Phage display and kinetic selection of antibodies that specifically inhibit amyloid self-replication

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The aggregation of the amyloid β peptide (Aβ) into amyloid fibrils is a defining characteristic of Alzheimer’s disease. Because of the complexity of this aggregation process, effective therapeutic inhibitors will need to target the specific microscopic steps that lead to the production of neurotoxic species. We introduce a strategy for generating fibril-specific antibodies that selectively suppress fibril-dependent secondary nucleation of the 42-residue form of Aβ (Aβ42). We target this step because it has been shown to produce the majority of neurotoxic species during aggregation of Aβ42. Starting from large phage display libraries of single-chain antibody fragments (scFvs), the three-stage approach that we describe includes (i) selection of scFvs with high affinity for Aβ42 fibrils after removal of scFvs that bind Aβ42 in its monomeric form; (ii) ranking, by surface plasmon resonance affinity measurements, of the resulting candidate scFvs that bind to the Aβ42 fibrils; and (iii) kinetic screening and analysis to find the scFvs that inhibit selectively the fibril-catalyzed secondary nucleation process in Aβ42 aggregation. By applying this approach, we have identified four scFvs that inhibit specifically the fibril-dependent secondary nucleation process. Our method also makes it possible to discard antibodies that inhibit elongation, an important factor because the suppression of elongation does not target directly the production of toxic oligomers and may even lead to its increase. On the basis of our results, we suggest that the method described here could form the basis for rationally designed immunotherapy strategies to combat Alzheimer’s and related neurodegenerative diseases.

Significance

The promise of antibody-based strategies to combat Alzheimer’s disease by inhibiting the aggregation of the amyloid β peptide (Aβ) has not yet been realized in clinical trials. In part, this situation has arisen because the antibodies explored so far have been developed without the benefit of the mechanistic information needed to block specific steps in Aβ aggregation. We introduce a strategy to target specifically fibril-dependent secondary nucleation, the microscopic step found to generate most neurotoxic Aβ assemblies. Phage display libraries are screened to find antibodies that bind to Aβ fibrils, followed by affinity ranking and chemical kinetic analysis. Our results suggest that such antibodies may serve as starting points for the development of effective therapeutics for neurodegenerative diseases.

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The misfolding and aggregation of protein molecules is associated with a range of debilitating human disorders including Alzheimer’s disease (AD), Parkinson’s disease, and type II diabetes (1, 2). Common to these diseases are deposits of protein aggregates that proliferate and spread through the brain or other organs, processes that are currently very difficult to combat. AD leads to the degeneration of neurons and synapses and is the most common process that is currently very difficult to combat. AD leads to the progression of AD, although some compounds such as cholinesterase inhibitors help to manage the symptoms for a limited time. The main approaches in the search for therapies in recent years have been the development of β- and γ-secretase inhibitors and of immunotherapy (16). The first approach aims at reducing Aβ production or lowering the proportion of the more toxic Aβ42 variant relative to the other isoforms. The latter strategy includes active immunization with Aβ and passive immunization using anti-Aβ antibodies (17). Clinical trials of antibody-based therapeutics have proved challenging and in some cases have revealed significant complications such as adverse inflammatory reactions (18). Recent advances include the use of autoantibodies derived from elderly individuals who are cognitively normal, with some favorable indications for aggregate-specific antibody Aducanumab (19). These results generate optimism that new strategies for deriving Aβ species-specific antibodies might become attractive.

Anti-Aβ antibodies (SI Appendix, Table S1) are reported to have three main modes of action: (i) modulation of phagocytosis, (ii) alteration of the distribution of Aβ between the CNS and plasma, and (iii) inhibition of aggregation through binding to Aβ oligomers. Here we propose an alternative strategy aimed at deriving fibril-specific antibodies based on the idea that such antibodies may block fibril-dependent aggregation processes. Our strategy has emerged from recent advances in the understanding of the Aβ42 aggregation mechanism in vitro, with the discovery of the secondary nucleation of monomers on fibril surfaces as the major contributor to aggregate proliferation and toxicity (20, 21). Such secondary nucleation dominates the formation of new aggregates from early stages of the aggregation process and provides an autocatalytic feedback reaction, which appears to generate the

Alzheimer | antibody | inhibitor | drug development | self-assembly
large majority of toxic oligomers (20–22). Inhibition of secondary nucleation can therefore lead to a dramatic decrease in the total number of oligomers formed during the aggregation process (21–24). By contrast, inhibiting the other two steps in the underlying mechanism, primary nucleation and elongation (20), will lead only to a delay in the formation of oligomers and, in the case of elongation, may increase the total quantities of these toxic species.

In a previous study, we have shown that the molecular chaperone pro-SPC Brichos inhibits secondary nucleation in a highly selective manner by binding to the fibril surfaces, thereby reducing substantially the toxicity resulting from the aggregation process (21). Inspired by the efficacy of this natural molecule in curtailing Aβ toxicity, we have here developed a systematic approach to the generation of single-chain antibody fragments (scFvs) that bind specifically to the fibrillar form of Aβ42. We demonstrate that this approach allows identification of antibody fragments that inhibit selectively the secondary nucleation of Aβ42 on fibril surfaces.

**Results**

**Generation of ScFvs with High Affinity for Aβ42 Fibrils.** The strategy used here for deriving antibody fragments with the ability to inhibit fibril-dependent microscopic steps in the Aβ42 aggregation process consists of three stages: (i) three rounds of selection of fibril-specific scFvs from phage display libraries after removal of monomer binders (Fig. 1 A and B); (ii) ranking by means of SPR assays of the fibril-binding affinity of scFvs (Fig. 1C), and (iii) kinetic screening for scFvs with the ability to cause selective suppression of fibril-catalyzed secondary nucleation (Fig. 1D). The kinetic screening relies on the fact that very different scenarios are predicted to be associated with the inhibition of primary nucleation, secondary nucleation, and elongation, respectively (Fig. 2 A–C). For proof-of-principle, we used two commercially available libraries (Tomlinson I and J) (25) of scFvs displayed on protein III of the filamentous phage (26, 27).

**Monomer-binder removal and selection of fibril-specific scFvs.** Monomer-binding phages were eliminated by mixing each library with streptavidin-coated magnetic beads with biotinylated Aβ42 monomers (Fig. 1A). This step also removes phages with affinity for the beads or the test tube surfaces. The beads were removed and the supernatant added to beads coupled with biotinylated Aβ42 fibrils (Fig. 1B). After extensive washing, the members of each library with high affinity for the fibrils were eluted with acid, neutralized, and used to infect Tg1 Escherichia coli cells to produce enriched scFv phage display libraries. The whole procedure of monomer-binder removal and fibril-binder capture was repeated three times. The infected E. coli cells were then spread on LB agar plates, and 150 colonies were picked for production of single-clone phages in liquid cultures.

**Affinity ranking.** Surface plasmon resonance (SPR) technology was used for preliminary ranking of phage-scFvs in terms of their apparent affinity for Aβ42 fibrils. The 150 single-clone solutions were used to identify putative high-affinity fibril binders (Fig. 1C) by injecting them over a sensor surface with immobilized Aβ42 fibrils. Seven clones (I2, I48, I68, J44, J46, J7, and J57) were chosen for further analysis in this work and used to infect the HB2151 E. coli strain for expression and purification of isolated scFvs free in solution rather than displayed on phages. SPR analysis verified in each case a high affinity binding to fibrils with Kd values in the range of 0.3–2 μM (Fig. 1D and SI Appendix, Fig. S1).

**Effects on microscopic steps in the Aβ42 aggregation process.** Measurements of the Aβ42 aggregation kinetics were used to analyze the effects of the scFvs on distinct microscopic steps in the fibril formation process. As an illustration of possible outcomes, we used the AmyloFit interface (28) to simulate kinetic curves resulting from the selective reduction of each of the microscopic rate constants—that is, those for primary nucleation (kα), secondary nucleation (kβ), and elongation (kγ). A reduction of kα leads to a progressive increase in the lag time without any change in the steepness of the sigmoidal transition (Fig. 2A). The overall production of nuclei is effectively unchanged, although shifted to later times (Fig. 2D). By contrast, a selective reduction of kβ leads mainly to a change in the slope of the aggregation plots (Fig. 2B). It also significantly reduces the overall nucleation rate (Fig. 2E). Finally, a selective reduction of kγ leads to both an extended lag phase and a decrease in slope (Fig. 2C). The maximum rate of nucleation remains the same as absence of an inhibitor; however, the high nucleation rate persists for longer as a result of a slower depletion of free monomer through elongation (Fig. 2F). These simulations emphasize that secondary nucleation is indeed the key process that needs to be inhibited to limit the production of high levels of oligomers. To attempt to

**Fig. 1.** Schematic outline of the phage display selection strategy. (Step 1) Monomer-binding members of the libraries were captured by Aβ42 monomers on magnetic beads that were removed using a magnet (negative selection, A). Ca. 1015 virions (16 pmol) were added to 100 pmol of monomers (blue line) on beads, thus providing capacity to bind all phages displaying scFvs with high affinity for monomers. (Step 2) The solutions with unbound phages were added to magnetic beads with Aβ42 fibrils to capture phages with fibril-binding scFvs (positive selection, B), followed by extensive washing to discard nonbinding members. Fibre-bound phages were eluted with acid, neutralized, and used to infect E. coli to produce an enriched scFv phage display library. Step 3 = step 1, and step 4 = step 2, except that the enriched libraries from step 2 were used. Step 5 = step 1, and step 6 = step 2, except that the enriched libraries from step 4 were used. (Step 7) Fibril-bound phages were eluted with acid, neutralized, and used to infect E. coli, spread on LB agar plates and single clones picked for production of phages with displayed scFvs. The medium after removal of E. coli cells by centrifugation was used in SPR experiments for ranking their affinity for Aβ42 fibrils immobilized on sensor chips (C). (Step 8) The strongest binding candidates were used to infect E. coli to produce isolated scFvs for more detailed SPR analysis and Aβ42 aggregation kinetics (D).
reduce the toxicity of Aβ42 through specific inhibition of secondary nucleation (Fig. 2E) (21), we set out to identify scFvs that modulate Aβ42 aggregation kinetics in a manner as close as possible to the scenario in Fig. 2B.

The intensity of ThT fluorescence as a function of time was used to monitor fibril formation for Aβ42 alone and in the presence of each of the seven scFvs (I2, I48, I68, J7, J44, J46, and J57) at concentrations ranging from 0.1 to 1.0 molar equivalents (Figs. 3A–D and 4A–C). These macroscopic aggregation curves are clearly affected by all four scFvs but not by randomly selected scFvs from the initial pool (Fig. 4D and E). The data obtained with each of the fibril-binding scFvs were fitted using the AmyloFit interface (28), with the rate constant for one microscopic process as a free parameter (different value allowed for each scFv concentration) and the other two as global parameters (same value used for all curves).

Identification of Specific Secondary Nucleation Inhibitors. For four of the candidate scFvs—I2, I48, J44, and J57—we found that the main effect of their presence in Aβ42 solutions was a change in the slope of the growth region (Fig. 3). The data are well fitted assuming a selective reduction of k2 (Fig. 3) but cannot be fitted at all if only kβ (SI Appendix, Fig. S2), or only kν (SI Appendix, Fig. S3), is varied. The strongest inhibitory effect of these scFvs is thus likely to be on secondary nucleation, whereas primary nucleation and elongation remain essentially unaffected, in agreement with the scenario shown in Fig. 2B. The fits to the data at the higher scFv concentrations indicate a very low value of k2. The effect saturates at ~1–2 μM scFv in solutions containing initially 3 μM Aβ42 monomers, suggesting that secondary nucleation is effectively completely suppressed in the presence of high concentration of these scFvs, and the secondary nucleation rate is much lower than the primary nucleation rate essentially during the full time course of the aggregation reaction.

The plots of the secondary nucleation rate constants obtained from the fitting procedure as a function of scFv concentration (Fig. 3E and F) are qualitatively similar to binding curves, where I48, J44, and J57 appear to have high enough affinities to saturate the binding sites for the scFvs at the highest scFv concentrations used. The scFv I2 appears to have somewhat lower affinity (SI Appendix, Fig. S1), and at the highest concentrations used, its inhibitory effect corresponds to ca. 20-fold reduction in k2, thus some secondary nucleation still occurs at a detectable
rate. We find that the fibril-specific scFvs from clones I2, I48, J44, and J57 most likely inhibit Aβ42 aggregation through selective reduction of the rate constant for secondary nucleation. In the presence of scFvs I48, J44, and J57 at molar ratios above ca. 1:2 scFv:Aβ42, secondary nucleation has no detectable kinetic significance, although for I2 there is a residual contribution from this microscopic step.

**Morphology of the Resulting Fibrils by Cryo-EM.** The Aβ42 fibrils formed in the absence of scFv appear to have two filaments wound around each other, and there are both well-dispersed fibrils (Fig. 3G) and lumps of fibrils (SI Appendix, Fig. S4A). Aβ42 fibrils formed in the presence of scFvs J57 (Fig. 3B)—I44, I48, and I2 (SI Appendix, Fig. S4 B–D)—are overall longer, and with a reduced tendency to form larger assemblies compared with fibrils of Aβ42 alone.

**Seeded Aggregation Kinetics.** Seeded aggregation kinetics experiments were performed for Aβ42 alone and for Aβ42 in the presence of scFvs (I2, I48, J44, or J57). The monomer solution was supplemented at time 0 with preformed fibrils at a total concentration of 1%, 3%, 10%, or 30% in monomer equivalents (Fig. 5). At 1% seeds, the need for primary nucleation is bypassed and the aggregation kinetics of Aβ42 in the absence of scFv are greatly accelerated by secondary nucleation (20, 22) as shown by a decrease in the length of the lag phase (Fig. 5E). However, scFvs I2, I48, J57, and J44 diminish this seeding effect (Fig. 5A–D) and 1% and 3% seeds have much lower catalytic effect than for Aβ42 alone. The aggregation in the presence of these scFvs is dominated by primary nucleation and still very strongly retarded relative to the behavior observed in the absence of scFv. At 30% seed, the aggregation kinetics of Aβ42 in the absence of scFv is greatly accelerated due to rapid elongation of the seeds, seen as the emergence of observable growth from time 0 (Fig. 5 E and F). The growth rate at early times can reveal effects on the elongation rate constant, and we find that I2, I48, J57, and J44 cause at most a factor of 1.3 change in this rate (Fig. 5 F and G), compared with the more than 10-fold (I2) or more than 100-fold (I48, J44, and J57) reduction in the rate constant for secondary nucleation (Figs. 3 E and F and 5G).

**Discarding ScFvs That May Inhibit Elongation.** In the presence of scFvs J7, J46, and I68, the aggregation kinetics indicate that the elongation rate constant may also be affected (Fig. 4). The curves at low concentration of scFvs J7 and I68 can be fitted if only k\textsubscript{on} is reduced (Fig. 4 A and B). The data at a higher concentration of scFv J7 and I68 can only be fitted by assuming that k\textsubscript{on} or k\textsubscript{off} are reduced as well (SI Appendix, Fig. S5), suggesting that elongation may be affected. J46 appears to affect the elongation rate at all concentrations (Fig. 4C). As a result of these findings, none of these three scFvs are considered further for further studies.

**The Amino Acid Sequences of the Selected ScFvs.** DNA sequencing was used to elucidate the amino acid sequences of the scFvs that selectively inhibit secondary nucleation (I2, I48, J44, and J57; SI Appendix, Table S2). The amino acid compositions in the variable positions of the complementarity-determining regions (CDRs) of these scFvs were compared with the respective libraries from which they originate (Fig. 6). Although the sample size is small, for the clones from the Tomlinson I library, we find an enrichment of N and T residues. For the selected Tomlinson J clones, we find an enrichment of N, T, R, K, Y, and A, whereas the largest hydrophobic residues in this library (F, L, I, M, and W) are totally absent. The enrichment of R and K can be rationalized on the basis of the net negative charge of Aβ42 fibrils. Positively charged or large hydrophobic residues are not present in the I library. Cysteine is present in both libraries but is not found in any of the selected clones, meaning that none of the observed effects are due to aberrant disulfide-bond formation.

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**Fig. 4.** Kinetic analyses to discard scFvs that affect other processes in addition to secondary nucleation. The aggregation kinetics of 3 μM solutions of Aβ42 monomers in the absence (black) and presence (colors) of scFvs I68 (A), J7 (B), and J46 (C) and two control scFvs selected randomly from the I and J libraries (D and E) at 0.3–3.0 μM. The fitted curves in A–C were generated by fixing k\textsubscript{on} and k\textsubscript{off}, allowing k\textsubscript{on} to have different values at each scFv concentration. Note that the data for these three scFvs cannot be fitted on this assumption nor on the assumption of a selective reduction of k\textsubscript{off}, making it likely that they also inhibit elongation.

**Fig. 5.** Seeded aggregation kinetics. Shown are the aggregation kinetics of solutions of 3 μM Aβ42 monomer in the absence (E) or presence of 2 μM scFv I2 (A), I48 (B), J44 (C), and J57 (D) supplemented with no (black), 30 nM (cyan), 90 nM (green), 300 nM (yellow), or 900 nM (red) seeds at time 0. The initial slopes in the presence of 30% (900 nM) seed fibrils (F) were fitted using straight lines. (G) The obtained elongation rate constant, k\textsubscript{on}, relative to the case of no seed is shown as bars, and the rate constant for secondary nucleation, k\textsubscript{off}, relative to the case of no seed is shown as bars in lighter colors.
**Discussion**

We discuss in this paper a strategy to derive scFvs that specifically bind amyloid fibrils and inhibit fibril-dependent microscopic processes. This strategy relies on the detailed mechanistic understanding of the aggregation process of Ab42 associated with the onset and development of AD (20). Previous studies have identified a series of microscopic steps in the process of aggregation that together contribute to the overall macroscopic kinetic profile of the reaction and have revealed the importance of the catalytic conversion of monomeric species into aggregates as a result of secondary nucleation on fibril surfaces. This process results in the production of neurotoxic oligomeric species (20, 21) with distinct intracellular targets (29).

Previous studies have shown that molecular chaperones (21, 30), metal ions (31), and small molecules (32, 33) may inhibit one or more of the underlying microscopic steps in the overall Ab42 aggregation process, and the selective inhibition of secondary nucleation has been observed with both proteins and small molecules. At the molecular level, a given inhibitor may interact particularly favorably with one of the species present during the aggregation reaction (34). Some inhibitors suppress a single microscopic step, whereas others act on more than one step, depending on whether the inhibitor interacts with monomers, oligomers, or fibrils (23, 24, 34). If this species is the monomer, the primary and secondary nucleation and elongation events will all be affected, albeit to different degrees. If the inhibitor interacts with one of the aggregated species, by contrast, the effect will be more specific. In particular, if binding occurs specifically to the fibrils, either elongation or secondary nucleation, or both, may be inhibited.

The search for secondary nucleation inhibitors was motivated by their ability to reduce dramatically the number of Ab42 oligomers produced in their presence (Fig. 2E) (21) and their ability to reduce Ab42 toxicity as demonstrated through electrophysiology measurements of γ-oscillations in brain slices (21). By contrast, suppression of elongation does not alter the maximum rate of oligomer generation and indeed could lead to an increase in toxicity because oligomer generation can maintain significant levels during a longer time frame when monomer consumption through elongation is inhibited. The total number of oligomers generated due to secondary (and primary) nucleation may increase several-fold if elongation is inhibited because fewer monomers are consumed in the elongation process (Fig. 2F).

Our search for secondary nucleation inhibitors is facilitated by their distinct macroscopic signature of a reduced slope of the growth phase of the aggregation reaction (Fig. 2B) and from the saturation of the observed effects at high inhibitor concentrations when the scFvs cover all of the catalytic sites on the fibril surface (21). Moreover, we exploit the fact that very different scenarios can be expected upon inhibition of primary nucleation or elongation (Fig. 2A and C). The identification of scFvs I2, I48, J44, and J57 as secondary nucleation inhibitors relies on the much-enhanced fits to the data of the integrated rate laws when rate constants for secondary (Fig. 3) rather than primary nucleation or elongation (SI Appendix, Figs. S2 and S3) are varied and the direct confirmation of these findings through analysis of seeded aggregation reactions (Fig. 5).

As a consequence of secondary nucleation inhibition, the reactive flux is redirected to a pathway including primary nucleation and elongation only. This means that a much smaller number of new aggregates are created (Fig. 2E) and a larger fraction of monomers are consumed in elongation of those fewer aggregates, leading to longer fibrils on average. Although length estimates from cryo-EM images are likely biased by the greater ease of looking at dispersed fibrils, it is obvious that many of the fibrils formed in the presence of scFvs span the entire field of view (Fig. 3H and SI Appendix, Figs. S4 B–D), meaning they are over 1 μm long, whereas many of the fibrils formed in the absence of scFvs are around 200–500 nm long (Fig. 3G and SI Appendix, Fig. S4A). Another interesting feature of fibrils formed in the presence of scFv is their apparent lower tendency toward higher order assembly in lumps. This suggests that their surface properties are altered due to binding of scFvs to the fibrils.

**Monovalent ScFvs May Be Selected as Fibril-Specific.** The use of scFvs, which have a single epitope-binding site (Fig. 6), and removal of monomer binders (Fig. 1) are important features of our methodology; this enables targeting of epitopes present in fibrils but not in monomers. The single binding site avoids the generation of fibril specificity as a result of the chelate effect (avidity), which was recently found to explain the apparent fibril specificity of intact antibodies that recognize with low affinity an epitope found in the monomer; each IgG molecule may bind simultaneously to two epitopes in species consisting of more than one monomer; each IgG molecule may bind simultaneously to two epitopes in species consisting of more than one monomer (35). An scFv can only be fibril-specific if it binds to an epitope that is present in fibrils but not in monomers and can therefore not be mimicked by short fragments of the Ab42 peptide (SI Appendix, Figs. S6 and S7). In addition to the relatively disordered N terminus (residues 1–15), side chains of K16, V18, A21, E22, D23, S26, K28, V40, and A42 are exposed on the surface of Ab42 fibrils (11, 12). Fibril-specific epitopes might include several of these residues, or features containing copies of the same amino acid in multiple fibril planes (11). The apparent enrichment of uncharged hydrophilic residues—N, T, and Y (Fig. 6)—sugests that hydrogen bonding features may be important determinants of the fibril specificity.

**Secondary Nucleation and Elongation Occur at Separate Sites.** The discovery of a group of scFvs that selectively inhibits secondary nucleation, and another that also inhibits elongation, implies that...
distinct 3D epitopes are likely to exist along the lengths and at the ends of fibrils and that separate features of the fibril are involved in elongation and in secondary nucleation. Indeed, if the sites for secondary nucleation and elongation were to be identical, it would not be possible to block selectively secondary nucleation without affecting elongation and vice versa. The scFvs I2, I48, I44, and I57 act in a manner similar to Brichos, which binds along the fibril surface (21) and blocks selectively secondary nucleation. These scFvs are likely to bind to epitopes presented along the sides of the fibrils in a manner that prevents Aβ42 monomer binding or nucleation. The scFvs I66 and J7 are intriguing in that they seem to show a duality of behaviors, with selective inhibition of secondary nucleation processes at low concentration, whereas at higher concentrations the elongation step also appears to be affected (Fig. 4 A and B and SI Appendix, Fig. S5). These scFvs are likely to have affinity for epitopes both along the fibrils and at the fibril ends, where scFv binding will inhibit elongation.

Potential Significance of the Present Findings. A large number of studies have reported that oligomeric species, formed during the process of aggregation, are the most highly cytotoxic species associated with protein misfolding (14, 15, 36). Secondary nucleation may represent a potential feedback mechanism that can lead to the rapid amplification of the number of aggregates once an initial population has been formed (20, 22) and also be very effective in the production of toxic oligomers (20, 21). Inhibition of secondary nucleation, therefore, appears likely to be an important approach to reduce the pathogenicity associated with protein aggregation (34). Indeed, the discovery that specific molecular chaperones can act in a highly selective manner to suppress secondary nucleation suggests that this approach is exploited by living systems to reduce the risks of protein aggregation in vivo. Selection of fibril-binding antibodies from phage display libraries is therefore a fruitful route toward identifying inhibitors with the potential both to act as therapeutics and to increase our understanding of the mechanism of protein aggregation and also to elucidate further the origins and means of progression of some of the most highly debilitating of all human diseases.

Conclusions

We have shown in this study that screening of phage display libraries for antibody fragments (scFvs) that bind to amyloid fibrils, combined with affinity ranking and kinetic screening, enables the identification of antibodies with the capacity to inhibit fibril-dependent secondary nucleation processes. Importantly, our approach allows scFvs that inhibit elongation to be detected and eliminated from further investigations, as such species can cause significant apparent inhibition of the overall aggregation reaction but are unlikely to result in a significant reduction of toxic species and could even lead to an increase in the overall production of oligomers. The results suggest further that scFvs generated from such a systematic approach are likely to be favorable starting points for the development of future therapeutic strategies designed to combat AD. In addition, the approach described in this study of Aβ42 should be generally applicable to other pathologic amyloid proteins for which secondary nucleation leads to autocatalytic amplification and the rapid proliferation of toxic oligomers and may represent a general strategy to find potential therapeutic agents directed at the wider range of protein misfolding diseases.

Materials and Methods

Aβ42 purification, monomer isolation, bead-conjugation, phage display studies, scFv production and purification, SPR experiments, aggregation kinetics measurements and analysis, and cryo-EM were performed essentially as described (20, 21, 25, 37). See SI Appendix, Materials and Methods.

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