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Authors

Yoo, Stan

Zhang, Sheng

Kreutzer, Adam G

et al.

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An efficient method for the expression and purification of A β (M1–42)

Stan Yoo^{#1}, Sheng Zhang^{#1}, Adam G. Kreutzer¹, and James S. Nowick^{1,*}

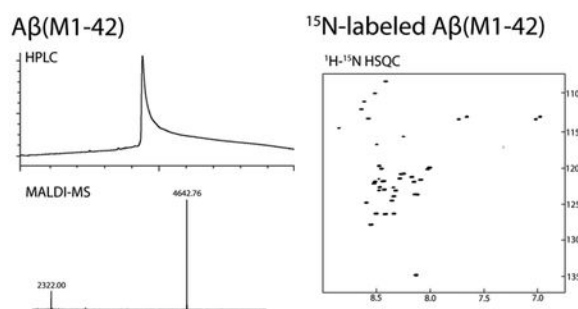
¹Department of Chemistry, University of California, Irvine, Irvine, California 92697-2025, USA.

[#] These authors contributed equally to this work.

Abstract

Advances in amyloid research rely on improved access to the β -amyloid peptide, A β . N-terminal methionine extended A β , A β (M1–42), is a readily expressed and widely used form of A β with comparable properties to the natural A β (1–42) peptide. Expression of A β (M1–42) is simple to execute and avoids an expensive and often difficult enzymatic cleavage step associated with expression and isolation of A β (1–42). This paper reports an efficient method for expression and purification of A β (M1–42) and ¹⁵N-labeled A β (M1–42). This method affords the pure peptide at about 19 mg per liter of bacterial culture through simple and inexpensive steps in three days. This paper also reports a simple method for construction of recombinant plasmids, and the expression and purification of A β (M1–42) peptides containing familial mutations. We anticipate that these methods will enable experiments that would otherwise be hindered by insufficient access to A β .

Graphical Abstract



*To whom correspondence should be addressed: James S. Nowick. Department of Chemistry, University of California, Irvine, Irvine, California 92697-2025, USA. jsnowick@uci.edu.

Supporting Information

The Supporting Information contains procedures for the expression, purification, sample preparation, and characterization of A β (M1–42) and ¹⁵N-labeled A β (M1–42), and A β (M1–42) peptides with familial mutations.

The Supporting Information is available free of charge on the ACS Publications website at <http://pubs.acs.org>.

Notes

The authors declare no competing financial interest.

Introduction

The β -amyloid peptide, A β , is central to the pathology of Alzheimer's disease.¹⁻³ The 40- and 42-amino acid alloforms of A β aggregate to form fibrils and oligomers in Alzheimer's disease. The 42-amino acid alloform, A β (1–42) aggregates more rapidly and is more toxic than A β (1–40).⁴

A plentiful source of pure A β peptides, including isotopically labeled A β and various mutants associated with familial Alzheimer's disease, is essential to progress in research in A β aggregation and Alzheimer's disease. Early efforts to generate A β focused on chemical synthesis of the peptide. Chemical synthesis of A β can lead to impurities, such as amino acid deletion products, that are difficult to eliminate during purification.⁵ Within the past fifteen years, expression has emerged as a useful alternative for preparing A β of superior purity. Expressed A β has been reported to aggregate three times faster and be significantly more toxic toward neuronal cells than synthetic A β .⁵ Expressed A β is typically generated as a fusion protein that is cleaved after expression using a protease.⁵⁻⁷ This approach requires expression and purification of the protease and an affinity purification step, which can make the preparation of A β costly and time-intensive.

Walsh and co-workers have introduced an A β expression system that circumvents the need for protease cleavage and affinity chromatography. In this expression system, A β (1–40) and A β (1–42) are expressed as variants A β (M1–40) and A β (M1–42) that contain an N-terminal methionine residue that originates from the translational start codon (Figure 1).⁸ A β (M1–40) and A β (M1–42) behave almost identically to the native peptides in aggregation and toxicity assays, and the additional N-terminal methionine has little impact on the fibril structure.^{9, 10} Because of these characteristics, A β (M1–40) and A β (M1–42) have emerged as widely used alternatives to the native A β peptides.¹¹⁻¹⁶ Although this expression system provides ready access to A β (M1–40) with yields of 5-20 mg per liter of bacterial culture, the preparation of A β (M1–42) gives substantially lower yields.⁸

Here we report an efficient method for expression and purification of A β (M1–42) and associated homologues, including the uniformly ¹⁵N-labeled peptide and familial mutants. Our method simplifies the construction of plasmids containing mutant A β sequences and bypasses cumbersome steps in previously reported purification procedures. Our approach offers several major advantages over previous procedures: (1) a short preparation time of only three days, (2) minimal expense, (3) easier laboratory techniques, and (4) production of substantial amounts of highly pure A β peptides at about 19 mg per liter of bacterial culture.

Material and Methods

All chemicals were used as received unless otherwise noted. Deionized water (18 M Ω) was obtained from a Thermo Scientific Barnstead Genpure Pro water purification system. The pET-Sac-A β (M1–42) was a gift from Dominic Walsh (Addgene plasmid # 71875).⁸ DNA sequences that encode A β (M1–42) familial mutants were purchased in 500 ng quantities from Genewiz. *NdeI* and *SacI* restriction enzymes, CutSmart buffer, and shrimp alkaline phosphatase (rSAP) were purchased from New England Biolabs (NEB). TOP10 Ca²⁺-

competent *E. coli* and BL21 DE3 PLYS Star Ca²⁺-competent *E. coli*, T4 ligase, and ethidium bromide were purchased from Thermo Fisher Scientific. Zymo ZR plasmid miniprep kit was purchased from Zymo Research. Zymoclean Gel DNA Recovery Kit was purchased from Zymo Research. Carbenicillin and chloramphenicol were purchased from RPI Research Products. The carbenicillin was added to culture media as a 1000X stock solution (50 mg/mL) in water. The chloramphenicol was added to culture media as a 1000X stock solution (34 mg/mL) in EtOH. ¹⁵NH₄Cl was purchased from Cambridge Isotope Laboratories.

The concentration of DNA was measured using a Thermo Scientific NanoDrop spectrophotometer. *E. coli* were incubated in a Thermo Scientific MaxQ Shaker 6000. *E. coli* were lysed using a QSonica Q500 ultrasonic homogenizer. Analytical reverse-phase HPLC was performed on an Agilent 1200 instrument equipped with a Phenomenex Aeris PEPTIDE 2.6u XBC18 column with a Phenomenex SecurityGuard ULTRA cartridges guard column for C18 column. Preparative reverse-phase HPLC was performed on a Rainin Dynamax instrument SD-200 equipped with an Agilent ZORBAX 300SB-C8 semi-preparative column (9.4 × 250 mm) with a ZORBAX 300SB-C3 preparative guard column (9.4 × 15 mm). During purifications, the C8 column and the guard column were heated to 80 °C in a Sterlite plastic bin equipped with a Kitchen Gizmo Sous Vide immersion circulator. (Any water heater large enough to submerge a HPLC column should be sufficient.) HPLC grade acetonitrile and deionized water (18 MΩ), each containing 0.1% trifluoroacetic acid (TFA), were used for analytical and preparative reverse-phase HPLC. MALDI-TOF mass spectrometry was performed using an AB SCIEX TOF/TOF 5800 System. ¹H-¹⁵N HSQC NMR was performed using a Bruker DRX500 500 MHz spectrometer equipped with a cryogenic probe.

For details of the expression and purification of Aβ(M1–42) and ¹⁵N-labeled Aβ(M1–42), the construction of recombinant plasmids, and the expression and purification of mutant Aβ(M1–42) peptides, see the Supporting Information.

Results and Discussion

The following describes the procedures that we have developed for the preparation of the Aβ(M1–42) wild-type and mutant peptides: For expression of the wild-type Aβ(M1–42) peptide, we use the commercially available plasmid, pET-Sac-Aβ(M1–42).⁸ For expression of mutant Aβ(M1–42) peptides, we construct recombinant plasmids containing mutant Aβ(M1–42) gene sequences using standard cloning techniques. Upon expression in *E. coli*, the peptides form inclusion bodies. The inclusion bodies are subjected to multiple rounds of washing, followed by solubilization in urea buffer. The resulting solution is filtered using a hydrophilic syringe filter and then immediately applied to a reverse-phase HPLC column. Pure HPLC fractions are then combined and lyophilized to give the peptide as a white powder. For biophysical and biological studies, the purified peptide is further treated with NaOH and then re-lyophilized. Yields are assessed both gravimetrically and by UV absorption. The composition and purity of the peptides are assessed by analytical HPLC, MALDI-MS, and SDS-PAGE with silver staining.

Expression of A β (M1–42)

To express A β (M1–42), pET-Sac-A β (M1–42) plasmid is transformed into BL21(DE3)pLysS competent *E. coli*. Expression is induced by isopropyl β -D-1-thiogalactopyranoside (IPTG). The expressed peptide is pelleted with the inclusion bodies, which are washed several times and then solubilized with 8 M urea. The yield of A β (M1–42) depends on the extent of cell growth prior to IPTG induction, with an OD₆₀₀ of ca. 0.45 proving optimal for wild-type A β (M1–42) production. Growth to substantially higher or lower OD₆₀₀ values gives lower yields of peptide.

Purification of A β (M1–42) by preparative HPLC

At this point in the procedure, the expressed peptide is handled like a synthetic peptide, and HPLC is used to purify it. The solution of the inclusion bodies in 8 M urea is filtered to prevent damaging the HPLC column. Initially, a 0.22 μ m nylon syringe filter was used, but doing so resulted in substantial loss of peptide. The hydrophobicity and propensity of A β to aggregate appear to make A β particularly prone to loss in filters. We screened several types of syringe filters to optimize peptide recovery, monitoring the relative concentrations of peptide by UV absorbance at 280 nm (Table 1).¹⁷ We found that a 0.22 μ m hydrophilic filter, such as hydrophilic polyvinylidene fluoride (PVDF) or polyethersulfone (PES), provided satisfactory peptide recovery.

A typical HPLC trace of unpurified A β (M1–42) shows three major peaks (Figure 2A). The first peak is the largest and contains mostly monomer, and the second and the third peaks appear to be oligomers (Figure S1). For preparative HPLC, a reverse-phase silica-based C8 column is used as the stationary phase, and a gradient of water and acetonitrile containing 0.1% trifluoroacetic acid is used as the mobile phase. To enhance resolution and reduce peak tailing, it was necessary to heat the column. Without heating, the resolution and yield of peptide are substantially lower. A β (M1–42) peptide monomer generally elutes at around 34% acetonitrile when the C8 column is heated to 80 °C in a water bath. HPLC fractions containing pure peptide were combined, and the purity was confirmed by analytical HPLC (Figure 2B). Acetonitrile was removed by rotary evaporation, and the aqueous solution of pure peptide was then frozen and lyophilized. These procedures typically yield about 19 mg of A β (M1–42) as the trifluoroacetate salt from one liter of bacterial culture.

This purification procedure does not require specialized equipment or costly reagents and is not time-consuming. It avoids the use of specialized and costly columns, such as cation-exchange chromatography columns and size-exclusion chromatography columns. Another advantage of this procedure is that it yields lyophilized powder as the final peptide product. Working with lyophilized peptide is convenient for subsequent studies as it can be dissolved in any appropriate buffer at a desired concentration.

The purity and composition of the A β (M1–42) peptide were further assessed through MALDI-MS, and SDS-PAGE with silver staining. MALDI-MS confirms that the observed mass of A β (M1–42) matches the expected mass (Figure 2C). The silver-stained SDS-PAGE gel shows that at low concentrations, the A β (M1–42) peptide exists as a monomer. At higher

concentrations, A β (M1–42) begins to form oligomers with molecular weights consistent with trimers and tetramers (Figure 2D).

Sample preparation for biophysical and biological studies

The propensity of A β to aggregate necessitates the preparation of monomeric A β for subsequent studies.¹⁸ Without any sample preparation, studies are reported to be irreproducible.¹⁹ Fezoui and co-workers reported that treatment of A β with NaOH disrupts aggregates and generates A β that is monomeric or nearly monomeric.²⁰ This NaOH-treated A β is widely used in subsequent aggregation studies.¹⁸

We applied this procedure to each batch of expressed A β to generate aliquots for further studies. Thus, the lyophilized powder was dissolved in 2 mM NaOH, and the pH was adjusted, if necessary, by addition of 0.1 M NaOH, to give a pH 10.5 solution. The solution was sonicated for one minute, the concentration was determined by UV absorbance at 280 nm, and the yield of A β (M1–42) was calculated. The solution was then aliquoted in 0.0055 or 0.020 micromole portions into small tubes, and these samples were frozen and lyophilized. The lyophilized aliquots are stored in a desiccator at –20 °C.

Expression of ¹⁵N-labeled A β (M1–42)

¹⁵N-labeled A β peptides are useful tools for structural studies by NMR and for studying binding profiles of A β . For expression of ¹⁵N-labeled A β (M1–42), *E. coli* are grown to an OD600 of ca. 0.45 in LB media, then the LB media is exchanged to M9 minimal media containing ¹⁵NH₄Cl. Expression is induced in the ¹⁵N-enriched M9 media for 16 hours with IPTG. Purification and sample preparation of ¹⁵N-labeled A β (M1–42) is performed identically to unlabeled A β (M1–42). The composition of the ¹⁵N-labeled A β (M1–42) was assessed by MALDI-MS (Figure 3A). A ¹H-¹⁵N HSQC NMR spectrum of 160 μ M ¹⁵N-labeled A β (M1–42) in 50 mM potassium phosphate buffer in 10% D₂O was recorded at 5 °C with a 500 MHz NMR spectrometer equipped with a cryogenic probe (Figure 3B). This spectrum matches the NMR spectrum reported by Macao and co-workers.⁹

The yield of the ¹⁵N-labeled A β (M1–42) peptide is comparable to that of the unlabeled A β (M1–42) peptide, at around 19 mg per liter of bacterial culture. Access to such amounts of the ¹⁵N-labeled peptide at low cost is enabling for performing experiments such as SAR by NMR.²¹

Construction of recombinant plasmids for expression of mutant A β (M1–42) peptides

To express A β (M1–42) peptides containing familial mutations, we construct recombinant plasmids by ligating enzymatically digested pET-Sac-A β (M1–42) and DNA sequences that encode A β (M1–42) mutants (Figure 4). In this procedure, pET-Sac-A β (M1–42) is first digested with *NdeI* and *SacI* restriction enzymes to remove the wild-type A β (M1–42) sequence. Next, the digested pET-Sac vector is treated with shrimp alkaline phosphatase (rSAP) to remove the terminal phosphate groups. The digested vector is isolated by agarose gel electrophoresis purification using a commercially available kit. Synthetic DNA encoding each mutant A β (M1–42) is purchased and then digested with *NdeI* and *SacI* to generate the insert. The vector and insert are ligated using T4 ligase and then transformed into TOP 10

competent *E. coli*. *E. coli* transformed with ligated plasmid form colonies on agar containing carbenicillin. Plasmids are isolated from colonies, and the sequences are verified by DNA sequencing. For this paper, we constructed five plasmids with familial mutations: A21G, E22G, E22K, E22Q, and D23N.

This cloning strategy is inexpensive and is simpler to execute than site-directed mutagenesis. The entire cloning procedure takes two days, and many mutants can be generated concurrently. Another advantage of this strategy is that A β (M1–42) plasmids containing multiple point mutations can be prepared as easily as plasmids containing single point mutations.

The purification and preparation of A β (M1–42) containing familial mutations is performed identically to that of A β (M1–42). The composition of familial mutant A β (M1–42) peptides was assessed using MALDI-MS (Figure 5A). The expression levels and yields of the A β (M1–42) familial mutants varied due to different aggregation propensities of the peptides. Analytical HPLC traces of crude samples of the A21G and E22Q mutants showed smaller first peaks and larger second and third peaks, suggesting that more oligomers are formed after dissolving the inclusion bodies. Figure 5B shows typical yields of the peptides. Our expression and purification procedures proved unsuitable for the E22Q mutant, which showed very little monomer in the HPLC trace.

Conclusion

The procedures described herein provide an efficient method for expression and purification of A β (M1–42), ¹⁵N-labeled A β (M1–42), and A β (M1–42) containing several familial mutations. Our method employs the most convenient features of protein expression and peptide purification to provide ready access to good quantities of the pure peptides. We anticipate that our method will provide new opportunities to pilot experiments that require large amounts of A β . We also anticipate that this method can be adjusted for the expression and purification of other amyloidogenic proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Benilova I, Karran E, and De Strooper B (2012) The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes, *Nature neuroscience* 15, 349–357. [PubMed: 22286176]

2. Haass C, and Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide, *Nature reviews. Molecular cell biology* 8, 101–112. [PubMed: 17245412]
3. Chiti F, and Dobson CM (2017) Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade, *Annual review of biochemistry* 86, 27–68.
4. Jarrett JT, Berger EP, and Lansbury PT (2002) The carboxy terminus of the .beta. amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease, *Biochemistry* 32, 4693–4697.
5. Finder VH, Vodopivec I, Nitsch RM, and Glockshuber R (2010) The recombinant amyloid-beta peptide Abeta1-42 aggregates faster and is more neurotoxic than synthetic Abeta1-42, *Journal of molecular biology* 396, 9–18. [PubMed: 20026079]
6. Hortschansky P, Schroeckh V, Christopeit T, Zandomenighi G, and Fandrich M (2005) The aggregation kinetics of Alzheimer's beta-amyloid peptide is controlled by stochastic nucleation, *Protein science : a publication of the Protein Society* 14, 1753–1759. [PubMed: 15937275]
7. Liao YH, and Chen YR (2015) A novel method for expression and purification of authentic amyloid-beta with and without (15)N labels, *Protein expression and purification* 113, 63–71. [PubMed: 25969353]
8. Walsh DM, Thulin E, Minogue AM, Gustavsson N, Pang E, Teplow DB, and Linse S (2009) A facile method for expression and purification of the Alzheimer's disease-associated amyloid beta-peptide, *The FEBS journal* 276, 1266–1281. [PubMed: 19175671]
9. Macao B, Hoyer W, Sandberg A, Brorsson AC, Dobson CM, and Hard T (2008) Recombinant amyloid beta-peptide production by coexpression with an affibody ligand, *BMC biotechnology* 8, 82. [PubMed: 18973685]
10. Silvers R, Colvin MT, Frederick KK, Jacavone AC, Lindquist S, Linse S, and Griffin RG (2017) Aggregation and Fibril Structure of AbetaM01-42 and Abeta1-42, *Biochemistry* 56, 4850–4859. [PubMed: 28792214]
11. Sandberg A, Luheshi LM, Sollvander S, Pereira de Barros T, Macao B, Knowles TP, Biverstal H, Lendel C, Ekholm-Peterson F, Dubnovitsky A, Lannfelt L, Dobson CM, and Hard T (2010) Stabilization of neurotoxic Alzheimer amyloid-beta oligomers by protein engineering, *Proc Natl Acad Sci U S A* 107, 15595–15600. [PubMed: 20713699]
12. Cohen SI, Linse S, Luheshi LM, Hellstrand E, White DA, Rajah L, Otzen DE, Vendruscolo M, Dobson CM, and Knowles TP (2013) Proliferation of amyloidbeta42 aggregates occurs through a secondary nucleation mechanism, *Proc Natl Acad Sci U S A* 110, 9758–9763. [PubMed: 23703910]
13. Bertini I, Gonnelli L, Luchinat C, Mao J, and Nesi A (2011) A new structural model of Abeta40 fibrils, *Journal of the American Chemical Society* 133, 16013–16022. [PubMed: 21882806]
14. Colvin MT, Silvers R, Ni QZ, Can TV, Sergeyev I, Rosay M, Donovan KJ, Michael B, Wall J, Linse S, and Griffin RG (2016) Atomic Resolution Structure of Monomorphic Abeta42 Amyloid Fibrils, *Journal of the American Chemical Society* 138, 9663–9674. [PubMed: 27355699]
15. Meisl G, Yang X, Hellstrand E, Frohm B, Kirkegaard JB, Cohen SI, Dobson CM, Linse S, and Knowles TP (2014) Differences in nucleation behavior underlie the contrasting aggregation kinetics of the Abeta40 and Abeta42 peptides, *Proc Natl Acad Sci U S A* 111, 9384–9389. [PubMed: 24938782]
16. Munke A, Persson J, Weiffert T, De Genst E, Meisl G, Arosio P, Carnerup A, Dobson CM, Vendruscolo M, Knowles TPJ, and Linse S (2017) Phage display and kinetic selection of antibodies that specifically inhibit amyloid self-replication, *Proc Natl Acad Sci U S A* 114, 6444–6449. [PubMed: 28584111]
17. Syringe filters with large pore sizes (0.45 µm) result in incomplete filtration of the peptide and risk damaging HPLC columns.
18. Teplow DB (2006) Preparation of Amyloid β-Protein for Structural and Functional Studies, 413, 20–33.
19. Wood SJ, Maleeff B, Hart T, and Wetzel R (1996) Physical, morphological and functional differences between pH 5.8 and 7.4 aggregates of the Alzheimer's amyloid peptide Abeta, *Journal of molecular biology* 256, 870–877. [PubMed: 8601838]

20. Fezoui Y, Hartley DM, Harper JD, Khurana R, Walsh DM, Condron MM, Selkoe DJ, Lansbury PT, Fink AL, and Teplow DB (2009) An improved method of preparing the amyloid β -protein for fibrillogenesis and neurotoxicity experiments, *Amyloid* 7, 166–178.
21. Shuker SB, Hajduk PJ, Meadows RP, and Fesik SW (1996) Discovering High-Affinity Ligands for Proteins: SAR by NMR, *Science* 274, 1531–1534. [PubMed: 8929414]

	1 42
Aβ(1–40)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV
Aβ(1–42)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
Aβ(M1–42)	MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Figure 1.
Sequences of Aβ(1–40), Aβ(1–42), and Aβ(M1–42).

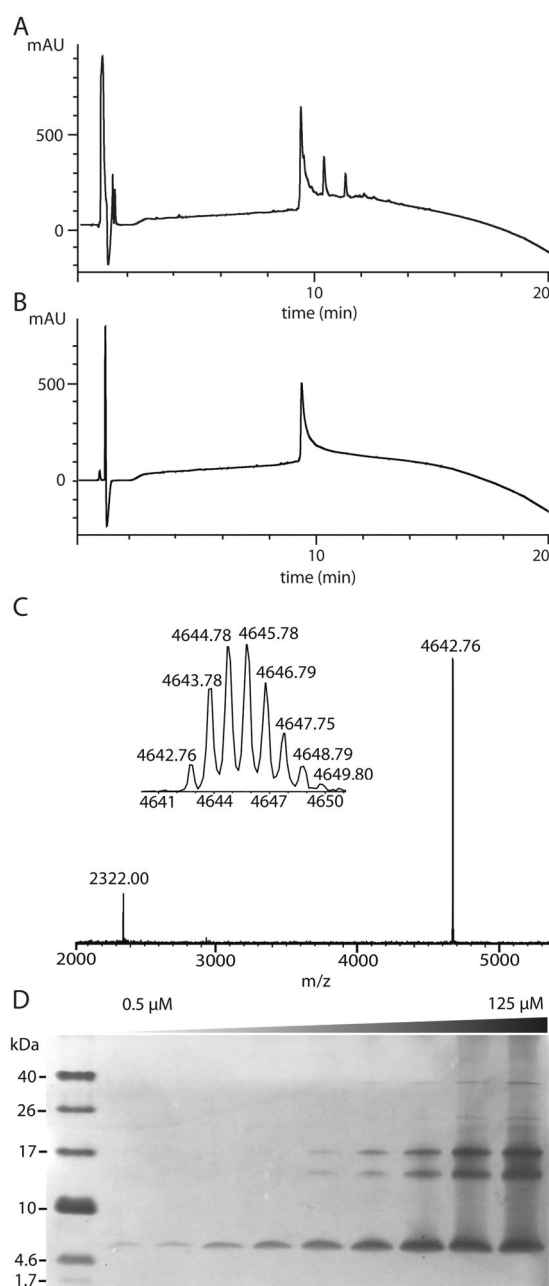


Figure 2.

Purification and characterization of Aβ(M1-42). (A) Typical analytical HPLC trace of filtered crude Aβ(M1-42) sample. (B) Typical analytical HPLC trace of purified Aβ(M1-42). (C) MALDI mass spectrum of purified Aβ(M1-42). (D) Silver-stained SDS-PAGE gel (16% polyacrylamide) of increasing concentrations of Aβ(M1-42) from 0.5 to 125 μM. A 12-μL aliquot was loaded in each lane of the gel.

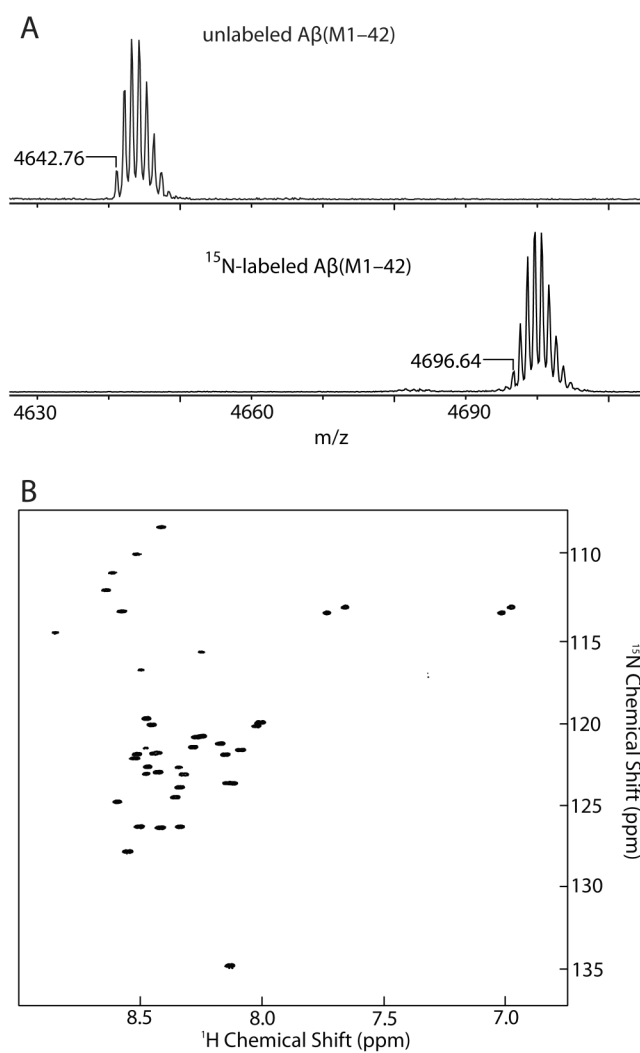


Figure 3. (A) MALDI spectra of unlabeled Aβ(M1-42) and ¹⁵N-labeled Aβ(M1-42) peptides. (B) ¹H-¹⁵N HSQC NMR spectrum of 160 μM ¹⁵N-labeled Aβ(M1-42) peptide at 5 °C at 500 MHz equipped with a cryogenic probe.

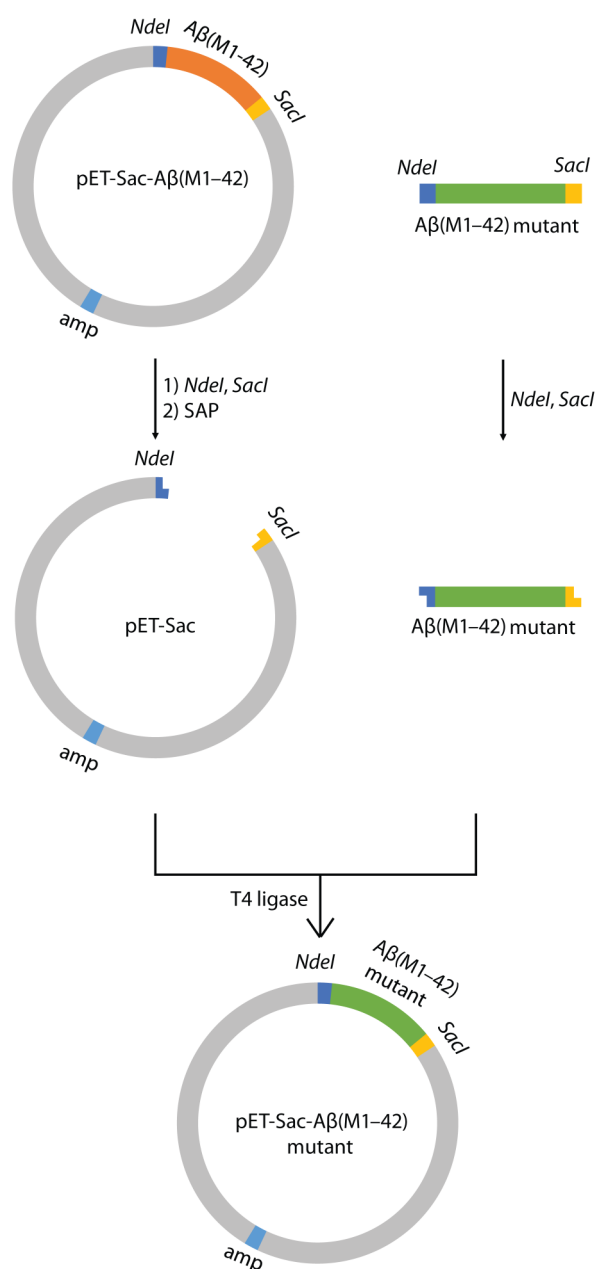


Figure 4.
Molecular cloning strategy to construct recombinant plasmids of Aβ(M1-42) containing familial mutations.

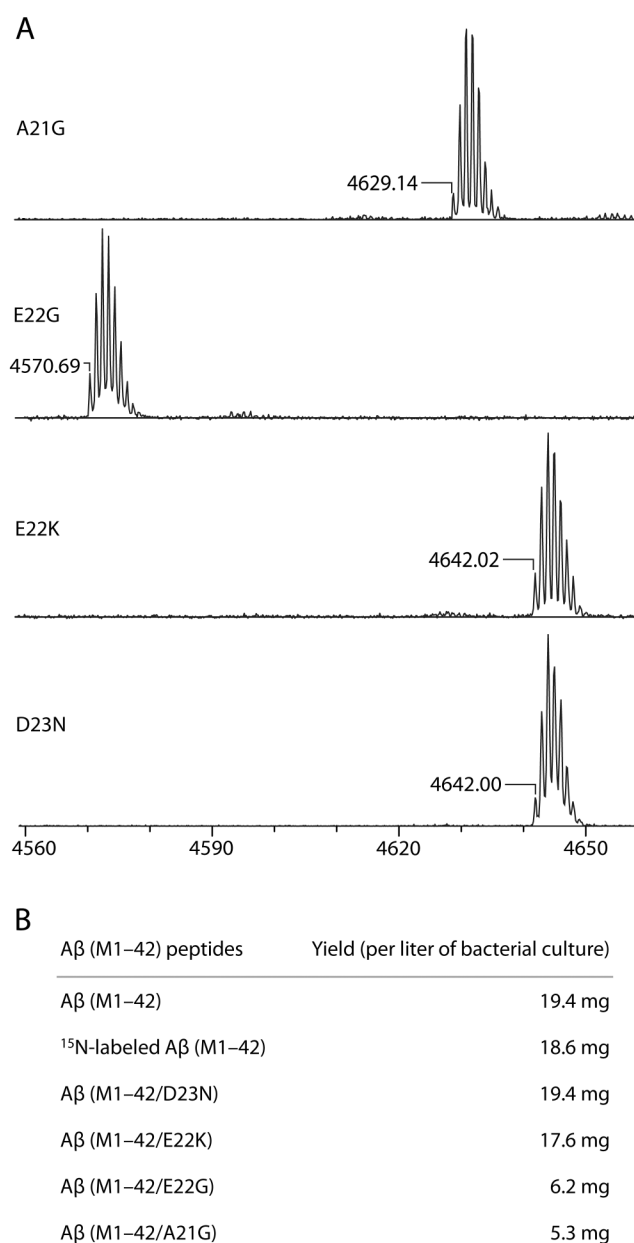


Figure 5.
(A) MALDI mass spectra of Aβ(M1–42) peptides with A21G, E22G, E22K, and D23N mutations. (B) Typical yields of Aβ(M1–42), ¹⁵N-labeled Aβ(M1–42), and mutant Aβ(M1–42) peptides.

Table 1.The effect of different syringe filters on A β (M1–42) recovery.

Filter type	UV absorbance at 280 nm	Peptide recovery
non-filtered A β sample	0.7279 $\pm$ 0.0052	N/A
Millex-HV PES (0.22 μ m)	0.6294 $\pm$ 0.0001	86.5%
Fisher hydrophilic PVDF (0.22 μ m)	0.6279 $\pm$ 0.0009	86.3%
Millex-GV hydrophilic PVDF (0.22 μ m)	0.5703 $\pm$ 0.0003	78.3%
Fisher nylon (0.22 μ m)	0.2907 $\pm$ 0.0001	39.9%
Millex-GV MCE (0.22 μ m)	0.2273 $\pm$ 0.0001	31.2%