Supporting Information

Construction of plasmids

The plasmid pET 21 GST HRV dL5 was constructed by amplification of L5 (E52D L91S referred to as L5 in this work) monomer and ligation into the bacterial expression vector pET21 GST HRV from pPNL6 dL5 NP138 using the primers AGATCCAAGCTTCAGGCCGTCGTTACCCAA and ATTCGACCATGGCGGACAGAACCGTCAGTTGT with 5' restriction site Hind III and 3' restriction site NcoI in the PCR primers and ligated into the plasmid pET 21 GST HRV (obtained from Dr. Joseph Franke) digested with the same restriction enzymes. pET21 GST HRV is a modified form of pET21 in which a 10X His Glutathione S-Transferase (GST) sequence was inserted using NdeI and EcoRI and an HRV 3C protease recognition sequence was inserted using EcoRI and HindIII (manuscript describing pET21 GST HRV in preparation) Synthetic 5' phosphorylated oligonucleotides of the sequences CATGGGCGGTGGTGGCGGCTCTGGTGGCGGTGGCAGCGGCGGTGGTGGTTCCGGAG GCGGCGGTTCTG and GATCCAGAACCGCCGCCTCCGGAACCACCACCGCCGCTGCCACCGCCACCAGAGCC GCCACCACCGCC

encoding the amino acids $(G_4S)_4$ were annealed together by heating 10 μM of each oligonucleotide to 95ºC and allowing cooling to room temperature. This annealed DNA was ligated into pET21 GST HRV L5 digested with the restriction sites NcoI and BamHI to create pET21 GST HRV L5 GS. A second L5 encoding PCR product was made using the primers GGTGGT TCTGGATCCCAGGCCGTCGTTACCC and

AAGGACGCGGCCGCGGAGAGGACGGTCAGCTG, digested with BamHI and NotI and ligated into pET21 GST HRV L5 GS digested with the same restriction enzymes. pET 21 GST HRV dL5_EAK was constructed by ligating double stranded DNA of the sequence GCGGCCGCGGTGGTGGCGGCTCTGGTGGCGGTGGCAGCGGCGGTGGTGGTTCCGCC GAAGCGGAAGCTAAAGCAAAAGCTGAAGCAGAAGCCAAAGCAAAAGCAGAAGCTG AAGCGAAAGCGAAATAACTCGAG (ordered from Blue Heron Biotechnologies, Bothell, WA) encoding $(G_4S)_3$ and $(AEAEAKAK)_3$ into pET 21 GST HRV dL5 using the restriction enzymes NotI and XhoI

Protein expression and purification

Expression and purification of dL5 and dL5_EAK were carried out by transforming pET21 GST- HRV-dL5 and pET21 GST-HRV-dL5_EAK into calcium competent Rosetta-Gami 2(DE3) *E. coli*. (Novagen, Madison, WI). Starter cultures of transformed Rosetta-Gami bacteria were grown overnight in 5 ml of LB media with 100 µg/ml ampicillin (Sigma-Aldrich, St. Louis, MO) and 34 µg/ml chloramphenicol (Sigma-Aldrich, St. Louis, MO) at 30ºC, then transferred to 500 ml of LB + (LB media (Difco, Detroit, MI) with 100 mM phosphate, 20mM succinic acid and 0.4% glycerol) and grown for 5-6 hours until the OD600 of the culture was at 0.6. Cultures were then moved to 20ºC shaking incubators for 1 hour and induced using 500 µM IPTG (Research products international corp, Mount Prospect, IL) and 0.4% glucose (Fisher Scientific, Hampton, NH) and grown overnight in 20ºC shaking incubators. Cells were pelleted by centrifugation at 5,000 rpm in a RC5C centrifuge with rotor SLA-1500 for 10 minutes and suspended in 30 ml FAP purification buffer (50 mM Tris HCl, 750 mM NaCl, 50 mM imidazole, 0.1% TritonX-100 and 0.025% Tween 20) and frozen at -20ºC. Bacteria were lysed using an EmulsiFlex-C3

Homogenizer (Avestin, Ottawa, ON, Canada) using 15,000 psi of pressure for 10 minutes. Lysates were clarified by centrifugation at 13,000 rpm for 30 minutes in an RC5C centrifuge equipped with a SA-600 rotor. Lysates were added to 600 µl of Ni-NTA agarose (Qiagen, Valencia, CA) and bound for 2 hours at 12ºC on a neutator mixing device. Following binding, lysates with Ni-NTA agarose were centrifuged for 10 minutes at 6,000 rpm in a Beckman TJ-6 centrifuge and the Ni-NTA agarose was transferred to a TALON 2-mL disposable gravity column (Clontech, Mountain View, CA). Bound Ni-NTA agarose was washed with 9 ml FAP purification buffer, after which the columns were capped and 0.6 ml of FAP purification buffer and 125 µl of 1mg/ml 10X His HRV 3C protease (obtained from Dr. Joseph Franke) were added to the Ni-NTA agarose. Columns were capped and incubated overnight at 12ºC. Eluted proteins were collected the following day by adding 50 µL of Ni-NTA agarose in order to bind remaining 10X His HRV protease, followed by incubation for 1 hour at 12ºC. Columns were uncapped and eluent collected. An additional 0.6 mL of FAP protein purification buffer was used to wash out residual protein, collected along with the elutant. Purified protein was dialyzed against 3 L of Phosphate buffered saline (PBS) overnight using a 10,000 Mw cutoff Slide-A-Lyzer dialysis device (Pierce, Rockford, IL) Protein concentration was determined by absorbance measurements at 280 nm and calculated using the extinction coefficient of 36,040 M^{-1} cm^{-1} for both dL5_EAK and dL5.

dL5 and dL5_EAK membrane EAK16-II membrane preparation and fluorescence quantification.

Insoluble protein/peptide membranes were made by mixing 20 µl of 5 mg/ml EAK16-II peptide (final concentration = 125 µM) with 1.25 µM dL5 or dL5_EAK for 1:100 protein to peptide ratios or 2.5 µM dL5 or dL5_EAK for 1:50 protein to peptide ratios in 500 µ~~u~~l of PBS+

(Phosphate buffered saline with 0.1% Pluronic F-127 (Invitrogen, Carlsbad, CA)) with 10 µl 0.01% Congo Red (Thermo Fisher, Waltham, MA). Mixtures were incubated for 2 hours at room temperature on a neutator mixing device then centrifuged at 16.1x g for 10 minutes in an Eppendorf model 5415 centrifuge (Eppendorf, Happauge, NY). The supernatant was pipetted off leaving pellets of visible red membrane. Membranes were washed with 500 µl of PBS+ and re-centrifuged for 1 minute at 16.1 x *g*. This wash step was repeated two additional times with pellet suspended in 225 µl PBS+ after the third wash by pipetting up and down to break up the membrane pellet. 200 µl of the pellet in PBS+ was used in a Costar 3596 96-well plate with 125 µM MG-2p added for excitation and emission spectra or 1 µM MG-2p added for protein to peptide ratio single point measurements. Fluorogen was incubated with suspended peptide/pellet for 10 minutes at room temp prior to analysis. A control of PBS+ with 125 µM MG-2p was also performed to measure fluorogen background. Spectral analysis of membrane pellets were done using 1:100 ratios of protein to peptide while single point measurements were done at both 1:100 and 1:50. Excitation spectra was performed on a TECAN Saphire 2 instrument with emission at 680 nm +/- 10nm from 400 nm to 660 nm in 1 nm increments with an instrument gain of 75 and a Z position of 8720 µm. Emission spectra was performed with 480 nm +/_10 nm excitation with emission measured from 510 nm to 800 nm in 1 nm increments with an instrument gain of 75 and a Z position of 8120 nm. Single point measurements of 1:100 and 1:50 ratios of protein/peptide were performed with excitation wavelength of 635 nm +/- 10 nm and emission wavelength of 680 nm +/- 10 nm with an instrument gain of 158 and Z position of 7220 µm using 10 reads per well with a 20 µs integration time.

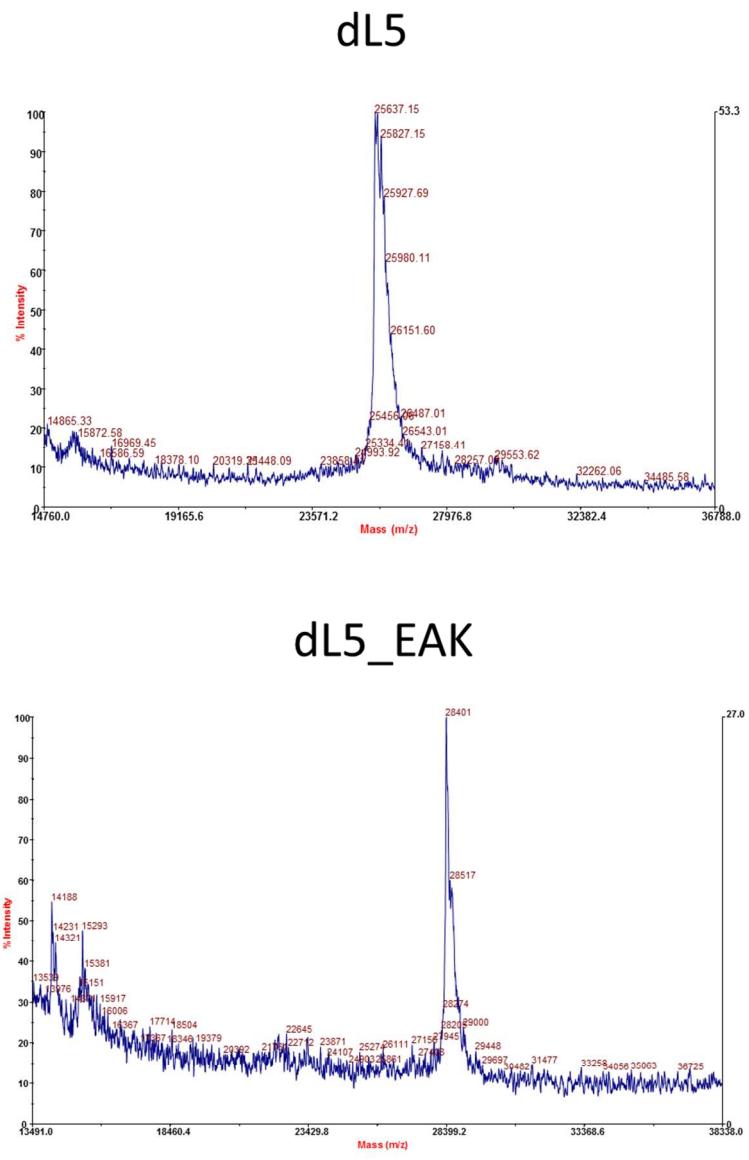
Fluorescence microscopy sample preparation

Fluorescence microscopy was performed using a Zeiss Axioplan 2 imaging microscope exciting

with a mercury lamp. . Samples were prepared using PET track-etched membrane 24 well format cell culture insert filters with 8.0 µm pore sizes (Becton Dickinson, Franklin Lakes, NJ). Filters were pre-soaked in PBS for 30 minutes and drained by gravity. Insoluble membranes for fluorescence microscopy were created by mixing 7.2 µl of 174 µM dL5 or 22 µl of 58 µM dL5_EAK with 40 µl of 5 mg/ml EAK16-II peptide along with 60 µl of 0.01% Congo Red and 1 µl of 745 µM MG-11p in approximately 50 µl of PBS remaining on the filter after soaking. The liquid was allowed to drain through the filter by gravity over the period of 20-30 minutes, leaving insoluble films of EAK16-II on the filter, visible by CR. The filter was washed 3 times with 500 µl volumes of PBS, allowed to drain through the filter by gravity, then removed using a scalpel and mounted on a glass microscope slide (Fisher Scientific, Hampton, NH) and covered with a cover slip (Fisher Scientific, Hampton, NH) for microscopic analysis. Congo red fluorescence was visualized using a 605/55 filter (Chroma, Lititz, PA) while MG/dL5 fluorescence was visualized using a 710/75 filter (Chroma Lititz, PA). Images were taken using a Zeiss Axio cam MRm camera.

Electron microscopy sample preparation

Samples for SEM microscopy were prepared as described for fluorescence microscopy with the exception of 54 µl of 58 µM dL5_EAK was used with 100 µl of 5mg/ml EAK16-II added to give a molar ratio of 1:100 for the dL5_EAK sample and no CR or MG was used in either sample.



Supporting figure S1. MALDI-TOF mass spectroscopy of dL5 and dL5_EAK proteins. Proteins were prepared as described above with an additional round of dialysis in 3L of 10 mM Tris-HCl pH 7.4 following the PBS dialysis. MALDI-TOF confirmed the mass of dL5 at 25.6 kDA and dL5_EAK at 28.4 kDA verifying the expected amino acid sequences. MALDI-TOF was performed on an Applied Biosystems Voyager DE-STR MALDI-TOF instrument by Dr. Mark Bier at the Center for Molecular Analysis of Carnegie Mellon University.