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A Chemical Screening Approach Reveals that Indole Fluorescence is Quenched by Pre-Fibrillar But Not Fibrillar Amyloid- β

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Abstract

Aggregated amyloid- β (A β) peptide is implicated in the pathology of Alzheimer's disease. *In vitro* and *in vivo*, these aggregates are found in a variety of morphologies, including globular oligomers and linear fibrils, which possess distinct biological activities. However, known chemical probes, including the dyes thioflavin T and Congo Red, appear to lack selectivity for specific amyloid structures. To identify molecules that might differentiate between these architectures, we employed a fluorescence-based interaction assay to screen a collection of 68 known A β ligands against pre-formed oligomers and fibrils. In these studies, we found that the fluorescence of five indole-based compounds was selectively quenched (~15%) in the presence of oligomers, but remained unchanged after addition of fibrils. These results suggest that indoles might be complementary to existing chemical probes for studying amyloid formation *in vitro*.

Keywords

Alzheimer's disease; neurodegeneration; protein misfolding; thioflavin T; fluorescence

Introduction

Alzheimer's disease (AD) is a severe neurodegenerative disorder characterized by the age-dependent aggregation of amyloid- β (A β) peptide in the brain.^{1,2} A β self-assembles into distinct conformations both *in vitro* and *in vivo*, giving rise to structures such as globular oligomers and linear fibrils.^{1,3} Despite being composed of the same monomer peptide, these conformations are strikingly different in shape and size.^{4–6} In addition to these architectural differences, evidence from both cell culture and animal models indicates that oligomers are more neurotoxic than fibrils.^{7–12} For example, A β oligomers permeate lipid membranes more readily than fibrils, a function that is thought to be involved in the neurotoxicity in AD.^{13–15}

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Supporting Information Available

The following supplementary material is available for this article: Figure S1: The chemical structures of the library members and the fluorescence screening results. Figure S2: Indole does not inhibit A β aggregation.

Further, oligomers disrupt long-term potentiation and impair memory in AD mouse models.^{16,17} Although it is clear that different conformations of A β exert independent biological activities, the structural basis for these unique properties has not been established.

Conditions such as temperature, time, salinity, and pH, have been established that promote the progression of A β monomers into predominantly oligomers or fibrils *in vitro*.^{4,18} The oligomers formed in this way share the properties of soluble A β preparations from AD patients, namely high levels of toxicity and spherical appearance by transmission electron microscopy (TEM) and atomic force microscopy (AFM).^{6,19,20} One of the powerful uses of fabricated oligomers is in studies of their structure. For example, recent NMR and hydrogen-deuterium exchange (HDE) reports show that A β oligomers are predominantly composed of β -sheets, but that the exposure of side chains as well as the packing of this β -sheet character in these species is distinct from fibrils.^{12,21,22} These studies also show that oligomers are less stable than fibrils, which are known to be more densely packed and resistant to denaturation. Collectively, these reports suggest that different A β conformations possess unique structural and biological properties. Yet, the molecular features that distinguish A β oligomers from fibrils have not been clearly established, and, surprisingly, reagents that discriminate between these structures are few.^{23–25} However, it is clear that cellular components (*e.g.* proteins, lipids) are somehow able to distinguish between oligomers and fibrils and, thus, it is important to identify how differences in their molecular surfaces might be recognized.

The spectral properties of small molecules, such as thioflavin T (ThT) and Congo Red (CR), are influenced by aggregated A β and, thus, these probes are often employed to quantify A β self-assembly.^{26–28} Recent models predict that these ligands interact with the pleated β -sheets of self-assembled A β .²⁹ Interestingly, although these compounds can readily detect the extent of aggregation, they do not distinguish between A β conformations.^{30,28} This finding might be expected, based on the similarities in the secondary structure of A β oligomers and fibrils; however, Necula *et al.* recently reported that certain chemical inhibitors selectively block formation of A β oligomers.^{24,23} These results suggest that small molecules might exploit subtle differences in architecture to differentially engage pre-fibrillar and fibrillar A β . To test this hypothesis, we screened 68 structurally diverse small molecules against pre-formed oligomers and fibrils in a fluorescence assay and, interestingly, discovered that the intrinsic fluorescence of five indole-containing compounds was sensitive to A β oligomers but not fibrils. To our knowledge, these are the first small molecules able to distinguish between preformed A β oligomers and fibrils.

Results

Preparation and Characterization of A β (1–42) Fibrils and Oligomers

We were interested in testing whether small molecules could differentiate between oligomers and fibrils. Toward this goal, we employed known conditions to generate relatively homogeneous populations of A β oligomers and fibrils.^{4,18,6} Briefly, A β (1–42) oligomers were prepared by incubation in DMEM-F12 at 4 °C, and fibrils were prepared by incubating in PBS at 37 °C (see Experimental Procedures). After 48 hours, these samples were analyzed by transmission electron microscopy (TEM), which confirmed that oligomer samples were free of the elongated, linear structures commonly observed in the fibril preparations (Figure 1A). To independently confirm these findings, the samples were cross-linked using glutaraldehyde and analyzed by non-denaturing gel electrophoresis followed by Western blotting using an anti-A β antibody (6E10) (Figure 1B). Consistent with previous reports,⁶ oligomer solutions lack the high molecular weight species that are present in the fibril preparation. Finally, the relative stabilities of the structures were probed by denaturation. Because the A β (1–42) peptide lacks a convenient tryptophan for monitoring integrity, we employed ThT reactivity to follow the response of the amyloid structure to denaturant. These studies confirmed that A β oligomers

are less stable than fibrils ($EC_{50} = 3.1 \pm 1.0$ M for oligomers and 8.0 ± 2.0 M for fibrils; Figure 1C), generally consistent with previous findings.^{12,21}

Bis-ANS fluorescence is increased by both A β oligomers and fibrils

Although the common dyes, thioflavin T (ThT) and Congo Red (CR), are unable to differentiate between A β oligomers and fibrils,^{28,26} we first considered whether another common fluorescent probe, Bis-ANS, might possess this activity (Figure 2A). Bis-ANS fluorescence increases upon binding hydrophobic regions of proteins, and is therefore widely employed to probe this property.^{31,32} Based on this literature, we reasoned that Bis-ANS may reveal differences between in A β oligomers and fibrils. To test this model, we measured changes in Bis-ANS fluorescence in the presence of pre-formed A β (1–42) structures. In these experiments, fluorescence increased 4-fold upon addition to A β fibrils, consistent with previous studies.^{33–35} However, we found that the fluorescence increase in the presence of A β oligomers was indistinguishable from the fibril-induced response (Figure 2B and C). Thus, Bis-ANS does not discriminate between oligomers and fibrils, suggesting that it interacts with either a shared structural element or that it is otherwise insensitive to differences between A β structures.

Fluorescence screen for A β conformation-specific small molecules

Because Bis-ANS failed to distinguish between A β conformations, we turned to a screening approach. Specifically, we collected a library of 68 structurally diverse small molecules, which included more than 11 distinct chemical scaffolds, including sulfonated dyes, curcumin, phenothiazines, tetracyclines, benzophenones, monophenyls, flavonoids, indoles, chalcones, azo dyes and quinones (Supplemental Figure 1). These compounds were largely selected based on their reported ability to inhibit A β aggregation or bind A β fibrils,^{23,24,36–38} in the hopes that these probes might be enriched for those with the potential to recognize features unique to A β structures. Importantly, in the selection of these compounds, we also favored low molecular mass (< 500 Da) compounds because we were interested in understanding the response of amyloids to small, organic probes. Finally, many of the chosen compounds contained conjugated ring systems that, like Bis-ANS and ThT, might provide convenient fluorescence signatures.

Guided by these principles, we screened the chemical collection for fluorescence changes in the presence of preformed A β oligomers or fibrils (Figure 3). For most of the compounds, we found that their fluorescence was insensitive to amyloids. This result is not interpreted as a failure to bind, only a failure of A β to impact the compound's fluorescence. Other members of the collection, such as **16** and **76**, displayed behaviors reminiscent of Bis-ANS; their fluorescence was indiscriminately altered in the presence of both oligomers and fibrils. However, we found that the fluorescence of nine compounds (**1**, **25**, **45**, **57**, **58**, **62–64** and **82**) was sensitive to either fibrils or oligomers. Interestingly, five of these compounds (**57**, **58**, **62–64**) contained an indole and their fluorescence was partially quenched in the presence of A β oligomers but not fibrils. To our knowledge, these are the first molecules shown to discriminate between pre-formed amyloid forms *in vitro*. Based on the structural similarity between five of the nine active compounds, we further explored the activity of indole (**58**).

Indole fluorescence is quenched by A β oligomers but not fibrils

To confirm the screening result, we performed a spectral scan on indole (**58**). Consistent with the finding at a single wavelength, no significant change in the indole spectra was observed upon addition of 25 μ M A β (1–42) fibrils (Figure 4A). However, the excitation and emission intensities decreased in the presence of 25 μ M oligomers (Figure 4B); excitation was quenched by approximately 8% at 280 nm and emission at 350 nm was reduced by 17%. After exposure to aggregated A β , the fluorescence of other amyloid probes, such as ThT, requires a short

incubation to achieve maximal signal.^{26,27} To explore the kinetics of indole quenching, we monitored the fluorescence immediately after addition of A β (Figure 4C). Specifically, indole was added to oligomers, fibrils, or low molecular weight A β peptide (LMW-A β) and fluorescence was recorded every 30 seconds for one hour. LMW-A β contains no visible aggregates by TEM analysis (data not shown) and we used this preparation to exemplify pre-oligomeric species. To compare the samples, we expressed the results as the percent change in total indole fluorescence. Using this approach, we found that both oligomer and LMW-A β quenched fluorescence, and that this effect reached a maximum of 12% after 15 minutes. Consistent with our previous experiment, only a minor (0.5%) change was observed in the fibril sample. Using a one-phase exponential fit, we calculated the observed rate constants (k_{obs}) and showed that LMW-A β ($0.130 \pm 0.002 \text{ min}^{-1}$) and oligomers ($0.272 \pm 0.011 \text{ min}^{-1}$) share similar kinetics. Together, these findings suggest the presence of a binding site that is exposed in both oligomeric and LMW-A β architectures but inaccessible or otherwise unavailable in fibrils.

Because TEM analysis shows that fibril preparations contain a fractional amount of oligomers after two days (data not shown), we hypothesized that the minor quenching observed in the fibril sample might be due to contaminating oligomers or LMW-A β structures. Given that the prevalence of these species decreases with incubation time, we permitted fibril preparations to aggregate for 17 days prior to recording the change in indole fluorescence (Figure 4D and 5D). Using this approach, we found that the quench is insignificant in the aged samples, consistent with minor LMW or oligomer contribution at earlier incubation times.

Characterization of indole quenching by A β oligomers versus fibrils

To further characterize the interaction between the parent compound indole (**58**) and A β conformations, we varied parameters of the fluorescence assay, including pH, indole concentration and structure, A β incubation time, and A β concentration (Figure 5). In these experiments, we observed no significant trends over the pH range tested (pH 2.2–8.2), which is expected based upon the pK_{a} of indole (~ 21 in DMSO) (Figure 5A).³⁹ When indole concentration was increased, an overall decrease in activity was observed, an effect that is likely caused by self-quenching interactions at high concentrations (Figure 5B). Interestingly, the substituted indoles (**57**, **58**, **62–64**) behaved similarly in the presence of oligomers, suggesting that oligomers are able to accommodate indoles decorated with various functional groups (Figure 5C). To explore the sensitivity of the interaction, we determined the lowest concentration of oligomers at which we could detect quenching of indole (**58**) and found that this value was between 5–15 μM (Figure 5E). Finally, amyloid experiments are often subject to heterogeneity in sample preparations, so we wanted to explore whether the observed indole activity was reproducible across independent samples. To test this idea, four separate aliquots of oligomers and fibrils were prepared and we found good reproducibility (less than 5% variance) between these trials (Figure 5F). Finally, using TEM and ThT approaches, we found that indole has no effect on aggregation of fresh A β (Supplementary Figure 2). Together, these experiments help define the experimental parameters for indole interactions with A β oligomers.

Small amounts of A β oligomer in fibril preparations can be detected by indole

Common amyloid probes, such as ThT, cannot distinguish between amyloid forms and, therefore, they can't be used to quantify the percentage of oligomer content in a heterogeneous amyloid preparation. Towards that goal, we were interested in testing whether quenching of indole fluorescence by oligomers could be detected in a background of fibrils. Specifically, oligomers and fibrils were prepared separately and then mixed at known ratios, while maintaining the total protein concentration (Figure 5G). Using this approach, we observed that even a minor percentage of oligomer ($\sim 25\%$) could be measured in a mixed population. One interesting aspect of these results was that there was a delay in the quenching of samples that

contained fibrils. Specifically, we found that the length of the lag phase was proportional to the percentage of fibrils present, suggesting that, despite failure to trigger a quench, indole might transiently interact with fibrils. Because ThT and indole do not compete for the same site (see Supplemental Information), these probes might be used in concert to study amyloid formation. It is important to note that experiments in this area would likely be restricted to *in vitro* studies because indole is known to bind non-amyloid targets that would be present in biological settings.

Discussion

To date, different A β aggregates have been largely defined by their solubility, appearance in TEM, reactivity with select antibodies, and their differential neurotoxicity. The goal of this effort was to determine if small molecules could differentiate between these preformed structures. We expected that molecules with this property could be used in studies of amyloid formation *in vitro* – much like how ThT and its derivatives are used to monitor the extent of aggregation. Towards this end, we employed A β samples enriched for oligomers or fibrils and screened 68 structurally diverse small molecules. These studies revealed that five indole-containing compounds were sensitive to pre-fibrillar structures but not fibrils. In contrast, the majority of chemical probes, such as Bis-ANS and Congo Red, failed to differentiate between them under these conditions. These results suggest that indoles might be complementary to existing chemical probes for studying amyloid formation *in vitro*.

Experimental Section

Materials

Amyloid- β (1–40) and (11–40) peptides were purchased from Anaspec (San Jose, CA). A β (1–42) peptide was purchased from EZBiolab (Westfield, IN). DMSO and HFIP were purchased from Sigma-Aldrich. Compounds used in the fluorescence screen were synthesized internally³⁸ or purchased from Sigma-Aldrich, Fluka, Fisher Scientific, and Cayman Chemicals (Ann Arbor, MI). Anti-amyloid β antibody (6E10) was purchased from Calbiochem (San Diego, CA). Secondary antibodies were purchased from Bio-Rad (Hercules, CA). All fluorescence readings were taken on a SpectraMax M5 multi-mode plate reader (Molecular Devices, Sunnyvale, CA).

Amyloid- β Stock Preparation

A one milligram samples of A β (1–42) peptide were dissolved in 200 μ L hexafluoroisopropanol (HFIP) and aliquoted to obtain 0.1 mg stocks. HFIP was removed under nitrogen to provide a thin film and these stocks were stored at -30°C until ready for use. Immediately prior to the start of each experiment, an aliquot was dissolved in DMSO (see below). Fibrils were obtained by adding phosphate buffered saline (PBS; pH 7.4) to a final concentration of 25 μM (1% final DMSO concentration). These solutions were vortexed, sonicated for 75 seconds, and agitated for 48 hours at 37°C . Oligomers were obtained by adding DMEM-F12 media (Gibco) to a final concentration of 25 μM (1% final DMSO concentration), followed by vortexing, sonicating for 75 seconds, and incubating for 48 hours at 4°C without agitation. LMW-A β was obtained by adding PBS (pH 7.4) to a final concentration of 25 μM (1% final DMSO concentration), vortexing, sonicating for 75 seconds and using these samples immediately.

Transmission Electron Microscopy

Freshly suspended A β or aggregated sample (25 μM ; 5 μL) was added to glow-discharged, Formvar/carbon 300-mesh copper grids (Electron Microscopy Sciences, Hatfield, PA) and incubated for 1.5 minutes at room temperature. Excess sample was blotted off with filter paper and each grid was washed twice with ddH₂O. Uranylacetate (1%, 3 μL) was added to each grid

and incubated for one minute. Excess sample was blotted off and grids were then dried for 15 minutes. Samples were visualized on a Phillips CM-100 transmission electron microscope at 80 kV and 94,000 $\times$ magnification.

Native Gel Electrophoresis and Western blotting

A β fibrils and oligomers were prepared at 25 μ M as described and cross-linked using glutaraldehyde (0.04% final concentration) for 40 minutes at room temperature. The reaction was quenched with glycine pH 8.2 (final concentration of 10 mM) and SDS-loading dye (non-reducing) was added (25% final volume). Each sample (8 μ g) was separated on a 10–20% gradient tris-tricine gel (Bio-Rad), and the gel was then transferred to a nitrocellulose membrane. The nitrocellulose was blocked in 10% non-fat dried milk in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBS-T) for one hour at room temperature, incubated in anti-A β 6E10 antibody (1:1000) in 3% BSA in TBS-T for one hour at room temperature, and probed with HRP-conjugated goat anti-mouse antibody (1:10,000) in 3% BSA in TBS-T for one hour at room temperature. Protein bands were visualized using the Bio-Rad ECL kit according to the manufacturer's instructions.

Urea denaturation of preformed A β aggregates

A β fibrils and oligomers were prepared at (25 μ M) as described and an aliquot (10 μ L) dispensed into 96-well plate format (black Corning). Urea (30 μ L) at varying concentrations (0 M – 6 M) was added to these samples, mixed thoroughly, and the solutions were incubated for 40 minutes at room temperature. Following this treatment, 200 μ L ThT (5 μ M in 50 mM glycine, pH 8.2) was added and these samples were then incubated for 15 minutes and the fluorescence recorded (E_x 446 nm; E_m 490 nm). Control experiments confirmed that urea did not impact the intrinsic fluorescence of the ThT reagent and TEM experiments confirmed a disruption in ultrastructure (not shown).

Bis-ANS Fluorescence

4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonate (Bis-ANS) (100 μ L; 25 μ M in 30 mM citrate pH 2.4, 1% DMSO) was added to 9 μ L A β fibrils or oligomers (25 μ M) or 9 μ L PBS or DMEM-F12 (1% DMSO) in a black 96-well plate (Corning Costar). Fluorescence was measured after 2 minutes (E_x 385 nm, E_m 520 nm, cutoff 515 nm). Experiments were performed using six replicates. Background fluorescence of fibrils, oligomers, PBS, and DMEM-F12 in the presence of 100 μ L buffer alone (30 mM citrate pH 2.4, 1% DMSO) was subtracted.

Fluorescence Screen

Each compound was dissolved in DMSO to a final concentration of 100 mM and then diluted to 50 μ M with ddH₂O (1% final DMSO concentration). 100 μ L of each compound was added to 9 μ L A β fibrils or oligomers (25 μ M) or 9 μ L PBS or DMEM-F12 (1% DMSO) in triplicate to a black 96-well plate and incubated for 10 minutes. Fluorescence spectra were then recorded at four excitation and emission values: *a*) E_x 290 nm, E_m 320–520 nm; *b*) E_x 350 nm, E_m 380–650 nm; *c*) E_x 400 nm, E_m 430–620 nm; *d*) E_x 450, E_m 480–650. Background fluorescence of fibrils, oligomers, PBS, and DMEM-F12 in the presence of 100 μ L ddH₂O only (1% DMSO) was subtracted. For indole time-course experiments, the fluorescence of six replicates was measured every 30 seconds for 1 hour (E_x 280 nm, E_m 350 nm, cutoff 325 nm) and this data was fit using a one-phase exponential association in GraphPad Prism software. For the detailed studies of the indoles (**57**, **58**, **62–34**) (5-methylindole-2-carboxylic acid, indole, melatonin, tryptamine, and tryptophan), each compound was dissolved in DMSO to a final concentration of 100 mM and diluted to 100 μ M in 50 mM glycine pH 8.2 (1% final DMSO concentration). To determine pH effects, pH was varied in either PBS (pH 2.2–7.0) or 50 mM glycine (pH 8.2). For the oligomer titration experiment, fibrils and oligomers were

prepared as described and mixed in varying percentages (0, 5, 10, 25, 50, 75, 95, and 100% oligomer), while maintaining a constant protein concentration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AD, Alzheimer's disease; A β , amyloid- β peptide; HCR, hydrophobic core region; TEM, transmission electron microscopy; AFM, atomic force microscopy; LMW, low molecular weight; 5-MI-2-CA, 5-methylindole-2-carboxylic acid; CR, Congo Red; ThT, thioflavin T; PET, positron emission tomography.

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A) Fibrils and oligomers have distinct ultrastructures by TEM

B) Fibril preparations contain high MW structures by native gel electrophoresis

C) Oligomers are more sensitive to chemical denaturation than fibrils

Figure 1. Characterization of the differences in shape, size, and stability of A β (1–42) fibrils and oligomers

A) Analysis of 25 μ M A β (1–42) fibrils (left) and oligomers (right) by transmission electron microscopy (TEM). Fibrils were prepared in PBS at 37 °C; oligomers were prepared in DMEM-F12 at 4 °C (see Experimental Procedures). Samples were incubated for 48 hours. Scale bar = 100 nm. B) Native gel electrophoresis and Western blot of fibrils and oligomers using anti-A β antibody (6E10). Oligomer preparations lack the high molecular weight material present in fibrils (red box). C) Oligomers are less stable than fibrils. Chemical denaturation of preformed aggregates with urea shows that A β oligomers are more sensitive to destabilizing conditions than fibrils.

A) Chemical structure of Bis-ANS

B) The fluorescence of Bis-ANS is enhanced by both fibrils and oligomers

Figure 2. Bis-ANS fluorescence increases upon addition of both A β (1–42) oligomers and fibrils
A) Chemical structure of Bis-ANS. B) Fluorescence of 25 μ M Bis-ANS (in 30 mM citrate pH 2.4) alone and in the presence of 25 μ M A β fibrils or oligomers (Ex 385 nm, Em 520 nm). Fluorescence of fibrils and oligomers alone is shown for comparison. Error is expressed as the standard deviation of six replicates. C) Fluorescence spectra of Bis-ANS alone and in the presence of oligomers or fibrils (Ex 350–450 nm, Em 520; Ex 385 nm, Em 450–600 nm). For each experiment, fluorescence was read 2 minutes after A β addition.

#			#			#		
fibrils			fibrils			fibrils		
oligomers			oligomers			oligomers		
congo-red-like class	1		29			57		
	2		30			58		
	3		31			59		
	4		32			60		
	5		33			61		
	6		34			62		
	7		35			63		
	8		36			64		
	9		37			65		
	10		38			66		
	11		39			67		
	12		40			68		
	13		41			69		
	14		42			70		
	15		43			71		
	16		44			72		
	17		45			73		
	18		46			74		
	19		47			75		
phenothiazines	20		48			76		
	21		49			77		
	22		50			78		
	23		51			79		
	24		52			80		
tetracyclines	25		53			81		
	26		54			82		
	27		55			83		
	28		56			84		

	non-fluorescent
	< 4% change in intrinsic fluorescence
	decrease in intrinsic fluorescence
	increase in intrinsic fluorescence

Figure 3. Screen for A β conformation-specific small molecules

The fluorescence of each compound (100 μ M) was recorded alone or in the presence of 25 μ M A β (1–42) fibrils or oligomers. Four wavelength ranges were used: Ex 290 nm, Em 320–520 nm; Ex 350 nm, Em 380–650 nm; Ex 400 nm, Em 430–650 nm; Ex 450 nm, Em 480–650 nm. Compounds were scored if changes in fluorescence behavior were observed in any of these regimes. Based on this analysis, compounds were categorized as weakly fluorescent (light gray boxes), fluorescent, but with minimal (< 4%) sensitivity for A β (dark gray boxes), or fluorescent and sensitive (>4%) for A β (red box for quenched signal; green box for increased signal). See the Supplemental Information for compound names, chemical structures and raw values.

A) The fluorescence of indole (**58**) does not change in the presence of A β fibrils

B) The fluorescence of indole (**58**) is quenched by A β oligomers

C) Fluorescence quenching by LMW and oligomeric A β is time-dependent

D) Indole fluorescence at 30 minutes

Figure 4. Indole fluorescence is quenched by A β (1–42) oligomers but not fibrils

Excitation and emission spectra of indole in the absence and presence of 25 μM A β (1–42) (A) fibrils and (B) oligomers (Ex 250–320 nm, Em 350; Ex 280 nm, Em 310–410 nm).

Fluorescence was read 30 minutes after A β addition. C) Indole fluorescence was monitored kinetically in the presence of low molecular weight (LMW) A β , oligomers, and fibrils (Ex 280 nm, Em 350). Results are plotted as percent quench in fluorescence. D) Percent decrease in indole fluorescence following a 30 minute incubation with LMW A β , oligomers, day 2 fibrils, or day 17 fibrils. In panels C and D, error is expressed as the standard deviation of six replicates.

Figure 5. Characterization of indole quenching by Aβ oligomers versus fibrils

(A–E) Indole fluorescence was recorded following a 30 minute incubation with Aβ (1–42) oligomers or fibrils (Ex 280 nm, Em 350 nm) and all results are expressed as percent decrease in indole fluorescence. Unless noted, indole and Aβ concentrations remained constant at 100 μM and 25 μM, respectively, in 50 mM glycine (pH 8.2). In panel A, indole was prepared in PBS or *50 mM glycine. In panel F, four aliquots of Aβ fibrils and oligomers were prepared independently to test the reproducibility of the indole fluorescence quenching. In panel G, oligomers were titrated into fibril preparations and the percentages indicate the amount of oligomer added to fibrils (0% = fibril only; 100% = oligomer only). For all experiments, errors bars are expressed as the standard deviation of three replicates. (5-MI-CA = 5-methylindole-2-carboxylic acid).