Ectodomain shedding at the cell surface is a major mechanism to regulate the extracellular and circulatory concentration or the activities of signaling proteins at the plasma membrane. Human meprin β is a 145-kDa disulfide-linked homodimeric multidomain type-I membrane metalloproteinase that sheds membrane-bound cytokines and growth factors, thereby contributing to inflammatory diseases, angiogenesis, and tumor progression. In addition, it cleaves amyloid precursor protein (APP) at the β-secretase site, giving rise to amyloidogenic peptides. We have solved the X-ray crystal structure of a major fragment of the meprin β ectoprotein, the first of a multidomain oligomeric transmembrane shedding, and of its zymogen. The meprin β dimer displays a compact shape, whose catalytic domain undergoes major rearrangement upon activation, and reveals an exosite and a sugar-rich channel, both of which possibly engage in substrate binding. A plausible structure-derived working mechanism suggests that substrates such as APP are shed close to the plasma membrane surface following an “N-like” chain trace.

Physiological processes in the extracellular milieu and the circulation require finely tuned concentrations of signal molecules such as cytokines, growth factors, receptors, adhesion molecules, and peptidases. Many of these proteins are synthesized as type-I membrane protein variants or precursors consisting of a glycosylated N-terminal ectoprotein, a transmembrane helix, and a C-terminal cytosolic tail. Their localization at the cell surface restricts their field of action to autocrine or juxtacrine processes. However, to act at a distance in paracrine, synaptic, or endocrine events, they have to be released from the plasma membrane into the extracellular space as soluble factors through “protein ectodomain shedding” (1, 2). This entails limited proteolysis and is a major posttranslational regulation mechanism that affects 2–4% of the proteins on the cell surface, occurs at or near the plasma membrane (3), and apparently follows a common release mechanism (2). It may also proteolytically inactivate proteins to terminate their function on the cell surface (4). Peptidases engaged in such processing are “sheddases” and the most studied transmembrane sheddases are members of the adamalysin/ADAMs (4, 5) and matrix-metalloproteinase (MMP) (6) families within the metzincin clan of metalloproteinases (MPs) (7–9). These include ADAM-8, -9, -10, -12, -15, -17, -19, -28, and -33 (1, 4) and membrane-type I (MT1)-MMP, MT3-MMP, and MT5-MMP (2, 6, 10). Other confirmed transmembrane sheddases are the aspartic proteinases BACE-1 and -2 (ref. 11 and references therein) and the malarial parasite serine proteinases, PSUB2, PIRO1M, and PIROM4 (12). Distinct sheddases may participate in intercalating processes with disparate physiological consequences: ADAM-9, -10 (α-secretases), and -17 contribute to the nonamyloidogenic pathway of human amyloid precursor protein (APP) processing, whereas BACE-1 (β-secretase) participates in the amyloidogenic pathway. Whereas the former generates innocuous peptides, the latter gives rise to the toxic β-amyloid peptides believed to be responsible for Alzheimer’s disease (11). In several instances, shedding at the membrane surface is followed by a “regulated intramembrane proteolysis” step within the membrane (1). This is the case for the processing of Notch ligand Delta1 and of APP, both carried out by γ-secretase after action of an α/β-secretase (11), and for signal-peptide peptidase, which removes remnants of the secretory protein translocation from the endoplasmic membrane (13).

Recently, human meprin β (Mβ) was found to specifically process APP in vivo, which may contribute to Alzheimer’s disease (14, 15). It was also reported to activate cell-anchored α-secretase ADAM-10 and to be widely expressed in brain, intestine, kidney, and skin (14, 16–18). Disruption of Mβ in mice affects embryonic viability, birth weight, and renal gene expression profiles (19). The enzyme was further identified as a sheddase or proteolytic regulator at the plasma membrane of interleukin-1β (20), interleukin-18 (21), tumor growth factor α (22), procollagen III (23), epithelial sodium channel (24), E-cadherin (25), tenascin-C (26), and vascular endothelial growth factor A (27). Further substrates include fibroblast growth factor 19 and connective tissue growth factor. Altered expression and activity of the enzyme are associated with pathological conditions such as inflammatory bowel disease (28), tumor progression (29), nephritis (30), and fibrosis (32).

Mβ is a 679-residue secreted multidomain type-I membrane MP that belongs to the astacin family within the metzincins (7, 9, 16, 31, 32). The enzyme is glycosylated and assembles into either disulfide-linked homodimers or heterodimers with the closely related meprin α-subunit (33). Mβ homodimers are essentially membrane bound but may also be shed from the surface by ADAM-10 and -17 (34–35). To assess function, working mechanism, and activation of Mβ, we analyzed the structure of the major ectoprotein of mature Mβ (MβΔC) and of its zymogen, promeprin β (pMβΔC). With regard to transmembrane sheddases, to date only the structures of the isolated monomeric catalytic domains of ADAM-17 [Protein Data Bank (PDB) access code 1BK], ADAM-33 (PDB 1R55), MT1-MMP (PDB 1BUV), MT3-MMP (PDB 1R8), BACE-1 (PDB 1FKN), and BACE-2 (PDB 2EYW) have been described. Accordingly, this is a unique structural report of a multidomain oligomeric transmembrane sheddase. This report has allowed us a better understanding of the structural basis for latency and activation of this MP and to derive a plausible working mechanism for shedding of glycosylated type-I membrane substrates such as APP at the extracellular membrane surface.
**Results and Discussion**

**Multidomain Structure of Promeprin β.** We solved the crystal structures of pMβΔC (with two molecules in the asymmetric unit) and MβΔC (with one molecule) (SI Materials and Methods and Table S1). The pMβΔC monomer has overall dimensions of 80 × 60 × 70 Å (Fig. 1A–C) and a four-domain architecture (Fig. S2A and B) spanning an N-terminal propeptide (PD; T23–E25–R61; Mβ residue numbering as superindices according to UniProt Q16820), a catalytic MP domain (CD; N62–L259), a MAM domain (S260–C427), and a C-terminal TRAF domain (P428–S593/Q597).

![Fig. 1. Structure of promeprin β.](image-url)

(A) The structure of the pMβΔC monomer shown in front (Left) (CD in standard orientation according to ref. 41) and top (Right) reference views. The latter is along the sugar channel (green arrow). Glycans are depicted as stick models and the respective asparagine residues are numbered. The corresponding surface models are depicted above each picture (glycans in white). PD is shown in ochre, CD in aquamarine, MAM in red, and TRAF in purple. The zinc and the sodium ions are shown as magenta and blue spheres, respectively. (B) Close-up view in stereo of A, Left, to highlight residues engaged in PD–CD interactions. (C) Same as B in mono for the interaction between PD and TRAF. The first two residues of the structure (P23–W24) are actually T23–P24 in the natural protein (SI Materials and Methods). (D) pMβΔC dimer superposed with its Connolly surface shown in the front (Left) (in a plane with the membrane) and bottom (Right) dimer reference views. PDs are shown in ochre and yellow, CDs in aquamarine and blue, MAMs in red and green, TRAFs in purple and pink, and sugar moieties in white and gray. The intermolecular twofold axis is shown in red and green arrows point at the sugar channels. (E) Cartoon depicting the dimer as a ribbon in front (Left) and bottom (Right) views as in D. Green arrows run along the sugar channels and a pink arrow highlights the cleft of one CD with the adequate orientation of a substrate. The segment containing the intermolecular disulfide bond between C295 residues is disordered in the zymogen and its approximate position is highlighted by orange ellipses.
The polypeptide starts on the front surface of TRAF (Fig. 1A and C) with the first PD residues facing bulk solvent. From $E^1$ to $D^4$, which includes a conserved segment among meprins (PDB 3LQ0), the polypeptide progresses right to left along the TRAF surface in a nearly extended conformation and includes strand $\beta 1$ (Fig. 1C; see Fig. S1 A and B for nomenclature and extent of regular secondary structure elements), which is engaged in a parallel $\beta$-ribbon interaction with TRAF strand $\beta 2$. In addition, $F^2$ leans toward a hydrophobic pocket generated by $Y^{557}$, $F^{32}$, and $M^{232}$ of TRAF; $D^{28}$ interacts with $S^{560}$, $V^{29}$ with $Y^{557}$ and $R^{316}$, and $D^{40}$ with both $R^{316}$ and $R^{446}$, the latter from CD. TRAF residues $Y^{476}$, $H^{479}$, and $A^{561}$ further contribute to binding. This interaction of the N-terminal segment of PD with TRAF, which buries $\sim 460$ Å$^2$, reveals a novel potential exosite on the TRAF surface that would affect genuine substrates at subsites $P_1^\prime$–$P_0$ when bound at the active-site cleft in reverse orientation to PD (see Zymogen Determinants in Promeprin $\beta$ and Activation to Mature Meprin $\beta$). Indeed, these positions are conserved among phylogenetically related substrates (36). An example is APP, which is cleaved by M$\beta$ at the $\beta$-secretase site ($M^{671}$–$D^{672}$; APP residue numbers as subindices according to UniProt P05067) in vivo and in vitro to generate amyloidogenic $A_42$ and $A_41$ peptides (14, 15). This process entails that upon Michaelis-complex formation, APP segment $D^{672}$AEFRHDSGYE$^{682}$ occupies substrate positions $P_1^\prime$–$P_1$, the tyrosine in $P_1^\prime$ would spatially overlap with PD residue $F^{17}$, and peptide in $P_2^\prime$ with $V^{28}$, and asparagine in $P_3^\prime$ with $D^{46}$. Generally, exostis distal from the cleavage site contribute to efficient cleavage and have been previously reported for other peptidases such as thrombin (37) and ADAM family members (38).

Zymogen Determinants in Promeprin $\beta$. At $V^{29}$ of PD, the chain sharply kinks downward and runs vertically until $G^{42}$ (Fig. 1C). Here, the chain turns again and progresses horizontally at $G^{42}$–$D^{46}$ to approach CD. From there on, the protein folds across the front surface of CD in reverse orientation to a substrate, thus blocking the cleft. This segment includes a helix ($\alpha 1$) perpendicular to the cleft. Altogether, the interaction of PD with CD buries an interface of $\sim 1,225$ Å$^2$ and includes three salt bridges on the prime side of the cleft ($D^{32}$–$R^{446}$ and $D^{34}$–$R^{446}$) and two more on the nonprime side ($R^{2}$–$E^{37}$ and $D^{30}$–$R^{33}$). A loop in the central part of the segment enables $D^{42}$ to chelate in a bidentate manner the catalytic zinc ion from above (Fig. S1; Fig. 1C). In the zymogen, this residue replaces the catalytic solvent molecule of mature astacins following the “aspartate-switch” mechanism (39). At $R^{57}$, the chain turns down and reaches the final maturation point of $pM^\beta$, $R^{61}$–$N^{62}$. The first residue of CD is buried in the zymogen in an internal cavity framed by $F^{60}$ and $F^{33}$ of PD and $W^{16}$ and $Y^{491}$ of CD, and its side chain interacts with $E^{50}$ and $S^{165}$. Overall, the fold of PD is reminiscent of that of the propeptide of astatin except that in the latter the N terminus is anchored to the catalytic moiety (in the absence of further domains) and helix $\alpha 1$ is rotated by $\sim 70^\circ$ around a vertical axis so that it rather parallels the active-site cleft (PDB 3LQ0) (39). This means that the PDs of the two structures are superposable only at $F^{60}$EGDIKL$^{29}$ ($F^{18}$EGDIKL$^{29}$ in proastacin) (39), which includes the zinc-binding aspartate. This is consistent with sequence similarity among PDs of general astacin family members being restricted to a short consensus sequence, FXGDX (X stands for any residue) (32). The short PD of $M^\beta$ and other astacins contrasts with the large prosequences found in ADAMs, which actually constitute separate domains capable of inhibiting or activating CD$\prime$s in trams (40).

Catalytic Domain in Promeprin $\beta$. The 198-residue CD is a compact ellipsoidal reminiscent of a pac-man (Fig. S1 A and B). A deep and narrow active-site cleft, which harbors the catalytic zinc ion at midwidth (Fig. S1C), separates an upper N-terminal and a lower C-terminal subdomain (NTS and CTS, respectively) of similar size when viewed in standard orientation (Fig. S1 A) (41). CD is cross-linked by two disulfide bonds within the NTS: $C^{102}$–$C^{255}$ connects the C terminus of the domain with a loop, which links helix $\alpha 2$ and strand $\beta 3$ (L2p13), and $C^{242}$–$C^{242}$ connects $\beta 5$ with Lp603 and contributes thus to shaping the upper rim of the active-site cleft on its prime side. NTS harbors a central twisted five-stranded $\beta$-sheet ($\beta$2–$\beta$6) whose lowest and only antiparallel strand ($\beta 5$) shapes the upper rim of the active-site cleft. The sheet is decorated on its concave bottom by two helices, the “backing helix” ($\alpha 2$) and the “active-site helix” ($\alpha 3$), which run nearly parallel to the strands of the sheet. Helix $\alpha 3$ includes the first part of a long zinc-binding consensus sequence, H$^{525}$EXXXGX$^{532}$ (Fig. S1C), which is found in astacins but also metzincins in general (7–9, 42, 43). This helix ends at $G^{159}$, which allows for a sharp turn of the polypeptide chain to enter the CTS. The latter contains the third zinc-binding residue, $R^{162}$, and the “family-specific” residue of astacins, $E^{163}$ (43, 44). Also typical for astacins and metzincins, a tight 1,4-$\beta$–type “Met turn” is located below the catalytic zinc site, featuring a strictly conserved methionine, M$^{329}$ (Fig. S1C) (8, 45). The rest of the CTS has little regular secondary structure farther to the major “C-terminal helix” ($\alpha 4$) (Fig. S1 A and B). Of particular interest is that the polypeptide chain is disordered at Y$^{70}$/D$^{184}$–S$^{191}$/I$^{187}$. This segment corresponds to the “activation domain” in astacins (39).

MAM and TRAF Domains in Promeprin $\beta$. After CD, the polypeptide chain enters the 168-residue MAM domain, which lies behind TRAF and performs no contact with the MP moiety with the exception of some residues near the interdomain junction (Fig. 1A, Right). MAM is a $\beta$-sandwich consisting of two five-stranded antiparallel $\beta$-sheets rotated away from each other by $\sim 25^\circ$. The sandwich consists of a front sheet twisted by $\sim 70^\circ$ (p10–p13–p18–p15–p16; Fig. S1 A and B, Center) and a back sheet twisted by $\sim 40^\circ$ and curled (p11–p9+–p12–p19–p14–p17), whose second strand (p9+–p12) is interrupted by $\alpha 3$–helix n3 and strands p10 and p11. Overall, the $\beta$-sandwich is built following a “jelly-roll” architecture made of two four-stranded Greek-key motifs (Fig. S1B, Center, in red and magenta, respectively), in which the second motif is inserted after the first $\beta$-ribbon (p9+–p12) of the first motif. The jelly roll is decorated by the aforementioned insertion (Fig. S1B, Center, in pink), which includes Lp10b11—partially undefined in one of the two molecules in the asymmetric unit—and Lp11b12, the “dimerization loop” (see below). In addition, the pairs C$^{565}$–C$^{737}$ and C$^{340}$–C$^{427}$ are at adequate distance and geometry for disulfide bonding but, contrary to the SS bonds in CD, the respective $\alpha 7$ atoms are 2.9 Å apart. We attribute this to a radiation-damage artifact due to the long exposure time required to collect a complete dataset in space group P1. In addition, the $\alpha 3$–p10–p11 insertion contributes to an octahedral cation-binding site tentatively interpreted as a sodium site. The ion is coordinated by the side chains of E$^{268}$, D$^{298}$, S$^{300}$, and D$^{416}$ and the main-chain oxygen atoms of S$^{266}$ and F$^{310}$ (Fig. S1D). Overall, the topology and architecture of this domain are reminiscent of receptor-type tyrosine-protein phosphatase $\mu$ (PDB 2V5Y), which belongs to the MAM protein family of adhesive proteins initially identified by bioinformatic searches in meprin $\alpha$ and $\beta$, A5 protein, and receptor protein tyrosine phosphatase $\mu$ (46). In particular, the MAM domain of tyrosine phosphatase $\mu$ was shown to play a major role in homodimerization of the phosphatase ectoprotein and in cell adhesion (47).

Downstream of MAM, the 170-residue TRAF domain interacts with the former, burying an interface of $\sim 650$ Å$^2$ (Fig. 1A, Right). TRAF also interacts with CD through a surface spanning $\sim 950$ Å$^2$ in one protomer and $\sim 1170$ Å$^2$ in the other as the polypeptide chain could be traced for four residues more in the latter. TRAF features the second type of all-$\beta$ structure found in mpM$\beta$ (Fig. S1 A and B, Right), with a five-stranded front sheet (p21–p22–p23–p29–p28) and a four-stranded back antiparallel sheet (p20–p30–p24–p25) rotated by $\sim 40^\circ$ against each other and arranged in a $\beta$-sandwich as in MAM. The front sheet is twisted
by ~5°, arched and curled, whereas the back sheet is twisted just by ~50°. Altogether, the strands are arranged as two Greek-key motifs (Fig. S1B, Right, in purple and violet), in which the second one is inserted between strands 3 (β30) and 4 (β23) of the first one instead of after the first β-ribbon in MAM (see above). Again contrary to MAM, which features a jelly roll with parallel Greek keys, in TRAF the second Greek key is rotated by ~180° relative to the first one around an axis perpendicular to the plane of the β-sheets. In TRAF, the double Greek key is decorated with a β-ribbon (β26-β27) after β25, an additional short strand (β28) for the front sheet, and a helix (α5) between β29 and β30 (all in blue in Fig. S1B, Right). The only cysteine found in this domain, C692, is buried and unbound, and the C terminus of the molecule (J599/Q599) protrudes from the top surface of the monomer (Fig. L4, Left). Overall, Mβ TRAF is structurally similar to tumor-necrosis factor receptor-associated factors 2, 3, and 6 (e.g., PDB 1LBS). These gave rise to the TRAF family, which comprises major mediators of cell activation engaged in homo- and heterodimerization (48).

**Glycosylation Sites and "Sugar Channel."** The pMβΔC and MβΔC structures contained sugar moieties attached to residues N218 and N254 of CTS; N370 of MAM; and N436, N445, N547, and N592 of TRAF. The observed N-glycosylation patterns, which are consistent with those found in other recombinant proteins produced in *Trichoplusia ni* insect cells (49), are similar to those found in mammalian glycosylation pathways (50) (Fig. S2). Accordingly, it is assumed that the glycosylations, which were able to be modeled to up to 10 hexose moieties at a single site (N67) and a maximum of 26 hexoses per protein monomer (Fig. S1B), represent a bona fide mimic of the authentic glycosylation pattern of the enzyme. Whereas the sugar moieties attached to N218 and N254 point into the center of the particle. They are disordered at Y211 to approach the catalytic ion (replaced in the MAM structure, which were obtained in different crystal forms, with regard to its side chain and occupies the space of D36′—E37′). Further inspection of the active-site cleft and the electron density on the prime side of the active-site cleft of the mature enzyme (36)—as in MAM (see above). Further evidence for the consistency of this orientation is based on the proximity of the CDs and their active-site clefts to the membrane surface, which is required if membrane-anchored substrates are to be cleaved close to the membrane.

**Activation of Mature Meprin β.** Activation of meprins requires proteolysis of the N-terminal PD, which is catalyzed by trypsin in the intestinal lumen and kallikrein-related peptidases (KLK-4, -5, and -8) in other tissues (17, 51). Comparison of the zymogenic and mature structures, which were obtained in different crystal forms, reveals that in general the dimers (established in MβΔC between symmetry equivalents) fit together with a global rmsd of 1.2 Å for 1,006 common Cα atoms (of 561 + 554 residues in pMβΔC and 533 + 533 in MβΔC; Fig. S3A). Detailed inspection, however, shows that CTSs undergo major rearrangement upon maturation through a hinge rotation of ~25° toward the cleft around E163, H155, and G156, which entails a maximum displacement of ~8 Å (at G156 Cα; Fig. S3 B–D). In this way, the space exposed by PD removal is subsequently occupied in part by the CTS. This closing hinge motion further causes displacement of the mature N terminus of Mβ and a rotation of its first three residues by ~180°. Thus, N62 becomes completely buried inside the mature CD moiety and hydrogen bonded through its N52 atom to the side chain of the family-specific residue E163. The latter has the same side-chain conformation as in the zymogen and is likewise bound to K286 through a buried salt bridge. Maturation further entails rigidification of the formerly flexible activation segment (see above) and its upward shift toward the cleft by ~4 Å (at Y191 Cα). This leads to a competent conformation that enables D190 and S190G to bind the α-amino group of N62 (Fig. S3 C and D). The stiffening of CD contributes to the traceability of MβΔC structure along its entire polypeptide chain (N65—T934). This holds true also for the flexible loop Lβ11β12, which encompasses the intermolecular disulfide bond, although this is based on weak electron density. A further key role in CTS rearrangement is played by W161, whose side chain rotates by ~150° around its χ1 angle and becomes sandwiched by the ascending side chain of Y185 of the activation segment. Overall, the major rearrangement observed is compatible with the gross particle structure, indicating that the zymogen is already in a preformed conformation adequate for catalysis, which requires a rigid-body rearrangement of a subdomain spanning only one-seventh of the full-length protein for full competence.

Maturation also constricts the active-site cleft and this affects the side chain of Y191, which is engaged in zinc and substrate binding, and catalysis in mature astacin CD5 (i.e., the "tyrosine-switch" residue) (52) and some other metzincins such as serinyls and pappalysins (8). In the zymogen, it is pulled away from its competent position by the intercalation of PD helix α1, in particular the side chains of I37 of CTS and ref. 36). Further inspection of the active-site cleft and the adjacent exosite provided by TRAF (see Multidomain Structure of Promeprin β) reveals that R140 and R216 (to the right of R235 in Fig.
I B and C), and R

194 from Lβ7n2 of CD, which interacts with E

32 in thezymogen and becomes reoriented upon maturation (Fig. S3 B–D), could explain the preference of Mβ for acidic side chains also in downstream prime-side subsites (see Multidomain Structure of Promeprin β), pinpointing a unique cleavage specificity among extracellular proteases (36).

Finally, the substrate specificity of Mβ is complementary to that of other physiologically relevant transmembrane sheddases. The most studied ones, ADAM-17, ADAM-10, and MT1-MMP, as well as other ADAM and MMP family members, have specificity for large and medium-sized hydrophobic residues in P

′ as found, for example, in ADAM-17 substrates (53) and the α-secretase site of APP (K

689–L

690). BACE-2 also cleaves at the α-secretase site (54). Accordingly, Mβ would be the only transmembrane sheddase capable of cleaving before acidic residues in general and at the β-secretase site of APP in particular in addition to BACE-1 (15).

Working Mechanism for Shedding at the Plasma Membrane. The structures of both pMβΔC and MβΔC reveal a dimer with a membrane-proximal face (see above). We constructed a homology model for the remaining ~60 residues of the ectoprotein, encompassing an EGF-like domain, the 20-residue transmembrane anchor, and the 27-residue cytosolic tail of each monomer (SI Materials and Methods), which enabled us to propose a molecular mechanism for Mβ-mediated shedding at the plasma membrane (Fig. 2). This is visualized using a tentative model for APP region F

624–L

723, which includes the final segment of the ectoprotein and the transmembrane helix (55) and, thus, the β-secretase cleavage site (see Multidomain Structure of Promeprin β). Following this model, a substrate chain would be bound at the membrane-distal part (F

624–V

640 of APP) by the sugar channel of Mβ monomer one, whose two major glycosylations at N

647 of MAM and N

790 of TRAF would act like a bat-winged saloon door to admit and retain the substrate chain. This would be consistent with the general function of such domains in protein–protein interactions. Downstream, the substrate chain would run along the back surface of the cognate CD until position L

650 to approach the interface between CDs within the dimer and, eventually, enter the active-site cleft of Mβ monomer two (Fig. 2). An intramolecular mechanism involving all three domains of one monomer is unlikely as the substrate would have to undergo a long excursion after passing through the sugar channel and the back surface of the cognate CD to reach its active-site cleft with the correct N-to-C polarization (Fig. IE). The first residue after the β-secretase cleavage site, D

722, would be located in the S1′ pocket of Mβ, thus matching its substrate specificity. Further downstream subsites of the substrate would run across the cleft and the prime-site exosite on the TRAF surface (see Multidomain Structure of Promeprin β) of Mβ monomer two and then turn down to reach the APP transmembrane segment at G

700. Generally, the substrate would follow an “N-like” trajectory (Fig. 2, Upper Right) and involve domains from both monomers within the Mβ dimer. In addition, this mechanism would be sugar assisted. The majority of potential shedding substrates (87% of single-pass transmembrane proteins) (56) are glycosylated, and this holds true also for APP and other Mβ substrates. In particular, APP is glycosylated at T

633, T

651, T

659, T

663, S

667, and Y

651 with regard to the segment under inspection here (57). Of these sites, the proposed model predicts that the latter three could be at or close to subsites P

6, P

6′, and P

6′′, i.e., they would not interact with the enzyme but rather be solvent exposed. By contrast, the glycans attached to T

633, T

651, and T

659 could potentially interact with the site created by the symmetric N

436 glycosylations of Mβ, and the one at T

633 could interact with that of Mβ N

436 (Fig. 2). Overall, this mechanism would be compatible with other type-I transmembrane substrates that are shed at sites at least 20–25 residues above the membrane. The different N-glycans found on the surface of the Mβ particle along the proposed substrate path (Fig. 2) could provide alternative anchor points for the particular sugar moieties of each substrate.

Materials and Methods

A detailed description of procedures is provided in SI Materials and Methods. Briefly, pMβΔC was produced by recombinant baculovirus-induced expression in insect cells and activated by trypsin as reported in ref. 58. The structure of pMβΔC was solved by a combination of single-wavelength anomalous diffraction and Patterson search. The structure of MβΔC was solved by Patterson search. A model for full-length Mβ was obtained by connecting a homology model for the EGF-like domain with the experimental structure and a modeled transmembrane helix plus cytosolic tail by linkers of stereochemically reasonable conformation.

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Supporting Information

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SI Materials and Methods

Protein Production. The propepin β ectoemoytia lacking the N-terminal signal peptide and the C-terminal 87 residues (num- bering according to the prepro-sequence, UniProt Q16820), hereafter referred to as pMβΔC, was produced by recombinant baculovirus-induced overexpression in Trichoplusia ni insect cells and activated by trypsin to yield mature meprin β (MβΔC) as reported in ref. 1. In the expression construct T23–P23 were replaced with P13–W24.

Crystallization and Structure Solution. Crystallization assays were performed by the sitting-drop vapor diffusion method. Reservoir solutions were prepared by a Tecan robot and 100-nL crystallization drops were dispensed on 96 x 2-well MRC plates (In- novadyne) by a Phoenix nanodrop robot (Art Robbins) at the High-Throughput Automated Crystallography Platform of the Barcelona Science Park. Plates were stored in Bruker steady-temperature crystal farms at 4 °C and 20 °C. Successful condi- tions were scaled up to the microliter range with 24-well Cry- schem crystallization dishes (Hampton Research). MβΔC at ~7.0 mg/mL in 20 mM Hepes, pH 7.5, was crystallized at 21°C from equi volumetric drops with 18.2% (wt/vol) polyethylene glycol 8,000, 1 M LiCl, and 0.1 M bicine, pH 9.0, as reservoir solution. MβΔC at ~6.8 mg/mL in 20 mM Hepes (pH 7.5), 1 mM phenylmethylsulfonyl fluoride, and 100 μM actinomycin was crys- tallized using 20% (wt/vol) polyethylene glycol 3,350, 0.2 M so- dium malonate, 0.1 M bis-Tris propane (pH 8.5) as a reservoir solution, and 0.1 M CdCl2 as an additive. Crystals were cryo- protected by immersion in harvesting solutions [25% (wt/vol) polyethylene glycol 3,350, 1 M LiCl, 0.1 M bicine (pH 9.0) for MβΔC and 25% (wt/vol) polyethylene glycol 3,350, 0.2 M so- dium malonate, 0.1 M bis-Tris propane (pH 8.5) for MβΔC] supplemented stepwise with glycerol [0–15% (v/v) for MβΔC and 0–20% (v/v) for MβΔC]. Complete diffraction data sets were collected from liquid-2flash-cryo-cooled crystals at 100 K (provided by an Oxford Cryosystems 700 series cryostreamer) on an ADSC Q315R CCD and a Pilatus 6M pixel detector at beam lines ID23-1 (pMβΔC and ID29 (MβΔC), respectively, of the European Synchrotron Radiation Facility (Grenoble, France) within the Block Allocation Group “BAG Barcelona.” Crystals were triclinic (pMβΔC and hexagonal (MβΔC), with two and one molecules per asymmetric unit, respectively. Diffraction data were integrated, scaled, merged, and reduced with programs XDS (2) and SCALA (3) within the CCP4 suite of programs (4) (Table S1).

The structure of pMβΔC was solved by a combination of single-wavelength anomalous diffraction and Patterson search. The latter was performed with the program PHASER (5) with the structure of crayfish proastain [Protein Data Bank (PDB 3LQO)] (6), adequately trimmed and modified for its side chains with program CHAINSAW (7), as a searching model for the catalytic domain and part of the prosegment; this gave two un- ambiguous solutions with space group P1. Diffraction data to 2-Å resolution of a crystal collected at the zinc K-edge peak wavelength, as inferred from a previous XANES fluorescence scan, enabled program SHELXD (8) to independently identify the two zinc sites of the dimer present in the asymmetric unit. Subsequent phasing with these two sets using program SHELXE and higher-resolved diffraction data to 1.85-Å resolution as a pseudonative dataset, combined with the calculated phases of the two Patterson-search solutions, yielded a suitable electron density map for chain tracing on a Silicon Graphics Octane2 Workstation, using program TURBO-FRODO (9). Model building of the four domains—propeptide (PD), catalytic do- main (CD), MAM domain, and TRAF domain—of the two molecules alternated with crystallographic refinement with the program BUSTER/TNT (10), which included Translation Li- bration Screw-motion refinement and, initially, Non-Crystallo- graphic Symmetry restraints, until completion of the model. The final model of pMβΔC comprised residues 25–193+200–301+ 308–597 of molecule A and 23–190+199–277+282–301+307– 593 of molecule B. Of the 10 cysteine residues of the sequence, 4 made two disulfi de bonds in the CD (C103–C173 and C124–C144); two more pairs of MAM (C265–C373 and C340–C427) were at adequate distance for disulfi de bonding but were unbound, probably due to a radiation-damage artifact resulting from the long exposure time required to collect a complete dataset in space group P1; C606 of MAM was in a flexible segment but was engaged in an intermolecular disulfi de bond as revealed by nonreducing SDS/PAGE; and C492 of TRAF was unpaired. In addition, each protomer contained a zinc and a (tentative) so- dium cation. N-linked glycosylations were found attached to residues 218, 254, 436, 445, and 547 of each monomer. These comprised 10 (10) N-acetylgalactosamine, 8 (7) α-D-mann- nase, 5 (3) α-L-fucose, and 4 (2) β-D-mannose moieties in total for monomer A (monomer B) according to the overall scheme depicted in Fig. S2. Five tentative chloride ions, one glycerol molecule, and 808 solvent molecules completed the model.

The structure of MβΔC was solved by Patterson search with the program PHASER. Each of the three domains shared with the zymogen was used as an independent searching model. Model completion and refinement with BUSTER/TNT proceeded as aforementioned except that structurally equivalent parts of the higher-resolution zymogen structure were used to restrain re- finement of the low-resolution mature enzyme structure. The final model of MβΔC comprised residues 62–594 of the single crystallographically independent molecule (monomer A) plus a glycerol molecule bound to the active-site cleft, a (tentative) sodium cation (by analogy with the zymogen structure) and a (tentative) cadmium replacing the catalytic zinc, which was assigned on the basis of electron-density maps, approximate distances to the protein li- gands, and its presence in the crystallization conditions. The in- termediate MAM domain is less well defined by electron density than the flanking ones, as revealed by a larger average thermal- displacement parameter (123 Å2 vs. 109 Å2 and 102 Å2). Despite the lower resolution of the diffraction data, the same asparagine residues as in the zymogen plus position 592 showed evident N- linked glycosylations, which total 12 N-acetylgalactosamine, 9 α-D-mannose, 3 α-L-fucose, and 2 β-D-mannose moieties. Therefore, we assume that position 592 is likewise glycosylated in the zymogen but that these sugars are flexible. All four cysteine pairs at adequate distance for disulfi de bonding were built as disulfi de bonds. Seg- ment 300–310, which contains the C605-mediated intermolecular disulfi de bond, was traced on the basis of weak electron density and should be treated with caution. Extra electron density in the active- site cleft was conservatively interpreted as a glycerol molecule.

Miscellaneous. Figures were prepared with program CHIMERA (11). Interaction surfaces (taken as half of the surface area buried at a complex interface) were calculated with CNS (12). Structure similarities were investigated with DALI (13). Model validation was performed with MOLPROBITY (14) and the WHAT- CHECK routine of WHATIF (15). To obtain a working model for the complete active dimer, a homology model for the EGF-like
domain of Mβ (residues 606–647) was constructed with SWISSMODEL (16) on the basis of a receptor-binding region of Notch ligand (PDB 2VJ3; sequence identity 36%; E-value 7.6E-6). This model was connected with constructed models for the transmembrane helix (residues 653–673) and the cytosolic region (674–701) and with the experimental structure by linkers of stereochemically reasonable conformation. A type-I transmembrane substrate was constructed on the basis of the amyloid precursor protein (APP) sequence (residues 624–723; UniProt P05067) and the solution structure of Aβ42 in high organic solvent (PDB 1IYT) (17), keeping its helical conformation only for the transmembrane region. The final coordinates of the experimental structures of human pMβΔC and MβΔC have been deposited with the PDB at www.pdb.org (access codes 4GWM and 4GWN).

Fig. S1.  (A) Ribbon-type plot of the constituting domains of pMβΔC: PD+CD (Left, ochre and aquamarine; shown in standard orientation according to ref. 1), MAM (Center, red), and TRAF (Right, purple). The regular secondary structure elements are labeled (helices α1–α5, strands β1–β30, and 310-helices η1–η3). The two ions present in the structure, zinc and sodium, are shown as magenta and blue spheres, respectively, as are the cysteine residues (①–⑤). The anchor points of the flexible activation domain are shown by black arrows, and scissors highlight the final maturation cleavage point. (B) Topology scheme of the four domains of pMβΔC as in A. The regular secondary structure elements are depicted and labeled, and their terminal residues are indicated. MAM (Center) features two intercalated Greek-key motifs (in red and magenta, respectively) and additional elements in pink. TRAF (Right) also consists of two intercalated Greek-key motifs (in lilac and purple, respectively) decorated with additional elements in blue. Cysteine residues are shown as yellow sticks and labeled in orange. The seven N-glycosylation sites are also shown, with the corresponding asparagine residues labeled in blue. In each case, the most complete sugar structure found in any of the twozymogen molecules or the single mature-enzyme molecule present in the respective crystal asymmetric units is shown; a gray square represents N-acetylglucosamine, a circle α-D-mannose, a hexagon β-D-mannose, and a triangle α-L-fucose. See also Fig. S2. Dashed lines represent regions disordered in the zymogen structure. (C) Zinc binding-site environment, with distances ①, 2.24/2.33 Å; ②, 2.06/2.12 Å; ③, 2.00/2.06 Å; ④, 1.96/1.96 Å; and ⑤, 2.13/2.22 Å. (D) Sodium-binding site, with distances ①, 2.24/2.37 Å; ②, 2.47/2.54 Å; ③, 2.21/2.22 Å; ④, 2.43/2.47 Å; ⑤, 2.87/3.07 Å; ⑥, 2.67/2.74 Å; and ⑦, 2.51/2.63 Å. The two values refer to the two molecules in the crystal asymmetric unit of pMβΔC. Residue E268 is not fully defined by electron density.

Fig. S2. Scheme depicting the N-glycan patterns found in the structure of (p)Mβ. They are in accordance with glycans found in recombinant proteins produced in *Trichoplusia ni* insect cells (table 1 in ref. 1). BMA, β-D-mannose; FUC, α-L-fucose; MAN, α-D-mannose; NAG, N-acetylglucosamine. The respective type of glycosidic bond is further shown.

Fig. S3. (A) Superposition of the pMβΔC dimer (ochre/aquamarine) onto the active MβΔC dimer (red) in two orthogonal views. The ions correspond to those of the zymogen. (B) Superposition in stereo of the CDs of pMβΔC (ochre/aquamarine) and MβΔC (red) in standard orientation (1, 2). (C) Close-up view of B in stereo, displaying the pMβΔC structure and the residues mainly involved in rearrangement upon activation. The segments flanking the disordered activation segment are pinpointed by black arrows. (D) Same as C for the MβΔC structure.

Table S1. Crystallographic data

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Promeprin β ΔC</th>
<th>Promeprin β ΔC, Zn²⁺ edge</th>
<th>Meprin β ΔC, Zn²⁺ edge</th>
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<tr>
<td>Space group/cell constants: a, b, c, in Å; α, β, γ, in °</td>
<td>P1/69.62, 71.12, 85.74</td>
<td>P1/69.50, 70.70, 85.74</td>
<td>P6₁/22/75.0, 75.0, 502.7</td>
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<td>Wavelength, Å</td>
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<td>No. measurements/unique reflections</td>
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<td>398,689/18,106</td>
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<td>Resolution range, Å (outermost shell)*</td>
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<td>48.5–2.00 (2.11–2.00)</td>
<td>48.2–3.00 (3.16–3.00)</td>
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<td>Completeness/anomalous completeness, %</td>
<td>96.6 (95.6)/—</td>
<td>94.7 (93.3)/89.8 (89.7)§</td>
<td>99.9 (99.3)/—</td>
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<tr>
<td>Rmerge†</td>
<td>0.069 (0.827)</td>
<td>0.055 (0.494)§</td>
<td>0.106 (0.912)</td>
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<td>0.078 (0.699)/0.055 (0.494)§</td>
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<td>Average intensity, &lt;I&gt;/σ(I)&gt;</td>
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<td>17,972 (711)</td>
<td>17,972 (711)</td>
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<td>No. protein atoms/solvent molecules/ions</td>
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<td>4,271/1 Cd³⁺, 1 Na⁺</td>
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<td>Sugar moieties/ligands</td>
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<td>Rmsd from target values</td>
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<td>Residue main-chain conformational angle and side-chain rotamer analysis§</td>
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<td>Favored regions/outliers/all residues/bad rotamers</td>
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<td>505/1/531/4.7%</td>
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</tbody>
</table>

FUC, fucose; GOL, glycerol; MAN, α- or β-mannose; NAG, N-acetyl glucosamine.

*Values in parentheses refer to the outermost resolution shell.
†For definitions, see table 1 in ref. 1.
§According to MOLPROBITY (2).
Friedel pairs were treated separately.