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## Screening methods for identifying pharmacological chaperones

Min Hyeon Shin and Hyun-Suk Lim\*

Protein folding is crucial for most proteins to achieve their correct three-dimensional conformations and function properly. Defects in protein folding frequently caused by mutations lead to a range of protein misfolding diseases, including Alzheimer's disease, Parkinson's disease, cystic fibrosis, amyloidosis, Gaucher disease, etc. One approach to treat these devastating diseases would be to use pharmacological chaperones, which are small-molecules that bind to and stabilize misfolded proteins, thereby correcting their pathogenic misfolding and rescuing their functions. As such, pharmacological chaperone therapy holds great promise for the treatment of numerous protein misfolding diseases. In this review, we highlight recent strategies for identifying small-molecules that act as pharmacological chaperones and revert protein misfolding diseases, with a focus on reports within the last five years.

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### Introduction

Protein folding is crucial for most proteins to attain their correct three-dimensional structures and function properly. If proteins are folded unsuccessfully, they are detected by intracellular quality-control systems (QCS) and subjected to proteasome-mediated degradation. Defects in protein folding are frequently caused by mutations and involved in numerous pathologies known as conformational diseases.<sup>1–5</sup> For example, protein misfolding diseases caused by loss-of-function mutations, such as cystic fibrosis,  $\alpha$ 1-antitrypsin, and lysosomal storage diseases, can induce excessive degradation of unstable misfolded proteins, leading to low activity of the proteins. In contrast, in protein misfolding diseases associated with gain-of-toxicity mutations, high levels of misfolded proteins are produced. These unstable aggregation-prone proteins form insoluble aggregates and amyloid fibrils, which are accumulated in tissues and hamper normal functions. These diseases include many neurodegenerative diseases such as Alzheimer's disease (AD), amyloidosis, familial amyloid polyneuropathy (FAP), and prion disease.

Given that errors in protein folding are known to cause many conformational diseases, a great deal of effort has been made to develop therapies. For instance, one approach used to correct defects in protein function is enzyme replacement therapy, in which correctly folded enzymes or proteins are added intravenously to replace the misfolded proteins.<sup>6</sup> Serving a similar purpose, gene therapy could hold potentially high clinical promise, but it has safety and delivery issues.<sup>7</sup> An alternative promising strategy is to use pharmacological chaperones (also known as pharmacoperones),

which are small molecules that can bind to and stabilize proteins against proteolytic degradation and thermal denaturation.<sup>1,3–5</sup> Upon binding to target proteins, pharmacological chaperone molecules can stabilize the native conformation of correctly folded proteins, preventing their misfolding. In addition, some pharmacological chaperones can promote the native folding conformation of disease-related mutant proteins, correcting their pathogenic misfolding and rescuing their functions. Thus, pharmacological chaperones have great potential as therapeutic interventions for the treatment of a number of protein misfolding diseases. Indeed, two such pharmacological chaperone molecules have been approved by the FDA and used clinically,<sup>8</sup> and several molecules are currently being evaluated in clinical trials.<sup>2,9</sup> Despite the successful discovery of several pharmacological chaperones, the development of such molecules for the treatment of protein misfolding diseases is a daunting task, mainly because of the lack of well-defined binding sites in many pathogenic proteins and the unclear mechanism of action of pharmacological chaperones. When a three-dimensional structure for a target protein (e.g., high-resolution X-ray crystal structure) is available, ligands as potential pharmacological chaperones can be rationally designed. For example, Kelley and colleagues developed pharmacological chaperones targeting a tetrameric conformation of transthyretin (TTR) protein.<sup>9–11</sup> These compounds have been identified by a structure-based design approach based on a crystal structure of TTR. These small-molecules were shown to bind to and stabilize the native folded tetrameric TTR, blocking dissociation of the tetramer to a misfolded monomer and thereby preventing amyloidogenesis and failure of the nervous system. In many cases, however, there is no structural information on the target proteins (either correctly folded proteins or misfolded proteins) and the interactions

Departments of Chemistry and Advanced Material Science, Pohang University of Science and Technology, Pohang 37676, South Korea. E-mail: hslim@postech.ac.kr

between pharmacological chaperones and proteins are not well characterized. Moreover, pathogenic proteins are often short-lived, making it challenging to obtain structural information using currently available methods such as NMR and crystallography. Despite these challenging issues, several pharmacological chaperones have been successfully identified. In this review, we focus on highlighting recent strategies for identifying small-molecules that act as pharmacological chaperones and revert conformational diseases.

### *In vitro* functional screens

*In vitro* functional assay methods have been successfully used to directly detect the activity of pharmacological chaperones. In particular, differential scanning fluorimetry (DSF) is an inexpensive and convenient screening method used to identify ligands that stabilize target proteins.<sup>12,13</sup> One can measure the thermal denaturation temperature at which a target protein is unfolded in the presence of a fluorescent dye.<sup>12</sup> These dyes are highly fluorescent when binding to a hydrophobic pocket of unfolded proteins, while they are not in the presence of native folded proteins (Fig. 1A). Therefore, a DSF technique can be applied for not only determining the conformational stability of proteins, but also discovering pharmacological chaperones.

Sampson *et al.* used DSF-based high-throughput screening (HTS) to identify pharmacological chaperones targeting cystic fibrosis transmembrane conductance regulator (CFTR) protein whose mutations cause cystic fibrosis, a common autosomal recessive disease.<sup>14</sup> CFTR is a multidomain protein composed of two membrane-spanning domains, two nucleotide-binding domains (NBD1 and NBD2), and a regulatory region. A deletion

mutation in NBD1 (phenylalanine deletion at the 508 position, called F508del) is the most common mutation found in cystic fibrosis and leads to the formation of misfolded CFTR. Thus, F508del-CFTR is unable to translocate to the plasma membrane and stays in the endoplasmic reticulum (ER).<sup>15</sup> The trafficking of F508del-CFTR to the plasma membrane can be corrected by using pharmacological chaperones that directly bind to F508del-CFTR. These molecules promote native folding and thus block proteasomal degradation by avoiding ER quality control.

To identify pharmacological chaperones for F508del-CFTR, DSF-based HTS was employed. Sampson *et al.* screened 224 compounds, which are hit compounds selected from previous screening to identify small-molecule correctors of the F508del-CFTR trafficking defect. From this screening, only one compound (called RDR1) was identified as a hit (Fig. 1B). RDR1 was found to thermally stabilize F508del-CFTR in a dose-dependent manner at concentrations of higher than 5  $\mu$ M. RDR1 did not compete with ATP for stabilizing F508del-CFTR, indicating that it bound to a different site from the ATP-binding site on F508del-CFTR. From further cellular assays and *in vivo* mouse experiments, it was demonstrated that this compound was able to not only correct trafficking of F508del-CFTR, but also potentiate F508del-CFTR function. As a result, RDR1 provides a promising starting point for further development of a new class of cystic fibrosis treatments.

More recently, Makley *et al.* reported DSF-based HTS to discover a pharmacological chaperone for  $\alpha$ -crystallin protein.<sup>16</sup>  $\alpha$ A-Crystallin (cryAA) and  $\alpha$ B-crystallin (cryAB) are molecular chaperone proteins belonging to a family of small heat shock

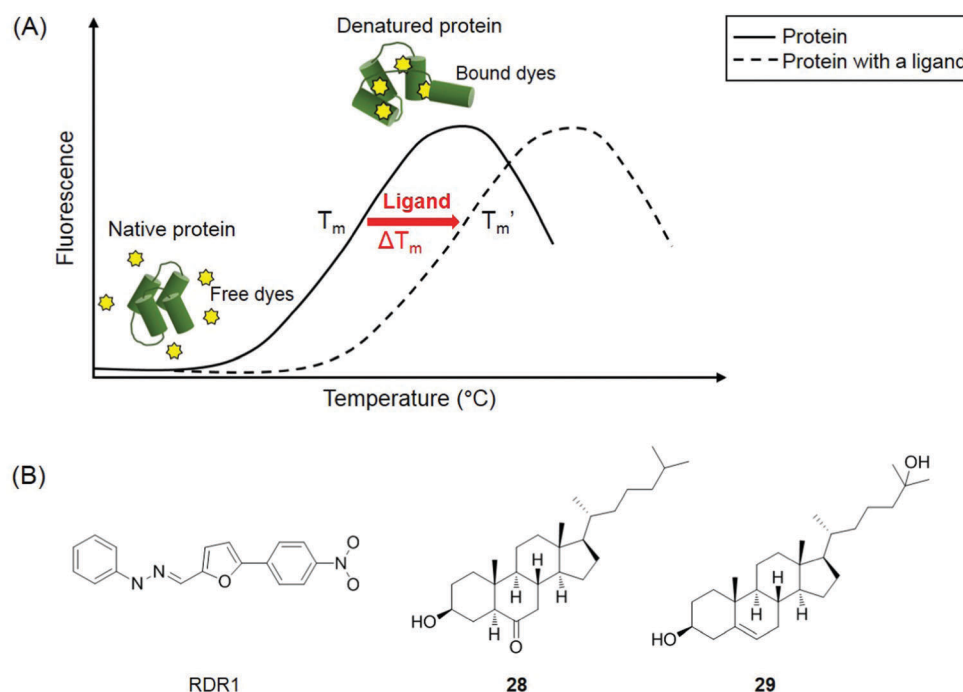


Fig. 1 (A) Ligand-induced changes in protein thermal stability as determined by DSF. (B) Chemical structures of pharmacological chaperones for F508del-CFTR (RDR1) and  $\alpha$ -crystallin protein (compounds **28** and **29**).

proteins. These lens proteins play a critical role in maintaining the solubility of the other lens proteins. Accumulated damage to these proteins or mutations (*e.g.*, R120G mutation in cryAB) lead to their misfolding and aggregation, thereby inducing the formation of amyloid-like fibers. These aggregated crystallin proteins cause a physical barrier to light and thus develop cataracts.<sup>17</sup> Thus, a pharmacological chaperone capable of stabilizing crystallin would restore transparency in cataracts by reversing crystallin aggregation.<sup>18</sup> Makley *et al.* set out to discover small-molecules that bind and stabilize the soluble form of cryAB as pharmacological chaperones. To this end, DSF was used as a HTS method. They screened 2450 compounds from NIH clinical collections and MS2000 to isolate compounds that can decrease the apparent  $T_m$  of R120G cryAB, providing 45 compounds. Further dose-dependence experiments confirmed 32 molecules as hit compounds. Interestingly, they found that 12 out of the 32 compounds have sterol-related structures. By testing these and additional sterol-like molecules, two compounds (28 and 29) showing reduction of the apparent  $T_m$  by  $>2$  °C were selected for further

evaluation (Fig. 1B). *In vitro* binding assays using bio-layer interferometry and subsequent HSQC NMR experiments showed that 29 bound R120G cryAB at the dimer interface ( $K_D = 10.1$   $\mu$ M). Electron microscopy (EM) experiments revealed that 29 at 100  $\mu$ M not only suppresses amyloid formation of R120G cryAB, but also partially reverses amyloid formation. In addition to R120G cryAB, compound 29 was found to have a similar effect on the other mutants in cryAA (*e.g.*, R49C), but not the mutants of structurally unrelated lens proteins (*e.g.*, P23T  $\gamma$ -crystallin), suggesting that compound 29 bound to a specific region of cryAA and cryAB. Compound 29 increased protein solubility and improved lens transparency in mouse model studies. This study provides a new paradigm for the treatment of cataracts, and the DSF method demonstrates its utility in discovering pharmacological chaperones.

Thioflavin T (ThT) dye fluorescence assays are useful for monitoring amyloid fibrillation. ThT, a benzothiazole dye, is able to detect  $\beta$ -amyloid fibril formation by inducing increased fluorescence upon its binding to  $\beta$ -sheets of amyloid fibrils,

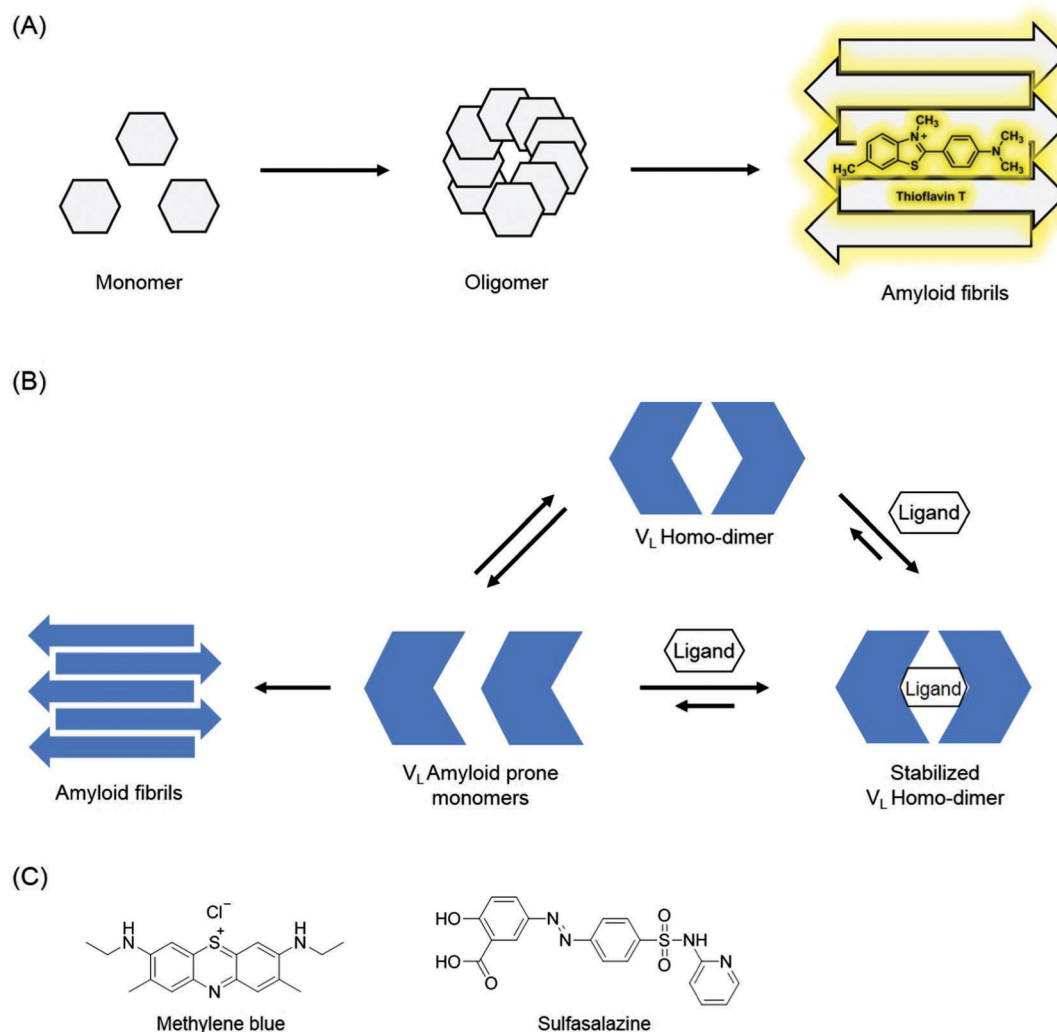


Fig. 2 (A) Thioflavin T (ThT) dye fluorescence assay used to monitor amyloid fibrillation. (B) A proposed mechanism for using ligands to hinder the aggregation of immunoglobulin  $V_L$ s into amyloid fibrils. (C) Chemical structures of pharmacological chaperones that can inhibit  $V_L$  amyloid formation.

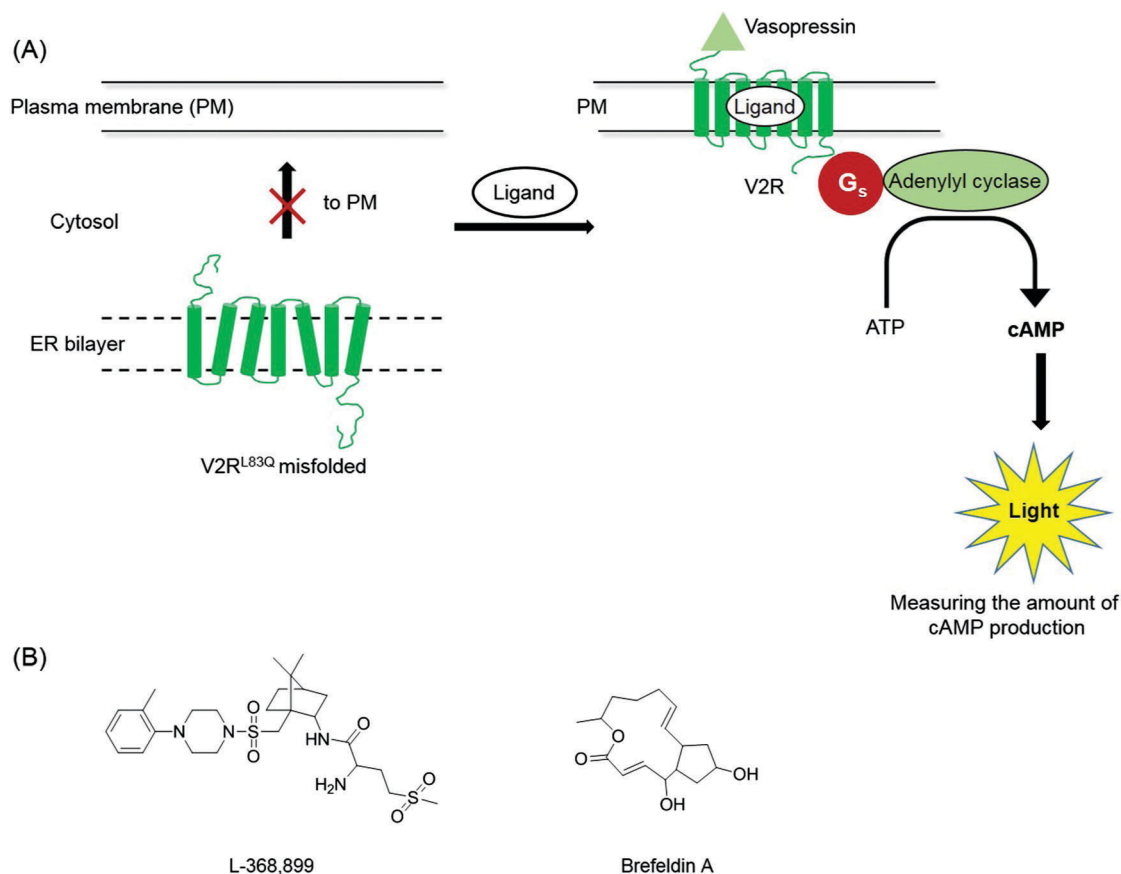
while free ThT in aqueous solutions has only weak fluorescence (Fig. 2A).<sup>19</sup> This is presumably because of the intercalation of ThT into the grooves between solvent-exposed side chains of the amyloid fibril, providing rigidity of the ThT molecule in the excited state. Given its ability to analyze amyloid fibril formation conveniently and quantitatively, ThT fluorescence assays represent one of the most attractive methods to identify ligands that inhibit the formation of amyloid fibrils.<sup>20</sup>

Brushtein *et al.* reported the identification of small molecules that stabilize the Mcg light chain (LC) variable domain ( $V_L$ ) homo-dimer by using ThT fluorescence assays and EM micrographs.<sup>21</sup> Overexpression of monoclonal LCs is associated with systemic amyloidosis. It is known that  $V_L$ s exist as either homodimers or monomers.<sup>22</sup> The monomer is an amyloid-prone form, and some mutations induce dimer dissociation or monomer unfolding, promoting the formation of amyloid fibrils.<sup>23</sup> These amyloid fibrils are deposited in patient tissues such as the heart and kidneys, causing organ failure and death. Thus, small molecules that prevent amyloid fibrillation by stabilizing  $V_L$  homo-dimers could be candidates for the treatment of amyloidosis (Fig. 2B).<sup>24</sup> In order to obtain such molecules, the authors utilized ThT assays along with EM micrographs. Initially, 27 compounds were tested for their ability to inhibit the formation of amyloid fibrils. Four compounds including methylene blue,

Chicago Sky Blue 6B, phenol red, and 8-aniline-1-naphthalene sulfonic acid exhibited weak inhibitory effects. These compounds were used to search for approved biomedical compounds with similar chemical structures and properties from Drug Bank. Finally, they selected sulfasalazine and methylene blue as hit compounds (Fig. 2C), which had  $EC_{50}$  values of 0.5 and 0.1 mM, respectively. Importantly, they demonstrated their binding to Mcg by solving the crystal structures of Mcg in the complex with methylene blue and sulfasalazine, uncovering that these compounds bound at the cavity formed by the two  $V_L$  domains. Based on analytical ultracentrifugation, the two compounds were found to bind  $V_L$  dimers, but not  $V_L$  monomers. Despite their weak activities, this study illustrated that the two ligands may serve as a starting point for the development of effective medications for the treatment of systemic light chain amyloidosis.

### Cell-based screens

In contrast to *in vitro* biochemical assays, cell-based phenotype screens have been employed to obtain pharmacological chaperones. For instance, Conn *et al.* developed a cell-based high-throughput system that allows for the identification of pharmacological chaperones of the vasopressin type 2 receptor (V2R).<sup>4,25</sup> Mutations in G-protein-coupled receptors (GPCR) like V2R often result



**Fig. 3** (A) The principle of cell-based HTS that monitors the rescue of vasopressin 2 receptor (V2R) function by a pharmacological chaperone. (B) Chemical structures of pharmacological chaperones for V2R.

in misfolded proteins, which are detected by the cellular QCS as defective ones.<sup>26</sup> As a result, these unfolded proteins (although they still possess functional activity) are retained in the ER without reaching the plasma membrane. In this regard, pharmacological chaperones may rescue the misfolded proteins and improve the trafficking of the target proteins to the proper site without being disturbed by the QCS. Given that, upon activation in response to vasopressin, V2R stimulates adenylate cyclase *via* G-protein (Gs type)-coupled pathway, converting ATP to cAMP, it would be possible to monitor the levels of suitably folded V2R on the membrane by measuring cAMP production (Fig. 3A). They developed a high-throughput assay designed to identify molecules that increased the amount of cAMP in HeLa cells constitutively expressing a misfolded mutant V2R (V2R<sup>L83Q</sup>) (Fig. 3B).<sup>25</sup> This cell-based screening system represents a convenient and useful tool to discover pharmacological chaperones rescuing the misfolding and mistrafficking of V2R mutants. The same research group also developed a similar cell-based HTS method for identifying pharmacological chaperones of gonadotropin-releasing hormone receptor (GnRHR), a GPCR stimulated by its ligand, GnRH.<sup>27</sup> In this case, they measured Ca<sup>2+</sup> concentration to monitor the levels of folded GnRHR rather than cAMP in the V2R case.

Very recently, a novel pharmacological chaperone attenuating hearing loss has been identified *via* cell-based screening.<sup>28</sup> Patients with Usher syndrome type III (USH3) experience progressive deafness. This is caused by mutations in the CLRN1 gene, which encodes a transmembrane protein that plays an

important role in the development and homeostasis of the inner ear and retina. For example, CLRN1<sup>N48K</sup> is one of the most commonly found forms of the USH3 mutations. This loss-of-function mutation is thought to result in improper folding and instability of the CLRN1 protein, thereby interfering with its translocation to the plasma membrane and causing hearing loss.<sup>29,30</sup> Therefore, one can expect that restoring the CLRN1 function with pharmacological chaperones could treat deafness in USH3 patients by improving the stability of CLRN1<sup>N48K</sup>. The authors used D6 cells expressing human CLRN1<sup>N48K</sup> fused to a hemagglutinin (HA) tag for a cell-based HTS method (Fig. 4A).<sup>28</sup> Using this screening format, ~50 000 compounds were screened for their ability to stabilize the CLRN1<sup>N48K</sup> protein. The stabilizing effect of compounds was determined by analyzing CLRN1<sup>N48K</sup> levels at the membrane with immunofluorescence assays using anti-HA antibody and subsequently secondary antibody conjugated with Cy3. The initially selected 48 compounds from this screen were further tested. Among them, they chose one compound (O03) as a final hit compound (Fig. 4B), which exerted selective stabilizing activity of CLRN1<sup>N48K</sup> with EC<sub>50</sub> of 2 μM, but did not have a significant effect on proteasome-mediated CLRN1<sup>N48K</sup> degradation. Next, they optimized the structure of O03 to improve its potency and physicochemical properties, resulting in the discovery of BF844 (Fig. 4B), which has improved potency (EC<sub>50</sub> of 0.3 μM) as well as metabolic stability. In subsequent experiments, BF844 was shown to mitigate CLRN1<sup>N48K</sup>-associated hearing loss in a mouse model with USH3 without affecting the growth of mice. Although the cellular mechanism of BF844 remains unclear, this

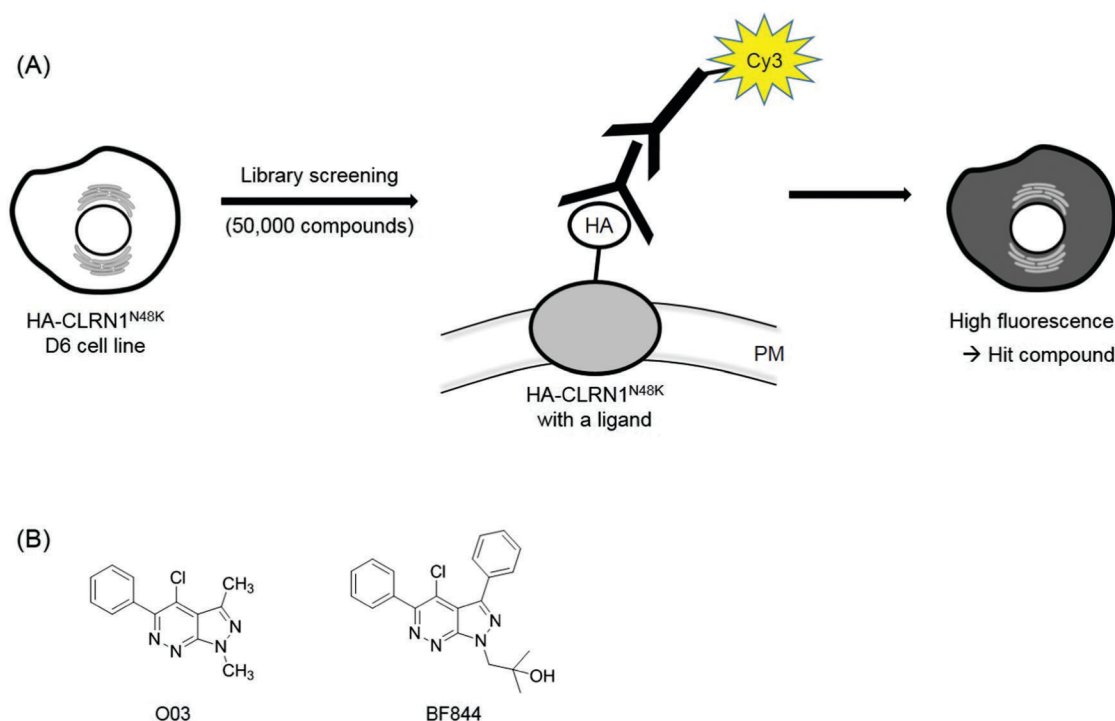


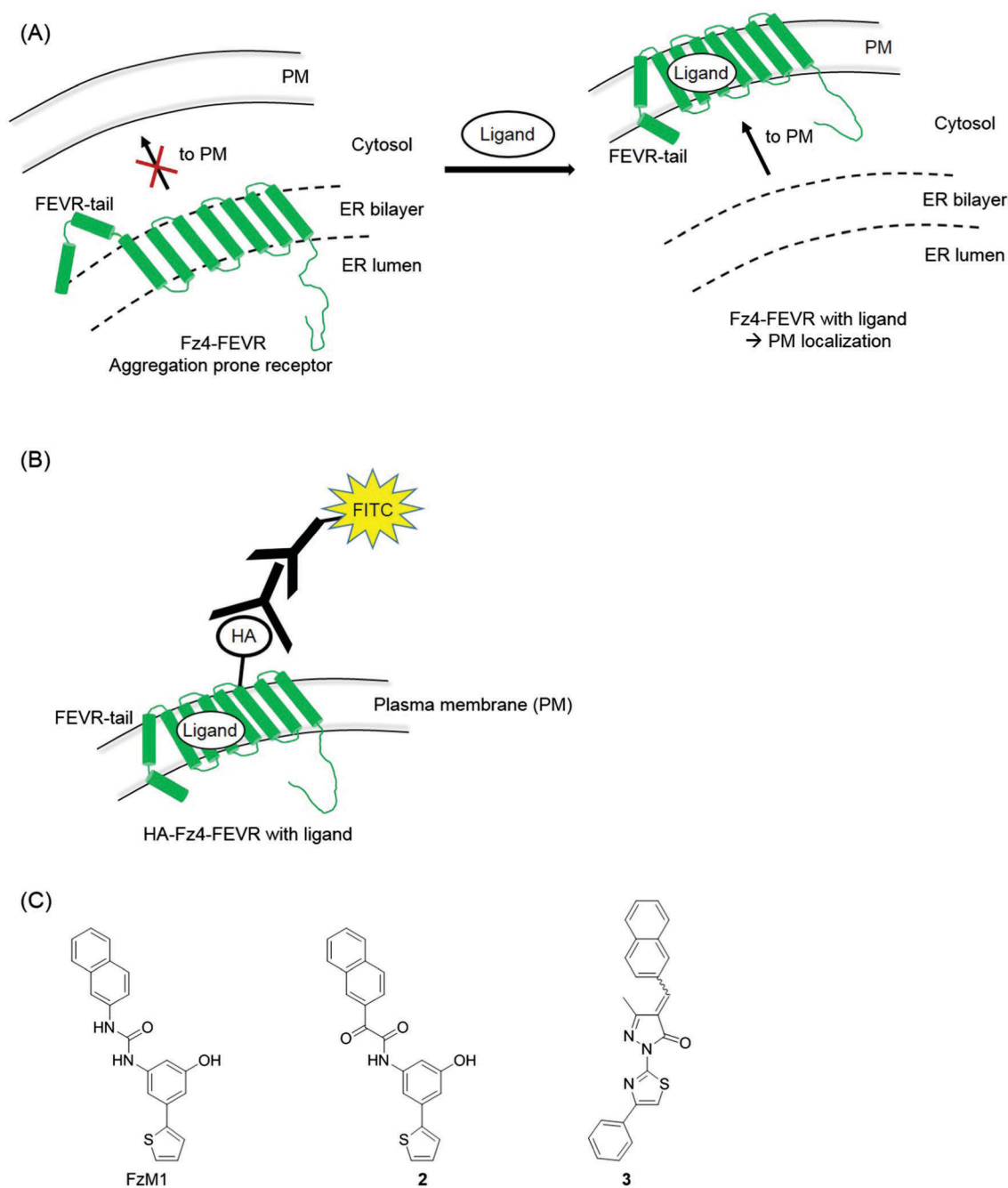
Fig. 4 (A) Illustration for a cell-based screen used to identify pharmacological chaperones that promote the trafficking of CLRN1<sup>N48K</sup> to the plasma membrane as determined by immunofluorescence. (B) Chemical structures of identified pharmacological chaperones for CLRN1<sup>N48K</sup>.



result demonstrates that stabilizing CLRN1 by small-molecules would be a promising and valid strategy to treat hearing loss associated with Usher syndrome.

In addition, an efficient cell-based screening platform has recently been developed to discover a pharmacological chaperone targeting Frizzled4 (Fz4).<sup>31</sup> Fz4 belongs to the Frizzled cell surface receptors, a GPCR class F family. Several mutations in the Fz4 gene are associated with familial exudative vitreoretinopathy (FEVR), which is a rare hereditary disorder causing

progressive vision loss. For example, the frameshift mutation (L501fsX533) in Fz4 induces defects in the Fz4 conformation, preventing its proper folding and localization to the cell plasma membrane.<sup>32</sup> Pharmacological chaperones that bind to Fz4 would rescue Fz4 folding and trafficking to the plasma membrane (Fig. 5A). In order to identify such pharmacological chaperone molecules, the authors developed a cell-based assay using cell lines expressing HA-tagged Fz4-FEVR. They screened a library of small molecules for their ability to rescue HA-Fz4-FEVR



**Fig. 5** (A) Pharmacological chaperones may rescue defects in Fz4-FEVR membrane localization. (B) Illustration of a cell-based assay to detect the improved trafficking of Fz4-FEVR to the plasma membrane by pharmacological chaperones. (C) Chemical structures of pharmacological chaperones for Fz4-FEVR.

trafficking to the plasma membrane (Fig. 5B). The membrane localization of HA-Fz4-FEVR was determined by immunofluorescence, resulting in the discovery of a compound called FzM1 (Fig. 5C). In addition, they found that two structurally related compounds (2 and 3) also showed similar activities, indicating the specificity of FzM1 (Fig. 5C). These compounds recovered the localization of HA-Fz4-FEVR at the plasma membrane in >10% of the cells with EC<sub>50</sub> in the micromolar range. FzM1 was shown to promote proper folding of HA-Fz4-FEVR during its folding process but did not rescue already aggregated ones.

Since Fz4 plays a key role in the Wnt- $\beta$ -catenin signaling pathway and its misregulation is related to tumor progression,<sup>33</sup> they examined whether FzM1 had effects on Wnt- $\beta$ -catenin signaling. FzM1 indeed inhibited Wnt- $\beta$ -catenin signaling by blocking  $\beta$ -catenin nuclear translocation, thereby affecting U87MG glioblastoma cell (a brain tumor cells) growth, migration, and differentiation. Based on this result, FzM1 represents a novel class of inhibitor of the Wnt- $\beta$ -catenin pathway.

### *In silico* screening

As mentioned above, when structural information (e.g., high-resolution X-ray crystal structures) for target proteins is available, a rational structure-based design strategy could be possible to identify ligands that are able to dock into a potential binding site on the protein surface. Moreover, such three-dimensional structures could be utilized for virtual screening, which is a widely used technique in drug discovery to search for small-molecules that are likely to bind to a target protein. This computational technique enables a vast number of molecules to be screened rapidly and inexpensively.

Mecozzi *et al.* reported an *in silico* screening method to find a pharmacological chaperone that can stabilize the retromer protein complex.<sup>34</sup> The retromer complex is composed of three core proteins, Vps35–Vps29–Vps26. This protein complex is thought to traffic the amyloid precursor protein (APP) from endosomes and mediate APP pathogenic processing. Defects in the retromer complex are shown to impair APP trafficking to

the membrane from endosomes where APP is degraded by  $\beta$ -site APP-cleaving enzyme 1 (BACE1). Because many studies showed that protein–protein interactions between the trimeric core proteins (Vps35–Vps29–Vps26) play a critical role in stabilizing the complex, the authors envisaged that pharmacological chaperones that are capable of increasing the interaction between retromer proteins would promote the formation of complex and retromer-mediated trafficking.

For *in silico* docking screening, they first identified ten potential docking sites located at the interfaces of the three core proteins based on the crystal structure of the retromer complex. Ligands that bind to the sites at the interfaces would enhance the interaction between the proteins, stabilizing the protein complex. A library of 46 000 small molecules was screened to obtain molecules that fit well into the pockets at the interface between Vps29 and Vps35, leading to the discovery of twenty-four hit compounds. After testing their activity on the denaturation temperature of the retromer complex as determined by DSF, only one compound was selected as a hit called R55 (Fig. 6). As anticipated, they found that this compound bound to the retromer complex with a  $K_D$  value of  $\sim 5 \mu\text{M}$  and increased retromer levels in neurons without significant toxicity. Moreover, R55 reduced the accumulation of amyloid  $\beta$  and APP processing. Notably, this is the first example of a pharmacological chaperone that stabilizes a multiprotein complex, while others are targeting a single protein. The results showed the power of virtual screening in identifying pharmacological chaperone molecules. This pharmacological chaperone offers a promising starting point for further drug development for the treatment of AD.

### Affinity-based screening

Given that pharmacological chaperones need to bind to a pathogenic protein for exerting their stabilizing activity, affinity-based screens would be an efficient strategy to discover such direct-binding molecules. Compared to conventional functional assays, affinity-based screens offer several advantages as a method for

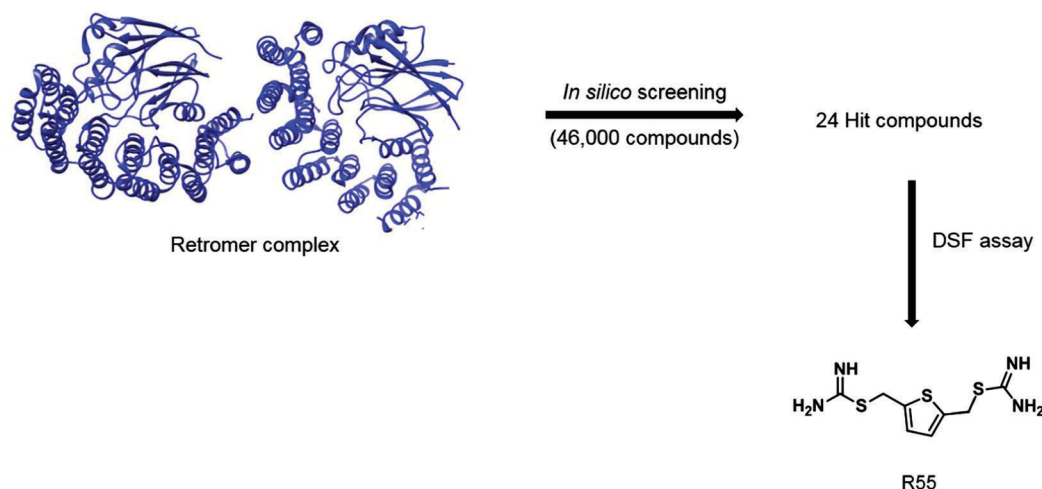


Fig. 6 *In silico* virtual screening based on the crystal structure of the retromer complex (PDB ID code: 2R17) identified a pharmacological chaperone molecule, R55.

identifying protein ligands.<sup>35</sup> While conventional screens generally use multi-well plate formats that require tremendous amounts of reagents and resources such as robotic systems, affinity-based screening needs only small amounts of reagents and no expensive instrumentation. Moreover, this screening format relies solely on the physical interaction between small-molecules and proteins. As a result, in principle, this method does not need any prior knowledge of the protein structure, while structure-based drug design approaches require detailed 3D structural information about ligand binding pockets on the target protein surface. In addition, an affinity-based screening method is able to examine a vast number of molecules (*e.g.*, over one million compounds) simultaneously by incubating whole library molecules with a target protein in a test tube. Therefore, affinity-based approaches represent a rapid and

inexpensive screening method for discovering protein-binding molecules.

Recently, Lim and colleagues have reported a convenient affinity-based HTS strategy that allows for the rapid discovery of potential pharmacological chaperones stabilizing  $\alpha$ -synuclein.<sup>36</sup> Studies suggest that  $\alpha$ -synuclein exists as folded tetramers and becomes an aggregation-prone form by pathogenic mutations, leading to its self-association and subsequent formation of Lewy bodies, which is the hallmark of Parkinson's disease (Fig. 7A).<sup>37,38</sup> Thus, pharmacological chaperones able to stabilize the folded tetrameric  $\alpha$ -synuclein would prevent  $\alpha$ -synuclein aggregation. To obtain such small-molecules, they developed an affinity-based screening format. They postulated that the use of  $\alpha$ -helix mimetic small molecules as a library source could increase the chance of isolating direct-binding molecules based

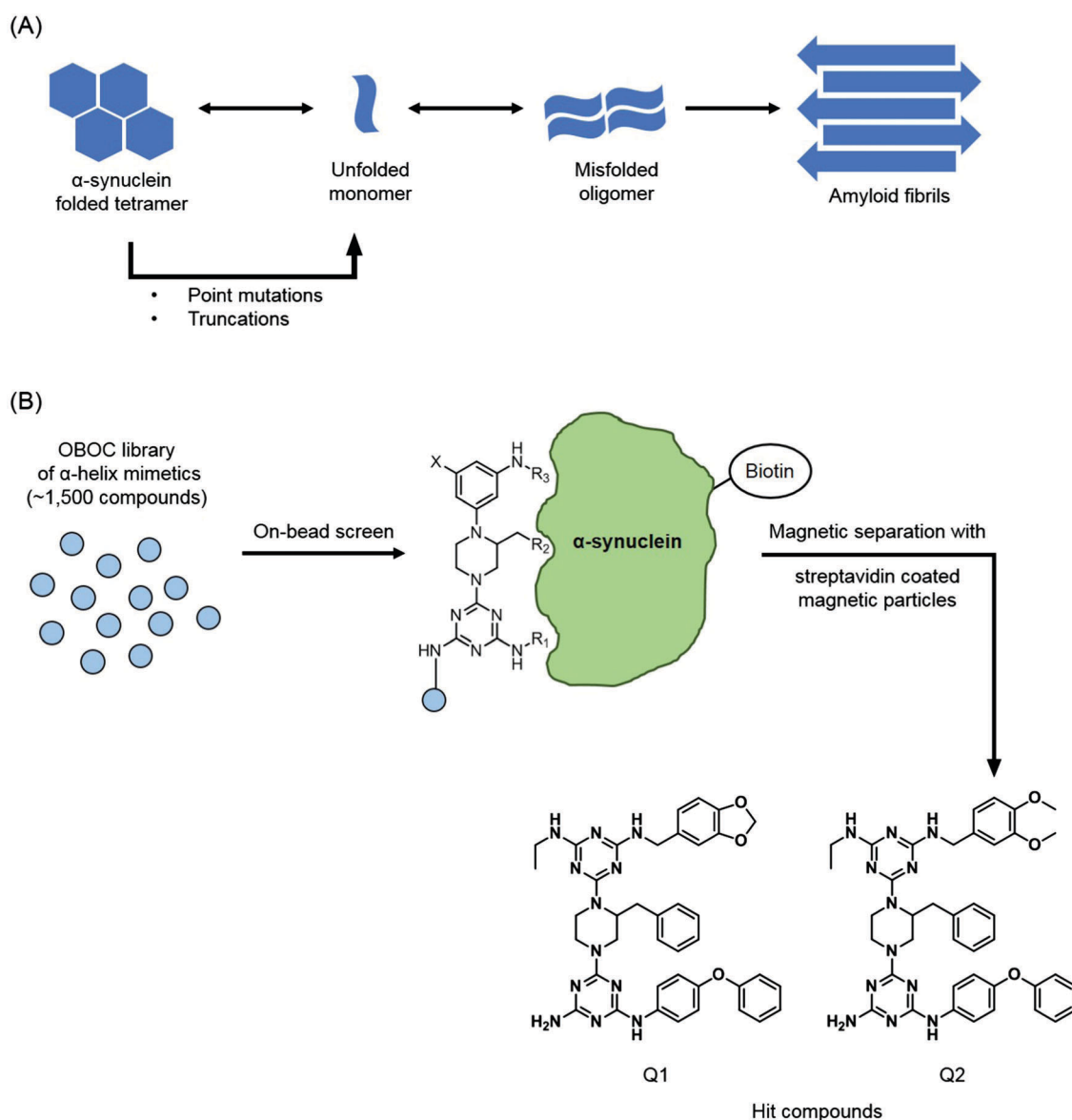


Fig. 7 (A) A proposed mechanism for the amyloid fibril formation of  $\alpha$ -synuclein. (B) Affinity-based screening of a one-bead one-compound combinatorial library of  $\alpha$ -helix mimetics against  $\alpha$ -synuclein and chemical structures of pharmacological chaperones for  $\alpha$ -synuclein.



on the fact that  $\alpha$ -synuclein is composed of mostly  $\alpha$ -helices.<sup>37,38</sup> Using a standard split-and-pool method,<sup>39</sup> a one-bead one-compound combinatorial library of  $\alpha$ -helix mimetic small molecules with structural diversity of  $\sim 1500$  molecules was synthesized. By incubating the bead library molecules with a biotinylated  $\alpha$ -synuclein, followed by magnetic separation with streptavidin-coated magnetic particles, two hit compounds (Q1 and Q2) were identified (Fig. 7B). Based on a fluorescence polarization assay, they were found to bind to  $\alpha$ -synuclein with  $K_D$  values of 68 and 148 nM, respectively. As expected, these compounds were shown to stabilize  $\alpha$ -synuclein in thermal denaturation assays and inhibit  $\alpha$ -synuclein aggregation as determined by ThT fluorescence assay. It should be noted that although these compounds improved  $\alpha$ -synuclein stability, their functional activities as pharmacological chaperones should be verified in further studies. Nonetheless, the results suggest that this affinity-based screening format will be a powerful tool to rapidly identify potential pharmacological chaperones for any given pathologic proteins.

## Conclusions

Protein misfolding is associated with various pathogenic conditions, leading to numerous conformational diseases. Pharmacological chaperones have proven to rescue defects in protein misfolding and restore their function. As such, pharmacological chaperone therapy represents a relatively new but highly promising strategy for the treatment of conformational diseases. Indeed, various technologies have been developed, producing a number of lead compounds serving as pharmacological chaperones for protein misfolding diseases. Among them, several molecules have been clinically used, and some are being evaluated in clinical trials, demonstrating their therapeutic potential. Here we have highlighted recent screening technologies that enables the discovery of pharmacological chaperone small-molecules, including *in vitro* functional screening, cell-based screening, *in silico* high-throughput screening, and affinity-based screening. We believe that further improvement in current technologies and development of new technologies, along with the development of new chemical space, will facilitate the discovery of useful pharmacological chaperone molecules for many challenging drug targets associated with protein misfolding.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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