Supporting Information

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SI Materials and Methods

Screening for TAPBPR RNA Expression. Human Total RNA master panel II was purchased from Clonetech. The cell line RNA panel was made by TRNA extraction using TRI REAGENT method (Sigma). First-strand cDNA was synthesized from 1 μg of extracted RNA using Transcriptor Reverse Transcriptase (Roche). The PCR primer sequences were as follows: tapasin-related protein (TAPBPR) forward, 5′-GCAGCCTCCATGGGCACACA-3′; TAPBPR reverse, 5′-AGGTCAGCTGGGCTGGCTTACA-3′; GAPDH forward, 5′-CCACCATGGAGAAGGCTGGGGCTCA-3′; GAPDH reverse, 5′-ATCACGCCACAGTTTCCCGGA-3′. Amplification was performed in BioMix Red (Bioline) with 0.4 μM primer and 1 μL of the cDNA reaction. PCR cycling was performed as follows for TAPBPR: 96 °C for 10 min; 3 cycles of 96 °C for 24 s, 65 °C for 45 s, and 72 °C for 45 s; 27 cycles of 96 °C for 25 s, 62 °C for 45 s, and 72 °C for 45 s; and 3 cycles of 96 °C for 25 s, 55 °C for 1 min, 72 °C for 2 min, 72 °C for 10 min, and 4 °C for 10 min. PCR cycling was performed as follows for GAPDH: 96 °C for 10 min and 25 cycles of 95 °C for 55 s, 55 °C for 55 s, and 72 °C for 40 s, 4 °C 10 min. PCR products were electrophoresed in 1% agarose gels containing ethidium bromide, and predicted size products were visualized under UV light. Expected products sizes for TAPBPR and GAPDH PCR were 1,419 and 287 bp, respectively.

Constructs. cDNA encoding amino acids 22–468 of TAPBPR was cloned into pK1, a mammalian cell vector containing an N-terminal generic signal sequence, a GFP cassette and myc tag in pcDNA3.0 backbone (1). TAPBPR was also cloned into a vector containing two protein A cassettes in place of the GFP vector (named ZZ). These plasmids were transiently transfected into HeLa cells using *Trans*IT-HeLaMonster transfection kit (Mirus). Full-length untagged TAPBPR was cloned into the lentiviral vector pHRSIN-C56W-UbEM, producing TAPBPR under the SFFV promoter and a GFP derivative emerald under the ubiquitin promoter. In addition, GFP-TAPBPR was cloned into the lentiviral vector pHRSINcPPT-SGW. For RNA interference, lentiviral shRNA plasmids specific for TAPBPR were purchased from Open Biosystems. Plasmids used were oligo ID TRCN0000060732 on pLKO.1 backbone and V2LHS_135531 on the pGIPZ backbone. Lentiviral plasmids were transfected into HEK-293T cells using *Trans*IT-293 (Mirus), along with pCMVR8.91 packaging vector and pMD-G envelope vector. These supernatants were used to produce stable transduced cells lines. TAPBPR shRNA depleted transduced cell lines were selected with puromycin. KBM-7 cells depleted of β2-microglobulin (β2m), tapasin, and transporter associated with antigen processing (TAP)2 were produced as described previously (2).

Affinity Chromatography Using ZZ-Tagged TAPBPR. Approximately 1×10^7 HeLa cells were transiently transfected with a protein A tagged form of TAPBPR (ZZ-TAPBPR). Cells were lysed at 4 °C in 1% Triton X-100 in Tris-buffered saline (TBS) (20 mM Tris HCl, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA) plus 1 mM PMSF and protease inhibitor mixture tablet (Roche). Lysates were cleared by centrifugation at 17,000 × *g* for 10 min at 4 °C and then precleared on Sepharose beads. ZZ-TAPBPR and associated proteins were immunoprecipitated by incubating with IgG-Sepharose beads for 2 h at 4 °C. The beads were washed thoroughly in 0.1% Triton X-100 TBS and then once in 5 mM ammonium acetate before being eluted in 0.5 M acetic acid at pH 3.4. Proteins were lyophilized, resuspended in reducing sample buffer separated by gel electrophoresis, and then stained with Coomassie blue.

IFN-γ Treatment of Cells. Cells were treated with 50 units/mL IFN-γ (Roche) at 37 °C for the indicated time.

Flow Cytometry and Immunofluorescence. For flow cytometry, cells were stained at 4 °C with MHC I-specific antibodies. Isotype control antibodies were used as negative controls. Antibodies were subsequently detected with species-specific Alexa Fluor 647 secondary antibodies (Molecular Probes). Cells were analyzed on a BD Bioscience FACS Calibur four-color analyzer. Immunofluorescence was performed as described previously (1).

Protein Identification with Mass Spectrometry. Identification of proteins was achieved with the standard techniques of in-gel trypsin digestion, peptide mass fingerprinting, and fragmentation. Gel bands were excised and digested with trypsin according to the method of Wilm et al. (3). Samples of tryptic peptide mixtures were mixed with α-cyano-4-hydroxy-transcinnamic acid matrix and analyzed with an ABI 4700 MALDI-TOFTOF. For peptide mass fingerprints, mass calibration was performed with features internal to the spectrum when available, specifically the matrix-related ion peak at 1,060.048 Da and the trypsin autolysis peaks at 2,163.057 and 2,273.160 Da. For peptide fragmentation spectra, an "external" mass calibration was generated from the 2,163.057-Da trypsin autolysis peak, using the y10, y14, and y16 fragment ions. Spectra were interpreted both manually and with the Mascot sequence database search engine (www.matrixscience.com). Mascot was configured to consider the presence of the common gel-derived variable modifications "Propionamide Cysteine" and "Methionine Sulfoxide," and the version of the NCBInr database as of November 11, 2005 (3,023,944 sequences; 1,040,428,944 residues) was searched. A mass tolerance of 70 ppm was routinely used and increased to 160 ppm when calibration was suspected to be suboptimal.

1. Boyle LH, Gillingham AK, Munro S, Trowsdale J (2006) Selective export of HLA-F by its cytoplasmic tail. *J Immunol* 176(11):6464–6472.
2. Duncan LM, et al. (2012) Fluorescence-based phenotypic selection allows forward genetic screens in haploid human cells. *PLoS ONE* 7(6):e39651.
3. Wilm M, et al. (1996) Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* 379(6564):466–469.

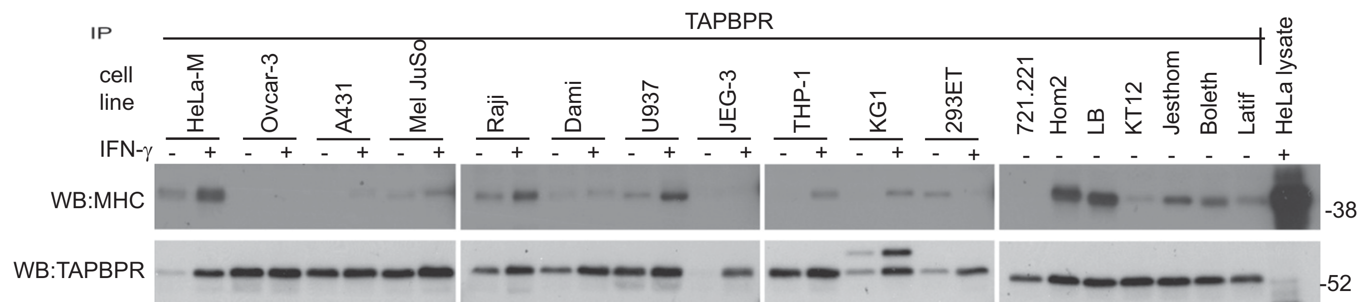


Fig. S1. Endogenously expressed TAPBPR interacts with MHC I in a number of human cell lines. Endogenous TAPBPR was immunoprecipitated from a panel of human cell lines lysed in 1% Triton X-100 using rabbit anti-TAPBPR. Western blotting was performed using the MHC I heavy chain (HC) specific mAb HCA2 and HC10. Blotting with mouse anti-TAPBPR is included to show the amount of endogenous TAPBPR immunoprecipitated.

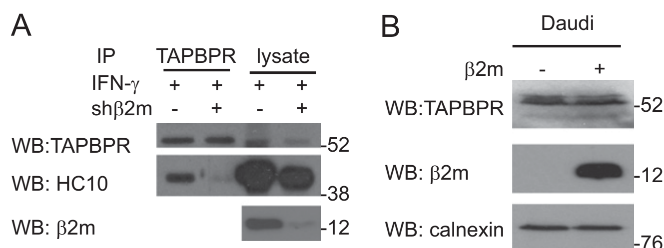


Fig. S2. β2m is essential for TAPBPR to associate with MHC I. (*A*) HeLa-S cells were depleted of β2m by transduction using pLVTHM shβ2m-GFP together with psPAX2 packaging and pMD2.G envelop vector [kind gifts from Ferreira de Figueiredo (Hannover Medical School, Hannover, Germany)]. TAPBPR was isolated by immunoprecipitation from IFN-γ–treated HeLa-S with and without stable depletion of β2m. Western blotting was performed with mouse anti-TAPBPR and HC10. Western blotting on lysates with rabbit anti-β2m is included to demonstrate efficiency of β2m depletion. (*B*) Expression of TAPBPR is not affected by the absence of β2m in Daudi cells. Lysates from Daudi and Daudi-expressing β2m [a kind gift from Peter Cresswell (Yale University School of Medicine) were blotted with mouse anti-TAPBPR, β2m, or calnexin as a loading control.

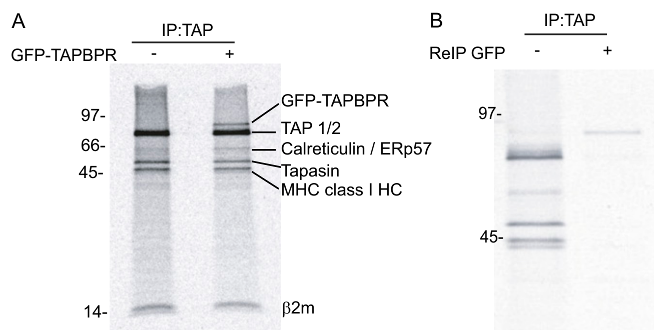


Fig. S3. Overexpressed TAPBPR can associate with the peptide loading complex. (*A*) The PLC was isolated from HeLa-M (–) or HeLa-M cells transfected with GFP-TAPBPR (+) using the TAP1-specific mAb 148.3. In samples overexpressing GFP-TAPBPR, an additional band at ~78 kDa was observed. (*B*) The PLC was isolated from HeLa-GFP-TAPBPR cells using the TAP1-specific mAb 148.3. A reimmunoprecipitation on 90% of this sample was performed using a GFP antibody. This confirmed contact of overexpressed GFP-TAPBPR with the PLC.

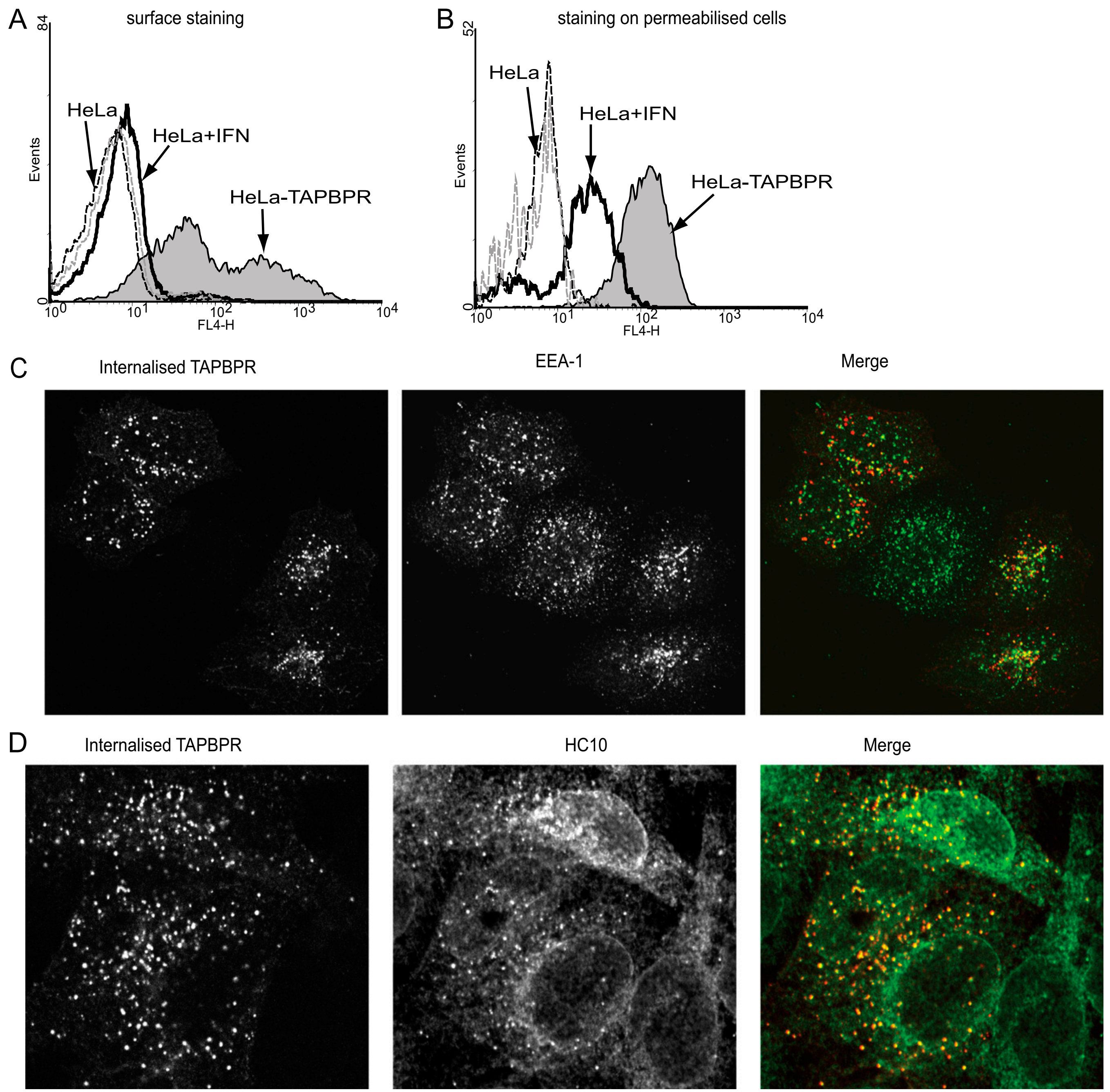
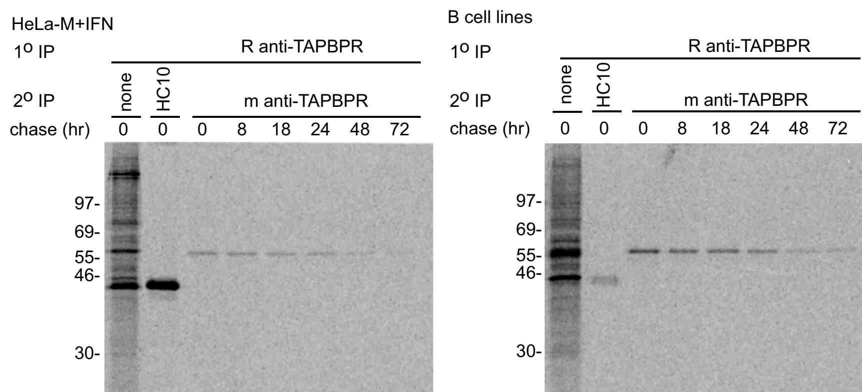


Fig. S4. TAPBPR is internalized into the endosomal system. (*A* and *B*) Cytofluorometric analysis of surface TAPBPR expression (*A*) or total TAPBPR expression on fixed and permeabilized cells (*B*). HeLa (black dotted line), IFN-γ treated HeLa (black solid line), and HeLa transduced with TAPBPR (gray solid fill) were stained using a TAPBPR mAb (Pete4). Staining with an isotype control antibody on IFN-γ treated HeLa is included as a negative control (gray dotted line). (*C* and *D*) HeLa cells transduced with TAPBPR were staining using rabbit anti-TAPBPR at 37 °C for 30 min. The cells were then fixed, permeabilized, and counter stained using a mAb specific for early endosome antigen 1 (*C*) or HC10 for MHC class I (*D*), followed by staining with species specific Alexa-fluorescent secondary antibodies.



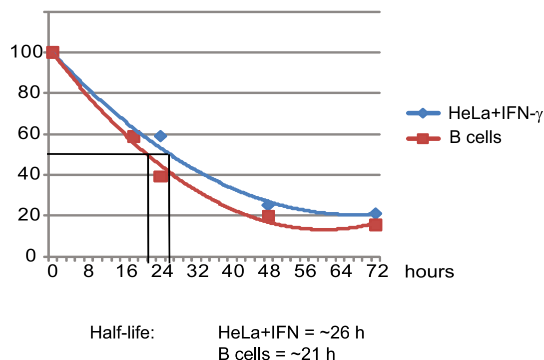


Fig. S5. Half-life of endogenous TAPBPR is ~24 h. HeLa cells treated for 24 h with IFN-γ and a mixture of EBV-transformed B-cell lines were metabolically labeled for 3 h with [^{35}S]methionine and cysteine and then chased for 0–72 h. Cells were solubilized in 1% Triton X-100 TBS. Precleared postnuclear supernatants were immunoprecipitated (1°) with rabbit anti-TAPBPR to isolated endogenous TAPBPR. Associated proteins were eluted in 1% SDS plus DTT. After quenching in 1% Triton-X 100–TBS and iodoacetamide, TAPBPR, and MHC I HC were reimmunoprecipitated (2°) with mouse anti-TAPBPR or HC10. Gels were dried and subjected to PhosphorImager analysis.

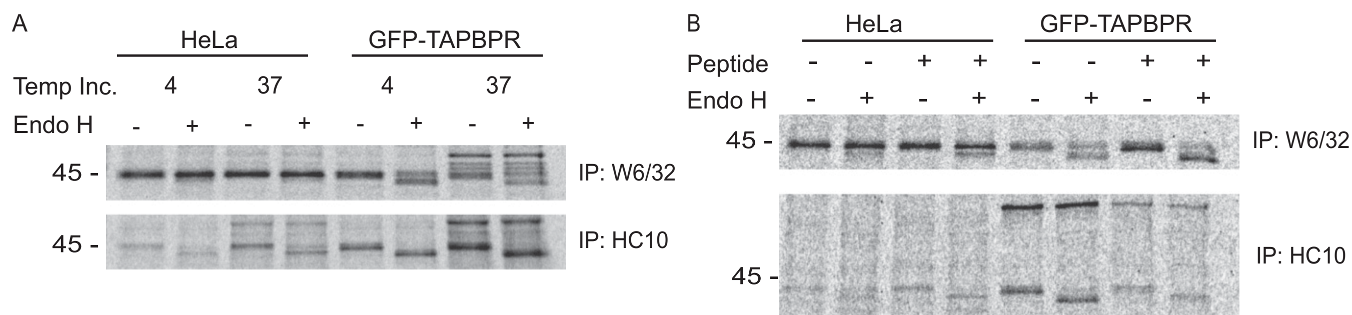


Fig. S6. MHC I molecules are thermolabile and peptide-receptive in the presence of TAPBPR. HeLa and HeLa cells expressing GFP-TAPBPR were labeled for 10 min with [^{35}S]cysteine/methionine and chased for 45 min. Triton X-100 lysates were maintained at 4 °C or incubated at 37 °C (*A*) or incubated with a high-affinity FEC peptide pool (from NIBSC) that comprises 32 peptides from flu, EBV, and CMV viral proteins with high affinity for numerous HLA-A and -B molecules (1) throughout the experiment at 4 °C as indicated (*B*). Immunoprecipitations were performed at 4 °C with W6/32 or HC10, and samples were treated with Endo H.

1. Currier JR, et al. (2002) A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. *J Immunol Methods* 260(1-2):157–172.

Table S1. Protein identification with mass spectrometry

Candidate (NCBInr accession no.)	MALDI-MS data: Peptide matches* [tol ppm]	Sequence coverage, %	Mascot PMF score†	Tandem (MS/MS) data: Peptide ion [MH+] m/z Observed	Expected	Amino acid residues‡	Sequence§	Mascot individual ions score†
1a. TAPBPR (31543801)	8/20 [160]	26	60/77	1,353.66	1,353.74	430–442	R.QAPTGLGLLQAER	45/44
				1,720.75	1,720.87	235–250	R.GQLVYSWTAGQGQAVR	105/44
				1,878.79	1,878.91	338–356	R.EELGGSPAQVSGASFSSLR	86/44
				2,173.76	2,173.91	443–460	R.WETTS(PamC)ADTQSSHLHEDR	29/44
1b. Protein A synthetic construct (1321813)	—	—	—	2,360.06	2,360.20	113–134	R.NAFIQSLKDDPSQSANLLAEAK	16/44
				2,485.03	2,485.20	93–112	K.EQQNAFYEILHLPNLNEEQR	94/44
1c. Recombinant construct-specific sequence	—	—	—	2,389.99	2,390.17	—	K.LISEEDLEGAGAKPHPAEGQWR	—
2. MHC class I antigen (46370049)	12/20 [70]	43	134/77	1,382.65	1,382.65	171–181	K.WEAAHVAEQWR	27/44
				1,454.77	1,454.77	268–280	K.WVAVVVPSGQEQR	76/44
				1,629.83	1,629.82	46–59	R.FIAVGYVDDTQFVR	99/44
				1,647.81	1,646.81	169–181	K.HKWEAAHVAEQWR	71/44
				1,775.80	1,775.79	73–86	R.APWIEQEGPEYWDR	81/44
				2,637.19	2,637.18	244–267	R.DGEDQTQDTELVETRPAGDGTFQK	25/44
3. β2-microglobulin (4757826)	5/20 [70]	64	68/77	827.35	827.33	96–101	K.DEYA(PamC)R	19/44
				1,122.63	1,122.62	102–111	R.VNHVTLSQPK	58/44
				1,148.56	1,148.55	69–78	K.VEHSDLSFSK	45/44
				1,655.86	1,655.86	47–61	Y.VSGFHPSDIEVDLLK	72/44

Proteins of interest with evidence collated from peptide mass fingerprinting (PMF) and peptide fragmentation (MS/MS) data.

*No. of mass matches/total no. of masses searched. tol, mass tolerance.

†Scores are shown with associated threshold score for *P* (match) < 0.05.

‡Numbers are indices of the database record identified by the given gi no.

§The residue immediately N terminal to the identified peptide is displayed before the period. PamC, propionamide cysteine.