Published in final edited form as:

Chembiochem. 2017 November 16; 18(22): 2205–2211. doi:10.1002/cbic.201700387.

Fluorescent Probe DCVJ Shows High Sensitivity for Characterization of Amyloid β -Peptide Early in the Lag Phase

Dr. Sureshbabu Nagarajan^{[a],[c]} and Prof. Lisa J. Lapidus^{[a],[b]}

^[a]Department of Physics and Astronomy, Michigan State University, 567 Wilson Road, Room 4227, East Lansing, MI 48824 (USA)

^[b]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824 (USA)

^[c]Centre for Drug Discovery and Development, Sathyabama University, Chennai, Tamil Nadu 600119 (India)

Abstract

The aggregation of intrinsically disordered and misfolded proteins in the form of oligomers and fibrils plays a crucial role in a number of neurological and neurodegenerative diseases. Currently, most probes and biophysical techniques that detect and characterize fibrils at high resolution fail to show sensitivity and binding for oligomers. Here, we show that 9-(dicyanovinyl)julolidine (DCVJ), a class of molecular rotor, binds amyloid beta ($A\beta$) early aggregates, and we report the kinetics as well as packing of the oligomer formation. The binding of DCVJ to $A\beta$ 40 increased its emission intensity with time at 510 nm and produced a second excimer peak at 575 nm. However, DCVJ did not bind to the prefibrillar aggregates of $A\beta$ 42, which indicated that the oligomers formed by $A\beta$ 40 and $A\beta$ 42 were not the same. The F4C F19W mutant of $A\beta$ 40, which did not form fibrils, also bound DCVJ, but the emission spectral profile varied from that of the wild-type (WT). Atomic force microscopy images of WT $A\beta$ 40, the F4C F19W mutant, and $A\beta$ 42 oligomers displayed differences in size and shape, confirming the difference in their DCVJ spectra. The effect of epigallocatechin-3-gallate (EGCG) on the reduction of $A\beta$ 42 fibrils was also observed with finer detail than with other techniques. The results of this study show that DCVJ detects early aggregates and provides valuable information regarding the oligomer kinetics, packing, and mechanism of formation.

Keywords

Alzheimer's disease; amyloid beta-peptides; DCVJ; lag phase; oligomers

Correspondence to: Lisa J. Lapidus.

Conflict of Interest

The authors declare no conflict of interest.

Supporting information and the ORCID identification numbers for the authors of this article can be found under <https://doi.org/10.1002/cbic.201700387>.

Introduction

The presence of amyloid aggregates in the form of fibrils has been associated with diseases such as Alzheimer's, Parkinson's, prion, cardiac amyloidosis, and diabetes.^[1–4] Although amyloid fibril deposits are linked to disease, the oligomers or the pre-fibrillar aggregates that are formed during the pre-transition growth phase or in the lag phase of the aggregation have been shown to be more toxic than fibrils or monomers.^[5–8] The kinetics of amyloid fibril formation in vitro follow a sigmoidal curve that has lag (nucleation), growth, and saturation phases, as detected by NMR, fluorescence, circular dichroism (CD), IR, and other biophysical methods.^[9–12] Several probes are available to detect, monitor, and characterize the growth and saturation phases.^[13,14] However, monomers and lower oligomers present predominantly in the lag phase are highly dynamic and heterogeneous in nature and render it very difficult to observe through any real-time techniques.^[15,16] Consequently, most of the drug development assays reveal a compound's activity to reduce inert fibril formation rather than toxic oligomer formation.^[17] Hence, there is a pressing need to develop a probe that is sensitive to the early phase of aggregation.

In this work, we tested several fluorescence probes previously reported for sensitivity to a change in microenvironment and aggregation during the A β lag phase (Figure S1 in the Supporting Information). Of these, only DCVJ fluorescence showed a drastic spectral change and provided high sensitivity for oligomer formation. DCVJ is part of a class of molecular rotors that has many applications in food processing, cellular imaging, and polymer chemistry due to its sensitivity to a change in viscosity of the local environment.^[18] The quantum yield of DCVJ can increase up to 30-fold upon transfer from propan-1-ol to glycerol.^[19] DCVJ contains a julolidine moiety connected through a sigma bond to an intramolecular quencher dicyano group. In a low viscosity solution, free rotation around the sigma bond decreases the quantum yield of DCVJ due to intramolecular quenching, but if rotation is restricted because of high viscosity or inclusion in a cavity or cage, the quantum yield increases at 510 nm.^[20,21] It was shown that DCVJ fluorescence could be employed to monitor the aggregation of actin and tubulin.^[19,22] DCVJ was also used to monitor oligomer formation in transthyretin and α -synuclein.^[21,23] In this work, two isoforms of amyloid beta peptide, A β 42 and A β 40, were chosen to investigate the kinetics of the lag phase. Although the isoforms share 96 % sequence similarity, A β 42 aggregates much faster than A β 40 and, as we conclude, through a different pathway.

Results and Discussion

Aggregation kinetics of wild-type A β 40 and A β 42

The kinetics of A β 42 and A β 40, monitored using thioflavin-T (ThT) at 482 nm, followed a sigmoidal curve that had three phases. The length of the lag phase for A β 42 (16 min) was approximately six times shorter than A β 40 (90 min), as reported in many previous studies^[24,25] (Figure S2). We compared ThT kinetics to those monitored by DCVJ. The emission spectrum of DCVJ in solution at 1 μ M showed a single peak maximum at 510 nm that corresponded to the monomeric form of DCVJ.^[21] This spectrum was unchanged by concentrations up to $\approx$ 6 μ M (Figure S3). The addition of A β 40 had no effect on the DCVJ spectrum over 20 min. This could be because either DCVJ did not bind to the monomer, or the chain was very flexible and did not restrict motion of the bound DCVJ. Between 20 and 30 min, there was a drastic increase in the intensity at 510 nm, and a new peak at 570 nm appeared. Both peaks increased for up to 60 min and then began to decrease (Figures 1 A, C and 2 D). After 90 min, the intensity at both wavelengths remained constant until at least 120 min. The increase in DCVJ intensity for A β 40 in the lag phase indicated that it was highly sensitive to the early oligomers or some other non-thioflavinophilic species. Measurement of DCVJ spectra with varying concentrations in the absence of A β 40 showed the emergence of a second peak at 570 nm above 6 μ M. Therefore, this peak was assigned as the excimer peak (Figure S3).^[20] The presence of this 570 nm peak confirmed the oligomers formed by A β 40 could accommodate two or more molecules of DCVJ, which together produced the excimer.

In contrast, the addition of A β 42 had no effect on DCVJ fluorescence, as monitored for the entire length of its lag phase (Figure 1 B). One explanation could be that the DCVJ/oligomer binding rate is slow compared to the A β 42 lag time. However, the addition of 1 μ M DCVJ to the preformed oligomers of WT A β 40 showed an instantaneous increase in fluorescence signal at 510 and 570 nm, thus confirming that the binding of DCVJ to A β was very rapid. Therefore, we concluded that predominant oligomers formed by A β 42 had different molecular structure than A β 40 and hence did not bind DCVJ in the lag phase (Figure S4).

The comparison of A β 40 DCVJ and ThT fluorescence intensity at 510 and 482 nm, respectively, shows that kinetics of oligomer formation exhibits both lag and growth phase similar to formation of amyloid fibrils (Figure 1 C). As DCVJ bound more strongly to the

early oligomers in the lag phase than the fibril, the DCVJ intensity underwent a sharp decrease of 35 % from its intensity during the pre-transition growth phase (Figure 1 C). In the pre-transition growth phase, the β -sheet aggregates that binds ThT start forming nuclei, either by orderly association of oligomers or by large structural changes in the oligomer, that act as a seed for the rapid conversion of other oligomers into fibrils.^[26,27] A recent study using *p*-cyanophenyl-alanine as a probe found that aromatic side chains in islet amyloid polypeptide (IAPP) remain exposed in the lag phase, and significant sequestering occurs when β -sheet aggregates that bind ThT form in the growth phase.^[28] The drastic decrease in DCVJ signal at 60 min did not seem to be a mere association of oligomers but indicated the large structural changes with concomitant formation of β -sheet aggregates that grew rapidly until the formation of more stable β -sheet fibrils.

Atomic force microscopy (AFM) images were taken for aliquots of A β 40 and A β 42 collected at 60 and 16 min, respectively. A β 40 revealed homogeneous spherical oligomers 10–20 nm in width, whereas A β 42 was a heterogeneous mixture of spherical, elongated, curved, and rod-shaped particles coexisting in solution (Figure 1E, F). Thus, the differences seen between the DCVJ spectra for A β 40 and A β 42 in the lag phase might be due to the difference in their kinetics as well as the structures formed. As there was no change in DCVJ fluorescence for A β 42 at 16 min, it appeared that DCVJ did not bind to any of these particles, even the rod-shaped protofibrils, despite binding to fibrils.

Approximately one decade ago, Bitan et al., investigated the differences between the oligomeric states of A β 40 and A β 42 by photoinduced crosslinking of aggregated free sample preparation.^[29] They showed that the critical difference in assembly is that A β 40 exists as a monomer, dimer, trimer, and tetramer in rapid equilibrium, whereas A β 42 forms pentamer/hexamer (*para*-nuclei) units that then assemble to form protofilaments. Upon further investigation into this aggregation mechanism, Bernstein et al. used ion mobility coupled with a mass spectrometry method to probe oligomerization of amyloid β -peptides. They tested WT A β 42, A β 40, and their mutants, Pro19 and Met35(O), and showed that all A β 40 peptides terminated at the tetramer, but A β 42 peptides further associated to form *para*-nuclei.^[30] The tetramers formed by A β 42 were more open than those formed by A β 40 and its mutants. Our DCVJ fluorescence and AFM data were in very good agreement with their results. The more open oligomeric structure for A β 42 when compared to A β 40's partially opened oligomeric structure might account for the differences observed in DCVJ spectra and kinetics, as A β 40 could cage two or more molecules of DCVJ in its cavity, restricting their free rotation and enhancing their monomeric and excimer fluorescence intensity. No further change was observed for the A β 40 DCVJ monomer signal at 510 nm in the growth or saturation phases; this suggests that the DCVJ monomer signal at 510 nm was not sensitive to the growth and stabilization of β -sheet-rich fibrils (Figure S5 A). The AFM image for A β 40 taken at 24 h predominantly showed two populations—spherical oligomers and fibrils—in agreement with the recent results from other groups (Figure S5 B).^[29,30] Furthermore, there was a continual increase in the excimer signal at 570 nm, as multiple DCVJ molecules were stabilized in close proximity within fibrils.

Aggregation kinetics of the A β 40 mutant

Recently, we reported that the F4C F19W mutant of A β 40 inhibits fibril formation.^[31] Similar types of experiments were carried out on this F4C F19W mutant to investigate the sensitivity of DCVJ binding. Thioflavin-T (ThT) kinetics showed that F4C F19W did not form β -sheet aggregates, even after 4 h of incubation (Figure 2A), whereas the WT predominantly formed β -sheet aggregates. However, DCVJ did show an increase in monomer peak intensity at 510 nm, immediately upon the addition of the F4C F19W mutant. Both the monomeric and excimer peaks intensity increased until 60 min and then reached saturation (Figure 2B). The plot of the DCVJ monomer and excimer kinetic profile for the WT and mutant are shown in Figure 2 C and D. The WT kinetics followed a sigmoidal curve with three phases: the lag, growth, and saturation phases. However, for the mutants, there appeared to be only two phases: growth and saturation. As the mutant formed stable oligomers and did not form fibrils, the intensity remained constant after the growth phase, whereas for the WT, there was a drop in the signal. Another difference in the DCVJ spectra between the WT and F4C F19W mutant was the ratio of the excimer (570 nm) to the monomer peak (510 nm). For the WT, the ratio of excimer to monomer peak intensity was much higher than for the mutant (compare spectra in Figures 1A and 2 B). This difference implied that the excimer formed by two DCVJ molecules in close proximity was more likely in the WT. The AFM images and the particle size analysis revealed that mutant F4C F19W formed homogeneous spherical oligomers that were 10–12 nm, smaller than the WT (Figures 2 E and S6).

Time-resolved fluorescence measurements

The fluorescence lifetime decay parameters for DCVJ in the presence of A β 40 monomers (0 min) and oligomers (20 and 60 min) were measured to determine the conformational dynamics of A β in these states. The fluorescence decay curve and fit of the decay curves monitored at 510 nm (monomer) and 575 nm (excimer) showed at least two components (Figure 3 A, B, E–H). The DCVJ decay curve for the free dye overlapped with the decay curve in the presence of both WT and the F4C F19W mutant of A β 40 at 0 min (data not shown). The fast component for the monomer peak at 510 nm (τ_1) increased from 0.03 to 0.3 ns, and the slow (τ_2) component increased from 0.04 to 1.7 ns for DCVJ in the presence of A β 40 at zero and 60 min, respectively (Figure 3 E, F). Similarly, for excimer fluorescence decay at 575 nm, both the fast and slow component increased from 0.03 to 0.3 ns and 0.2, to 1.15 ns, respectively (Figure 3 G, H). The increased DCVJ lifetime, with respect to both components in the oligomer-bound state (60 min) compared to the free dye or at 0 min, confirmed that the intra-molecular rotation around the sigma bond was hindered, and excimer packed into the oligomer cavity. For the F4C F19W mutant, the τ_2 value at 510 nm was higher than for the WT, whereas at 575 nm, both the τ_1 and τ_2 values were lower than those of the WT, showing that excimers formed by the association of DCVJ molecules were not tightly packed as compared to the WT (Figure 3E–F).

Time-resolved fluorescence anisotropy data for DCVJ was recorded in the presence of monomer and oligomers of A β 40 at 510 and 570 nm (Figure 3C, D). The rotational relaxation of the free DCVJ or with A β 40 at 0 and at 60 min could be well-fitted to a single exponential decay, and the values are shown in Figure 3 I and J. The anisotropy decay for

WT A β 40 was slower for DCVJ bound to oligomer (60 min) than to monomer (0 min) at 510 and 575 nm, confirming the hindrance to rotation in accordance with both steady-state and time-resolved fluorescence measurements. The F4C F19W mutant had a value that was slightly slower at 510 nm and faster at 575 nm compared to the WT, in agreement with fluorescence decay measurements.

Effect of epigallocatechin-3-gallate (EGCG) on A β aggregation

Epigallocatechin-3-gallate (EGCG) is a polyphenol abundantly present in green tea and has been shown to inhibit neurotoxicity both in vitro and in a mouse model.^[27,32,33] The effect of EGCG on the aggregation of A β 42 was measured using DCVJ and compared to ThT. In the presence of EGCG, A β 42 did not show any significant increase in ThT intensity when compared to A β 42 alone, confirming the effective role in inhibition of β -sheet-rich aggregates (Figure 4 A). However, DCVJ bound early in the lag phase to the oligomers of A β 42 treated with EGCG (Figure 4 B). Hence, binding of EGCG modulated the structure of A β 42 oligomers. The spectra of DCVJ recorded at 72 min for A β 42 treated with EGCG showed both monomer and excimer peaks similar to the A β 40 WT and F4C F19W mutant. The ratio of the monomer to excimer intensity was close to that of the F4C F19W A β 40 mutant (Figure 4C and D). The AFM images of A β 42 taken after 1 day of incubation showed a mixture of short fibrils and oligomers, whereas EGCG treatment appeared to stabilize the spherical oligomer (Figure 4 E and F). All of the above results clearly revealed that binding of EGCG modulates oligomer structure and packing but does not retain the peptide's monomeric nature.

Conclusion

Based on the DCVJ fluorescence data, in combination with AFM images, we proposed a mechanism for A β oligomerization and subsequent fibril formation (Figure 5). DCVJ is sensitive to the exact compaction of the oligomer. Thus, the data showed that WT A β 40 in the early lag phase formed oligomers that had a partially open structure that allowed formation of the DCVJ excimer. During the late lag phase or pre-transition growth phase, these oligomers underwent large structural changes into β -sheet-rich aggregates. The mutant A β 40 also formed oligomers with a similar structure that might be more compact and inhibited formation of the excimer. These oligomers did not go on to form fibrils or change in a way that was sensitive to DCVJ. In contrast, the oligomers of WT A β 42 in the early lag phase had structures that were more open compared to A β 40 and therefore did not bind and restrict DCVJ. However late in the lag phase or in the pre-transition growth phase, A β 42 formed spherical, curved, and rod-shaped particles that more rapidly transformed into β -sheet-rich aggregates. Binding of EGCG to A β 42 might modulate the open oligomeric structure into a closed structure, preventing further association into β -sheet-rich aggregates, as does the F4C F19W A β 40 mutant. DCVJ also clearly showed that EGCG does not retain the monomeric nature of A β 42, which makes it a useful assay for testing drug candidates. The sensitivity of DCVJ to oligomer structure and packing provides us with a suitable probe to detect the early lag phase of amyloid assembly.

Experimental Section

Peptide expression and purification

A β 42, A β 40, and the A β 40 mutant peptide were expressed in *E. coli* by using a fusion construct consisting of 19 soluble *N*-acetylneuraminic acid phosphatase (NANP) repeats, followed by a TEV protease cleavage site, followed by the A β sequence. The protein expression and purification steps were performed according to a recently reported protocol.^[16] Briefly, cells transformed with the corresponding plasmids were grown in lysogeny broth (1 L) at 37°C with constant stirring for 16 h. IPTG was added to induce protein expression, and the cells were harvested after 4 h. The cells were lysed, and the recombinant protein was purified by Ni-NTA affinity and HPLC columns. The purified protein was cleaved by TEV protease to obtain recombinant A β peptide and was lyophilized and stored at –80°C until use. The lyophilized peptide was monomerized, and ThT measurements were performed according to the previously reported protocol.^[16]

DCVJ fluorescence measurements

All equilibrium measurements were completed on a PTI QW4 spectrofluorimeter (Horiba Scientific, Edison, NJ). DCVJ (1 μ M) was used for steady-state and kinetics measurements. At this concentration, DCVJ spectra showed only a monomeric peak at 510 nm. Hence, this concentration was used for all measurements mentioned here except for the DCVJ concentration-dependent experiment. Aggregation kinetics were initiated by the addition of A β (80 μ M) from the stock solution to the DCVJ solution to obtain final concentrations of 8 μ M A β and 1 μ M DCVJ. The kinetics of A β aggregation were recorded by constantly stirring the mixture at 37°C and measuring fluorescence for every 4 min for A β 42 and every 10 min for A β 40 with an excitation wave-length of 465 nm and emission around 480–600 nm. For the EGCG inhibitor assay, a 1:1 ratio of peptide to EGCG was used.

Time-resolved fluorescence measurements

A time-correlated single-photon counting (TCSPC) system was used to obtain time-domain polarized fluorescence intensity decays. The light source was a CW mode-locked Nd:YAG laser (Coherent Antares 76-s, Santa Clara, CA) that produces 100 ps, 1064 nm pulses at a 76 MHz repetition rate. The third harmonic of the CW mode-locked Nd:YAG laser that produced a 1064 nm pulse was used to excite a cavity-dumped dye laser (Coherent 702-2, Santa Clara, CA), operated with Stilbene 420 dye (Exciton, Dayton, OH) at 460 nm. The collection wavelength (505 and 565 nm, 10 nm detection bandwidth) and polarization were computer-controlled using LabVIEW 7.1 code. Fluorescence lifetime data were collected at 54.7° with respect to the vertical excitation polarization; the reorientation data were collected at polarizations parallel (0°) and perpendicular (90°) to the vertically polarized incident light.

AFM measurements

AFM images on the A β peptides (WT A β 40, WT A β 42, and mutant F4C F19W A β 40) were collected by using a Cypher S instrument (Asylum Research, Santa Barbara, CA). Samples were prepared by adsorbing aliquots of the A β peptides (20 μ L) onto freshly

cleaved mica for 30 min. The salts and excess fluid from the mica surface were removed and dried overnight. Samples were imaged by using a high-precision silicon tip for ac mode imaging in air.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Plasmids for the A β fusion proteins were a kind gift from Dr. Rudi Glockshuber. We thank Terry Ball for assistance with protein purification, Dr. Reza Loloee for assistance with AFM measurements, and Dr. Gary Blanchard for assistance and discussion with time-resolved fluorescence and anisotropy measurements. This work was supported by the National Institutes of Health (R01 GM100908).

References

1. Eisenberg D, Jucker M. Cell. 2012; 148:1188–1203. [PubMed: 22424229]
2. Kellie JF, Higgs RE, Ryder JW, Major A, Beach TG, Adler CH, Merchant K, Knierman MD. Sci Rep. 2014; 4:5797. [PubMed: 25052239]
3. Mukherjee A, Morales-Scheihing D, Butler PC, Soto C. Trends Mol Med. 2015; 21:439–449. [PubMed: 25998900]
4. Ruberg FL, Berk JL. Circulation. 2012; 126:1286–1300. [PubMed: 22949539]
5. Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT Jr. Nature. 2002; 418:291.
6. Stroud JC, Liu C, Teng PK, Eisenberg D. Proc Natl Acad Sci USA. 2012; 109:7717–7722. [PubMed: 22547798]
7. Wolff M, Unuchek D, Zhang B, Gordeliy V, Willbold D, Nagel-Steger L. PloS One. 2015; 10:e0127865. [PubMed: 26024352]
8. Glabe CG. J Biol Chem. 2008; 283:29639–29643. [PubMed: 18723507]
9. Arosio P, Knowles TPJ, Linse S. Phys Chem Chem Phys. 2015; 17:7606–7618. [PubMed: 25719972]
10. Pauwels K, Williams TL, Morris KL, Jonckheere W, Vandersteen A, Kelly G, Schymkowitz J, Rousseau F, Pastore A, Serpell LC, Broersen K. J Biol Chem. 2012; 287:5650–5660. [PubMed: 22157754]
11. Sureshbabu N, Kirubakaran R, Jayakumar R. Eur Biophys J. 2009; 38:355–367. [PubMed: 19005650]
12. Nagarajan S, Ramalingam K, Neelakanta Reddy P, Cereghetti DM, Padma Malar EJ, Rajadas J. FEBS J. 2008; 275:2415–2427. [PubMed: 18422968]
13. LeVine H 3rd. Protein Sci. 1993; 2:404–410. [PubMed: 8453378]
14. Benditt EP, Eriksen N, Berglund C. Proc Natl Acad Sci USA. 1970; 66:1044–1051. [PubMed: 4097575]
15. Young LM, Saunders JC, Mahood RA, Revill CH, Foster RJ, Ashcroft AE, Radford SE. Methods. 2016; 95:62–69. [PubMed: 26007606]
16. Nag S, Sarkar B, Bandyopadhyay A, Sahoo B, Sreenivasan VK, Kombrabail M, Muralidharan C, Maiti S. J Biol Chem. 2011; 286:13827–13833. [PubMed: 21349839]
17. Thapa A, Jett SD, Chi EY. ACS Chem Neurosci. 2016; 7:56–68. [PubMed: 26529184]
18. Viriot ML, Carre MC, Geoffroy-Chapotot C, Brembilla A, Muller S, Stoltz JF. Clin Hemorheol Microcirc. 1998; 19:151–160. [PubMed: 9849928]
19. Kung CE, Reed JK. Biochemistry. 1989; 28:6678–6686. [PubMed: 2790023]
20. Gavvala K, Sasikala WD, Sengupta A, Dalvi SA, Mukherjee A, Hazra P. Phys Chem Chem Phys. 2013; 15:330–340. [PubMed: 23165311]
21. Lindgren M, Sorgjerd K, Hammarstrom P. Biophys J. 2005; 88:4200–4212. [PubMed: 15764666]

22. Sawada S, Iio T, Hayashi Y, Takahashi S. *Anal Biochem.* 1992; 204:110–117. [PubMed: 1514677]
23. Paslawski W, Andreasen M, Nielsen SB, Lorenzen N, Thomsen K, Kaspersen JD, Pedersen JS, Otzen DE. *Biochemistry.* 2014; 53:6252–6263. [PubMed: 25216651]
24. Hasegawa K, Yamaguchi I, Omata S, Gejyo F, Naiki H. *Biochemistry.* 1999; 38:15514–15521. [PubMed: 10569934]
25. Sandberg A, Luheshi LM, Sollvander S, Pereira de Barros T, Macao B, Knowles TP, Biverstal H, Lendel C, Ekholm-Petterson F, Dubnovitsky A, Lannfelt L, Dobson CM, Hard T. *Proc Natl Acad Sci USA.* 2010; 107:15595–15600. [PubMed: 20713699]
26. Lee J, Culyba EK, Powers ET, Kelly JW. *Nat Chem Biol.* 2011; 7:602–609. [PubMed: 21804535]
27. Marek P, Gupta R, Raleigh DP. *Chem Bio Chem.* 2008; 9:1372–1374.
28. Kuperstein I. *EMBO J.* 2010; 29:3408. [PubMed: 20818335]
29. Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, Teplow DB. *Proc Natl Acad Sci USA.* 2003; 100:330–335. [PubMed: 12506200]
30. Bernstein SL, Dupuis NF, Lazo ND, Wyttenbach T, Condrón MM, Bitan G, Teplow DB, Shea JE, Ruotolo BT, Robinson CV, Bowers MT. *Nat Chem.* 2009; 1:326–331. [PubMed: 20703363]
31. Acharya S, Srivastava KR, Nagarajan S, Lapidus LJ. *ChemPhysChem.* 2016; 17:3470–3479. [PubMed: 27490673]
32. Bieschke J, Russ J, Friedrich RP, Ehrnhoefer DE, Wobst H, Neugebauer K, Wanker EE. *Proc Natl Acad Sci USA.* 2010; 107:7710–7715. [PubMed: 20385841]
33. Lin CL, Chen TF, Chiu MJ, Way TD, Lin JK. *Neurobiol Aging.* 2009; 30:81–92. [PubMed: 17590240] Rezai-Zadeh K, Arendash GW, Hou H, Fernandez F, Jensen M, Runfeldt M, Shytle RD, Tan J. *Brain Res.* 2008; 1214:177–187. [PubMed: 18457818]

Figure 1.

Kinetics of A β aggregation in the lag phase. A) and B) DCVJ fluorescence emission spectra in the presence and absence of A β 40 and A β 42, respectively. All DCVJ fluorescence experiments were carried out at 1 μ M concentrations at 37 $^{\circ}$ C with constant stirring. Spectra were recorded for 10 μ M A β 42 and A β 40 peptides in the presence of DCVJ at λ_{ex} = 465 nm and λ_{em} = 480–600 nm. Comparison of DCVJ (510 nm) to ThT (482 nm) kinetics for C) A β 40 and D) A β 42 peptide, respectively. Data for (D) were collected every 2 min but are displayed every 4 min for clarity. AFM image of F) A β 40 (80 min) or G) A β 42 (16 min) aliquots collected at the end of the lag phase.

Figure 2.

A) Measurement of ThT fluorescence emission intensity at 482 nm for the WT and F4C F19W mutant of Aβ40 at time intervals of 10 min up to 230 min (●: F4C F19W mutant, ●: WT Aβ40). B) DCVJ fluorescence spectra for F4C F19W mutant of Aβ40 at time intervals of 10 min up to 80 min. Measurement of DCVJ emission intensity at C) 510 nm and D) 570 nm, respectively, for the WT and F4C F19W mutant of Aβ40 at time intervals of 10 min up to 90 min (●: F4C F19W mutant, ●: WT Aβ40). E) AFM image for the F4C F19W mutant for the aliquot collected at 80 min.

Figure 3.

Time-resolved fluorescence decay overlays for DCVJ in the presence and absence of A β 40 WT and the F4C F19W mutant at A) 510 nm and B) 575 nm, respectively (●: DCVJ, ●: WT A β 40 20 min, ●: WT A β 40 60 min, ●: F4C F19W mutant 60 min). DCVJ anisotropy decay for A β 40 after 0 (●) and 60 min (●), monitored at C) 510 nm and D) 575 nm. Fluorescence decay parameters τ_1 (E, F) and τ_2 (G, H) [ps] for A β 40 at 0, 20, and 60 min and the F4C A β 40 mutant at 60 min, monitored at 510 (E, G) and 575 nm (F, H). Anisotropy decay parameters τ_{1r} [ps] for A β 40 (at 0, 20, and 60 min) and the F4C A β 40 mutant at 60 min monitored at I) 510 and J) 575 nm.

Figure 4.

Measurement of fluorescence intensity for Aβ42 in the presence and absence of EGCG. A) ThT kinetics at 482 nm. B) DCVJ kinetics at 510 nm (Aβ42 in the ● absence and ● presence of EGCG, respectively). DCVJ fluorescence emission spectra at 0 h (●) and 72 h (●) for Aβ42 in the C) absence and D) presence of EGCG, respectively. E) and F) AFM images for Aβ42 in the presence and absence of EGCG, respectively.

Figure 5.

Scheme showing the sensitivity of DCVJ as a fluorescence probe to differentiate the oligomers and higher aggregates formed by WT A β 40, WT A β 42, and the A β 40 mutant.