

ization. Rapid changes in the polarity of PIN proteins in response to developmental or environmental cues have been shown to redirect auxin flow and mediate multiple developmental processes (1, 3, 5), but the mechanism underlying polarity control is largely unresolved. These rapid relocations are enabled by constitutive cycling of PIN proteins. GNOM, an endosomal regulator of vesicle budding that mediates this process, is required for the recycling but does not seem to directly determine the polarity of PIN targeting, because *gnom* mutants show randomized changes in PIN1 polar localization (15). In line with this more general function of GNOM in polar auxin transport, most, if not all polar auxin transport-dependent developmental processes are disturbed in *gnom* mutants (19). In contrast, the role of PID in controlling PIN polarity is more specific. It appears that PID acts as a binary switch, with subthreshold PID levels leading to basal PIN localization and above-threshold PID levels leading to apical PIN localization. Consistently, the defects observed in both loss- and gain-of-function of *PID* lines can each be ascribed to a reversion in auxin flow. The observation that auxin controls cellular PID levels (9) raises the intriguing possibility that PID is involved in a feedback mechanism by which auxin influences the polarity of its own flow. Such a regulatory network has been proposed in the classical

“canalization” model to explain the self-organizing properties of vascular tissue differentiation (20) and would also account for the dynamic auxin-dependent changes in auxin redistribution underlying phyllotaxis (3, 18). Even though endogenous PID acts in a limited subset of PIN-dependent development processes, many more cells are competent to respond to PID overexpression. In *Arabidopsis*, PID belongs to a family of 23 protein kinases, the members of which are differentially expressed. It is therefore likely that ectopic PID expression reveals functions that are normally represented by these different PID family members. Although further experimentation is needed to determine whether PIN proteins are direct targets of PID, it is clear that PID-dependent phosphorylation is an essential intermediate step in the control of polar PIN protein targeting, and thus of directional auxin flow regulating patterning processes.

References and Notes

1. J. Friml *et al.*, *Nature* **426**, 147 (2003).
2. J. Mattsson, W. Ckurshumova, T. Berleth, *Plant Physiol.* **131**, 1327 (2003).
3. E. Benkova *et al.*, *Cell* **115**, 591 (2003).
4. J. Friml, K. Palme, *Plant Mol. Biol.* **49**, 273 (2002).
5. J. Friml, J. Wisniewska, E. Benkova, K. Mendgen, K. Palme, *Nature* **415**, 806 (2002).
6. N. Geldner *et al.*, *Cell* **112**, 219 (2003).
7. S. R. M. Bennett, J. Alvarez, G. Bossinger, D. R. Smyth, *Plant J.* **8**, 505 (1995).
8. S. K. Christensen, N. Dagenais, J. Chory, D. Weigel, *Cell* **100**, 469 (2000).

9. R. Benjamins, A. Quint, D. Weijers, P. Hooykaas, R. Offringa, *Development* **128**, 4057 (2001).
10. A. Muller *et al.*, *EMBO J.* **17**, 6903 (1998).
11. J. Friml *et al.*, *Cell* **108**, 661 (2002).
12. I. Ottenschlager *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 2987 (2003).
13. J. Friml *et al.*, data not shown.
14. K. Ljung *et al.*, *Plant Mol. Biol.* **50**, 309 (2002).
15. T. Steinmann *et al.*, *Science* **286**, 316 (1999).
16. D. Weijers, N. Geldner, R. Offringa, G. Jurgens, *Nature* **414**, 709 (2001).
17. D. Weijers *et al.*, *Development* **128**, 4289 (2001).
18. D. Reinhardt *et al.*, *Nature* **426**, 255 (2003).
19. N. Geldner *et al.*, *Development* **131**, 389 (2004).
20. T. Berleth, T. Sachs, *Curr. Opin. Plant Biol.* **4**, 57 (2001).
21. Materials and methods are available as supporting material on Science Online.
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Harnessing Chaperones to Generate Small-Molecule Inhibitors of Amyloid β Aggregation

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Protein aggregation is involved in the pathogenesis of neurodegenerative diseases and hence is considered an attractive target for therapeutic intervention. However, protein-protein interactions are exceedingly difficult to inhibit. Small molecules lack sufficient steric bulk to prevent interactions between large peptide surfaces. To yield potent inhibitors of β -amyloid ($A\beta$) aggregation, we synthesized small molecules that increase their steric bulk by binding to chaperones but also have a moiety available for interaction with $A\beta$. This strategy yields potent inhibitors of $A\beta$ aggregation and could lead to therapeutics for Alzheimer's disease and other forms of neurodegeneration.

Aggregation of $A\beta$ peptide, generated by proteolytic cleavage of amyloid precursor protein (APP), is a key event in the pathogenesis of Alzheimer's disease (1–3). Genetic studies on Alzheimer's disease implicate mutations

in APP and in genes involved in APP processing (4) in disease development. Because genetic factors that contribute to Alzheimer's disease also promote $A\beta$ aggregation, inhibition of polymerization may have therapeutic benefit. However, this process has proven extremely difficult to prevent (5, 6). One hurdle to overcome is the poor capacity of low molecular weight drugs to interfere with the protein-protein interactions that generate toxic $A\beta$ aggregates. Blocking protein-

protein interactions is a general problem and, despite the importance of these interactions in biologic processes, few inhibitors have been identified (7, 8). The binding energy that drives protein-protein contacts is typically distributed over a large area and, unlike enzymes, these surfaces lack a defined “hotspot” for pharmacological intervention. Additionally, protein surfaces possess a high level of plasticity that can serve to accommodate small molecules, thereby avoiding inhibition.

To approach this problem, we envisioned a Trojan Horse strategy in which a small bifunctional molecule would gain access to the relevant biologic compartment, bind tightly to a chaperone, and thereby gain the steric bulk needed to disrupt a protein-protein interaction (Fig. 1A). Chaperones are ideally suited for this approach, as they bind folding or misfolded proteins via exposed hydrophobic surfaces.

In this strategy, one end of the bifunctional molecule binds the FK506 binding protein (FKBP) chaperone family of peptidyl prolyl cis-trans isomerases. FKBP are encoded by 23 different genes, are highly expressed in all mammalian cells (9, 10), and are good candidates for recruitment by bifunctional molecules (11–15). The other end

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of the bifunctional molecule is free to interact with aggregating A β . A number of organic compounds, including Congo Red (CR), function as amyloid ligands. These compounds are unsuitable for therapeutic use because high molar ratios of drug to A β are required to inhibit aggregation (5, 6). To investigate whether chaperone recruitment could empower these ineffective small molecules with the ability to interfere with A β aggregation and toxicity, we synthesized SLF-CR (Fig. 1B) (fig. S1), which is composed of CR covalently tethered to a synthetic ligand for FKBP (SLF) (16).

To assess the impact of chaperone recruitment on the potency of aggregation inhibitors, we incubated A β (1-42) in the presence of drug and FKBP. Fibrillogenesis was followed in real time by turbidity measurements. SLF-CR/FKBP (10 μ M) completely blocked the formation of A β aggregates capable of scattering light (Fig. 1C). Even at nanomolar concentrations, SLF-CR/FKBP [median inhibitory concentration (IC₅₀) = 0.43 μ M] delayed amyloid aggregation (Fig. 1D). Inhibition by SLF-CR required FKBP, but the chaperone alone did not change A β aggregation. In contrast, CR was a modest inhibitor of turbidity and FKBP did not alter its potency.

As a second measure of inhibition, we explored the aggregation of A β by the thioflavin T assay. Thioflavin T fluorescence increases in the presence of A β aggregates, which makes this an attractive assay for screening inhibitors (17). The IC₅₀ of SLF-CR/FKBP for decreasing the concentration of thioflavin T-binding species was lower than that of CR/FKBP by a factor of about 5 (Fig. 1E). Increasing the molar equivalents of FKBP further enhanced the potency of SLF-CR (Fig. 1F). In the absence of FKBP, SLF-CR and CR had similar activity. These results suggest that drug-mediated recruitment of FKBP is required for enhanced potency.

Recent evidence has suggested a role for nonfibrillar A β aggregates in the pathology of Alzheimer's disease (1-3). Preventing formation of these intermediates is therefore an important measure of inhibitory potency (2, 18). To explore the ultrastructure of A β aggregates, we examined drug-treated mixtures by transmission electron microscopy (TEM) and atomic force microscopy (AFM) (19). At a concentration of 1 μ M, CR, SLF-CR, and CR/FKBP were ineffective at reducing the size and abundance of A β fibrils (Fig. 2, A and B). However, consistent with the thioflavin T and turbidity experiments, SLF-CR/FKBP almost fully blocked fibril formation. By AFM, smaller, less elongated species were observed in samples treated with SLF-CR/FKBP; these probably represent an intermediate trapped before the formation of fibrils. The particles were approximately uni-

form in size (28 \pm 5 nm²; N = 50), which suggested that SLF-CR/FKBP interrupted fibril formation at a discrete step in the aggregation pathway. Because these species were not observed in samples treated with CR/FKBP, they might represent a qualitative difference between the mechanisms of action of CR and SLF-CR/FKBP. Furthermore, electrophoretic analysis of A β oligomers showed a predominance of small aggregates in A β samples treated with SLF-CR/FKBP but not CR/FKBP (fig. S2B).

Although a strategy that interrupts the aggregation process provides mechanistic insight, the most important criterion of therapeutic potential is the capacity to prevent the formation of neurotoxic aggregates. We examined whether the bifunctional drugs could in-

hibit neurotoxicity of in vitro aggregated A β . The cell viability of P0 hippocampal neurons was assessed with the 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazoliumbromide (MTT) assay (Fig. 3A). SLF-CR/FKBP-treated A β samples were substantially less toxic than untreated or CR/FKBP-treated samples (Fig. 4A). The EC₅₀ of SLF-CR/FKBP was lower than that of CR/FKBP by a factor of about 4 (0.9 \pm 1.3 μ M and 4.2 \pm 1.4 μ M, respectively). SLF-CR's ability to prevent A β -induced neuronal death was dependent on the concentration of FKBP (Fig. 3B). Thus, drug-mediated recruitment of FKBP not only blocked aggregation but could also inhibit A β toxicity.

To examine the morphology of the treated neurons, we applied A β and drug-protein combinations to cultured hippocampal neurons.

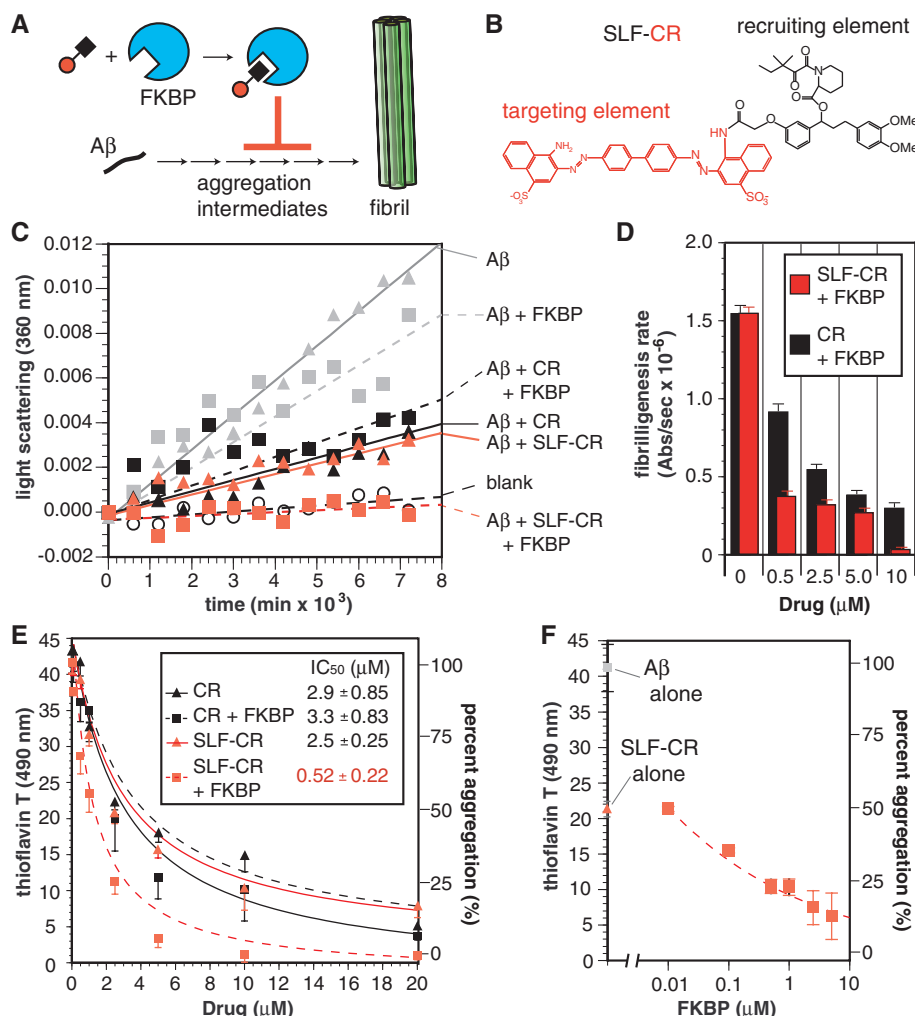


Fig. 1. The combination of SLF-CR and FKBP inhibits aggregation of A β (1-42). (A) Model of A β fibrillogenesis and interruption by a bifunctional small molecule recruiting FKBP to increase the drug's steric bulk. (B) The chemical structure of SLF-CR is shown with the targeting domain, CR, in red and the recruiting domain, SLF, in black. (C) Inhibition of A β fibrillogenesis as measured by light scattering. Drug was added to a concentration of 10 μ M, FKBP to 1 μ M, and A β to 25 μ M. (D) The initial rate of turbidity as a function of drug concentration was assumed to be linear, which yielded the fibrillogenesis rate. (E) Inhibition of A β fibrillogenesis as measured by thioflavin T fluorescence. Results are averages (\pm SEM) of at least three independent experiments performed in duplicate. (F) Impact of FKBP availability on inhibition of thioflavin T reactivity mediated by 2.5 μ M SLF-CR. Results are averages (\pm SEM) of two experiments performed in triplicate.

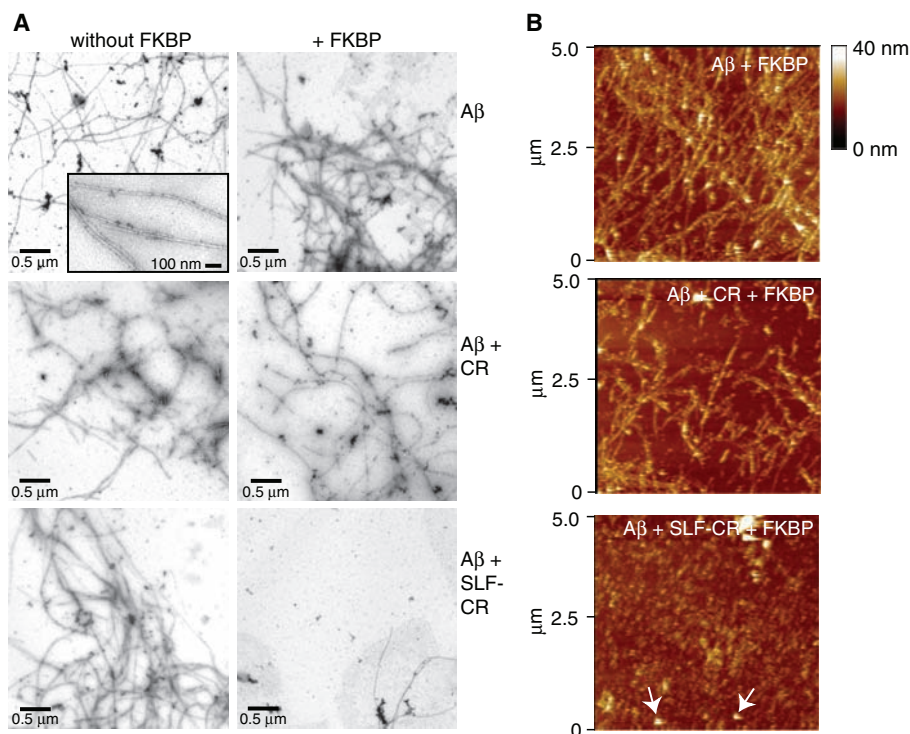
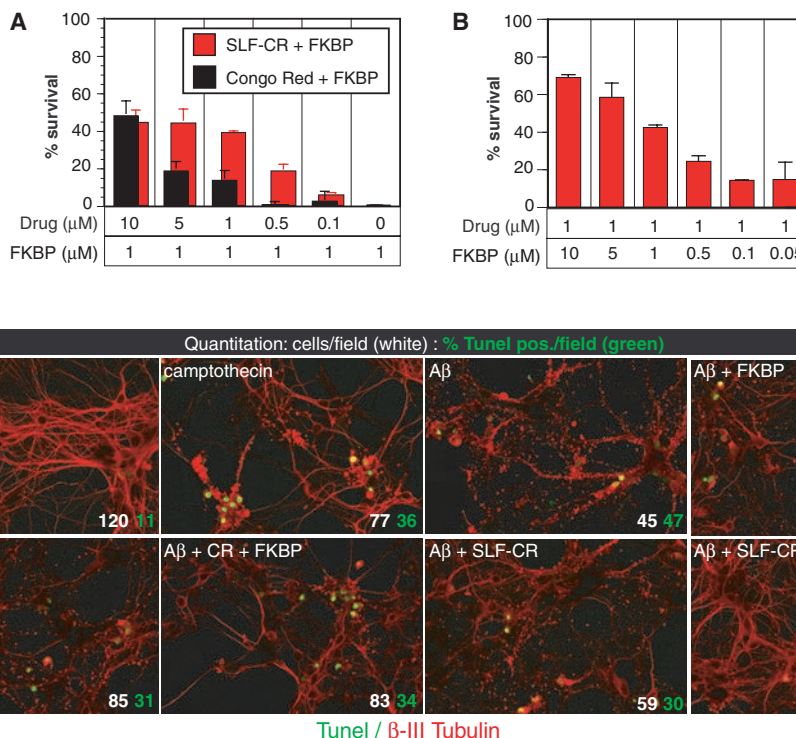


Fig. 2. Direct visual investigation of A β (1-42) fibrils. **(A)** TEM images depict the ultrastructure and distribution of amyloid fibrils. Images are representative of at least three independent experiments. The inset shows fibrils in greater detail. Fibrils in samples treated with SLF-CR and FKBP were uncommon, but one is shown in the figure to illustrate the similarity to those in other samples. **(B)** Representative AFM images show A β treated with FKBP alone, CR/FKBP, or SLF-CR/FKBP. The x-y distances are shown at the left; color scale at right indicates height (z direction). An arrow indicates the position of species common to samples treated with SLF-CR/FKBP.

Cell death was measured by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay, and cell morphology was assessed with a cytoskeletal immunostain. As expected, treatment with A β induced cell death as well as abnormal cell morphology (Fig. 3C) (fig. S3); neurites became dystrophic, some neurons detached from the slides, and those remaining showed signs of nuclear fragmentation and membrane blebbing. Incubation of A β with FKBP, CR, CR/FKBP, or SLF-CR failed to reduce the number of TUNEL-positive cells or prevent changes in cell morphology. The SLF-CR/FKBP combination, however, markedly blocked toxicity. Moreover, SLF-CR/FKBP-treated neurons displayed mostly normal morphology (Fig. 3C). These studies show that FKBP recruitment can protect cultured neurons from cell death triggered by toxic A β aggregates.

Our strategy is designed to allow bifunctional drugs to wield the steric bulk of FKBP and prevent A β from joining the nascent fibril. Improved coverage of the A β surface might provide superior inhibition, possibly by altering the orientation of FKBP relative to the A β surface. Our synthetic strategy permits the assembly of bifunctional compounds from a collection of modular targeting domains, recruiting domains, and interchangeable linkers. Therefore, we envisioned that by installing linkers that vary in length and flexibility, we might identify compounds that

Fig. 3. The combination of SLF-CR and FKBP reduces the toxicity of A β (1-42). **(A)** Cell viability of hippocampal neurons as measured by the conversion of MTT. Results are averages (\pm SEM) of at least two experiments performed in triplicate. At the concentrations used, neither the compounds (up to 20 μ M) nor FKBP (1 μ M) were toxic in the absence of A β (fig. S2C). **(B)** Dependence of cell viability on the concentration of FKBP. In these experiments, A β (1-42) (100 μ M) was treated with varying levels of FKBP at constant drug concentrations (1 μ M). Results are averages (\pm SEM) of two experiments performed in triplicate. **(C)** The viability and morphology of hippocampal neurons were determined by labeling with TUNEL (green) and antibody to β -III tubulin (red). In the lower right corner of each panel, the total number of cells per 40 \times field (white) and percent TUNEL-positive cells in the same fields (green) are shown ($n = 200$ to 600 cells). Cells treated with phosphate-buffered saline (PBS) or the toxin camptothecin (0.6 mg/ml) are shown for comparison.



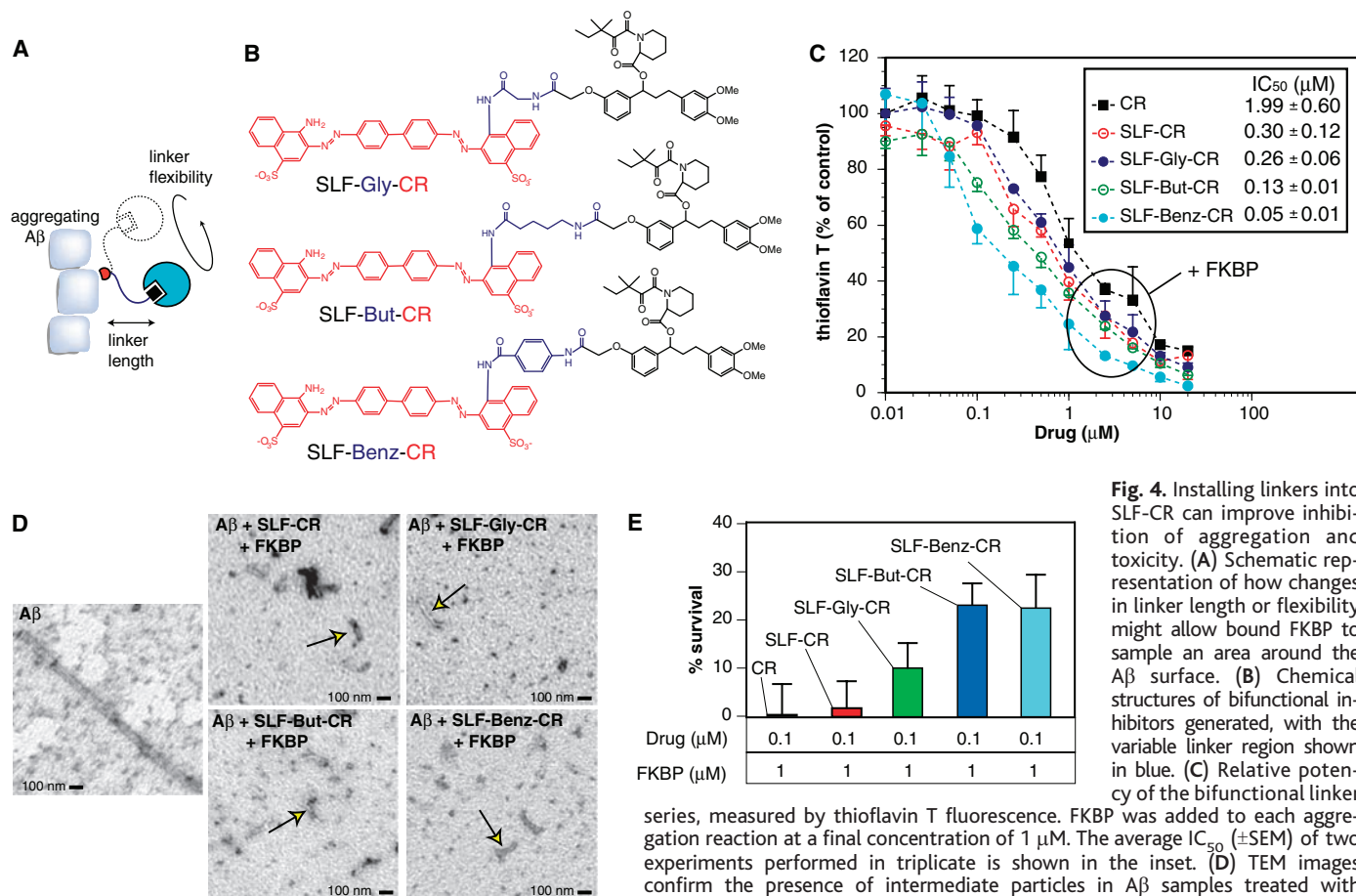


Fig. 4. Installing linkers into SLF-CR can improve inhibition of aggregation and toxicity. (A) Schematic representation of how changes in linker length or flexibility might allow bound FKBP to sample an area around the Aβ surface. (B) Chemical structures of bifunctional inhibitors generated, with the variable linker region shown in blue. (C) Relative potency of the bifunctional linker series, measured by thioflavin T fluorescence. FKBP was added to each aggregation reaction at a final concentration of 1 μM. The average IC₅₀ (±SEM) of two experiments performed in triplicate is shown in the inset. (D) TEM images confirm the presence of intermediate particles in Aβ samples treated with compounds from the linker series. (E) Survival of hippocampal neurons treated with Aβ and compounds from the bifunctional linker series. Results are averages (±SEM) of one or two experiments performed in triplicate.

permit FKBP to scan the Aβ surface for favorable arrangements (Fig. 4A).

In a search for more potent inhibitors, we generated a series of bifunctional compounds that vary in the linker (Fig. 4B) (fig. S3A). These molecules were named according to the reagent used to create the linker (i.e., the amino acid glycine was used to generate SLF-Gly-CR). When we tested these compounds in conjunction with FKBP in the thioflavin T assay, we found that SLF-But-CR/FKBP and SLF-Benz-CR/FKBP were potent inhibitors (Fig. 4C) (fig. S3B). The most active compound, SLF-Benz-CR/FKBP, had an IC₅₀ of about 50 nM, which is lower than that of CR by a factor of 40 and represents an improvement over the parent combination, SLF-CR/FKBP, by a factor of 6. Like SLF-CR, the potency of the compounds was dependent on the availability of FKBP (fig. S3B). TEM revealed that the size and shape of Aβ intermediates were similar in samples treated with each of the bifunctional molecules (Fig. 4D). Thus, regardless of the properties of the linker, a common FKBP-drug-Aβ complex formed.

To explore the role of the linker in reduction of Aβ toxicity, we measured neuronal viability. Consistent with the rank order

as measured by thioflavin T fluorescence, SLF-Benz-CR/FKBP and SLF-But-CR/FKBP were, at nanomolar concentrations, significantly more potent than CR/FKBP or SLF-CR/FKBP (Fig. 4E) (fig. S3C). Even at the high molar concentrations of Aβ used in these experiments, SLF-Benz-CR remained active at 100 nM. Despite the modest number of compounds in the linker series, potent inhibitors could be uncovered by assembling modular components.

Our results indicate that recruitment of chaperones can block Aβ fibril formation and substantially reduce Aβ toxicity. Whereas other inhibitors of Aβ aggregation, such as CR and short peptides, are active in the 2 to 10 μM range (5, 6), our best compound was effective at 50 nM. This improvement is likely a result of the inhibitory mechanism used, because the bifunctional molecules were not superior to CR in the absence of chaperone. Analogs based on the SLF-CR model may have potential as therapeutics for Alzheimer's disease. The advantage of therapeutic intervention at the aggregation step is that it targets a purely pathological event in disease development. Thus, directly inhibiting Aβ aggregation with the recruited-chaperone approach might provide a viable

complement to recent efforts to reduce the rate of Aβ release (20, 21), enhance its clearance (22, 23), or template nontoxic aggregates (24–26).

References and Notes

1. J. Hardy, D. J. Selkoe, *Science* **297**, 353 (2002).
2. W. L. Klein, *Neurochem. Int.* **41**, 345 (2002).
3. J. D. Harper, S. S. Wong, C. M. Lieber, P. T. Lansbury Jr., *Biochemistry* **38**, 8972 (1999).
4. D. J. Selkoe, M. B. Podlisny, *Annu. Rev. Genomics Hum. Genet.* **3**, 67 (2002).
5. V. M.-Y. Lee, *Neurobiol. Aging* **23**, 1039 (2002).
6. M. A. Findeis, *Biochim. Biophys. Acta* **1502**, 76 (2000).
7. A. V. Veselovsky et al., *J. Mol. Recognit.* **15**, 405 (2002).
8. W. L. Delano, M. H. Ultsch, A. A. De Vos, J. A. Wells, *Science* **287**, 1279 (2000).
9. A. Galat, *Curr. Top. Med. Chem.* **3**, 1315 (2003).
10. R. F. Standaert, A. Galat, G. L. Verdine, S. L. Schreiber, *Nature* **346**, 671 (1990).
11. R. Briesewitz, G. T. Ray, T. J. Wandless, G. R. Crabtree, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1953 (1999).
12. D. M. Spencer, T. J. Wandless, S. L. Schreiber, G. R. Crabtree, *Science* **262**, 1019 (1993).
13. K. Stankunas et al., *Mol. Cell* **12**, 1615 (2003).
14. P. D. Braun et al., *J. Am. Chem. Soc.* **125**, 7575 (2003).
15. R. Pollock, M. Giel, K. Linher, T. Clackson, *Nature Biotechnol.* **20**, 729 (2002).
16. D. A. Holt et al., *J. Am. Chem. Soc.* **115**, 9925 (1993).
17. H. LeVine III, *Methods Enzymol.* **309**, 274 (1999).
18. E. H. Koo, P. T. Lansbury Jr., J. W. Kelly, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9989 (1999).
19. S. S. Wong, J. D. Harper, P. T. Lansbury Jr., C. M. Lieber, *J. Am. Chem. Soc.* **120**, 603 (1998).
20. M. Citron, *Neurobiol. Aging* **23**, 1017 (2002).
21. M. L. Michaelis, *J. Pharmacol. Exp. Ther.* **304**, 897 (2003).

22. C. Janus, M. A. Chishti, D. Westway, *Biochim. Biophys. Acta* **1502**, 63 (2000).
 23. R. Kayed et al., *Science* **300**, 486 (2003).
 24. J. Ghanta, C.-L. Shen, L. L. Kiessling, R. M. Murphy, *J. Biol. Chem.* **271**, 29525 (1996).
 25. J. C. Sacchettini, J. W. Kelly, *Nature Rev. Drug Discov.* **1**, 267 (2002).
 26. F. E. Cohen, J. W. Kelly, *Nature* **426**, 905 (2003).
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Ciliary Photoreceptors with a Vertebrate-Type Opsin in an Invertebrate Brain

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For vision, insect and vertebrate eyes use rhabdomeric and ciliary photoreceptor cells, respectively. These cells show distinct architecture and transduce the light signal by different phototransducing cascades. In the marine ragworm *Platynereis*, we find both cell types: rhabdomeric photoreceptor cells in the eyes and ciliary photoreceptor cells in the brain. The latter use a photopigment closely related to vertebrate rod and cone opsins. Comparative analysis indicates that both types of photoreceptors, with distinct opsins, coexisted in Urbilateria, the last common ancestor of insects and vertebrates, and sheds new light on vertebrate eye evolution.

In animal photoreceptor cells (PRCs), the surface membrane is enlarged for the storage of opsin photopigment (1). Two major types of PRCs are recognized by electron microscopy (table S1). In rhabdomeric PRCs, the apical cell surface folds into microvilli. This is the predominant type of PRC used for vision in invertebrates. In contrast, in vertebrates, the rods and cones of the retina and the PRCs of the pineal eye, a light-sensitive structure in the dorsal diencephalon, are of the ciliary type. In ciliary PRCs, the membrane of the cilium is folded for surface enlargement. To elucidate the evolution of ciliary PRCs, we investigated the photosensitive system in *Platynereis dumerilii* (Polychaeta, Annelida, Lophotrochozoa) (2, 3). This species shows ancestral anat-

omy and development and an ancestral gene inventory (2, 4). In addition, polychaetes and vertebrates are evolutionarily far apart (2), and thus any feature specifically shared between them as a result of their common evolutionary heritage necessarily existed in Urbilateria, the last common ancestor of all animals with bilateral symmetry.

Platynereis develops different pairs of eyes (5), as demarcated by opsin expression in Fig. 1A: "larval eyes," implicated in larval phototaxis, and "adult eyes," active in adult vision. All these eyes use rhabdomeric PRCs. The two pairs of adult eyes originate from a single anlage that is not yet split at the stage of the analysis. To identify ciliary PRCs, we used an antibody directed against acetylated α -tubulin (Fig. 1A) (3), which specifically labels stabilized microtubules in cilia and axons. In addition to the axonal scaffold, we detected two paired structures in the developing median brain, on the dorsal side of the apical organ (Fig. 1A, arrows). Electron microscopy (3) revealed that these structures represent multiple cilia (Fig. 1, B to E) that branch out into digits, each inheriting one of the peripheral microtubule doublets (Fig. 1, C and D). Such ciliated cells have been described for the brain of adult nereidids (6) and other polychaetes (7) and nemertines (8),

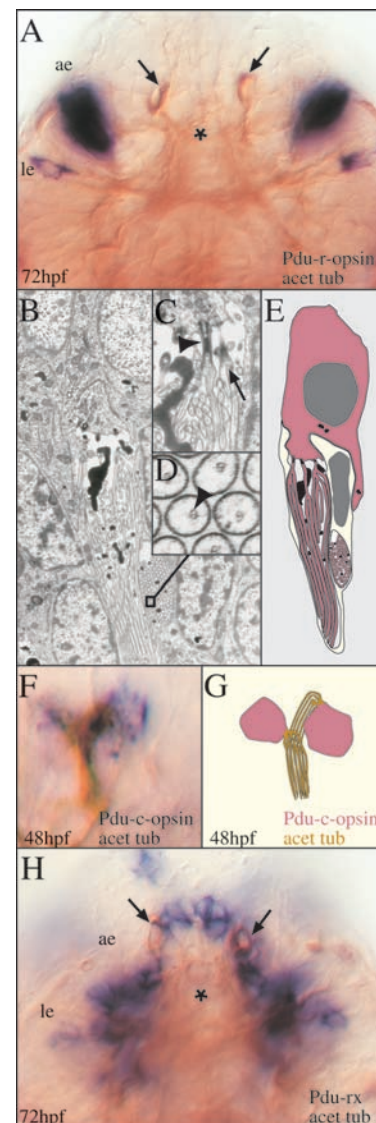


Fig. 1. *Pdu-c-opsin* and *Pdu-rx* expression in *Platynereis* ciliary photoreceptors (cPRCs). (A, F, H) Apical views of in situ hybridizations (blue) double-stained with an antibody to acetylated tubulin (brown). (A) *Pdu-r-opsin* expression (blue) 72 hours after fertilization (72 hpf), localized to larval and adult eyes (le, ae). (B) Electron micrograph of brain cPRCs. (C) Basal bodies of cilia (arrowhead), ramification in longitudinal section (arrow). (D) Ramifications in cross section with one doublet of microtubules (arrowhead). (E) Schematic based on (B). (F) *Pdu-c-opsin* expression (blue) in cPRCs (brown) at 48 hpf. (G) Schematic of (F). (H) Brain cPRCs (arrows) show high expression of *Pdu-rx*. Asterisks demarcate the apical organ. Magnification [(A) and (H)] $\times 300$, (B) $\times 3000$, (C) $\times 3800$, (D) $\times 25,000$, and (F) $\times 1200$.

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