Cleavage of amyloid precursor protein by an archaeal presenilin homologue PSH

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Aberrant cleavage of amyloid precursor protein (APP) by γ-secretase contributes to the development of Alzheimer’s disease. More than 200 disease-derived mutations have been identified in presenilin (the catalytic subunit of γ-secretase), making modulation of γ-secretase activity a potentially attractive therapeutic opportunity. Unfortunately, the technical challenges in dealing with intact γ-secretase have hindered discovery of modulators and demand a convenient substitute approach. Here we report that, similar to γ-secretase, the archaeal presenilin homolog PSH faithfully processes the substrate APP C99 into Aβ42, Aβ40, and Aβ38. The molar ratio of the cleavage products Aβ42 over Aβ40 by PSH is nearly identical to that by γ-secretase. The proteolytic activity of PSH is specifically suppressed by presenilin-specific inhibitors. Known modulators of γ-secretase also modulate PSH similarly in terms of the Aβ42/Aβ40 ratio. Structural analysis reveals association of a known γ-secretase inhibitor with PSH between its two catalytic aspartate residues. These findings identify PSH as a surrogate protease for the screening of agents that may regulate the protease activity and the cleavage preference of γ-secretase.

intramembrane protease | Alzheimer’s disease | PSH | gamma-secretase | beta-amyloid peptide

Amyloid precursor protein (APP) is initially cleaved by β-secretase in the extracellular space, producing a membrane-tethered, 99-residue carboxyl-terminal fragment known as APP C99 (1). APP C99 then undergoes sequential cleavages by γ-secretase, first at the ε sites, yielding Aβ49/Aβ48, and eventually at the γ sites, generating Aβ42/Aβ40/γ-38 (2–4) (Fig. 1A). The 230-kDa γ-secretase contains four components: presenilin (PS), Pen-2, Aph-1, and nicastrin (NCT), of which PS1 is the target of most mutations derived from early-onset familial Alzheimer’s disease (FAD) patients. Rather than abolishing the protease activity of γ-secretase, these mutations are thought to increase the molar ratio of Aβ42 over Aβ40 (2).

Among the cleavage products of γ-secretase, Aβ42 is particularly prone to aggregation, leading to formation of β-amyloid plaque in the brain and presumably causing Alzheimer’s disease (3). Other FAD-derived mutations map to APP and PS2, lending support to the causal relationship between formation of β-amyloid plaque and Alzheimer’s disease (5). Therapeutic intervention of Alzheimer’s disease may directly benefit from in vitro investigation of γ-secretase and discovery of its potential modulators (6). Unfortunately, such effort has been hampered by the difficulty in expression, purification, and manipulation of the complex protease. This problem persists despite recent breakthroughs in structural elucidation of γ-secretase and nicastrin (7, 8).

The intramembrane aspartate protease PSH from the archaean Methanoculleus marisnigri JR1 shares 19% sequence identity and 53% sequence similarity with human PS1 (hPS1). The signature motifs for catalysis, ΦYDΦΦ (Φ for a hydrophobic residue) on transmembrane segment 6 (TM6) and ΦGΦGD on TM7, are identical between PSH and hPS1. PSH can be readily overexpressed in Escherichia coli and purified in large quantity (9, 10). Importantly, the crystal structure of PSH is available and offers a valuable guide for the design and improvement of protease modulators (10). Therefore, PSH may represent an attractive surrogate of γ-secretase for modulator screening if PSH cleaves APP C99 and such cleavages recapitulate those by the intact γ-secretase. Notably, due to the presence of two different presenilin variants (hPS1 and hPS2) and two Aph-1 variants (Aph-1A and Aph-1B), there are at least four distinct human γ-secretase complexes, which exhibit different signature Aβ profiles (11). In this study, we compare PSH to the most prevalent γ-secretase complex comprising hPS1 and Aph-1A.

Results

Cleavage of APP C99 by PSH. We reconstituted an in vitro bulk cleavage assay, using recombinant PSH as the protease and APP C99 as the substrate. To facilitate purification and detection, APP C99 was fused to a maltose-binding protein (MBP) at the amino terminus and tagged by an octa-histidine (8xHis) at the carboxyl terminus (Fig. 1A). MBP can be conveniently released through thrombin cleavage. The MBP-tagged APP C99 serves as the substrate for PSH throughout this study. Incubation of this substrate with PSH at 37°C resulted in cleavage of APP C99 into a large amino-terminal fragment and a small carboxyl-terminal peptide, as judged by SDS-PAGE analysis (Fig. 1B, lanes 1–3).

Significance

Amyloid precursor protein (APP) is cleaved by β-secretase to produce APP C99, which undergoes additional, sequential cleavages by γ-secretase to generate amyloid-β peptides including Aβ40 and Aβ42. Increased ratios of Aβ42 over Aβ40 are thought to cause Alzheimer’s disease. Screening of γ-secretase modulators is hindered by the technical challenges in expression and biochemical manipulation of γ-secretase. In this study, we demonstrate that the archaeal intramembrane protease PSH represents an excellent surrogate of γ-secretase in terms of cleavage of APP C99. Ratio of Aβ42 over Aβ40, and modulation of cleavage preferences by known modulators of γ-secretase. Our finding may facilitate discovery of γ-secretase inhibitors and modulators.


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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 4Y6K).

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Addition of the γ-secretase–specific inhibitor III-31C (12) led to concentration-dependent inhibition of the protease activity of PSH (Fig. 1B, lanes 4–14). Quantitation of the results revealed an inhibitory constant (IC$_{50}$) of ~10 μM for III-31C (Fig. 1C).

The band that corresponds to the small carboxyl-terminal peptide of APP C99 was excised from the SDS-PAGE gel, eluted, and subjected to amino-terminal peptide sequencing. This analysis revealed NH$_2$-Leu-Val-Met-Leu-Lys-COOH as the first five amino acids of the major peptide species and NH$_2$-Val-Met-Leu-Lys-COOH as the first five residues of the minor species (Fig. 1D). These sequences exactly match those in APP C99 and identify the first cleavage sites by PSH to be at the carboxyl terminus of Thr48 and Leu49, coinciding with the C99 and identify the first cleavage sites by PSH to be at the carboxyl terminus.

Next, we subjected the substrate APP C99 to overnight incubation with PSH, which led to complete cleavage of APP C99. The carboxyl-terminal peptides of the digested products and the uncleaved substrates were removed by Ni$_2^+$-NTA resin; the remaining fragments were liberated through thrombin cleavage and the MBP moiety was removed by the maltose-binding resin (Fig. S1A). Analysis of the remaining fragments by mass spectrometry (MS) identified two major cleavage products, whose molecular weights perfectly matched those of Aβ38 and Aβ40 (Fig. S1B). This result demonstrates that, similar to γ-secretase, PSH cleaves APP C99 into Aβ38 and Aβ40. Our MS results also identified Aβ42 and suggested Aβ40 to be a more abundant cleavage product compared with Aβ42 (Fig. S1C).

**Cleavage Preference of PSH.** To detect the less abundant cleavage products of PSH, we used an antibody-based, spectroscopic AlphaLISA assay (13–15). In this assay, the presence of a specific Aβ peptide brings the donor and acceptor beads to close proximity, triggering the transfer of an oxygen molecule from the donor to the acceptor and consequent detection of emission light from the acceptor (Fig. 2A). Using this sensitive method, we found that the cleavage products of MBP-tagged APP C99 by 3 μM PSH not only included Aβ40, but also Aβ42 (Fig. 2B, Left). The amount of Aβ40 is ~4.8-fold more than Aβ42.

In our hands, human γ-secretase exhibited a low level of protease activity toward the MBP-tagged APP C99, which might be explained by a compromised ability for nicastrin to recognize the amino terminus of the MBP-tagged substrate (16–19). To overcome this problem, we expressed and purified the substrate APP C99 with its native amino terminus exposed and its carboxyl terminus attached to a poly-His tag. In sharp contrast to the MBP-tagged substrate, the amino terminus exposed APP C99 was readily cleaved by 1 μM γ-secretase in the AlphaLISA assay, with considerably more Aβ40 than Aβ42 (Fig. 2B, Right). Importantly, the relative ratio of Aβ42 over Aβ40 by 3 μM PSH (0.1 mg/mL) is similar to that by 1 μM γ-secretase, further validating PSH as a surrogate protease of γ-secretase. The Aβ42/Aβ40 ratio decreased slightly with increasing concentrations of PSH (Fig. 2C). Production of Aβ40 and Aβ42 by both PSH and γ-secretase was also confirmed by Western blots using monoclonal antibodies against these peptides (Fig. S2).

The FAD-derived mutation N135D in PS1 (N141I in PS2) was reported to increase the relative ratio of Aβ42 over Aβ40 (20–22). We confirmed this result by quantifying the cleavage products of wild-type (WT) γ-secretase and the PS1-N135D–containing γ-secretase (Fig. 2B, Right). Compared with WT PS1, the main change associated with PS1-N135D was a 93% reduction of the Aβ40 level, although the Aβ42 level was also reduced by ~52%. Next, we generated the corresponding mutation N46D in PSH, purified the recombinant protein PSH-N46D to homogeneity, and...
spectroscopically. (A) A schematic diagram of the AlphaLISA assay. A monoclonal antibody attached to the donor bead recognizes a common region in Aβ peptide. Upon binding of the specific Aβ peptide by a second monoclonal antibody attached to the acceptor bead, an oxygen molecule is generated in the donor bead by a 680-nm excitation wavelength and transferred to the acceptor bead, triggering an emission wavelength of 615 nm. The emission signal is detected spectroscopically. (B) Production of Aβ40 and Aβ42 by PSH (Left) and γ-secretase (Right). Shown here are results of AlphaLISA assay. The spectroscopic reading of Aβ40 by wild-type (WT) γ-secretase is normalized as 1.0. Asn46 in PSH corresponds to Asn135 in hPS1. The ratio of Aβ42 over Aβ40 by PSH is close to that by γ-secretase. The Aβ42/Aβ40 ratio by γ-secretase is normalized as 1.0. The Aβ42/Aβ40 ratio by PSH decreases slowly with increasing concentrations of PSH.

examined its cleavage preferences by using the AlphaLISA assay. The result showed that, similar to γ-secretase, the mutation N46D in PSH markedly compromised its ability to generate Aβ40, resulting in an increased ratio of Aβ42 over Aβ40 (Fig. 2B, Left). These observations identify PSH as a faithful surrogate of γ-secretase in terms of the impact on cleavage preferences by the mutation N46D.

Modulation of PSH Cleavages. The increased ratio of Aβ42 over Aβ40 is thought to have a causal link with Alzheimer’s disease (23). Considerable effort has been dedicated to the discovery of modulators that, upon binding to γ-secretase, may decrease the Aβ42/Aβ40 ratio. The small molecules GSM-1 and E2012 are known to achieve this effect by inhibiting the production of Aβ42 (24–27) (Fig. 3A). This conclusion was confirmed by our analysis using the AlphaLISA assay and the amino terminus exposed APP C99 as the substrate (Fig. 3A and B). For γ-secretase, the presence of 125 and 250 μM GSM-1 led to marked reduction of the Aβ42 levels by ∼53% and ∼68%, respectively; production of Aβ40 was also decreased by 26% and 37% (Fig. 3A). Consequently, the Aβ42/Aβ40 ratio by γ-secretase was decreased from 1.0 to 0.65 ± 0.11 and 0.52 ± 0.08, respectively, in the presence of 125 and 250 μM GSM-1 (Fig. 3A). In contrast, the decreased Aβ42/Aβ40 ratio by the modulator E2012 was mainly caused by increased levels of Aβ40 and, to a lesser extent, by decreased production of Aβ42 (Fig. 3B).

Next, we investigated the impact of these modulators on the cleavage preferences of PSH. The Aβ42/Aβ40 ratio was slightly reduced in the presence of GSM-1 (Fig. 3C) but sharply decreased in the presence of E2012 (Fig. 3D). The decreased Aβ42/ Aβ40 ratio by E2012 was contributed by higher levels of Aβ40 and lower levels of Aβ42 (Fig. 3D). These results indicate that the modulators GSM-1 and E2012 have a generally similar effect on γ-secretase and PSH.

Impact of Mutations on PSH Cleavage Activity. Our experimental findings predict that, in addition to N46D (Fig. 2B, Left), other mutations in PSH that correspond to FAD-derived mutations in hPS1 may also have a similar consequence on the cleavage preference of PSH compared with γ-secretase. We examined this prediction. The high degree of sequence homology between PSH and hPS1 allowed identification of a number of FAD-derived mutations in hPS1 that affect invariant or conserved residues between PSH and hPS1 (Fig. S4A). We chose three such representative hPS1 mutations: L85P (25), F237I (29), and L420R (30), which correspond to L17P, F142I, and V261R, respectively, in PSH. These three PSH variants were individually expressed, purified, and examined for their cleavage preferences.

Both WT PSH and the variants were able to generate the Aβ42 and Aβ40 cleavage fragments, as judged by Western blots using monoclonal antibodies against these peptides (Fig. S4B). To accurately determine changes of the Aβ42/Aβ40 ratio, we used the AlphaLISA assay. Compared with WT PSH, all three variants exhibited varying degrees of compromised ability to generate Aβ40 (Fig. 4A). The PSH variants F142I and V261R produced more Aβ42, whereas the variant L17P exhibited a decreased ability to generate Aβ42. In all three cases, however, the Aβ42/ Aβ40 ratio is elevated compared with WT PSH (Fig. 4B). These findings further corroborate the notion that PSH is a bona fide surrogate for γ-secretase in terms of substrate cleavage preference.

Structure of PSH Bound to an Inhibitor. In addition to III-31C, there are a large number of other γ-secretase–specific inhibitors. Given the conserved biochemical properties between γ-secretase and PSH, we speculated that such inhibitors may also specifically target PSH. Supporting this notion, two such inhibitors L-682,679 (referred to as L679 hereafter) and JCS (31), which share a similar chemical structure (Fig. S3), were able to inhibit the protease activity of PSH in a concentration-dependent manner (Fig. 5A). L679 at 0.2 mM inhibited ∼50% cleavage activity of PSH toward the substrate APP C99, whereas 1 mM L679 abrogated the activity (Fig. 5A, lanes 2–5). In contrast, 0.2 mM JC-5 inhibited nearly 90% of PSH cleavage activity (Fig. 5A, lane 7).

To understand the mechanism of inhibition, we sought to determine the crystal structure of PSH bound to these small-molecule inhibitors. After numerous attempts, we crystallized PSH bound to L679 and solved the structure at 3.85 Å resolution (Fig. 5B and Table S1). The presence of L679 was unambiguously determined by the Fc−Fc electron density that is located between TM6 and TM7. Determination of the relative orientation of the inhibitor was facilitated by the Br derivative of JC-5 (Fig. 5C, Fig. S5, and Table S1). The moderate resolution of 3.85 Å and the highly anisotropic diffraction gave rise to electron density maps that are insufficient for accurate assignment of atomic interactions between L679 and specific residues in PSH. Nonetheless, the mechanism of inhibition is clearly suggested by the current structure: The two catalytic residues of PSH, Asp162 and Asp220,
are separated by a chunky electron density from L679, which was assigned to a phenol group from the amide end of L679 (Fig. 5B). This binding mode explains how L679 inhibits the protease activity of PSH and suggests a similar mechanism for hPS1. Notably, no significant conformational changes were observed between free PSH and L679-bound PSH.

**Discussion**

In this study, we demonstrate that the enzymatic properties of PSH are strikingly similar to those of γ-secretase. PSH cleaves APP C99 first at the ε sites, producing Aβ48 and Aβ49 (Fig. 1), and then successively at the γ-sites, generating Aβ42, Aβ40, and Aβ38 (Fig. 2 and Fig. S1). Importantly, PSH preferentially produces Aβ40 over Aβ42, with a nearly identical ratio of Aβ42/Aβ40 compared with that by γ-secretase (Fig. 2). Despite their chemical divergence, the γ-secretase modulators GSM-1 and E2012 exhibit a generally similar effect on PSH, decreasing the Aβ42/Aβ40 ratio (Fig. 3). The detailed mechanisms by GSM-1 and E2012 may differ for PSH versus γ-secretase. For example, GSM-1 decreases the production of Aβ42 by γ-secretase, but not by PSH; in contrast, E2012 markedly reduces Aβ42 production by PSH, but not by γ-secretase (Fig. 3). Mutations in PSH that correspond to FAD-derived mutations in hPS1 have a similar consequence on the cleavage preference by PSH (Fig. 4). Together, these findings identify PSH as a surrogate for the investigation of γ-secretase. PSH recapitulates nearly all enzymatic behaviors of γ-secretase, including the Aβ42/Aβ40 ratio and its modulation by known modulators (27, 32). These biochemical mimics strongly suggest a highly conserved active site arrangement and a similar pattern of substrate recognition between PSH and hPS1.

![Fig. 3. Modulators of γ-secretase exhibit a similar effect on the cleavage preferences of PSH.](image)

![Fig. 4. Alteration of the Aβ42/Aβ40 ratio by PSH variants.](image)
Despite generally similar biochemical properties, γ-secretase and PSH exhibit subtle, but important differences. For example, the inhibitor III-31C is considerably more potent toward PSH (12) (Fig. 1C), which likely reflects the fine conformational differences between γ-secretase and PSH. In addition, PSH is relatively insensitive to GSM-1 and only becomes sensitive to E2012 at high concentrations of 500 μM, whereas PSH is relatively insensitive to GSM-1 and only becomes sensitive to E2012 at 30 μM. We suggest that PSH may be made more γ-secretase–like through dedicated protein engineering effort in which select sets of amino acids in PSH can be replaced by those in hPS1. Such protein engineering effort is likely facilitated by the crystal structure of PSH (10), and by the atomic structure of hPS1 in the future.

It should be noted that the human γ-secretase comes in four different forms, each with a different Aβ cleavage profile (11). hPS1 appears to reduce the endopeptidase activity of γ-secretase (γ-sites) compared with hPS1, and Aph-1 variants affect the carboxypeptidase-like activities (gamma-sites) (11). In this study, we demonstrate that the enzymatic properties of PSH closely resemble those of the most abundant γ-secretase complex that comprises hPS1, Pen-2, Aph-1A, and nicastrin. It remains to be investigated to what extent PSH can be compared with the other three distinct γ-secretase complexes. Regardless of the outcome of these investigations, the use of a single protease PSH as a surrogate no longer allows assessment of the impact on Aβ generation by two related γ-secretases, each with a different variant subunit (such as Aph-1A versus Aph-1B) (11).

In this study, we used five different methods for the detection of APP C99 cleavage by PSH and γ-secretase. Direct visualization of cleavage products by SDS-PAGE (Figs. 1B and 5A), amino-terminal peptide sequencing analysis (Fig. 1D), and the conventional Western blots based assay (Figs. S2 and S4B) offer a qualitative assessment. In contrast, mass spectrometry (Fig. S1) and the AlphaLISA assay (Figs. 2 B and C, 3, and 4 A and B) tend to be more accurate and quantitative. Notably, in all cases, the cleavage reactions were carried out in detergent micelles, as opposed to lipid bilayers or liposomes. Published results show little difference for reactions performed in detergent micelles versus lipid bilayers (33).

Although Aph-42 and the ratio of Aph42/Aph40 are treated as the main culprits of Alzheimer’s disease, the real situation is considerably more complex. For example, Aph43 was exhibited to exhibit more potent amyloidogenicity and pathogenicity (34). Existing data suggest that absolute levels of Aph42 are not as important as the altered ratios of the Aph peptides, most notably the ratio of Aph42/Aph40 (22, 33, 35). These considerations have significant ramifications on the discovery of novel drugs that may target mutant γ-secretases. Nevertheless, the ease of expression, purification, and biochemical manipulation of PSH may greatly facilitate discovery of its enzymatic inhibitors and modulators that may prove to have a similar effect on the intact γ-secretase. Such small-molecule inhibitors and modulators may not just serve as investigative tools for γ-secretase but also find potential therapeutic applications for Alzheimer’s disease.

**Materials and Methods**

**Preparation of PSH and γ-Secretase Variants.** All PSH variants were derived from the PSH construct that was crystallized (10). The cloning and purification of PSH variants were performed as described (10). The variants were desalted on a Hitrap desalting column (GE Healthcare) to PBS buffer, pH 7.4, with 0.02% (wt/vol) NaN₃, 0.02% DDM. The inhibitor was then incubated with the HRP substrate (Supersignal West Pico; Thermo Scientific), with 1:5,000 secondary antibody (Goat-anti-Mouse IgG; CWBio) for 1 h, incubated with the HRP substrate (Supersignal West Pico; Thermo Scientific), and exposed to film by using Universal Hood II Imaging System (Bio-Rad). The raw images were processed by Image Lab software (Bio-Rad).

**Activity Assay for PSH Variants and γ-Secretase.** APP C99 was fused with an N-terminal MBP tag and a C-terminal 6xHis tag. A unique thrombin cleavage site (LVPRGS) was introduced between MBP and APP C99. The final construct was cloned into pET-21b (Novagen), overexpressed in E. coli strain BL21(DE3), and purified. The PSH variants (0.1 mg mL⁻¹) were mixed with this substrate (1 mg mL⁻¹) in PBS, in the presence of 50 mM citrate, pH 5.1, and 0.02% DDM. APP C99 with a C-terminal 6xHis tag was used as the substrate for γ-secretase. In the reaction, γ-secretase variants (0.15 mg mL⁻¹) were mixed with APP C99 (0.5 mg mL⁻¹) in 0.25% CHAPSO, 0.1% (wt/vol) phosphatidylcholine, 0.025% (wt/vol) phosphatidylethanolamine, 25 mM Hepes, pH 7.4, and 150 mM NaCl. The reaction was conducted at 37 °C for 16 h and stopped by SDS sample buffer. Inhibitor or modulator diluted by DMSO was added at indicated concentrations. The same volume of DMSO was added as a negative control for each batch of assay.

**Western Blotting.** For detection of Aph40 and Aph42, the reaction samples were applied onto 16% SDS-PAGE and transferred to PVDF membranes. Membranes were blocked by 4% (wt/vol) BSA followed by incubation with 1:500 primary antibody (Aph1-40 or Aph1-42 monoclonal antibody; Covance) in 2% (wt/vol) BSA at 4 °C overnight. After washing, the membrane was incubated with 1:5,000 secondary antibody (Goat-anti-Mouse IgG; CWBio) for 1 h, incubated with the HRP substrate (Supersignal West Pico; Thermo Scientific), and exposed to film by using Universal Hood II Imaging System (Bio-Rad). The raw images were processed by Image Lab software (Bio-Rad).

**AlphaLISA Assay.** The assay was performed as described in the AlphaLISA Kit (PerkinElmer). Briefly, 2-μL reaction products were incubated with 8-μL AlphaLISA Apt-40/Aph42 Acceptor beads at 23 °C for 1 h. After another 30-min incubation with 10-μL AlphaLISA Apt-40/Aph42 Donor beads in the dark at 23 °C, the samples were read by using Envision-Alpha Reader (PerkinElmer). The readings were expressed in arbitrary unit.

**Cleavage Sites Identification by Mass Spectrometry.** The reaction products were flowed through Ni⁺²-NTA affinity resin (Qiagen) to remove the C-terminal peptides of the digested products and the uncleaved products. The flow-through was incubated with thrombin. The C-terminal fragments of thrombin-cleaved products were collected by further flowing through chitin resin followed by incubation with 15% trichloroacetic acid. The pellets were


Fig. S1. Major cleavage products of APP C99 by PSH include Aβ40 and Aβ38. (A) A schematic diagram for detection of the major cleaved fragments of APP C99. Following the first cleavage after Thr48 or Leu49 by PSH, the remaining substrate is further cleaved into several distinct products. These products were separated from the amino-terminal MBP moiety by thrombin cleavage and subjected to mass spectrometry (MS). (B) Identification of Aβ40 and Aβ38 as the major cleavage products by PSH. Shown here are the MS results, which conclusively identify Aβ40 (Right) and Aβ38 (Left). (C) MS analysis suggests Aβ42 to be a less abundant cleavage product than Aβ40. Shown here is the original MS data. The signal intensity for Aβ40 is approximately an order of magnitude higher than that for Aβ42.
Fig. S2. Generation of Aβ40 and Aβ42 by both PSH and γ-secretase is confirmed by conventional Western blots. Cleavage products were recognized by monoclonal antibodies against Aβ40 and Aβ42. The substrate for PSH is MBP-tagged APP C99, whereas the substrate for γ-secretase is the amino terminus exposed APP C99.

Fig. S3. Chemical formula of four inhibitors and modulators used in this study. Note that L-682,679 (abbreviated as L679) is chemically similar to JC-5.

Fig. S4. Alteration of the Aβ42/Aβ40 ratio by PSH variants. (A) Sequence alignment between PSH and hPS1. Identical amino acids are highlighted by red background, and conserved residues are boxed. The three residues chosen for mutations are identified by background colors: Leu17 (cyan), Phe142 (green), and Val261 (blue). (B) The three PSH variants remained the ability to generate Aβ42 and Aβ40. Shown here are results of Western blots. Monoclonal antibodies against Aβ42 and Aβ40 were used to identify the cleavage products.
Fig. S5. Synthesis of brominated JC-5. Shown here is a schematic procedure for the chemical synthesis of bromide (Br)-derivatized JC-5. The synthesis of brominated dipeptide \(16\) started from commercially available amino acid \(13\) without complication. Thus, temporary masking the free amine with Boc allowed the installation of first the primary amide and then the leucine amide in dipeptide \(15\). Deprotection of the Boc group with trifluoroacetic acid provided the desired product \(16\). Finally, coupling of carboxylic acid \(12\) with dipeptide \(16\) gave tripeptide \(17\) in 85% yield, and TBAF treatment provided Br-JC-5 as a white solid in 80% yield. The identity of Br-JC-5 is confirmed by NMR spectroscopy.
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Values in parentheses are for the highest resolution shell. \(R_{merge} = \sum_{h} \sum_{i,j} |I_{h,i} - I_{h,j}| / \sum_{h} \sum_{i} I_{h,i}\), where \(I_{h,i}\) is the mean intensity of the \(i\) observations of symmetry related reflections of \(h\). \(R = \sum |F_{o} - F_{c}| / \sum F_{o}\), where \(F_{o}\) is the observed structure factor and \(F_{c}\) is the calculated structure factor from the atomic model (\(R_{free}\) was calculated with 5% of the reflections selected).

*The data completeness is affected by the highly anisotropic diffraction, with one dimension reaching to only approximately 6 Å.