

Supplementary Information

Structure-based peptide inhibitor design of amyloid- β aggregation

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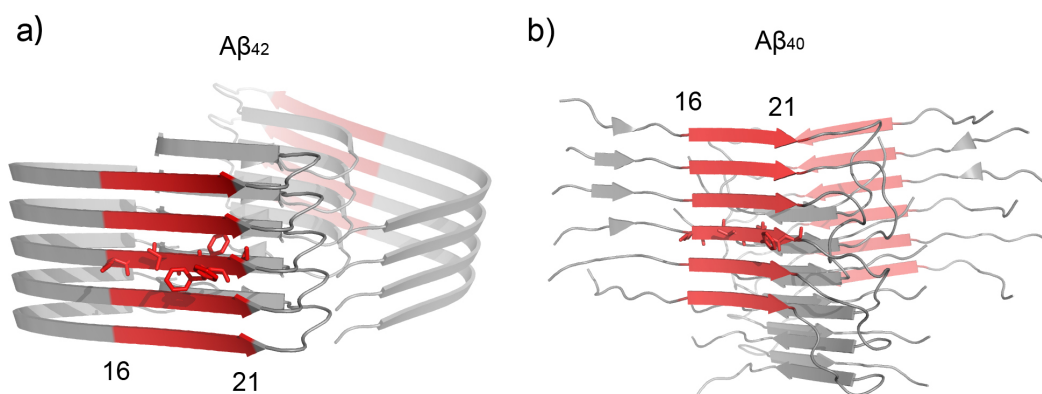
Supplementary Table 1

Supplementary Figures 1 to 14

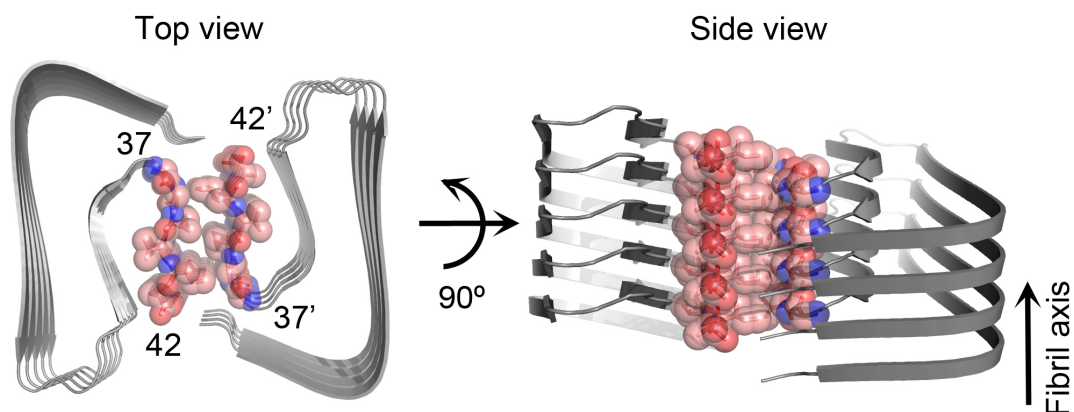
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Supplementary Table 1. Secondary structure contents of designed peptide inhibitors in solution measured by CD spectroscopy. Data were analyzed by using CDPro¹.

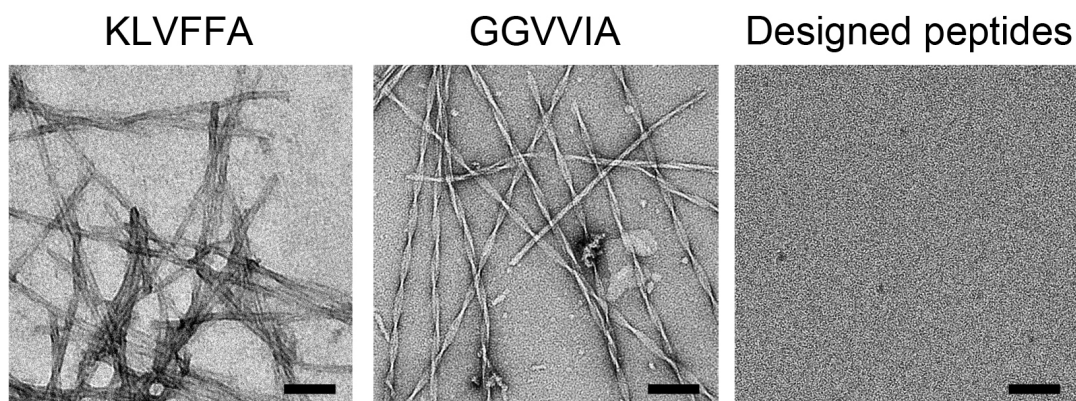
Peptide ID	Sequence	Secondary structure (%)		
		α -Helix	β -Sheet	Unstructured
K6A1	TLWYK	2.9	25.1	72.0
K6A2	EHWYH	4.3	23.3	72.4
G6A1	HYFKY	0.4	48.1	51.5
G6A2	HYYIK	0.1	33.2	66.7
G6A3	KYYEI	0	56.9	43.1



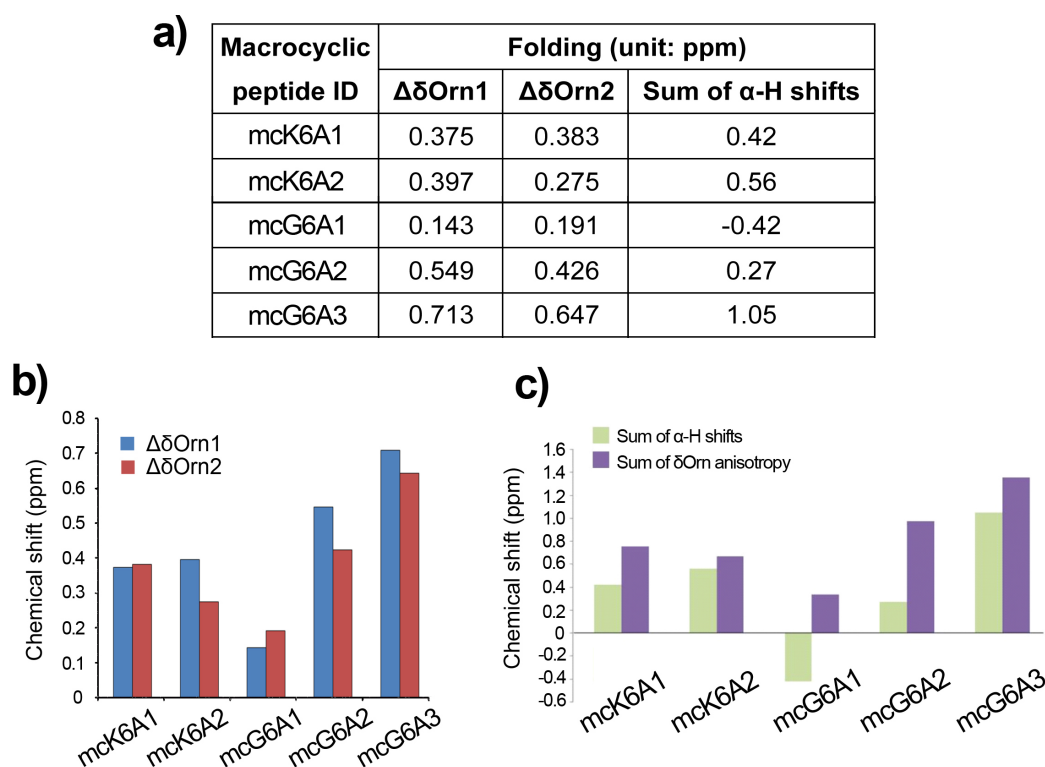
Supplementary Figure 1. Structures of segment ¹⁶KLVFFA²¹ in the full-length Aβ structures. (a) The structure of Aβ₄₂ fibril determined by cryo-EM (PDB ID: 5OQV). (b) The structure of Aβ₄₀ fibril determined by solid-state NMR (PDB ID: 2LMN). Segment ¹⁶KLVFFA²¹ is highlighted in red.



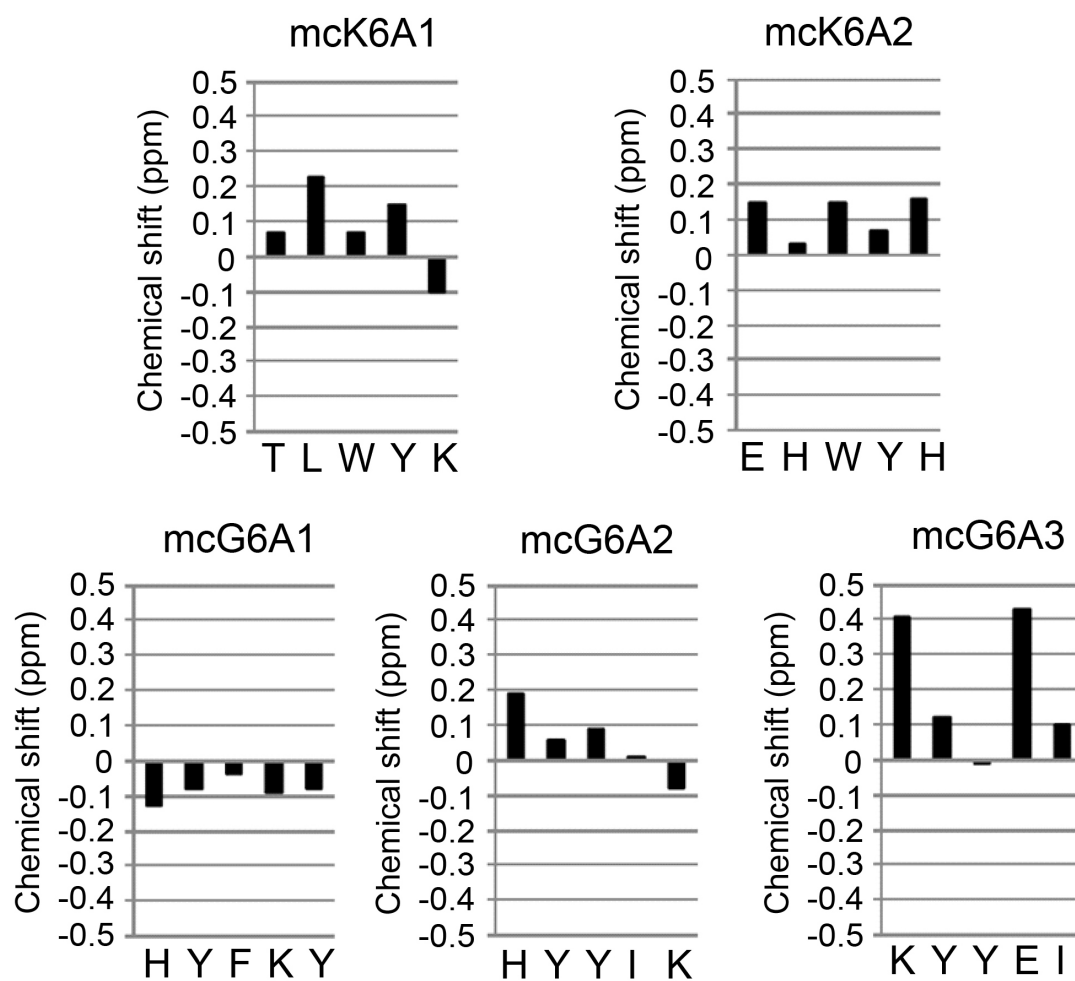
Supplementary Figure 2. The structure of segment $^{37}\text{GGVVIA}^{42}$ in the fibril structure of full-length $\text{A}\beta_{42}$ determined by cryo-EM (PDB ID: 5OQV). The $^{37}\text{GGVVIA}^{42}$ segment is shown as sticks and spheres. Hydrophobic interactions between $^{37}\text{GGVVIA}^{42}$ side chains contribute dominantly to the interface of the two protofilaments of the mature fibril.



Supplementary Figure 3. TEM images of amyloid fibrils formed by target segments KLVFFA and GGVVIA. Fibrils were formed by dissolving 5 mg/ml of peptides with water and incubating at 37 °C. The designed peptide inhibitors including free and macrocyclic peptides do not form fibrils. The scale bars are 100 nm.

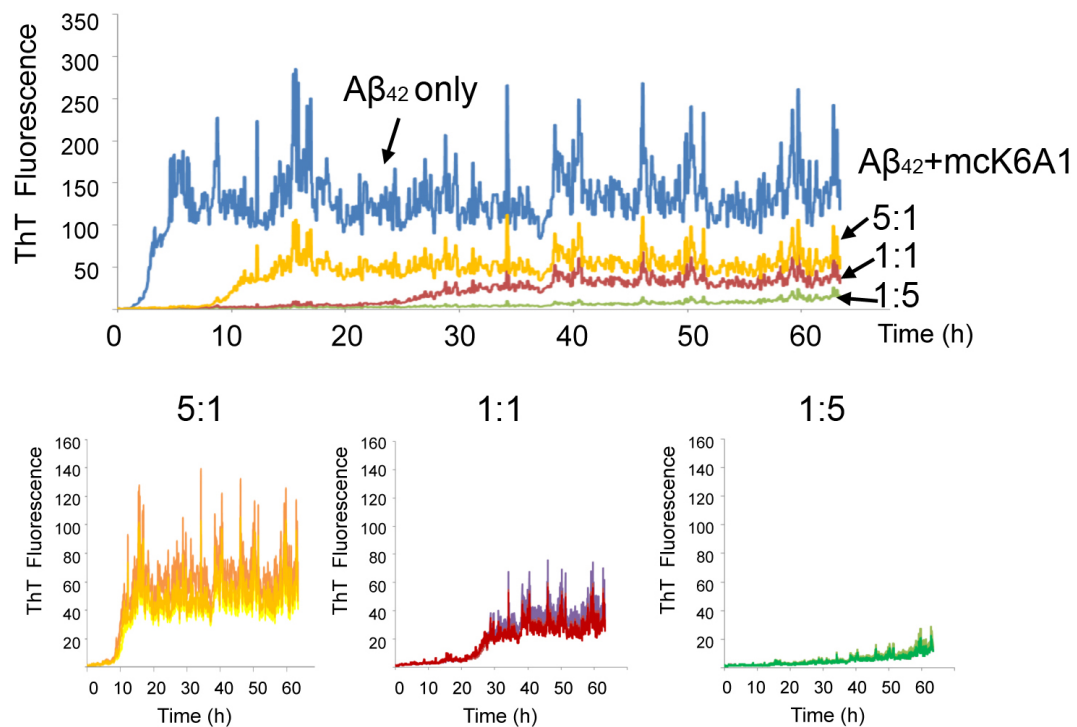


Supplementary Figure 4. ^1H NMR study validates the designed macrocyclic peptide inhibitors generally adopt folded β -strand structures in solution. The degree of β -strand folding of macrocyclic peptides can be assessed by measuring the magnetic anisotropy of the diastereotopic δOrn δ -protons ($\Delta\delta\text{Orn}$) and downfield shifting of the amino acid α -protons of the macrocycles³⁻⁵. (a) The values of α -H shifts and $\Delta\delta\text{Orn}$ anisotropy of the designed macrocyclic peptides indicates that mcG6A2, mcG6A3, mcK6A1 and mcK6A2 exhibit moderate to good folding, while mcG6A1 exhibit partial folding in solution. (b) The $\Delta\delta\text{Orn1}$ and $\Delta\delta\text{Orn2}$ anisotropy of each macrocyclic peptide. (c) The sum of α -H shifts and $\Delta\delta\text{Orn}$ anisotropy of each macrocyclic peptide. All NMR data were collected at the peptide concentration of 2 mM in D_2O at 298 K.

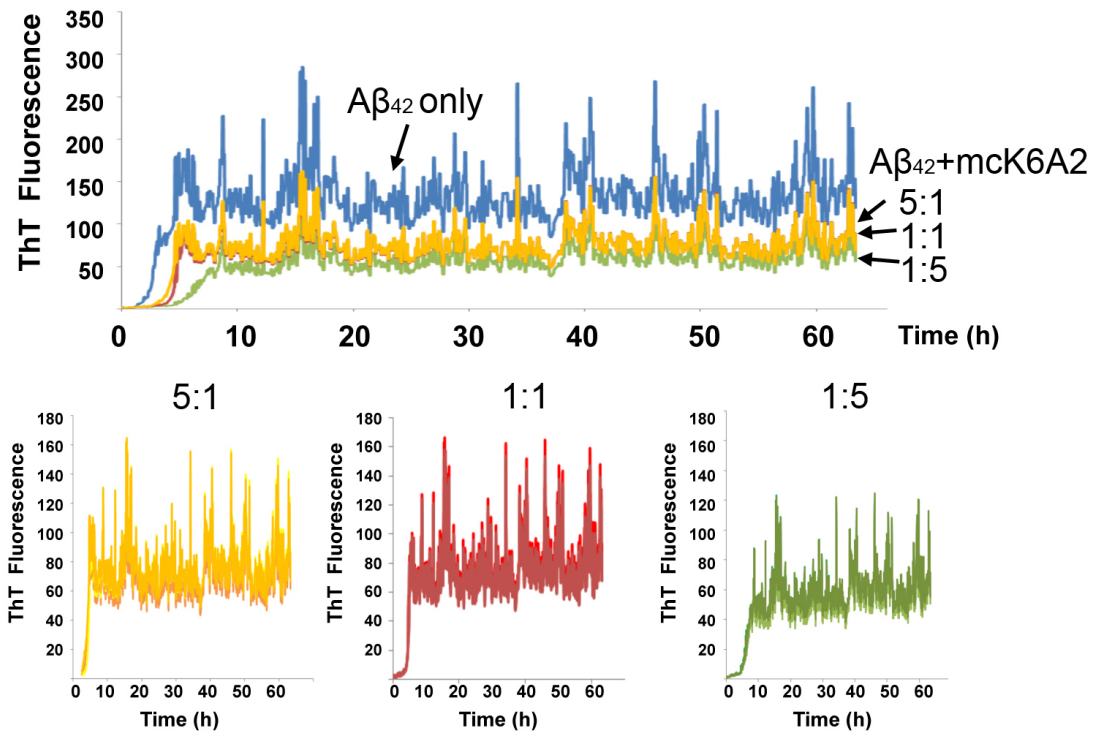


Supplementary Figure 5. Folding of macrocyclic peptides evaluated by the α -H shifts "fingerprint" of the individual amino acid of the inserted designed sequences.

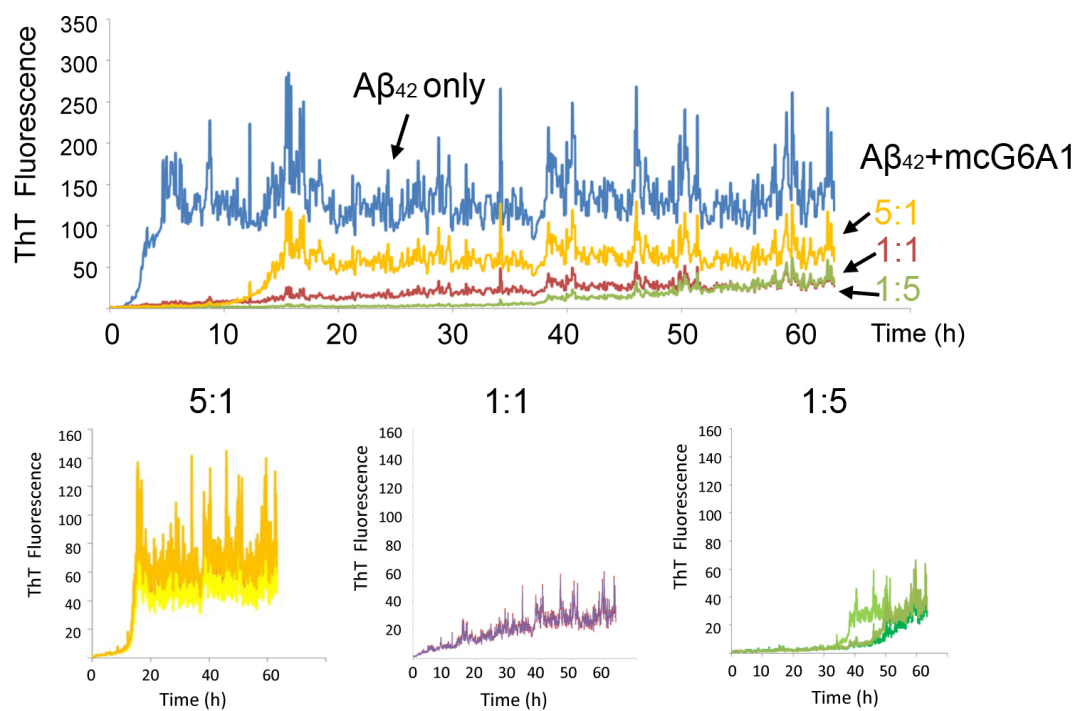
All NMR data were collected at the peptide concentration of 2 mM in D₂O at 298 K.



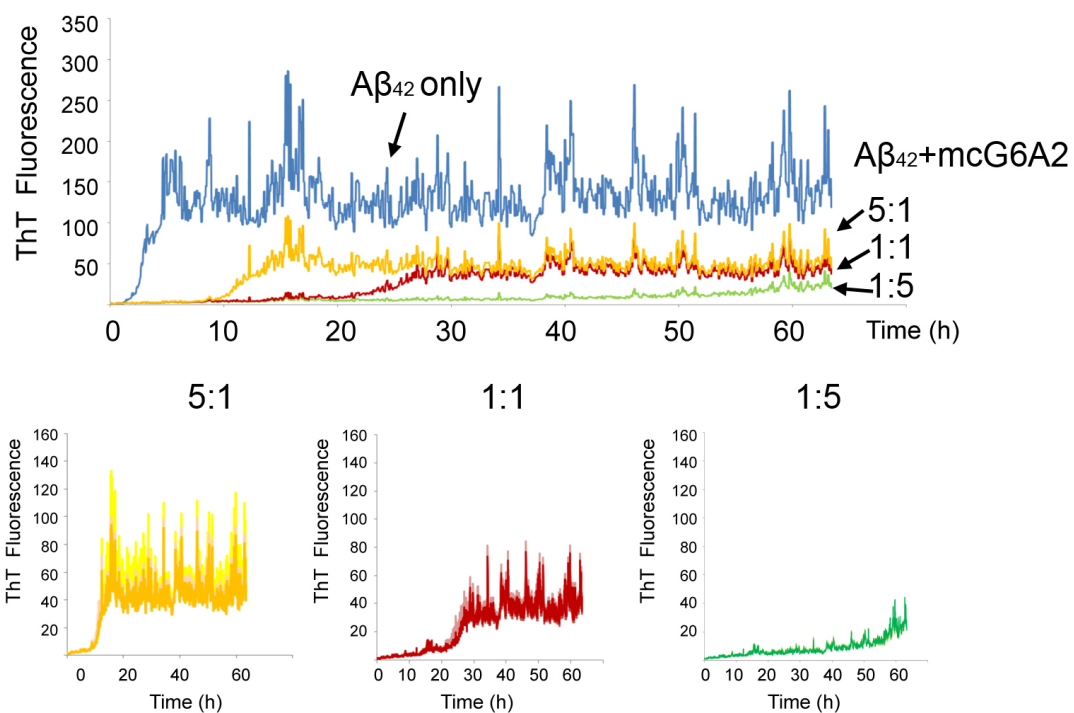
Supplementary Figure 6. Dose-dependent inhibition of mcK6A1 on A β_{42} aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



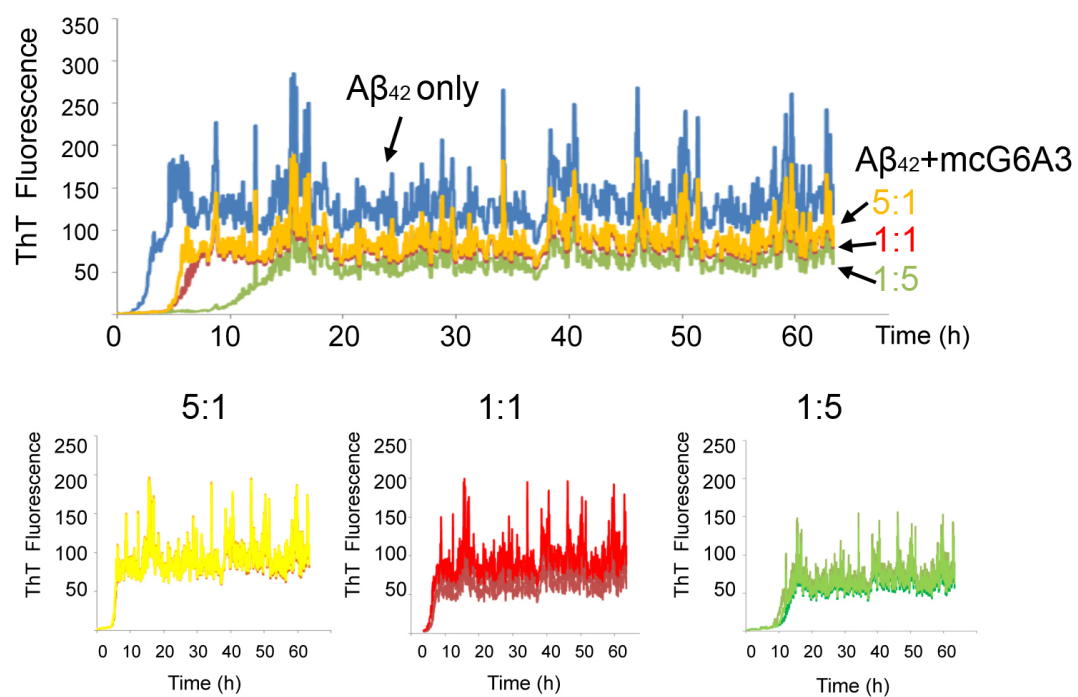
Supplementary Figure 7. Inhibition of mcK6A2 on $A\beta_{42}$ aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



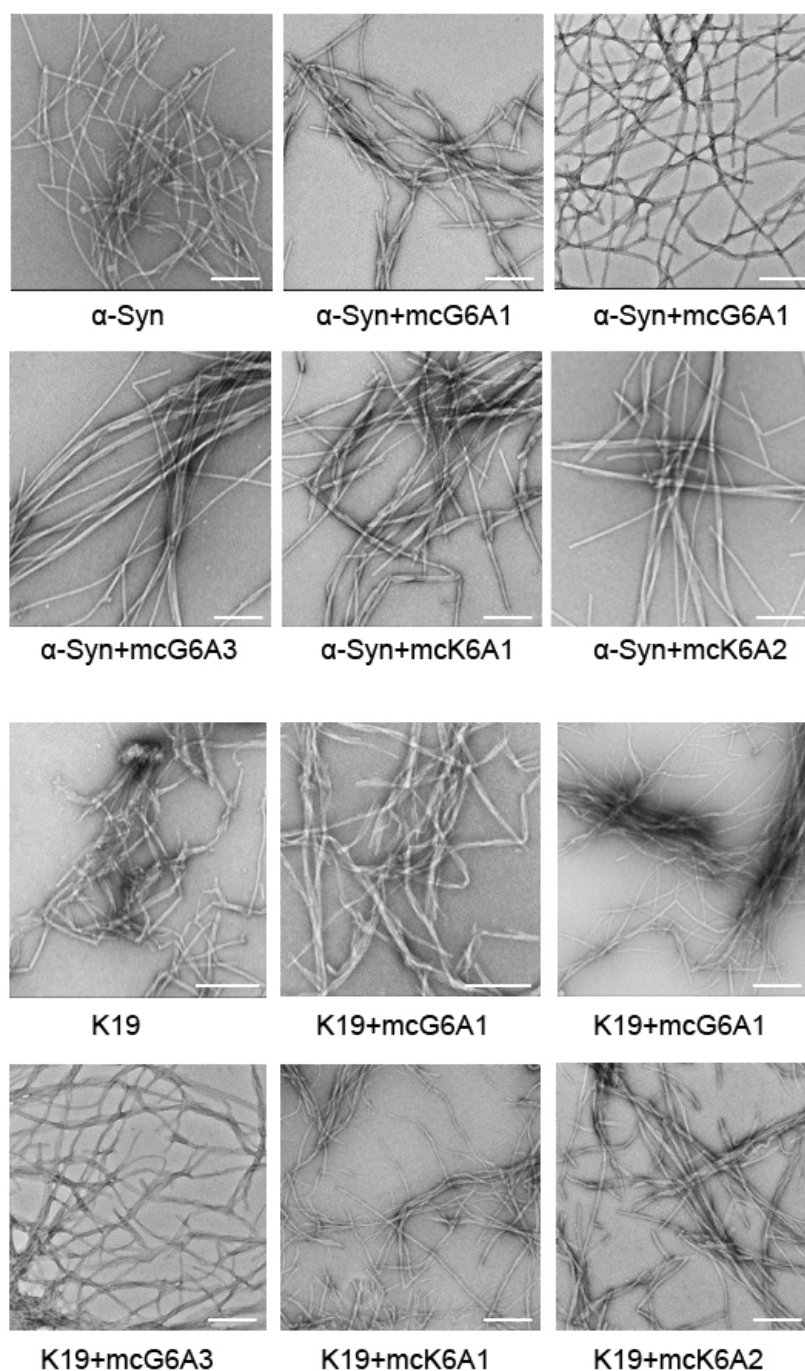
Supplementary Figure 8. Dose-dependent inhibition of mcG6A1 on A β_{42} aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



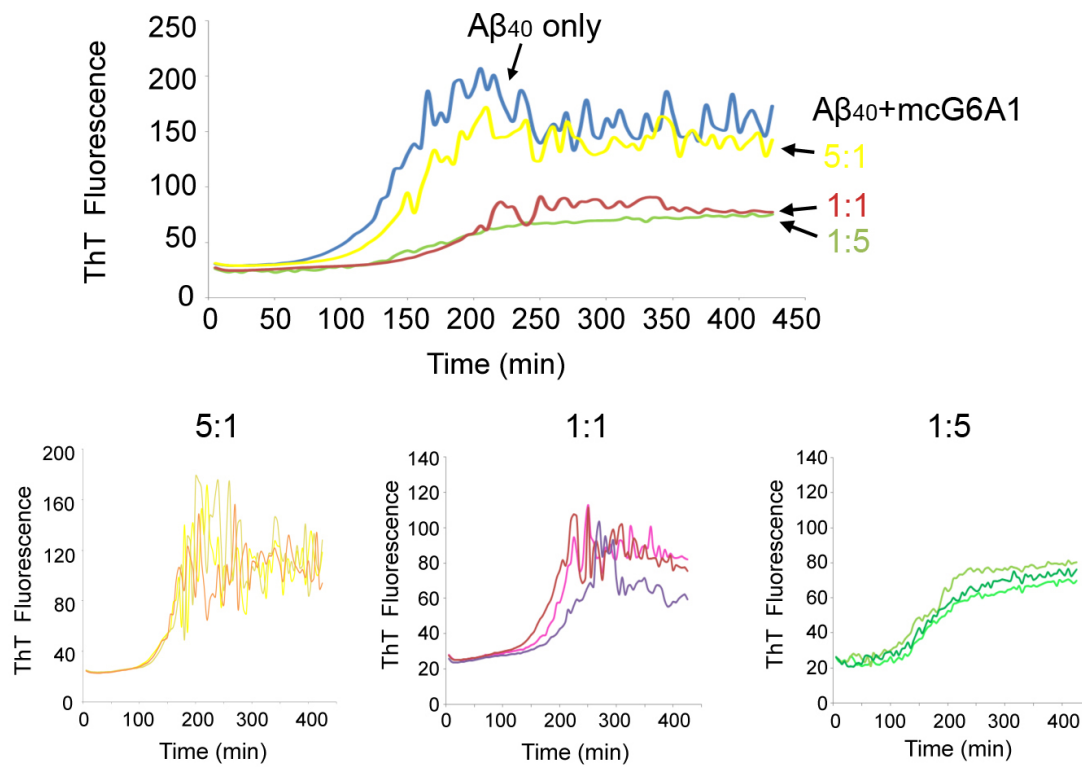
Supplementary Figure 9. Dose-dependent inhibition of mcG6A2 on A β_{42} aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



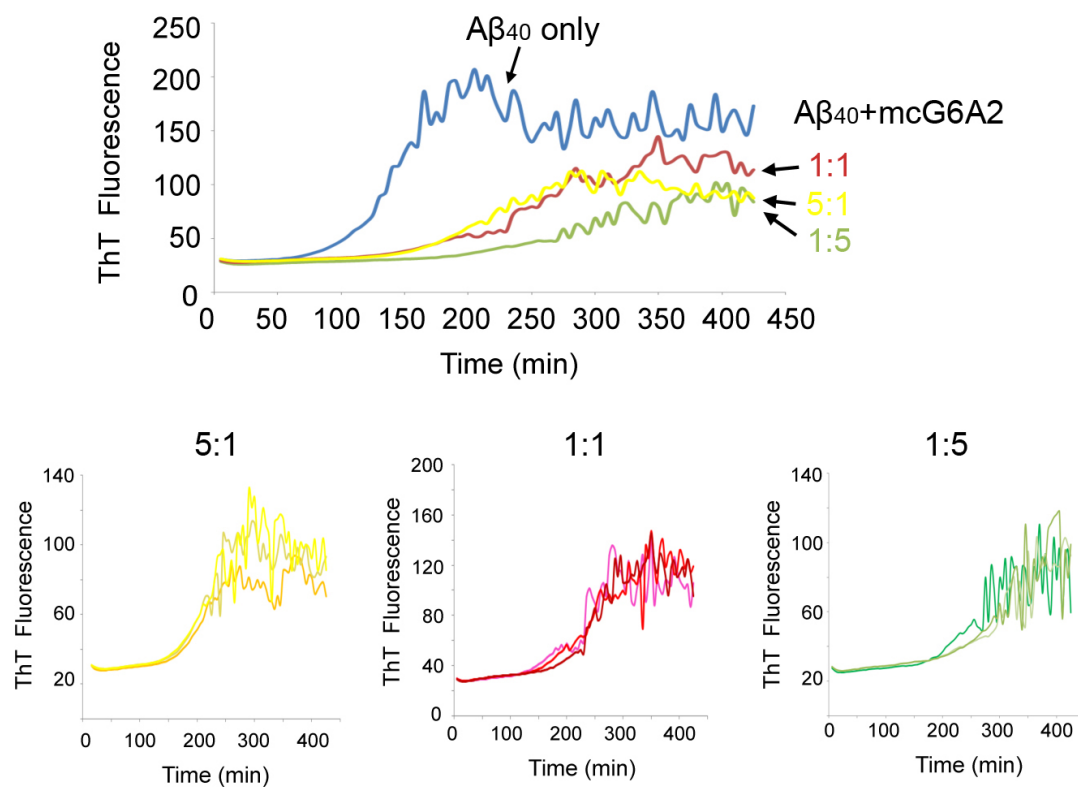
Supplementary Figure 10. Inhibition of mcG6A3 on $A\beta_{42}$ aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



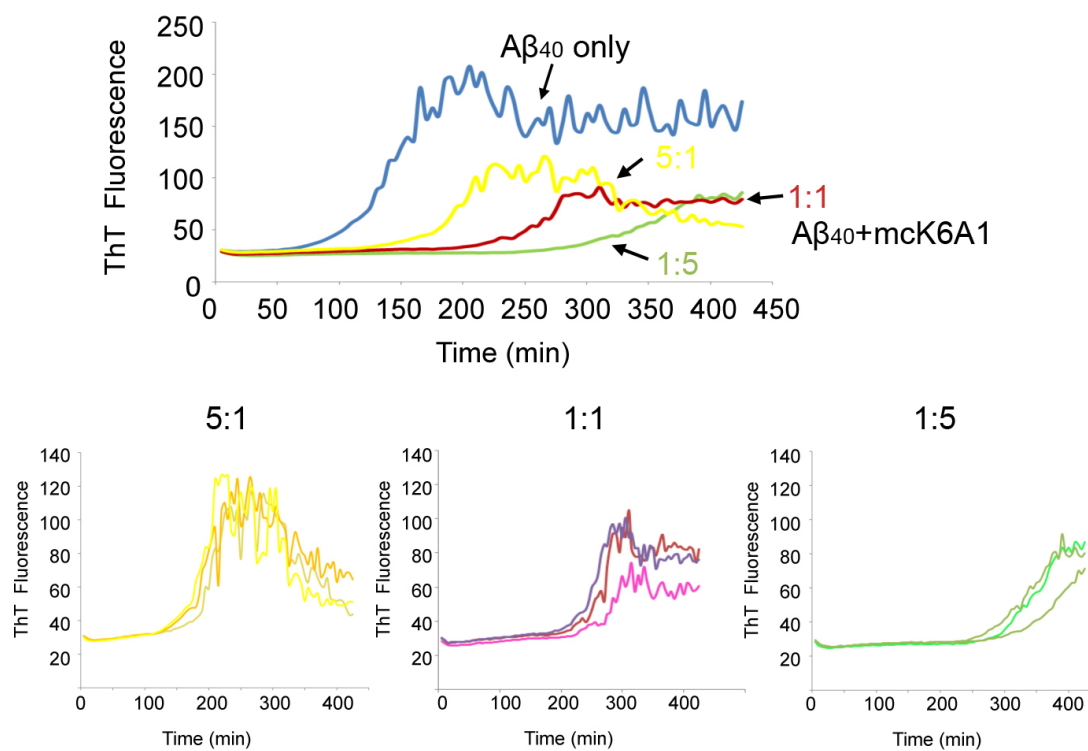
Supplementary Figure 11. Effects of designed peptides on the amyloid fibril formation of α -syn and tau K19. Peptides designed for inhibiting A β aggregation showed no significant influences on the amyloid fibril formation of α -syn and tau K19 imaged by TEM. α -Syn and tau K19 (50 μ M) were incubated with 1.0 equiv. of designed peptides for 15 h. The scale bars are 200 nm.



Supplementary Figure 12. Effect of mcG6A1 on $A\beta_{40}$ aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



Supplementary Figure 13. Effect of mcG6A2 on $A\beta_{40}$ aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.



Supplementary Figure 14. Dose-dependent inhibition of mcK6A1 on Aβ₄₀ aggregation measured by ThT fluorescence assay. Three replicates for each dosage are shown below at the bottom.

References

- [1] W. C. Johnson, *Proteins* **1999**, *35*, 307-312.
- [2] V. H. Finder, I. Vodopivec, R. M. Nitsch, R. Glockshuber, *J. Mol. Biol.* **2010**, *396*, 9-18.
- [3] J. Zheng, C. Liu, M. R. Sawaya, B. Vadla, S. Khan, R. J. Woods, D. Eisenberg, W. J. Goux, J. S. Nowick, *J. Am. Chem. Soc.* **2011**, *133*, 3144-3157.
- [4] T. V. Khasanova, O. Khakshoor, J. S. Nowick, *Org. Lett.* **2008**, *10*, 5293-5296.
- [5] S. Levin, J. S. Nowick, *Org. Lett.* **2009**, *11*, 1003-1006.