Modulating the aggregation of amyloid proteins by macrocycles

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Abstract
The aggregation of amyloid proteins has been suggested to be the main cause of multiple human disorders; for example, amyloid β aggregates in Alzheimer’s disease and α-synuclein aggregates in Parkinson’s disease. In the search for therapeutic medicines, many molecules have been discovered and developed to modulate the aggregation of amyloid proteins. This century has witnessed the flourishing growth of supramolecular chemistry, and some biocompatible macrocycles have been proven to inhibit the aggregation of some amyloid proteins via host-guest interactions and could thus be used for the prevention or treatment of related diseases. Here, we review the application of macrocycles in modulating the aggregation of amyloid proteins.

KEYWORDS
amyloid protein, calixarene, cucurbituril, cyclodextrin, inhibitor, macrocycle

1 | INTRODUCTION
Because proteins are the primary participants in cellular activities, protein dysfunction leads to many types of diseases. In addition to genetic factors that may cause the abnormal expression of proteins, the misfolding and/or aggregation of some proteins will also reduce the level of normal proteins and/or increase the level of harmful species. Abnormal protein aggregates are often termed amyloid fibrils, and the proteins that form these fibrils can be defined as amyloid proteins. Although the direct link between the aggregation of amyloid proteins and the pathological processes of some diseases remains elusive, it is widely accepted that the major causative factors are the oligomers and fibrils that form during the aggregation process and not the soluble monomers.

The amyloid cascade hypothesis, a theory approximately 30 years old regarding Alzheimer’s disease, believes that amyloid β (Aβ) aggregates can not only impair synaptic activity but also trigger the aggregation and downstream toxicity of tau protein. The accumulation of α-synuclein (α-syn) has been observed in Parkinson’s disease and other Lewy body diseases, and α-syn oligomers and fibrils are believed to play key roles in these diseases. Moreover, recent studies suggest that some amyloid proteins, including Aβ and α-syn, have prion-like properties, which means that their aggregates can self-replicate and spread from cell to cell.

In addition to the above-described disease-related amyloid proteins, some protein drugs tend to aggregate and can also form amyloid fibrils. These tendencies hinder their formulation and decrease their effective concentration in the...
body.\[^{[10]}\] For example, insulin, which is the cornerstone for the treatment of diabetes,\[^{[11]}\] aggregates to form fibrils under various conditions.\[^{[12,13]}\] Inhibiting the aggregation of such drugs to form amyloid fibrils and stabilizing them as effective monomers will increase their efficiency in the treatment of related diseases.

Therefore, modulating the aggregation of amyloid proteins has been recognized as a potential therapeutic method for many diseases. Some inhibitors of Aβ, including EGCG (NCT03978052, NCT00951834) and trehalose (NCT04663854), have entered clinical trials for Alzheimer’s disease.\[^{[14]}\] Tafamidis meglumine, which can stabilize the amyloid protein transthyretin (TTR) in tetramer form and inhibit the formation of fibrils,\[^{[15]}\] has been approved by the food and drug administration (FDA) for the treatment of transthyretin amyloid cardiomyopathy.\[^{[16]}\]

Many other compounds, including small molecules,\[^{[17–19]}\] peptides,\[^{[20,21]}\] and nanoparticles,\[^{[22]}\] have also been found to modulate the aggregation of amyloid proteins. Here, we focus on synthetic macrocycles that possess interior cavities and exterior functional groups.\[^{[23]}\] The characteristic structural properties of these macrocycles make them suitable for the recognition and hosting of diverse guest molecules, particularly peptides/proteins and other biomolecules.\[^{[24–27]}\]

The macrocycles are somewhat similar to antibodies because they exhibit strong and specific binding with target molecules but have lower molecular weights. Compared with small molecules, macrocycles may exhibit better specificity in binding with proteins due to their larger binding surfaces.\[^{[28]}\]

The rigid skeletons of macrocycles also make them different from flexible peptides. These widely used macrocycles, including cucurbiturils, calixarenes (CAs), and cyclodextrins (CDs), are generally safe and biocompatible, which supports their broad application in biological systems.\[^{[24–27]}\]

Amyloid fibrils generally possess β-sheet structures that are stabilized mainly by hydrogen bonds and π–π stacking.\[^{[29]}\] Some macrocycles can bind with specific functional groups of amyloid proteins through host-guest interactions and subsequently interfere with the interactions needed for fibril formation or sterically hinder the associations between amyloid proteins. Therefore, these macrocycles can modulate the aggregation of amyloid proteins and may be used for the prevention or treatment of related diseases. Here, we summarize the macrocycles that could function as modulators of amyloid aggregation and offer some personal opinions regarding their future development.

2 | AGGREGATION OF AMYLOID PROTEINS

The detailed aggregation processes of amyloid proteins have not been fully determined due to the difficulties in characterizing and quantifying transient and metastable oligomers. In general, monomers of amyloid proteins first aggregate to form oligomers, and the oligomers are then converted to fibrils, which is a process called primary nucleation. Then, fibrils can subsequently bind monomers and grow in length, which is a process termed elongation. The fibrils can also act as catalysts to generate new fibrils from monomers, and this process is termed surface-catalyzed nucleation or secondary nucleation. Therefore, the modulators (mainly inhibitors) of amyloid aggregation may bind to monomers, oligomers, or fibrils and then interfere with various microscopic steps (Figure 1A).\[^{[30,31]}\]

Several conventional biophysical methods are widely used to characterize the aggregation of amyloid proteins, including thioflavin T (ThT) kinetics, CD spectroscopy, transmission electron microscope (TEM), and atomic force microscope (AFM).\[^{[32]}\] ThT is a fluorescent dye that yields approximately 1000-fold fluorescence intensity when incorporated into the β-sheet structures of amyloid fibrils.\[^{[33]}\] Therefore, ThT is widely used to monitor the aggregation process of amyloid proteins. Results from CD spectroscopy reflect the secondary structures of peptides/proteins; thus, it can be used to confirm the formation of β-sheet structures. The morphology and nanostructures of amyloid aggregates (particularly fibrils) can be directly observed by TEM and AFM. These methods do not provide comprehensive information about oligomers and cannot reveal the detailed microscopic aggregation processes.

Other techniques have been developed in recent years to obtain a better understanding of oligomers and aggregation processes. For example, native mass spectrometry and ion mobility mass spectrometry enable the detection and analysis of amyloid oligomers.\[^{[34,35]}\] Dot blot has been applied to detect antibody-specific oligomers,\[^{[36]}\] and solid-state nuclear magnetic resonance spectroscopy has been used to reveal the structures of amyloid oligomers.\[^{[37]}\] In addition, single-molecule fluorescence spectroscopy offers quantitative information about the formation of oligomers at the single-molecule level.\[^{[38]}\] Many molecular probes, including Congo red derivatives and aggregation-induced emission molecules, have been developed to complement ThT in studies of the aggregation of amyloid proteins.\[^{[39]}\]

Knowles et al. developed kinetic models to describe the microscopic steps of fibril formation.\[^{[30,40]}\] Microscopic aggregation processes can be viewed as chemical reactions; thus, chemical kinetics can be applied to determine the rate laws of different steps. With the rate law and reaction time in hand, theoretical predictions of the reaction profiles, including quantities of monomers, oligomers, or fibrils, can be obtained through mathematical calculations. The data obtained from chemical kinetics simulations are consistent with the results from some biophysical experiments, particularly ThT kinetics. The inhibitory mechanisms of different molecules could be confirmed by chemical kinetics because the effects on different microscopic steps will induce different changes in the observed ThT kinetics curves (Figure 1B).\[^{[31,41]}\] In fact, a single molecule might interfere with multiple microscopic steps because the molecule may interact with more than one amyloid species during the aggregation process.\[^{[42]}\]

3 | CUCURBITURILS FOR MODULATING AMYLOID AGGREGATION

Pumpkin-shaped cucurbit[n]urils (CB[n], n = 5–8, 10, 14)\[^{[26]}\] are products from the reaction of glycoluril with formaldehyde (Figure 2A).\[^{[25]}\] Increases in n from 5 to 10 can increase the cavity volume from 82 Å³ to 870 Å³, which allows cucurbiturils to bind with guest molecules of various sizes.\[^{[25]}\] Cucurbiturils have one nonpolar cavity and two electronegative portals, which facilitates their strong
binding with molecules containing both hydrophobic groups and positively charged groups. Both the hydrophobic interactions inside the cavity and the electrostatic interactions at the exterior contribute to the specific and strong binding of cucurbiturils with some guest molecules. For example, CB[7] binds to adamantylamine with $K_a = 4.23 \times 10^{12} \text{ M}^{-1}$, whereas its binding affinity for 1-adamantanecarboxylic acid decreases to $K_a = 3.23 \times 10^8 \text{ M}^{-1}$.\[43\]

Because the side chains of aromatic amino acids are hydrophobic, and some basic amino acids are positively charged under physiological conditions, cucurbiturils have the capability to bind with different peptides and proteins.\[26,28,44,45\] Therefore, it is reasonable to infer that cucurbiturils could interact with some amyloid proteins and may modulate their aggregation.

Urbach et al. reported the interactions between insulin and CB[7].\[47\] Isothermal titration calorimetry (ITC) results suggest that the N-terminal Phe is the major contributor to the binding affinity observed ($K_a = 1.5 \times 10^6 \text{ M}^{-1}$). By analyzing the crystal structure of the CB[7]-insulin complex, the researchers also found that the N-terminal Phe in the B1-chain of insulin is included in the cavity of CB[7]. The binding affinity to CB[7] for peptides with midchain Phe residues\[44,47\] is approximately $10^4 \text{ M}^{-1}$, which suggests the importance of ion-dipole interactions (Figure 2B).\[46\]

Kim et al. reported the first work using cucurbituril to modulate the aggregation of amyloid proteins.\[48\] The researchers found that CB[7] could significantly inhibit the aggregation of 10 $\mu$M insulin even at a ratio of 1:0.5 (insulin:CB[7]) based on ThT kinetics, TEM, and CD spectroscopy. The ITC and native polyacrylamide gel electrophoresis (PAGE) results also confirmed the binding between insulin and CB[7]. The researchers also found that CB[7] could inhibit the aggregation of $\beta_\text{1-40}$ and $\beta_\text{1-42}$, which contain Phe residues in the middle of the chain, although the effective ratio of $\beta_\text{CB[7]}$ was higher than 1:10. Mutation of the Phe residues in the sequence of insulin or $\beta_\text{CB}$ decreased the binding affinity with CB[7]. The researchers also revealed that CB[7] effectively decreased the cytotoxicity of insulin, $\beta_\text{1-40}$ and $\beta_\text{1-42}$ fibrils. CB[7] is water soluble and nontoxic, which makes it a good candidate for further application in biological systems.\[25\]

Bowers et al. studied the effects of CB[7] on the aggregation of $\beta_{25-35}$ and found inhibition of the formation of $\beta$ fibrils (1:10, $\beta$-CB[7]).\[49\] Ion-mobility spectrometry–mass spectrometry results suggest that CB[7] suppresses the formation of $\beta_{25-35}$ homo-oligomers. It should be noted that $\beta_{25-35}$ contains no Phe residues. Molecular dynamics (MD) simulations reveal that CB[7] binds to Lys28 of $\beta_{25-35}$ mainly through electrostatic interactions.

Anderson et al. developed a method named supramolecular PEGylation to stabilize biopharmaceuticals, including insulin.\[10\] The researchers first synthesized CB[7]-N$_2$ using a previously reported method.\[50\] Then, strain-promoted click chemistry\[51\] was used to conjugate CB[7]-N$_2$ with polyethylene glycol (PEG), and CB[7]-PEG was obtained (Figure 3). CB[7]-PEG still preserved the ability to bind with insulin. Owing to the good solubility of both CB[7] and PEG, the aggregation of insulin was greatly inhibited by CB[7]-PEG under physiological conditions with continuous agitation (1:1, insulin:CB[7]). After aging for 100 d with CB[7]-PEG, no significant aggregation was observed, and the activity of insulin was retained. In addition to insulin, the stabilization of glucagon and an antibody for human CD20 was also achieved with CB[7]-PEG. The researchers also found that the formulation of insulin with CB[7]-PEG could maintain a low blood glucose level in STZ diabetic mice, which suggests a depot effect of CB[7]-PEG on insulin in vivo. The supramolecular PEGylation method has been further extended to insulin analogs\[52\] and a coformulation of insulin with pramlintide.\[53\]
Li et al. demonstrated that CB[7] inhibits the fibrillation of another potential protein drug, human calcitonin (hCT).[54] hCT could be a good substitute for salmon calcitonin, which is used for the treatment of some bone-related diseases. However, hCT suffers the same shortcomings as insulin. The researchers found that 25-fold CB[7] is needed to fully suppress the aggregation of hCT because hCT contains no N-terminal Phe residues. Mutating the aromatic amino acids (Tyr and Phe) in hCT to Ala decreases its binding affinity (1:10, protein:CB[7]) into rats and observed better calcitonin release in the latter group. Moreover, the authors proved that CB[7] is not immunogenic and could decrease the immunogenicity of hCT.

In addition to CB[7], other cucurbiturils have also been used to modulate the aggregation of amyloid proteins. Kim et al. reported that CB[6], which is barely soluble in water, could form host-guest complexes with Lys-containing peptides/proteins with Phe residues, particularly N-terminal Phe residues. A greater number of phenol units are synthesized by the reaction of phenol derivatives with formaldehyde.[24,68] A greater number of phenol units are associated with a larger cavity size. A large amount of CAs with different structures can be obtained due to the multiple sites available for modification. Different substitutions on both the upper and the lower rims can be easily achieved. CAs also possess different conformational structures, which results in an almost unlimited numbers of applications.[24,70,71] Several CAs have been reported to inhibit the aggregation of Aβ and insulin.[24] More biocompatible CAs are expected to interfere with the aggregation of amyloid proteins, which offers potential therapeutic methods for related diseases.

Mohanty et al. reported the inhibitory effects of p-sulfonatocalix[4/6]arene (SC[4/6]A) on the aggregation of insulin (Figure 6).[72] Not only was the aggregation process suppressed, but the mature insulin fibrils could also be disintegrated by SC[4/6]A (1:1, insulin:SC[4/6]A). The cytotoxicity of insulin was decreased when incubated with SC[4/6]A (1:1, insulin:SC[4/6]A). The chemical functionalization of cucurbiturils is expected to increase their water solubility and make their in vivo applications possible (Table 1).

4 | CAs FOR MODULATING AMYLOID AGGREGATION

Calix crater-like CAs (CnA, n = 4, 5, 6, 8...) are synthesized by the reaction of phenol derivatives with formaldehyde.[24,68] A greater number of phenol units are associated with a larger cavity size. A large amount of CAs with different structures can be obtained due to the multiple sites available for modification. Different substitutions on both the upper and the lower rims can be easily achieved. CAs also possess different conformational structures, which further extend their structural diversity (Figure 5). Changing the starting materials from phenol derivatives to aromatic heterocycles leads to heterocalixaromatics.[69,70]

The structural diversity of CAs makes it possible for them to recognize and bind to different types of molecules, including cations, anions, organic molecules, and biomacromolecules, which results in an almost unlimited numbers of applications.[24,70,71] Several CAs have been reported to inhibit the aggregation of Aβ and insulin.[24] More biocompatible CAs are expected to interfere with the aggregation of amyloid proteins, which offers potential therapeutic methods for related diseases.
at pH 2. The addition of SC[4/6]A to the fibrils gradually changed the fibril surface charge from positive to negative, which suggests interactions with sulfonate. The cavity also contributes to the inhibitory effects observed because p-hydroxybenzenesulfonic acid could not inhibit the aggregation of insulin. Later, Sun et al. reported that SC[n]A (n = 4, 6, 8) could also inhibit the aggregation of Aβ1-42, among which SC[8]A was the most effective (1:50, Aβ1-42:SC[8]A). Compared with insulin, a higher ratio of SC[n]A is needed to achieve significant inhibition of Aβ1-42. Leblanc et al. demonstrated that resorcinarene strongly inhibited the aggregation of Aβ1-40/42 at low concentrations (1:5, Aβ1-40/42:resorcinarene). Resorcinarenes, unlike SC[n]A, are the reaction products of resorcinol derivatives with aldehydes. In addition to sulfonate on the aromatic rings, the resorcinarene used is modified by thiomethyl groups at the methylene bridge carbon. The results from ThT kinetics, CD spectra and AFM confirm that the resorcinarene inhibits the fibrillation of Aβ1-40/42 at a 1:1 ratio. Moreover, cytotoxicity experiments using sea urchin embryos have revealed that the resorcinarene is not toxic at the effective concentrations. The cytotoxicity of Aβ1-42 is also reduced by the resorcinarene. MD simulations and molecular docking have been performed to study the binding modes between Aβ1-42 fibrils and the resorcinarene. The researchers have found that the thiomethyl groups of the resorcinarene form nonpolar interactions with Ala, Leu, and Val residues on Aβ1-42 fibrils. More recently, the same research group studied the effects of different tail-engineered resorcinarenes on the aggregation of insulin. The resorcinarene itself exerts a strong inhibitory effect on insulin at a ratio of 1:0.2 (insulin:resorcinarene). However, changing the tail from...
thiomethyl to propyl or butyl results in disappearance of the inhibitory effect. In fact, butyl derivatives of resorcinarene accelerate the aggregation process. MD simulation and docking results suggest that the resorcinarene may bind to both insulin monomers and dimers and then prevent the dimerization and fibrillation of insulin.

Pappalardo et al. constructed a conjugate of peptide and calix[4]arene, and this conjugate exerts better inhibitory effects on the aggregation and cytotoxicity of Aβ₁₋₄₂ than the peptide or calix[4]arene alone (1:5, Aβ:conjugate). The sequence of the peptide is gly-pro-gly, lys-leu-val-phe-phe (GPGKLVFF). The KLVFF (Aβ₁₆₋₂₀) motif is widely used to bind to Aβ and inhibit its aggregation. The GPG motif is added to prevent the aggregation of the conjugate itself because the KLVFF motif has the ability to aggregate. A derivative of p-amino-calix[4]arene has been used for coupling with the peptide at the lower rim. Mass spectrometry and sodium dodecyl sulfate (SDS)-PAGE results suggest that the conjugate could decrease the formation of Aβ oligomers.

Guo et al. synthesized an amphiphilic sulfonatocalixarene and evaluated its effect on the aggregation of insulin. Four dodecyl groups have been added to SC₄A to yield amphiphilic SC₄CE, which contains hydrophilic sulfonate on the upper rim and hydrophobic alkyl chains at the lower rim. SC₄CE has been reported to form micelles at concentrations higher than 0.02 mM. The researchers found that the SC₄CE micelle significantly inhibits the aggregation of insulin at a 1:1 ratio, whereas SC₄A and sodium dodecyl benzenesulphonate (SDBS) exert weaker effects. The results from a competitive fluorescence titration assay indicate that the binding affinity between SC₄CE and insulin is 2.0 × 10⁷ M⁻¹, although direct binding sites have not been revealed.

Guo et al. recently developed a heteromultivalent peptide recognition method to inhibit amyloid fibrillation and further applied this method to transgenic mice with Alzheimer’s disease. The authors constructed a coassembly of CA and CD amphiphiles that formed a hetero-assembly in solution. CA and CD were simultaneously distributed on the outside of the coassembly, exposing two types of recognition sites for different guest molecules. Owing to heteromultivalency and synergistic effects, peptides with multiple side chains that can bind with CA and CD separately are expected to bind selectively and strongly with the coassembly (Figure 7). Specifically, CD binds preferentially to Tyr, whereas CA binds more strongly to Lys. Therefore, the researchers first tested their hypothesis using some model peptides containing Lys and Tyr and proved the importance of heteromultivalency. Afterward, the authors determined that the binding affinity between the CD-CA coassembly and Aβ₁₋₄₂ is 7.9 × 10⁷ M⁻¹, whereas no interactions could be observed if the Lys and Tyr residues of Aβ₁₋₄₂ were knocked out. The coassembly exerts better inhibitory effects than either CD or CA assembly alone or their mixture. Moreover, the coassembly decreases the cytotoxicity of Aβ and effectively disintegrates preformed Aβ fibrils at a ratio of 1:1. The effectiveness and biocompatibility of the CD-CA coassembly indicates its potential as a therapy for Alzheimer’s disease, as was confirmed recently. Researchers have injected the coassembly into 5xFAD mice through intrahippocampal or intranasal administration and found that both the amyloid plaques and the level of Aβ monomers were significantly reduced. The results from multiple behavioral experiments suggest that the administration of the coassembly reduces cognitive decline and restores the memory of 5xFAD mice. Analysis of brain slices revealed that the coassembly reduces neuroinflammation and neuronal apoptosis. These results support the notion that heteromultivalent peptide recognition by the coassembly should be a

![Figure 6](image-url) Molecular structures of the calixarenes reported to modulate the aggregation of amyloid proteins.
good candidate for the treatment of multiple amyloid-related
diseases. Guo et al. reported the construction of another coassembly
of CD and guanidinium-modified CA (GCA) amphiphiles. Unlike the CA amphiphiles used in the previous studies, GCA exhibits preferential binding to Glu and Asp. The coassembly GCA-CD exhibits higher binding affinity with Aβ1-42 than CD-CA, which guarantees its effectiveness in reversing cognitive decline in 5xFAD mice. Guo et al. also constructed nanoparticles composed of GCA, PEG, and the photothermal material poly-5,5′-(2,5-bis(-2-octyldodecyl)3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione. The nanoparticles exert great inhibitory effects toward the aggregation and cytotoxicity of Aβ1-42. After light irradiation, the nanoparticles cross the blood-brain barrier (BBB) and decrease Aβ plaques in Alzheimer’s disease (AD) model mice.

The above-described studies show that CAs are significantly effective in inhibiting the aggregation of amyloid proteins. The disintegration of mature fibrils has been reported by different groups. Guo and coworkers have demonstrated that the coassembly of CD-CA amphiphiles is effective in AD model mice. Further in vivo studies of CAs are urgently needed for bench-to bedside translation (Table 2).

5 | CYCLODEXTRINS AND CROWN EThERS FOR MODULATING AMYLOID AGGREGATION

5.1 | CDs

CDs, which were initially separated from the enzymatic reaction products of starch, are cyclic oligosaccharides linked by α-1,4-glucosidic bonds. α-, β-, and γ-CDs contain 6, 7, and 8 units of D-glucose, respectively (Figure 8). CDs are water soluble, and their multiple hydroxyl groups are easily to be modified. Different substituted CDs with different cavity sizes can then be applied to recognize and encapsulate a number of molecules particularly some therapeutic drugs. β-CD has been approved by the FDA for use in humans as an oral excipient in some drugs. The effects of CDs on the aggregation of amyloid proteins have been extensively investigated, and comprehensive reviews were performed approximately 5 years ago. The readers are advised to refer to these papers, and we summarize some recent progress here.

Zheng et al. reported that hydroxypropyl (HP)-β-CD effectively inhibits the aggregation of Aβ1-42 at a ratio of 1:2 (Aβ1-42:HP-β-CD), which is lower than the concentrations used in previous studies involving β-CD. Fewer fibrils and reduced β-sheet structures have been observed in the presence of HP-β-CD. Occupation of the cavity of HP-β-CD by ferulic acid results in disappearance of its inhibitory effects, which suggests the importance of its hydrophobic cavity. The results from molecular docking and MD simulations have revealed that HP-β-CD interacts with the Phe4, Lys16, Phe19, Ala21, Ile31, and Met35 residues in the Aβ pentamer. Vecchio et al. synthesized a variety of β-CD derivatives and evaluated their effects on the aggregation of Aβ1-42. CD-5-carboxy-8-hydroxyquinoline (IOX) and CD-dimethylaminobenzene exert better inhibitory effects than β-CD (1:5, Aβ-CD derivatives). The conjugate of porphyrin with β-CD (CDTHPP) effectively decreases the cytotoxicity of Aβ1-42 (1:1, Aβ-CD derivatives). Dot blot results have revealed that CDTHPP inhibits the formation of toxic oligomers. Enzymatic digestion experiments have suggested that CDTHPP interacts mainly with the central region of the Aβ sequence, although the proof was not direct. Vecchio et al. also conjugated both carnosine and 8-hydroxyquinoline with β-CD and obtained CDCarHQ, which almost completely inhibits the aggregation of Aβ at a ratio of 1:5 (Aβ:CDCarHQ). Circular dichroism results have indicated that Aβ forms α-helix structures and cannot be converted to β-sheet structures in the presence of CDCarHQ. Moreover, the carboxymethyl-β-CD
### Table 2
Summary of the effects of calixarenes on different amyloid proteins

| Peptides/proteins | Macrocycles | Binding sites | Effects | Ref |
|-------------------|-------------|---------------|---------|-----|
| Insulin           | SC[n]A (n = 4, 6) | Basic residues | Disintegrating mature fibrils. | [72] |
| Aβ₁₋₄₂           | SC[n]A (n = 4, 6, 8) | Multiple binding sites | Decreasing cytotoxicity in SH-SY5Y cells. | [73] |
| Aβ₁₋₄₀A₂        | Resorcinarene | Nonpolar interactions | Decreasing cytotoxicity in sea urchin embryos. | [74] |
| Insulin           | Resorcinarene derivatives | Dimer interface | Different resorcinarenes had different effects. | [75] |
| Aβ₁₋₄₂           | Peptide-calix[4]arene | β-sheet sequence | Better inhibitory effects than the peptide or CAA. | [76] |
| Insulin           | SC4CE        |                            | Better inhibitory effects than SC4A. | [77] |
| Aβ₁₋₄₂           | Coassembly of CD and CA amphiphiles | Lys and Tyr residues | Disintegrating fibrils. Reversing cognitive decline in 5xFAD mice. | [79,80] |
| Aβ₁₋₄₂           | Coassembly of CD and GCA amphiphiles | Glu, Asp, and Tyr residues | Disintegrating fibrils. Reversing cognitive decline in 5xFAD mice. | [81] |
| Aβ₁₋₄₂           | Nanoparticles containing GCA | Glu and Asp residues | Disintegrating fibrils. Decreasing Aβ plaques in 5xFAD mice. | [82] |

Abbreviations: CA, calixarene; CD, cyclodextrin; GCA, guanidinium-modified calixarene.

### Figure 8
Molecular structures of unmodified cyclodextrins (CDs) and representative molecular structures of the CDs reported to modulate the aggregation of amyloid proteins

![Figure 8](DOI: 10.1002/anie.201202990)

polymers (pACD) has been constructed. Subsequently, the pACD has been modified with 5-(aminomethyl)-8-hydroxyquinoline (AMHQ) to yield pCDHQ, which exerts stronger inhibitory effects than pACD and AMHQ (1:2, Aβ:CD derivatives). In addition, pCDHQ has the capability to chelate copper ions, which in turn inhibits copper-induced Aβ aggregation.\(^{[91]}\)

Mohanty et al. reported that SBE\(_{7}\)β-CD, which possesses similar functional groups to SC[n]A,\(^{[72]}\) inhibits the aggregation of insulin and lysozyme.\(^{[92]}\) The effective ratio is 1:1 (protein:inhibitor). The researchers also proved that SBE\(_{7}\)β-CD disintegrates insulin and lysozyme fibrils based on circular dichroism, AFM, and dynamic light scattering results. Incubation with SBE\(_{7}\)β-CD decreases the cytotoxicity of insulin and lysozyme. Based on their studies with SC[n]A and SBE\(_{7}\)β-CD, the researchers believed that both the sulfonate groups and the cavity of β-CD play important roles in inhibiting the aggregation of amyloid proteins.

Sun et al. synthesized a conjugate of β-CD with peptide Ac-LVFFARK-NH\(_2\) (LK7).\(^{[93]}\) The researchers previously found that LK7 partly inhibits the aggregation of Aβ but is itself toxic.\(^{[94]}\) The conjugate LK7-β-CD exerts better inhibitory effects on Aβ₁₋₄₀ than LK7 alone or the simple mixture of LK7 and β-CD. LK7-β-CD is less toxic than LK7 and reduces the cytotoxicity of Aβ₁₋₄₀ at a ratio of 1:1. ITC results have confirmed that LK7-β-CD binds more strongly to Aβ₁₋₄₀ monomers than LK7 and β-CD.

In addition to conjugation with other molecules, β-CD has also been mixed with polyphenols and used to inhibit the aggregation of some amyloid proteins. Chowdhury et al. studied the effects of mixtures of β-CD with different polyphenols on α-synuclein aggregation.\(^{[95]}\) Curcumin mixed with β-CD exhibits the highest efficiency in inhibiting fibrillation and disaggregating preformed fibrils. Dubey et al. demonstrated that a 1:2 mixture of curcumin and β-CD significantly inhibits the aggregation of silk fibroin.\(^{[96]}\)

Khan et al. reported that α-CD could reverse surfactant-induced amyloid fibrillation.\(^{[97,98]}\) The researchers found that 0.2 mg/ml lysozyme forms aggregates in the presence of 0.3 mM SDS, whereas the addition of 1 mM α-CD completely eliminates the aggregates. Molecular docking results have suggested that α-CD has the capability to bind with both
lysozyme and SDS. Similar results have been obtained for the SDBS-induced fibrillation of insulin.

### 5.2 Crown ethers

Crown ethers are well known for their ability to bind with different cations, particularly metal ions. Recent studies have suggested that some crown ethers could bind with positively charged Lys, His, and Arg residues, which expands their applications to biomolecules.

Tian et al. reported the effects of 12-crown-4 on the aggregation of Aβ

The researchers found that 12-crown-4 could change the surface charges of Aβ and partly inhibit its aggregation (1:2, Aβ:12-crown-4). The authors then conjugated PiB, a positron emission tomography ligand for Aβ, to 12-crown-4 and obtained PiB-C (Figure 9), with the aim of achieving specificity for Aβ. The conjugate exerts better inhibitory effects on the aggregation and cytotoxicity of Aβ than PiB. The researchers have proved that the conjugate could effectively cross the BBB and stain amyloid plaques in AD model mice.

Yokoyama and coworkers investigated the effects of a variety of crown ethers and 18-crown-6 (18C6) derivatives on the aggregation of V30M-mutated transthyretin (V30M-TTR). 18-crown-6 may interact with the Lys residues on TTR. 4′-carboxybenzo-18C6 (Figure 9), which stabilizes the 2 μM TTR tetramers at a concentration of 4 mM, has been identified as the best inhibitor tested. The crystal structure of V30M-TTR complexed with 4′-carboxybenzo-18C6 suggests that the 18C6 derivative is an allosteric inhibitor.

The easily available and biocompatible CDs are popular drug candidates. However, derivatizations of CDs are needed to achieve higher binding affinity and selectivity toward amyloid proteins. The combination of crown ethers and other macrocycles may yield better results on the aggregation of amyloid proteins (Table 3).

### 6 CONCLUSION AND OUTLOOK

In summary, all the above-described studies support the notion that macrocycles efficiently modulate the aggregation of different amyloid proteins. Considering the capability of macrocycles to stabilize protein drugs or decrease toxic aggregates, these compounds have the potential for further use in the treatment of multiple diseases.

In addition to the abovementioned macrocycles, many other synthetic macrocycles, including heteroalcalixaromatics, calixphyrins, pillararenes, and cyclophanes have been developed in recent years. Their distinctive structural properties make them potential candidates for modulating the aggregation of different amyloid proteins, although no related studies have been reported thus far.

The conjugation of macrocycles with other molecules increases their capability to inhibit the aggregation of amyloid proteins. Heteromultivalent recognition methods with two types of macrocycles have also demonstrated great potential for inhibiting fibrillation. Novel derivatives of the macrocycles are expected to be developed for different amyloid proteins. The combined administration of different host molecules is likely to achieve effectiveness and specificity at the same time.

For the most efficient design of amyloid modulators, a detailed understanding of the inhibitory mechanisms of different macrocycles is needed. More experimental structural efforts are needed to clarify the binding modes between different macrocycles and amyloid proteins. Moreover, classic methods for characterizing the aggregation of amyloid proteins, including ThT kinetics, offer little information for the microscopic steps of amyloid aggregation. Thus, chemical kinetics analysis is recommended to clarify the inhibitory mechanisms of different macrocycles, which may guide the development of novel inhibitors.

The macrocycles reviewed here are effective amyloid modulators in vitro, but few of these molecules have been tested in vivo. Although most macrocycles are generally safe, various molecules in the human body easily interfere the interactions between macrocycles and amyloid proteins. For example, CB[8] can bind spermidine and some steroid hormones with $K_a = 10^6 \sim 10^8 \text{M}^{-1}$. To achieve effectiveness in vivo, macrocycles need to exhibit stronger binding affinities with the targeted molecules. Moreover, their capability to cross the BBB remains to be tested when studying amyloid proteins in the brain. If the above problems could be resolved by the development of novel macrocycle derivatives, these macrocycles might not only serve as amyloid modulators but also be used for in vivo diagnostics.
TABLE 3 Summary of the effects of cyclodextrins and crown ethers on different amyloid proteins

| Peptides/proteins | Macrocycles | Binding sites | Effects | Ref |
|-------------------|-------------|---------------|---------|----|
| αP1-42            | HP-β-CD     | Phe4, Lys16, Phe19, Ala21, Ile31, Met35 | Decreasing cytotoxicity in SH-SYSY cells. | [86] |
| αP1-42            | CD-IOX, CD-DMAB | Central region of Aβ | Better inhibitory effects than β-CD. | [87,88] |
| αP1-42            | CDTTHPP     | N-terminal region | Decreasing formation of toxic oligomers. | [89] |
| αP1-42            | CDCarHQ     | N-terminal region | Freezing the α-helix intermediate. | [90] |
| αP1-42            | pCDHQ       |                | Inhibiting metal-induced aggregation. | [91] |
| Insulin, lysozyme | SBE2-β-CD   |                | Decreasing cytotoxicity in CHO cells. | [92] |
|                   |             |                | Disintegrating fibrils. | [93] |
| αP1-40            | LK7-β-CD    |                | Decreasing cytotoxicity in SH-SYSY cells. | [94] |
| α-synuclein       | β-CD + polyphenols |                | Disaggregating fibrils. Decreasing cytotoxicity. | [95] |
| Silk fibroin      | β-CD + curcumin |                | Inhibitor. Preventing fibril formation. | [96] |
| Lysosome, Insulin | α-CD complexed with SDS |                | Solubilizing surfactant-induced amyloid fibrils. | [97,98] |
| αP1-40            | 12-crown-4, PiB-C | Basic residues | BBB permeable and staining amyloid plaques. | [100] |
| V30M-TTR          | 18-crown-6 derivatives | Lys residues | Stabilizing V30M-TTR tetramers. | [101] |

Abbreviations: BBB, blood-brain barrier; CA, calixarene; CD, cyclodextrin; DMAB, dimethylamino benzene; GCA, guanidinium-modified calixarene; TTR, transthyretin.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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