Electronic Supplementary Material (ESI) for Biomaterials Science.
This journal is © The Royal Society of Chemistry 2018

Supporting Information

Cationic Cell Penetrating Peptide Modified SNARE Protein VAMP8 as Free Chains for Gene Delivery

Baizhu Chen[†,*], *Chi Wu*[†,§]

†Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

§The Hefei National Laboratory of Physical Science at Microscale; Department of Chemical physics, The University of Science and Technology of China, Hefei, Anhui 230026, China

1. Expression and purification of precession protease

The plasmid encoding the GST-Precession protease was transferred into the *Escherichia coli* strain BL21 (DE3) for expression. The colonies were grown overnight at 37 °C in LB media supplemented with 100 µg/mL ampicillin. The overnight culture was added to LB media with antibiotics and continued culturing with shaking at 37°C until reaching an OD600 of ~0.6. Then, 1 M IPTG solution was added to the cell cultures at the final working concentration of 0.1 mM to induce protein expression overnight at 16℃. Cells were harvested by centrifugation at 6000g and resuspended in binding buffer (20 mM sodium phosphate, 150 mM NaCl, 3 mM DTT, and 5% Glycerol). Then, cells lysed by ultra-sonication and centrifuged at 20,000 g for 1 h to remove cell debris and collect the supernatant containing the target protein. The supernatant was loaded onto a binding-buffer equilibrated GSTrap HP column (GE Healthcare Life Science) for 1 h circular binding. After sufficient binding has occurred, the column was washed with about 50 times the column's volume of the binding buffer

to remove contaminations. The protein was then eluted with binding buffer containing 10 mM GSH. The SDS-PAGE analysis of of the precession protease during expression and purification is shown in Figure S1. Gel filtration was then performed using a PBS-equilibrated Superdex 200 10/300 GL column (GE Healthcare Life Science) to further purify the precession protease using the ÄKTA protein purification system (GE Healthcare Life Science). After collection the elution peak, protein was concentrated and stored in storage buffer (PBS containing 50% Glycerol) for further use.

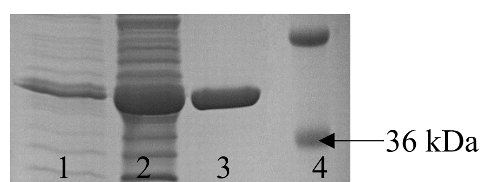

Figure S1. SDS-PAGE analysis of the precession protease during expression and purification. Lane 1 is the bacterial culture without IPTG induction, lane 2 is the bacterial culture induced by 0.1 mM IPTG overnight, lane 3 is the precession protease after purification and lane 4 is the protein marker.

2. Gene sequencing results

Table S1. Gene sequencing results for gene segments of designed fusion proteins detected by the pGEX-R primer, where the enzyme sites (marked as blue), the target sequence and the precession protease site (mark as italic type) were presented.

Protein	Sequence
R8-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC

	GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGGCGAC GGCGACGACGACGACGGCGGGATCCCAG*GGGCCCCTGGA* *ACAGAACTTCCA*GATC
R12-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGACGAC GGCGACGACGACGGCGACGACGACGACGGCGGGATCCCA G*GGGCCCCTGGAACAGAACTTCCAG*ATC
TAT-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGGCGAC GACGCTGACGACGTTTTTGCGGCCGGATCCCAG*GGGCCC* *CTGGAACAGAACTTCCAG*ATC
H5R8-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGACAGTG GTGGTGGTGGTGGCGACGGCGACGACGACGACGGCGATG ATGATGATGATGGCAGGATCCCAG*GGGCCCCTGGAACAGA*

	*ACTTCCAG*ATC
VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGATGAACAGAATGATGATGAAAACGATGACACAGATCAGCACAATCATTTTGACGTTTTTCCACCAGAATTTGCGGCGACTTTTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAGGTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGCGCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTTAACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCATTACCACCACCTTCACTGGCTTCTTCCATGGATCCCAG*GGGCCCCTGGAACAGAACTTCCAG*ATC

Table S2. Gene sequencing results for gene segments of designed fusion proteins detected by the T7-R primer, where the enzyme sites (marked as blue), the target sequence and the precession protease site (mark as italic type) were presented.

Protein	Sequence
R8-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGATGAACAGAATGATGATGAAAACGATGACACAGATCAGCACAATCATTTTGACGTTTTTCCACCAGAATTTGCGGCGACTTTTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAGGTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGCGCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTTAACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCATTACCACCACCTTCACTGGCTTCTTCCATCCATGGGCGACGGCGACGACGACGACGGCGCATGGATCC
R12-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGATGAACAGAATGATGATGAAAACGATGACACAGATCAGCACAATCATTTTGACGTTTTTCCACCAGAATTTGCGGCGACTTTTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAGGTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGCGCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTTAACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCATTACCACCACCTTCACTGGCTTCTTCCATCCATGGACGAC

	GGCGACGACGACGGCGACGACGACGACGGCGCATGGATC C
TAT-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGGCGAC GACGCTGACGACGTTTTTGCGGCCCATGGATCC
H5R8-VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATCCATGGACAGTG GTGGTGGTGGTGGCGACGGCGACGACGACGACGGCGATG ATGATGATGATGGCACATGGATCC
VAMP8	CTCGAGTTAGCTGAACGCACCCGTAGCGAACAGGACGAT GAACAGAATGATGATGAAAACGATGACACAGATCAGCAC AATCATTTTGACGTTTTTCCACCAGAATTTGCGGGCGACTT TTTGAGACGTGGTTTTAAAGTGTTCGCTCGTCGCTTCCAG GTCTTCGGTTTTATTACGCAGATGTTCCAGGTTTTCACCGC GCGCCAGGATACGTTCAACATTCTGGGTCATAATGTTTTT AACGCCTTCCACTTCCGATTGCAGATTACGAACACGGTCA TTACCACCACCTTCACTGGCTTCTTCCATGGATCC

3. Primers used for construction of CPPs modified VAMP8

Table S3. Primers design for different CPPs modified VAMP8 in pGEX-6P-3 vectors.

CPPs	*Primer Sequence*
R12	5’-ATA***GGATCC***CGCCGTCGTCGTCGTCGCCGTCGTC GTCGCCGTCGT -3’ 5’-AAT***CCATGG***ACGACGGCGACGACGACGGCGACG ACGACGACGGCG-3’
TAT	5’-TAT***GGATCC***GGCCGCAAAAAACGTCGTCAGCGTC GTCGC-3’ 5’-ATA***CCATGG***GCGACGACGCTGACGACGTTTTTG CGGCC-3’
H5R8	5’-TAT***GGATCC***TGCCATCATCATCATCATCGCCGTCGT CGTCGTCGCCGTCGCCACCACCACCACCACTGT-3’ 5’-ATA***CCATGG***ACAGTGGTGGTGGTGGTGGCGACG GCGACGACGACGACGGCGATGATGATGATGATGGCA-3’
VAMP8 control	5’-CTG***GGATCC***ATGGAAGAAGCCAGTGA-3’ 5’-CCG***CCATGG***GCTGAACGCACCCGTAG-3’

Table S4. Primers design for different CPPs modified VAMP8 in pcDNA3.1 vectors.

CPPs	*Primer Sequence*
R8	5’-TCT**GGATCC**ATGCGCCGTCGTCGTCGTCGCCGT -3’
TAT	5’-TCA***GGATCC***ATGGGCCGCAAAAAACGTCGTCAG-3’
H5R8	5’-CAT***GGATCC***ATGTGCCATCATCATCATCATCGC-3’
Reverse primer	5’-TTA***CTCGAG***TTAGCTGAACGCACCCGTAGCGAA-3’