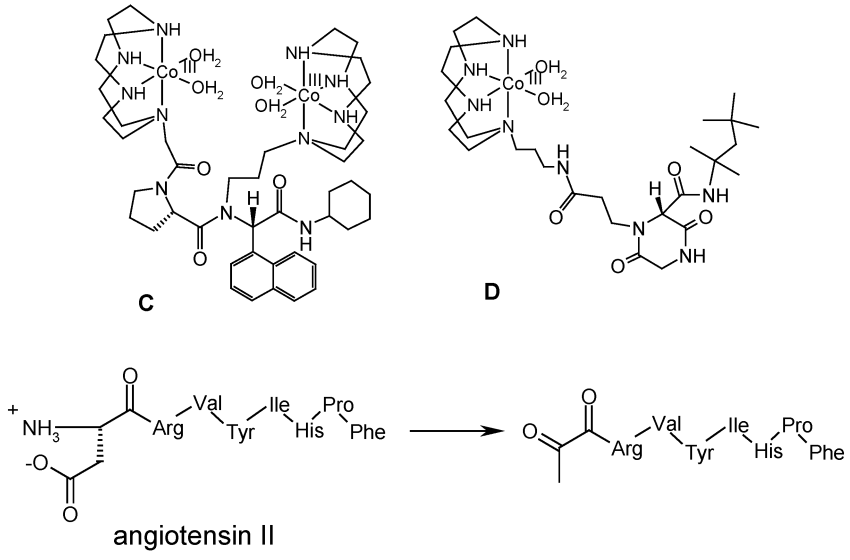
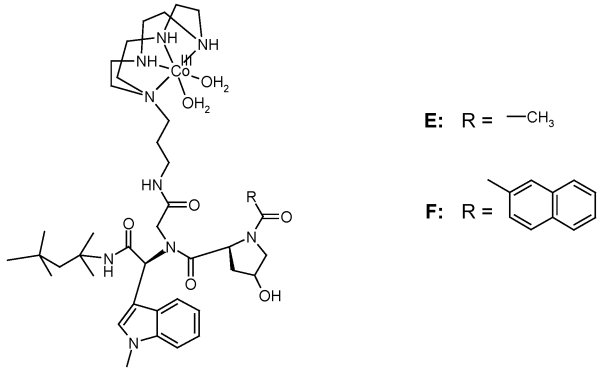



**Fig. 3** Oxidative decarboxylation of angiotensin II.

Melanin-concentrating hormone, consisting of 19 amino acid residues, is another oligopeptide which contains an *N*-terminal aspartate residue. Catalysts (**E**, **F**) for oxidative decarboxylation of melanin-concentrating hormone, a potential target for designing drugs for obesity [11], were also synthesized [12,13].




Catalytic efficiency for the oxidative decarboxylation by **C–F** is low and requires millimolar concentrations of the catalysts to observe considerable reaction rates. The low catalytic efficiency limits the application of the catalysts as drugs. Instead, the catalysts may be exploited in searching for their inhibitors which form complexes with the oligopeptide substrates snugly. Small molecules that can be tightly bound to angiotensin II or melanin-concentrating hormone may be used as substitutes for receptor antagonists.

## Artificial proteases selective for oligomers of amyloid β-42 peptide

AD is characterized by the presence of senile plaques containing amyloid β (Aβ) peptides in the brain [14]. Aβ is primarily composed of amyloid β-40 ($A\beta_{40}$) and amyloid β-42 ($A\beta_{42}$) that contain 40 and 42 amino acid residues, respectively. Soluble oligomers of $A\beta_{42}$ instead of the monomer or insoluble amyloid fibrils are believed to be responsible for synaptic dysfunction in the brains of patients of AD [15–19].

The association process of amyloidogenic peptides or proteins (AMPs) such as $A\beta_{40}$ or $A\beta_{42}$ involves formation of several oligomers, protofibrils, and fibrils as summarized in Fig. 4 [19,20]. Here, the species placed in the rectangle stands for the soluble oligomers.

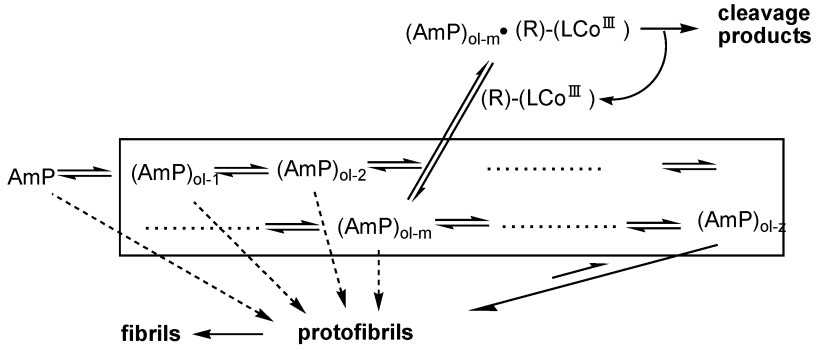
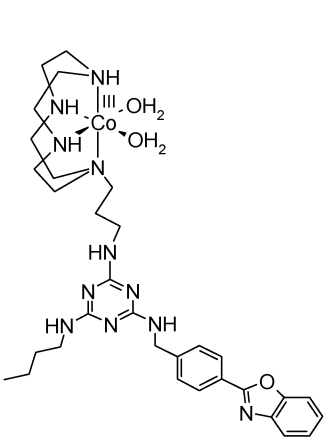



**Fig. 4** Formation of various assemblies of AMP and reduction of the assemblies by the action of cleavage agents.

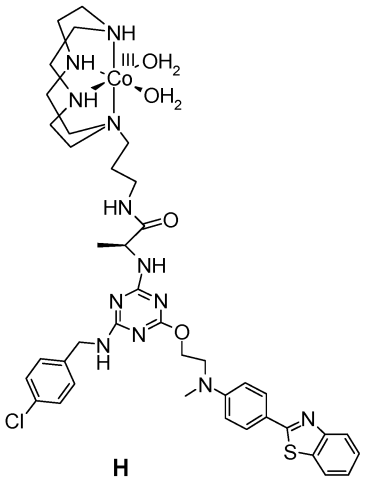
As a therapeutic option to alleviate $A\beta_{42}$-induced neurotoxicity, various attempts have been made to lower the level of the $A\beta_{42}$ assembly in brain [20–25]. Artificial proteases led to a novel method of reducing the level of $A\beta_{42}$ oligomers by cleavage of $A\beta_{42}$ included in an oligomer [26]: As shown in Fig. 4 where the cleavage agent is indicated as (R)-(LCoIII), cleavage of $A\beta_{42}$ included in a target

oligomer reduces the concentration of the target oligomer, lowering the concentrations of other oligomers which are readily transformed into the target oligomer.

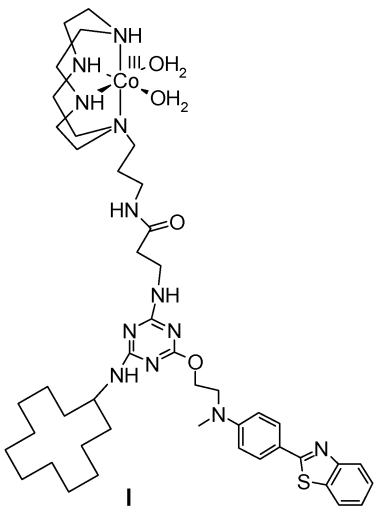
Four catalysts (**G–J**) were selected from a chemical library [26]. Agents **G** and **H** exhibited cleavage activity for both $A\beta_{40}$ and $A\beta_{42}$, whereas **I** and **J** manifested activity only for $A\beta_{42}$.



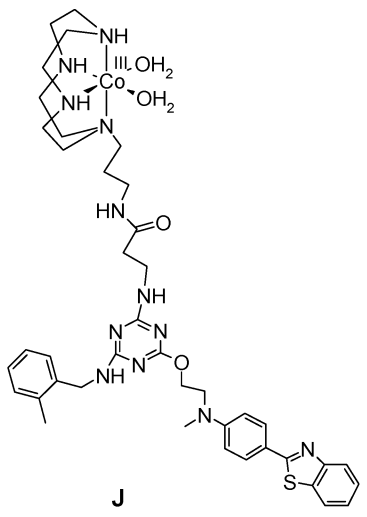

**G**




**H**




**I**




**J**

The cleavage yields measured after reaction with various initial concentrations ($C_o$) of **G–J** for 36 h at 37 °C and pH 7.50 are plotted against log $C_o$/M in Fig. 5. Plateau values of the cleavage yields are 10–30 %, and significant yields were observed at 100 nM $C_o$. Under the conditions of the AD patients, the efficiency for cleavage of $A\beta_{42}$ by **G–J** is expected to be much higher than that presented in Fig. 5 [26].

Other experiments were performed, which verified that the fragments were formed mainly by cleavage of oligomers instead of monomer, protofibrils, or fibrils for cleavage of both $A\beta_{40}$ and $A\beta_{42}$ by **G–J**. At present, the identity of oligomers of $A\beta_{40}$ or $A\beta_{42}$ cleaved by **G–J** is unknown.

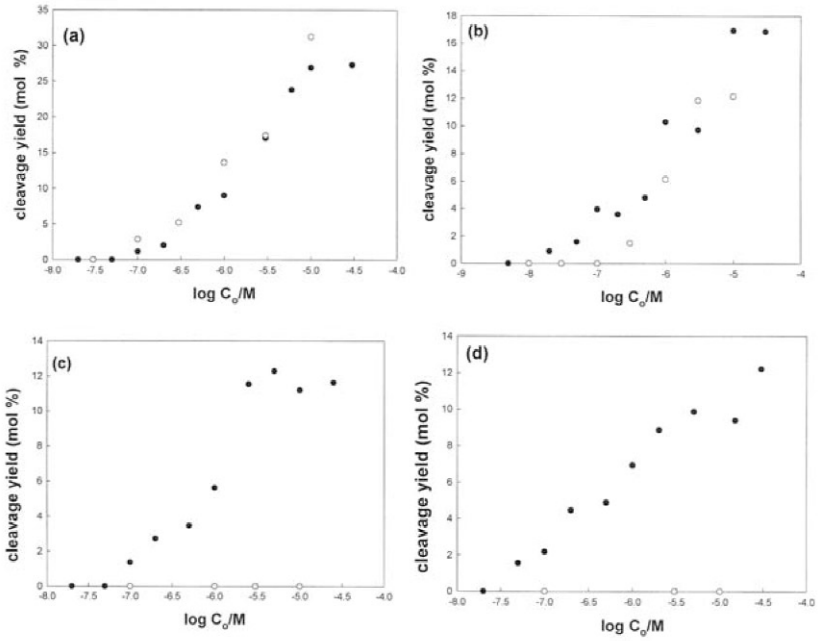



**Fig. 5** Plot of the cleavage yield against log $C_o$/M for cleavage of $A\beta_{40}$ (○) or $A\beta_{42}$ (●) (4.0 μM) by **G** (a), **H** (b), **I** (c), and **J** (d) measured after reaction for 36 h at 37 °C and pH 7.50.

Nevertheless, reduction of the concentration of the target oligomer would decrease concentrations of other oligomers, since the oligomers are in equilibria with one another.

Agents **G** and **H** cleaved not only $A\beta_{42}$ but also $A\beta_{40}$ although they were selected from the combinatorial library by screening against $A\beta_{42}$. Excessive cleavage of $A\beta_{40}$ may interfere with its normal functions. The level of soluble $A\beta_{40}$ in the brains of patients of AD is 30–40 times higher than those of nondemented elderly controls and, therefore, partial cleavage of soluble oligomers of $A\beta_{40}$ during cleavage of $A\beta_{42}$ may not cause considerable side effects.

## CONCLUSIONS

Proteins related to cancers or originating from bacteria or viruses could be the primary targets of peptide-cleaving agents since destruction of those proteins would induce few side effects. Many soluble protein toxins are known [27], and protein-cleaving agents based on metal complexes would provide a new method for their detoxification.

Another challenging area of the protein-cleaving agents is the cleavage of oligomers of amyloidogenic proteins or oligopeptides causing amyloidoses [28] such as AD, type 2 diabetes mellitus, Parkinson's disease, and mad cow's disease. Catalytic drugs based on artificial metalloproteases would be particularly effective for the amyloidogenic proteins or oligopeptides because conventional drugs targeting active sites cannot be designed for them. Many more cleavage agents for the $A\beta_{42}$ oligomers can be synthesized in addition to **G–J** by combining a Co(III)-ligand complex and a binding auxiliary with affinity for the $A\beta_{42}$ oligomers. After performing proper in vivo tests, some of the synthetic cleavage agents may be found suitable for therapeutic treatment of AD patients. Then, the concept of catalytic drugs based on artificial metalloproteases could be extended to other amyloidoses and the value of the artificial proteases as catalytic drugs could be widely recognized.

## ACKNOWLEDGMENTS

This work was supported by the Korea Science and Engineering Foundation through the National Research Laboratory Program (No. M10500000001-06J0000-00110) funded by the Ministry of Science and Technology.

## REFERENCES

1. J. Suh. *Acc. Chem. Res.* **36**, 562 (2003).
2. W. S. Chei, J. Suh. *Prog. Inorg. Chem.* **55**, 79 (2007).
3. J. W. Jeon, S. J. Son, C. E. Yoo, I. S. Hong, J. B. Song, J. Suh. *Org. Lett.* **4**, 4155 (2002).
4. J. W. Jeon, S. J. Son, C. E. Yoo, I. S. Hong, J. Suh. *Bioorg. Med. Chem.* **11**, 2901 (2003).
5. B. Jang, J. Suh. *Bull. Korean Chem. Soc.* **29**, 202 (2008).
6. P. S. Chae, M.-s. Kim, C.-s. Jeung, S. D. Lee, H. Park, S. Y. Lee, J. Suh. *J. Am. Chem. Soc.* **127**, 2396 (2005).
7. P. T. Ravi Rajagopalan, S. Grimme, D. Pei. *Biochemistry* **39**, 779 (2000).
8. A. Dömling, I. Ugi. *Angew. Chem., Int. Ed.* **39**, 3168 (2000).
9. M.-s. Kim, J. W. Jeon, J. Suh. *J. Biol. Inorg. Chem.* **10**, 364 (2005).
10. W. C. De Mello (Ed.). *Renin Angiotensin System and the Heart*, John Wiley, New York (2004).
11. C. G. Bell, D. Meyre, C. Samson, C. Boyle, C. Lecoeur, M. Tauber, B. Jouret, D. Jaquet, C. Levy-Marchal, M. A. Charles, P. Froguel, A. J. Walley. *Diabetes* **54**, 3049 (2005).
12. M. G. Kim, M.-s. Kim, S. D. Lee, J. Suh. *J. Biol. Inorg. Chem.* **11**, 867 (2006).
13. M. G. Kim, M.-s. Kim, H. Park, S. Lee, J. Suh. *Bull. Korean Chem. Soc.* **27**, 1151 (2007).
14. D. J. Selkoe. *Physiol. Rev.* **81**, 741 (2001).
15. J. Hardy, D. J. Selkoe. *Science* **297**, 353 (2002).
16. R. E. Tanzi. *Nat. Neurosci.* **8**, 977 (2005).
17. E. M. Snyder, T. Nong, C. G. Almeida, S. Paul, T. Moran, E. Y. Choi, A. C. Nairn, M. W. Salter, P. J. Lombroso, G. K. Gouras, P. Greengard. *Nat. Neurosci.* **8**, 1051 (2005).
18. S. Barghorn, V. Nimmrich, A. Striebinger, C. Krantz, P. Keller, B. Janson, M. Bahr, M. Schmidt, R. S. Bitner, J. Harlan, E. Barlow, U. Ebert, H. Hillen. *J. Neurochem.* **95**, 834 (2005).
19. S. Lesné, M. T. Koh, L. Kotilinek, R. Kayed, C. G. Glabe, A. Yang, M. Gallagher, K. H. Ashe. *Nature* **440**, 352 (2006).
20. G. Bitan, M. D. Kirkitadze, A. Lomakin, S. S. Vollers, G. B. Benedek, D. B. Teplow. *Proc. Natl. Acad. Sci. USA* **100**, 330 (2003).
21. D. Schenk, R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko1, M. Lee, Z. Liao1, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandevert, S. Walker, M. Wogulis, T. Yednock, D. Games, P. Seubert. *Nature* **400**, 173 (1999).
22. R. B. DeMattos, K. R. Bales, D. J. Cummins, J.-c. Dodart, S. M. Paul, D. M. Holtzman. *Proc. Natl. Acad. Sci. USA* **98**, 8850 (2001).
23. C. M. Dobson. *Science* **304**, 1259 (2004).
24. T. Cohen, T. Frydman-Marom, M. Rechter, E. Gazit. *Biochemistry* **45**, 4727 (2006).
25. D. S. Choi, D. Wang, G.-q. Yu, G. Zhu, V. N. Kharazia, J. P. Paredes, W. S. Chang, J. K. Deitchman, L. Mucke, R. O. Messing. *Proc. Natl. Acad. Sci. USA* **103**, 8215 (2006).
26. J. Suh, S. H. Yoo, M. G. Kim, K. Jeong, J. Y. Ahn, M.-s. Kim, P. S. Chae, T. Y. Lee, J. Lee, J. Lee, Y. A. Jang, E. H. Ko. *Angew. Chem., Int. Ed*. **46**, 7064 (2007).
27. M. W. Parker (Ed.). *Protein Toxin Structure*, Landes, Austin (1996).
28. G. Bittan, E. A. Fradinger, S. M. Spring, D. B. Teplow. *Amyloid* **12**, 88 (2005).