

**Structure, Volume 23**

**Supplemental Information**

**Crystal Structures of the Extracellular Domain  
from PepT1 and PepT2 Provide Novel Insights  
into Mammalian Peptide Transport**

**John H. Beale, Joanne L. Parker, Firdaus Samsudin, Anne L. Barrett, Anish Senan, Louise E. Bird, David Scott, Raymond J. Owens, Mark S.P. Sansom, Stephen J. Tucker, David Meredith, Philip W. Fowler, and Simon Newstead**

## **Supplementary Figures**

**Crystal structures of the extracellular domain from PepT1 and PepT2  
provide novel insights into mammalian peptide transport.**

MousePepT1/1-709 ..... **M**G**M**S**K** ..... **S**R**G**C**F**G ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 HsPepT1/1-708 ..... **M**G**M**S**K** ..... **S**H**S**F**F**G ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 RatPepT1/1-710 ..... **M**G**M**S**K** ..... **S**R**G**C**F**G ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 DogPepT1/1-708 ..... **M**G**M**S**K** ..... **S**Y**G**C**F**G ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 RabbitPepT1/1-707 ..... **M**G**M**S**K** ..... **S**L**S**C**F**G ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 RatPepT2/1-729 ..... **M**N**P**F**Q****K**N**E**S**K**E**T**L**F**S**P**V**S**T**E**M**L**P**R**P**P**S**P**P**K**K**S** ..... **S**P**T**I**C**S**N** ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R  
 HsPepT2/1-729 ..... **M**N**P**F**Q****K**N**E**S**K**E**T**L**F**S**P**V**S**I**E**V**P**P**R**P**P**S**P**P**K**K**P** ..... **S**P**T**I**C**S**N** ..... **Y**P**I**S**I**F**F**I**V**V**N**E**F**C**E**R

H1

MousePepT1/1-709 **F**S**Y****Y****G**M**R**A**L**L**V**L**Y**F**R**N**F**L**G**W**D**D**D** **L**S**T**A**I****Y**H**T**F**V**A**L**C**Y**L**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 HsPepT1/1-708 **F**S**Y****Y****G**M**R**A**L**L**V**L**Y**F**R**N**F**I**S**W**D**D**D** **L**S**T**A**I****Y**H**T**F**V**A**L**C**Y**L**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 RatPepT1/1-710 **F**S**Y****Y****G**M**R**A**L**L**V**L**Y**F**R**N**F**L**G**W**D**D**D** **L**S**T**A**I****Y**H**T**F**V**A**L**C**Y**L**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 DogPepT1/1-708 **F**S**Y****Y****G**M**R**A**L**L**V**L**Y**F**R**R**F**I**G**W**D**D**D** **L**S**T**A**I****Y**H**T**F**V**A**L**C**Y**L**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 RabbitPepT1/1-707 **F**S**Y****Y****G**M**R**A**L**L**V**L**Y**F**R**N**F**I**G**W**D**D**D** **L**S**T**A**I****Y**H**T**F**V**A**L**C**Y**L**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 RatPepT2/1-729 **F**S**Y****Y****G**M**R**A**V**L**L**L**Y**F**L**Y**F**L**H**W**N**E**D**T**S**T**S****Y**H**A**F**S**S**L**C**Y**F**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I  
 HsPepT2/1-729 **F**S**Y****Y****G**M**R**A**V**L**L**L**Y**F**L**Y**F**L**H**W**N**E**D**T**S**T**S****Y**H**A**F**S**S**L**C**Y**F**T**P**I**L**G**A**I**A**D**S**W**L**G**K**F**K**T**I

H1

H2

MousePepT1/1-709 **V**S**L**S**I****V****Y**T**I**G**Q**A**V**T**S**V**S**S**I**N**D****L**T**D**H**D**H**N**G**S**P**D**S**L**P**V**H**V**A**L**S**M**V**G**L**A**L**I**A**L**G**T**G**G**I**K**P  
 HsPepT1/1-708 **V**S**L**S**I****V****Y**T**I**G**Q**A**V**T**S**V**S**S**I**N**D****L**T**D**H**H**N**D**G**T**P**D**S**L**P**V**H**V**V**L**S**L**I**G**L**A**L**I**A**L**G**T**G**G**I**K**P  
 RatPepT1/1-710 **V**S**L**S**I****V****Y**T**I**G**Q**A**V**T**S**V**S**S**I**N**D****L**T**D**H**D**H**D**G**S**P**N**N**L**P**L**H**V**A**L**S**M**I**G**L**A**L**I**A**L**G**T**G**G**I**K**P  
 DogPepT1/1-708 **V**S**L**S**I****V****Y**T**I**G**Q**A**V**T**A**V**S**S**I**N**D****L**T**D**Y**N**K**D**G**T**P**D**N**L**S**V**H**V**A**L**S**M**I**G**L**A**L**I**A**L**G**T**G**G**I**K**P  
 RabbitPepT1/1-707 **V**W**L**S**I****V****Y**T**I**G**Q**A**V**T**S**L**S**S**V**N**L**T**D**N**N**H**D**G**T**P**D**S**L**P**V**H**V**A**V**C**M**I**G**L**L**L**I**A**L**G**T**G**G**I**K**P  
 RatPepT2/1-729 **I**Y**L**S**L****V**V**L**G**H**V**F**K**S**L**G**A**I**P**T**L**G**.....**G**K**M**L**H**T**I**L**S**L**V**G**L**S**L**I**A**L**G**T**G**G**I**K**P**  
 HsPepT2/1-729 **I**Y**L**S**L****V**V**L**G**H**V**I**K**S**L**G**A**L**P**T**L**G**.....**G**Q**V**V**H**T**V**L**S**L**I**G**L**S**L**I**A**L**G**T**G**G**I**K**P**

H3

H4

MousePepT1/1-709 **C**V**S**A**F**G**G**D**O**F**E**E**G**Q**E**K**Q**R**N**R**F**S**I**F**Y**L**A**I**N**A**G**S**L**S**T**I**T**P**I**M**R**V**Q**Q**C**G**T**H**S**Q**Q**A**C**Y  
 HsPepT1/1-708 **C**V**S**A**F**G**G**D**O**F**E**E**G**Q**E**K**Q**R**N**R**F**S**I**F**Y**L**A**I**N**A**G**S**L**S**T**I**T**P**I**M**R**V**Q**Q**C**G**T**H**S**K**Q**A**C**Y  
 RatPepT1/1-710 **C**V**S**A**F**G**G**D**O**F**E**E**G**Q**E**K**Q**R**N**R**F**S**I**F**Y**L**A**I**N**A**G**S**L**S**T**I**T**P**I**M**R**V**Q**Q**C**G**T**H**S**Q**Q**A**C**Y  
 DogPepT1/1-708 **C**V**S**A**F**G**G**D**O**F**E**E**G**Q**E**K**Q**R**N**R**F**S**I**F**Y**L**A**I**N**A**G**S**L**S**T**I**V**T**P**M**R**V**H**E**C**G**I**Y**S**Q**K**A**C**Y  
 RabbitPepT1/1-707 **C**V**S**A**F**G**G**D**O**F**E**E**G**Q**E**K**Q**R**N**R**F**S**I**F**Y**L**A**I**N**A**G**S**L**S**T**I**T**P**M**V**R**V**Q**Q**C**G**I**H**V**K**Q**A**C**Y  
 RatPepT2/1-729 **C**V**A**A**F**G**G**D**O**F**E**E**H**A**E**A**R**T**R**Y**F**S**V**F**Y**L**A**I**N**A**G**S**L**S**T**F**I**T**P**M**R**G**D**V**K**C**F**G.....**Q**D**C**Y  
 HsPepT2/1-729 **C**V**A**A**F**G**G**D**O**F**E**E**K**H**A**E**E**R**T**R**Y**F**S**V**F**Y**L**S**I**N**A**G**S**L**I**S**T**F**I**T**P**M**R**G**D**V**Q**C**F**G.....**E**D**C**Y

H5

MousePepT1/1-709 **P**L**A**F**G**V**P**A**L**M**A**V**A**L**I**V**F**V**L**G**S**G**M**Y**K**K**F**Q**P**Q**G**N**I**M**G**K**V**A**K**C**I**G**F**A**I**K**N**R**F**R**H**R**S**K**A**Y  
 HsPepT1/1-708 **P**L**A**F**G**V**P**A**A**L**M**A**V**A**L**I**V**F**V**L**G**S**G**M**Y**K**K**F**K**Q**P**Q**G**N**I**M**G**K**V**A**K**C**I**G**F**A**I**K**N**R**F**R**H**R**S**K**A**F  
 RatPepT1/1-710 **P**L**A**F**G**V**P**A**A**L**M**A**V**A**L**I**V**F**V**L**G**S**G**M**Y**K**K**F**Q**Q**P**Q**G**N**I**M**G**K**V**A**K**C**I**G**F**A**I**K**N**R**F**R**H**R**S**K**A**F  
 DogPepT1/1-708 **P**L**A**F**G**V**P**A**A**L**M**A**V**S**L**I**V**F**V**I**G**S**G**M**Y**K**K**F**Q**Q**P**Q**G**N**V**M**G**K**V**V**K**C**I**G**F**A**I**K**N**R**F**R**H**R**S**K**Q**F  
 RabbitPepT1/1-707 **P**L**A**F**G**V**P**A**I**L**M**A**V**S**L**I**V**F**I**I**G**S**G**M**Y**K**K**F**Q**Q**P**Q**G**N**I**L**S**K**V**V**K**C**I**G**F**A**I**K**N**R**F**R**H**R**S**K**Q**F  
 RatPepT2/1-729 **A**L**A**F**G**V**P**G**L**L**M**V**L**A**L**V**V**F**A**M**G**S**K**M**Y**R**K**P**P**E**G**N**I**V**A**Q**V**I**K**C**I**W**F**A**L**C**N**R**F**R**N**R**S**G**D**L  
 HsPepT2/1-729 **A**L**A**F**G**V**P**G**L**L**M**V**I**A**L**V**V**F**A**M**G**S**K**I**Y**N**K**P**P**E**G**N**I**V**A**Q**V**F**K**C**I**W**F**A**I**S**N**R**F**K**N**R**S**G**D**L

H6

MousePepT1/1-709 **P**K**R**E**H**W**L**D**W**A**K**E**K**Y**D**E**R**L**I**S**Q**I**K**M**V**T**K**V**M**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**T**I**M**N  
 HsPepT1/1-708 **P**K**R**E**H**W**L**D**W**A**K**E**K**Y**D**E**R**L**I**S**Q**I**K**M**V**T**R**V**M**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**T**I**M**S  
 RatPepT1/1-710 **P**K**R**N**H**W**L**D**W**A**K**E**K**Y**D**E**R**L**I**S**Q**I**K**I**M**T**K**V**M**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**T**I**M**T  
 DogPepT1/1-708 **P**K**R**E**H**W**L**D**W**A**K**E**K**Y**D**E**R**L**I**S**Q**I**K**M**V**T**K**V**M**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**T**I**M**S  
 RabbitPepT1/1-707 **P**K**R**A**H**W**L**D**W**A**K**E**K**Y**D**E**R**L**I**A**Q**I**K**M**V**T**R**V**L**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**T**I**M**S  
 RatPepT2/1-729 **P**K**R**Q**H**W**L**D**W**A**A**E**K**Y**P**K**H**L**I**A**D**V**K**A**L**T**R**V**L**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**I**R**M**N  
 HsPepT2/1-729 **P**K**R**Q**H**W**L**D**W**A**A**E**K**Y**P**K**Q**L**I**M**D**V**K**A**L**T**R**V**L**F**L**Y**I**P**L**P**M**F**W**A**L**D**Q**O**G**S**R**W**T**L**O**A**I**R**M**N

H7

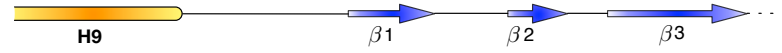
MousePepT1/1-709 **G**K**I**G**A**L**E**I**O**P**D**Q**M**O**T**V**N**A**I**L**V**I**M**V**P**I**V**D**A**V**V**Y**P**L**A**K**C**G**F**N**F**T**S**L**K**K**M**T**V**G**M**F**L**A**S**  
 HsPepT1/1-708 **G**K**I**G**A**L**E**I**O**P**D**Q**M**O**T**V**N**A**I**L**V**I**M**V**P**I**F**D**A**V**V**Y**P**L**A**K**C**G**F**N**F**T**S**L**K**K**M**A**V**G**M**F**L**A**S**  
 RatPepT1/1-710 **G**K**I**G**T**I**E**I**O**P**D**Q**M**O**T**V**N**A**I**L**V**I**M**V**P**I**V**D**A**V**V**Y**P**L**A**K**C**G**F**N**F**T**S**L**K**K**M**T**V**G**M**F**L**A**S**  
 DogPepT1/1-708 **G**K**I**G**L**L**E**V**O**P**D**Q**M**O**T**V**N**A**I**L**V**I**M**V**P**I**V**D**A**V**V**Y**P**L**A**K**C**G**F**N**F**T**S**L**K**K**M**T**V**G**M**F**L**A**S**  
 RabbitPepT1/1-707 **G**R**I**G**L**L**E**I**O**P**D**Q**M**O**T**V**N**T**L**I**I**L**V**P**I**M**D**A**V**V**Y**P**L**A**K**C**G**L**N**F**T**S**L**K**K**M**T**I**C**M**F**L**A**S  
 RatPepT2/1-729 **G**D**L**G**F**F**V**L**O**P**D**Q**M**O**V**L**N**P**F**L**V**L**I**F**I**P**L**D**L**V**I**Y**R**L**S**K**R**I**N**F**S**S**L**R**K**M**A**V**G**M**I**L**A**C  
 HsPepT2/1-729 **R**N**L**G**F**F**V**L**O**P**D**Q**M**O**V**L**N**P**L**L**V**L**I**F**I**P**L**D**E**V**I**Y**R**L**V**S**K**C**G**I**N**F**S**S**L**R**K**M**A**V**G**M**I**L**A**C

H8

H9

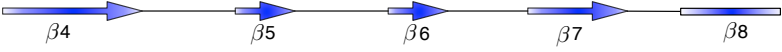
370 380 390 400 410 420

MousePepT1/1-709 MAFVVAIVQVEIDKTLVPVPGGNQVQIKVLNIGNNMTVHFPGN.....SVTLAQ  
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RatPepT1/1-710 MAFVVAIVQVEIDKTLVPVPSGNQVQIKVLNIGNNDMAVYFPGK.....NVTVAQ  
DogPepT1/1-708 MAFVVAIVQLEIDKTLVPVPGKNEVQIKVLNIGNGAMNVSFPCA.....VTVSQ  
RabbitPepT1/1-707 MAFVVAAILQVEIDKTLVPVPKANEVQIKVLNVGSENMIISLPGQ.....TVTLNQ  
RatPepT2/1-729 LAFVAALVETKINGMIHPQASQEIFLQVLNLADGDVKKVTVLGSRRNSSLVSVSS  
HsPepT2/1-729 LAFVAALVETKINEMAPAQPGPQEVFLQVLNLADDEVKVTVVGENNSSLIESVKS



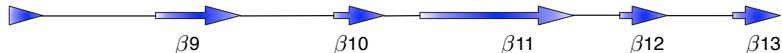
430 440 450 460 470

MousePepT1/1-709 MSQTDTFMTFDIDKLTSTINISSSGSPGVTTVAHDFEQGHRHTLLVWNPFSQ...YRVV  
HsPepT1/1-708 MSQTDTFMTFDVVKLSTRINISSPGSPVTAVTDDFKQGQRHTLLVWAFNH...YQVV  
RatPepT1/1-710 MSQTDTFMTFDVDQLTSINVSPPGSPGVTTVAHEFEPGHRHTLLVWGFNL...YRVV  
DogPepT1/1-708 MSQSDGFMTFDVDKLTSTINISSTGSPVIPVTYNFEPGHRHTLLVWAFNN...YRVV  
RabbitPepT1/1-707 MSQTDTFMTFNEDTSTINISSTGSPVTMITPSLEAGQRHTLLVWAFNN...YRVV  
RatPepT2/1-729 FQNTTHYSKHLLEAKSQDLHFHLKYNLSLVHNDHSVEEKNCYQLLIHQDGESISSML  
HsPepT2/1-729 FQKTPHYSKHLKTKSQDLHFHLKYNLSLVYTEHSVQEKNWYSLVIRBEDGNSISSMM



480 490 500 510 520 530

MousePepT1/1-709 KDGILNOKPEKENGIRFVNITL NEMVITKMSGKVYENVVTS.HNAGSYQFFPSGEKQYGT  
HsPepT1/1-708 KDGILNOKPEKENGIRFVNITL NEMVITKMSGKVYANISS.YNASTYQFFPSGIKGFT  
RatPepT1/1-710 KDGILNOKPEKENGIRFVSTL NEMVITKMSGKVYENVVTS.HSASNYQFFPSGQKDYT  
DogPepT1/1-708 KDGILNOKPEKENGIRFVNSL NESLNIITMGDKVYVNVVTS.HNASEYQFFSLGTKNIT  
RabbitPepT1/1-707 NDGLTQKSDKENGIRFVNITYSQPINVTMSGKVYEHIAS.YNASEYQFFTSVKGFT  
RatPepT2/1-729 VKDTGIKPANGMAAIRFVNITLHKDLNITSLDTDAFLSVGKDYGVSAVRYVLRGKYPAV  
HsPepT2/1-729 VKDTESRITNEMTTVRFVNITLHKDVNITSLDTSLSLVGEVYGVSAVRYVLRGNSISSMM



540 550 560 570 580

MousePepT1/1-709 INT.TAVAPTCLTDFKSSNLD FGSAYTYVIR.RASDGCLEVKEFEDIIPNTVNMALQ  
HsPepT1/1-708 ISS.TEIPPOCPNFNTFYVLE FGSAYTYIVQ.RKNDSCPEVKVFEDIISANTVNMALQ  
RatPepT1/1-710 INT.TEIAPNCSDFKSSNLD FGSAYTYVIRSRASDGCLEVKEFEDIIPNTVNMALQ  
DogPepT1/1-708 ISSTQQISQNTKVLQSSNLE FGSAYTYVIG.TQSTGCPPELHMFEDIIPNTVNMALQ  
RabbitPepT1/1-707 VSS.AGISEQCRDDFESPLYE FGSAYTYLIT.SQATGCPQVTEFEDIIPNTMNMALQ  
RatPepT2/1-729 HCE....TEDKVFSLDLGLD FGTTYLVFVIT.NITSQGLQAWKAEDIPANKLSIAWQ  
HsPepT2/1-729 HCR....TEDKNFSLNLGLD FGAAYLVFVIT.NNTNQGLQAWKIEDIPVKNKLSIAWQ



590 600 610 620 630 640

MousePepT1/1-709 IPQYFLITCGEVVFSVTGLEFSYSQAPS NMKSVLQAGWLLTAVAGNIIVLIVAGAGH  
HsPepT1/1-708 IPQYFLITCGEVVFSVTGLEFSYSQAPS NMKSVLQAGWLLTAVAGNIIVLIVAGAGQ  
RatPepT1/1-710 IPQYFLITCGEVVFSVTGLEFSYSQAPS NMKSVLQAGWLLTAVAGNIIVLIVAEAGH  
DogPepT1/1-708 IPQYFLITCGEVVFSVTGLEFSYSQAPS NMKSVLQAGWLLTAVAGNIIVLIVAGAGQ  
RabbitPepT1/1-707 IPQYFLITSGEVVFSITGLEFSYSQAPS NMKSVLQAGWLLTAVAGNIIVLIVAGAGQ  
RatPepT2/1-729 LPQYVLLVTAAGVVMFVSVTGLEFSYSQAPS SMKSVLQAAWLLTAVAGNIIVLVVAQFSG  
HsPepT2/1-729 LPQYALVTAAGVVMFVSVTGLEFSYSQAPS SMKSVLQAAWLLTAVAGNIIVLVVAQFSG



650 660 670 680 690

MousePepT1/1-709 FPKQWAEYILFASLLLVCVIFAIMARFYTYINPAEIEAQFDEDEKKGIGKENPYS  
HsPepT1/1-708 FSKQWAEYILFAALLLVVCVIFAIMARFYTYINPAEIEAQFDEDEKKNRLEKSNPYF  
RatPepT1/1-710 FDKQWAEYVLFASLLLVCVIFAIMARFYTYINPAEIEAQFDEDEKKGVVGKENPYS  
DogPepT1/1-708 FSEQWAEYILFAALLLVVCVIFAIMARFYTYINPAEIEAQFDEDEKKN.LEKMNVYS  
RabbitPepT1/1-707 INKQWAEYILFAALLLVVCVIFAIMARFYTYINPAEIEAQFDEDEKKNPEKNDLYP  
RatPepT2/1-729 L.AQWAEFVLFSCLLLVCVIFSVMAYYVVPKLSEDTREATD...KQIPAVQGNMI  
HsPepT2/1-729 L.VQWAEFVLFSCLLLVCVIFSIMGYVVPVKTEDMRGPAD...KHIPHIQGNMI

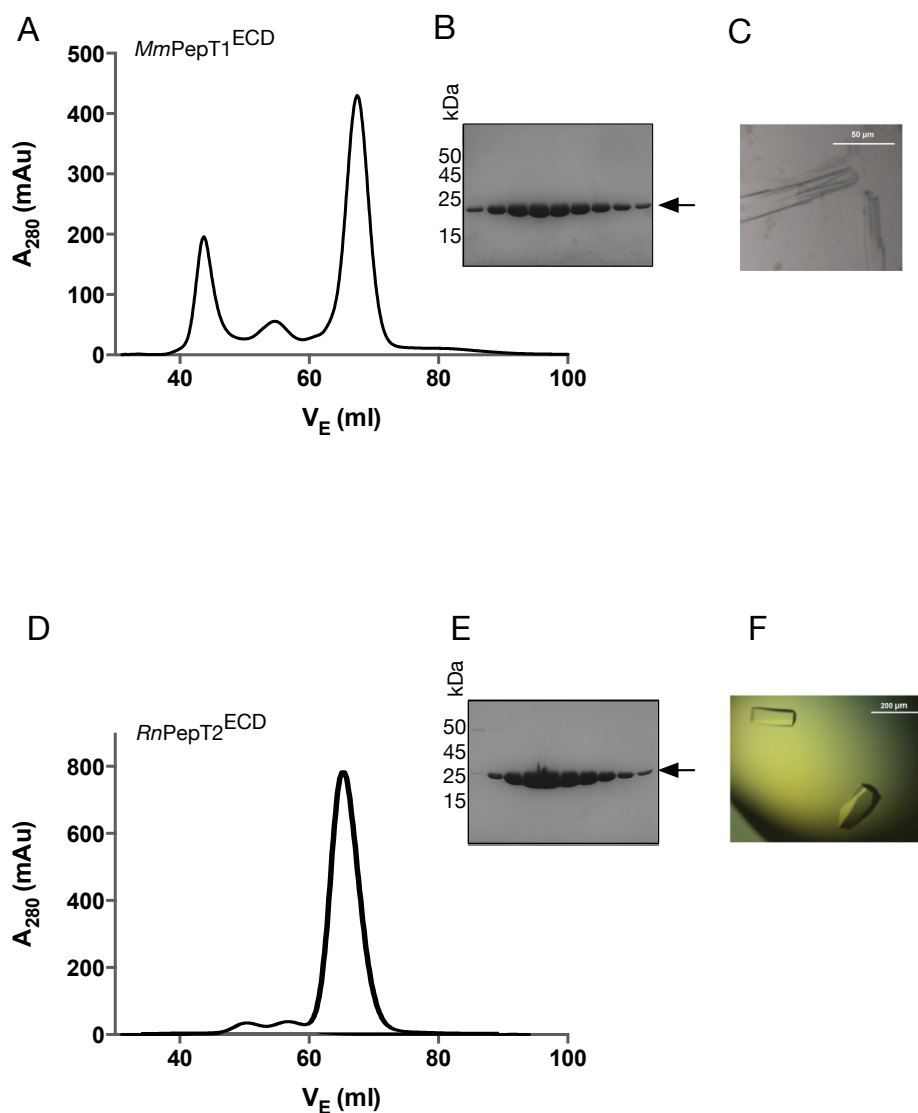


700

MousePepT1/1-709 SLEPVSTNM  
HsPepT1/1-708 MSGANSQKQM  
RatPepT1/1-710 SLEPVSTNM  
DogPepT1/1-708 TVTVPVSTQM  
RabbitPepT1/1-707 SLAPVSTQM  
RatPepT2/1-729 NLETKNTRL  
HsPepT2/1-729 KLETKTKL

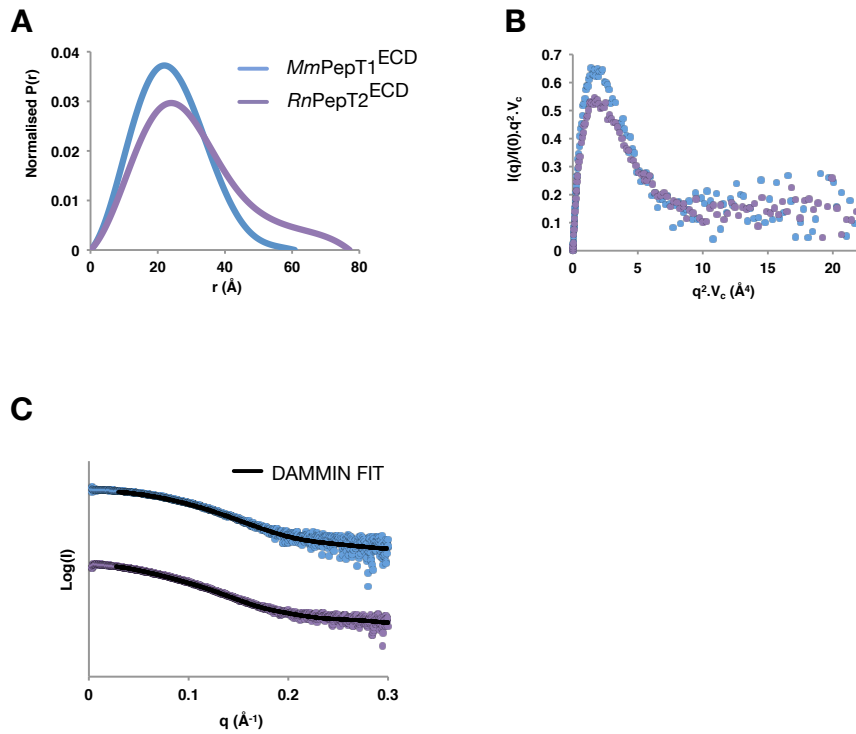
**Figure S1, related to Figure 1 - Sequence alignment and secondary structure of the mammalian POT family peptide transporters.**

An amino-acid sequence alignment between the mammalian peptide transporters; mouse PepT1 (Q9J1P7), human PepT1 (P46059), Rat PepT1 (Q75YE4), Dog PepT1 (Q8WMX5), Rabbit PepT1 (P36836), Rat PepT2 (Q63424) and human PepT2 (Q16348). The sequences were aligned using CLUSTALW as implemented in Jalview (Clamp et al., 2004; Waterhouse et al., 2009) and the figure produced using ESPript (Robert and Gouet, 2014). Identical residues are highlighted in red. The locations of the trans-membrane  $\alpha$ -helices were determined following sequence alignments with the bacterial POT family transporter PepTSo (Newstead et al., 2011) and are shown as orange tubes below the alignment. The secondary structure of the extra-cellular domain region, as reported here, is highlighted as arrows. Green stars highlight the two residues identified as interacting with trypsin.



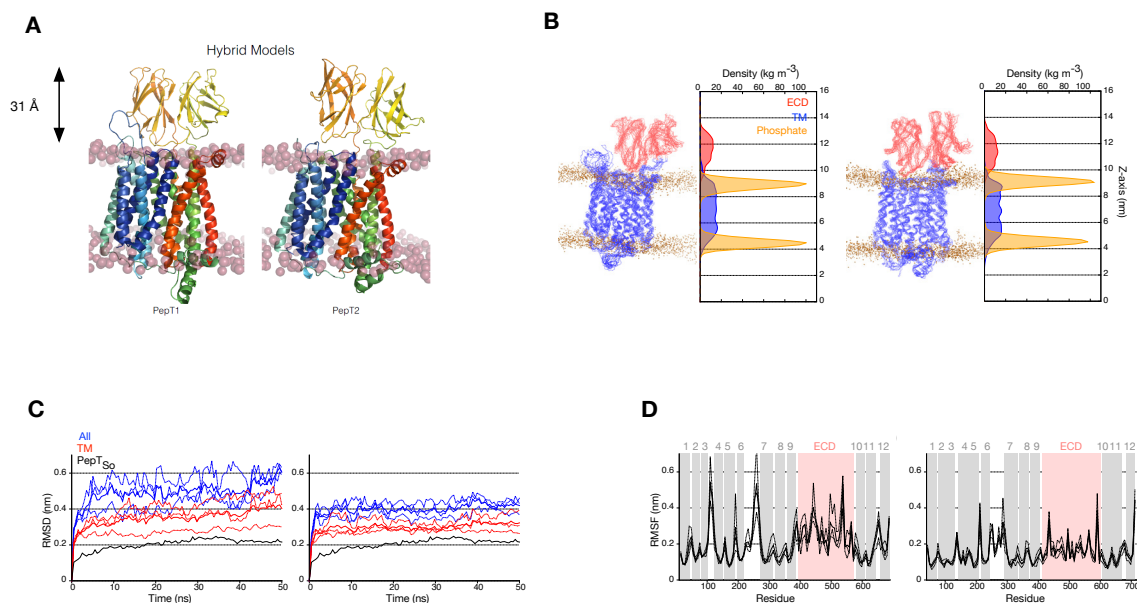
**Figure S2, related to Figure 2 - Purification and crystallization of *MmPepT1*<sup>ECD</sup> & *RnPepT2*<sup>ECD</sup>.**

**A-C.** The final step in the *MmPepT1*<sup>ECD</sup> purification showing the UV trace from the S75 16/60 size exclusion column. A 15 % Tris-Gly SDS PAGE showing the peak fractions and a representative crystal obtained from the hanging drop vapor diffusion plates. **D-F.** The same data for *RnPepT2*<sup>ECD</sup>.



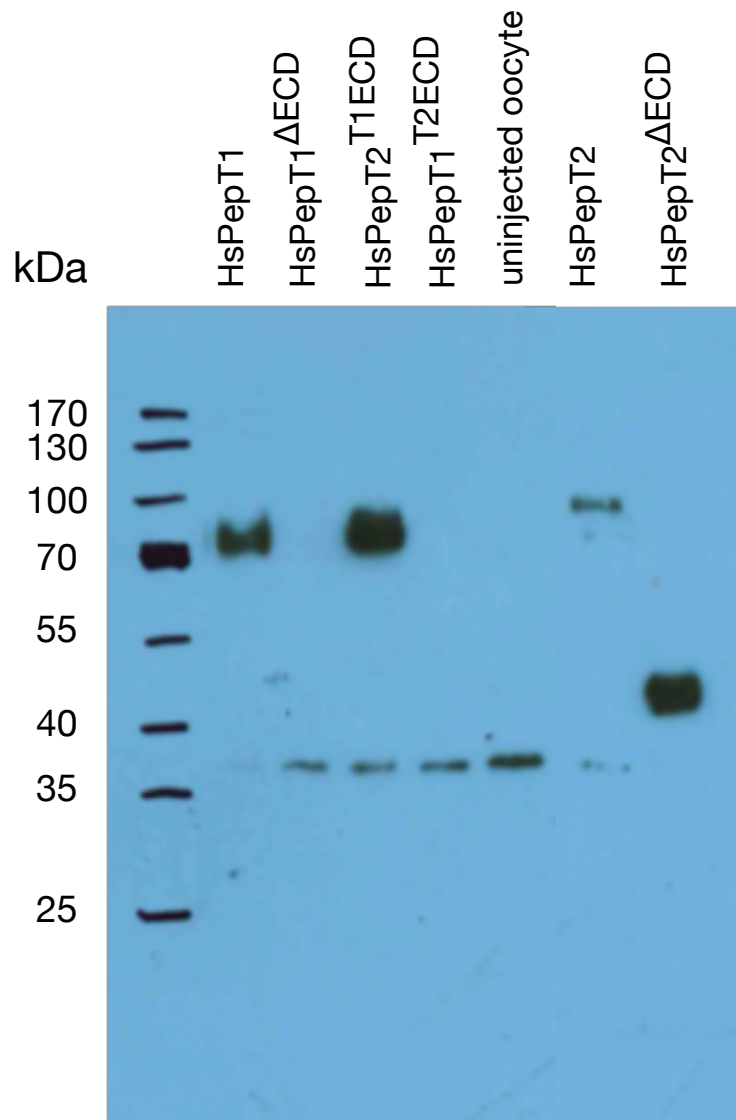
**Figure S3, related to Figure 3 – SAXS data analysis of *MmPepT1*<sup>ECD</sup> and *RnPepT2*<sup>ECD</sup> (shown in blue and purple respectively).**

**A.** Scattering pair distribution functions of the PepT1<sup>ECD</sup> and PepT2<sup>ECD</sup> calculated using ScÅtter and normalized to the sum of the paired distances. The distributions shown an increase in the PepT2  $D_{MAX}$  and a shift away from the spherical shape observed for PepT1<sup>ECD</sup> to a wider, more elongated shape. **B.** The dimensionless  $V_c$  based Kratky plot (Rambo and Tainer, 2011, 2013) of the ECDs data curves. The plot clearly shows a reduction in the main peak height for *RnPepT2*<sup>ECD</sup> compared to *MmPepT1*<sup>ECD</sup> indicating an increase the surface area to volume ratio and therefore a large particle. **C.** Shows the stacked scattering curves of the ECDs with the plotted DAMMIN fits of the averaged spherical harmonic models shown in Figure 3C. The DAMMIN  $\chi^2$  fits for PepT1<sup>ECD</sup> and PepT2<sup>ECD</sup> were 1.09 and 1.27 respectively.

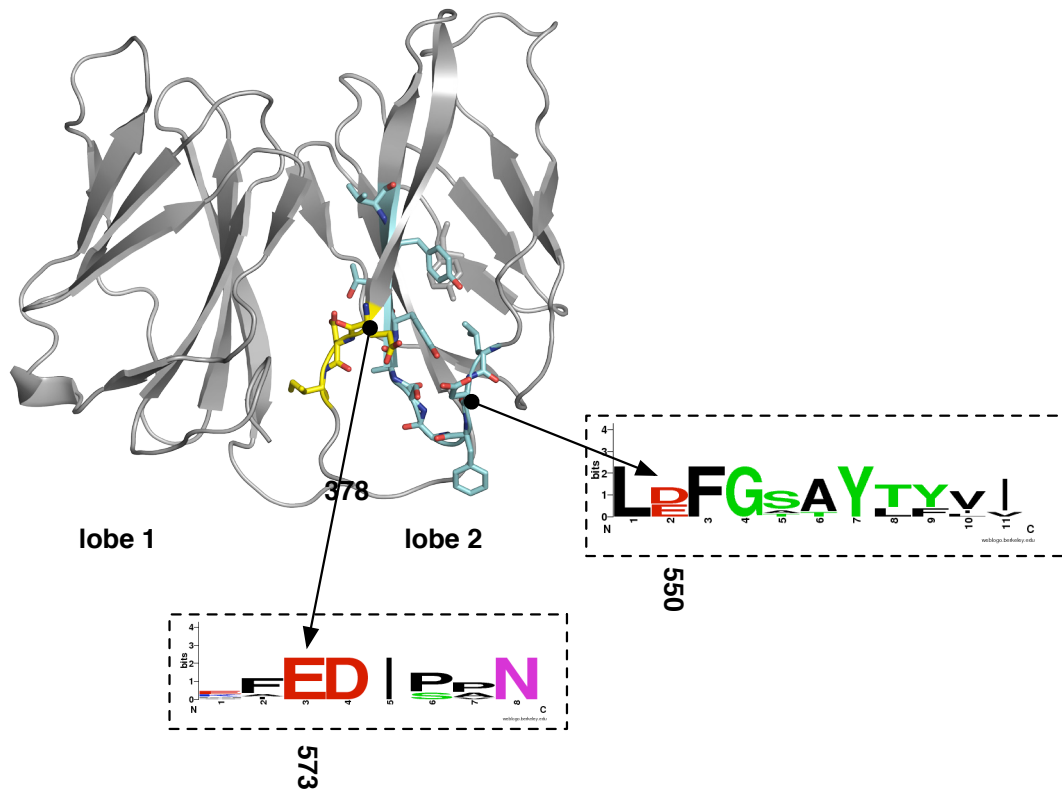


**Figure S4, related to Figure 4 - Homology models of human PepT1 and PepT2.** **A.** Cartoon representation of the homology models of the human transporters. The trans membrane domains are coloured blue to red, with the extracellular domains shown in yellow. In each subsequent panel PepT1 is on the right and PepT2 the left. **B.** Each hybrid model was inserted into an equilibrated 381-molecule POPC bilayer using GROMACS `g_membed` protocol. The simulations were run for 50 ns and clearly showed the ECD remaining in the upright position, away from the membrane and the phospholipid head groups. **C.** Simulations were analyzed using `g_rmsd` and `g_rmsf` tools in GROMACS and showed the models were stable. As a control the crystal structure of PepTSo is shown for reference (black line). **D.** The RMSF analysis shows that the ECD structure is stable and has similar backbone fluctuations to the homology model of the transmembrane domain.

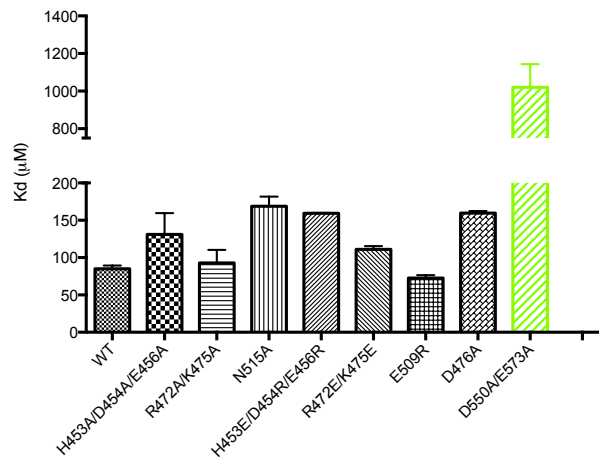
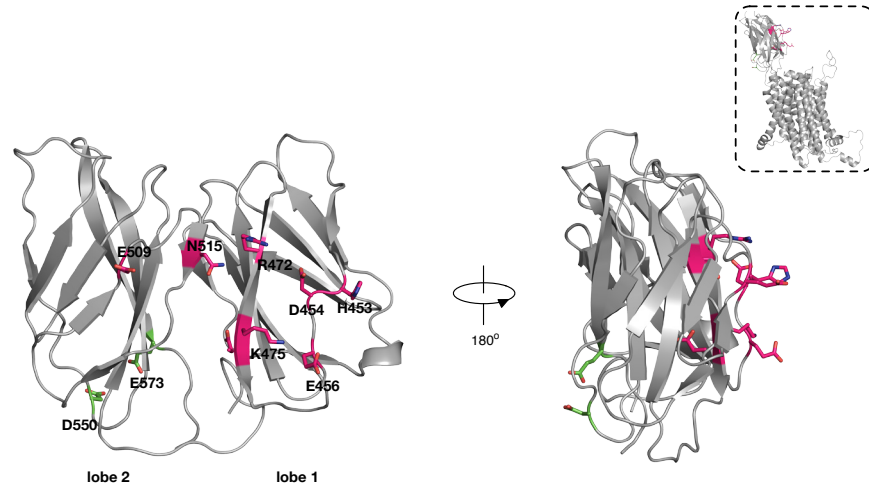




**Figure S5, related to Figure 4 - Relative expression levels of *HsPepT1* and *HsPepT2* constructs in *Xenopus leavis* oocytes.** Western blot of a 10% Tris-Glycine SDS-PAGE gel probed using an anti-FLAG antibody. Each lane contains 5 *Xenopus* eggs after 4 days incubation with either injected mRNA or water control. A cross-reactive band at ~38 kDa was observed in some of the eggs. *HsPepT2*<sup>ΔECD</sup> runs ~ 10 kDa smaller than the expressed protein, most likely due to known faster migration of transmembrane proteins in SDS-PAGE gels.



**Figure S6, related to Figure 5 – Conserved motifs in the extracellular domains of the mammalian PepT1 and PepT2 proteins.** The residues identified as playing an important role in binding to the trypsin protease are located in very conserved regions of the primary structure. Illustrated here the crystal structure of the mouse PepT1<sup>ECD</sup> as it is thought to exist in solution. Highlighted are the two conserved regions of the sequence, in cyan the region containing the first acidic residue D550 and in yellow the region containing the second acidic residue, E573. Of note is that the second residue in this motif, D574, form part of the salt bridge network identified as playing a role in stabilizing the interface between lobe1 and lobe 2 of the ECD in solution.



**Figure S7, related to Figure 5 – Analysis of binding sites for trypsin on mouse PepT1 ECD.** Residues on both the front (magenta) and back (green) face of the mouse PepT1 ECD were analyzed for their interaction with bovine trypsin using SPR. Only residues on the back face of the ECD (D550/E573) resulted in a significant reduction of binding affinity compared to WT. D476A forms part of the salt bridge network stabilizing the interface between lobe1 and lobe 2. Top right - homology model of full length mouse PepT1 showing the location of the residues analyzed with respect to the trans membrane helices and peptide translocation pathway.

### **Supplementary References:**

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