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Expression of N-Terminal Cysteine Aβ$_{42}$ and Conjugation to Generate Fluorescent and Biotinylated Aβ$_{42}$

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Abstract

Fluorescent derivatives of the β-amyloid peptides (Aβ) are valuable tools for studying the interactions of Aβ with cells. Facile access to labeled expressed Aβ offers the promise of Aβ with greater sequence and stereochemical integrity, without impurities from amino acid deletion and epimerization. Here, we report methods for the expression of Aβ$_{42}$ with an N-terminal cysteine residue, Aβ$_{(C1–42)}$, and its conjugation to generate Aβ$_{42}$ bearing fluorophores or biotin. The methods rely on the hitherto unrecognized observation that expression of the Aβ$_{(MC1–42)}$ gene yields Aβ$_{(C1–42)}$ peptide, because the N-terminal methionine is endogenously excised by *E. coli*. Conjugation of the Aβ$_{(C1–42)}$ with maleimide-functionalized fluorophores or biotin affords the N-terminally labeled Aβ$_{42}$. The expression affords ca. 14 mg of N-terminal cysteine Aβ from one liter of bacterial culture. Subsequent conjugation affords ca. 3 mg of labeled Aβ from one liter of bacterial culture with minimal cost for labeling reagents. HPLC analysis indicates the N-terminal cysteine Aβ to be more than 97% pure and labeled Aβ peptides to be 94–97% pure. Biophysical studies show that the labeled Aβ peptides behave similarly to unlabeled Aβ and suggest that labeling of the N-terminus does not substantially alter the properties of the Aβ. We further demonstrate applications of the fluorophore-labeled Aβ peptides, by using fluorescence microscopy to visualize their interactions with mammalian cells and bacteria. We anticipate that

these methods will provide researchers convenient access to useful N-terminally labeled Aβ, as well as Aβ with an N-terminal cysteine that enables further functionalization.

Graphical Abstract
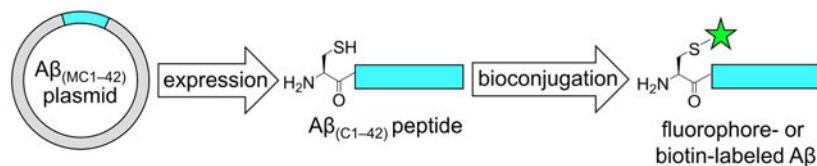
INTRODUCTION

Peptides and proteins bearing an N-terminal cysteine residue are valuable tools in chemical biology research, because the unique reactivity of N-terminal cysteine permits native chemical ligation, conjugation, and other site-specific modifications.[1–4] Amyloid-β (Aβ) peptides bearing an N-terminal cysteine offer promise in Alzheimer's disease research through N-terminal labeling with fluorophores or biotin, as well as other N-terminal modifications. N-terminally labeled Aβ peptides have been widely used to investigate cellular phagocytosis, transport, and clearance of Aβ through optical fluorescence microscopy, and to screen anti-Aβ antibodies through enzyme-linked immunosorbent assays (ELISA).[5–29] Site-specific labeling on the N-terminus of Aβ minimizes perturbation in the structure and function of the peptide, as the central and the C-terminal regions of Aβ are more involved in fibril and oligomer formation.[16,27,30] Although some N-terminally labeled derivatives of Aβ are commercially available, they are expensive (ca. $1000/mg) and limited to biotin and a few common fluorophores. Ready access to good quantities of pure N-terminally labeled Aβ can advance Alzheimer's disease research by enabling experiments that would otherwise be hindered by insufficient access to these peptides.

Here we describe an efficient method for recombinant expression and purification of the Aβ peptide with an N-terminal cysteine, Aβ$_{(C1–42)}$, and the preparation of labeled Aβ$_{(C1–42)}$ peptides. Expressed Aβ peptides are superior to chemically synthesized Aβ, because they are free from the amino acid deletions or epimers that form through amino acid racemization during each coupling step.[31,32] Expressed Aβ peptides have been found to aggregate more quickly and be more toxic than synthetic Aβ, and thus are widely used in amyloid research.[33–35] Although Aβ peptides bearing fluorescent and biotin labels can be prepared by chemical synthesis, the expressed Aβ peptide bioconjugates should also be preferable to those derived from synthetic Aβ.

We recently reported an efficient method for expression and purification of N-terminal methionine Aβ, Aβ$_{(M1–42)}$.[36] In the current paper, we set out to adapt this method to include a cysteine residue for further functionalization by attempting to express Aβ$_{(MC1–42)}$. When we expressed an Aβ$_{(MC1–42)}$ plasmid in *E. coli*, we were surprised to find that the N-terminal methionine is endogenously excised by the *E. coli* methionyl aminopeptidase (MAP), leaving cysteine on the N-terminus and affording Aβ$_{(C1–42)}$ (Figure 1).[37–39] This hitherto unrecognized observation provides a method of preparing the Aβ$_{(C1–42)}$

peptide, without the additional methionine group that originates from the translational start codon.[30,40] The absence of methionine is significant, because $A\beta_{(C1–42)}$ represents the minimal modification of native $A\beta_{(1–42)}$ that permits further functionalization through robust cysteine-maleimide conjugation. Our method to prepare $A\beta_{(C1–42)}$ is straightforward and does not require any additional treatment with enzymes or reagents after the initial expression and reverse-phase preparative HPLC purification. This expression method affords ca. 14 mg of $A\beta_{(C1–42)}$ with > 97% purity from one liter of bacterial culture.

The $A\beta_{(C1–42)}$ can be further functionalized to create a variety of derivatives using thiol-maleimide chemistry and readily available maleimide-based labeling reagents.[41] We have developed an optimized labeling protocol to allow $A\beta_{(C1–42)}$ to be labeled with fluorophores or biotin in 15 minutes without Aβ oligomer or fibril formation, and we demonstrate this protocol through the preparation of $A\beta_{(C1–42)}$ labeled with fluorophores (TAMRA and FAM) and biotin. Only two equivalents of the labeling reagents are required to achieve complete labeling, which minimizes the cost of the maleimide reagents. This labeling protocol affords ca. 3 mg of labeled Aβ from one liter of bacterial culture with minimal cost for reagents. HPLC analysis indicates the labeled peptides to be 94–97% pure. We also demonstrate that these labeled Aβ peptides behave similarly to unlabeled $A\beta_{(M1–42)}$ in aggregation and fibrillization assays, which indicates that the labeling of the N-terminus of Aβ does not substantially alter the properties of the Aβ. We further demonstrate the application of the fluorophore-labeled $A\beta_{(C1–42)}$ by using fluorescence microscopy to visualize its interactions with mammalian cells and bacteria.

These methods represent a novel and valuable contribution to the Aβ toolbox, because they exploit a hitherto unrecognized cleavage of methionine in expressed Aβ42, provide tailored conjugation conditions to enable rapid labeling of this highly aggregation-prone peptide, and allow choice of wide variety of fluorophores and other labels. We anticipate that these methods will provide researchers with convenient access to useful Aβ peptides bearing N-terminal labels or a free N-terminal cysteine residue.

MATERIALS AND METHODS

The materials and procedures for molecular cloning, peptide expression, conjugation, purification, and characterization, as well as oligomerization and fibrilization studies and fluorescence microscopy studies are described in detail in the Supporting Information.

RESULTS AND DISCUSSION

We expressed $A\beta_{(C1–42)}$ in *E. coli* using a plasmid for $A\beta_{(MC1–42)}$ that we constructed and have subsequently made available through Addgene.[42] The expressed peptide forms inclusion bodies that are isolated by multiple rounds of washing. The inclusion bodies are then solubilized in urea buffer, and the solubilized $A\beta_{(C1–42)}$ is purified by reverse-phase HPLC. The pure HPLC fractions of $A\beta_{(C1–42)}$ are combined, and the conjugation reactions are performed directly in the combined pure fractions at alkaline pH. The conjugated peptides are purified by another round of HPLC purification. The pure HPLC fractions are then combined and lyophilized. The yields of the labeled peptides are assessed

gravimetrically, and the composition and purity of the peptides are assessed by analytical HPLC and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). For subsequent biophysical and biological studies, the purified peptide is rendered monomeric by treatment with hexafluoroisopropanol (HFIP). Biophysical experiments show that the labeled $A\beta_{(C1-42)}$ behaves similarly to unlabeled $A\beta_{(M1-42)}$, and fluorescence microscopy studies with mammalian cells and bacteria further demonstrate applications of the labeled $A\beta_{(C1-42)}$.

Expression and Purification of $A\beta_{(C1-42)}$.

We prepared a pET-Sac plasmid encoding $A\beta_{(MC1-42)}$ in the same fashion as we had previously described,[36] and we deposited the plasmid with Addgene to make it available to others.[42] We also provide procedures to prepare the plasmid in the Supporting Information (Figure S1). To express $A\beta_{(C1-42)}$, we transform the pET-Sac-$A\beta_{(MC1-42)}$ plasmid into BL21(DE3)-pLysS competent *E. coli* and induce expression with isopropyl β-D-1-thiogalactopyranoside (IPTG) when the cultured *E. coli* reach an optical density (OD_{600}) of 0.45. After expression, we lyse the cells, wash the inclusion bodies containing $A\beta_{(C1-42)}$ with Tris buffer, and then solubilize the inclusion bodies with 8 M urea. We then filter the solution through a 0.22 μm hydrophilic polyethersulfone (PES) syringe filter or a 0.22 μm hydrophilic polyvinylidene fluoride (PVDF) filter (to prevent damaging the HPLC columns) and purify the $A\beta_{(C1-42)}$ by preparative reverse-phase HPLC using a C8 column at 80 °C with water and acetonitrile containing 0.1% trifluoroacetic acid (TFA). A typical analytical HPLC trace of the filtered lysate shows four major peaks, with the first peak corresponding to the $A\beta_{(C1-42)}$ monomer (Figure S2). The $A\beta_{(C1-42)}$ monomer elutes at ca. 34% acetonitrile during the preparative HPLC purification. We then combine the HPLC fractions containing pure $A\beta_{(C1-42)}$ monomer, and the combined pure fractions typically show >97% purity, as assessed by analytical HPLC (Figure 2A and Figure S7). We measure the concentration of $A\beta_{(C1-42)}$ in the combined the HPLC fractions by UV absorbance at 280 nm with an estimated extinction coefficient for tyrosine of 1490 $M^{-1}cm^{-1}$. On the basis of the spectrophotometrically determined concentration, we typically obtain ca. 14 mg of $A\beta_{(C1-42)}$ as the trifluoroacetate salt from one liter of bacterial culture.

MALDI-MS shows that the mass of the peptide is consistent with $A\beta_{(C1-42)}$, not $A\beta_{(MC1-42)}$ (Figure 2B), and tandem mass spectrometry (MS/MS) peptide sequencing confirms that the N-terminus of the peptide begins with cysteine, rather than methionine (Figures S9). The loss of the N-terminal methionine is consistent with a mechanism in which methionyl aminopeptidase (MAP) excises the N-terminal methionine.[39–41] The combined pure HPLC fractions of $A\beta_{(C1-42)}$ can be directly used for subsequent labeling, or stored at −80 °C for at least three weeks without forming detectable aggregates.

Labeling of $A\beta_{(C1-42)}$.

Proteins are often labeled with fluorophores or biotin by dissolving the lyophilized protein powder in buffer and adding a reactive derivative of the fluorophore or biotin to the solution of the protein. We found that when we lyophilized the $A\beta_{(C1-42)}$, it became unsuitable for labeling, because it aggregated during the lyophilization and redissolution process. All of our attempts to label lyophilized $A\beta_{(C1-42)}$ gave rise to turbid solutions of aggregated

Aβ$_{(C1-42)}$ that were unsuitable for subsequent purification and use. To address this problem, we have developed a protocol to rapidly label the Aβ$_{(C1-42)}$ in the combined HPLC fractions, where Aβ remains mostly monomeric.

In this protocol, we first we determine the concentration of Aβ$_{(C1-42)}$ in the combined HPLC fractions by UV absorbance at 280 nm. We then calculate the amount of the maleimide-based labeling reagent needed for a 2:1 molar ratio of the reagent to the Aβ$_{(C1-42)}$ peptide. Although typical thiol-maleimide labeling protocols use a 5–20-fold molar excess of the maleimide reagent, we found that a 2-fold molar excess is sufficient to achieve complete labeling of the Aβ$_{(C1-42)}$. Use of only a 2-fold molar excess of the reagent not only minimizes the cost, but also simplifies subsequent purification of the Aβ$_{(C1-42)}$ conjugate. Typical thiol-maleimide labeling protocols suggest treating the cysteine-containing unlabeled peptide with a 10–100-fold molar excess of tris(2-carboxyethyl)phosphine (TCEP) to reduce any disulfide bonds that might have formed. We found that TCEP interferes with the labeling of Aβ$_{(C1-42)}$ and is unnecessary for our Aβ labeling protocol.[43]

We found that the labeling reaction must be performed in alkaline conditions to proceed rapidly and avoid aggregation of the Aβ. The pH of the combined HPLC fractions of Aβ$_{(C1-42)}$ is ca. 2.2 because of the presence of TFA in the mobile phases, which is unfavorable for thiol-maleimide conjugation chemistry. At acidic pH, the thiol-maleimide reaction does not reach completion after overnight incubation, and Aβ$_{(C1-42)}$ aggregates before being completely labeled. Common protein labeling protocols recommend doing the thiol-maleimide conjugation at ca. pH 7.4. Aβ$_{(C1-42)}$ aggregates rapidly at this pH, which is close to its isoelectric point (pI = 5.6), where Aβ$_{(C1-42)}$ is not heavily charged and is thus prone to aggregation.[44] We observed that at pH 7.4, Aβ aggregates in several minutes, which hinders the labeling of Aβ. We thus developed a basification protocol to rapidly raise the pH of the acidic HPLC fractions to ca. 9.0. It is essential to bring the pH from ca. 2.2 to ca. 9.0 rapidly to bypass the pI quickly thus prevent Aβ aggregation.[45] In this protocol, we first titrate a 1 mL portion of the combined HPLC fractions with pH 9.0 sodium borate buffer until a pH of 9.0 is reached. This portion of the combined HPLC fractions is discarded. We then calculate the quantity of borate buffer needed to basify the remainder of the combined HPLC fractions to pH 9.0 and add it in a single portion. We then add the appropriate amount the maleimide reagent as a solution in DMSO and allow the reaction to proceed for 15 minutes at room temperature to achieve complete labeling of the Aβ.

Purification, Characterization, and HFIP Treatment of Labeled Aβ$_{(C1-42)}$.

We purify the labeled Aβ$_{(C1-42)}$ by preparative HPLC to remove unreacted maleimide reagent, borate buffer, and oligomers that formed during the labeling reaction. Before HPLC purification, we must filter the reaction mixture to prevent damaging the HPLC column. We have found both hydrophilic polyvinylidene fluoride (PVDF) syringe filters and hydrophilic polyethersulfone (PES) syringe filters are suitable for filtering expressed Aβ$_{(M1-42)}$ and Aβ$_{(C1-42)}$ before HPLC purification while minimizing peptide loss.[36] Nevertheless, we find that *only* PES filters should be used for filtering the labeling reaction mixture, as the use of PVDF filters resulted in substantial loss of fluorophore or biotin labeled Aβ$_{(C1-42)}$ peptide.

The filtered solution is then subjected to purification by preparative reverse-phase HPLC on a C8 column at 80 °C. The labeled $A\beta_{(C1-42)}$ elutes at ca. 34–38% acetonitrile. The pure fractions of labeled $A\beta_{(C1-42)}$ peptide are combined.

Using these procedures, we labeled Aβ with three different maleimide reagents: maleimide-6-carboxyfluorescein (FAM), maleimide-5-tetramethylrhodamine (TAMRA), and maleimide-PEG_2-biotin (Figure 3). MALDI-MS corroborated the composition of each labeled Aβ peptide (Figure 4). HPLC analysis indicated the purity of each peptide to be 94.0–96.6% (Figures 5, S10, S12, S14). After lyophilizing the combined pure HPLC fractions, we gravimetrically determined the yield of each of the labeled peptides as the trifluoroacetate salt to be 2.6–3.6 mg per liter of bacterial culture (Table S15). To provide aliquots of monomeric labeled Aβ for subsequent biophysical and biological studies, we dissolve and incubate the lyophilized powder of labeled Aβ in HFIP and portion the labeled Aβ into 0.02 μmol (ca. 0.1 mg) aliquots. We then lyophilize the aliquots to remove HFIP and store the lyophilized aliquots in a desiccator at −20 °C until further use.

Oligomerization and Fibrilization Studies of Labeled $A\beta_{(C1-42)}$.

To determine whether N-terminal labeling perturbs the aggregation properties of the $A\beta_{(C1-42)}$, we compared labeled Aβ to unlabeled Aβ by SDS-PAGE, thioflavin T (ThT) fluorescence assays, fluorescence suppression assays, and transmission electron microscopy (TEM). In these studies we used $A\beta_{(M1-42)}$, an expressed homologue with properties similar to native $A\beta_{(1-42)}$, as an unlabeled Aβ control.[36]

SDS-PAGE shows that the labeled Aβ peptides behave similarly to $A\beta_{(M1-42)}$ (Figure 6). We ran SDS-PAGE at a range of concentrations to better observe both the monomeric and oligomeric species, and we visualized the peptides by silver staining. We also visualized the FAM- and TAMRA-labeled Aβ directly by fluorescence imaging. At lower concentrations (e.g., 4 and 8 μM), the Aβ shows up exclusively or predominantly as the monomer, which appears as a band between the 4.6 kDa and 10 kDa ladder bands. At higher concentrations (e.g., 125 and 250 μM), bands associated with trimers and tetramers appear just below and above the 17 kDa ladder band. In spite of the differences among the N-terminal substituents and visualization techniques for the SDS-PAGE gels, all of the gels show remarkably similar patterns of monomers and oligomers among the four peptides.

ThT fluorescence assays are widely used to study the onset of fibril formation by amyloidogenic peptides. In these assays, Aβ or a related amyloidogenic peptide is incubated with ThT. After a lag time, the onset of fibril formation is marked by the rapid increase in ThT fluorescence and a subsequent plateau. In a ThT assay, the $A\beta_{(M1-42)}$ exhibited a lag time of ca. 100 minutes, followed by a rapid increase in fluorescence and a plateau, reflecting the formation of fibrils (Figure 7A). The biotin-labeled $A\beta_{(C1-42)}$ exhibited a shorter lag time (ca. 20 minutes), followed by a more rapid increase in fluorescence and a plateau (Figure 7B). The FAM label absorbs light at wavelengths similar to ThT and does not permit a conventional ThT fluorescence assay to be performed. Aggregation of the FAM- and TAMRA-labeled Aβ can be observed by a diminution of fluorescence as the labeled Aβ begins to aggregate, even before the onset of fibril formation.[27,29] We thus studied the aggregation of these fluorophore-labeled Aβ peptides through a fluorescence

suppression assay. In this assay, the FAM-labeled $A\beta_{(C1-42)}$ exhibited a slight initial diminution in the fluorescence signal, followed by a lag time, and then a rapid drop in fluorescence. The initial diminution in fluorescence may reflect the formation of small oligomers, while the subsequent drop likely reflects the formation of fibrils (Figure 7C). In contrast, the TAMRA-labeled $A\beta_{(C1-42)}$ exhibited an immediate substantial drop in fluorescence, suggesting more rapid aggregation and fibril formation (Figure 7D). The differences observed between the biotin-labeled $A\beta_{(C1-42)}$ and the $A\beta_{(M1-42)}$, and between the FAM- and TAMRA-labeled $A\beta_{(C1-42)}$, indicate that the choice of label is important in the aggregation of the $A\beta$.

To directly visualize amyloid fibrils formed by labeled $A\beta$, we performed transmission electron microscopy (TEM). TEM studies reveal that $A\beta_{(M1-42)}$, biotin-labeled $A\beta_{(C1-42)}$, FAM-labeled $A\beta_{(C1-42)}$, and TAMRA-labeled $A\beta_{(C1-42)}$ all form fibrils after one day of incubation (Figure 8 and Figure S18). The fibrils differ in morphology: $A\beta_{(M1-42)}$ forms wide, twisted, multi-fibrillar assemblies. Biotin-labeled $A\beta_{(C1-42)}$ forms long, thin fibrils. FAM- and TAMRA-labeled $A\beta_{(C1-42)}$ form more heterogenous fibrils. The TEM studies further confirm that the choice of label is important in the aggregation of $A\beta$. We performed atomic force microscopy (AFM) to further characterize the fibrils. AFM shows that the fibrils formed by $A\beta_{(M1-42)}$, biotin-labeled $A\beta_{(C1-42)}$, FAM-labeled $A\beta_{(C1-42)}$, and TAMRA-labeled $A\beta_{(C1-42)}$ range in height from ca. 2–5 nm and exhibit some morphological differences (Figure 9 and S19). Although there are differences among the rates of fibril formation and the morphologies of the fibrils, the TEM and AFM experiments and the ThT and fluorescence suppression experiments collectively show that all of the labeled $A\beta$ peptides retain the propensity to form fibrils.

Fluorescence Microscopy Studies of Fluorophore-Labeled $A\beta_{(C1-42)}$.

To evaluate the applicability of the labeled $A\beta$ in fluorescence microscopy, we visualized the interaction of the FAM- and TAMRA-labeled $A\beta_{(C1-42)}$ with mammalian cells and bacteria. The interaction of $A\beta$ with neuronal cell membranes is thought to be important in neurodegeneration in Alzheimer's disease.[28,46] When we treated SH-SY5Y neuroblastoma cells with the fluorescent $A\beta$, we observed binding to the cell membranes, internalization of the fluorescent $A\beta$ (Figure 10A, 10B, and S17). These interactions occurred rapidly (within 3 hours) for the TAMRA-$A\beta$ and more slowly (within 48 hours) for the FAM-$A\beta$. In both cases, we observe small punctate features within the cells, suggesting that internalization occurs through interaction with the cell membranes and the formation of endosomes. Macrophages have been shown to play an important role in $A\beta$ clearance.[47,48] When we treated RAW 264.7 macrophage cells with fluorescent $A\beta$, we also observed uptake of the $A\beta$, with the uptake of the TAMRA-$A\beta$ occurring more extensively. The punctate features associated with the internalized peptides are larger, consistent with phagocytosis of the labeled $A\beta$ by the macrophage (Figure 10C, 10D, and S17). $A\beta$ is also thought to have antimicrobial activity and play a role in innate immune response.[49,50] When we treated *B. subtilis* (Gram-positive) and *E. coli* (Gram-negative) with the fluorescent $A\beta$, we observed interaction of what appeared to be aggregated $A\beta$ with the bacterial cell walls (Figure 10E and 10F). These results indicate that the fluorescent $A\beta$ peptides prepared by this

method can be used to visualize and monitor interactions between Aβ and cells, with the TAMRA-Aβ giving somewhat more rapid and more uniform staining.[51]

CONCLUSIONS

Our discovery that expression of an $Aβ_{(MC1–42)}$ plasmid in *E. coli* leads to endogenous excision of methionine has enabled us to develop methods to express and purify $Aβ_{(C1–42)}$ and then label the Aβ at the N-terminus with fluorophores or biotin through cysteine-maleimide chemistry. The expression and labeling of $Aβ_{(C1–42)}$ provide an efficient and economical route to obtain ca. 14 mg of N-terminal cysteine Aβ or ca. 3 mg of labeled Aβ per liter of bacterial culture. Analytical HPLC and MALDI analyses confirm that the $Aβ_{(C1–42)}$ and labeled Aβ are pure and homogeneous. SDS-PAGE, ThT fluorescence assays and related fluorescence suppression assays, and TEM studies establish that N-terminally labeled Aβ retains aggregation properties similar to unlabeled Aβ. Fluorescence microscopy shows that the labeled Aβ can be used to visualize interactions between Aβ and mammalian or bacterial cells.

Expression offers advantages over solid phase peptide synthesis, because the expressed Aβ is free of deletion or epimerization impurities. The addition of a single cysteine residue at the N-terminus enables labeling of the expressed Aβ with complete site specificity using maleimide-based reagents. Although NHS esters are widely used to label lysine residues or the N-terminus of proteins, *site specific* labeling of expressed Aβ is not possible using NHS-ester based reagents, because multiple labeling sites are present.

The expression, purification, and conjugation methods that we report here should enable other researchers to produce milligram quantities of pure labeled Aβ in their own laboratories. Ready access to labeled Aβ peptides will advance amyloid and Alzheimer's disease research by facilitating experiments that might otherwise be hindered by insufficient access to these valuable peptides.[52–64] We anticipate these fluorophore and biotin labeled Aβ peptides will be especially useful in investigating cellular interactions, transport, and clearance of Aβ, as well as screening anti-Aβ antibodies.[65–67] Researchers may also apply our methods to label Aβ with other popular fluorophores, such as Alexa Fluor™ 488 and BODIPY™ FL.[68,69] The potential to install a variety of fluorophores may enable studies of Aβ peptide interactions through Förster resonance energy transfer (FRET) studies in which Aβ has been labeled with FRET donors and acceptors, such as Cy3 and Cy5. The N-terminal cysteine Aβ formed through N-terminal methionine excision also promises to be valuable in other applications, such as native chemical ligation or other site-specific modifications. We anticipate that this method of incorporating an N-terminal cysteine residue can be adapted to the expression, purification, and conjugation of other variants of Aβ, such as familial mutants,[36] further enabling a variety of experiments in Alzheimer's disease research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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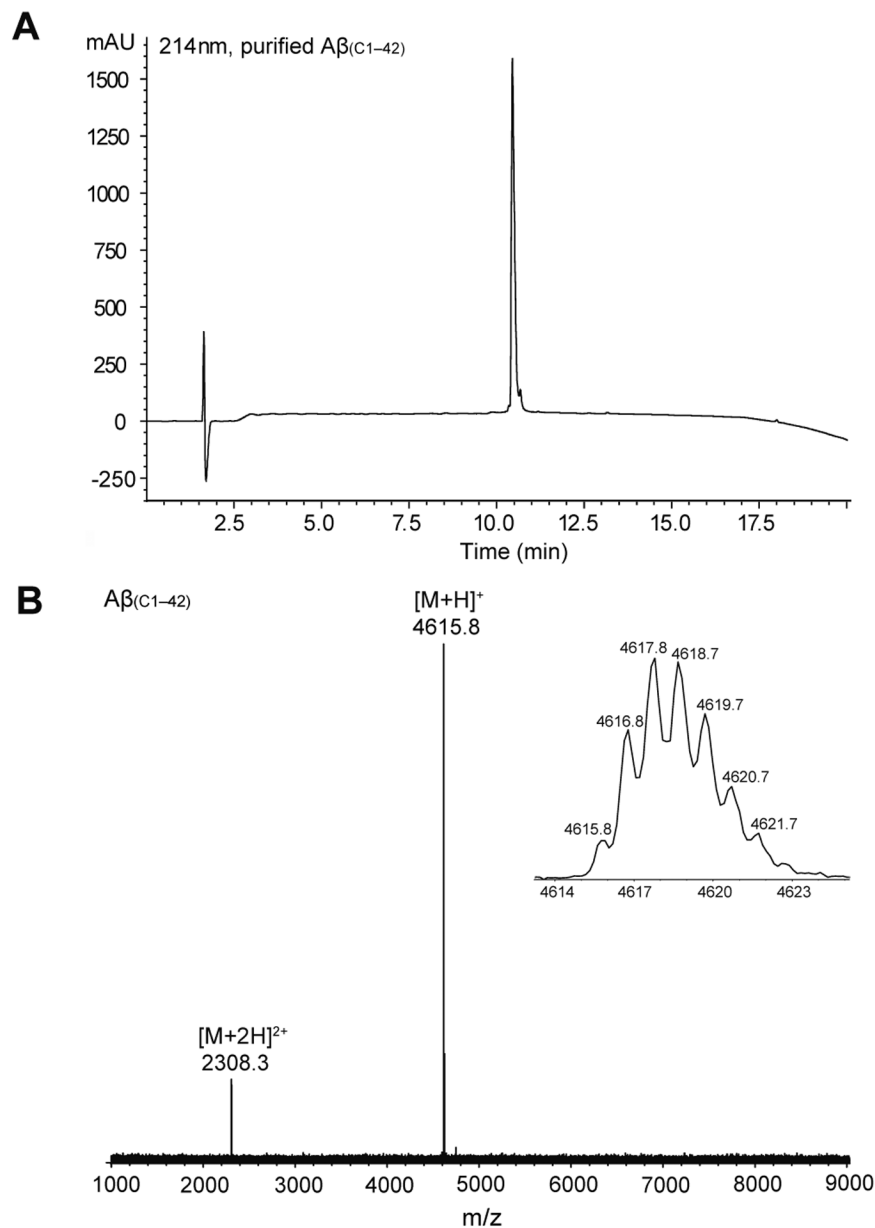
1   42

Aβ(1–42)   DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Aβ(MC1–42)   MCDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Aβ(C1–42)   CDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
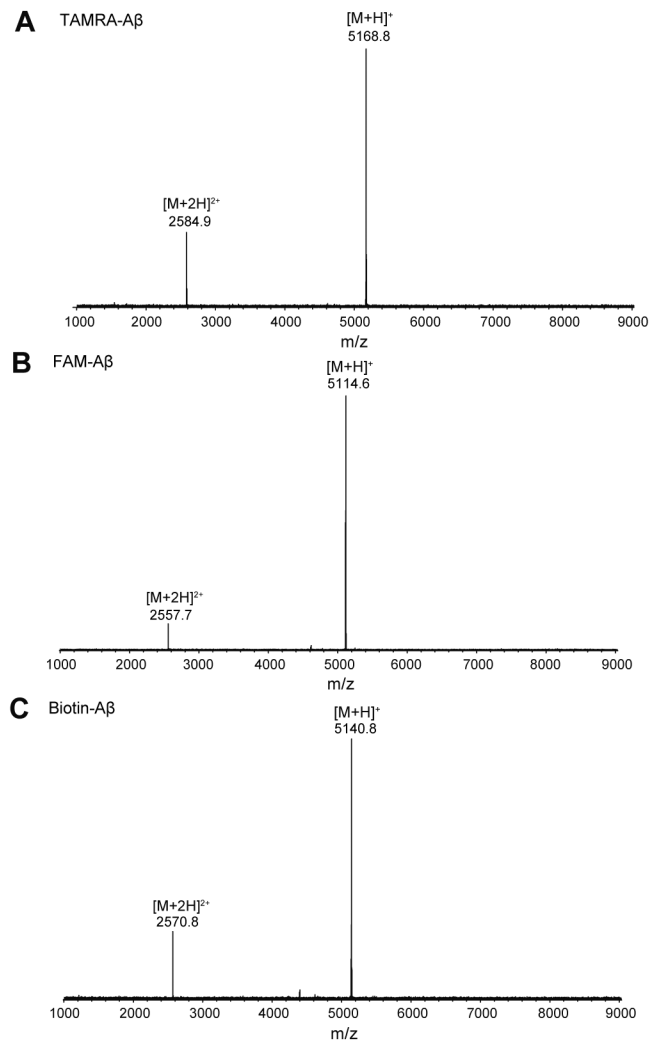
Figure 1.
Sequences of Aβ(1–42), Aβ(MC1–42), and Aβ(C1–42).

Figure 2.
Characterization of Aβ(C1–42). (A) Analytical HPLC trace of purified Aβ(C1–42). HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes. (B) MALDI mass spectrum of Aβ(C1–42). Mass-to-charge (m/z) labels on peaks correspond to [M+H]+ and [M+2H]2+.
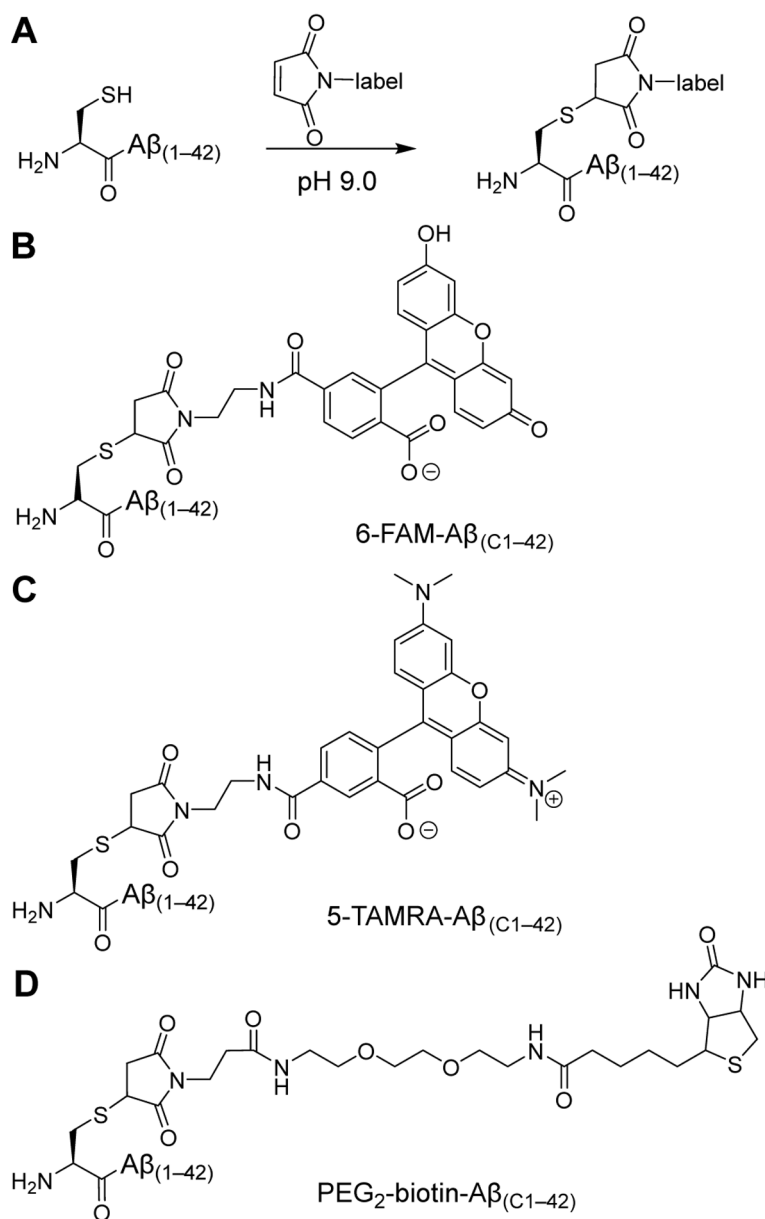
Figure 3.
Conjugation of Aβ(C1–42) with maleimide reagents (A). Structures of labeled Aβ products: 6-FAM-Aβ(C1–42) (B), 5-TAMRA-Aβ(C1–42) (C), and PEG2-biotin-Aβ(C1–42) (D).

Figure 4.
MALDI mass spectra of fluorophore and biotin labeled Aβ$_{(C1–42)}$: (A) TAMRA-labeled Aβ. (B) FAM-labeled Aβ. (C) Biotin-labeled Aβ. Mass-to-charge (m/z) labels on peaks correspond to [M+H]$^+$ and [M+2H]$^{2+}$.
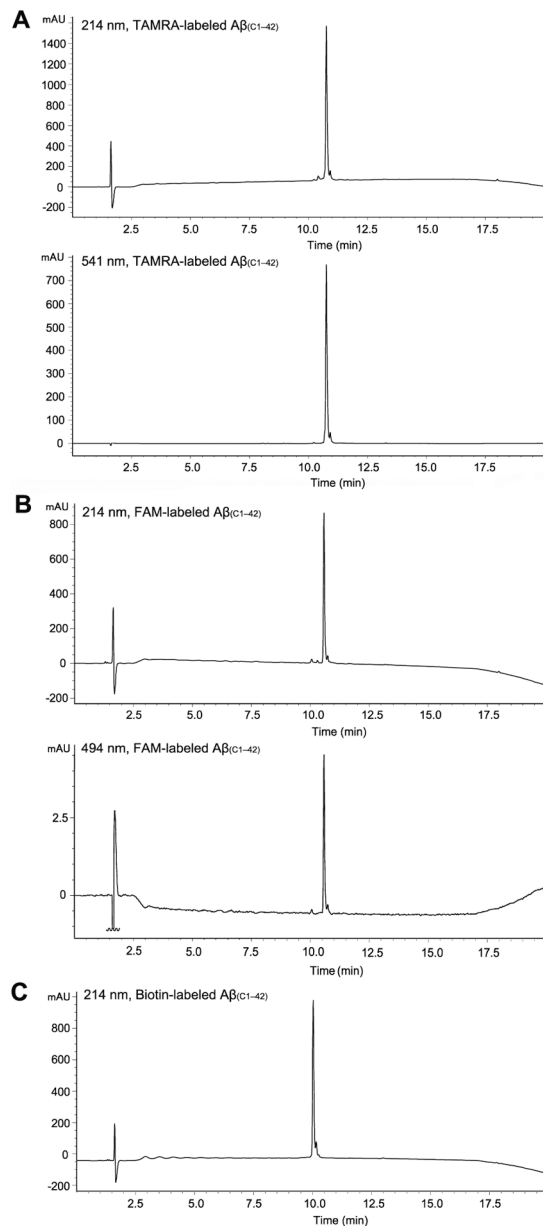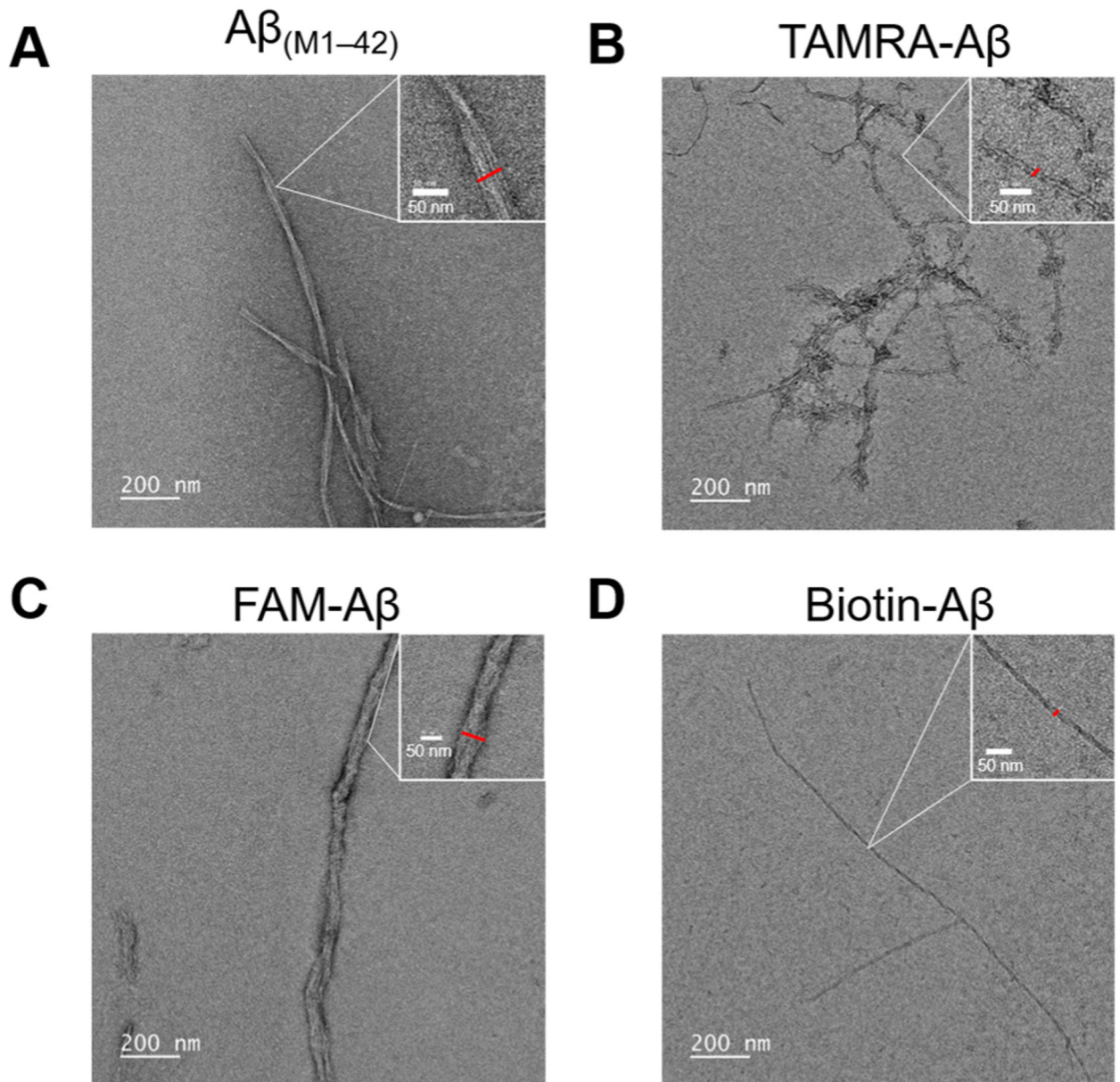
Figure 5.
Analytical HPLC traces of purified fluorescent and biotin labeled $A\beta_{(C1-42)}$: (A) HPLC trace of TAMRA-labeled $A\beta$ at 214 nm and 541 nm. (B) HPLC trace of FAM-labeled $A\beta$ at 214 nm and 494 nm. (C) HPLC trace of biotin-labeled $A\beta$ at 214 nm. HPLC was performed on a C18 column at 60 °C with elution with 5–67% acetonitrile over 15 minutes. The absorbance of FAM-labeled $A\beta$ at 494 nm is low in the acidic (0.1% TFA) HPLC mobile phase.
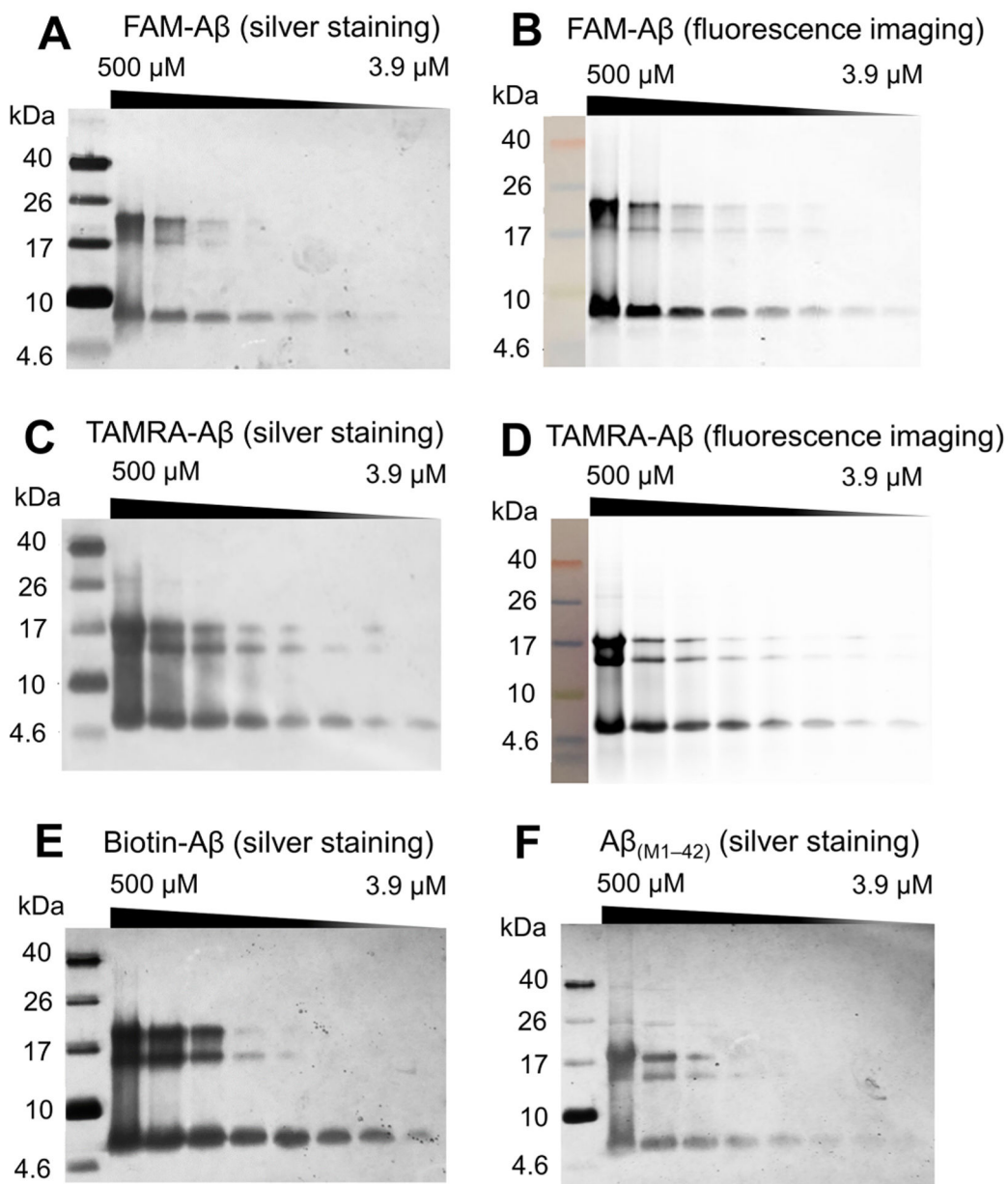
Figure 6.
SDS-PAGE of labeled $A\beta_{(C1-42)}$ and $A\beta_{(M1-42)}$ at a range of concentrations, visualized by silver staining and fluorescence imaging. (A and B) FAM-labeled $A\beta_{(C1-42)}$ visualized by silver staining and fluorescence imaging. (C and D) TAMRA-labeled $A\beta_{(C1-42)}$ visualized by silver staining and fluorescence imaging. (E and F) Biotin-labeled $A\beta_{(C1-42)}$ and $A\beta_{(M1-42)}$ visualized by silver staining. The contrast of images 6A, 6C, 6E, and 6F have been increased by 40% to enhance the visualization of the silver staining.
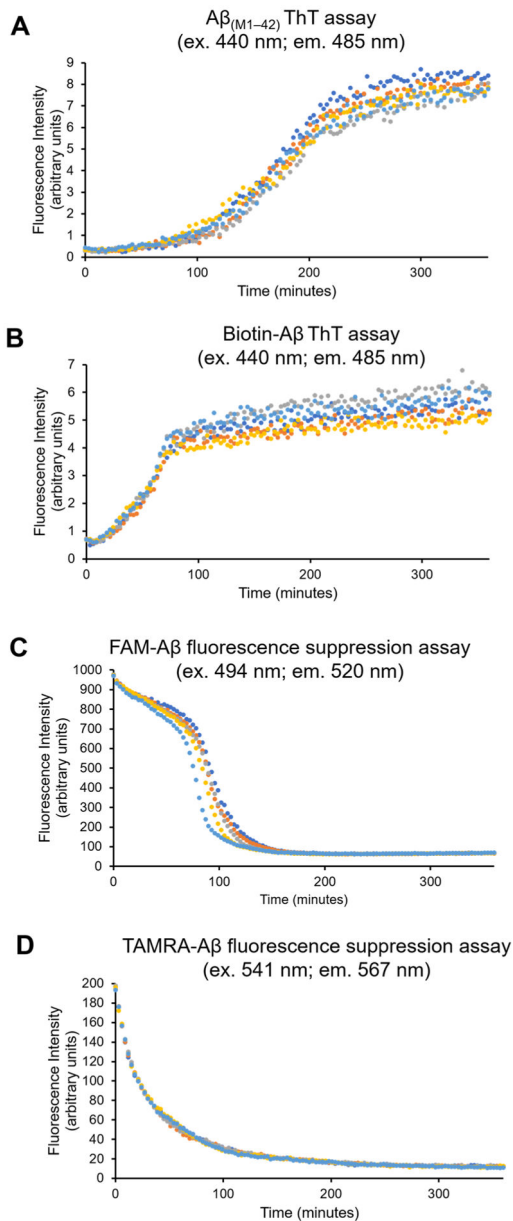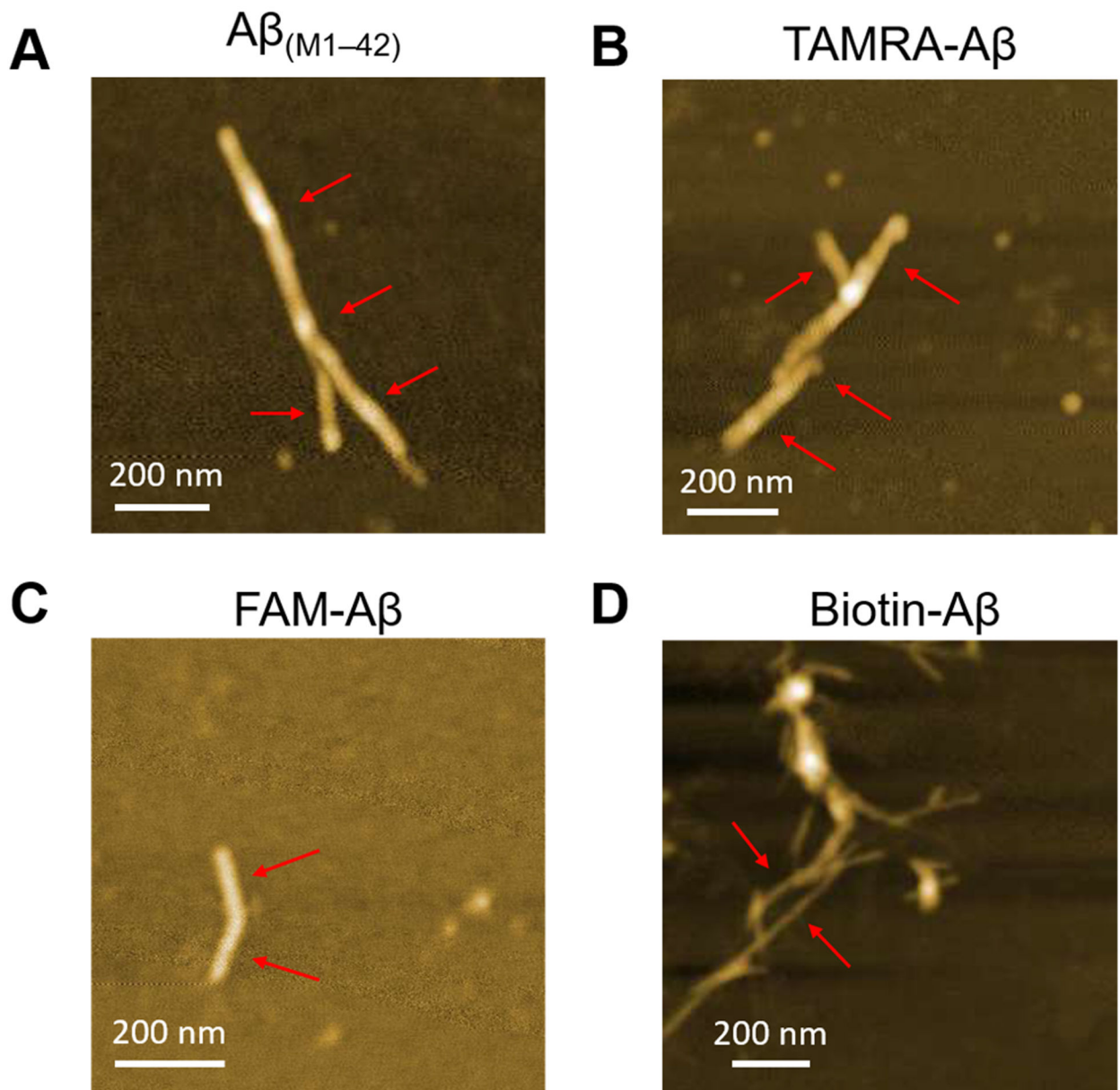
Figure 7.
ThT fluorescence assays and fluorescence suppression assays of labeled $A\beta_{(C1-42)}$ and $A\beta_{(M1-42)}$. (A and B) ThT fluorescence assays of $A\beta_{(M1-42)}$ and biotin-labeled $A\beta$ (40 μM peptide, 40 μM ThT, 37 °C, quiescent, 6 hours). (C and D) Fluorescence suppression assays of FAM-labeled $A\beta$ and TAMRA-labeled $A\beta$ (40 μM peptide, 37 °C, quiescent, 6 hours). These assays were performed in five replicates (light blue, dark blue, orange, yellow, and gray).
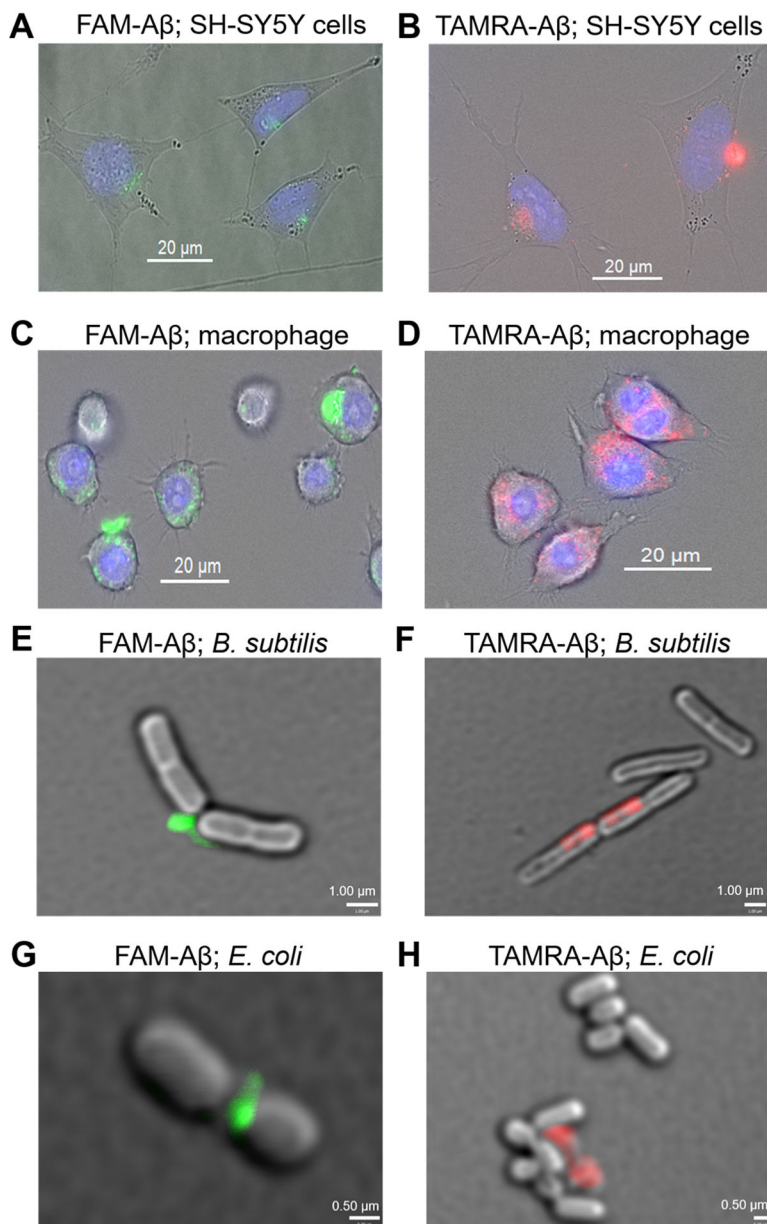
Figure 8.
Transmission electron micrographs of fibrils formed by Aβ peptides: (A) Aβ$_{(M1-42)}$, fibril width ca. 36 nm; (B) TAMRA-labeled Aβ, fibril width ca. 15 nm; (C) FAM-labeled Aβ, fibril width ca. 56 nm; (D) Biotin-labeled Aβ, fibril width ca. 12 nm. Fibrils were formed by incubating 40 μM of each peptide in PBS buffer (pH 7.4, 37 °C, 1 day, 225 rpm shaking). The red bars indicate the locations where the widths of the fibrils were measured.

Figure 9.
Atomic force micrographs of fibrils formed by Aβ peptides: (A) Aβ$_{(M1–42)}$, fibril height ca. 4.5 nm; (B) TAMRA-labeled Aβ, fibril height ca. 5.2 nm; (C) FAM-labeled Aβ, fibril height ca. 2.3 nm; (D) Biotin-labeled Aβ, fibril height ca. 4.1 nm. Fibrils were formed by incubating 40 μM of each peptide in PBS buffer (pH 7.4, 37 °C, 1 day, 225 rpm shaking). The red arrows indicate the locations where the heights of the fibrils were measured.

Figure 10.
Fluorescence micrographs of labeled Aβ with mammalian cells and bacteria. (A and B) SH-SY5Y neuroblastoma cells treated with FAM- and TAMRA-labeled Aβ (10 μM peptides, 37 °C, 48 and 3 hours, respectively). The nuclei are shown in blue through Hoechst 33342 staining. (C and D) RAW 264.7 macrophage cells treated with FAM- and TAMRA-labeled Aβ (10 μM peptides, 37 °C, 4 hours). (E and F) *B. subtilis* treated with FAM- and TAMRA-labeled Aβ (5 μM peptides, 37 °C, 225 rpm, 225 rpm shaking, 2 hours). (G and H) *E. coli* treated with FAM- and TAMRA-labeled Aβ (5 μM peptides, 37 °C, 225 rpm, 225 rpm shaking, 2 hours).