Journal of Materials Chemistry B

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Journal:	*Journal of Materials Chemistry B*
Manuscript ID	TB-REV-08-2019-001871.R1
Article Type:	Review Article
Date Submitted by the Author:	24-Sep-2019
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Fundamentals and Introductory of Cross-seeding of Amyloid Proteins

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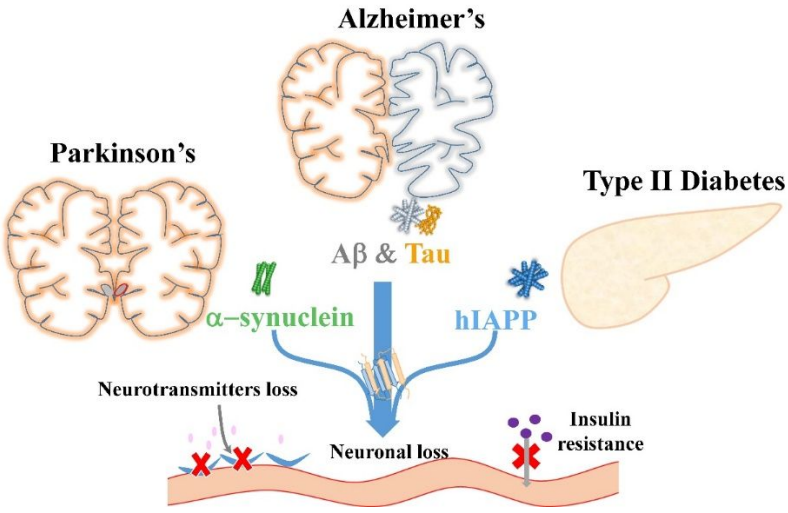

Abstract

Misfolded protein aggregates formed by the same (homologous) or different sequences (heterologous/cross) sequences are the pathological hallmarks of many protein misfolding diseases (PMDs) including Alzheimer disease (AD) and type 2 diabetes (T2D). Different from homologous-amyloid aggregation that is solely associated with a specific PMD, cross-amyloid aggregation (i.e. cross-seeding) of different amyloid proteins are more fundamentally and biologically important for understanding and untangling not only the pathological process of each PMD, but also a potential molecular cross-talk between different PMDs. However, the cross-amyloid aggregation is still a subject poorly explored and little is known about its sequence/structure-dependent aggregation mechanisms, as compared to the widely studied homo-amyloid aggregation. Here, we review the most recent and important findings of amyloid cross-seeding behaviors from in vitro, in vivo, and in silico studies. Some typical cross-seeding phenomena between Aβ/hIAPP, Aβ/tau, Aβ/α-synuclein, and tau/α-synuclein are selected presented, and the underlying specific or general cross-seeding mechanisms are also discussed to better reveal their sequence-structure-property relationship. The potential use of cross-seeding concept to design amyloid inhibitors is also proposed. Finally, we offer some personal perspectives on current major challenges and future research directions in this less-studied yet important field, and hopefully will stimulate more research to explore all possible fundamental and practical aspects of amyloid cross-seeding.

1. Introduction

The misfolding and aggregation of the same (homologous) amyloid peptide into cytotoxic species are pathologically associated with a specific protein misfolding disease (PMD), e.g., amyloid-β (Aβ) aggregation in Alzheimer disease (AD), human islet polypeptide (hIAPP) aggregation in type 2 diabetes (T2D), and α-synuclein aggregation in Parkinson disease (PD). These homologous aggregates have been widely studied for their molecular structures, aggregation kinetics, and pathological/physiological functions in PMDs. More interestingly, recent studies in vitro and in vivo have found that some misfolded proteins of different (heterologous) sequences associated with different diseases can cross-interact with each other to promote amyloid aggregation[1, 2]. This process is known as amyloid cross-seeding aggregation. The coexistence of heterologous protein aggregates, such as Aβ and α-synuclein[3], Aβ and tau[4], Aβ and transthyretin[5], hIAPP and insulin[6], has been described in patients with several PMDs.

Considering that all amyloid proteins share many structural, kinetic, and even cytotoxic characteristics during amyloid aggregation, it is intuitive to speculate that molecular cross-talk between different PMDs is attributed to direct interactions between their corresponding disease-causative proteins, leading to amyloid cross-seeding aggregation. But in other cases, the interactions between different amyloid peptides lead to cross-amyloid inhibition. Both cross-amyloid aggregation and inhibition come from the same origin of cross-amyloid interactions, but such interactions indicate different energy barriers for heterogeneous protein aggregation. In addition, a number of studies have also found that amyloid proteins/aggregates can be transported between different types of cells, which also increases a possibility not only for cross-seeding between different amyloid proteins/aggregates, but also for the spread of disease-related amyloids to different tissues/organs involved in different PMDs. From a structural viewpoint, common structural characteristics of different amyloid seeds of distinct sequences, origins, and biological functions enable a possibility to realize the amyloid cross-seeding through the conformational selection and population shift of different amyloid seeds at compatible states[7]. Amyloid seeds of one species with stackable β-strands, unsatisfied hydrogen bonds, increased hydrophobic surface area, and in-registered backbone packings could facilitate the template-assisted growth of another species. Alternatively, amyloid seeds formed by different amyloid proteins could mutually adjust and optimize their conformations to achieve binding preference, leading to cross-seeding.

In a broader view, similar cross-seeding behaviors may not only limit to the naturally-occurring amyloid peptides, but also occur between non-amyloid-peptides and amyloid peptides, i.e. between bacterial curli and amyloid peptides of SEVI, Aβ, and hIAPP[8]. The cross-seeding between bacterial-produced amyloidogenic curli and the HIV-involved amyloid aggregates indicates that the curli cross-seeding not only affect the nucleation and ensuing amyloidogenic aggregation of IAPP and $A\beta_{1-40}$, but also induce biological exogenous infections[8], which may provide the molecular clue for

the exogenously-triggering amyloid diseases. These important findings imply that amyloid cross-seeding aggregation is speculated as a main mechanism for the spread of amyloidogenesis across different cells and tissues between different diseases[9]. Significant efforts and progress have been made to understand the homologous protein aggregation and its biological impacts, but less is known about the cross-amyloid interaction mechanism and its underlying sequence-structure-aggregation relationship. As shown in **Table 1**, we summarize all typical amyloid cross-seeding behaviors between different amyloid proteins from computational and experimental studies over the past decade. Since amyloid cross-seeding is more fundamentally and biologically important in the pathology of each disease and a potential link between different diseases, we expect that more and continuous research efforts will be devoted to the better study and understanding of amyloid cross-seeding.

Table 1. Summary of cross-seeding between different amyloid proteins by computational and experimental approaches.

	Experiment		
Disease	Cross-Seedings	Main Results	Reference
Prion Disease	$PrP_{120-144}$ various species-$PrP_{120-144}$ various species	PrP (human) could cross-seed with PrP (bank vole), *vice versa*, and both have weak tendency to adopt the conformation of the PrP (Syrian hamster) seed	[10]
	$PrP_{23–144}$ various species-$PrP_{23–144}$ various species	$PrP_{23–144}$ from different species adopt distinct secondary structures and morphologies, while the cross-seeding of $PrP_{23–144}$ from one species with fibrils from another species may overcome natural sequence-based structural preferences, resulting in a new amyloid strain.	[11]
	PrPC-α-synuclein	α-synuclein aggregates trigger misfolding of PrPC, produce self-replicating PrP states. Non-fibrillar a-synuclein or fibrillar A beta failed to cross-seed misfolding of PrPC	[12]
Parkinson's Disease	α-synuclein (human)-α-synuclein (mouse)	Cross-seeded aggregation of human and mouse α-synuclein is bidirectionally restricted.	[13]
	α-synuclein (mouse)-N-ter truncted α-synuclein (human)	N-terminal, not C-terminal truncation switches its conformational preference	[14]

	α-synuclein (mouse)-C-ter truncted α-synuclein (human)	with structural properties similar to those of mouse α-synuclein fibrils, markedly enhance cross-seeding activity	
	α-synuclein (human)-Aβ	Seeding effects of $A\beta_{40/42}$ fibrils in α-synuclein aggregation pathway are lower than that of α-synuclein fibrils. While the seeding effects of α-synuclein fibrils are higher than those of $A\beta_{40/42}$ fibrils in the $A\beta_{40/42}$ aggregation pathway.	15
	α-synuclein (human)-Quinolinic acid	Quinolinic acid assemblies co-localize with α-synuclein aggregates in neurons, which can facilitate the aggregation of soluble α-synuclein monomers	16
Alzheimer's Disease	$A\beta_{42}$-PrP^{SC}	In an Alzheimer's transgenic mouse model, prions inoculation shows a dramatic acceleration and exacerbation of both pathologies	2
	$A\beta_{40}$-$A\beta_{42}$	Misfolding of E22G pathogenic mutated $A\beta_{40}$ was enhanced by adding wild type Ab_{42} fibril as seed, whereas wild type $A\beta_{40}$ was unaffected by $A\beta_{42}$ fibril seed.	17
	$A\beta_{40}$-$hIAPP_{37}$	In vitro, IAPP seeds accelerates Aβ aggregation and the resulting fibrils are composed of both peptides. In vivo, inoculation of IAPP aggregates into the brains of AD transgenic mice resulted in more severe AD pathology and significantly greater memory impairments.	18
	$A\beta_{24-34}$-$hIAPP_{19-29}$ S20G	With 64% sequence similarity, fibrils of the two segments induced amyloid formation through self- and cross-seeding	19

	Aβ-ASC specks	Exposure of microglia to $A\beta_{42}$ caused the formation and release of ASC specks, which in turn accelerated Aβ aggregation and spreading, in vitro and in vivo	20
	Aβ- exogenous amyloidogenic proteins: casein, fibroin, sericin, actin, IAPP	The activity of various fibrillar seeds on the Aβ assembly was in the order of: Aβ = actin >casein = IAPP = sericin >fibroin. NMR studies revealed that E3, R5, H13, H14, and Q15 of Aβ are common binding regions between the Aβ monomer and the fibrillar seeds of other proteins	21
	Tau-$A\beta_{42}$	Pre-aggregated Aβ can directly seed Tau-aggregation and strongly accelerate propagation of Tau-pathology in vitro and in vivo, while monomeric Aβ did not induce significant Tau-aggregation	22
	Tau-α-synuclein	Recombinant, preformed α-synuclein fibrils cross-seed intracellular tau to promote the formation of neurofibrillary tangle-like aggregates	23
Type 2 Diabetes	$hIAPP_{37}$-$A\beta_{42}$	Aβ and hIAPP oligomers can efficiently cross-seed each other via the association of two highly similar U-shaped β-sheet structures.	24
	$rIAPP_{37}$-$hIAPP_{37}$	All preformed hIAPP aggregates can cross-seed nonaggregating rIAPP to promote the final fibril formation, and hIAPP seeds preformed at a growth phase show the strongest cross-seeding potential to rIAPP	25

Simulation

Disease	Cross-Seeding Complex	Result	Reference
Prion Disease	$PrP_{120-144}$ various species-$PrP_{120-144}$ various species	Hydrophobic sidechain-sidechain attraction, along with the backbone hydrogen bonding interaction, are the driving forces for seeding and	10

		cross-seeding aggregation. HuPrP(120–144) could cross-seed with BV seed and vice versa. Hu and BV weakly cross-seed with SHa seed.	
	$PrP_{106-126}$-hIAPP $PrP_{106-126}$-rIAPP	The four-stranded-β-sheet and the compact helix-4-stranded-β-sheet are the favorable species	26
	$PrP_{106-126}$-hIAPP	The driving forces of hIAPP-$PrP_{106-126}$ cross-seeding are mainly hydrophobic interactions. The palindromic region of $PrP_{106-126}$ and SNNFGAIL region of IAPP were found to play important roles in the interaction	27
Parkinson's Disease	NAC/AS-$A\beta_{42}$	NAC oligomers prefer to interact with $A\beta_{42}$ oligomers to form double-layer over single-layer conformations due to electrostatic/hydrophobic interactions. Among the single-layer conformations, the NAC oligomers induce formation of new β-strands in $A\beta_{42}$ oligomers, thus leading to new Aβ oligomer structures. NAC oligomers stabilize the cross-β structure of Aβ oligomers.	28
Alzheimer's Disease	$A\beta_{42}$-Tau K18 $A\beta_{42}$-Tau K19	Aβ oligomer stretches tau conformation and drastically reduces the metastable secondary structures/hydrogen bonding/salt-bridge networks in tau monomers and exposes their fibril nucleating motifs ^{275}VQIINK^{280} and ^{306}VQIVYK^{311}	29
	Mutated Tau (R2)-$A\beta_{17-42}$	The preferred mechanisms of the interactions between $A\beta_{17-42}$ oligomers and mutated tau repeat R2 oligomers occur via interactions of a single-layer of $A\beta_{17-42}$ oligomers and a single-layer of mutated tau repeat R2 oligomers to form a double-layer conformation along the fibril axis.	30

$A\beta_{42}$-A2T $A\beta_{42}$	Heterodimeric system of $A\beta_{42}$-A2T $A\beta_{42}$ has tendency to weaken transient, intrachain contacts formed between the central and C-terminal hydrophobic residues. Heterodimer is lacking in significant secondary structure and displays a weak interchain interface. The A2T N-terminus, particularly residue F4, is frequently engaged in tertiary and quaternary interactions with central and C-terminal hydrophobic residues in those distinct structures, leading to hydrophobic burial.	31
$A\beta_{17–42}$-Tau (R2, R3, R4)	Aβ oligomers are likely to interact with the R2 domain to form a stable complex with better alignment in the turn region and the β-structure domain.	32
Tau K18-Tau K19	Formation of stable R2 and R3 conformations contributes to K18 aggregation, and R3 contributes to K19 fibrillization. The different core units in K18 and K19 may create a cross-seeding barrier for the K18 seed to trigger K19 fibril growth because R2 is not available for K19.	33
$A\beta_{40}$-$hIAPP_{37}$	Aβ is a good template for the growth of amylin and vice versa. Water molecules permeate the β-strand–turn −β-strand motif pore of the oligomers, supporting a commonly accepted mechanism for toxicity of β -rich amyloid oligomers.	34
$A\beta_{42}$-$hIAPP_{37}$	Aβ and hIAPP can indeed associate with each other to form stable hybrid aggregates via different β-sheet	35

		arrangements. The double-layer and elongation models are more favorable than other two models.	
	$A\beta_{17-42}$-$hIAPP_{37}$	Aβ-hIAPP assemblies with different interfacial β-sheet packings exhibited high structural stability and favorable interfacial interactions in both oligomeric and fibrillar states. Aβ-hIAPP association depends on interfacial polarity and geometry. Salt bridges are critical for the formation of cross-seeding assemblies.	36
Type 2 Diabetes	hIAPP-Aβ	Aβ and hIAPP have fairly good backbone and side chain interactions with each other. hIAPP-Aβ interface is mainly governed by hydrophobic contacts and salt bridges. $_aCN_h$ is more engerentically favorable than $_aNN_h$. Electrostatic interactions clearly play a dominant role in interlayer interactions.	24
	hIAPP-rIAPP	Laterally antiparallel stacking of hIAPP and rIAPP oligomers with each other are energically favorable for cross-seeding.	37
	hIAPP-rIAPP	hIAPP monomer and oligomers can interact with conformationally similar rIAPP to form stable complexes and to co-assemble into heterogeneous structures. Interactions between hIAPP and rIAPP were arise from hydrophobic contacts and hydrogen bonds at the interface, particularly at N- and C-terminal β-sheet regions.	38
	$hIAPP_{37}$-$A\beta_{17-42}$	Aβ-hIAPP cross-seeding assembly associates with lipid bilayers strongly via the N-terminal strands of Aβ. Electrostatic interactions are the major forces governing peptide-lipid	39

interactions.

In this review, we aim to summarize recent progress and future direction in amyloid cross-seeding aggregation from both computational and experimental viewpoints. This review mainly covers fundamental principles of cross-seeding phenomena, selectively highlights some cross-seeding systems, and points out some of the persistent technological barriers and the research directions that should be undertaken to overcome these barriers. Finally, we will offer some personal opinions to highlight the challenges and opportunities at the interface of amyloid peptides and model membranes. Hopefully, this review will provide a different perspective to stimulate further research efforts for exploring all possible aspects of amyloid cross-seeding aggregation *in silico*, *in vitro*, and *in vivo* at different levels.

2. Mechanistic models of amyloid cross-seeding

All amyloid proteins generally undergo the nucleation-polymerization aggregation process, in line with their structural conversion and aggregation from unstructured monomers to critical seeds, and eventually to amyloid fibrils containing dominant β-sheet structures[40]. From a similarity viewpoint, both homogenous-seeding and cross-seeding always involve the same or similar competing folding and binding events between different polymerized forms via complex interaction pathways. Such commonalities in amyloid structure, dynamics, and functionality appear to suggest a potential interactive basis for different amyloid proteins to enable cross-seeding interactions. In some studies, different amyloid proteins, including tau-k18 and tau-k19[41-44], Aβ and tau[32], and hIAPP and rIAPP[38], were found to interact with each other to form hybrid amyloid fibrils containing cross-β-structures similar to those amyloid fibrils formed by pure amyloid peptides[45]. However, different from homogenous-seeding aggregation that always occurs, not any two different amyloid proteins enable cross-seeding behaviors, suggesting that certain cross-species barriers exist along the folding pathways of different amyloid proteins. Several reports also showed that different amyloid proteins had a lower efficiency of cross-seeding than homo-seeding, further implying the existence of cross-species barriers[46, 47]. Thus, this difference between homo- and cross-seeding further raises a fundamental question: if cross-species barriers exist, what are sequence and structural characteristics critical for amyloid cross-seeding? Considering that the polymorphic nature of amyloid proteins, even a given amyloid protein exists as conformational enables with different structural populations at a vast number of transit states, where the conformational differences can be small or large, and some conformations are more populated than others. Due to such conformational complexity and polymorphism of amyloid proteins, the exact cross-seeding mechanisms are still unclear, but it is likely that cross-seeding of amyloid species is governed by the "conformational selection and population shift" model, in which both partners of different amyloid species need to dynamically and mutually adjust their conformations to achieve compatible binding states. Different from the traditional "lock-and-key" model for protein-protein binding that only targeting protein

induces the conformational change of its binding partner, amyloid cross-seeding admits that both binding partners are flexible and have conformational distributions, so that both bound conformations could be induced by their binding partners.

Amyloid cross-seeding involves competing folding and binding events between the same and different amyloid species with different populations. The "conformational selection and population shift" model could enable different cross-seeding scenarios (**Figure 1**). First, since different amyloid proteins have different folding and aggregation kinetics, one amyloid protein could form the more populated seeds (i.e. conformers), which serves as template to recruit the lower populated seeds for homologous and heterologous aggregation. The ability of amyloids to efficiently cross-seed also depends on its self-aggregation state. From a free energy viewpoint, such highly populated seeds from one species can overcome cross-species barriers to conformationally select and drive the lower population seeds of the other species to be shifted and accommodated into the specific structures along the energy-downhill aggregation pathways (**Figure 1a**)[48]. In the second cross-seeding scenario (**Figure 1b**), if both amyloid species have similar populated seeds, the structural equilibrium will select those heterogeneous seeds with high conformational similarity to lower the cross-species barriers and promote mutual binding and recognition between different species, leading to amyloid cross-seeding. If the dominant conformations of two species are sufficiently different, they will grow into different fibrils, reflecting species barriers. As a proof-of-concept example, both scenarios have been observed for human tau proteins[33, 49]. Cross-seeding of two types of tau isoforms - four-repeat K18 (R1, R2, R3, and R4 units) and three-repeat K19 (R1, R3, and R4 units) human tau proteins – demonstrates conformational selection concept that K19 can seed K18, but not vice versa. When K19 acts as seeds, the R3 is a common catalytic center for conformational selection, so that R3 in K19 can cross-seed conformationally similar R3 in K18 to form hybrid K18-K19 fibrils. However, when using K18 as seed, the R2 is the catalytic center in K18, but is missing in K19, so K18 can not recruit K19 due to conformationally dissimilar-induced barrier. Therefore, in principle, the high structural complexity and polymorphism of amyloid proteins is considered as a key energetic and physical barrier to greatly impede the cross-seeding ability, because the diverged structural forms would decrease effective templates for hybrid amyloid fibril growth.

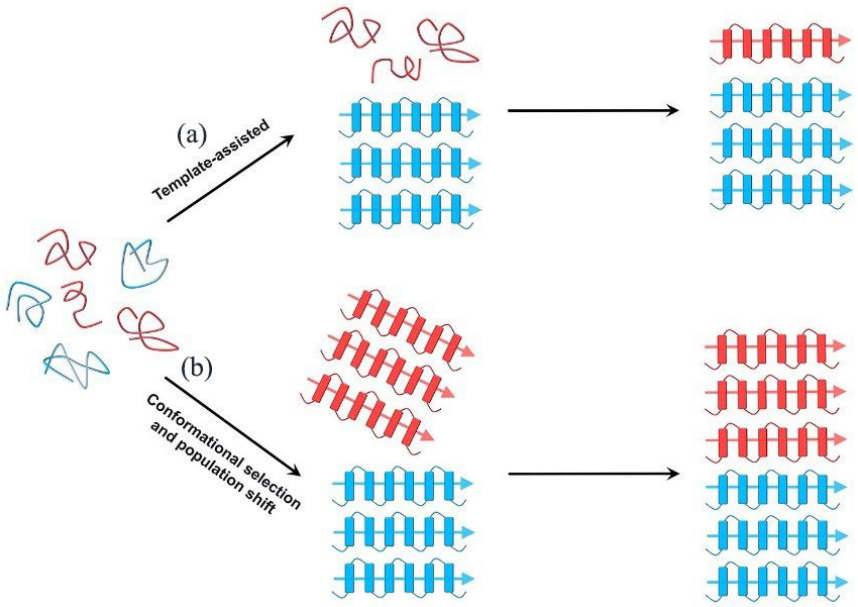

Figure 1. Hypothetical cross-seeding models via "template-assisted" and "conformational selection and population shift" mechanisms.

3. Cross-seeding barriers for determining directional amyloid aggregation

It is generally believed that prior to the formation of mature amyloid fibrils, numerous small oligomeric assemblies, also known as oligomeric aggregates (i.e. amyloid seeds), are formed at the early nucleation stage. These seeds have highly polymorphic structures with different sizes, conformations, and morphologies, and some of them contain certain degrees of β-sheet structures[50-53]. It is intuitive to speculate that amyloid cross-seeding may favor some directional interactions and specific conformations. Some experimental and computational studies have reported that amyloid seeds of different sequences can interact with each other to form hybrid amyloids containing conformational cross-β-sheet structures similar to homo-amyloids[7, 54-56]. *In vitro* cross-seeding of $hIAPP_{37}$ and $A\beta_{42}$ monomers underwent conformational transition from random structures to α-helix to β-sheet. Molecular dynamics (MD) simulations reveal that Aβ and hIAPP proteins can form a similar U-bend β-strand-turn-β-strand conformation, which serves as a basic template nucleus for mutual amyloid growth via either monomer attachment for elongation or lateral stacking[57-62]. Thus, given the striking similarities in the pathological mechanisms and structural properties of distinct amyloid seeds, conformational compatibility between different amyloid seeds appears to play a key role in determining cross-seeding barriers.

From an interaction viewpoint, cross-seeding has a directional effect, i.e. the interaction between different amyloid proteins may work in both directions or in a single direction. For example, Aβ aggregates act as a good seed for promoting both prion and α-synuclein aggregation, similarly both prion and α-synuclein aggregates also accelerated Aβ aggregation[2, 15]. This indicates that the aggregation of one amyloid species could be mutually triggered or promoted by structural templates from another amyloid seeds, showing a bidirectional cross-seeding effect (**Figure 2a**). Differently,

unidirectional cross-seeding was also observed (**Figure 2b**). It was reported that while monomeric Aβ and hIAPP can cross-seed to form hybrid amyloid fibrils containing morphologically similar β-sheet-rich structures to pure Aβ and hIAPP[24], use of one pre-existing seeds to cross-seed another one exhibited unidirectional cross-seeding barriers, i.e. Aβ fibrils were able to serve as very efficient seeds to interact with hIAPP and thus promote hIAPP aggregation, but hIAPP fibrils had little or no effect on Aβ fibrillization[63].

Of note, different from homologous seeding that generally results in the acceleration of amyloid aggregation through providing a nucleus to bypass the lag phase[64], not all cross-seeding of different amyloid proteins leads to the acceleration of amyloid formation. Instead in some cases, the interactions between different amyloid proteins lead to cross-amyloid inhibition (**Figure 2c**), or a more complex scenario of the co-existence of cross-seeding and cross-inhibition behaviors. The former example of the cross-seeding of Aβ and hIAPP monomers showed the coexistence of both the retarded process at the initial nucleation stage and the accelerated process at the fibrillization stage. This suggests that the cross-seeding of Aβ and hIAPP mixtures was less efficient than homologous seeding of pure Aβ or hIAPP, but such cross-seeding does not necessarily prevent either Aβ or hIAPP aggregation[65]. Another complex example is that apolipoprotein A-II and serum amyloid A can both cross-seed and cross-inhibit amyloid formation, depending upon the experimental conditions (seeding concentrations, sequence specificity, even agitation). In all studied cases, the cross-seeding and homo-seeding of different and same amyloid proteins are likely to be occurred in a competitive manner, thus leading to different cross-species barriers for cross-seeding or cross-inhibition behaviors.

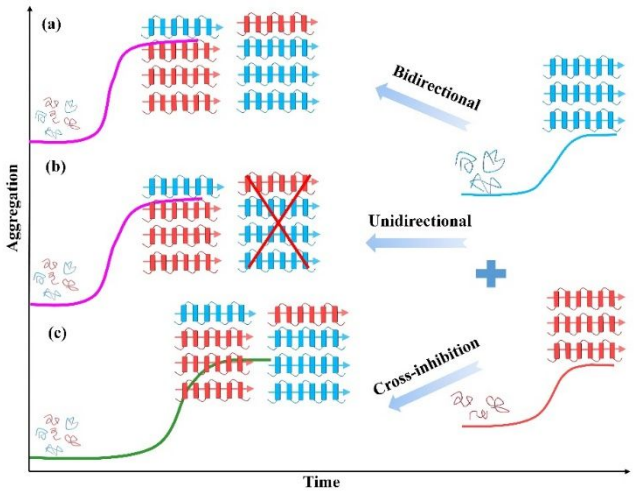

Figure 2. Amyloid cross-seeding with different cross-seeding barriers, leading to (a) bidirectional aggregation for both amyloid proteins; (b) unidirectional aggregation for a single amyloid protein; and (c) the slowdown of the aggregation of both amyloid proteins.

It still remains unclear how specific sequences alter the cross-seeding behavior of amyloid proteins. It is generally true that sequence similarity is critical for cross-seeding,

so that small sequence variations by different fragments (e.g. PrP[66] fragments), different lengths (e.g. $A\beta_{40}$ and $A\beta_{42}$[67]), different point-mutations (e.g. wild-type α-synuclein and mutant α-synuclein[68]), and different organisms (e.g. hIAPP and rIAPP[69]) are able to interact and potentiate their aggregation processes. However, some counterexamples also showed the sequence identity effect on the cross-seeding between hen lysozyme and other five proteins[46]. In contrast to our common intuition, neither the highest nor lowest sequence identity between hen lysozyme and the proteins can lead to the higher cross-seeding efficiency. This observation provides a hint on the importance of amyloid core structures for cross-seeding. If amyloid proteins or their variations can form a similar β-core structure, their cross-seeding barriers would be lower even with large differences in their sequences. On the other hand, if the differences in sequence are located in regions of the amyloid chain that are not involved in the amyloid core structure or small sequence variations alter the core structure, the cross-seeding efficiency is likely to be much lower though both cross-seeding species have the high sequence identity. These findings highlight the importance of conformational compatibility between different amyloid seeds, thus the structural differences between amyloid seeds is more important for determining the cross-seeding efficiency between amyloid proteins with or without high sequence similarity.

4. Cross-seeding of different amyloid proteins

The presence and interaction of two different amyloid proteins have been considered as a main cause to induce, spread, and explain the pathophysiology and co-existence of different PMDs in the same person. Here, we mainly focus on the cross-seeding between several representative amyloid proteins of Aβ, tau, hIAPP, and α-synuclein, which critically involve in the most widely recognized PMDs of AD, type 2 diabetes (T2D), and Parkinson disease (PD).

4.1. Cross-seeding of Aβ and tau

The presence of intracellular Aβ amyloid plagues and extracellular tau neurofibrillary tangles (NFTs) is considered as a major hallmark of AD (**Figure 3a**). Multiple in vitro studies have demonstrated the cross-seeding between Aβ and tau, and its synergistic promotion effect on amyloid aggregation bidirectionally (**Figure 3b**). It was found that Aβ binds to multiple tau peptides, especially those in exons 7 and 9, while tau binds to multiple Aβ peptides in the mid to C-terminal regions of Aβ[70]. Such binding affinity between Aβ and tau was almost 1,000-fold higher than tau for itself. However, upon tau phosphorylation by GSK-3β, the interactions between Aβ and tau were significantly reduced, leading to the dissociation of the Aβ/tau complex[70]. Similarly, use of Src family tyrosine kinase inhibitor of PP1 and phosphatidylinositol-3-kinase inhibitor of LY294002 enabled to block Aβ oligomer-induced, not Aβ monomer-induced, tau phosphorylation[71]. These findings provide strong evidences for identifying the role of Aβ oligomers in the induction of tau hyperphosphorylation in AD.

In parallel to *in vitro* studies, *in vivo* studies provide more in-depth insights into

the cross-seeding between Aβ and tau. Similar Aβ/tau complexes were also found in soluble extracts from the brain tissues of AD patients. It was reported that in P301L mutant tau transgenic mice, injection of $A\beta_{42}$ fibrils can significantly accelerate NFT formation in P301L mice, which further induced phosphorylation of tau[72], indicating that the cross-seeding interaction of $A\beta_{42}$ with the P301L mutation generate the much higher numbers of NFTs than either $A\beta_{42}$ or P301L mutant alone. In another study, the introduction of the Tau in the Tg2576 transgenic mice similarly enhanced the expression of mutant β-amyloid precursor protein (APP) and subsequent amyloidogenisis[73]. Further, the double-mutant JNPL3 transgenic mice expressed both Aβ precursor protein (APP) and tau proteins, and developed robust NSFs and amyloid plaques in the spinal cord and brain vulnerable to these lesions, both of which caused progressive motor and behavioral abnormalities of mice. These in vivo results further support the cross-seeding interaction between APP or Aβ and tau, leading to the increased production of these amyloid aggregates in tissues affected individuals, in transfected cells, and in transgenic animals, which could explain the Aβ and tau pathologies in AD.

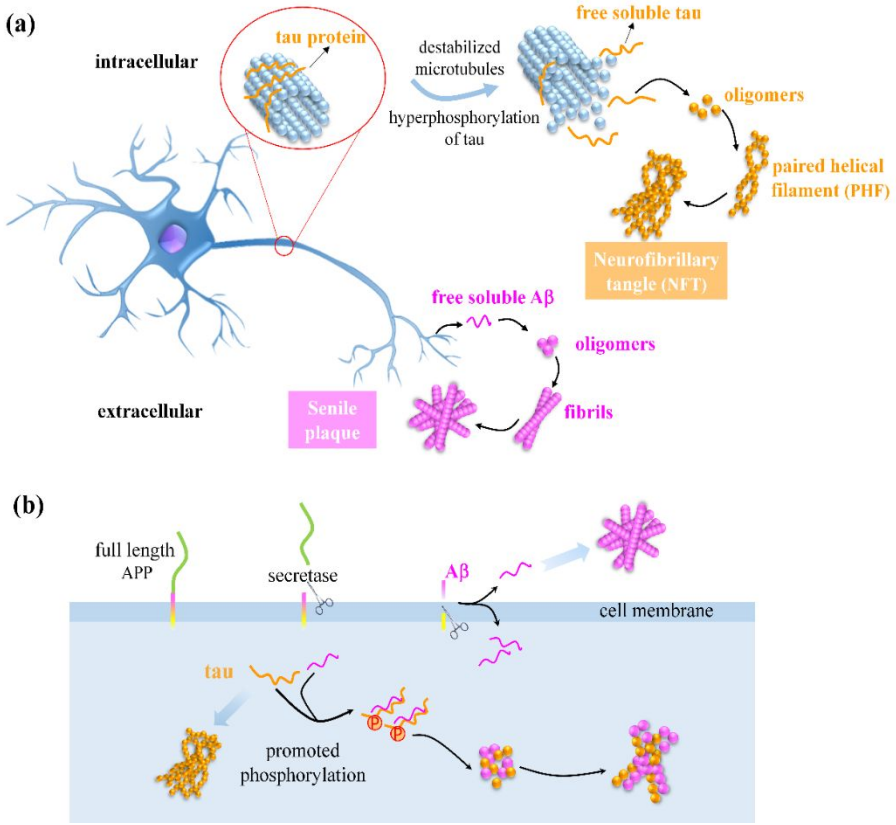

Figure 3. Cross-seeding between Aβ and tau. (a) Pathological process of disease-related core proteins of Aβ and tau in AD, leading to intracellular Aβ amyloid plagues and extracellular tau neurofibrillary tangles (NFTs). (b) Schematic of potential cross-seeding interactions between Aβ and tau.

4.1. Cross-seeding of Aβ and hIAPP

Among 20+ different PMDs, AD and T2D are the two common chronic disorders[74, 75], both have affected millions of people globally[76]. Clinical and

epidemiological studies have showed a potential link between AD and T2D[77, 78], e.g. ~80% of AD patients are affected by T2D or glucose-related disorders, while AD patients also show a higher risk to develop islet amyloidosis than healthy aged people[79]. While it is not clear how the two diseases are connected, several lines of evidences appear to support the hypothesis that the AD-T2D link could arise from the cross-amyloid interactions between Aβ and hIAPP: (1) Aβ and hIAPP are found to be co-existence in blood vessels and cerebrospinal fluids with similar nanomolar concentrations[80]; (2) hIAPP, normally co-secreted with insulin, can also be expressed by the sensory neurons and has the high affinity binding sites at hindbrain[81-83]; (3) Aβ is found to co-localize with hIAPP in pancreatic islet amyloid deposits of T2D patients[84]; (**Figure 4a**) (4) hIAPP and Aβ show high degrees of sequence identify (25%) and similarity (50%), especially these identical and similar sequences are mainly located at the β-strand forming region[80, 85]; (5) under disease conditions, both Aβ and hIAPP can misfold and self-aggregate into similar U-bend fibrillar structures[78], which may provide a common structural basis to initiate the cross-amyloid aggregation.

While the exact correlation between the Aβ-hIAPP interactions and the AD-T2D link is still under investigation[1, 2, 47], a number of *in silico*, *in vitro*, and *in vivo* studies have evidenced the cross-seeding of Aβ and hIAPP, but with different cross-seeding efficiencies (even some inconsistent data from different studies), depending on seed states, solution conditions, and even agitation (**Figure 4b**). Several studies have consistently shown that the cross-seeding of Aβ and hIAPP monomers delayed the nucleation of Aβ/hIAPP mixtures. However, at the final fibrillization stage, different cross-seeding-induced fibrillization was observed. We reported that once the critical seeds were formed, the cross-seeding accelerated and promoted amyloid fibril formation of $A\beta_{42}$/hIAPP. But, Yan et al.[65] observed the retard of the fibrillization of $A\beta_{40}$/hIAPP mixtures. Such differences could be caused by the use of different Aβ ($A\beta_{40}$ vs. $A\beta_{42}$). Both studies demonstrated the cross-seeding of Aβ and hIAPP monomers. Further seeding experiments showed that Aβ fibrils can seed with hIAPP to promote hIAPP aggregation, but hIAPP fibrils were very poor seeds for Aβ aggregation[63]. Moreover, the cross-seeding of $A\beta_{40}$ and hIAPP at 1:1 molar ratio on lipid membranes was also observed[86], and $A\beta_{40}$/hIAPP mixtures can aggregate into β-sheet-rich fibrils, and the cross-seeding fibrils were morphologically similar to pure hIAPP fibrils, but different from pure Aβ fibrils. No cross-inhibition of the fibrillation process in the presence of lipid membrane was observed, and this behavior is similar to the one observed in the bulk[65]. Further *in vivo* study showed that injection of Aβ seeds into hIAPP transgenic mice potentiated hIAPP deposition to the same level as injection of proIAPP fibrils, but hIAPP did not recruit Aβ[87]. Tissue samples extracted from the AD patients presented a combination of anti-Aβ antibody and anti-IAPP antiserum on cortical brain sections, suggesting that the amyloid plaques in the AD brains are the mixture of hIAPP and Aβ. Both *in vitro* and *in vivo* studies have shown that the cross-seeding of Aβ and hIAPP was less efficient than homologous seeding of pure Aβ or hIAPP, and such difference in seeding and cross-seeding ability indicate the existence of different cross-species barriers that are likely depended on structural similarity

between different amyloid seeds. Moreover, cross-seeding does not necessarily prevent the homo-seeding of Aβ or hIAPP, while they are likely to be occurred in a competitive manner.

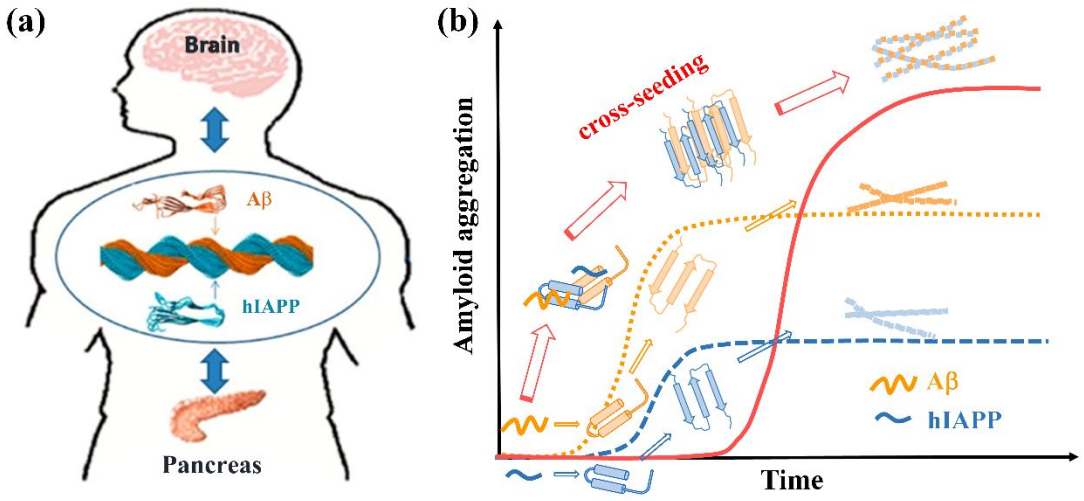

Figure 4. Cross-seeding of Aβ and hIAPP. (a) Aβ and hIAPP can co-exist and co-deposit in human blood serum, cerebrospinal fluid, human brain, and pancreas, making cross-seeding possible. (Reprinted with permission[24], Copyright 2015 American Chemical Society) (b) Cross-seeding of Aβ and hIAPP with different cross-seeding efficiencies, depending on seed states, solution conditions, and even agitation.

4.3. Cross-seeding of Aβ and α-synuclein

The aggregation of misfolded Aβ into extracellular senile plaques and of α-synuclein into intraneuronal Lewy bodies (LB) are associated with AD and Parkinson disease (PD) and Lewy body Disease (LBD), respectively[99, 100]. Many AD patients develop signs of PD and some PD patients become demented, suggesting that both diseases could involve overlapping pathological pathways. In the early 1990s, a distinctive dementia (a mixture of AD and LB), instead of pure AD was first reported, and then accumulating evidences showed that different non-amyloid components of α-synuclein (NAC) and pathological LB were found in senile plaques[88]. The co-existence of senile plaques and remarkable NFTs in LB could explain the overlapping symptoms from AD and PD patients[89, 90]. The presence of α-synuclein in transgenic mice induced Aβ-dependent neuronal deficits in specific brain regions, while overexpression of $A\beta_{42}$, in turn, interfered with the processing of α-synuclein, promoted the intraneuronal accumulation of α-synuclein, and accelerated the development of motor deficits in transgenic mice[91]. Such mutual cross-seeding effect between α-synuclein and Aβ indicates that α-synuclein and Aβ could interact more directly to promote toxic conversion *in vivo* by engaging synergistic neurodegenerative pathways. Moreover, a multi-dimensional NMR study revealed the structural-based interactions between $A\beta_{40}$ or $A\beta_{42}$ and α-synuclein in the presence of membrane mimic sodium dodecyl sulfate (SDS) environment [92]. The synaptic membrane-bound α-synuclein interacted more strongly with $A\beta_{42}$ to produce more toxic oligomers than $A\beta_{40}$. Upon interactions, $A\beta_{42}$

oligomers cleaved the NAC fragment from α-synuclein, which is clinically observed in senile plaques in DLB patients. So, the identification of NAC component in the amyloid plaques is another indicator of the interactions of α-synuclein with Aβ. Another in vivo study showed that Aβ and α-synuclein can co-immunoprecipitate together to form complexes in the brains of patients with AD/PD and in transgenic mice[93]. Further in vitro cell culture analysis showed that Aβ directly interacted with α-synuclein to form hybrid pore-like oligomers, which serve as cation channels in cell membrane to induce calcium influx and cellular ionic homeostasis. All of these *in vitro* and *in vivo* studies above mainly demonstrated the cross-seeding interactions between monomeric Aβ and α-synuclein. To better understand the preformed seeds effect of Aβ and α-synuclein on their aggregation pathways, a series of preformed fibrils and crosslinked oligomers of $A\beta_{40}$, $A\beta_{42}$, and α-synuclein were co-incubated with freshly prepared $A\beta_{40}$, $A\beta_{42}$, and α-synuclein monomers[15]. Aggregation kinetics showed that while both fibrils and crosslinked oligomers of $A\beta_{40}$, $A\beta_{42}$, and α-synuclein had seeding effects on the aggregation pathways of different species and the same species, fibrils had the higher seeding efficiency than oligomers. Cross-seeding effects indicate that aggregates of Aβ and α-synuclein acted as seeds can promote the aggregation of each other, but α-synuclein exhibited the higher cross-seeding efficiency to promote Aβ aggregation. Also, different cross-seeding efficiencies between $A\beta_{40}$, $A\beta_{42}$, and α-synuclein further confirm the existence of cross-seeding barriers.

4.4. Cross-seeding between tau and α-synuclein

Clinical diagnosis and distinguish of LBD, PD, and AD remains as an extremely challenging task, because all these diseases possess similar dementia symptoms that could occur alone or in combination with different brain disorders[94, 95]. Tau at a normal condition is to stabilize abundant microtubules in neurons and central nerve system, but the hyperphosphorylation of tau proteins at a disease condition is closely associated with the pathologies and dementias of AD and PD[96]. At the same time, PD and LBD are typical α-synucleinopathies, characterized by abnormal accumulation of misfolded α-synuclein in neurons[97, 98]. With similar biological consequences but different on-set organs in human brains, patients may develop one neurodegenerative disease after another and frequently present the clinical and pathological features of both diseases, implying that tau and α-synuclein may share the overlapping pathogenetic pathways[99]. Consistently, some clinical studies showed that α-synuclein aggregates were also detected in AD, and *vice versa*[100-102]. α-synuclein immunological activity exists in the on-set organs of ~50% of AD patients[103-105], suggesting the co-existence or cross-talk between the two proteins (**Figure 5**).

Unlike most neurodegenerative proteins, the monomeric tau protein does not spontaneously misfold, instead a hyperphosphorylation is required to induce its aggregation[106, 107]. Studies have found that α-synuclein directly stimulates the phosphorylation of tau by protein kinase A (PKA)[108] and glycogen synthase kinase-3β (GSK-3β). The non-amyloid β-component (NAC) domain and acidic region of α-synuclein are responsible for the stimulation of GSK-3α-mediated tau

phosphorylation[109]. Apart from α-synuclein-mediated phosphorylation, small amount of preformed α-synuclein aggregates induced tau fibrillation. Cellular study showed that hyperphosphorylated tau can intermingle with endogenously expressed α-synuclein aggregates[23]. In addition to the accelerated aggregation rate as induced by cross-seeding effect, α-synuclein seeds also promoted the toxic conversion of tau oligomers in SH-SY5Y and CV-1 cells and spine retraction in primary neurons. From the comparison between brain-derived tau oligomers isolated from pure tauopathy and brain-derived α-synuclein/tau oligomers complexes from PD cases, α-synuclein seeds surprisingly induced a distinct toxic tau oligomeric strain, which extended toxic lifetime of tau oligomers and averted tau fibrillization[110].

Genomic studies have revealed a potential link between PD and gene-encoding tau[111]. In a synucleinopathy mouse model study, small NAP peptide decreased tau hyperphosphorylation and thus exhibited neuron protection effect on PD, demonstrating a protective role of tau in synucleinopathies[112]. Different cell models of synucleinopathy also showed that the overexpression of tau changed the pattern of α-synuclein aggregation by increasing the number of aggregates with the smaller sizes. Meanwhile, co-transfection of tau increased the secreted α-synuclein and its cytotoxicity. This observation suggest that tau enhances α-synuclein aggregation and toxicity and disrupts α-synuclein inclusion formation[113]. Solution NMR results revealed that monomeric tau selectively interacted with the C-terminal region of α-synuclein, accelerating α-synuclein aggregation. The non-aggregated tau and α-synuclein can interact strongly to form hybrid oligomers and distinct twisted thicker filaments at molar ratios of 1:10 and 1:100[114]. A recent study has shown that cross-seeding between α-synuclein and tau impairs the eyes and dopaminergic neurons in the fruit fly model, implying a broader impact of cross-seeding on the tau and α-synuclein pathologies[115].

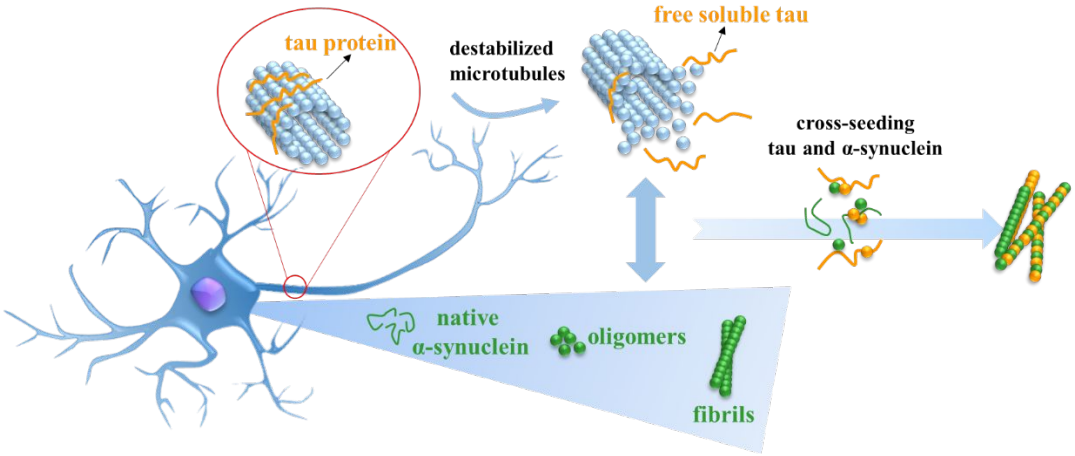

Figure 5. Cross-seeding between tau and α-synuclein.

Apart from the cross-seeding of the four pairs of amyloid proteins, there still remains other or unexplored cross-seeding possibilities between different amyloid proteins, and the underlying cross-seeding-induced pathological mechanisms. For

instance, cross-seeding between apolipoprotein A-II and protein A amyloid revealed a complex scenario, i.e. the interactions between the two amyloid proteins may promote the overall fibrillation, but may also prevent the aggregation of one of them or both[116], depending on not only the aggregation states of both proteins, but also environmental conditions including temperature, pH, agitation, and ionic strength.

5. Computational modeling and simulations of cross-seeding

While *in vitro* and *in vivo* experiments have demonstrated the existence of cross-seeding between Aβ/hIAPP, Aβ/tau, Aβ/α-synuclein, and tau/α-synuclein, it still remains a great challenge to resolve atomic-resolution structures of any cross-seeding aggregates, which will help to reveal how different amyloid aggregates interact with each other and how their interactions impact the aggregation kinetics and structures of hybrid amyloid formation. Less efforts and research have been devoted to the computational modeling and simulations of the cross-seeding of different amyloid proteins, presumably due to structural heterogeneity and transit nature of hybrid assemblies. While structural polymorphism is a general feature of amyloid aggregates, β-sheet-rich conformation is still a dominant and conserved structural domains as observed in $A\beta_{42}$ (PDB: 2MXU, 2BEG) [117, 59, 118], $A\beta_{40}$ (PDB: 2M4J, 2LMN, 2LMO, 2LMP, 2LMQ)[119, 120], αB-crystallin (PDB: 4M5T)[121], α-synuclein (PDB: 2N0A)[122], and different fragments of Aβ (PDB: 2Y3J, 2Y3K, 2Y3L, 3PZZ, 3OW9)[123], hIAPP (PDB: 5E5V, 5E5X, 5E5Z, 5E61)[124], Tau (PDB: 3OVL, 4NP8)[125, 126], human Transthyretin (PDB:4XFN, 4XFO)[127], α-synuclein (PDB: 4ZNN, 4RIK, 4RIL, 4R0U, 4R0W) [145, 146], and prion proteins[128, 129] (3NVE, 3NVG, 3NVH) [145, 146]. In principle, highly populated β-sheet conformation provides a structural basis and interaction template for amyloid cross-seeding. Also, high structural similarity allows to lower the cross-species barriers and promote mutual binding and recognition between different species, leading to amyloid hetero-assembly.

The cross-seeding behavior of Aβ and hIAPP at monomeric and oligomeric states in solution and on lipid bilayers was recently studied using coarse-grained (CG) replica-exchange molecular dynamics (REMD) and all-atom molecular dynamics (MD) simulations. A number of computational studies have shown that the cross-seeding Aβ-hIAPP assemblies adopted a wide range of polymorphic structures via different combinations of β-sheet associations and orientations[130]. Aβ and hIAPP can associate together through peptide addition along the fibril axis[34] or lateral stacking on the top of each other via β-sheet packings. Among them, a double-layer Aβ-hIAPP assembly exhibited highly structural stability and favorable interfacial interactions at both oligomeric and fibrillar states (**Figure 6a**), highlighting the importance of salt bridges and β-sheet packings for in stabilizing cross-seeding assemblies[35, 36]. More importantly, the Aβ-hIAPP assembly, regardless of its initial orientations, interacted more strongly with POPC/POPG bilayer than POPC bilayer, indicating that electrostatic interactions are the major forces governing peptide-lipid interactions[39]. Upon adsorption of Aβ-hIAPP assembly on lipid membranes, Aβ peptides are fully buried under hIAPP peptides, producing the hIAPP-like fibril morphologies. This may also explain

experimental observation that Aβ-hIAPP assembly on the raft-like membrane exhibits a similar structure to pure hIAPP, but not to pure Aβ.

A number of computational studies have shown that Aβ can act as seeds and affect α-synuclein aggregation, and vice versa. Aβ conformers had ability to bind α-synuclein monomers, homodimers, and trimers, forming hybrid ring-like pentamers and hexamers in solution via interactions between the N-terminus of Aβ and the N-terminus and C-terminus of α-synuclein[93]. The formation of pentamers and hexamers in the POPC bilayer facilitated the conformation change to a ring-like structure, leading to the increased calcium influx and ionic homeostasis consistent with experiments[15]. Another statistical mechanical model showed the formation of a co-oligomer formed by mixtures of α-synuclein with Aβ is more favorable than self-oligomer formation.[131] Furthermore, cross-seeding between Non-β-component of α-synuclein (NAC) and Aβ preferred to adopting double-layer conformation over single-layer conformation and NAC enables new β-content formation in Aβ (**Figure 6b**).[28] Among different Aβ/α-synuclein complexes, $_{18}$VFFAED$_{23}$ in Aβ and $_{38}$LYVGSK$_{43}$ in α-synuclein are found to be key binding residues for cross-seeding between Aβ and α-synuclein.[132]

It is interesting to observe from in vitro experiments that for the two K18 and K19 tau protein isoforms, K19 can seed K18, but not vice versa. To obtain insight into the cross-seeding between K18 and K19 aggregates, MD simulation results showed that the formation of stable R2 and R3 conformations is the critical step for K18 aggregation and R3 is critical for K19 fibrillization. The different core units in K18 and K19 lead to different cross seeding barriers for promoting K18 growth on K19 seeds, but preventing K19 growth on K18, because R2 is not available for K19 (**Figure 6c**)[41]. All of these simulation results confirm that the formation of a common β-structure provides a structural basis and possibility for realizing amyloid cross-seeding via different complex interaction pathways[48].

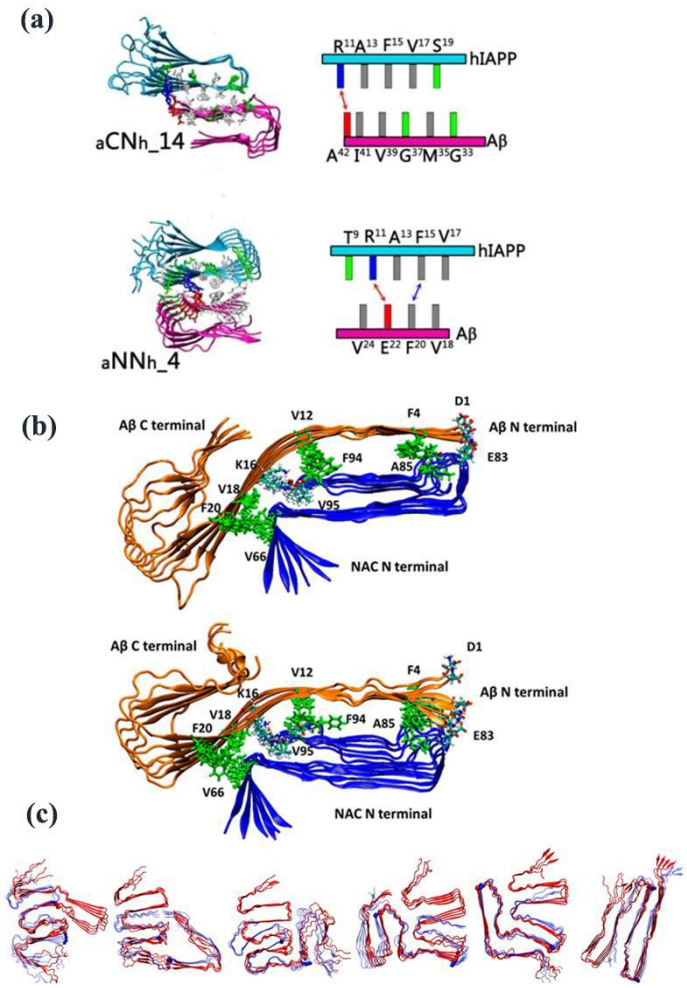

Figure 6. Molecular modeling and structures of cross-seeding between (a) Aβ and hIAPP (Reprinted with permission[36], Copyright 2015 Royal Society of Chemistry) (b) Aβ and α-synuclein (NAC) (Reprinted with permission[28], Copyright 2016 American Chemical Society) and (c) K18 and K19 tau protein isoforms (Reprinted with permission[41], Copyright 2012 American Society for Biochemistry and Molecular Biology.) All of cross-seeding assemblies display structural polymorphism, but with a common β-structure as cross-seeding building blocks.

6. Potential cross-seeding design for amyloid inhibitors/promotors

From a different viewpoint, the cross-seeding barrier offers a potential strategy for the de novo design of cross-seeding-based amyloid inhibitors. A straightforward design strategy is to select the truncated fragments from amyloid proteins and test their inhibition ability to prevent the amyloid aggregation of their parent and/or counterpart amyloid proteins via cross-seeding interactions. Since these fragments are highly homologous to their parents in both sequences and structures, it is not surprising that they could cross-seed and interfere with the folding and aggregation of amyloid proteins. Such fragmental inhibitors have been well developed to target Aβ, hIAPP, α-synuclein, serum amyloid protein, and β2M. Interestingly, some amyloid-derived fragments with

central hydrophobic clusters or abundant hydrogen bonding contributors can also bind to unrelated amyloid proteins and inhibit their aggregation. It was reported that as identified from the hot regions of Aβ-hIAPP cross-seeding interface, some hIAPP-derived fragments, i.e., $hIAPP_{30-37}$, $hIAPP_{1-18}$, $hIAPP_{8-18}$, can specifically bind to $A\beta_{40}$, retard its aggregation rates, and reduce the fibrillization to different extents[80, 133]. Similar inhibition effect of hIAPP-derived fragments on insulin aggregation was also observed[134].

Short amyloid fragments can be easily synthesized and modified by point-mutations and anchoring other functional molecules to introduce new functionality (e.g. anti-enzyme cleavage stability, biocompatibility, targeting ability) while still retaining or even strengthening their amyloid inhibition capacity. For instance, the N-methylation is considered as another design strategy to simultaneously amplify the inhibitory effect and weakens the amyloidogenesis of amyloid fragments. N-methylation modifications often lead to the elimination of amide bonds on one side of the peptides to prevent the hydrogen bond formation by NH and CO that are prerequisite for the association of the β-sheet oligomers into the high-order amyloid aggregates, while remaining another side of the peptides almost intact for the cross-seeding with other amyloid aggregations (**Figure 7a**). It was found that the N-methylation of $A\beta_{25-35}$ fragment at each residue site can effectively reduce the aggregation and cytotoxicity of the parent $A\beta_{25-35}$ peptides, independent of their N-methylation sites[135]. Later, a series of double N-methylated hIAPP were developed and demonstrated their dual inhibition ability to fully eliminate the fibrillization and cytotoxicity of both $hIAPP_{1-37}$ and $A\beta_{1-40}$ by converting them into less toxic amorphous aggregates[146, 149, 150]. These studies broaden the application potentials of the N-methylation of cross-seeding.

Another design strategy is to fuse the amyloid recognition sequences into native proteins to achieve new biological functions. For instance, a family of robust β-sheet macrocyles containing a variety of heptapeptide sequences derived from different amyloid proteins including Aβ, β2M, α-synuclein, hIAPP, human and yeast prion protein, and Tau was constructed and used as amyloid β-sheet mimics (ABSMs) to bind to their parent amyloid proteins, leading to the delay of their aggregation lag phases and the reduction of final fibrillization (**Figure 7b**)[136]. ABSMs containing Aβ fragments also greatly reduced cell toxicity induced by both $A\beta_{1-40}$ and $A\beta_{1-42}$[136]. Since the fused amyloid sequences in the macrocyles always fold into the β-sheet structure, the inhibition activity of AMSMs is likely contributed by their β-sheet interactions with amyloid aggregates. In another study, the insertion of amyloid fragments into amyloid-targeting antibody allowed to design the conformation- and sequence-specific antibodies against amyloid aggregations and toxicity[137]. Similarly, the grafting of amyloidogenic motifs into the complementarity determining regions (CDRs) of small γ-antibodies endowed the amyloid-grafted γ-antibodies to have amyloid-targeting functions via the cross-seeding interactions between the grafted amyloid sequences and amyloid proteins (**Figure 7c**)[138-140]. The γ-antibodies grafted by hydrophobic Aβ, α-synuclein, and hIAPP fragments enabled to specifically recognize and inhibit the

fibrillation of the corresponding amyloid proteins. So, amyloid fragment-modified native proteins and antibodies also demonstrate a possibility to develop amyloid inhibitors via the cross-seeding design concept.

Computer-aided structural design of new peptide inhibitors with non-amyloidogenic sequences is another interesting strategy to introduce cross-inhibition effect on amyloid aggregation[141]. A series of short peptides with 5-7 residues were computationally designed and served as structural templates to form steric zippers with different amyloid fibers[142]. Assisted with an in-house rotamer library, the sidechain orientation and chemistry of the inhibitors can be precisely controlled and optimized to achieve the best intermolecular packing with the targeted amyloid proteins via cross-seeding interactions (**Figure 7d**). As proof-of-concepts, D- (TLKIVW) and L- (WW61) peptides contained asymmetrical sidechain interacting surfaces, i.e., one side of peptides contains the attractive side chains to strongly interact with amyloid proteins via steric-zippers, while another side with the repulsive side chains to block the growth of β-sheet at the edge and lateral directions. Moreover, we proposed a "like-interacts-like" design principle to design and identify a new class of β-sheet-forming self-assembling peptides (SAPs) with non-amyloidogenic sequences and SAP-nanoparticle conjugates as Aβ inhibitors, including several SAP inhibitors (CTLWWG, GTVWWG, CTIYWG, LVFFARKHH, LVFFARK, CVVIA, CLPFFD, VVIACLPFFD, LPFFDCVVIA)[143, 144], and SAP-nanoparticle conjugate inhibitors (LVFFARK-β-cyclodextrin, CVVIA-AuNPs, CLPFFD-AuNPs, VVIACLPFFD-AuNPs, LPFFDCVVIA-AuNPs, LVFFARK-CuNPs)[145-148] (**Figure 7e**). Some of them possess a dual inhibition function to prevent the aggregation and toxicity of both Aβ and hIAPP peptides. The underlying cross-seeding inhibition mechanism is that if SAP derivatives exhibit strong self-aggregation ability to form β-structure-rich aggregates, they are likely to interact strongly with conformationally similar motifs of amyloid proteins and to competitively reduce amyloid-amyloid interactions, thus preventing amyloid aggregation. The "like-interacts-like" design principle may generate a new class of peptide-based inhibitors without including any amyloid sequences.

Different from the design of cross-seeding amyloid inhibitor, it is also possible for the design of cross-seeding promotors to accelerate amyloid aggregation by bypassing the most toxic oligomeric aggregation stage. Increasing evidences have shown that amyloid oligomers, instead of mature amyloid fibrils, are main toxic species responsible for the onset of PMDs. Alternative to the inhibition of amyloid aggregation, the acceleration of amyloid aggregation could represent a potential strategy to ameliorate the toxicity of amyloidogenesis through bypassing oligomeric intermediates. However, only a few compounds have been reported to promote amyloid aggregation and reduce amyloid cytotoxicity simultaneously. A star-shaped poly(2-hydroxyethyl acrylate) (PHEA) was designed to promote aggregation and ameliorate the toxicity of hIAPP[149]. Highly rigid, long-arm, and aromatic-rich moieties in PHEA facilitated the rapid sequestration and fibrillization of hIAPP monomers into amyloid fibrils. Such PHEA-induced amyloid acceleration significantly reduced toxicity in a pancreatic β-

cell line and in mouse islets. Trodusquemine, an aminosterol which consists of polyamine spermine and a fused sterol ring, was proved to promote $A\beta_{42}$ aggregation and reduce its toxicity to neuroblastoma cells by rapidly converting low molecular weight oligomers to less toxic higher order aggregates[150]. We recently identified a flavanonol molecule, which can simultaneously promote the aggregation of both hIAPP and Aβ (not published yet). Different from non-peptide amyloid promotors, even less studies have reported the peptide-based amyloid promotors. *N*-acetylated and *C*-amidated hexapeptide AcPHF6 (MeCO-VQIVYK-NH_2) derived from native tau-hexapeptide sequence was designed and synthesized to promote both $A\beta_{40}$ and $A\beta_{42}$ fibrillogenesis and reduce neuronal toxicity[151]. We found that a hexapeptide GNNQQNY derived from yeast prion protein Sup35 can cross-seed with both Aβ and hIAPP. The presence of GNNQQNY can remodel aggregation kinetics of Aβ and hIAPP with the greater aggregation propensity to form nontoxic hybrid GNNQQNY/Aβ and GNNQQNY/hIAPP fibrils (not published yet). In conclusion, cross-seeding offers two different design strategies for the design of amyloid inhibitors or amyloid promotors against amyloidogenesis and PMDs.

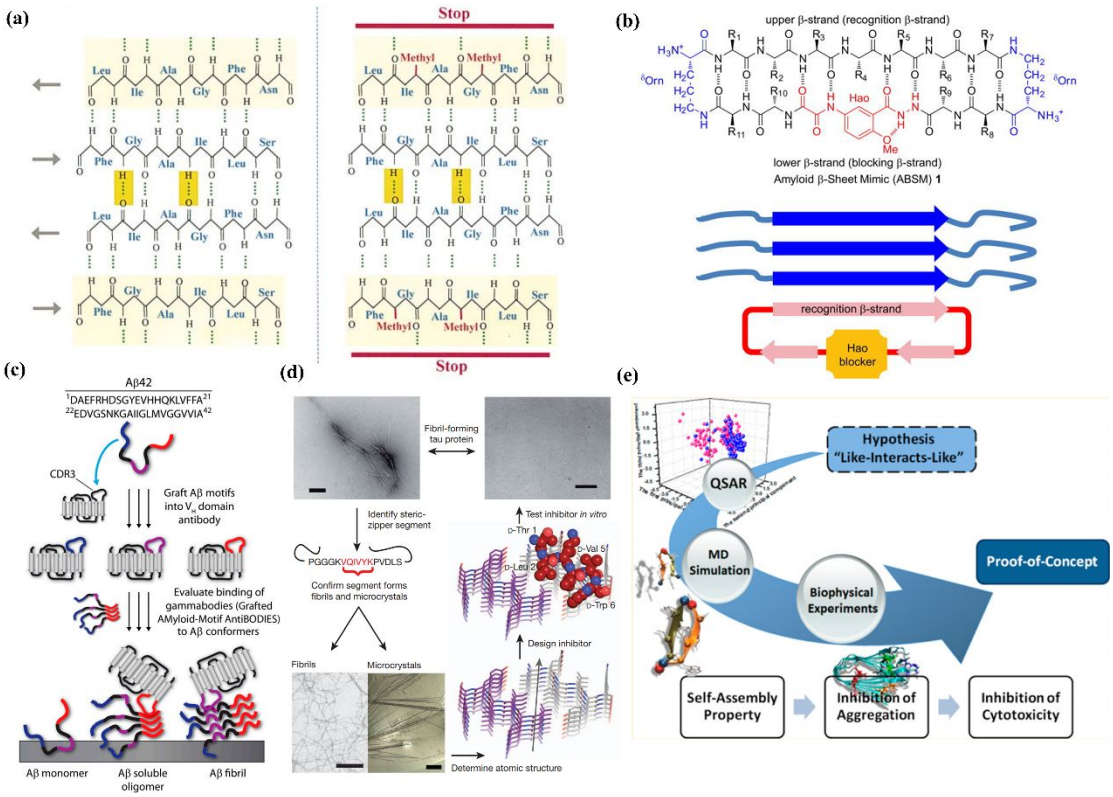

Figure 7. Cross-seeding-inspired design for amyloid inhibitors. (a) Double N-methylated IAPP sequences. The N-methylation is applied to Ile and Gly of hIAPP core amyloidogenic sequences to block the fibrillization of hIAPP (Reprinted with permission[152], Copyright 2002 Elsevier); (b) Grafting of amyloid fragmental sequences into macrocyles to produce engineered amyloid-macrocyles, enabling to specifically interact with different amyloid proteins including $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, $h\beta_2M$, and α-synuclein (Reprinted with permission[136], Copyright 2012 Springer Nature); (c) Grafting amyloid

sequences into γ-antibodies, enabling to achieve conformational- and sequence-specific binding and inhibition of Aβ (Reprinted with permission[153], Copyright 2012 National Academy of Science); (d) Computer-aided design of the steric-zipper peptide inhibitors to prevent amyloid formation (Reprinted with permission[154], Copyright 2011 Springer Nature). (e) Computational design of β-sheet-forming self-assembling peptides (SAPs) with non-amyloidogenic sequences as amyloid inhibitors. (Reprinted with permission[143], Copyright 2014 American Chemical Society).

7. Conclusions and Perspectives

Given that all amyloid proteins can misfold and aggregate into similar β-structure at disease conditions, it seems a reasonable observation that some amyloid proteins, probably not all of them, can interact with each other to form hybrid amyloid aggregates containing conformationally cross-β-sheet structures similar to homo-amyloids, (namely cross-seeding behaviors). These cross-seeding species can travel between different cells and tissues via cerebrospinal fluids and blood vessels to induce the overlapping amyloidogenesis of different PMDs and to accelerate the progress of PMDs. Hypothetically, the cross-seeding between different amyloid proteins strongly depends on their conformational compatibility, which create different cross-seeding barriers to determine the fate of cross-seeding. High conformational similarity between heterogeneous seeds would lower the cross-species barriers to promote mutual binding and recognition between different species, leading to bidirectional cross-seeding. Such bidirectional cross-seeding to promote the synergistic aggregations of different amyloid proteins may explain the overlapping pathology of different PMDs. On the other hand, if the two heterogeneous seeds have sufficiently large conformational differences, either highly populated conformers (HPCs) will select and recruit the less populated conformers (LPCs) of different proteins, drive LPCs to fit this to the HPCs, and finally stabilize the complexes, resulting in unidirectional cross-seeding; or they will grow into their own fibrils, reflecting non-cross-seeding. All different scenarios reflect structural-dependent cross-seeding barriers. Ironically, it seems that all preformed amyloid seeds contain β-sheet-rich conformations, which likely serve as general building blocks for cross-seeding, as observed in many cases. But, such general structural-dependent interactions do not explain the existence of cross-species barriers. Polymorphic nature of amyloid aggregation could be magnified in the cross-seeding process, which could prevent cross-seeding formation. Moreover, cross-seeding barriers also depend on experimental conditions (seeding concentrations, sequence specificity, even agitation).

"Seeing is understanding" remains as a great challenge for directly capturing the structures and interaction modes of cross-seeding species at molecular level. Iterative and seamless integration between computational and experimental works would be extremely necessary for uncovering new fundamental knowledges and developing new techniques for amyloid cross-seeding. While there always exists some "system-gaps" between molecular modellings/simulations and amyloid cross-seeding experiments due to huge timescale and lengthscale differences and inadequate computational sampling issue for a complex biological process (not only limited to the cross-seeding process),

molecular modeling/simulations would still be able to obtain some coherent clues. The rapid advance and integration of computational technologies (e.g. artificial intelligent, hardware chips, graphic process units, and data science and mining), high-resolution structural microscopy techniques (e.g. cryo-EM, x-ray, NMR), and molecular simulation techniques (e.g. coarse-grained simulations, multiple-resolution models, enhanced sampling algorithms, and accurate atomistic force fields) are highly promising to explore different aspects of amyloid cross-seeding, including the more accurate prediction of the atomic-resolution structures, dynamics, interactions, and misfolding/aggregation pathways of different amyloid peptides in different environments (e.g. aqueous solution, lipid bilayers, cell-mimic membranes), passive or active membrane permeation to illustrate the toxic potentials of cross-seeding species, and even transport pathways and mechanisms of cross-seeding species between different cells via the assistance of additional membrane components or other drugs. In parallel, experimental data to characterize numerous modes of amyloid cross-seeding always aid in the development of realistic molecular models and the validation of molecular simulation predictions. We hope that this review will stimulate more research towards the amyloid cross-seeding – which is studied less intensively than amyloid homogenous seeding – to decipher a possible molecular cross-talk and disease pathophysiology between different PMDs. Additionally, cross-seeding concept could guide the drug design and peptide engineering to fight against PMDs.

Acknowledgement. J.Z. thanks financial supports, in partial, from NSF (CBET-1510099, DMR-1806138, and CMMI-1825122).

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