Crystal structure of the γ -secretase component nicastrin

Tian Xie^{a,b,1}, Chuangye Yan^{b,c,1}, Rui Zhou^{a,b}, Yanyu Zhao^{a,b}, Linfeng Sun^{a,b}, Guanghui Yang^{b,c}, Peilong Lu^{a,b}, Dan Ma^{a,b}, and Yigong Shi^{a,b,2}

^aMinistry of Education Key Laboratory of Protein Science, ^cState Key Laboratory of Bio-Membrane and Membrane Biotechnology, and ^bTsinghua-Peking Joint Center for Life Sciences, Center for Structural Biology, School of Life Sciences and School of Medicine, Tsinghua University, Beijing 100084, China

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γ -Secretase is an intramembrane protease responsible for the generation of amyloid- β (A β) peptides. Aberrant accumulation of A β leads to the formation of amyloid plaques in the brain of patients with Alzheimer's disease. Nicastrin is the putative substrate-recruiting component of the γ -secretase complex. No atomic-resolution structure had been identified on γ -secretase or any of its four components, hindering mechanistic understanding of γ -secretase function. Here we report the crystal structure of nicastrin from *Dictyostelium purpureum* at 1.95-Å resolution. The extracellular domain of nicastrin contains a large lobe and a small lobe. The large lobe of nicastrin, thought to be responsible for substrate recognition, associates with the small lobe through a hydrophobic pivot at the center. The putative substrate-binding pocket is shielded from the small lobe by a lid, which blocks substrate entry. These structural features suggest a working model of nicastrin function. Analysis of nicastrin structure provides insights into the assembly and architecture of the γ -secretase complex.

An intramembrane protease, γ -secretase, cleaves the type I integral membrane proteins within their transmembrane domains (1, 2). One of the most prominent substrates is the amyloid precursor protein (APP). Sequential cleavage of APP by β -secretase and γ -secretase gives rise to the amyloid- β (A β) peptides, particularly those containing 40 and 42 amino acids (A β 40 and A β 42). The A β peptides are the main constituent of the amyloid plaques found in the brains of patients who have Alzheimer's disease (AD). Modulation of the activity and specificity of γ -secretase represents a potential therapeutic strategy for the treatment of Alzheimer's disease (3–6).

γ -Secretase consists of four components: presenilin (PS), presenilin enhancer 2 (Pen-2), anterior pharynx-defective 1 (Aph-1), and nicastrin (7–9). PS is an aspartyl protease and functions as the catalytic component of γ -secretase (10, 11). PS contains nine transmembrane helices (TMs); the two catalytic aspartate residues are located in the sixth and seventh TMs (12). Pen-2, bearing two TMs, is thought to facilitate the maturation of PS and enhance the γ -secretase activity (13). Aph-1 is a seven-transmembrane protein known to stabilize the γ -secretase complex (13, 14). Nicastrin is a type I transmembrane glycoprotein with a large extracellular domain (ECD) and a single TM at the C terminus. As the largest component of γ -secretase with 709 amino acids and 30- to 70-kDa glycosylation (15), nicastrin accounts for approximately two-thirds of the 230-kDa apparent molecular mass of the intact human γ -secretase. The nicastrin ECD is thought to play a critical role in the recruitment of γ -secretase substrate (16–19).

At present, there is no atomic-resolution structure for the intact γ -secretase or any of its four components. The limited structural information comes from low-resolution electron microscopic (EM) analysis of γ -secretase (20–24), an NMR structure of the C-terminal three TMs of PS1 (25), and a crystal structure of a PS homolog from archaea (12). Consequently, mechanistic understanding of γ -secretase has been slow to emerge. Our recent cryo-EM structure of human γ -secretase, at 4.5-Å resolution, revealed its overall 3D architecture and most

secondary structural elements, including 19 TMs (26). In this study, we present the high-resolution crystal structure of the nicastrin ECD from the eukaryote *Dictyostelium purpureum* and discuss its functional implications.

Results

Overall Structure of the Nicastrin ECD. The ECD of human nicastrin (HsNCT), containing residues 1–669, accounts for 94% of the full-length sequence. Similar to other higher organisms, *D. purpureum* contains all four components of the γ -secretase, and its endogenous γ -secretase was reported to process human APP into A β 40 and A β 42 (27). The nicastrin ECD sequences from *D. purpureum* and human share 23% identity and 40% similarity. Both proteins were overexpressed and purified to homogeneity for crystallization. The ECD of *D. purpureum* nicastrin (DpNCT; residues 19–611) yielded well-diffracting crystals in the space group P4₁2₁2 (Table S1). The structure was determined by a combination of molecular replacement and bromide-based single-wavelength anomalous dispersion (SAD). The atomic model was refined to 1.95-Å resolution (Fig. 1A, Fig. S1, and Table S1). Residues 33–605 were assigned in the structure; 20 residues at the N and C termini are disordered in the crystals.

The nicastrin ECD comprises a large lobe and a small lobe (Fig. 1A). The large lobe consists of 12 α -helices and 14 β -strands

Significance

γ -Secretase is a four-component intramembrane protease associated with the onset of Alzheimer's disease. Nicastrin is the putative substrate-recruiting component of the γ -secretase complex, but no atomic-resolution structure had been identified on γ -secretase or any of its four components. Here we report the first atomic-resolution crystal structure of a eukaryotic nicastrin which shares significant sequence homology with human nicastrin. This structure reveals the fine details of nicastrin and allows structure modeling of human nicastrin. Analysis of the structural details yields a working model showing how nicastrin might function to recruit substrate protein. The nicastrin structure also allows reevaluation of the previously proposed transmembrane helix assignment in the γ -secretase complex. Our structural analysis provides insights into the assembly and function of γ -secretase.

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Data deposition: Crystallography, atomic coordinates, and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 4R12).

¹T.X. and C.Y. contributed equally to this work.

²To whom correspondence should be addressed. Email: shi-lab@tsinghua.edu.cn.

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Fig. 1. Structure of the nicastrin ECD from *D. Purpureum*. (A) The overall structure of the nicastrin ECD from *D. Purpureum* is shown in surface representation (Left) and ribbon diagram (Right). The structure can be divided into a large lobe (blue) and a small lobe (green). An extended loop from the small lobe (red) forms a lid to cover an otherwise exposed surface region on the large lobe. The highly conserved Trp145 in the lid is indicated in red ball-and-stick representation. Five N-linked glycans are displayed in light gray; six disulfide bonds are shown in orange. All structural figures were prepared with PyMOL (42). (B) Structure of the large lobe of nicastrin. The large lobe contains a core and a number of additional structural motifs on the surface, notably a pair of antiparallel β -strands (purple oval) and a small globular domain (orange oval). (C) The structure of the small lobe of nicastrin. As does the large lobe, the small lobe contains two prominent structural elements beyond the core: a small globular domain (orange oval) and a lid that interacts with the large lobe (purple oval).

exhibiting an α/β fold (Fig. 1B and Fig. S2). A nine-stranded β -sheet in the center of the large lobe is surrounded by seven α -helices on one side and four on the other side; these secondary structural elements form the core of the large lobe. The core contains three glycosylation sites (on Asn333, Asn385, and Asn584) and two disulfide bonds between Cys308 and Cys318 and between Cys479 and Cys486. Beyond the core, a pair of antiparallel β -strands caps the surface loops on one side of the β -sheet, whereas a small globular domain, consisting of three β -strands and one α -helix, stacks against helices $\alpha 15$ and $\alpha 16$ on the other side. The small globular domain is stabilized by two additional disulfide bonds, between Cys540 and Cys551 and between Cys546 and Cys556 (Fig. 1B and Fig. S2).

The small lobe contains five α -helices and 10 β -strands. The structural core of the small lobe is a seven-stranded β -sandwich closely stacked by three α -helices and surrounding loops, with

two glycosylation sites (on Asn96 and Asn166) and one disulfide bond between Cys42 and Cys54 (Fig. 1C and Fig. S2). An extended loop protrudes out of the core, forming a lid that covers the putative substrate-binding site in the large lobe (discussed in detail later). Away from the lid and on the other side of the core is another small globular domain comprising a three-stranded β -sheet and an α -helix. As in the large lobe, the small globular domain in the small lobe is stabilized by a disulfide bond between Cys204 and Cys210 (Fig. 1C). Notably, the four Cys residues involved in the formation of the disulfide bond in the small lobe are invariant among different organisms (Fig. S2), suggesting a conserved pattern of disulfide bonds.

Interface Between the Large and Small Lobes. The large lobe associates with the small lobe through a striking pattern of interactions: a high density of van der Waals contacts at the center of the

Fig. 2. A unique pattern of interactions at the interface between the large and small lobes of DpNCT. (A) A schematic diagram of the interface between the large and small lobes of nicastrin. At the center of the interface is a high density of van der Waals interactions. At the periphery of the interface are a number of H-bonds that appear in a C-shaped distribution. These interactions are conserved between HsNCT and DpNCT. (B) A close-up view of the H-bonds between the large and small lobes of nicastrin. The lid from the small lobe is highlighted in red. H-bonds are represented by red dashed lines. The same coloring scheme is used in all figures. (C) A close-up view of the hydrophobic pivot at the center of the interface between the large and small lobes of nicastrin. Phe244 and Phe245 from the large lobe are nestled in a greasy pocket formed by hydrophobic residues from the small lobe. (D) A close-up view of the interactions between Trp145 from the lid of the small lobe and residues from the large lobe.

interface and a half-circle of 11 hydrogen bonds (H-bonds) at the periphery (Fig. 2*A* and Fig. S3). These 11 H-bonds are mostly solvent-exposed and exhibit a C-shaped distribution (Fig. 2*B* and Fig. S3). Of the 11 H-bonds, six map to the interface between the cores of the large and small lobes. In particular, the side chains of both Asp436 and Tyr432 in the large lobe make H-bonds to the side chains of Lys119 and Lys120, respectively, in the small lobe. The other four H-bonds involve main-chain groups (Fig. 2*B*). In addition, four H-bonds come from the interface between the lid in the small lobe and the core of the large lobe (Fig. 2*B*). The last H-bond, involving main-chain groups, is mediated by Phe507 from the core of the large lobe and Pro212 from the small globular domain in the small lobe (Fig. 2*B*).

At the center of the interface, the phenyl rings of Phe244 and Phe245 in the core of the large lobe insert into a greasy pocket formed by the side chains of Phe95, Phe152, Phe157, and Ile161 in the core of the small lobe (Fig. 2*C*). These hydrophobic residues are highly conserved (Fig. S2), suggesting that these van der Waals contacts between the large and small lobes are likely preserved among different organisms.

In addition to these interfaces, the lid from the small lobe reaches above to cover an otherwise solvent-exposed, hydrophilic region in the large lobe (Fig. 2*D*). Specifically, Trp145 in the lid is nestled in a hydrophobic pocket formed by His363, Pro399, His421, and Tyr423 in the large lobe (Fig. 2*D*). Trp145 in DpNCT, corresponding to Trp164 in HsNCT, is highly conserved among different organisms (Fig. S2). As discussed later, Trp164 in HsNCT also is located in a similar lid that directly contacts the large lobe.

Position of the Small Lobe Relative to the Large Lobe. To identify structural homologs of nicastrin, we searched the Protein Data Bank (PDB) using DALI (28). The search was performed with the large lobe alone, the small lobe alone, or the entire ECD. Although many structural homologs such as the glutamate carboxyl peptidase PSMA (PDB ID code 2XEF) (26) were identified, none can be aligned to nicastrin for more than half of its amino acid sequences. The structurally similar regions are restricted to the cores of the large and small lobes. The closest entry by all three searches is the structure of a bacterial aminopeptidase (BAP) (PDB ID code 2EK9).

Similar to nicastrin, BAP contains a large and a small lobe (Fig. 3*A*). The cores of the large and small lobes of nicastrin are individually quite similar to the corresponding lobes of BAP, with rmsds of 2.4 Å and 3.0 Å over 190 and 108 aligned Cα atoms, respectively (Fig. 3*A* and Fig. S4*A*). Intriguingly, however, when the large lobes of nicastrin and BAP are superimposed, the small lobe of nicastrin is separated from that in BAP by a rotation of ~100° (Fig. 3*A*). Consequently, the lid of nicastrin partially overlaps the small lobe of BAP (Fig. S4*B*). In fact, the large and small lobes of BAP are loosely joined, with few interactions between them. Thus, it appears that the positioning of the small lobe in nicastrin is linked to the interaction between the lid and the large lobe.

Features of the Putative Substrate-Binding Site. The large lobe of BAP contains the active site. In the BAP structure (PDB ID code 2EK9), two zinc ions and a competitive inhibitor bestatin (BES) are located in the substrate-binding pocket (Fig. 3*B*). The corresponding region in DpNCT likely constitutes the substrate-binding site, which is covered by the lid from the small lobe. Nonetheless, the local structural elements surrounding the putative substrate-binding pocket in DpNCT generally are similar to those in the corresponding region of BAP (Fig. 3*B*). Two zinc ions, which are essential for the catalytic activity of the aminopeptidase, are coordinated by the side chains of His228, Asp240, Glu273, Asp301, and His371 in BAP (Fig. 3*C*). In contrast, there is no zinc in the nicastrin structure, because the five corresponding zinc-binding residues in BAP have been replaced by Pro238,

Fig. 3. Identification and features of the putative substrate-binding pocket in DpNCT. (A) Structural comparison between BAP (PDB ID code 2EK9) and nicastrin. Overlaying the large lobe of BAP with that of nicastrin reveals quite different positions for their small lobes, which are separated from each other by a rotation of ~100°. The large and small lobes of BAP are colored yellow and magenta, respectively. (B) Identification of the putative substrate-binding pocket in nicastrin. The structure of BAP is superimposed here to illustrate its substrate-binding pocket, which is occupied by the competitive inhibitor BES (orange). The two bound zinc ions in BAP are shown as gray spheres. The putative substrate-binding pocket in nicastrin is located in the large lobe and is shielded by the lid from the small lobe. (C) A close-up view of zinc coordination in BAP. The nicastrin structure is superimposed here for comparison. The two zinc ions in BAP are missing in nicastrin. The residues that coordinate the zinc ions in BAP are colored magenta; the corresponding residues in nicastrin are shown in gray. (D) A close-up view of the putative substrate-binding pocket in the large lobe of nicastrin. These residues are mostly polar. The amino acids Glu289 (corresponding to Glu333 in HsNCT) and Tyr293 (corresponding to Tyr337 of the DYIGS motif in HsNCT) are both located in the putative substrate-binding pocket.

Gln254, Arg290, Asn344, and Tyr430 in DpNCT (Fig. 3*C*). This observation may explain why nicastrin exhibits no protease activity.

In BAP, the competitive inhibitor BES is bound in the substrate-binding pocket (Fig. S4*C*). Intriguingly, with omission of the lid, the corresponding region of nicastrin also contains a similar pocket (Fig. S4*D*). It is possible that this pocket may serve to recruit the substrate of γ -secretase, perhaps by anchoring the hydrophilic N terminus of the substrate such as the β -secretase cleavage product APP-C99. Consistent with this analysis, this pocket is surrounded by a number of hydrophilic amino acids (Fig. 3*D*). Importantly, the amino acids Glu289 (corresponding to Glu333 in HsNCT) and Tyr293 (corresponding to Tyr337 of the DYIGS motif in HsNCT) are both located in the putative substrate-binding pocket. This finding provides a plausible explanation for the observed critical role of Glu333/Tyr337 in the modulation of substrate cleavage by human γ -secretase (16, 17).

Structural Features of Human Nicastrin. DpNCT shares 40% sequence similarity with HsNCT (Fig. S2). The 1.95-Å resolution crystal structure of DpNCT, together with the 4.5-Å resolution EM map of the human γ -secretase (26), allowed us to build a

considerably improved model for HsNCT (Fig. 4*A* and Fig. S5). This updated atomic model of HsNCT, containing ~100 more amino acids than the old model (26), fits the EM density well and contains a number of important features that were unavailable in our previous study (Fig. 4*B*). These features include a number of secondary structural elements, two newly identified small globular domains in the large and small lobes of HsNCT, and, importantly, a lid from the small lobe that covers the putative substrate-binding site on the large lobe (Fig. 4*A* and *B*). As discussed in detail later, these features have significant functional implications.

The key structural features of nicastrin are highly conserved between DpNCT and HsNCT. The striking pattern of interactions at the interface between the large and small lobes is nearly identical. As does DpNCT (Fig. 2*C*), HsNCT contains a high density of van der Waals contacts at the center of the interface between the large and small lobes, involving a nearly identical set of amino acids (Fig. 4*C*). Phe287, corresponding to Phe244 in DpNCT, is nestled in a greasy pocket formed by four hydrophobic amino acids: Phe103, Leu171, Phe176, and Ile180. Three of the four amino acids are invariant between DpNCT and HsNCT, and Phe152 in DpNCT is replaced by Leu171 in HsNCT. Additionally, as in DpNCT, an extended loop sequence from the small lobe forms a lid that hovers right above the putative substrate-binding site in HsNCT (Fig. 4*D*). In the lid of HsNCT, Trp164 corresponds to Trp145 in DpNCT, which plays a key role in binding to the large lobe (Fig. 2*D*).

A Working Model of Nicastrin. The primary sequences of nicastrin are highly conserved among slime mold, nematode worm, fruit fly, mouse, and human, with 31 invariant residues and 273 residues

conserved in at least three of the five organisms (Fig. S2). Sequences in the small lobe appear to be more conserved than those in the large lobe. The invariant and highly conserved residues account for 8.2% and 53.8%, respectively, of the total sequences in the small lobe and for 4.0% and 45.3%, respectively, of the sequences in the large lobe. These conserved amino acids were mapped to the structures of DpNCT and HsNCT (Fig. 5*A* and Fig. S6). Notably, the small globular domain in the small lobe of DpNCT, containing six invariant and 14 highly conserved residues, makes direct interactions with TMs at the thin end of γ -secretase (Fig. 5*B*). This analysis suggests that the overall assembly and specific interactions are likely conserved among γ -secretases from different organisms.

Our structural analysis of nicastrin provides a tantalizing clue about its function. The large and small lobes associate with each other via three generally conserved interfaces: a central hydrophobic pivot, an H-bond-rich C-shaped strip at the periphery, and a lid over the putative substrate-binding pocket. In addition, the interface between the nicastrin ECD and the TMs appears to be conserved for γ -secretase in *D. purpureum* and humans (Fig. 5). The observed conformation of nicastrin, both in the EM map and in the current crystal structure, likely represents an inactive conformation in which substrate access is blocked by the lid. In contrast, the aminopeptidase BAP contains the large and small lobes, but not the lid; the two lobes are separated, with the active site on the large lobe solvent-exposed. This analysis suggests a working model in which the presence of the lid blocks access to the substrate-binding site. We speculate that the large and small lobes may rotate relative to each other around the central hydrophobic pivot, thus causing the closure and opening of the lid (Fig. 6*A*). This model predicts that, in the activated

Fig. 4. Structural features of the modeled HsNCT. (*A*) Overall structure of the modeled HsNCT. The atomic model for HsNCT was generated on the basis of the crystal structure of DpNCT and fitted into the 4.5-Å resolution EM density of human γ -secretase (26). The large and small lobes of HsNCT model are colored blue and green, respectively. The lid from the small lobe is highlighted in red. (*B*) The modeled structure of HsNCT contains important previously unidentified features compared with the previous partial model. These features include two small globular domains, one in each lobe, and a lid from the small lobe that covers the putative substrate-binding site on the large lobe. The previous partial model of HsNCT is colored gray. (*C*) A close-up view of the hydrophobic pivot at the center of the interface between the large and small lobes of HsNCT. Phe287 from the large lobe interacts with four hydrophobic residues from the small lobe. These residues are highly conserved in different organisms. (*D*) A close-up view of the lid from the small domain of HsNCT. As is Trp145 in DpNCT, Trp164 in HsNCT is located in the lid of the small lobe, making contacts with residues in the large lobe.

Fig. 5. Structural conservation between DpNCT and HsNCT. (A) Comparison of conserved structural features in DpNCT and HsNCT. Invariant residues among all five organisms (Fig. S2) are highlighted in red; conserved amino acids are colored yellow. (B) The bottom surface of the small lobe, which is in contact with the TMs of γ -secretase, is highly conserved. The small globular domain from the small lobe, which is in contact with the thin end of the TMs, is particularly conserved. The TMs of γ -secretase are shown in surface representation.

conformation of nicastrin, the lid opens and is relocated away from the putative substrate-binding site, thus allowing substrate recruitment. Supporting this conjecture, nicastrin in γ -secretase was reported to undergo a marked conformational switch in response to inhibitor/substrate binding (24), resulting in the closure of the upper subdomain (likely the large lobe) onto the lower subdomain (the small lobe) and compaction of the overall conformation.

Discussion

The 4.5-Å cryo-EM structure of the human γ -secretase revealed its overall architecture, in which 19 TMs form a horseshoe-shaped assembly with a thick end and a thin end (26). On the basis of structural information about the PS1 homolog mmPSH (12), the thick end was tentatively assigned to PS1 and Pen-2 (26). Understanding the nicastrin structure allows us to revisit TM assignment. In our updated model of the HsNCT ECD, its C terminus is clearly identified (Fig. S7A). The most C-terminal residue in the HsNCT model is Leu662, whereas the predicted single TM of HsNCT spans from Leu670 to Ile690. This analysis leaves only seven residues between the C terminus of the modeled HsNCT ECD and the N terminus of the TM. This information, together with the EM density, facilitated the putative assignment of the connection between the C terminus of the HsNCT ECD and the candidate TM of nicastrin. The TM of nicastrin is likely located at the far edge of the thick end (Fig. S7B).

γ -Secretase is thought to consist of two subcomplexes (24, 29), one containing Pen-2 and PS1-NTF (the N-terminal six TMs), bearing eight TMs, and the other comprising PS1-CTF (the C-terminal three TMs), Aph-1, and nicastrin, possessing 11 TMs. The putative assignment of the lone TM from nicastrin suggests that the subcomplexes Pen-2/PS1-NTF and PS1-CTF/Aph-1/nicastrin may occupy the thin and thick ends, respectively. Based on this rationale, we examined the reported EM density of human γ -secretase (26) and identified seven TMs that very nicely fit

the predicted structure of PS1, which was modeled after the structure of the PS1 homolog mmPSH (12). These seven TMs include TM1, TM3-5, and TM7-9, each matching the EM density nearly perfectly (Fig. 6B and Fig. S7B). The predicted TM2 and TM6 of PS1 exhibit relatively poor EM density, likely because they are located on the outside face of the TM horseshoe. The arrangement and position of the clearly assigned seven TMs of PS1 are identical to those of the corresponding TMs in mmPSH (Fig. 6C). The remaining seven TMs in the thick end likely belong to Aph-1, whereas the remaining three TMs (including two short TMs) at the far edge of the thin end were tentatively assigned to Pen-2 (Fig. S7B). Consistent with previously reported biochemical data (30, 31), the TMs of Pen-2 interact with TM4 of PS1 in the reassigned structural model.

Nicastrin is heavily glycosylated. In the structure of DpNCT, five N-glycosylation sites were identified (Fig. 1A). All these N-glycosylation sites are located on the surface of nicastrin and might help stabilize the overall structure. In HsNCT, there are 16 potential N-glycosylation sites. The glycosylation of HsNCT is reported to be important for the formation and stabilization of the γ -secretase complex, although glycosylation may not be essential for the protease activity of γ -secretase (32, 33). Consistent with this conclusion, the potential N-glycosylation sites of nicastrin are not conserved across different organisms.

In summary, the 1.95-Å crystal structure of the nicastrin ECD represents, to our knowledge, the first piece of atomic-resolution information on any component of γ -secretase and serves as an important framework for future mechanistic understanding. Analysis of the nicastrin structure reveals striking features that may be of functional significance. On the basis of such analysis, we proposed a conserved working model for the substrate-recruitment function of nicastrin. This model awaits experimental scrutiny, and comprehensive understanding of γ -secretase function requires elucidation of the structure of the entire complex at an atomic resolution.

Fig. 6. Implications for nicastrin function and reassignment of the TMs in PS1. (A) A working model of nicastrin. In this hypothetical model, binding the lid to the large lobe blocks access to the substrate-binding site. Rotation of the large lobe relative to the small lobe around the central hydrophobic pivot may cause the lid to open, exposing the putative substrate-binding site and allowing substrate access. (B) Reassignment of the TMs in PS1 of human γ -secretase. The EM density for TM2 and TM6 of PS1 was relatively poor. The EM density for the seven remaining TMs of PS1 was clearly assigned. (C) Structural comparison of PS1 (rainbow) with the archaeal homolog mmPSH (gray). The clearly assigned seven TMs of PS1 (TM1, TM3, TM4, TM5, TM7, TM8, and TM9) superimpose well with the corresponding TMs of mmPSH.

Materials and Methods

Protein Preparation. The ECD (residues 19–611) of nicastrin from *D. Purpureum* was subcloned into pFastBac Dual (Invitrogen) after the polyhedrin promoter. Nicastrin was fused with the gp67 signal peptide at the N terminus, followed by an 8xHis tag. Bacmids were generated in DH10Bac cells, and the resulting baculoviruses were generated and amplified in Sf9 insect cells. After transfection of Hi5 cells by the baculovirus for 48 h, the secreted protein was purified from the medium by nickel affinity chromatography (Qiagen). Nicastrin was further purified to homogeneity by anion exchange chromatography (Source-15Q; GE Healthcare) and gel filtration chromatography (Superdex-200; GE Healthcare). An additional step of deglycosylation by endoF3 was performed to remove the N-linked glycans just before gel filtration. The purified nicastrin was kept in a buffer containing 25 mM Tris (pH 8.0) and 150 mM NaCl. The protein was concentrated to 10 mg/mL for crystallization.

Crystallization and Data Collection. The crystals were generated at 18 °C by the hanging-drop vapor-diffusion method. Crystals of nicastrin appeared overnight in a well buffer containing 0.1 M Tris (pH 8.0), 0.2 M sodium chloride, and 20% (wt/vol) PEG 3000, and grew to full size in 3 d. Derivative crystals were obtained by soaking crystals for 1 min in a buffer containing 0.1 M Tris (pH 8.0), 1 M sodium bromide, 20% (wt/vol) PEG 3000, and 10% (vol/vol) ethylene glycol. Both native and bromide-derived crystals were flash-frozen in a cold nitrogen stream at 100 K. All datasets were collected at the Shanghai Synchrotron Radiation Facility beamline BL17U and were processed with the HKL2000 package (34). Further data processing was carried out using programs from the CCP4 suite (35). Data collection statistics are summarized in Table S1.

Structure Determination and Refinement. Molecular replacement was carried out with PHASER (36) using a partial model of the HsNCT ECD (26) as the initial search model. The poor phases of the solution made refinement of the atomic model very difficult. To improve the phases, a SAD dataset of the bromide-soaked nicastrin crystal was collected. Using the previously obtained model as an input, the positions of the Br atoms were determined by the PHASER SAD experimental phasing module. With identification of the Br positions and the molecular-replacement model, better phases were generated using PHENIX AutoSol (37). The automated model building was performed with ARP/wARP (38) using the improved map. Manual model rebuilding and refinement were iteratively performed with COOT (39) and PHENIX (40), respectively. The structure refinement statistics are summarized in Table S1.

The structure of nicastrin ECD from *D. Purpureum* reveals a number of previously unidentified structural features compared with the previous partial model of HsNCT derived from the cryo-EM studies (26). The *D. Purpureum* nicastrin ECD was docked to the EM density map of human γ -secretase, and the fit was excellent. On the basis of the docking and sequence homology, we generated an updated model for HsNCT by adding ~100 amino acids. The model was fitted to the EM density with VMD (41) and refined with PHENIX (40) against artificial X-ray reflection data generated from the cryo-EM map.

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