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Rational design of potent domain antibody inhibitors of amyloid fibril assembly

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Antibodies hold significant potential for inhibiting toxic protein aggregation associated with conformational disorders such as Alzheimer's and Huntington's diseases. However, near-stoichiometric antibody concentrations are typically required to completely inhibit protein aggregation. We posited that the molecular interactions mediating amyloid fibril formation could be harnessed to generate antibodies with potent antiaggregation. Here we report that grafting small amyloidogenic peptides (6–10 residues) into the complementarity-determining regions of a single-domain (V_H) antibody yields potent domain antibody inhibitors of amyloid formation. Grafted AMYloid-Motif AntiBODIES (gammabodies) presenting hydrophobic peptides from A β (Alzheimer's disease), α -Synuclein (Parkinson's disease), and islet amyloid polypeptide (type 2 diabetes) inhibit fibril assembly of each corresponding polypeptide at low substoichiometric concentrations (1:10 gammabody:monomer molar ratio). In contrast, sequence- and conformation-specific antibodies that were obtained via immunization are unable to prevent fibrillization at the same substoichiometric concentrations. Gammabodies prevent amyloid formation by converting monomers and/or fibrillar intermediates into small complexes that are unstructured and benign. We expect that our antibody design approach—which eliminates the need for immunization or screening to identify sequence-specific domain antibody inhibitors—can be readily extended to generate potent aggregation inhibitors of other amyloidogenic polypeptides linked to human disease.

beta-amyloid | misfolding | protein design | IAPP

The cytotoxicity of protein aggregates (e.g., prefibrillar oligomers and amyloid fibrils) linked to several neurodegenerative diseases has motivated the search for molecules that can inhibit and/or reverse protein aggregation (ref. 1 and references therein). The remarkable specificity of antibodies makes them particularly attractive as inhibitors of protein aggregation (1–3). Sequence-specific antibodies that bind to continuous or discontinuous sequence epitopes within amyloidogenic proteins can sequester monomers and prevent them from oligomerizing. A limitation of this approach is that low substoichiometric antibody concentrations ($\leq 1:10$ antibody:monomer molar ratios) are expected to be insufficient to sequester enough monomeric protein to prevent aggregation. In contrast, antibodies that are conformation-specific can selectively bind to and sequester oligomeric nuclei. The strength of this approach is that such antibodies may be inhibitory at low substoichiometric concentrations because they do not bind to monomeric protein. However, a limitation is that aggregated conformers must form before antibody binding, and this binding may be unable to arrest further conformational maturation of oligomeric nuclei into amyloid fibrils.

We recently reported that domain antibodies specific for the Alzheimer's amyloid β (A β) peptide can be designed by grafting hydrophobic A β peptide segments into the complementarity-determining regions (CDRs) of a single-domain (V_H) antibody (4). These Grafted AMYloid-Motif AntiBODIES (gammabodies) bind to A β oligomers and fibrils with nanomolar affinity and recognize A β monomers weakly. Gammabodies presenting the central hydrophobic A β motif (residues 18-VFFA-21) preferentially bind to

A β fibrils, whereas gammabodies presenting the C-terminal hydrophobic A β motif (residues 34-LMVGGVVIA-42) preferentially bind to A β oligomers and fibrils. Moreover, each A β gammabody uses homotypic interactions between the grafted A β motif and the same motif within A β aggregates to mediate binding. Interestingly, gammabodies bind to A β oligomers and fibrils noncompetitively with antibodies obtained via immunization that are specific for oligomeric [A11 (5) antibody] and fibrillar [OC (6) and WO1 (7) antibodies] conformers.

The unusual ability of A β gammabodies to bind precisely to the hydrophobic peptide segments that mediate A β aggregation led us to hypothesize that these domain antibodies would inhibit fibrillization either by interfering with the nucleation of A β monomers into prefibrillar oligomers or the conversion of amyloidogenic intermediates into fibrils (Fig. 1). We also posited that gammabodies would be more effective at inhibiting A β aggregation than conventional conformation-specific antibodies that do not target hydrophobic linear epitopes recognized by A β gammabodies. Finally, we posited that our domain antibody design strategy could be readily extended to other amyloidogenic polypeptides to generate potent sequence-specific inhibitors of amyloid formation. To evaluate these hypotheses, we designed gammabodies that display hydrophobic peptide segments from three polypeptides [A β , α -Synuclein, and islet amyloid polypeptide (IAPP)] that form amyloid fibrils and whose aggregation is linked to human disease. Here we report that gammabodies potentially inhibit amyloid formation of each polypeptide in a sequence-specific manner at substoichiometric concentrations (1:10 gammabody:monomer molar ratio), whereas sequence- and conformation-specific antibodies obtained via immunization are noninhibitory at the same substoichiometric concentrations.

Results

A β Gammabodies Potently Inhibit Amyloid Formation. To evaluate our hypotheses related to the design of domain antibody inhibitors of amyloid assembly, we first sought to optimize the biophysical properties of a V_H antibody scaffold for CDR grafting (4, 8, 9). We find that gammabodies presenting hydrophobic A β peptide segments (e.g., A β residues 15–24 and 33–42) within their third CDR (CDR3) readily aggregate when heated, stick to size-exclusion columns, and express at relatively low levels (5–7 mg/L) (4, 9). However, inserting a triad of negatively charged residues (Asp-Glu-Asp) at each edge of the hydrophobic CDR3 loops results in gammabodies that fail to aggregate when heated, elute as monomeric peaks from size-exclusion columns, and express at relatively high levels (15–20 mg/L; Fig. S1) (9). Importantly, the charged A β gammabodies bind with much higher affinity to A β fibrils (IC_{50}

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The authors declare no conflict of interest.

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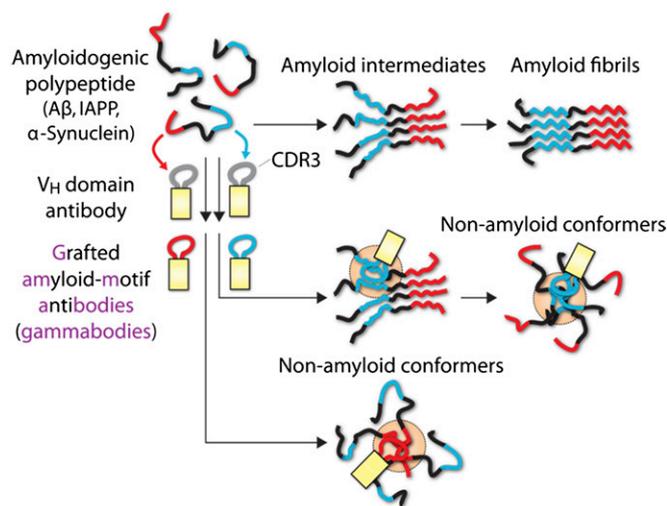


Fig. 1. Proposed method for designing gammabody inhibitors of amyloid fibril formation. Small peptide segments (6–10 residues) from amyloidogenic polypeptides (A β , IAPP, and α -Synuclein) are grafted into CDR3 of a single-domain (V_H) antibody, and the resulting gammabodies are evaluated for their ability to inhibit nucleation of monomers and/or conversion of amyloid intermediates (prefibrillar oligomers or fibrillar intermediates) into fibrils.

values of 190–210 nM; K_d value of 320 ± 30 nM for the A β 33–42 gammabody) than they bind to A β monomers (IC_{50} values >3 μ M; Fig. S1), their binding affinity is similar to uncharged A β gammabodies (IC_{50} values of 330–520 nM; K_d values of 330–490 nM) (4, 9), and they fail to bind to soluble or aggregated conformers of other amyloidogenic polypeptides (IAPP and α -Synuclein; Fig. S1).

We next investigated the ability of the charged gammabodies (herein referred to simply as gammabodies) to inhibit A β amyloid formation (Fig. 2). We used antibodies specific for prefibrillar oligomers (A11) and fibrillar conformers (OC) to monitor the aggregation of A β 42 via immunoblotting (Fig. 2A), as we reported previously (4, 10). In the absence of gammabody inhibitors, A β forms prefibrillar oligomers (recognized by the A11 antibody) after 1 d; these oligomers convert into fibrillar conformers (recognized by the OC antibody) on the second day and persist for an additional 4 d (longer times not evaluated).

We find that A β gammabodies inhibit amyloid formation, which we first evaluated at substoichiometric gammabody concentrations (1:10 gammabody:A β molar ratio; Fig. 2A). As expected, the A β 1–10 gammabody (which fails to bind to A β) is noninhibitory. The A β 12–21 and A β 15–24 gammabodies also fail to inhibit the formation of prefibrillar oligomers (day 1) and fibrillar conformers (day 2) but convert fibrillar A β conformers into nonfibrillar ones (days 3–6). In contrast, the A β 30–39 and A β 33–42 gammabodies prevent formation of both oligomer and fibrillar A β conformers (days 0–6; Fig. 2A). This inhibitory activity is unchanged at higher gammabody concentrations (1:1 gammabody:A β molar ratio), whereas each active gammabody is inactive at a molar ratio of 1:100 gammabody:A β (Fig. S2). We also find that gammabodies presenting 6mer A β peptides (A β residues 16–21, 34–39, and 37–42) within CDR3 are as inhibitory as their parent gammabodies presenting 10mer A β peptides, whereas gammabodies presenting 4mer A β peptides (A β residues 36–39 and 39–42) are inactive (Fig. S2). Moreover, scrambling the grafted A β peptides (Fig. 2A) or mutating them with single proline substitutions or glycine insertions (Fig. S2) eliminates the inhibitory activity of gammabodies. Finally, A β gammabodies that are inhibitory when added before A β oligomerization (day 0) are noninhibitory when added after A β oligomerization (Fig. S3).

Although the inhibitory A β gammabodies eliminated A11- and/or OC-immunoreactivity, we sought additional evidence of their anti-aggregation activity. Atomic force microscopy (AFM) imaging and fluorescence analysis using two dyes sensitive to the conformation of

A β (8-anilino-1-naphthalene sulfonate, ANS; thioflavin T, ThT) confirmed that the A β 12–21 and A β 15–24 gammabodies fail to prevent formation of prefibrillar oligomers (day 1) or fibrillar intermediates (day 2), but both prevent fibril formation (days 3–6; Fig. 2B–D and Fig. S3). AFM and fluorescence analysis also confirmed that the A β 30–39 and A β 33–42 gammabodies prevent both A β oligomerization and fibrillization (Fig. 2B–D and Fig. S3). Importantly, the inhibitory activity of gammabodies presenting A β peptide segments that overlap (A β 12–21/A β 15–24 and A β 30–39/A β 33–42) is indistinguishable (Fig. 2 and Fig. S3). Finally, circular dichroism spectroscopy revealed that the A β 15–24 gammabody converts β -sheet fibrillar intermediates (day 2) into unstructured A β conformers (days 3–6), whereas the A β 33–42 gammabody maintains A β monomers (day 0) as unstructured conformers (days 1–6; Fig. S4).

These findings provide further evidence that gammabodies arrest A β in soluble conformers that are incompetent for amyloid formation, but they do not provide insight into the local structure

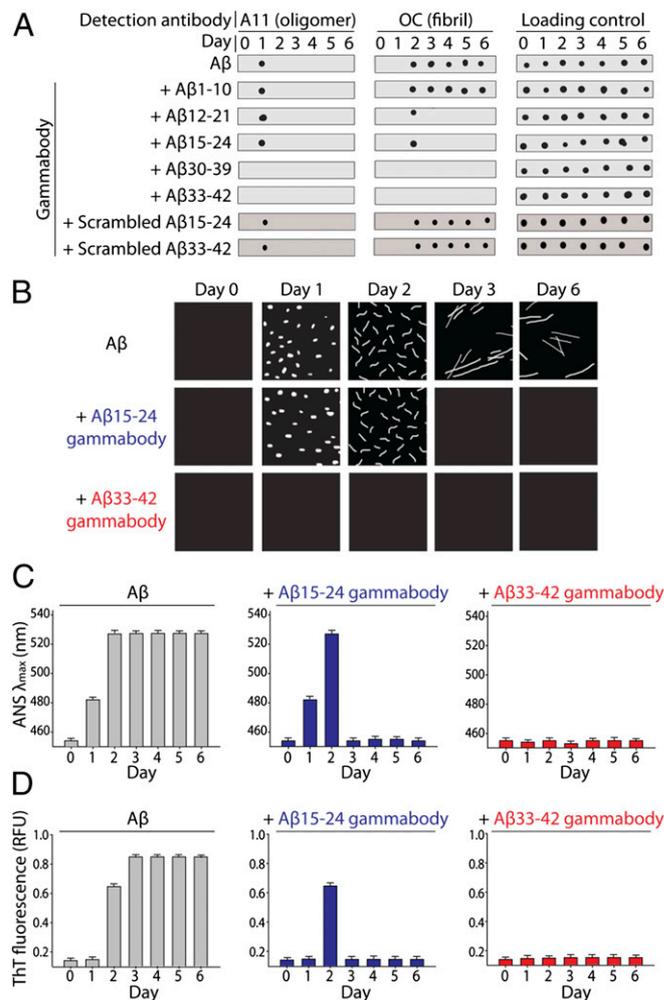


Fig. 2. Gammabodies inhibit A β amyloid formation at substoichiometric concentrations. A β 42 (25 μ M) was incubated in the absence (control) and presence of A β gammabodies (2.5 μ M; 1:10 gammabody:A β molar ratio), and the gammabody–A β samples were evaluated via (A) immunoblotting, (B) AFM, (C) ANS fluorescence, and (D) ThT fluorescence. In A, the blots were probed with antibodies specific for prefibrillar oligomers (A11), fibrillar conformers (OC), and the N terminus of A β (6E10; loading control). In B, the AFM images are 3×3 μ m, and the blank images are A β samples with heights <1 nm relative to the heights of the A β aggregates that are 7 – 19 nm (average heights of 7 ± 1 nm on day 1, 14 ± 5 nm on day 2, and 19 ± 7 nm on day 3). In C, the wavelength corresponding to the maximum emission fluorescence (λ_{max}) is reported. In C and D, the reported errors are the SDs of three replicates.

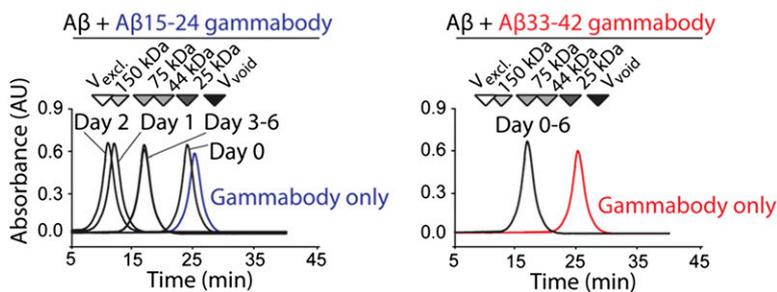


Fig. 3. Gammabodies inhibit amyloid formation by converting A β monomers or fibrillar intermediates into small gammabody–A β complexes. Size-exclusion chromatography analysis of A β 42 (25 μ M) in the presence of gammabodies (2.5 μ M; 1:10 gammabody:A β molar ratio). The chromatograms were obtained using an analytical size-exclusion column (TSK Gel G3000SWxl) and monitored at 280 nm.

of A β peptide segments within such conformers. Therefore, we evaluated the impact of the A β 12–21 and A β 33–42 gammabodies on the relative solvent accessibility of N-terminal (A β residues 3–10), middle (A β residues 18–22), and C-terminal (A β residues 30–36) A β peptide segments during fibrillization using a proteolytic assay that we have reported previously (10). We find that the solvent accessibility of the hydrophilic N terminus of A β is unchanged during A β fibrillization (days 0–6), and that the A β 12–21 and A β 33–42 gammabodies do not alter its solvent accessibility (Fig. S4). In the absence of A β gammabodies, the solvent protection of the hydrophobic C terminus of A β (residues 30–36) progressively increases upon conversion of A β monomers into prefibrillar oligomers (day 1) and fibrillar intermediates (day 2), at which point the A β C terminus fails to become more solvent protected upon conversion into fibrils (days 3–6). The A β 12–21 gammabody converts A β fibrillar intermediates (day 2) into A β conformers (days 3–6) whose C terminus is as unfolded as within A β monomers (Fig. S4). In contrast, the A β 33–42 gammabody maintains the hydrophobic C terminus of A β in an unfolded state without allowing A β to initially form solvent-protected aggregated conformers. Both A β gammabodies also increase the solvent exposure of the central hydrophobic region of A β (residues 18–22) in a similar manner as they do for the A β C terminus. Our findings collectively demonstrate that gammabodies inhibit aggregation either by arresting the conformational maturation of A β monomers or by converting fibrillar intermediates into unfolded conformers that possess biochemical properties indistinguishable from A β monomers.

Gammabodies Inhibit A β Amyloid Assembly by Forming Small Gammabody–A β Complexes. We next sought to determine how substoichiometric concentrations of inhibitory gammabodies (1:10 gammabody:A β molar ratio) render excess A β in a state that is incompetent for amyloid formation. Interestingly, some chaperones, aromatic small molecules, and peptides with antiaggregation activity have also been shown to completely prevent amyloid formation at low substoichiometric concentrations (\leq 1:10 inhibitor: monomer molar ratios) by converting monomers into unstructured, nonamyloid complexes (11–17). Thus, we posited that gammabodies convert A β fibrillar intermediates and monomers into similar complexes that are incompetent for amyloid formation.

To evaluate this hypothesis, we performed size-exclusion chromatography analysis of A β amyloid formation in the absence and presence of gammabodies (Fig. 3). In the absence of gammabodies, A β sticks to the column (TSKgel G3000SWxl; Tosoh Bioscience) regardless of its conformation and fails to elute in nondenaturing buffers. However, gammabody–A β complexes elute as single, symmetric peaks due to the hydrophilicity of gammabodies (Fig. 3). Therefore, we evaluated the increase in size of gammabodies (18–19 kDa) in the presence of A β conformers (1:10 gammabody:A β molar ratio; A β 42 molecular weight is 4.5 kDa) to further elucidate the mechanism used by gammabodies to inhibit amyloid formation. The A β 1–10 gammabody that fails to inhibit amyloid formation (Fig. 2A) does not bind to A β (days 0–6; Fig. S5). In contrast, the A β 15–24 gammabody binds to A β monomers (day 0), prefibrillar oligomers (day 1), and fibrillar intermediates (day 2; Fig. 3). Interestingly, on the third day, the A β 15–24 gammabody converts relatively large gammabody–fibrillar intermediate complexes

(>150 kDa) into small complexes (\sim 75 kDa), and the size of these complexes is invariant for an additional 3 d (longer times not evaluated). The A β 33–42 gammabody rapidly converts A β monomers into small complexes (day 0) that are indistinguishable in size relative to those formed by the A β 15–24 gammabody (days 3–6), and these complexes fail to change size for an additional 5 d (Fig. 3). We obtained similar sizes of gammabody–A β complexes (\sim 75 kDa) using a different size-exclusion column (Superdex 200; GE Healthcare), as well as via cross-linking and SDS/PAGE analysis (\sim 75 kDa; Fig. S5). Finally, size-exclusion analysis also revealed that the A β 15–24 and A β 33–42 gammabodies bind selectively to A β peptide fragments containing their cognate sequences (Fig. S5), suggesting that gammabodies bind to soluble A β via homotypic interactions.

We next investigated the impact of varying the gammabody:A β molar ratio on the size of gammabody–A β complexes. We posited that equimolar concentrations of gammabody and A β would decrease the size of gammabody–A β complexes relative to those formed at substoichiometric gammabody concentrations. However, cross-linking and SDS/PAGE analysis reveals that the size of gammabody–A β complexes is unchanged over a wide range of gammabody:A β molar ratios (1:1–1:20) for the A β 33–42 (Fig. 4) and A β 15–24 (Fig. S6) gammabodies, which we also confirmed via size-exclusion chromatography analysis in the absence of cross-linker (Fig. S6). Strikingly, only the molar ratio of 1:10 gammabody:A β resulted in complete complexation of A β and gammabody, whereas higher gammabody concentrations (1:9–1:1 gammabody:A β molar ratios) resulted in uncomplexed gammabody and lower gammabody concentrations (1:11–1:20 gammabody:A β molar ratios) resulted in uncomplexed A β (Fig. 4 and Fig. S6). We also measured the stoichiometry of the A β 33–42 gammabody

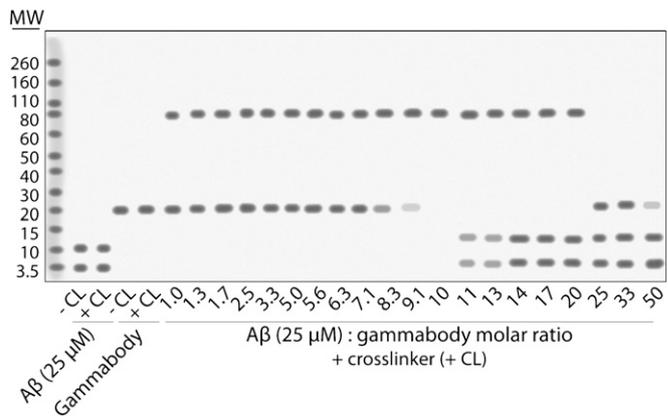


Fig. 4. Stoichiometric analysis of gammabody–A β complexes. A β 42 (25 μ M) was incubated in the presence of the A β 33–42 gammabody at different stoichiometries (1:1–1:50 gammabody:A β molar ratios), the resulting complexes were cross-linked with glutaraldehyde and analyzed via SDS/PAGE and silver staining. As controls, A β and gammabodies were separately combined with cross-linker to demonstrate that neither one was cross-linked in a nonspecific manner.

complexes that elute from size-exclusion columns via fluorescence labeling analysis and find that the stoichiometry of such complexes is 10.9 ± 1.3 A β molecules per gammabody. This stoichiometric analysis yields a size of gammabody–A β complexes (63–68 kDa) that is similar to their measured size (~ 75 kDa; Figs. 3 and 4 and Figs. S5 and S6). Collectively these results suggest that gammabodies form complexes composed of ~ 10 A β peptides per gammabody, and higher (e.g., stoichiometric) gammabody concentrations do not reduce the size of gammabody–A β complexes.

We also evaluated whether gammabody–A β complexes are toxic to mammalian cells (Fig. 5 and Fig. S6). In the absence of gammabodies, A β prefibrillar oligomers (day 1) are most toxic and A β monomers (day 0) are least toxic at high A β concentrations (2.5 μ M; Fig. 5 and Fig. S6), whereas only prefibrillar oligomers are toxic at lower A β concentrations (0.1 μ M; Fig. S6). Moreover, the A β gammabodies are nontoxic (Fig. 5 and Fig. S6). Addition of the A β 15–24 gammabody fails to inhibit the toxicity of A β monomers (day 0) or prefibrillar oligomers (day 1) but inhibits the toxicity of fibrillar intermediates (day 2). Importantly, the gammabody–A β complexes formed by the A β 15–24 (days 3–6) and A β 33–42 (days 0–6) gammabodies are nontoxic (Fig. 5, and Fig. S6). We conclude that gammabodies inhibit amyloid formation by converting A β into small complexes that are benign.

Conventional Sequence- and Conformation-Specific Antibodies Fail to Potently Inhibit A β Amyloid Formation. We suspected that the potent inhibitory activity of A β gammabodies is linked to their unusual mode of interaction with the hydrophobic peptide segments that mediate A β fibril formation. This led us to posit that conventional A β antibodies would be unable to inhibit A β fibrillization at similar low substoichiometric concentrations (1:10 antibody:A β molar ratio) because such antibodies would either only sequester a small fraction of A β monomer (sequence-specific antibodies) or fail to bind to linear hydrophobic A β epitopes that mediate aggregation (conformation-specific antibodies).

To test these hypotheses, we evaluated the antiaggregation activity of three sequence-specific monoclonal antibodies against the N-terminal (A β residues 3–10; 6E10), middle (A β residues 18–22; 4G8), and C-terminal (A β residues 35–39; 9F1) regions of A β , as well as two conformation-specific polyclonal antibodies against prefibrillar oligomers (A11) and fibrillar conformers (OC; Fig. 6 and Fig. S7). Importantly, none of these antibodies inhibit A β fibrillization at substoichiometric concentrations (1:10 antibody:A β molar ratio). Size-exclusion chromatography analysis confirmed that each noninhibitory antibody bound to A β in the expected manner (Fig. S7). Although the conformation-specific polyclonal antibodies A11 and OC fail to arrest amyloid formation, their binding to A β oligomers (A11) and fibrillar conformers (OC) inhibits toxicity (Fig. 6). The sequence-specific A β antibodies (6E10, 4G8, and 9F1) fail to prevent toxicity at the same substoichiometric concentrations (Fig. 6), as expected on the basis of the small fraction of A β sequestered by such

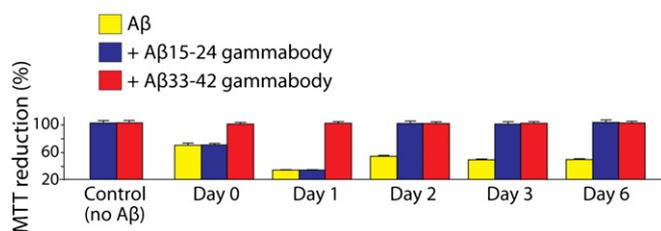


Fig. 5. Gammabodies inhibit A β -mediated cytotoxicity. A β 42 (25 μ M) was incubated in the absence (control) and presence of gammabodies (2.5 μ M; 1:10 gammabody:A β molar ratio), and the toxicity of gammabody–A β samples to PC12 cells was evaluated via an MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay. The final A β and gammabody concentrations after dilution into the cell culture media were 2.5 and 0.25 μ M, respectively. The reported errors are the SDs of three replicates.

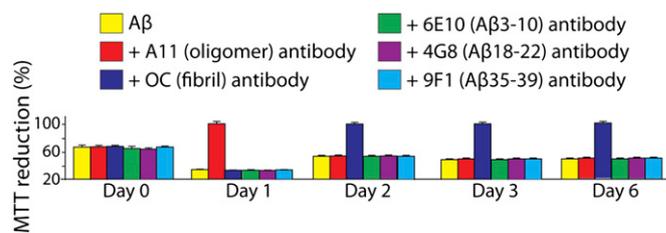


Fig. 6. Impact of substoichiometric concentrations of conventional sequence- and conformation-specific antibodies on A β -mediated cytotoxicity. A β 42 (25 μ M) was incubated in the absence (control) and presence of substoichiometric concentrations (2.5 μ M; 1:10 antibody:A β molar ratio) of monoclonal sequence-specific (6E10, A β residues 3–10; 4G8, A β residues 18–22; 9F1, A β residues 35–39) and polyclonal conformation-specific (A11, prefibrillar oligomers; OC, fibrillar conformers) antibodies. The antibody–A β samples were diluted 10-fold into PC12 cell cultures, and the toxicity was evaluated via an MTT reduction assay. The reported errors are the SDs of three replicates.

antibodies before oligomerization (Fig. S7). Our findings demonstrate that conventional conformation-specific (polyclonal) antibodies bind to aggregated A β conformers and prevent their toxicity without inhibiting amyloid formation.

IAPP and α -Synuclein Gammabodies Potently Inhibit Amyloid Formation in a Sequence-Specific Manner. The ability of A β gammabodies to potently inhibit amyloid formation led us to investigate whether gammabodies could be designed to inhibit fibrillization of other amyloidogenic polypeptides. Therefore, we selected the peptide hormone IAPP that forms amyloidogenic aggregates associated with type 2 diabetes (18), and the protein α -Synuclein that forms aggregates linked to Parkinson's disease (19). We identified 10-residue amyloidogenic peptide segments in IAPP (residues 22-NFGAILSSTN-31) and α -Synuclein (residues 69-AVVTGVTAVA-78) that are predicted to mediate amyloid formation of each polypeptide by multiple algorithms (20–24). Grafting these peptide segments into CDR3 (along with negatively charged residues at each edge of CDR3; *SI Methods*) yielded single-domain (V_H) gammabodies that are well-expressed (>20 mg/L) and fail to aggregate when heated. Each gammabody binds with higher affinities to its cognate fibrils (IC_{50} values of 204 ± 7 and 222 ± 10 nM for the IAPP and α -Synuclein gammabodies, respectively; K_d values of 1.37 ± 0.05 and 1.40 ± 0.08 μ M for the IAPP and α -Synuclein gammabodies, respectively) than to its monomers (IC_{50} values >3 μ M), and fails to cross-react with soluble or aggregated polypeptides that lack the corresponding peptide segments (Fig. S8).

We next evaluated the ability of the IAPP and α -Synuclein gammabodies to inhibit amyloid formation of each polypeptide at substoichiometric concentrations (Fig. 7 and Figs. S8 and S9). Strikingly, the IAPP and α -Synuclein gammabodies inhibit fibrillization in a sequence-specific manner at both 1:10 (Fig. 7) and 1:1 (Fig. S8) gammabody:monomer molar ratios. Single proline substitution or glycine insertion mutations in the grafted peptide segments eliminate the inhibitory activity of each gammabody (Fig. S8). We also find that the IAPP and α -Synuclein gammabodies inhibit amyloid formation by rapidly converting their respective amyloidogenic polypeptides into small complexes (<100 kDa) that are incompetent for amyloid formation (Fig. S9), as observed for the A β 33–42 gammabody (Fig. 3). Moreover, the conformation of IAPP and α -Synuclein in these small complexes is indistinguishable from the corresponding monomeric polypeptides, and the complexes are nontoxic (Fig. S9). Importantly, conventional sequence- and conformation-specific antibodies against α -Synuclein and IAPP that were obtained via immunization are noninhibitory at substoichiometric concentrations (1:10 antibody:monomer molar ratio; Fig. 7 and Fig. S9). We conclude that domain antibodies displaying amyloidogenic peptide sequences within their CDRs are more potent inhibitors of amyloid formation than typical conventional antibodies obtained via immunization.

IAPP are capable of potentially inhibiting amyloid formation when grafted into antibody loops. However, both α -Synuclein and IAPP contain additional peptide segments that are predicted to be amyloidogenic (e.g., IAPP residues 12–18 and α -Synuclein residues 85–95), which we would also expect to inhibit fibrillization when grafted into similar antibody loops. Moreover, we expect that grafting more than one amyloidogenic peptide into multiple loops within single- and multidomain antibodies will yield gammabodies with even higher potency for inhibiting amyloid formation. The ease of generating gammabodies will allow these and related hypotheses to be rapidly evaluated to further define principles for designing highly potent inhibitors of amyloid formation.

Methods

A β 42 peptide (American Peptide) was dissolved in 100% hexafluoroisopropanol (HFIP; Fluka), and the HFIP was evaporated overnight. A β was then dissolved in 50 mM NaOH (1 mg/mL A β), sonicated (30 s), and diluted in PBS (25 μ M A β). The peptide was incubated at 25 °C for 0–6 d. IAPP was synthesized and pu-

rified as described previously (39), and then dissolved in HFIP. After removal of HFIP, IAPP was dissolved in 20 mM Tris-HCl (pH 7.4) at 32 μ M and incubated at 25 °C for 0–4 d. α -Synuclein (residues 1–140) and a fragment thereof (residues 1–115) were expressed in bacteria, purified as described previously (40), diluted into buffer [20 mM HEPES, 0.1 M NaCl (pH 7.4)] at 50 μ M (α -Synuclein residues 1–115) and 100 μ M (α -Synuclein residues 1–140), and agitated (500 rpm) at 37 °C for 0–7 d.

Additional methods are described in *SI Methods*: cloning, expression and purification of gammabodies, immunoblot analysis, ThT and ANS fluorescence, AFM imaging, cell toxicity analysis, size-exclusion chromatography, cross-linking and SDS/PAGE analysis, circular dichroism spectroscopy, and proteolytic analysis.

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