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Molecular mechanisms of amyloid disaggregation

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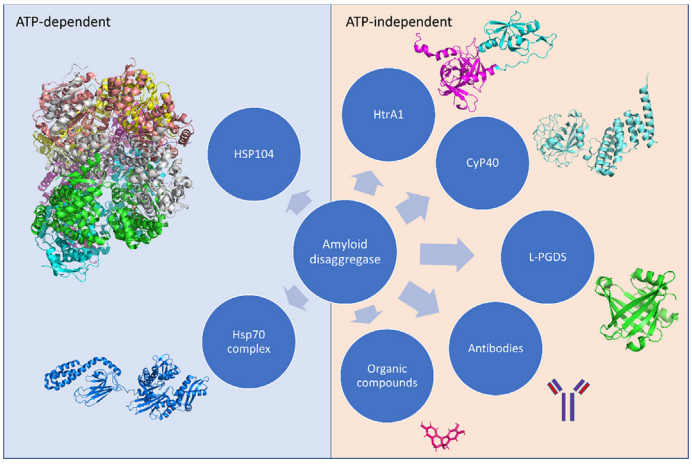
HIGHLIGHTS

- Importance of disaggregation mechanism and innate disaggregation in living systems.
- Different types and mechanism of disaggregation reported in literature.
- Structural details of the interactions and the disaggregation mechanisms.
- Amyloid disaggregation in protein aggregation disorders as a potential treatment.
- Proposed amyloid disaggregation mechanism of an ATP-independent chaperone (L-PGDS).

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Protein aggregation and deposition of uniformly arranged amyloid fibrils in the form of plaques or amorphous aggregates is characteristic of amyloid diseases. The accumulation and deposition of proteins result in toxicity and cause deleterious effects on affected individuals known as amyloidosis. There are about fifty different proteins and peptides involved in amyloidosis including neurodegenerative diseases and diseases affecting vital organs. Despite the strenuous effort to find a suitable treatment option for these amyloid disorders, very few compounds had made it to unsuccessful clinical trials. It has become a compelling challenge to understand and manage amyloidosis with the increased life expectancy and ageing population.

Objective: While most of the currently available literature and knowledge base focus on the amyloid inhibitory mechanism as a treatment option, it is equally important to organize and understand amyloid disaggregation strategies. Disaggregation strategies are important and crucial as they are present innately functional in many living systems and dissolution of preformed amyloids may provide a direct benefit in many pathological conditions. In this review, we have compiled the known amyloid disaggregation

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mechanism, interactions, and possibilities of using disaggregases as a treatment option for amyloidosis.
Methods: We have provided the structural details using protein-ligand docking models to visualize the interaction between these disaggregases with amyloid fibrils and their respective proposed amyloid disaggregation mechanisms.
Results: After reviewing and comparing the different amyloid disaggregase systems and their proposed mechanisms, we presented two different hypotheses for ATP independent disaggregases using L-PGDS as a model.
Conclusion: Finally, we have highlighted the importance of understanding the underlying disaggregation mechanisms used by these chaperones and organic compounds before the implementation of these disaggregases as a potential treatment option for amyloidosis.
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Introduction

Protein aggregation and amyloidosis:

Cellular deregulation of amyloid formation is implicated in many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Prion disease (PrD), and diseases affecting other parts of the body such as cataracts, Type II Diabetes and Corneal Dystrophy (CD) [1–4]. Fifty different proteins or peptides involved in such amyloid aggregation disorders are structurally and functionally characterized [5]. Typically amyloid fibrils are generated from highly amyloidogenic peptide regions of a protein as the result of protein misfolding, genetic mutations, or undesired proteolytic cleavage of that protein [6]. The resulting peptides may induce the formation of highly reactive seeds and nuclei [7,8]. Most amyloid fibrils share a common cross beta-structure where the beta-strand of each protofilament is perpendicular to the long axis of the fibrils [9]. In Alzheimer's disease, amyloid-beta (Aβ) peptides aggregate outside a cell forming Aβ fibrils deposited as extracellular plaques, while tau protein aggregation results in the deposition of intracellular tangles [10,11]. In Corneal Dystrophy, mutations in the Transforming Growth factor beta-induced gene (*TGFBI*) result in protein aggregation and amyloid fibril formation that are deposited in different layers of the cornea leading to complete loss of vision [12–14].

Functional Amyloids:

However, not all amyloid fibril formation results in detrimental diseases while some may be important to fulfil a biological function and take place in well-modulated and highly contingent conditions [15]. Amyloid fibril formation is important for certain cellular activities like hormone release, skin pigmentation (Pmel17) and memory (CPEB) [15–17]. In some cases, functional amyloids are controlled by a balance between peptides' production and clearance of amyloids, reduction in the production of oligomeric seeds, minimizing interaction of oligomeric seeds with other aggregation-prone proteins via compartmentalization and the presence of an inherent disaggregation mechanism [17–19]. Understanding why certain amyloids are toxic while others are biologically important may reveal important information on the function of these amyloids or develop novel treatment avenues in amyloid associated diseases.

Naturally occurring disaggregation machinery in protozoans and metazoans:

In order to remove toxic amyloid build-up in the cell during cellular stress, some protozoans such as yeasts are equipped with molecular machines capable of disaggregating diverse amyloid and nonamyloid structures [20]. In yeast, several types of heat shock proteins (HSPs) are discovered to work together to form disaggregation machinery [20,21]. This machinery reduces the toxic amyloid species present in the cell and restores the native function of the protein buried in the amyloids via an amyloid disaggregation process [20]. Metazoans such as mammals might experience less cellular stress resulting in the rapid build-up of toxic amyloid in the intracellular environment but are susceptible to accumulation of both intracellular and extracellular amyloids in various pathological conditions. To disaggregate these toxic amyloids in the extracellular environment, metazoans are equipped with ATP-independent chaperones such as HtrA1 [22] and the Lipocalin-type Prostaglandin D Synthase (L-PGDS) [23] instead of the ATP-dependent HSPs, found in yeast. To deal with intracellular amyloids, the metazoan cells are also equipped with other types of HSPs i.e., Hsp110, Hsp70, Hsp40, and other smaller proteins from the heat shock protein families [24–26]. These diverse disaggregation mechanisms evolved to reverse the formation of the toxic amyloids and survive through cellular stress and preclude amyloid-related pathogenicity.

Disaggregation in pathological condition as a treatment option:

In neurodegenerative diseases such as Alzheimer's disease, aggregates resulting from amyloidogenic peptides deposit into senile plaques which later leads to neurofibrillary tangles, synaptic dysfunction, and neuronal cell death [27]. In each disease, a specific peptide or protein aggregates to form amyloid fibrils. In AD, the Amyloid precursor proteins (APP) are proteolytically cleaved by secretases to form small peptides commonly known as Aβ peptides [28] (**Review**). These Aβ peptides tend to aggregate and result in the formation of the Aβ fibrils and plaques in the extracellular environment [11]. In PD, small intrinsically disordered proteins known as the α-synuclein (α-syn), clump together to form fibrils that are found in Lewy bodies of degenerating dopamine neurons [29]. The formation of these toxic oligomeric and fibrillar species seems to be functionally significant to the pathogenesis of these diseases. However, there is no effective therapeutic solution that is capable of reversing the formation of these aggregates. Amyloid disaggregation seems to be a viable option where these amyloid fibrils can be broken down into non-toxic aggregates and this would possibly help to mitigate the toxic effects caused by these amyloid fibrils [30]. However, it is important to note that the breaking down of fibrils might lead to the formation of products with higher toxicity than the fibrils as discussed in the later paragraphs [31]. Hence, it is important to examine the products of disaggregation before introducing amyloid disaggregation as a potential therapeutic option. In this review, we mainly focus of the disaggregation and the remodulation of the preformed fibrils into smaller molecular weight species by different disaggregating agents instead of the inhibition of fibril formation or aggregation. Many protein disaggregases have shown promising results in *in vitro* studies where pathogenic amyloids fibrils are solubilized

through the action of these disaggregases [22–24,32,33]. These studies will be discussed in this review to showcase the potential of using amyloid disaggregation as a treatment for several neurodegenerative diseases.

TGFBI associated Corneal Dystrophy (CD), is a genetically inherited disorder with 74 different mutations reported to date [34]. The resulting mutant protein is processed distinctively leading to abnormal proteolysis of the mutant proteins. The resulting peptides act as the nucleus that triggers the aggregation cascade and the formation of fibrils in the cornea [13]. The disease is bilateral, and these deposits build up in multiple layers of the cornea, making the cornea opaque leading to decreased visual acuity in patients. With time, in severe cases, the patient eventually loses their vision due to protein aggregation and amyloidosis [12,35]. The current treatment for vision loss due to CD involves the replacement of the affected cornea by surgical methods such as corneal transplantation or corneal excimer [12,35]. However, major limitations of the surgical methods include the recurrence of the disease and the global shortage of transplant grade donor cornea used for transplantation [35,36]. Hence, a non-invasive and effective treatment would be ideal for affected patients to restore vision and improve visual acuity. It has been suggested that the use of an ophthalmic solution containing compounds or molecules that are capable of clearing or disaggregating the aggregated fibrils could be a possible treatment for the disease [37]. This possibility highlights the importance of discovering and implementing an endogenous chaperone or other chemical compounds that can disaggregate the amyloid fibrils in the cornea of patients. Before the implementation of these disaggregases as a potential treatment option, it is necessary to understand the underlying molecular mechanism of disaggregation used by these chaperones and chemical compounds.

Disaggregases:

Disaggregases are defined as proteins or compounds that are capable of unfolding and solubilizing protein aggregates such as amyloids [38]. Different types of amyloid disaggregases have been reported. There are two main categories of amyloid disaggregases, i.e. (i), Heat shock proteins (Hsps) utilizing ATP hydrolysis as driving force for disaggregation and (ii), smaller proteins or compounds which directly interact and remodel the amyloid fibrils. These two types of disaggregases are capable of breaking down many different amyloid fibrils implicated in protein misfolded diseases (Table 1). Despite their potency in solubilizing protein aggregates, the detailed disaggregation mechanism for both types of disaggregation machinery remains unresolved.

The resulting products of disaggregation are not characterized in detail at the molecular level for most of the studied systems. According to the amyloid aggregation cascade hypothesis, amyloid fibrils are formed by the aggregation of monomers, followed by the aggregation of the resulting oligomers [39] (**Review**). Hence, the solubilization of amyloid fibrils could result in the formation of either the oligomeric or monomeric state of the aggregates. For Hsp104 disaggregases, the disaggregation process involves translocating of polypeptides loops through their central pore, and the resulting product is either sent for refolding or degradation [38]. Most disaggregation assays involve the 'disintegration' of the amyloid fibrils (reduction in ThT intensity or direct observation via Transmission Electron Microscopy) upon the addition of disaggregases without elucidating the aggregation state of the resultant products. The aggregation state of the resulting products forming higher-order oligomers could exacerbate the disease due to their elevated cytotoxicity as reported in the case of Alzheimer's disease [40]. The smaller size oligomers are more capable of diffusing in the body and are more flexible in terms of the interaction with other cell targets as compared to the elongated fibrils [41]. Thus, it is important to identify the resulting products after the disaggregation process before marking these disaggregases as a possible therapeutic approach for amyloid-related diseases such as TGFBI-associated Corneal Dystrophy.

In this review, we discuss different types of protein disaggregases with the view on their specific disaggregation mechanism using both high-resolution structures of the amyloid-disaggregase complex where available and the corresponding mechanisms using simplified illustrations. After reviewing and comparing the different models of disaggregation, we propose two different generalized models for the mechanism of ATP-independent disaggregation and suggest methods to test our hypothesis.

Innate disaggregase mechanism:

In several organisms, there is a ubiquitous expression of disaggregases and chaperones to maintain protein homeostasis under stressful situations [42]. The disaggregases are a protein quality control machinery responsible for solubilizing aggregates and rearranging them for refolding or degradation. The disaggregase mechanism helps to recover and salvage any functional protein molecule from aggregation to avoid additional energy expenditure, degrade the aggregated proteins and replace them with newly synthesized functional molecules [24,43]. Protein disaggregases can be further categorized as ATP-dependent and ATP-independent.

ATP-dependent disaggregation

Heat-shock protein 104 (Hsp104)/ caseinolytic peptidase B (ClpB) molecular chaperones

One of the main functions of yeast Hsp104 and its bacterial homolog caseinolytic peptidase B (ClpB), as a molecular chaperone,

Table 1
The list of molecular systems with disaggregase activities.

	Protein disaggregases						**Small compounds**			
	ATP-dependent		**ATP-independent**				**Polyphenols**	**Organic compounds e.g. osmolyte**	**β-strand breakers and amino acids hybrids**	**Nanoparticles and polymers**
	Hsp104 and Hsp70	Hsp110, Hsp70 and Hsp40	HtrA1	Cyp40	L-PGDS	Anti Aβ Antibodies				
Amyloid β	X (1)		X (2)		X (3)	X (4–7)	X (8–14)		X (15–17)	X (18, 19)
α-synuclein/ γ-synclein	X (1)	X (20, 21)		X (22)			X (23–25)		X (26)	X (27)
Tau	X (1)	X (28)	X (2, 29)	X (22)			X (30–32)	X (33)	X (33)	
Amylin (IAPP)	X (1)						X (34)		X (15)	
TGFBIp					X (manuscript in preparation)			X (35)		

is to dissolve aggregated proteins when the organism is under extreme stress conditions [42]. HSPs belong to the ATPases associated with diverse activities (AAA +) superfamily of ATPases. They are made up of six subunits (P1-P6) forming a hexamer AAA + ATPase ring with a central pore [44]. Each Hsp104 subunit consists of an amino-terminal domain (NTD), a middle domain (MD), two nucleotide-binding domain (NBD1 and NBD2) and a carboxy-terminal domain (CTD) [45–48]. To disaggregate amyloid fibrils efficiently, Hsp104/ClpB works collaboratively with the Hsp70/DnaK chaperone system, two co-chaperones, i.e., J-domain chaperone known as (Hsp40) and Nucleotide exchange factor (NEF) known as Hsp110, to form a large disaggregase complex [49].

The amyloid substrates are loaded onto the disaggregase complex, translocated through the central pore and released as a single polypeptide for refolding or degradation. This large disaggregation complex is capable of solubilizing a broad spectrum of fibrils including the Aβ fibrils, tau tangles, alpha-synuclein, and amylin fibrils [24]. The chaperone Hsp104 originated from yeast, but it is well-tolerated in neuroblastoma cell cultures and mouse models. The working ratio of 1:20 (peptide: protein) is required for effective amyloid fibrils disaggregation activity of the Hsp104 proteins [20,24]. The large Hsp 104 amyloid disaggregation complex is not only capable of disaggregating amyloid fibrils but also refolds the resultant polypeptide back to its non-toxic native functional state. The released monomers interact with the chaperone network in the cell to prevent the re-aggregation of monomers [21]. Hence, the resulting products of Hsp104 amyloid disaggregation are non-toxic and Hsp104 has shown protective effects against the fibril's toxicity in several animal models [20,50,51].

The power stroke model of disaggregation

One of the most ubiquitous disaggregation mechanisms employed by the Hsp100s disaggregases is the power stroke model. This model involves the AAA + ATPases (Hsp104) using the energy harnessed from ATP binding and hydrolysis to forcefully translocate peptide through the central pore. This results in the extraction and unfolding of the aggregates (amyloid fibrils) to form polypeptides thus, disaggregating the amyloids [38]. Each chaperone protein plays its unique role in the large disaggregase complex: Hsp70 and J domain-containing Hsp40 engage the amyloid substrates via a conformational change induced by ATP hydrolysis. The ADP bound Hsp70/Hsp40 has a higher affinity for the substrate and will simultaneously recruit the cooperating Hsp104 hexamer for disaggregation activity. The Hsp70/Hsp40 complex binds to the MD domain of the Hsp104 hexamer and loads the amyloid substrates to the central pore of Hsp104. The removal of the ADP molecule by nucleotide exchange factor (NEF) then triggers a conformational change in Hsp70 and releases the substrate inside the Hsp104 disaggregase complex [52].

Locking of the substrates and binding of ATP molecules in Hsp104 subsequently initiates a power stroke which is accompanied by a conformational change of Hsp104 from closed ring to open ring conformation [38,47]. During the transition of conformation, an ATP molecule binds to the NBD domains of P6 and induces binding between P6 and the amyloid substrate at the higher position. At the same time, hydrolysis of ATP at the neighboring protomer releases the amyloid substrate. This results in a ratchet-like translocation of the substrate in a step of two amino acids [38] along the central pore of Hsp104 allowing disaggregation to occur (Fig. 1A). After translocation, the release of bound ADP at P1 and ATP hydrolysis of the next protomer converts Hsp104 to a closed ring conformation. Then, the binding of the next ATP molecule at P1 again converts Hsp104 to the opened ring conformation state (Fig. 1A). The continuous and rotatory disaggregation of the amyloid substrate is achieved by translocation and conformational change of the Hsp104 hexamer coupled with the ATP hydrolysis cycle [38,48]. This mechanism is schematically shown in Fig. 7A. In this model, binding and removal of ATP molecule not only regulates amyloid substrate-binding affinity but also induces the conformational change required for the activation of the Hsp disaggregation complex.

The Brownian ratchet model

The Brownian ratchet model was also suggested as a possible mechanism for protein translocation and disaggregation by Hsp100s [53]. This model assumes that the polypeptide of the amyloid aggregate randomly slides back and forth in the central pore of the disaggregase machinery due to collision and fluctuation of the peptide. According to this model, Hsp70 has an additional role to play, e.g., while it binds to an unfolded polypeptide on one side of the central pore of Hsp104, it prevents backward movement and sliding of the substrate into the disaggregation complex. Conformational change resulting from ATP hydrolysis allows repeated binding and release of the substrate to Hsp70 [54,55] (**Review**). In this model, the pulling force applied to the substrate is generated by repeated binding of Hsp70 on one side of the substrate. In this aspect, this model differs from the power stroke model described in the previous section. This cycle continues until the entire amyloid aggregate is threaded through the central pore of the Hsp100s machinery.

Hsp70, Hsp 110 and J family of molecular chaperone in metazoans

The amyloid disaggregation function of Hsp104/ClpB is known to be well conserved in protozoans. Despite their efficient disaggregation function, these protein chaperones are not found in metazoans [56] (**Review**). The reason for the absence of Hsp104 in metazoans largely remains unknown despite the successful application of Hsp104 to effectively solubilize amyloid aggregates in the Parkinson's disease and Huntington's disease animal models [20,50,51]. It has been postulated that in metazoans, the protein quality assurance is focused on the prevention of protein aggregation rather than on the disaggregation itself [56]. Many reports suggest that high levels of Hsp70 in metazoans might be able to compensate for the missing Hsp104 function [57,58]. However, Hsp70 alone is incapable of disaggregating large amyloid fibrils found in metazoans when tested in a cell-free setting [24]. The Hsp70 protein only contains a substrate-binding domain and an ATP binding domain. As the result, it requires a co-chaperone (Hsp40 and NEF protein) activation of its ATPase activity in order to release bound ADP and to start a continuous cycle of amyloid disaggregation [24,59]. Hsp110 is a divergent relative of Hsp70 discovered in mammalian cytosol. It is capable of forming a disaggregase machinery with Hsp70 and acts as NEF to release the ADP molecule from Hsp70. Hence, Hsp70 with the help of Hsp40, Hsp110 and other different types of J proteins, promotes disaggregation of large amyloid fibrils found in metazoans [24,26,59].

Entropic pulling model

In the absence of a hexamer central pore from Hsp104, another disaggregation model was proposed to explain how force is generated in the Hsp70/Hsp110 disaggregation machinery. The entropic pulling model which involves the lowering of entropy when Hsp70 is bound to substrates was suggested by De Los Rios to explain the disaggregation mechanism observed in the Hsp70, Hsp40 and Hsp110 complexes [57,60]. This model also suggests that Hsp70 and Hsp40 directly bind to the amyloid substrates [57]. With the help of ATP hydrolysis by Hsp40 on the NBD of Hsp70, Hsp70 can bind tightly to the aggregated substrate with its substrate-binding domain (SBD). ATP hydrolysis at NBD of Hsp70 induces a conformational change in SBD of Hsp70 which helps to trap the amyloid substrates within the Hsp70 complex (Fig. 1B). To 'regain' the high entropy before binding, Hsp70 exerts some stretching

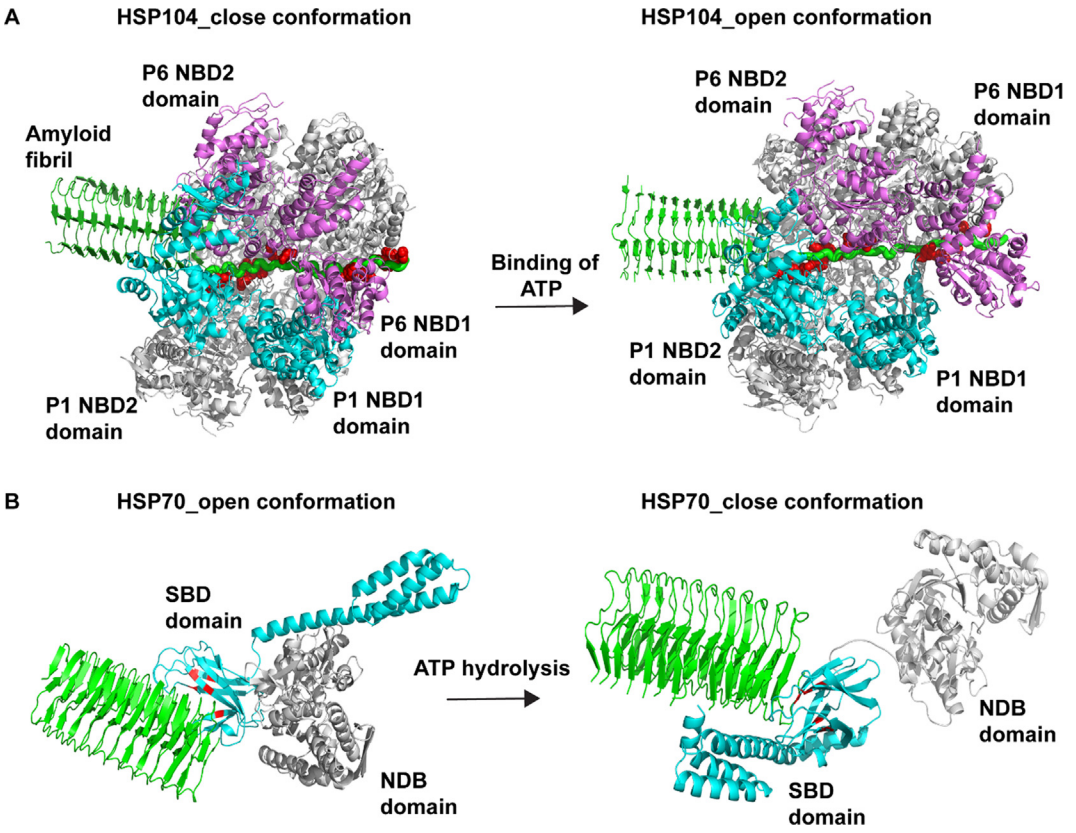

Fig. 1. Interaction between HSP and amyloid fibrils and the ATP induced conformational change in the HSPs (A) Interaction between closed configuration of HSP104 (PDB code: 5VJH) and amyloid fibril (PDB code: 2MXU). HSP104 consists of six protomers (P1-P6) forming a hexamer complex. Upon the binding of ATP molecules at nucleotide binding domains (NBD) of P6, the previously unbound NDB1 domain of P6 binds to the amyloid substrate at a higher position and induces an activation to the open conformation of the HSP104 hexamer (PDB code: 5VYA). Subsequently, the release of ADP molecule in the NBD1 domain of P1 becomes unbound to the substrate and induces a conformational change back to the closed configuration of HSP104. Another ATP molecule will then bind to the NBD1 of P1, allowing it to bind to the amyloid substrate an even higher position and repeats the cycle to drive the substrate translocation through the central pore and effectively disaggregate the amyloid substrate. Residues of HSP104 in contact with the substrate are colored in red and all of them are located within the NBDs of HSP104 protein. (B) Interaction between opened configuration of HSP70 (PDB code: 4B9Q) and amyloid fibril (PDB code: 2MXU). The substrate affinity of HSP is regulated by the binding and hydrolysis of ATP molecules in the NBD of HSP70. The hydrolysis of ATP molecule binding at the NBD will induce a conformation change in the SBD of HSP70 into a closed conformation (PDB code: 2KHO) and this will result in an increased in the affinity of HSP70 for amyloid substrate and locks the fibril in the HSP70-amyloid substate complex. Residues of HSP70 within the SBD which are in contact with the amyloid substrates are coloured in red. The docking models are obtained from online docking server, ClusPro (59–61).

force on the substrate [57,60]. This large directional force generated by favorable entropy of unfolding allows the disaggregation of the substrates to occur. Thereafter, Hsp110 acts as NEF to release ADP in Hsp70 by lowering its affinity to the substrate. This allows Hsp70 to release the amyloid substrate and undergo another pulling and unfolding cycle [57,60]. Working together, Hsp40, Hsp70 and Hsp110 form a powerful hetero-oligomer disaggregation complex that can break down amyloid fibrils such as alpha-synuclein [24]. Recently, the discovery of the molecular mechanism of amyloid disaggregation by Hsp70 and its co-chaperone (Hsp40/DNAJB1 and Hsp110) using biochemical tools and NMR spectroscopy further supported the entropic pulling model proposed by De Los Rio et al. [57]. Wentink et al. showed that the Hsp40/DNAJB1 first recognize the C-terminal of the α-synuclein and target them to the open ATP state of the Hsp70 protein. Then, the Hsp70 protein binds to the N terminal of the α-synuclein and the maximal stoichiometry of Hsp70 to α-synuclein is 1:2. As more Hsp70 proteins are recruited by the Hsp40/DNAJB1 onto the α-synuclein fibrils, this will result in a crowded environment for the Hsp70 proteins on the fibrils. The author hypothesized that the steric clashes due to the close proximity of Hsp70 proteins would result in a highly energetically unfavorable conformation which would then favour the disaggregation of the fibril via entropic pulling. Moreover, the high affinity between Hsp70 and the α-synuclein induced by the Hsp40/DNAJB1 provided the energy to create a more crowded environment to produce stronger entropic-pulling forces. The recycling of the Hsp70 is achieved by the ADP hydrolysis by the Hsp110 proteins. The high molecular weight of the Hsp110 further enhanced the density of Hsp70 on the fibril as they prefer to interact with free Hsp70 instead of the fibril bound Hsp70. This activity bias facilitated the crowding of Hsp70 on the fibrils thus resulting in a more productive amyloid fibril disaggregation process. The cycle of amyloid disaggregation will start again with the binding of Hsp70 to a new ATP molecule and the conversion to the open conformation of Hsp70 for the next round of substrate binding [61]. This mechanism is schematically illustrated in Fig. 7B.

Disaggregation via ATP-independent chaperone:

The previous examples of the Hsp100s proteins involve hydrolysis of several ATP molecules to drive the disaggregation of amyloid substrates into monomers. However, several amyloidogenic aggregates are only found in the extracellular environment where ATP is scarce [62]. Therefore, this ATP-dependent disaggregation machinery might not work as efficiently on the extracellular aggregates, as the supply of ATP molecules in the extracellular space is limited [63] (**Review**). As the result, ATP-independent disaggregation would most likely play a more important role than the Hsp100s proteins in breaking down extracellular aggregates such as amyloid fibrils found in AD.

The PDZ serine protease high temperature requirement family of serine proteases (HtrA1)

HtrA1 is a serine protease that consists of a flexible N terminal (a signal peptide and a partial insulin-like growth factor binding protein-70) domain, a protease domain and a C-terminal PDZ domain. HtrA1 is an ATP-independent protein disaggregase found abundantly in the human brain where it has been reported to disintegrate tau and Aβ fibrils effectively [22,64]. Poepsel et al. discovered that the PDZ domain is important for the disaggregase activity of HtrA1 as mutations in the PDZ domain of HtrA1 reduced disaggregase ability and abolished binding to tau protein [22]. The PDZ domain is responsible for amyloid fibril binding as it recognizes the beta-strand structure of the fibrils (Fig. 2). However, the PDZ domain does not act independently but collaborates with the protease domain to disintegrate fibrils efficiently. The authors suggested a two-part mechanism for HtrA1 ATP-independent disaggregation, first is the disintegration of amyloid fibrils by the PDZ domain followed by proteolysis of the substrates via the protease domain of HtrA1. HtrA1 binds along the axis of the amyloid fibrils. Therefore, it is speculated that the PDZ domain binds to the beta-strand structure of the amyloid fibrils like a finger where it grabs the strands and tugs them apart allowing access for the protease domain of the protein to cleave the amyloid fibrils. The specific binding to substrates via the PDZ domain also alters the conformation of HtrA1 resulting in the activation of the protease domain which degrades the bound amyloid fibrils such as tau filament and amyloid-beta fibrils [22]. This mechanism is represented in a schematic diagram in Fig. 8A.

The specific high-affinity binding of the PDZ domain to the fibrils helps to eliminate the need for ATP hydrolysis induced conformational change to increase the affinity between the protein and ligand as observed in the previous Hsp100 examples. After disaggregation, the lower affinity disaggregate products would then be released passively in the surrounding. The specific binding of the PDZ domain to the amyloid fibrils seems to be a crucial step in the amyloid disaggregation mechanism of HtrA1. However, the exact mechanism for the disintegration by the PDZ domain remains unknown. Therefore, it is interesting to further investigate how the specific binding leads to the pulling effect of the HtrA1 protein observed during the disaggregation process. An equimolar ratio of HtrA1 and amyloid fibrils has been utilized for effective amyloid disaggregation activity of HtrA1 [22]. Notably, HtrA1 is a protease. As the result, the products of the disaggregation are further degraded into smaller fragments, leading to the disintegration of the fibrils [22,64].

Cyclophilin 40 (CyP40)

CyP40 belongs to the family of cis/*trans*-peptidyl-prolyl isomerase (PPIases) and it isomerizes proline residues without any expenditure of the energy from ATP hydrolysis. Baker et al. reported that Cyp40 was able to disintegrate tau amyloid [32]. However, they found that Cyp40 is only capable of disaggregating proline-containing amyloids such as alpha-synuclein and tau filaments. To uncover the underlying mechanism for the specific disaggregation ability of CyP40, they removed the PPIase subunit of CyP40 by inducing a mutation. They concluded that the disaggregase mechanism employed by Cyp40 is proline dependent, as the disaggregation ability of CyP40 was diminished after the removal of the PPIase subunit. They further proposed that the PPIase domain of CyP40 interacts with proline residues located at the beta turns of these amyloid fibrils and isomerize these specific proline residues into an alternative conformation (from cis to trans or vice versa) (Fig. 3). This disrupts the beta-turn structure of the amyloid fibrils and results in the disaggregation of the amyloid fibrils. The schematic diagram of this mechanism is represented in Fig. 8B. Since the disaggregation reaction is ATP-independent, they also suggested that the specific binding of the PPIase domain with the proline residues results in a conformation change within the TPR domain to form an open conformation to accommodate the

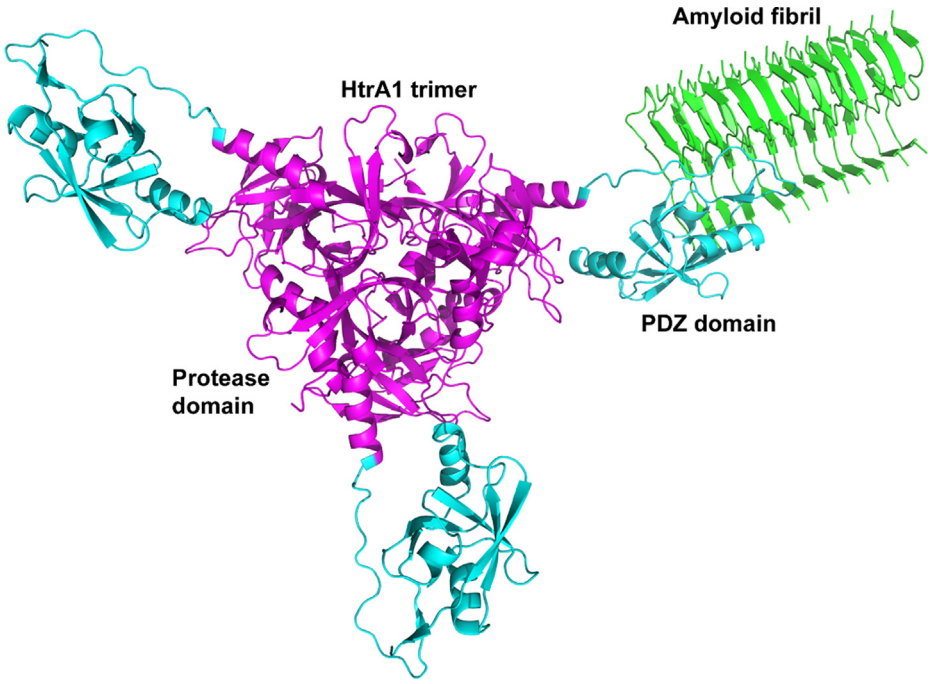

Fig. 2. Interaction between Htra1 and amyloid fibrils. The trimer of HtrA1 (PDB code: 3NZI) interact with the amyloid fibril (PDB code: 2MXU). The PDZ domain of HtrA1 (PDB code: 2YTW) (cyan) is responsible for the interaction with the amyloid fibrils by binding to specific sequence along the fibril. The exact binding interaction between PDZ domain and fibril remains unknown. In addition, the PDZ domain needs to be tethered with the protease domain of HtrA1 (magneta) to disaggregate amyloid fibrils effectively. The docking models are obtained from online docking server, ClusPro (59–61).

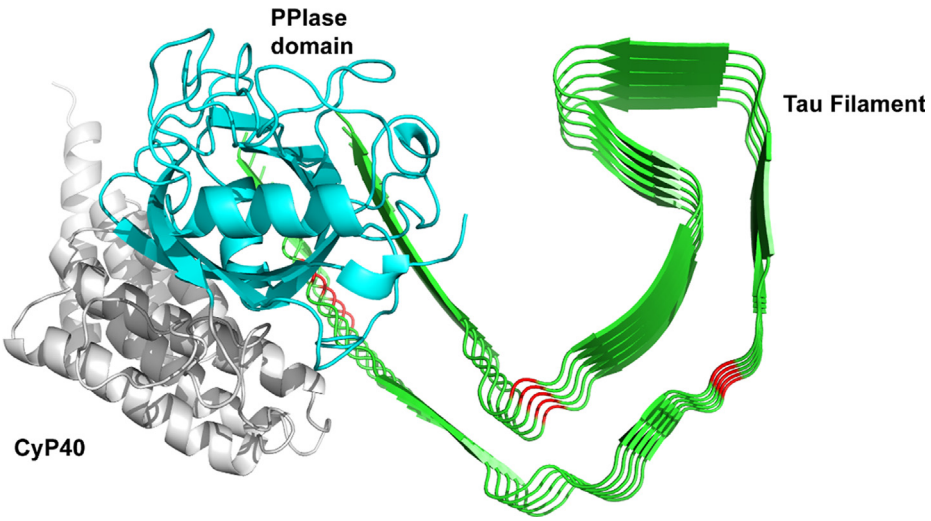

Fig. 3. Interaction between CyP40 and amyloid fibrils. The PPIase domain (cyan) of bovine CyP40 (PDB: 1IHG) will interaction with the proline residues (red) of the tau filaments (PDB code: 5O3T) and result in an isomerization of the proline residues on the tau fibril. Hence, destabilizing the tau fibril and lead to disaggregation of the fibril. The docking models are obtained from online docking server, ClusPro (59–61).

beta-turn of amyloid fibrils [32]. This conformational change may provide the energy required for the disaggregation reaction and allow the proline residues in the beta turns to be more accessible for the PPIase domain. In summary, CyP40 is capable of disaggregating proline containing amyloid fibrils via the proline isomerization mechanism. The disaggregating effects of CyP40 can be observed at the molar ratio of 1:5 (peptide: protein) and maximum disaggregation efficiency of CyP40 can be obtained in an equimolar ratio with the tau filaments. Even though the disaggregation products of CyP40 are not explicitly characterized, it is reported that overexpression of CyP40 can preserve the viability of neurons and rescue tau-induced cognitive decline in mouse models [32]. The disaggregation products of CyP40 are thought to be non-toxic and possibly beneficial to the neuronal cells.

Lipocalin-prostaglandin D synthase (L-PGDS)

Lipocalin-Type Prostaglandin D synthase (L-PGDS) is the second most abundant protein in cerebrospinal fluid with an estimated concentration of 15–30 mg/L [65]. L-PGDS is a multifunctional protein. It plays a role in the inflammatory pathway, catalyzing the isomerization of Prostaglandin H2 (PDH2) to Prostaglandin G2 (PDG2), and scavenging reactive oxygen species (ROS) by acting as a pseudo-peroxidase when in complex with heme. It is proposed that L-PGDS traps the resulting ROS by directing it to a secondary substrate. Thus, reducing the amount of ROS in the environment [66,67]. The deep calyx of L-PGDS allows L-PGDS to accommodate different types of ligands or targets [68]. As a result, L-PGDS is also known to be a lipophilic carrier for extracellular lipophilic ligands [68,69]. Furthermore, L-PGDS has been proposed as a drug delivery system for poorly soluble drugs such as Diazepam and anti-cancer drugs [70,71]. However, the interaction between L-PGDS and hydrophobic drugs needs to be taken into consideration as it is shown that these drugs might interfere with the neuroprotective functions of L-PGDS due to overlapping binding sites of the different ligands [72].

L-PGDS is a dual function Aβ chaperone where it is capable of inhibiting Aβ aggregation [73] and disaggregating preformed Aβ fibrils [23]. L-PGDS has a high affinity for Aβ fibrils where the dissociation constant is in the nanomolar range [73]. Hence, it has been suggested that specific interaction between L-PGDS and Aβ fibrils is important for the disaggregation reaction similar to the previously mentioned ATP-independent disaggregases [23,30]. L-PGDS was found to bind along the axis of the amyloid fibrils which is similar to the HtrA1 protease binding model [23]. Interestingly, L-PGDS does not contain any protease domain or isomerization domain to facilitate the release of the amyloid substrate. Hence, further studies would be necessary to understand the substrate release pathway of L-PGDS and the consequences of specific binding of L-PGDS to amyloid fibrils to aid in the elucidation of the ATP-independent amyloid disaggregation mechanism of L-PGDS. The disaggregation efficiency of L-PGDS is relatively high, as L-PGDS exhibits disaggregase activity even at a molar ratio of 1:10 (protein: peptide) [23]. Moreover, the disaggregation efficiency increases with a higher molar ratio of L-PGDS (Manuscript in preparation). From the analysis of the disaggregation products of L-PGDS, the number of monomeric Aβ species increased after L-PGDS disaggregation [23]. However, the exact species and the toxicity of the resulting products have not been characterized. Therefore, it is possible that the products of L-PGDS disaggregation will result in more toxic oligomers and further investigations are necessary to evaluate the relative toxicity of the disaggregation products of L-PGDS.

Antibodies raised against Aβ

There are various types of antibodies targeting different regions of Aβ peptides. The antibodies of interest are those that target the N terminal [31] and the mid-region of the Aβ peptides [74]. These antibodies bind and clear the preformed amyloid fibrils via the disaggregation of the assembly of Aβ fibrils. One example of the mid-region targeting antibodies is Crenezumab. Crenezumab is a fully-humanized immunoglobulin isotype G4 (IgG4) anti-Aβ monoclonal antibody that is capable of inhibiting Aβ aggregation and promoting Aβ disaggregation at the same time via the obstruction of the sequence required for aggregation and the disruption of the hairpin turn. Crenezumab binds to Aβ fibrils in an extended form, sequestering amino acids in Aβ fibril, important for the salt bridge at beta turns of the fibrils. It has also been proposed that the high-affinity binding between the antibodies and Aβ peptides disrupt the equilibrium/binding between the Aβ monomers resulting in the disaggregation of fibrils [74]. Recently, anti-amyloid antibodies such as Gantenerumab and Crenezumab were found to have multiple binding modes with Aβ fibrils using the online molecular

docking platform HADDOCK [75,76]. The Crenezumab was capable of binding to both the N-terminal and the cross-section of the Aβ fibrils. Furthermore, the contact numbers between each residue of Crenezumab and Aβ was calculated using (Molecular Dynamics) MD simulation. It revealed that the disaggregation mechanism was driven by the hydrophobic interactions between the tyrosine residues of the antibody and the Met35 residues of the Aβ fibril which disrupt the stability of the Aβ fibril [76].

Antibodies raised against the N terminal of Aβ are also capable of disaggregating amyloid fibrils [31]. There are many different types of antibodies specific for different regions of the N terminus of Aβ. In this review, we would only be discussing N terminus targeting antibodies that are capable of disaggregating preformed fibrils. An antibody targeting 4–10 amino acid of amyloid-beta is capable of partially inhibiting amyloid-beta aggregation and disaggregate preformed fibrils at a molar ratio of 1:20–1:50 (antibody: Aβ) [77]. Similarly, an antibody targeting the 1–11 aa of Aβ peptide was discovered to be able to prevent amyloid-beta aggregation and disaggregate preformed fibrils and other oligomeric species into non-filamentous and nontoxic aggregates at a molar ratio of 1:50 (Ab: Aβ) [78]. 6C6, another antibody targeting 1–16 aa of Aβ can modify amyloid fibrils into a nontoxic uncharacterized species of the peptide at a molar ratio of 1:10 (Ab: Aβ) [30]. Lastly, an N terminal targeting antibody (6E10 antigen epitope, Aβ residues 1–17) was found to be able to disaggregate preformed Aβ fibrils with the concentration of 10 μM of peptides with 2 μg in 1 μg of antibody. However, in the process of breaking down the preformed fibrils, this 6E10 antibody not only promotes the formation of toxic oligomers, but also increases the neurotoxicity of the fibrils in both *in-vitro* and *in-vivo* assays [31]. Regarding the disaggregation mechanism by anti-Aβ antibodies, it has been suggested that these N-terminal targeting antibodies bind to Aβ peptides at high affinity, which interferes with the aggregation of the peptides and the equilibrium between the Aβ peptide and amyloid fibrils [30]. It has also been proposed that these antibodies would bind along the length of the fibrils thus disrupting the noncovalent interaction between fibrils and leading to the disaggregation of the fibrils [78]. However, the exact disaggregation mechanism by anti-Aβ antibodies remains unresolved.

Disaggregation via small molecules

Besides protein disaggregases, small molecules such as polyphenols [79–83], tanshinones [84] and brazilin [85] are also capable of disaggregating amyloid fibrils into non-toxic amyloid aggregates. These small compounds are particularly interesting as they are mostly extracted from natural plants. Thus, these compounds have relatively lower cytotoxicity, and they hold great potential as therapeutics for diseases related to protein misfolding and aggregation. The exact molecular mechanism on how these small compounds exert disaggregation effects on the amyloid fibrils remains unknown. Li et al. and Wang et al. suggested that the direct interactions between the natural compounds and β-sheets of the fibrils might be crucial in understanding the disaggregation mechanism where these small compounds could interact with the β-turn of fibrils via hydrogen bond or hydrophobic interaction [84,86]. Using MD simulation, Du et al. showed that brazilin molecules remodel amyloid fibrils by preferentially forming hydrogen bonds with the amyloid fibrils. From their simulations, 3 brazilin molecules directly interact with the chains A and B of the amyloid via 3 hydrogen bonds (Fig. 4A). One of the brazilin molecules forms a hydrogen bond with Asp23 of chain A which disrupts the intermolecular salt bridge Asp23-Lys28 of the amyloid fibril (Fig. 4B). The intermolecular salt bridge between Asp23 and Lys28 of the amyloid fibrils is important for the stability of the fibrils [87,88] (**Review**). Thus, the disruption of the Asp23-Lys28 salt bridge would lead to instability of the hydrogen bonds within the amino acid backbone that supports the fibrils and results in remodeling or disaggregation of the fibrils [85]. Using molecular docking (AutoDock Vina) and MD simulation with the disease-relevant Aβ fibril (PDB code: 2NAO), Windsor et al. futher showed that the amentoflavone-type bioflavonoids disaggregate Aβ fibrils through the preferential binding to the N-terminal of the fibril via π-π interactions [89,90]. The aromatic rings of the compounds bind to the aromatic residues of the fibril and fit inside in the N-terminal pocket to stabilize the biflavonoid-fibril complex. Subsequently, the hydrogen bond formation between the hydroxy groups at R2/R3 of the compound with the peptide backbone resulted in a large decrease in β sheet content of the fibril and altered the structural conformation of the Aβ fibril. Thus, resulting in the disaggregation of the fibril [90]. The mechanism of the amyloid disaggregation by the small compounds is schematically shown in Fig. 8C.

Due to antioxidant abilities of most of the small compound used for disaggregation, other mechanisms such as covalent modification of the amyloid fibrils by the reactive oxidation products of these compounds [86] and chelation of metal ions important for fibril structure maintenance were also hypothesized [91,92]

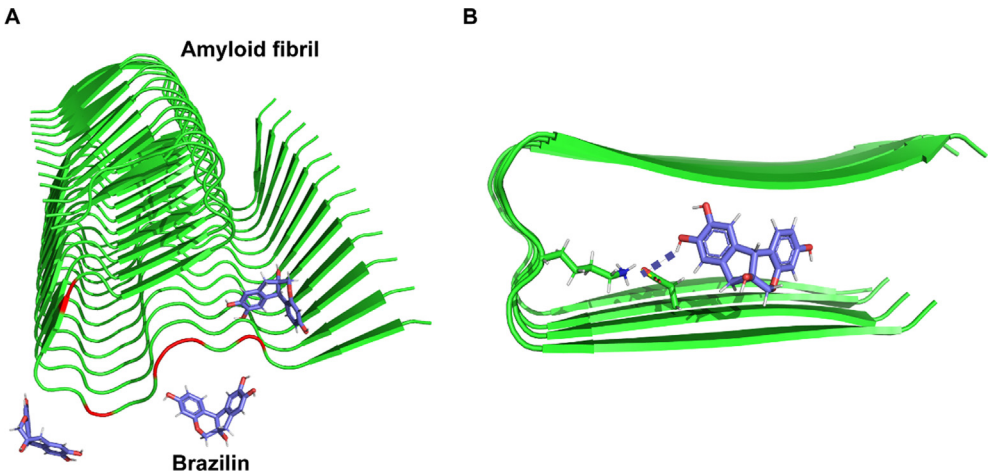

Fig. 4. Interaction between brazilin and amyloid fibril. (A) The interaction between 3 brazilin molecules and amyloid fibril (PDB code: 2MXU). Residues of amyloid fibril in contact with the brazilin molecules are coloured in red. (B) The zoom in of the interaction between one brazilin molecules with the Asp23-Lys28 salt bridge (red line). One of the brazilin molecules has been shown to form a hydrogen bond (blue dashed line) with Asp23. This will disrupt the salt bridge between Asp23-Lys28 which is important of the stability of fibril. Hence, resulting in the disaggregation of fibril. The docking models are obtained from online docking server, ClusPro (59–61).

(**Review**). The tanshiones and brazilin compounds required a molar ratio of 2:1 or 5:1 (compound: Aβ) to show effective disaggregation of the preformed amyloid fibrils while the curcumin only requires a molar ratio of 1:12.5 to show substantial disaggregation effects on the Aβ fibrils [79,84]. All three compounds have been shown to disaggregate amyloid fibrils into unstructured aggregates and reduced the Aβ fibril induced toxicity in cultured neuroblastoma cell lines [79,84,85].

Disaggregation by osmolytes

Osmolytes are small chemical organic compounds that are used by cells in response to stress conditions [93] as they can act as thermo protectants. Osmolytes can be classified as (i) polyhydric alcohols and sugars (polyols), (ii) amino acids and their derivatives and (iii) methyl ammonium compounds [93,94] and act as chemical chaperones to stabilize protein structure and aid in the proper folding of the proteins. Many osmolytes have been identified that can inhibit amyloid fibrillation and there has been little work on the disaggregation properties. The osmolyte betaine has been shown to disaggregate amyloid fibrils derived from GST-GFP, (glutathione-S-transferase-green fluorescent) in a dose-dependent manner (>10 M) into soluble assemblies and was able to partially recover the secondary structure of the protein [95], but it could not effectively restore the tertiary structure of the protein. The disaggregated products could then be accessed by chaperones or proteasome complexes, which can either recycle the functional protein or degrade and clear the proteins, that could not regain function [95]. The authors reported that higher concentrations of betaine can penetrate inside the cells, and the osmolytes activate the other chaperones inside the cells responsible for the disaggregation of amyloid aggregates [96].

The role of osmolytes to activate the innate chaperone network in disaggregation and correct refolding of proteins under stress, in E-coli cells, was reported by Diamant et al. [97]. In *in-vitro* salt-stressed E coli cells, the authors observed a natural accumulation of osmolytes K-glutamate and glycine-betaine (betaine). The osmolytes acted as thermo protectants and also activated the ClpB chaperone associated disaggregation machinery. The osmolytes K-glutamate and betaine were also efficient to counteract the compounds that inhibit the stability of ClpB oligomers thus enhancing the activity of the chaperones and the proper refolding of functional protein [97].

Recently our group also investigated the role of osmolytes to inhibit and disaggregate amyloid fibrils in *TGFBI*-corneal dystrophy. Based on our in-vitro peptide aggregation model (23 amino acid long peptide from the 4th-FAS1 domain of TGFBIp), we reported the effect of osmolytes, betaine, raffinose, sarcosine and taurine on the amyloid disaggregation [37]. The concentration of osmolytes used in the assays was 200 mM and the osmolytes did not show any toxicity to human corneal fibroblasts up to 1 M concentration. Raffinose was found to be most effective to disaggregate the amyloid fibrils with approximately 60% disaggregation efficiency. The second best osmolyte to show disaggregation was taurine with 55% efficiency followed by sarcosine and betaine. The molecular mechanism by which the osmolyte exerted disaggregation properties remains unexplored. In *TGFBI*-corneal dystrophy, both inhibitory and amyloid disaggregation properties are important for treatment. While the disaggregation of amyloid fibrils is used to treat patients with decreased visual acuity, the amyloid inhibitory activity plays an important role in the prevention of amyloid fibril formation in asymptomatic patients and prevent disease recurrence in patients following corneal tissue replacement surgery. Thus, the use of osmolytes in patients with *TGFBI*-corneal dystrophy serves as a simple yet effective treatment option to ameliorate protein aggregation and dissolve preformed protein aggregates [37]. Other types of osmolytes such as trehalose have also been shown to have the amyloid disaggregating ability using Hen Egg-white Lysozyme (HEWL) as a model. The molecular docking analysis between trehalose and FoldAmyloid for HEWL revealed that the osmolyte interacted with the amyloidogenic regions of fibrils through hydrophobic and hydrogen bonding interactions which led to the alteration of the fibril structure thus disaggregating the fibrils [98].

Disaggregation by peptides or amino acids hybrids

The hallmark of amyloid fibril formation is the conversion into beta-sheet structures and the presence of amyloidogenic amino acids in the protein sequence with a high propensity to form β-sheets [99] (**Review**). Furthermore, aromatic residues are crucial in the formation of amyloid fibrils. These residues were found to participate in π-π interactions which are important for the assembly process of amyloid fibril [100]. There have been reports on peptide sequences that have been designed to inhibit β-sheet formation and assembly, by interfering with the amyloid nucleation process or disruption of the π-π interactions within the fibrils. These peptides or amino acid hybrid act as β-sheet breakers that disaggregate the amyloid sheets in the complex amyloid fibrils [101]. A unique dipeptide sequence D-Trp-Aib (D-tryptophan-α aminoisobutyric Acid) was designed to recognize aromatic amino acids and Cα-methylation β-breakage strategy. The dipeptides were added (20-fold excess for IAPP, and calcitonin and 30-fold excess for –synuclein) to preformed amyloid fibrils that had attained fibrillation plateau. The dipeptides were able to disaggregate the amyloid fibrils, but the mechanism of disaggregation has not been discussed in detail by the authors. They hypothesized that the presence of aromatic amino acid in the dipeptide creates a competition for the monomeric proteins or peptides for any possible interactions for the elongation of the oligomers or amyloid fibrils. They postulated that the β-breakage component of the dipeptide could break the b-sheets or disrupt the assembly of the complex b-sheets [101]. Another β-sheet breaker which is a pentapeptide (Lys-Leu-Val-Phe-Phe), commonly known as KLVFF can also disaggregate preformed fibrils through the binding of the hydrophobic regions of Aβ fibrils [102,103].

Amino acids hybrids such as tryptophan-galactosylamine conjugates and naphthoquinone-tryptophan based hybrids were found to disaggregate preformed amyloid fibrils. Both hybrids reduced the toxicity induced by the fibrils where tryptophan-galactosylamine conjugates disaggregate the preformed fibrils into non-toxic intermediates [104,105]. Using MD simulations, the amyloid disaggregating mechanism of naphthoquinone-tryptophan based hybrids were found to be the intercalation between the aromatic rings of naphthoquinone and the Tryptophan residue of the hybrids with the Tyrosine residues of the fibrils. In addition to the intercalation effects, the hybrid molecule formed hydrogen bonds with key residues such as valine which are crucial for β strand formation and stability. These interactions disrupted the original π-π stacking in the fibrils and disruption of the inter-peptide hydrogen bonds within the fibrils. Thus, resulting in fibril disaggregation [105],

Disaggregation by nanoparticles and polymers

Nanoparticles are defined as particle matters with the size of 1–100 nm in at least one dimension of measurement [106,107] (**Review**). They have attracted many interests as one of the most promising therapeutic and diagnostic agents due to their small size [108], ability to penetrate the blood-brain barrier [109], rapid clearance [110] and high flexibility in terms of the ease of modification [106]. These nanoparticles can be applied in the field of

amyloid fibril disaggregation in two different ways. Firstly, the nanoparticles can act as the fibril disaggregating agents. Graphene quantum dots (GQD) which have low cytotoxicity and high biocompatibility levels, has been found to directly interact with preformed α-synuclein fibrils via charge-charge interactions between the N-terminal region of the fibrils and the carboxy groups of GQD. The detailed mechanism of the disaggregation was examined using molecular dynamics simulation to calculate the time-dependent secondary structure plot using the dictionary of secondary structure (DSSP) algorithm and the interaction energies of the fibril against time. It was revealed that the disaggregation action of GQD was mainly driven by hydrophobic interactions between the GQD's basal plane and valine residues of α-synuclein fibrils followed by structural changes of the β sheet component in the α-synuclein fibrils [111]. Other types of nanoparticles such as dendrimers [112], nanotubes [108] and chitosan-based nanoparticles [113] were also found to be capable of disaggregating preformed amyloid fibrils. The second application of nanoparticles is the conjugation of these particles to other disaggregating agents to increase their solubility or serves as an imaging tool for *in vivo* studies. Curcumin has been established as an amyloid fibril disaggregating agent where it was found to disaggregate tau filaments [114] and preformed Aβ fibrils [79,115]. However, due to its hydrophobic properties, curcumin is insoluble in water and has low bioavailability which restricts its therapeutic application. The conjugation of curcumin with gold nanoparticle not only increases the solubility of curcumin, but it also enhances the disaggregating ability of curcumin to dissolve amyloid fibrils [116]. Next, conjugation of amyloid disaggregating chaperone-like L-PGDS with nanoparticles such as Fe_3O_4 nanoparticles in the different coating was found to aid in the visualization and imaging of amyloids in the brain which allows early diagnosis and treatment of the amyloid-related disease [117]. Another branch of nanomedicine is polymer therapeutics. The conjugated polymer such as conjugated polymer-core thermoresponsive micelles (CPMs) was found to disaggregate preformed Aβ fibrils. Interestingly, these CPMs disaggregate fibrils via reactive oxygen species (ROS) generation after white light irradiation. Subsequently, the ROS formed disaggregate the Aβ fibrils into α helical structures [118]. Moreover, the polymer-peptide conjugate was reported to disaggregate Aβ fibrils via direct interaction with the fibrils and the rate disaggregation was discovered to be linked to the molecular weight of the polymer [119].

Computational methods in understanding the disaggregation mechanisms:

In many cases where direct experimental and structural methods are not possible to study the methods of disaggregation due to inherent practical difficulties, computational methods and simulations become increasingly important and ideal tool to understand the molecular mechanism and interactions. Numerous studies have utilized and reported the combination of molecular docking and molecular dynamics (MD) simulations to probe the molecular mechanism of fibril disaggregation. The purpose of molecular docking is to examine the binding mode between the amyloid fibrils and the disaggregating agent while the MD simulation aids in the detailed investigation of the detailed molecular interactions and the possible disaggregation mechanisms. These computational methods demonstrated that the direct interaction between disaggregating agents and amyloid fibrils which can either lead to instability within the fibril structure [76,85,90], the conversion of the β sheet content within the fibril [98,120] or the direct insertion into the cavity of fibrils to disrupt fibril structure leading to fibril disaggregation [121]. The computational methods have the greatest advantage as they serve to explain the dynamics and molecular mechanism of amyloid disaggregation at an atomic level [122] (**Review**).

Data collected from MD simulation can also be utilized in drug design specifically targeted for fibril disaggregation. For example, residues important for the stability of the amyloid fibril can be examined using standard MD and steered molecular dynamics simulations (SMD). Lemkul et al. used in-silico mutation and MD simulations on the core of the Aβ 42 amyloid fibril model to show that a definite level of hydration around the Asp23-Lys28 salt bridge is paramount to the stability of the fibril and increase solvation in that region resulted in instability of the fibril [123]. Using SMD, the authors discovered that most of the peptides disassociated from the protofibril when the interpeptide backbone hydrogen bonding between Gly33 and Met 25 was broken [123]. Similar computational methods were used by Dutta et al. on the solution NMR structure of Aβ protofibril L17-A42 (PDB ID: 2BEG) and hypothesized that the aromatic residues and hydrophobic residues within the beta-strands of fibrils were also critical for packing the amyloid core and thus provide stability of the fibrils [124]. SMD involves the determination of the breaking threshold of peptide disassociation from the protofibrils or in other words, the important interaction required for fibril stability by pulling a peptide away from the protofibrils as a function of time. Kouza et al. utilized the solvent all-atom SMD simulations to determine mechanical stability of the calmodulin N-lobe bound with ER-alpha peptide complex and they proposed that the application of the results from the simulations could be a novel method to test the efficacy of peptide ligands that could block the formation of protein aggregates [125]. The molecular information obtained from these simulations will help in the design of specific amyloid disaggregating agents and scaffolds that can destabilise the protofibril arrangement in the amyloid fibrils. The computational methods also provide molecular-level information on the critical residues that should be targeted in the amyloid fibrils to result in effective fibril destabilization.

Amyloid disaggregase-membrane interactions:

It has been suggested that one of the key components of amyloid toxicity is the disruption of the cell membranes by different assemblies of the amyloid. Pollard et al. have shown that the insertion of Aβ peptides into the lipid membranes formed calcium permeable channels which later resulted in the disruption of the membrane integrity and cell death [126,127]. Moreover, Aβ oligomers and fibrils were also found to interact with lipid bilayers resulting in membrane leakage [128]. Sciacca et al. proposed a two-step mechanism for membrane disruption by Aβ oligomers and fibrils. Firstly, the smaller sized and soluble oligomers interact with the membrane to form channels affecting the permeability of the cell membrane (Fig. 5A). Following that, the extension of the Aβ fibrils on the membrane acts as a detergent and resulted in the fragmentation of the membrane [129] (Fig. 5B). The membrane damaging effect of the amyloid-like fibrils was further highlighted using cryoelectron tomography by Milansi et al. They showed that the β2-microglobulin (β2m) fibril ends extracted lipid from the membrane and resulted in the disruption and breakage of the membrane [130]. On the other hand, interaction with a membrane-like environment can also affect the fibrillization process of Aβ fibrils where the fibrils grown in the presence of membranes exhibited a different fibril morphology than fibrils grown in an aqueous environment [131]. Hence, it can be postulated that the interactions between Aβ and lipid membrane affect each other significantly and this relationship might be applicable to the mode of action of the amyloid disaggregases in a lipophilic environment. Fig. 6.

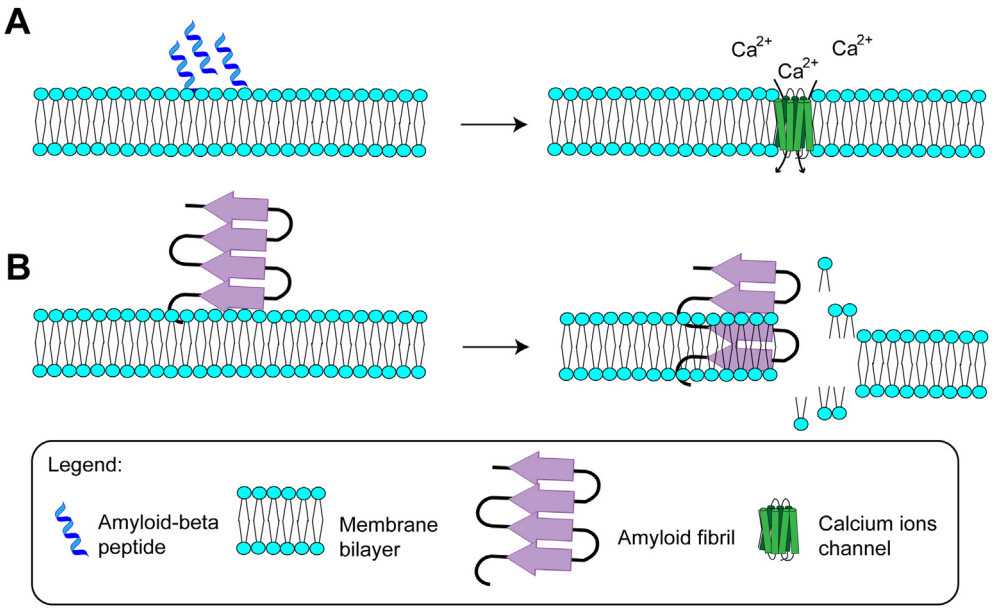

Fig. 5. Interaction between amyloid peptides or fibril with membrane. (A) Aβ peptides interact with the membrane to form a calcium ions channel that disrupt the integrity of the plasma membrane and resulting in cell death. (B) Aβ fibrils interacts and acts as a detergent and results in the fragmentation of the membrane.

Dopamine and L-dopa have been shown to disaggregate preformed amyloid fibrils via a Schiff-base formation with the Lysine residues found within the α-synuclein fibrils [86]. In a later study, Vu et al. went on to investigate the effects of different types of liposome membranes (normal liposomes, daft-forming liposomes, charged liposomes and oxidized liposomes) on the amyloid fibrils derived from peptide Aβ (1–40) and Aβ (1–42) to study the amyloid disaggregation rate of dopamine. They discovered that the addition of liposomes formed by the negatively charged phospholipids 1,2-dimirstoyl-*sn*-glycerol-3-phosphocholine (DMPC) can increase the rate of amyloid fibril disaggregation by dopamine. Subsequently, the authors proposed that the conversion of dopamine into its quinone form under a liposomal environment might be the possible mechanism for the enhancement of amyloid disaggregation by dopamine in the presence of these liposome membranes [132]. It has been reported that the quinone-forms of dopamine were the major active species in the fibril disaggregation action of dopamine [86]. Hence, it was suggested that the interaction between the liposome membrane and dopamine removed the proton from the dopamine molecules to promote the formation of the quinone forms. This conversion triggered the amyloid disaggregation ability of dopamine and increased the rate of amyloid disaggregation in Aβ peptides as observed by the authors [132].

Preclinical trials of amyloid disaggregases:

Some of the disaggregating agents and compounds have been successfully tested on preclinical studies and animal models. One classic example is the Hsp104 complex protein that originated from yeast. As a result, almost all *in vivo* studies regarding the complex involves the overexpression of the yeast Hsp104 in transgenic mouse models of protein misfolding related diseases such as PD and AD. These studies showed that an increased expression of the Hsp104 protein was safe in the animal models tested [50 133]. Lo Bianco et al. demonstrated that overexpression of Hsp104 not only decreased the level of phosphorylated α-synuclein but also reduced the degeneration of the dopaminergic neurons induced by α-synuclein in PD [50]. However, the major limitation of the Hsp104 complex in these preclinical studies and its future therapeutic application was the high concentration that was required for the optimal disaggregation of the fibrils. Different types of engineered Hsp104 variants were then designed to address this problem. It was reported that the engineered variants of Hsp104 exhibited higher efficacy when compared to WT Hsp104. The engineered Hsp104 variants required only nanomolar concentration to remodel the SEVI fibrils [134] and they also displayed enhanced disaggregating ability, proteotoxicity suppression and greater reduction of dopaminergic degeneration in the *C. elegans* PD model [135].

For the ATP independent disaggregases, adeno-associated virus serotype 9-CyP40 was injected in the rTg4510 mice to examine the *in vivo* effects of CyP40. Baker et al. demonstrated that the overexpression of CyP40 in these mice models resulted in a large decrease of sarkosyl-insoluble tau and simultaneously increasing the amount of soluble tau. Moreover, CyP40 reduced the amount of pathological relevant tau and rescued tau-induced cognitive deficits. This evidence suggests that the *in vivo* overexpression of CyP40 is likely to disaggregate tau filaments into nontoxic species and improve the cognitive function of the PD mice model [32]. Intraventricular injections of ferritin nanocages conjugated L-PGDS could also target amyloid rich regions of the 3xTg AD mice brain. Interestingly, intranasal administration of the conjugated L-PGDS was capable of crossing the blood-brain barrier and target different regions of the brain such as the hippocampus [117]. This non-invasive administration method used nanoparticle technology to deliver protein disaggregase. This innovative delivery system can be explored further to deliver other protein disaggregases to the central nervous system.

There are many reports on the administration of several synthetic and natural compounds for amyloid disaggregation. The administration or delivery of these compounds is relatively more straightforward as they are much smaller in size as compared to the protein disaggregases and gene overexpression is not required. Most of the small compounds showed very promising results in the preclinical studies [79,136,137] (**Review**). Epigallocatechin-3-gallate (EGCG) is one of the natural polyphenols that is capable of remodelling different types of amyloid fibrils such as Aβ fibrils [138], IAPP fibrils [139] and tau [140]. Intraperitoneal injection of TgAPPsw mice (a transgenic mouse model of AD) with EGCG (20 mg/kg) resulted in the decreased amount of insoluble Aβ found

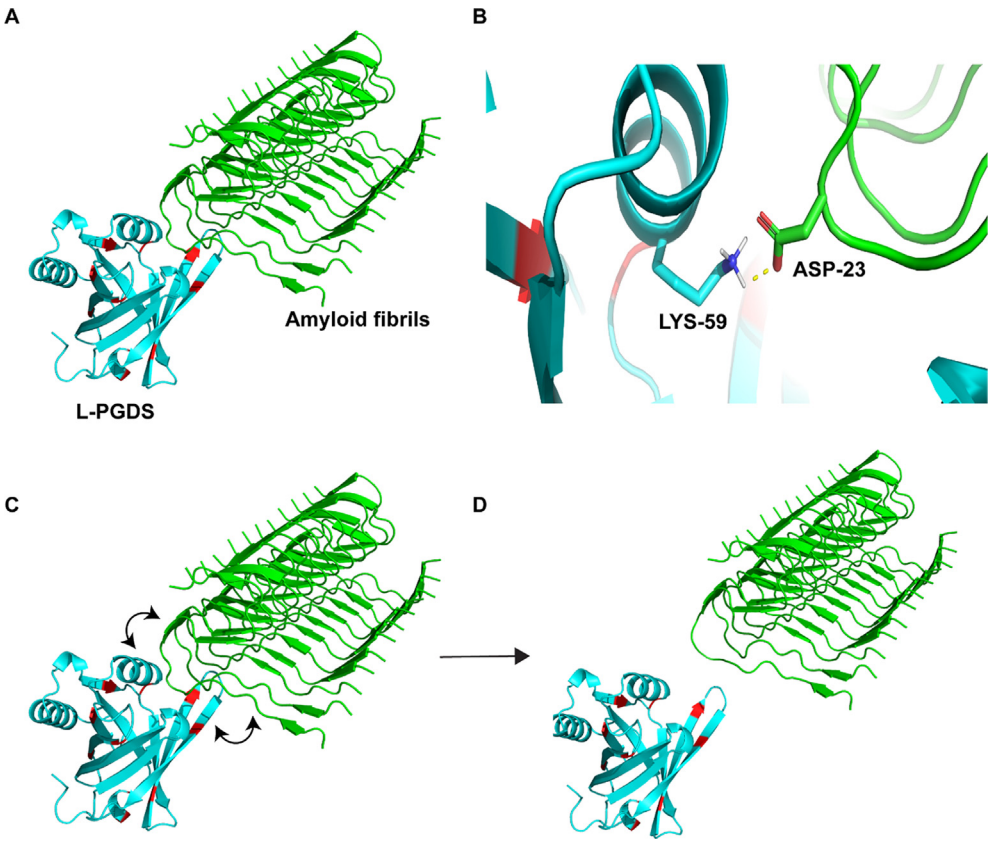

Fig. 6. Interaction between L-PGDS and amyloid fibril and possible amyloid disaggregation mechanisms for L-PGDS. (A) Predicted interaction between L-PGDS (PDB code: 4IMN) and amyloid fibril (PDB code: 2MXU) using online protein–protein docking platform, ClusPro. Residues in L-PGDS known to interact with amyloid peptides are highlighted in red. (B) The zoom in of the possible hydrogen bond between LYS59 of L-PGDS and ASP23 of amyloid fibril obtained from the docking model. This further support our claim where L-PGDS might work in a similar way as the small molecule by forming hydrogen bond with ASP23 of the amyloid fibril, thus disrupting the salt bridge between ASP23 and LYS28 of the amyloid fibril which important for fibril stability. Hence, disaggregating the amyloid fibril. (C) The proposed entropic pulling model which is adapted from HSP70 amyloid disaggregation mechanism where the attempt to "regain" freedom after the binding event will result in a large directional force which in turn will lead to the disaggregation of the fibril. The docking models are obtained from online docking server, ClusPro (59–61).

in the brain and reduction of cerebral amyloidosis [141]. Rezai-Zadeh et al. orally administered EGCG in drinking water (50 mg/ml) of the TgAPPsw mice model and they also observed a reduction of Aβ deposition in the brain [142]. Subsequently, Smith et al. designed nanolipidic EGCG particles to improve the oral bioavailability of the EGCG compound. The Sprague Dawley rats used in this study were fed with 100 mg/kg body weight of EGCG and blood samples were collected at different time points. It was found that NanoEGCG had a Cmax of 704.67 and relative bioavailability of 2.5 as compared to the control EGCG which was a Cmax of 116.57 and relative bioavailability of 1 [143].

The promising results of different disaggregation agents in preclinical studies as discussed earlier have led to the successful application of disaggregation agents into the various stage of clinical trials. Small compounds such as the EGCG has reached phase 2 of the clinical trial which aims to investigate the effects of EGCG in the early stage of AD [144]. Anti-Aβ antibodies such as Crenezumab have reached phase 2 (ABBY and BLAZE) [145,146] and phase 1b of a clinical trial [147] which involved the investigation of the safety and efficacy of Crenezumab in mild to moderate AD patients and safety and tolerability of Crenezumab in preparation for phase 3 clinical studies. These clinical studies highlight the relevance of other disaggregating agents that are discussed in this review where they might also serve as interesting drug candidates in future clinical trials on different types of neurodegenerative disorders.

Discussion

In this review, we have discussed the two different types of disaggregation machinery: the chaperone protein amyloid disaggregation and the amyloid disaggregation by small molecules. The chaperone protein disaggregases can be further categorized into ATP-dependent heat shock proteins and ATP-independent endogenous chaperones. The mechanism of ATP-dependent amyloid disaggregation has been intensively studied whereas the general and detailed amyloid disaggregation of ATP-independent chaperones mechanism remains inconclusive. For Hsp104 and Hsp70 proteins, ATP hydrolysis coupled with conformational change is the main driving force of the amyloid disaggregation process, where it is important for both substrate affinity of the Hsp70 protein and the coordinated movement of the domains which allows the subsequent translocation of the polypeptide through the central channel of the Hsp104 complex. Different force-generating models such as the power stroke model, the Brownian ratchet model and the entropic pulling model were also discussed to explain the source of energy used during Hsp chaperone amyloid disaggregation. On the other hand, the high substrate affinities and the activation of the disaggregation machinery in ATP-independent disaggregases such as HtrA1, CyP40, L-PGDS, antibodies and small molecules are achieved through specific binding to the amyloid substrates. Thus, eliminating ATP requirement during the amyloid

Table 2
Summary of the different amyloid disaggregases.

	Mechanism	Toxicity of disaggregase	Working ratio (chaperone/compound: peptide)	Product toxicity	References
Hsp104, Hsp110, Hsp70 and Hsp40 system	Substrate translocation through the central pore of Hsp104 mediated by ATP induced conformational change, the force can be generated could be explained by power stroke, Brownian rachet or entropic pulling model.	Well-tolerated in mammalian cell cultures and animal models.	20:1	Non-toxic native peptides	[1,20,36,37,38,39,40,41]
HtrA1	The binding of the fibril by the PDZ domain results in the proteolytic cleavage of the fibril by the protease domain of HtrA1.	Endogenous chaperone	1:1 (equimolar)	Uncharacterized	(2)
CyP40	Proline residue isomerization leads to fibril disaggregation	Endogenous chaperone	1:5 and 1:1 for maximum solubilizing effect (22)	Uncharacterized	(22)
L-PGDS	Unknown	Endogenous chaperone	1:10, increasing efficiency with increasing concentration	Uncharacterized	(3)
Anti Aβ antibodies	MD simulations showed that the disaggregation mechanism of Crenezumab is driven by hydrophobic interaction between the aromatic residues of Crenezumab and Met35 residue of Aβ fibrils.	Crenezumab: A fully humanized antibody N-terminal targeting antibodies: Well-tolerated in mice models.	Antibodies against residues 4–10 1:20–1:50 Antibody against residues 1–11 1:50 Antibody against residues 1–16 (6C6) 1:10 Antibody against residues 1–17 (6E10) 10 μM of peptides with 2 μg in 1 μg of antibody	Resulting products include non-filamentous and nontoxic species of peptides except for antibody 6E10 where toxic oligomers were produced.	[4,42,7,5,6,43,44]
Small molecules	MD simulations showed that the disaggregation mechanism is mostly driven by the direct interaction (hydrogen bonding) with the residues within the fibrils.	Polyphenols are extracted from natural plants, low cytotoxicity	Brazilin and Curcumin 2:1 and 5:1 Tanshinones 1:12.5	Short and unstructured aggregates which reduced the Aβ induced toxicity in neuroblastoma cell lines.	[45–47,8,9,32,48]
Osmolytes	Unknown	Relatively non-toxic, widely used to stabilize and facilitate protein folding.	>10 M betaine 200 mM betaine, raffinose, sarcosine and taurine	Uncharacterized	[35,49]
Short peptides	Amyloid disaggregation is driven by intercalation between aromatic rings and direct interaction with the residues within the fibrils.	Non-toxic to mammalian cell lines	20-fold excess for IAPP, and calcitonin and 30-fold excess for –synuclein Tau: NQTrp/Cl-NQTrp (5:1, 1:1, 1:5)	Reduced the toxicity of preformed fibrils. Amino acids hybrids: disaggregate fibrils into non-toxic intermediates.	[17,26,33,50]
Nanoparticle or polymer	Disaggregation is driven by hydrophobic interaction followed by structural alteration of the fibrils.	Some of the nanoparticles might be toxic and the toxicity depends on the size and chemical composition of the nanoparticles. Polymer: Showed no toxicity in mice models.	Graphene quantum dots (GQD): 1:1 (GQD:fibrils) 6.7-fold molar excess for Polymer-doxycycline conjugates (PDC)	GDQ: Production of high populations of monomers and protect against degeneration of dopamine neurons. PDC: Uncharacterized	[27,51,52]

disaggregation process [22,23,32]. However, the detailed mechanism of how this specific binding ultimately leads to the disaggregation of amyloid remains unknown. After reviewing different types of disaggregation complexes and their proposed amyloid disaggregation mechanism, we would like to propose two possible mechanisms for the disaggregation mechanism by ATP-independent chaperone. Amongst the different types of ATP-independent chaperone discussed, little is known about the disaggregation mechanism of L-PGDS. Thus, L-PGDS would be used as a model to facilitate the following discussion of the proposed mechanisms.

Similar to the small molecules with disaggregating effects, specific binding of the ATP-independent protein disaggregases e.g., L-PGDS to amyloid substrates might also introduce perturbation to the native hydrogen bond network important for the stability of the amyloid fibrils. Recently, a cryo-EM structure of Aβ42 fibrils has shown that the structure of the amyloid fibrils is stabilized by salt bridges formed between the Asp and Lys residues found in the β-turn region of the fibrils [88,148]. Small molecules such as Brazilin form hydrogen bonds with the Asp23 residue of the amyloid fibril which is involved in the Asp23-Lys28 salt bridge important for fibril stability (Fig. 4) [85]. Furthermore, this hypothesis is supported by the fact that the specific sequence recognized by ATP-independent disaggregases seems to correspond to the β-turns of the amyloid fibrils [23][32] (Figs. 2-5). Nuclear magnetic resonance (NMR) data has shown that L-PGDS preferentially

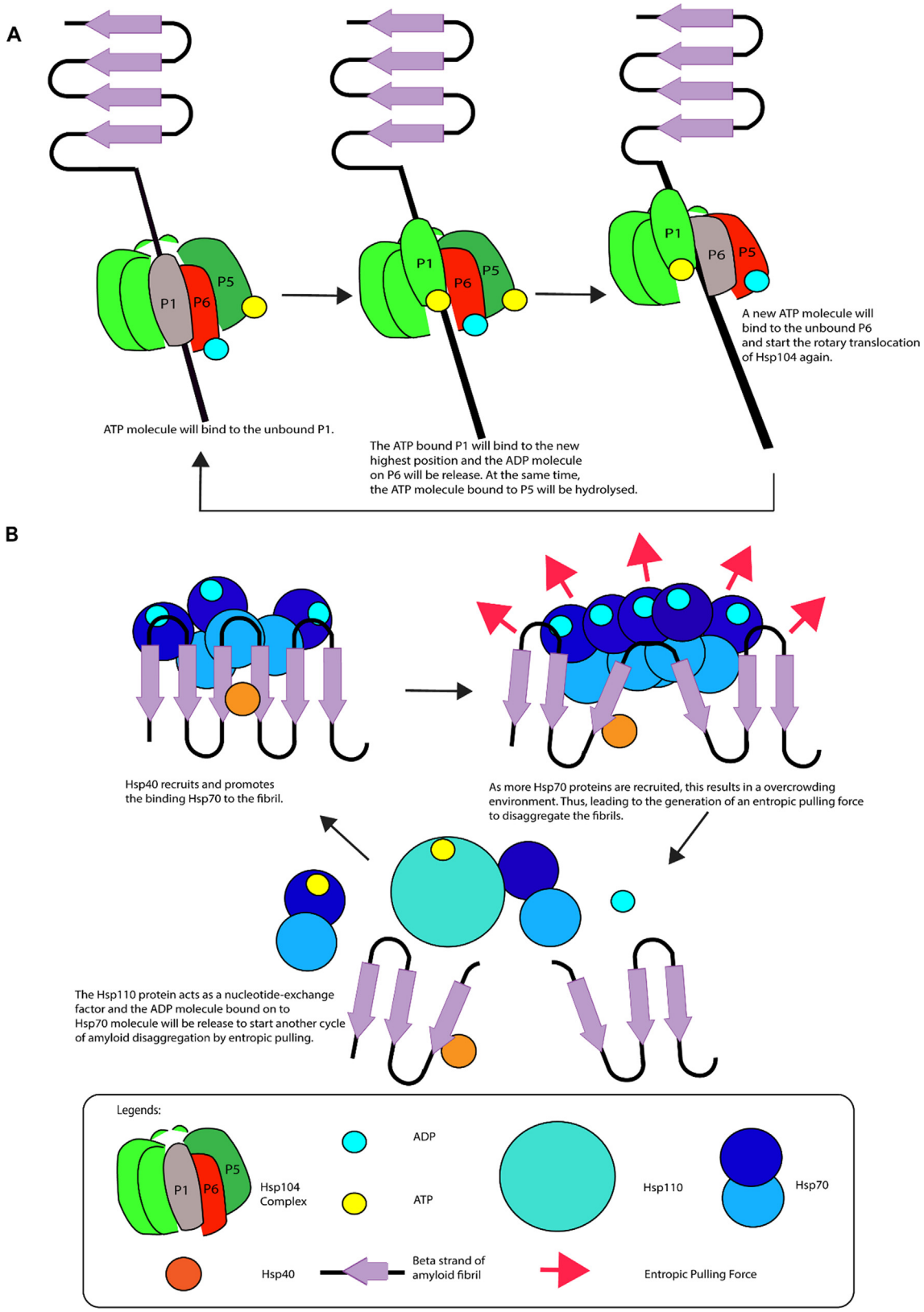

Fig. 7. The schematic diagram showing the disaggregation mechanism of the ATP dependent disaggregases (A) Hsp104 complex and (B) the Hsp70, Hsp40 and Hsp110 complex.

interacts with residues located in the similar regions of Aβ40 peptides [23]. Hence, it is likely that these disaggregases will interact and disrupt the hydrogen bond within the β turns, leading to the disaggregation of the amyloid fibrils. Our docking model of L-PGDS and amyloid fibril (Fig. 5A-B), it shows that the LYS59 of L-PGDS is capable of forming a hydrogen bond with ASP23 in the

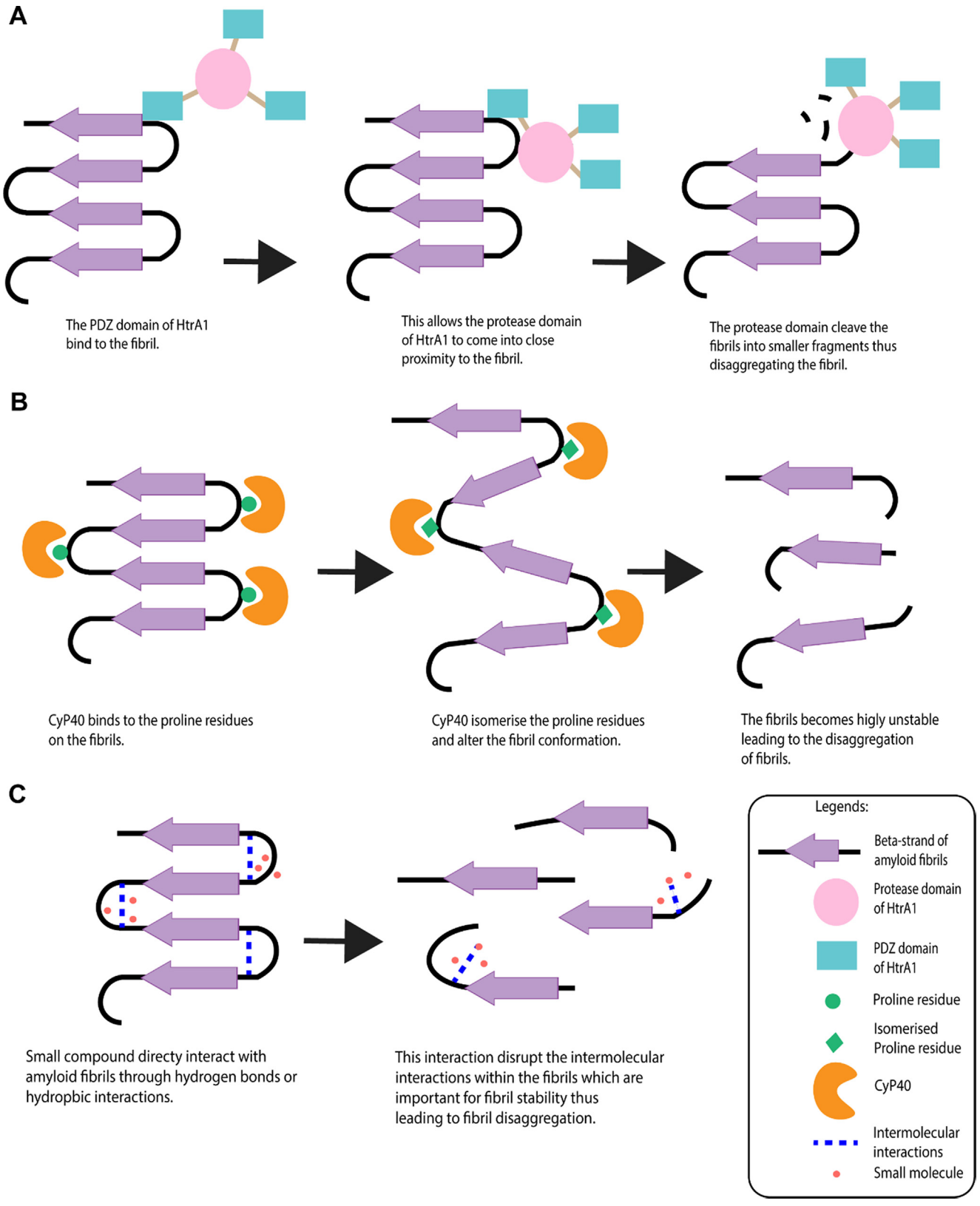

Fig. 8. The schematic diagram showing the disaggregation mechanism of the ATP independent disaggregases (A) HtrA1 protease, (B) CyP40 and (C) small compounds.

amyloid fibril [149–151]. This further highlights the possibility that ATP-independent disaggregases such as L-PGDS disaggregate amyloid fibrils through the formation of hydrogen bonds with the residues involved in salt bridge formation required for stable fibril formation. Furthermore, this helps to explain why these ATP-independent disaggregases appear to be a broad spectrum disaggregase in terms of amyloid targets as most of them recognize and interact with a certain common feature of the amyloid targets.

We propose that the entropic pulling model might also apply to the ATP-independent disaggregation mechanism [57,60] The input of energy in this hypothesis is generated from the attempt to regain entropy after substrate binding. The specific binding of the protein disaggregase helped to anchor the protein to the amyloid targets. However, the binding action restricts the movement of the protein disaggregases. To regain its degree of freedom and entropy before the binding event, a large directional force will be generated and results in the pulling action of the protein away from the amyloid target. Hence, the individual protofibrils will be separated from the amyloid fibrils. The continuous binding and pulling away of the amyloid fibrils will lead to the disaggregation of the amyloid aggregates. This hypothesis can be supported by the observation that most ATP-independent disaggregases can remain functional at a low molar ratio (Table 2) as the pulling action is a repeated cycle of binding and release of amyloid substrates.

Further studies should focus on the elucidation of ATP-independent disaggregation mechanism as it would be necessary to understand how ATP-independent disaggregation works before introducing these amyloid chaperones as a potential treatment for protein misfolding diseases such as AD and *TGFBI* associated corneal dystrophy. For instance, more experiments can be done to validate our proposed hypotheses mentioned earlier. Structural studies such as solid-state NMR (ssNMR) and cryo-EM experiments could be conducted to show the corresponding global structural change in the amyloid fibrils following the addition of ATP-independent chaperones [148,152,153]. In addition, the molecular mechanism in terms of the alteration in the hydrogen bond pattern within the amyloid fibril and chaperone complex can be reflected through the change in the chemical shift of the corresponding residues involved during amyloid fibril disaggregation [154]. Subsequently, the chemical shift data obtained via solid-state NMR spectra can be compared with simulated chemical shift data obtained from the quantum calculation of the docking model generated to verify the accuracy of the docking model [155,156]. Moreover, the detailed structural analysis will provide a substrate release mechanism such as inducing a conformational change in the substrate to reduce the substrate binding affinity of the chaperone.

All these amyloid disaggregating systems discussed in this review show great potential as a treatment for amyloid relating disease. However, each system has its strengths and weaknesses. The relative size of the Hsp104 protein complex and their yeast origin might pose a challenge in their therapeutic application in patients suffering from neurodegenerative diseases (Fig. 1). The selectivity of CyP40 for proline containing amyloid targets, the relative size of the HtrA1 trimer complex and the external origin of small compounds and antibodies might also hinder the discovery of a broad, safe and efficient treatment for all amyloid-related diseases [22,32]. Most importantly, the toxicity of the resulting products from the disaggregation are a major challenge towards the application of these disaggregases in disease treatment [63]. Disaggregation of amyloid fibrils into more toxic oligomers might exacerbate the effects of the aggregated peptides/proteins [31]. It is therefore important to closely examine and characterize the product toxicity of these disaggregases before their implementation as a possible treatment for amyloidosis. Even though it is impossible to test out different types of disaggregases in AD patients, it might be plausible to implement different disaggregase systems in *TGFBI*-associated patients as the disaggregases can be applied topically and the outcome measurements and effects of treatment can easily be monitored in corneal tissue of patients.

Despite the shortcomings of these disaggregases, amyloid disaggregation by chaperone protein and small compounds are still highly valued in their potential in amyloid associated disease treatment. However, more experiments such as the study of detailed amyloid disaggregation mechanisms and the evaluation of the toxicity of disaggregation products are necessary to pave the way for the application of these disaggregases as an effective treatment for amyloid-related diseases.

Conclusions

In summary, we have discussed different disaggregase systems currently available, their proposed disaggregation mechanism and their potential utility as treatments for several protein aggregation disorders. The ATP independent disaggegases utilize the energy obtain from ATP hydrolysis to forcefully translocate or pull the fibrils away from the aggregates. On the other hand, the ATP independent disaggregases such as the small molecules directly interacts with the fibrils and disrupt the important interactions required for fibril stability to disaggregate the fibrils. After reviewing different systems, we proposed two different hypotheses for ATP independent disaggregation using L-PGDS as a model for discussion. There are still many unknowns in the success of the therapeutic utility of disaggregases as a potential treatment option for amyloid accumulation in diseases. Given their great potential in these currently untreatable diseases, it is thus worthwhile to have a detailed understanding of the reported disaggregases and their corresponding amyloid disaggregation mechanisms.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without due reservation, to any qualified researchers.

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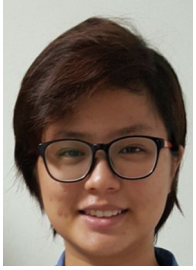

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