Structure-Based Peptide Inhibitor Design of Amyloid-β Aggregation

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Many human neurodegenerative diseases are associated with amyloid fibril formation. Inhibition of amyloid formation is of importance for therapeutics of the related diseases. However, the development of selective potent amyloid inhibitors remains challenging. Here based on the structures of amyloid β (Aβ) fibrils and their amyloid-forming segments, we designed a series of peptide inhibitors using RosettaDesign. We further utilized a chemical scaffold to constrain the designed peptides into β-strand conformation, which significantly improves the potency of the inhibitors against Aβ aggregation and toxicity. Furthermore, we show that by targeting different Aβ segments, the designed peptide inhibitors can selectively recognize different species of Aβ. Our study developed an approach that combines the structure-based rational design with chemical modification for the development of amyloid inhibitors, which could be applied to the development of therapeutics for different amyloid-related diseases.

Keywords: neurodegenerative diseases, Alzheimer’s disease, Aβ fibril, protein misfolding, structure-based inhibitor design

INTRODUCTION

Amyloid diseases, including many neurodegenerative diseases, are increasingly prevalent in aging societies (Eisenberg and Jucker, 2012; Dobson, 2017). The pathogenesis of these devastating diseases is closely associated with aberrant protein aggregation (Chiti and Dobson, 2006). In the progression of amyloid aggregation, soluble proteins undergo a series of conformational changes and self-assemble into insoluble amyloid fibrils (Riek and Eisenberg, 2016). Plaques containing amyloid fibrils are one of the histological hallmarks of Alzheimer’s and Parkinson’s diseases (Lee et al., 1991; Spillantini et al., 1997; Koo et al., 1999). Various strategies have been exploited to interfere with the process of amyloid aggregation by targeting different conformational species, including stabilizing monomers by antibodies (Ladiwala et al., 2012), redirecting monomers to nontoxic off-pathway oligomers by polyphenolic compounds (Ehrnhoefer et al., 2008), accelerating mature fibril formation by fibril binders (Bieschke et al., 2012; Jiang et al., 2013), inhibiting fibril growing by peptide blockers (Seidler et al., 2018), and disrupting amyloid assembly by nanomaterials (Hamley, 2012; Huang et al., 2014; Lee et al., 2014; Li et al., 2018; Han and He, 2018). Many of these strategies show promising inhibitory effects against toxic amyloid aggregation (Härd and Lendel, 2012; Arosio et al., 2014), but so far none has led to clinical drugs because of unsettled
issues such as target selectivity, side effects, membrane permeability and penetration of the blood-brain barrier.

Amyloid β (Aβ) has long been targeted for drug development and therapeutic treatment of Alzheimer’s disease (Caputo and Salama, 1989; Haass and Selkoe, 2007; Sevigny et al., 2016). In addition to the common difficulties in targeting amyloid proteins, Aβ is especially challenging since it contains multiple species with various lengths generated by γ-secretases (Acx et al., 2014; Kummer and Heneka, 2014; Szaruga et al., 2017).

Many studies have shown that Aβ42 rather than Aβ40 is more prone to form toxic aggregates, and the ratio of Aβ42/Aβ40 is better correlated with the pathology rather than the amount of each individual Aβ species (Lewczuk et al., 2004; Jan et al., 2008; Kuperstein et al., 2010). However, selective inhibition of Aβ42 is very difficult because it is only two residues longer than Aβ40 at the C-terminus. In this work, we targeted two key amyloid-forming segments of Aβ42 (16KLVFFA21 and 37GGVVI42) based on the cryo-EM structure of Aβ42 fibrils reported recently (Gremer et al., 2017). We designed peptide binders of these two segments using RosettaDesign with the atomic structures of these two segments as templates (Sawaya et al., 2007; Colletier et al., 2011). The designed sequences showed inhibitory effect to Aβ42 fibril formation. We further utilized a macrocyclic β-sheet mimic scaffold (Zheng et al., 2011; Cheng et al., 2012, 2013) to constrain the designed peptide inhibitors in β-conformation, which significantly enhanced the inhibitory effect on Aβ42 aggregation. Furthermore, we show that the peptide inhibitor designed to target the C-terminus of Aβ42 can selectively inhibit Aβ42 aggregation, but not to that of Aβ40 or other amyloid proteins. Our work shed light on the application of structure-based rational design combined with chemical modification in the development of therapeutics for Alzheimer’s disease and other amyloid-related diseases.

**MATERIALS AND METHODS**

**Structure-Based Design by Rosetta Software Package**

**Initial Structure Model for Design**

We chose two key amyloidogenic Aβ segments, 16KLVFFA21 and 37GGVVI42, for our inhibitor design. The design templates were taken from the crystal structures of KLVFFA (PDB ID: 2Y2A) and GGVVI (PDB ID: 2ONV). The backbone of the inhibiting pentapeptide was fully extended to mimic β-conformation. This extended peptide was aligned with the N, C, and O backbone atoms of the template.

**Rosetta Design of Fibril-Inhibiting Peptides**

The peptide inhibitors were subsequently designed to ensure maximal interaction, while keeping the template amino acid sequence fixed. Computational designs were carried out using the RosettaDesign software package1. This algorithm involves building side-chain rotamers of all L-amino acids onto a fixed peptide backbone. The optimal set of side-chain rotamers at each position with the best interaction energy is then identified, with the guidance of a full-atom energy function containing a Lennard-Jones potential, an orientation-dependent hydrogen bond potential, an implicit solvation term, amino acid-dependent reference energies, and a statistical torsional potential that depends on the backbone and side-chain dihedral angles. Finally, the entire structure was refined by simultaneously optimizing degrees of freedom on: (1) the rigid-body geometry between the inhibiting peptide and template; (2) backbone torsions of each peptide; and (3) side chain torsions of each peptide. The lowest-energy model was picked and the interaction energies of each final model from different peptide inhibitors are listed in Table 1.

**Circular Dichroism Spectroscopy (CD)**

Chirascan spectrometer (Applied Photophysics) equipped with a Peltier temperature controller (Quantum Northwest) is used to acquire the CD spectra. Far UV spectra (240–180 nm) are collected in 0.05 cm path-length quartz cells. Sample concentration is 600 μM. All measurements are conducted at 23°C. Water is used as blank for subtraction from corresponding samples. Secondary structure is predicted from CD using CDPro (Eisenberg and Jucker, 2012).

**Preparation of Aβ42 and Aβ40**

Both Aβ42 and Aβ40 were purified from E. coli expression system as reported previously (Dobson, 2017). The expression constructs contain an N-terminal His-tag, followed by 19 repeats of Asn-Ala-Asn-Pro, the Tobacco etch virus (TEV) protease site, and the sequence of Aβ42 or Aβ40. Purification of Aβ42 and Aβ40 follows the same experimental procedure. Briefly, the Aβ fusion protein was overexpressed into inclusion bodies in E. coli BL21(DE3) cells. The inclusion bodies were solubilized in 8 M urea, followed by washing in a high salt and detergent-containing solution. The Aβ fusion proteins were purified through HisTrap™ HP Columns, followed by reversed-phase high-performance liquid chromatography (RP-HPLC). After cleavage by TEV protease, Aβ was released from fusion protein, and purified through RP-HPLC followed by lyophilization. To disrupt preformed Aβ aggregates, lyophilized Aβ powder was resuspended in 100% HFIP and incubated at room temperature for 2 h. HFIP was fully removed by evaporation. Before used in ThT or MTT assay, Aβ was freshly dissolved in 10 mM NaOH, solubilized by sonication. Aβ is further diluted to 200 μM in phosphate buffer saline (PBS) as a stock solution.

**Synthesis of Designed Macroyclic Peptides**

Designed macrocyclic peptides were synthesized by standard Fmoc solid-phase peptide synthesis. In brief, with Boc-Orn(Fmoc)-OH attached onto 2-chlorotriyl chloride resin, the linear peptide was elongated by standard automated Fmoc solid-phase peptide synthesis. Then, the peptide was cleaved from the resin under mildly acidic conditions, followed by being cyclized to the corresponding protected cyclic peptide by slow addition to HCTU and DIEA in dilute (ca. 0.5 mM) DMF solution. Since

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1https://www.rosettacommons.org
the C-terminus of the protected linear peptide comprises an amino acid carbamate (Boc-NH-CHR-COOH), the cyclization condition efficiently avoids problematic epimerization. The final deprotection with TFA solution followed by RP-HPLC purification yielded macrocyclic peptides in 18%–43% overall yield, based on the loading of Boc- Orn(Fmoc)-OH attached onto the resin.

| Targeting sequence | Ap42 | Inhibitor ID | Inhibitor sequence | Predicted binding energy (kcal/mol) | Buried area (Å²) | Shape complementarity (Sc; Lawrence and Colman, 1993) |
|--------------------|------|--------------|--------------------|-------------------------------------|-----------------|---------------------------------------------------|
| 16KLVFFA21          | Ap42 | K8A1         | TLWYK              | –16                                 | 280             | 0.65                                              |
| 37GSGVIA42          | Ap42 | G6A2         | HYYIK              | –15                                 | 252             | 0.72                                              |
|                    | Ap42 | G6A3         | KYEYI              | –14                                 | 270             | 0.66                                              |

**TABLE 1 | Characteristics of designed peptide inhibitors.**

**1H NMR Spectroscopy**

1H NMR experiments for the designed macrocyclic peptides were performed in D2O with the internal standard 4,4-Dimethyl-4-silapentane-1-ammonium trifluoroacetate (DSA) at 500 MHz (Bruker Avance) or 600 MHz (Bruker Avance). All peptides were studied at 2 mM in D2O at 298 K. Sample solutions were prepared gravimetrically by dissolving the macrocyclic peptides directly in solvent. All amino groups were assumed to be protonated as the TFA salts for molecular weight calculation. The data were processed with the Bruker XwinNMR software.

**ThT Fluorescence Assay**

Thioflavin T (ThT) fluorescence assays were performed to monitor the real-time aggregation of Ap42 and Ap40 in the absence or presence of designed peptides. ThT assays were conducted in 96-well plates (black with flat optical bottom) in a Varioskan fluorescence plate reader (Thermo Scientific, 444 nm excitation, 484 nm emission). Each experiment was run in triplicates. The reaction solution contained 30 µM pre-disaggregated Ap42 or Ap40, 10 µM ThT, and designed peptides at indicated concentrations in PBS. The ThT assay was conducted at 37°C, without shaking for the Ap42 aggregation assay, and with shaking (300 rpm) for Ap40 aggregation assay. The fluorescence readings were collected every 2 min.

**Native Gel Electrophoresis**

Purified Ap42 powder was pre-treated by HFIP and dissolved in PBS buffer as described above. Ap42 solution was diluted to a final concentration of 10 µM with or without the macrocyclic peptides mcG6A1, mcG6A2, and mcK6A1 (the final concentration of the inhibitors was 50 µM), and incubated at 37°C for 7.5 h. The samples were separated by a NativePAGE 4%–16% BisTris Gel (Novex, USA) and transferred to a nitrocellulose membrane pre-packed in iBlot 2 NC Mini Stacks (Novex, USA) by iBlot 2 Dry Blotting System (Life technologies, USA). The membrane was probed by β amyloid, 1–16 (6E10) Monoclonal Antibody (Covance, USA) and secondary anti-mouse IgG-HRP (MBL, USA), and detected with SuperSignal West Pico Chemiluminescent Substrate (Thermo, USA). The freshly made Ap42 sample without inhibitors was loaded to a separated native gel and detected by the same method as a 0-h control. The molecular weight of the protein aggregates or monomer were accurately determined by the protein standard especially for native gel (Life technologies; cat. # LC0725).

**Transmission Electron Microscopy (TEM)**

For specimen preparation, 5 µl of each sample was deposited onto a glow-discharged carbon film on 400 mesh copper grids, followed by washing in water twice. The grids were then stained in 0.75% uranyl formate. A Tecnai G2 Spirit transmission electron microscope operating at an accelerating voltage of 120 kV was used to examine and visualize the samples. Images were collected by a 4k × 4k charge-coupled device camera (BM-Eagle, FEI).

**Cell Viability Assay**

We performed MTT-based cell viability assays to evaluate the toxicity of Ap42 in the absence or presence of the designed peptides. We used a CellTiter 96 aqueous non-radioactive cell proliferation assay kit (Promega cat. # G4100). PC-12 cell lines (ATCC; cat. # CRL-1721) were used to test the cytotoxicity of Ap42 under different conditions. PC-12 cells were cultured in ATCC-formulated RPMI 1640 medium (ATCC; cat. # 30-2001) with 5% fetal bovine serum and 10% heat-inactivated horse serum. Before the cell viability experiment, PC-12 cells were plated at 10,000 cells per well in 96-well plates (Costar; cat. # 3596), and cultured for 20 h at 37°C in 5% CO2. For the preparation of Ap42 and peptide inhibitors mixture solutions, purified and pre-disaggregated Ap42 samples were dissolved in PBS to a final concentration of 5 µM, followed by the addition of different peptide inhibitors at indicated concentrations. The mixture solution was filtered through a 0.22 µm filter, followed by incubation at 37°C without shaking for 16 h. To initiate the cell viability assay, 10 µl of pre-incubated mixture was added to each well containing 90 µl medium. After incubation at 37°C in 5% CO2 for 24 h. Fifteen microliter Dye solution (Promega; cat. # G4102) was applied into each well. After incubation for 4 h at 37°C, 100 µl solubilization Solution/Stop Mix (Promega; cat. # G4101) were added. After further incubation at room temperature for 12 h, the absorbance reading was collected at 570 nm with background reading at 700 nm. Four replicates were measured in parallel for each sample. The cell survival rate was normalized by using the PBS-treated cells as 100% and 0.02% SDS-treated cells as 0% viability.
RESULTS
Structure-Based Design of Peptide Inhibitors
To effectively inhibit Aβ fibril formation, we targeted two key amyloid-forming segments of Aβ42: 16KLVFFA21 and 37GGVVIA42 (Figure 1A). The 16KLVFFA21 segment has been identified as a key segment accounting for both Aβ42 and Aβ40 nucleation and fibrillation (Ahmed et al., 2010; Colletier et al., 2011; Fawzi et al., 2011; Lu et al., 2013). In the known structures of Aβ fibrils including the recent cryo-EM structure of Aβ42 and the previous solid-state NMR structure of Aβ40 (Paravastu et al., 2008; Ahmed et al., 2010), this segment forms extended β-strands and stacks repetitively along the fibril axis to form the Aβ fibril core (Supplementary Figure S1). Thus, we selected 16KLVFFA21 as one of our design targets. In addition, the cryo-EM structure of Aβ42 fibril shows that the C-terminal segment 37GGVVIA42 plays an essential role in the fibril formation (Supplementary Figure S2). 37GGVVIA42 of one protofilament interdigitates via side chains with its counterpart of the neighboring protofilament forming a steric-zipper-like interaction to compose the mature fibril. Therefore, preventing the self-assembly of either 16KLVFFA21 or 37GGVVIA42 may potentially inhibit the assembly of Aβ42 fibrils.

For structure-based computational design, we used the atomic structures of 16KLVFFA21 (PDB ID: 2Y2A) and 37GGVVIA42 (PDB ID: 2ONV) as templates. The atomic structures of these two segments represent their conformations in the context of the full-length Aβ fibrils (Figures 1A,B). Based on the structures of the two targeting templates, we designed pentapeptides

![Figure 1](image-url)
that bind the targeting segments to block the stacking of Aβ molecules along the fibril axis, thus inhibiting fibril growth (Figure 1C). We extracted a five-stranded layer from the steric-zipper structure of each segment, and docked a fully extended pentapeptide backbone on one end of the β-sheet. Then, we maximized the backbone interaction with the template by forming a backbone H-bonding network. To further increase the binding affinity and selectivity, we searched for the canonical L-amino acids at each position of the pentapeptide, using RosettaDesign (Leaver-Fay et al., 2011) for the side chains and their conformations, that provide maximal interactions with the template.

Next, we calculated the binding energy, buried surface area and shape complementarity of the binding interfaces of the predicted binding models, and proceeded with experimental validation for the top-ranking designs. Using ThT fluorescence assay, we observed that the top-5 designs showed inhibitory effects on Aβ42 amyloid aggregation by significantly delaying the aggregation lag time (Xue et al., 2008; Knowles et al., 2009; Figure 2). Among them, two peptide inhibitors (K6A1 and K6A2) were designed for targeting 16KLVFFA21 and three (G6A1-G6A3) were for 37GGV VIA42 (Table 1). Furthermore, unlike their targeting segments, the five designed peptides do not form amyloid fibrils by themselves (Supplementary Figure S3).

Constraining the Structures of Designed Peptides With a Chemical Scaffold

We next sought to enhance the potency of the peptide inhibitors. In our design, the peptide inhibitors were expected to adopt an extended β-strand conformation to maximize the interaction with the template (Figure 1C). However, in solution, the peptides are mainly unstructured (Supplementary Table S1). Thus, upon binding to the template, the peptides need to undergo conformational change to form extended β strands, which causes an entropy decrease and thus weakens the binding affinity of the peptides to the template. To overcome the entropy lost during the conformational change, we adopted a macrocyclic β-sheet mimic scaffold to fix the peptide binders into β strands (Figure 3A). The Knowles group has developed a series of macrocycles in different sizes as robust scaffolds for displaying peptides of interest in β-conformation (Liu et al., 2012; Cheng et al., 2013; Salveson et al., 2016; Kreutzer et al., 2017). According to the length of our designed peptides, we chose a 42-membered macrocyclic β-sheet mimic and grafted the designed sequence into the open strand of the macrocyclic scaffold with appropriate amino acids in the blocking strand for proper solubility and stability (Figure 3B). The β-strand conformation of the grafted sequence was validated by measuring the α-H shifts and δOrn anisotropy using 1H NMR experiments (Supplementary Figures S4, S5) in solution. Furthermore, we confirmed that the macrocycles carrying the designed peptides do not form amyloid aggregation in solution, while those carrying native amyloid-forming sequences may form amyloid fibrils with an out-of-register packing (Lu et al., 2013; Supplementary Figure S3).

Next, we tested the inhibitory effects of the macrocyclic peptides on Aβ42 amyloid aggregation. The result showed that, in comparison with the free peptides, the macrocyclic peptides remarkably enhanced the inhibition on Aβ42 aggregation (Figures 2B, 4A,B, and Supplementary Figures S6–S10). For instance, the macrocycle carrying K6A1 (mcK6A1) is about 10 times more potent than free K6A1 in prolonging the lag time of Aβ aggregation. The macrocyclic peptides inhibited the amyloid aggregation of Aβ42 in a dose-dependent manner. McK6A1, mcG6A1 and mcG6A2 showed remarkably strong inhibition with a 7–10-fold increase of the lag time at sub-stoichiometric concentrations of 0.2 molar equivalence to Aβ42 monomer (Figure 4A).

Moreover, we found that the designed macrocyclic peptides can inhibit the formation of Aβ42 oligomers, the toxic intermediates of Aβ aggregation, monitored by the native gel (Figure 4C). This result demonstrated that targeting 16KLVFFA21 and 37GGV VIA42 can prevent both oligomer and fibril formation, indicating the potential important role of these two segments in the early stage of Aβ42 aggregation. To further assess whether the designed peptides can reduce Aβ cytotoxicity,
we performed the MTT-based cell viability assay. The result showed that the designed macrocyclic peptides can significantly reduce the cytotoxicity of Aβ_{42} to PC-12 cells even with a molar ratio of inhibitor to Aβ_{42} as low as 0.2:1 (Figure 4D). Also, the designed macrocyclic peptides showed little toxicity to the PC-12 cells (Figure 4D). In addition, the designed inhibitors of Aβ_{42} showed no inhibition of the amyloid aggregation of other amyloid proteins (e.g., α-synuclein and the K19 variant of Tau), indicating that the designed peptides are highly sequence-specific (Supplementary Figure S11).

**Designed Peptides Selectively Inhibit the Aggregation of Aβ_{42} but Not Aβ_{40}**

Selective inhibition of Aβ_{42} aggregation over that of Aβ_{40} is challenging because Aβ_{42} is only two residues longer than Aβ_{40} at the C-terminus (Figure 5A). Since segment 37GGVVIAM exists only in Aβ_{42}, the designed peptides that target this segment may selectively inhibit the aggregation of Aβ_{42} but not that of Aβ_{40}. As shown in the designed models, mcG6A1 that is designed to target 37GGVVIAM forms extensive side-chain interactions with 37GGVVIAM (Figure 5B). The aromatic residues Tyr

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**FIGURE 3 | Design of macrocyclic peptide inhibitors.** (A) The schematic shows that as the macrocyclic β-sheet mimic scaffold constrains the designed peptide sequence into a β-strand, the entropy loss is diminished during the process of target binding. “f” represents free peptide; “mc” represents macrocyclic peptide. The zoom-in view shows the structure model of a macrocyclic inhibitor binding to the targeting segment. The targeting segment is in magenta. The designed sequence is in cyan. The macrocyclic scaffold is in gray. H-bonds between the designed sequence and the targeting sequence are labeled by yellow dotted lines. (B) The 42-membered macrocyclic scaffold used in this study. The open strand (positions R1 to R5) accommodates the designed peptides in β-conformation. Two δ-linked ornithine turn units are in blue. The Hao unit in the blocking strand is in red. Sequences of R1-R7 are listed in the table below.
and Phe of mcG6A1 interact with Ile41 of 37GGVIA42 via van der Waals forces. The absence of Ile41 and Ala42 in Aβ40 diminishes the binding of mcG6A1 to Aβ40. Indeed, the experimental data showed that mcG6A1 and mcG6A2 that strongly inhibit the amyloid aggregation of Aβ42, cannot effectively inhibit the aggregation of Aβ40, as measured by ThT assay (Figure 5C, Supplementary Figures S12, S13). Note that a weak inhibitory effect of mcG6A1 and mcG6A2 to Aβ40 remains, which might come from non-specific backbone interactions between the inhibitors and Aβ40 (Figure 5B). In contrast, mcK6A1 that was designed to target the 16KLVFFA21 segment, a segment important for the amyloid aggregation of both Aβ42 and Aβ40 aggregation (Figures 4A, 5C,D, and Supplementary Figure S14). However, the inhibitory efficiency of mcK6A1 on Aβ40 is weaker than that on Aβ42, indicating that 16KLVFFA21 may play a more important role in Aβ42 aggregation than that of Aβ40. This implication is in agreement with the hypothesis that Aβ42 and Aβ40 may employ different amyloid nucleation and aggregation process (Sánchez et al., 2011; Meisl et al., 2014).

**DISCUSSION**

Development of peptide-based drugs is gaining greater attentions. In general, peptide-protein interactions have a high density of hydrogen bonds and highly complementary packing via hot-spot binding residues, leading to high binding affinity and exquisite selectivity with fewer off-target side effects (Kaspar and Reichert, 2013). Many attempts have been made to rationally design peptide inhibitors of amyloid protein aggregation, including modified internal segments of parent amyloid proteins, non-natural amino-acid inhibitors, proline substitutions, and other methods (Abedini et al.,...
Specificity of designed macrocyclic peptides for the inhibition of Aβ42 and Aβ40 aggregation. (A) The sequences of Aβ42 and Aβ40. The amyloid-forming segment 16KLVFFA21 (highlighted in orange) is present in both Aβ42 and Aβ40, while segment 37GGVIA42 (highlighted in magenta) is present only in Aβ42. The consensus sequence of Aβ42 and Aβ40 is highlighted in gray. (B) The structure models of mcG6A1 (cyan) in complex with 37GGVIA42 (magenta) and 16KLVFFA21 (orange), respectively. McG6A1 was designed based on the structure of GGVIA. Residues Tyr and Phe of mcG6A1, and Ile41 of GGVIA (highlighted with a gray frame) engage in van der Waals interactions at the inhibitor-target interface. In contrast, mcG6A1 designed for GGVIA has no specific side-chain interactions, but merely non-specific backbone interactions with KLVFFA. (C) The effects of mcK6A, mcG6A1 and mcG6A2 on Aβ40 aggregation (30 µM by Aβ40 monomer equivalence), measured by ThT assay. Error bars correspond to standard deviations of three replicates of each experiment. ∗p-value < 0.05; **p-value < 0.01; ***p-value < 0.001; n.s. represents "not significant." (D) TEM images of Aβ40 (30 µM) after incubation without inhibitors (left), and with 1.0 equivalent of mcK6A1 to Aβ monome (right). The scale bars are 200 nm.

Macrocyclic β-sheet mimics have been shown to be a useful model system to study the structural basis of amyloid-like oligomers and fibrils (Liu et al., 2012; Cheng et al., 2013; Zheng et al., 2013; Salveson et al., 2016). A variety of key amyloidogenic segments from different amyloid proteins (e.g., Aβ, α-synuclein and prion) were constructed into the
macrocycles (Zheng et al., 2011; Cheng et al., 2012). However, the self-assembling and potential toxic properties of macrocyclic molecules that contain native amyloid-forming sequences hinder application of macrocycles in the development of amyloid inhibitors (Liu et al., 2012; Salveson et al., 2016). In this study, by using RosettaDesign approach, we developed novel sequences and incorporated them into macrocycles. These designed macrocyclic peptides resist self-assembly and exhibit small cytotoxicity. In addition to Aβ, the structures of many other pathogenic amyloid fibrils have been determined recently (Tuttle et al., 2006; Fitzpatrick et al., 2017; Murray et al., 2017). Thus, the strategy of combining RosettaDesign and chemical scaffolds may be useful for peptide inhibitor design of different amyloid proteins for a variety of amyloid-related diseases.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

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AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnmol.2019.00054/full#supplementary-material
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