A short peptide tag for covalent protein labeling based on coiled-coils

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Supporting Information

Abbreviation:

Fmoc: 9-fluorenylmenthoxycarbonyl

HBTU: 2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate

HOBt: N-hydroxybenzotriazole

DMF: N, N-dimethylformamide

DIPEA: N, N-diisopropylethylamine

5(6)-FAM (fl): 5-(and-6)-carboxyfluorescein

5(6)-TMR (tmr): 5-(and-6)-tetramethylrhodamine

Mtt: 4-Methyltrityl

DCM: dichloromethane

TFA: trifluoroacetic acid

EDT: 1,2-Ethanedithiol

TIS: triisopropylsilane

Dap: diamino propionic acid

RT: room temperature

Materials and Instruments

Unless otherwise noted, all reagents were used without further purification. Fmoc-protected amino acids for solid-phase peptide synthesis were obtained from GL Biochem Ltd (Shanghai, China). 5(6)-FAM (*fl*) and 5(6)-TMR (*tmr*) were purchased from Life Technologies (USA). Other reagents were purchased from commercial suppliers, Labscan Limited (Thailand), Meryer

Technologies Co., Ltd (Shenzhen, China), Chem-Impex International Inc. (USA), Sigma-Aldrich Co. (USA).

Peptide synthesis

Peptides were manually synthesized based on standard FMOC solid phase peptide synthesis protocol. Briefly, Rink Amide-ChemMatrix® resins (PCAS BioMatrix, Canada) with 0.1 mmol amine group were utilized in each synthesis. A solution containing Fmoc-protected amino acid, HBTU, HOBt, and DIPEA (with a ratio of 1: 1: 1: 2 and 5 fold excess) in 2 mL was added to the resin and stirred for 30 min at RT. After confirming the completion of the coupling reaction by Kaiser Test, the resins were washed with DMF and deprotected in 5 mL 20% piperidine in DMF (v/v) to remove the Fmoc group. To incorporate fluorescent dyes, 5(6)-FAM (*fl*), 5(6)-TMR (*tmr*) or Cy5 free acids were activated by 3-fold EDC/HOBt, and then added to deprotected resins. The mixture was incubated for overnight. In order to construct the unnatural amino acid X, (2S)-2-amino-3-[(2-chloroacetyl)amino]propanoic acid, the resins were pre-treated by 10% acetic acid in DCM (v/v), and then Mtt group was removed in 1% TFA and 5% TIS in DCM (v/v). The side chain amine was subsequently coupled with 10-fold excess of chloroacetic acid, EDC and HOBt in DMF for 1 hr. For the synthesis of CCK-1-dimer, Fmoc-Lys(Fmoc)-OH was first conjugated to a Gly-Gly sequence on the resin. The Fmoc group was removed to expose two branches of amino groups on which a CCK-1 sequence was coupled. The purity of the peptide was confirmed by reverse phase HPLC (**Figure S7**) and mass spectrometry (**Table S2**).

Peptide purification and characterization

To every 100 mg resins, 2 mL final cleavage cocktail containing EDT, TIS, phenol, H2O and TFA (with a ratio of 1: 2: 2: 2: 33, v/v/w/v/v) was added. The cleavage reaction was allowed to proceed for 2 hours at RT under stirring. After the resins were removed through filtration, ice-cold diethyl ether was added to the supernatant dropwise to precipitate the peptides. A final volume of 700 μL 50% ACN in H2O (v/v) was used to dissolve the peptide pellet. After being filtered through a 0.2 μm filter, the peptide solution was injected to RP-HPLC (Shimadzu, DGU 20A5, Japan) equipped with a C18 column (Shimadzu, 250L× 4.6, Japan). 0.1% TFA in H2O (v/v) and 0.1% TFA in ACN (v/v) were used as the mobile phase A and B respectively. For all the analytical HPLC trials, the total flow rate was set to be 1 mL/min and the B concentration raised from 0 % to 95 % over 16 min following a linear gradient. For the purification of peptides

in a larger scale by semi-prep HPLC columns (Grace, 218TP510, USA), the total flow rate was set to be 3 mL/min and the concentration of B raised linearly from 0 % to 45 % over 24 min. The peptide peaks were collected, lyophilized and validated by MALDI-TOF mass spectrometry analysis (Bruker, autoflex TOF/TOF, USA) (**Table S2**).

Construction of pET28m-EGFP-CCE-1 plasmid

Forward and reverse DNA fragments coding CCE-1 peptide (ECAALEKEVAALEKEVAALEK) were synthesized by Life technology: forward, 5'-CAAATCTGAAGAGTC-TTATGAATGTGCTGCCTTAGAGAAGGAAGTTGCAGCGTTAGAGAAGGAAGTTGCTGC ATTAGAGAAGTAGA-3'; reverse, 5'-AGCTTCTACTTCTCTAATGCAGCAACTTCCTTC-TCTAACGCTGCAACTTCCTTCTCTAAGGCAGCACATTCATAAGACTCTTCAGATTTGA GCT-3'. The two fragments were annealed in annealing buffer (50 mM HEPES, 100 mM NaCl, pH 7.4), and sub-cloned into Sac I and Hind III sites of the pET28m-EGFP plasmid (a kind gift from Prof. Kowk Fai Lau of CUHK) to yield pET28m-EGFP-CCE-1. The plasmid was confirmed by DNA sequencing (BGI, Shenzhen) (**Figure S10**).

Expression and purification of EGFP-CCE-1 proteins

The plasmids pET28m-EGFP and pET28m-EGFP-CCE-1 were transformed into E. coli. Rosetta 2 (DE 3) competent cells. Colonies were grown in LB medium at 37^{o}C for overnight and a starter culture was grown from a single colony for overnight. 600 mL LB medium was inoculated by the overnight starter culture (1:100 dilution) and allowed to grow at 37^{o}C until OD_{600} reached 0.4−0.6. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1mM for induction. After growing at 16^{o}C for 20 hours, the cells were harvested by centrifugation at 6000 rpm for 15 min, and re-suspended in 25 mL of lysis buffer (50 mM Tris, 300 mM NaCl, 4 mM β-mercaptoethonol, and 10 mM imidazole, pH 7.5). The cell suspension was sonicated on ice and centrifuged at 20000 g for 2 hr. The supernatant was collected, filtered, and incubated with Ni-NTA resins (GE healthcare, USA) on ice for 40 min to allow Histagged EGFP-CCE-1 protein to bind to the resins. After washes, the bound protein was eluted by elution buffers (50 mM Tris, 300 mM NaCl, 4 mM β-mercaptoethonol, , pH 7.5) containing increasing concentrations of imidazole ranging from 50 mM to 500 mM. The protein eluent was exchanged from elution buffer to storage buffer (50 mM Tris, 300 mM NaCl, 15% glycerol (v/v), pH 7.5) and stored at -

20 oC. Protein concentration was measured by Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., USA) based on an absorption coefficient constant ε_{484} of 56,000 $cm^{-1} \cdot M^{-1}$.

Construction of EGFR and hIP plasmids

The plasmid pDisplay-HA-E3-EGFR (a kind gift from Prof. Shiroh Futaki of Kyoto University) was used as the template to construct pDisplay-CCE-9-EGFR. The Ile to Cys mutation was introduced by a PCR reaction using primers EGFR-F (5'-GAGCTAGCGAATGCG-CCGCGTTAGAG-3') and EGFR-R (5'-GACACTCGAGTCATGCTCC-AATAAATTCAC-3') and sub-cloned into Nhe I and Xho I sites of pDisplay-HA-E3-EGFR to yield pDisplay-CCE-9-EGFR (**Figure S11**).

A CCE-9 peptide sequence (ECAALEKEIAALEKEIAALKE) was introduced to the N terminus of hIP in the pcDNA3.1-3HA-hIP plasmid (a kind gift from Prof. Helen Wise of CUHK) by PCR reaction using primers CCE-9-F (5'-CAAGCTTGCCACCATGGAATGTGCTGCCTTAGAGA-AGGAAATTGCAGCGTTAGAGAAGGAAATTGCTGCATTAGAGAAGATGTACCCATAC GATGTTCC-3') and hIP-R (5'-CGGGATCCTCAGCAGAGGGAGCAGG-CGACGCTG-3') and sub-cloned into Hind III and BamH I sites of pcDNA3.1 (+) plasmid to yield pcDNA3.1-CCE-9-3HA-hIP (**Figure S12**).

A plasmid expressing CCE-9-hIP-EGFP with the C terminal (cytosolic) end linked to an EGFP tag was also constructed by PCR reaction based on the pcDNA3.1-3HA-hIP using primers CCE-9-F (5'-CAAGCTTGCCACCATGGAATGTGCTGCCTTAGAGAAGGAAATTGCAGCGTTA-GAGAAGGAAATTGCTGCATTAGAGAAGATGTACCCATACGATGTTCC-3') and hIP-R-N1 (5'-CGGGATCCGCAGAGGGAGCAGGCGACGCTG-3') and sub-cloned into Hind III and BamH I sites of pEGFP-N1 to yield pEGFP-N1-(CCE-9-hIP-EGFP) (**Figure S13**).

Fitting the cross-linking reaction to second-order kinetics

The CCE/CCK conjugation reactions were fit into a second order kinetic equation.

peptide E + peptide K → heterodimer E-K

$$\frac{-\mathrm{d}[E]}{dt} = \frac{d[E-K]}{dt} = k[E][K]$$

The concentrations of peptides E and K were normalized to 100, $[E]_0=[K]_0$=50 μM, so

$$[E] = 1/(kt + 0.02\,\mu M^{-1}) \quad \cdots\cdots\cdots\cdots\cdots (1)$$

$$[E-K] = 50\,\mu M - 1/(kt + \mu M^{-1}) \quad \cdots\cdots\cdots\cdots\cdots (2)$$

The decrease of reactants E and K were then fit into equation (1) using Origin 8.1; the increase of product E-K was fit into equation (2). Half-life $t_{1/2}$ was calculated as $t_{1/2} = 0.02\,\mu M^{-1}/k$.

Extracting covalently labeled EGFR for gel electrophoresis

1.5×10^6 CHO cells were seeded in a 10 cm dish (Corinng, USA) one day prior to the scale-up transfection (transfection protocol was described in **Cell culture, transfection and labeling** section). 60 h after transfection, the cells were pre-treated with HEPES buffer containing 0.5 mM TCEP and then incubated with 100 μM *CCK-9-dimer* probe for 2 hours at 37ºC. After being rinsed in PBS, the cells were scraped into 1.5 mL PBS and pelleted by centrifugation at 12000 rpm for 15 min at 4ºC. The cells were then lysed in RIPA buffer (Sigma, USA. 50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing protease inhibitor cocktail (Promega, USA) for 45 min at 4ºC. The supernatant was collected by centrifugation at 12000 rpm at 4ºC, mixed, incubated with 5× protein loading dye for 40 min at RT, denatured, and subjected to SDS-PAGE and subsequent in-gel fluorescence scanning.

cAMP assay for Drug responsiveness of hIP transfected cells

Adenylyl cyclase activity was conducted based on previous reported protocols.[1, 2] 5×10^5 CHO cells were seeded in poly-D-lysine coated 12-well plates (Corning, USA) and cultured in DMEM with 10% FBS for 1 day. Cells were transfected using the protocol described in **Cell culture, transfection and labeling** section) for 40 h, then replace cell medium with 1 ml DMEM with 1% FBS (reducing serum to facilitate the uptake of [^{3}H]adenine). Add 10 μl [^{3}H]-adenine (1 μCi/10 μl) (Amersham Biosciences, Hong Kong) to each well and return plates to incubator for overnight. Aspirate the medium to remove free [^{3}H]adenine and wash each well twice with 1 ml complete HEPES buffer. Then 0.5 ml assay solution (HEPES buffer containing 1 mM 3-Isobutyl-1-methylxanthine (IBMX), to inhibit cyclic nucleotide phosphodiesterase activity) was added to each well and incubated for 15 min in 37ºC water bath. 10 μl compound stock solution (forskolin,

cicaprost or buffer) were added to each well, and incubated for 30 min in 37^{o}C water bath. The reaction was stopped by addition of ice-cold trichloroacetic acid and ATP, and [^{3}H]cAMP was separated from [^{3}H]ATP by column chromatography, and counted in a liquid scintillation counter using OptiPhase 'HiSafe' 3 scintillant (Pharmacia Biotech Far East Ltd, Hong Kong). The production of [^{3}H]cAMP from cellular [^{3}H]ATP was estimated as the ratio of radiolabeled cAMP to total AXP (i.e. adenosine, ADP, ATP and cAMP), and is expressed as [cAMP]/[total AXP]×100 (i.e. % conversion). Cicaprost was a gift from Schering AG, Berlin, Germany. All assays were performed in triplicate.

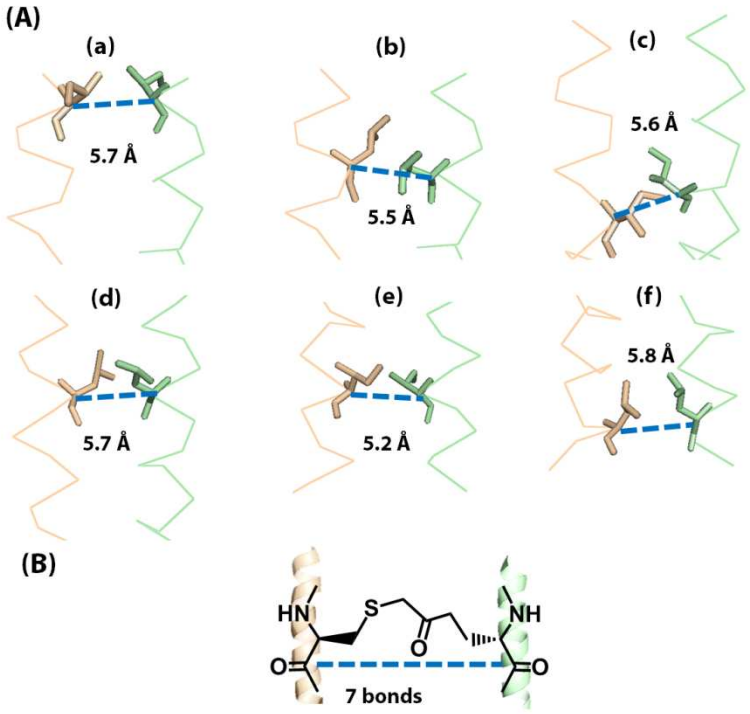


Figure S1. Structure-based design of the covalent linkage. (A) Distances between the α-carbons of the residues at *a* or *d* positions and those at corresponding *a*' and *d*' positions of a CCE/CCK heterodimer based on the structure (PDB ID 1U0I). (a), Ile-Ile at the first *a*-*a*' position; (b), Leu-Leu at the first *d*-*d*' position; (c), Ile-Ile at the second *a*-*a*' position; (d), Leu-Leu at the second *d*-*d*' position; (e) Ile-Ile at the third *a*-*a*' position; (d), Leu-Leu at the third *d*-*d*' position. (B) The structure of the thioether bond. (2S)-2-amino-3-[(2-chloroacetyl)amino]propanoic acid was chosen because the length of the resultant thioether bond matches the distance between the *a* and *a*' residues. For example, the distance between the α-carbons of the residues at *a* or *d* positions and those at corresponding positions is 5.6 ± 0.2 Å in the crystal structure of the CCE/CCK heterodimer (PDB ID 1U0I).[3] Containing 7 single bonds between the two α-carbons, the resultant thioether linkage mimics this distance.

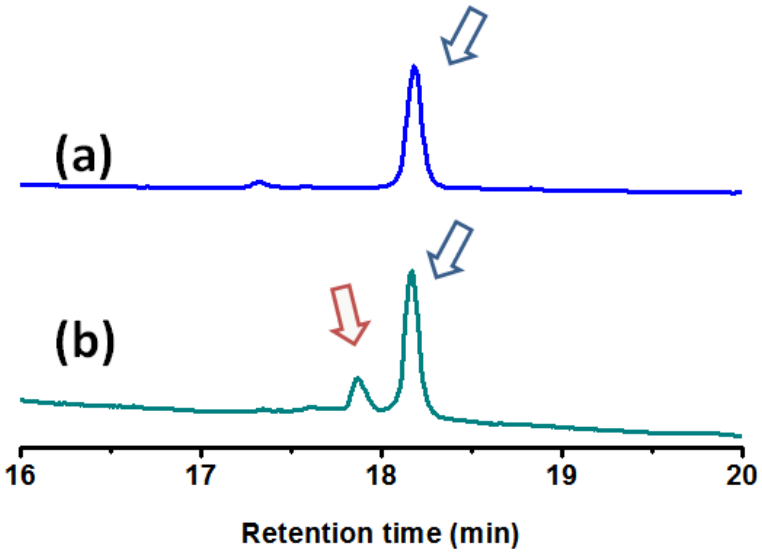


Figure S2. Covalent reaction between *CCK-1* peptide and 10 fold excess of glutathione. 50 μM *CCK-1* peptide containing an α-chloroacetyl moiety reacted with 10 fold excess, 500 μM reduced glutathione molecule for 10 hr at room temperature. Trace (a), *CCK-1* peptide before reaction; trace (b), reaction mixture after 10 h, both at 560 nm channel. Blue arrows indicate the unreacted *CCK-1* peptide; red arrow indicates the *CCK-1*-glutathione conjugate, which is about 16% of the total peptide content (or 8 μM).

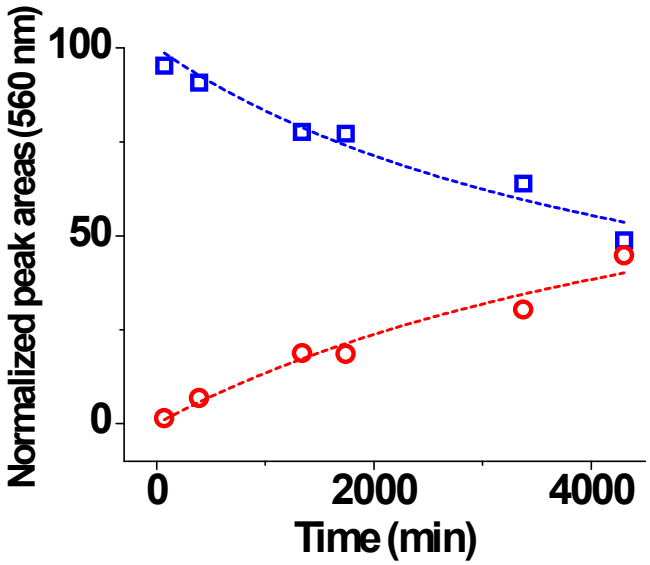


Figure S3. Reaction kinetics of the *CCK*-7−*CCE-7* pair as one example. [Peptide]=50 μM. Red circles represent heterodimer, and blue squires represent *CCE-7*. The dashed lines show curves fit to second-order reaction kinetics. Half-life $t_{1/2}$ was calculated to be >4000 min.

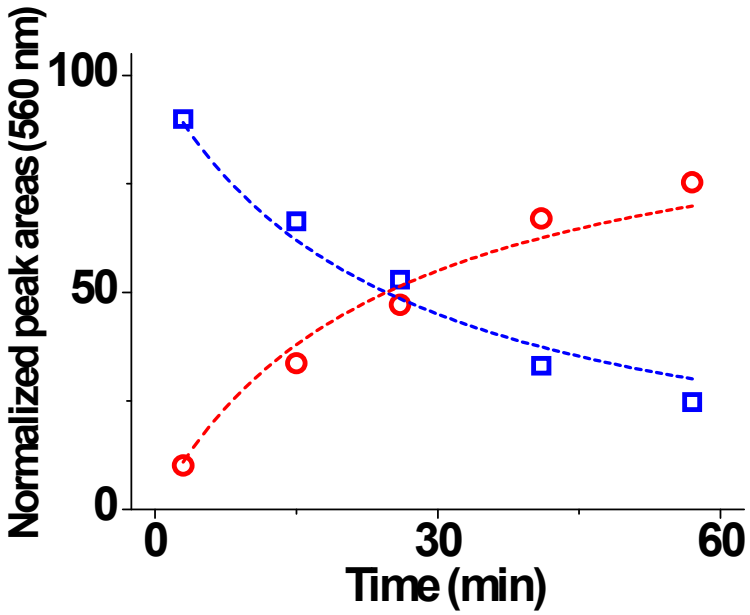


Figure S4. Reaction kinetics of the "out-of-heptad" pair shows a $t_{1/2}$ of 25 min. [Peptide]=50 μM. The sequences of the two peptides were *tmr*-KSEESY-C EKE VAALEKE NAALEKE VAALEK and *fl*-X KEK VAALKEK NAALKEK VAALKE (blue). The heterodimer was shown in red. The dashed lines show curves fit to second-order reaction kinetics. Half-life $t_{1/2}$ was calculated to be 25 min.

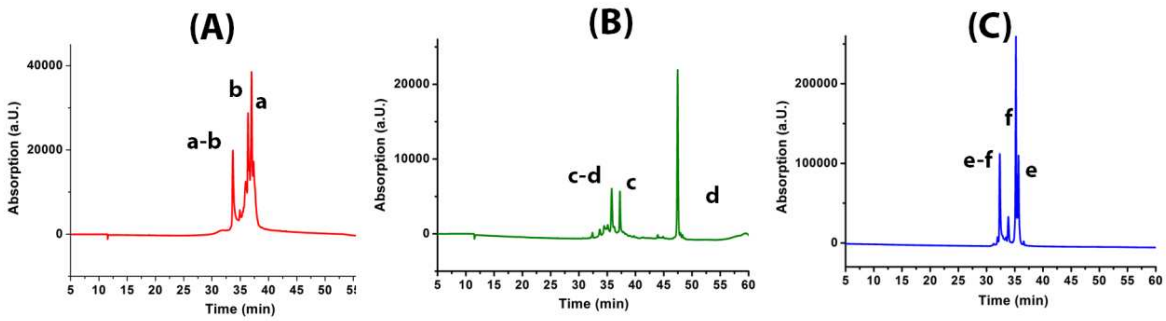


Figure S5. Covalent cross-linking of coiled-coil peptide pairs. (A) Incubation of two peptides, p_{1X} (peak **a**) and p_{2C} (peak **b**) resulted in the heterodimer p_{1X}–p_{2C} (peak **a-b**). (B) Incubation of p_{3X} (peak **c**) and p_{4C} (peak **d**) resulted in the heterodimer p_{3X}–p_{4C} (peak **c-d**). (C) Incubation of p_{5C} (peak **e**) and p_{6X} (peak **f**) resulted in the heterodimer p_{5C}–p_{6X} (peak **e-f**).

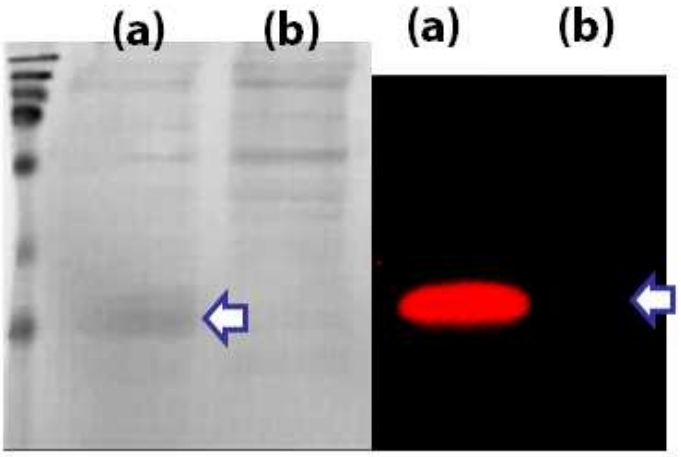


Figure S6. Covalent labeling of *EGFP-CCE-1* in mouse brain lysate (total protein concentration were 7.5 mg/ml). *EGFP-CCE-1* protein was added to brain tissue homogenate in lane (a). Lane (b) without doped protein serves as a control. The gel was imaged with a Typhoon Imager at the TRITC channel (right) and then stained with Coomassie blue dye (left).

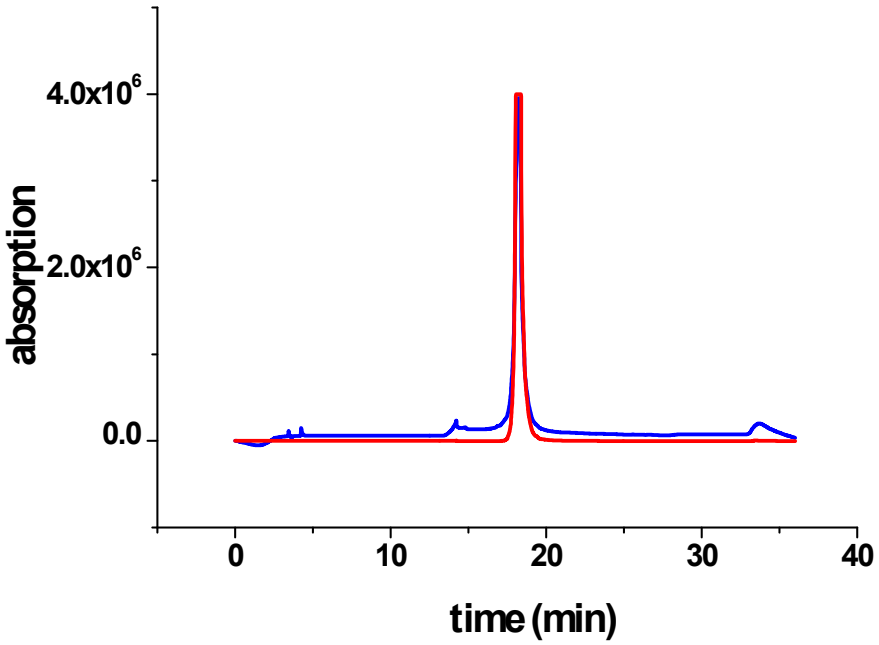


Figure S7. RP-HPLC trace of the purified *tmr-CCK-1-dimer* peptide at 215 nm (blue) and 560 nm (red).

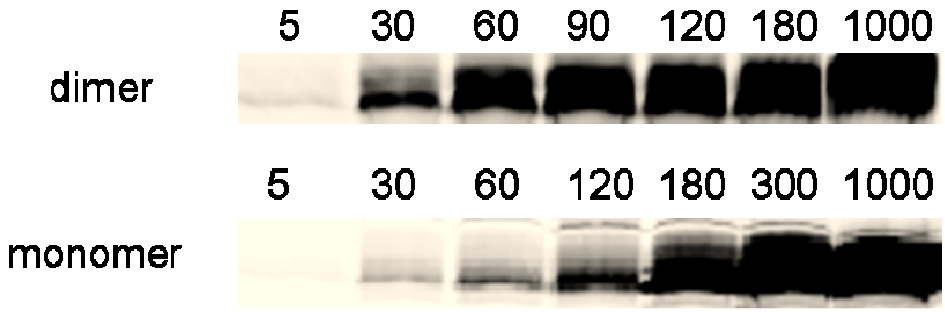


Figure S8. Covalent cross-linking reactions between *CCK-1-dimer* probe (dimer) or *CCK-1* probe (monomer) and *EGFP-CCE-1* protein. Peptide and protein were mixed at RT ([protein]= [peptide]=20 μM) to allow for covalent cross-linking reaction. At different time points (shown above in min), aliquots were taken and the reactions were quenched by heating at 95^{o}C for 10 min. The solutions were loaded in reducing SDS-PAGE. The gel was imaged by a Typhoon imager at TRITC channel.

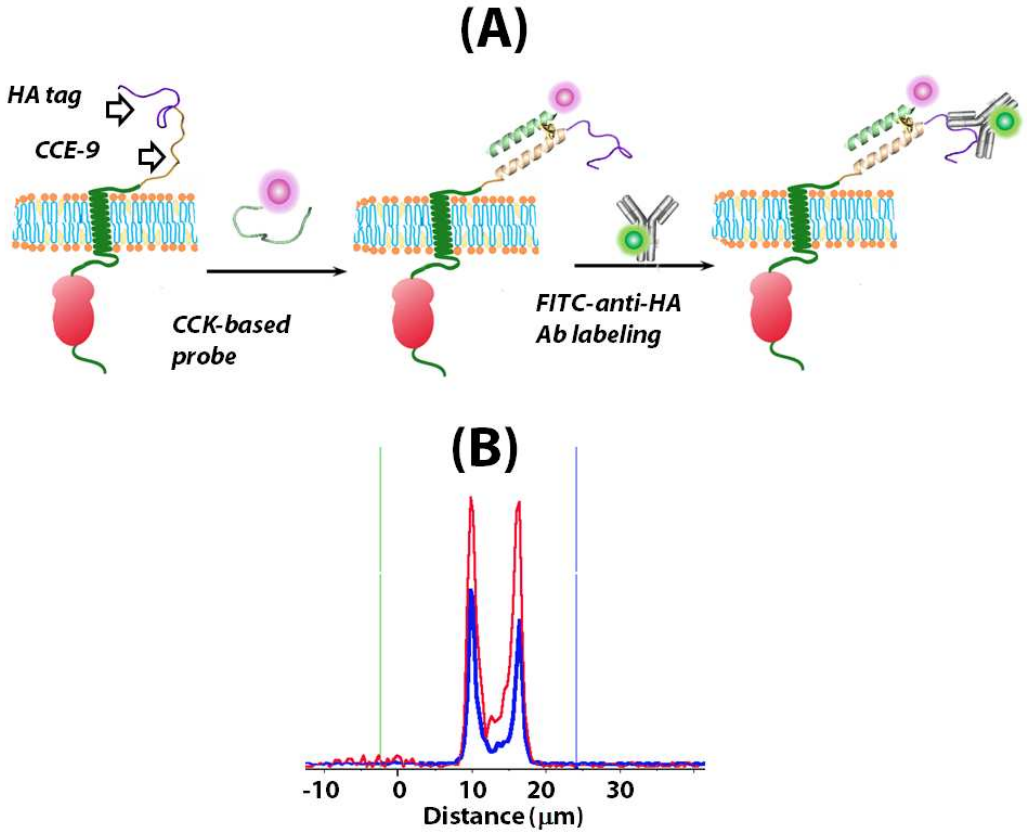
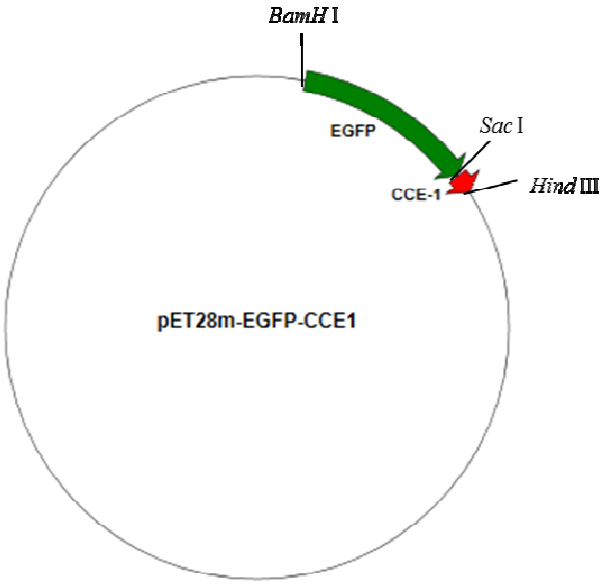


Figure S9. Covalent labeling of *CCE-9-EGFR* on a cell surface. (A) Scheme of the two-step labeling procedure. (B) Fluorescent signal of the cell intersection shows membrane labeling. Red, TRITC channel; blue, FITC channel.

Figure S10. C terminal tagging of hIP with an EGFP disrupt the membrane localization of the receptor. Also CHO cells transfected by pEGFP-N1-(CCE-9-hIP-EGFP) failed to be labeled by *tmr-CCK-9-dimer* probe. Scale bar = 10 μm.



pET28m-EGFP-CCE-1

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GGATCCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAG
CTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC
ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGG
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ATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
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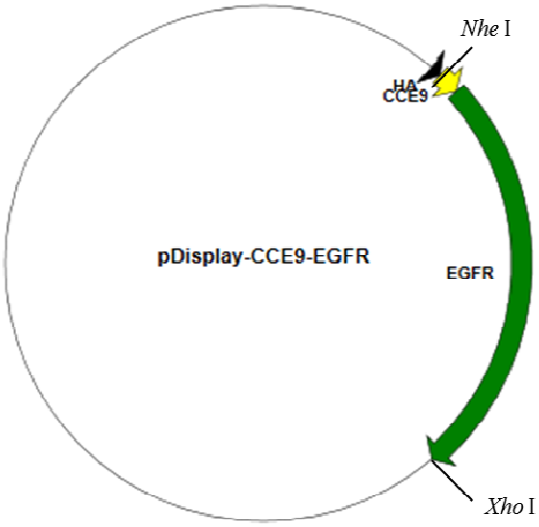
TTAGAGAAGGAAGTTGCTGCATTAGAGAAGTAGAAGCTT

Figure S11. Plasmid construct and sequencing result of pET28m-EGFP-CCE-1



pDisplay-CCE-9-EGFR

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CCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACT
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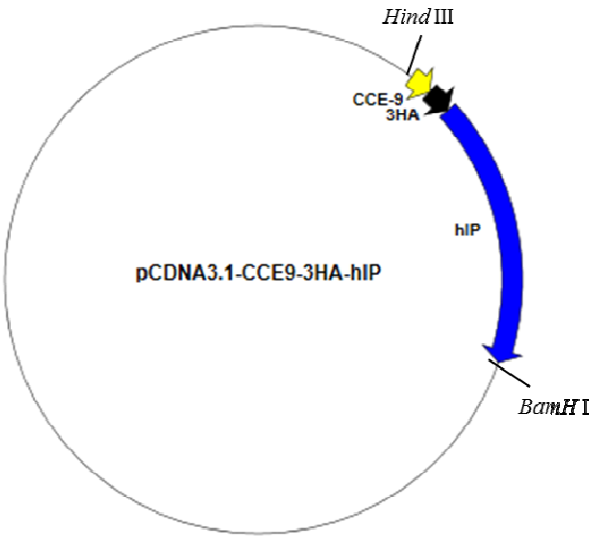
Figure S12. Plasmid construct and sequencing result of pDisplay-CCE-9-EGFR



pCDNA3.1-CCE-9-3HA-hIP

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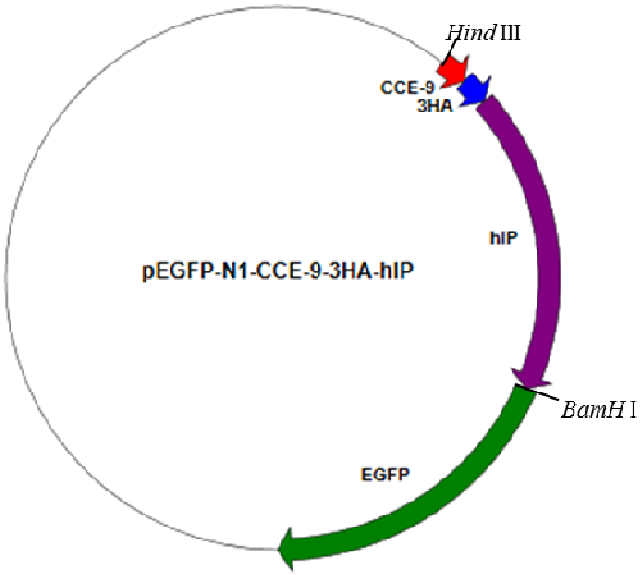
GGGGGACCTCCTTGCCTTCCGCTTCTACGCCTTCAACCCCATCCTGGACCCCTGGGTCTTCATCCTTTTCC
GCAAGGCTGTCTTCCAGCGACTCAAGCTCTGGGTCTGCTGCCTGTGCCTCGGGCCTGCCCACGGAGACTCG
CAGACACCCCTTTCCCAGCTCGCCTCAGGGAGGAGGGACCCAAGGGCCCCCTCTGCTCCTGTGGGAAAGGA
GGGGAGCTGCGTGCCTTTGTCGGCTTGGGGCGAGGGGCAGGTGGAGCCCTTGCCTCCCACACAGCAGTCCA
GCGGCAGCGCCGTGGGAACGTCGTCCAAAGCAGAAGCCAGCGTCGCCTGCTCCCTCTGCTGAGGATCCCG

Figure S13. Plasmid construct and sequencing result of pcDNA3.1-CCE-9-3HA-hIP



pEGFP-N1-CCE9-3HA-hIP

CAAGCTTGCCACCATGGAATGTGCTGCCTTAGAGAAGGAAATTGCAGCGTTAGAGAAGGAAATTG
CTGCATTAGAGAAGATGTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGTTCCAGAT
TACGCTTACCCATACGATGTTCCAGATTACGCTGATGCGGATTCGTGCAGGAACCTCACCTACGT
GCGGGGCTCGGTGGGGCCGGCCACCAGCACCCTGATGTTCGTGGCCGGTGTGGTGGGCAACGGGC
TGGCCCTGGGCATCCTGAGCGCACGGCGACCGGCGCGCCCCTCGGCCTTCGCGGTGCTGGTGACC
GGACTGGCGGCCACCGACCTGCTGGGCACCAGCTTCCTGAGCCCGGCCGTGTTCGTGGCCTATGC
GCGCAACAGCTCCCTGCTGGGCCTGGCCCGAGGCGGCCCCGCCCTGTGCGATGCCTTCGCCTTCG
CCATGACCTTCTTCGGCCTGGCGTCCATGCTCATCCTCTTTGCCATGGCCGTGGAGCGCTGCCTG
GCGCTGAGCCACCCCTACCTCTACGCGCAGCTGGACGGGCCCCGCTGCGCCCGCCTGGCGCTGCC
AGCCATCTACGCCTTCTGCGTCCTCTTCTGCGCGCTGCCCCTGCTGGGCCTGGGCCAACACCAGC
AGTACTGCCCCGGCAGCTGGTGCTTCCTCCGCATGCGCTGGGCCCAGCCGGGCGGCGCCGCCTTC
TCGCTGGCCTACGCCGGCCTGGTGGCCCTGCTGGTGGCTGCCATCTTCCTCTGCAACGGCTCGGT
CACCCTCAGCCTCTGCCGCATGTACCGCCAGCAGAAGCGCCACCAGGGCTCTCTGGGTCCACGGC
CGCGCACCGGAGAGGACGAGGTGGACCACCTGATCCTGCTGGCCCTCATGACAGTGGTCATGGCC
GTGTGCTCCCTGCCTCTCACGATCCGCTGCTTCACCCAGGCTGTCGCCCCTGACAGCAGCAGTGA
GATGGGGGACCTCCTTGCCTTCCGCTTCTACGCCTTCAACCCCATCCTGGACCCCTGGGTCTTCA
TCCTTTTCCGCAAGGCTGTCTTCCAGCGACTCAAGCTCTGGGTCTGCTGCCTGTGCCTCGGGCCT
GCCCACGGAGACTCGCAGACACCCCTTTCCCAGCTCGCCTCAGGGAGGAGGGACCCAAGGGCCCC
CTCTGCTCCTGTGGGAAAGGAGGGGAGCTGCGTGCCTTTGTCGGCTTGGGGCGAGGGGCAGGTGG
AGCCCTTGCCTCCCACACAGCAGTCCAGCGGCAGCGCCGTGGGAACGTCGTCCAAAGCAGAAGCC
AGCGTCGCCTGCTCCCTCTGCcgGGATCCCG

Figure S14. Plasmid construct and sequencing result of pEGFP-N1-(CCE-9-hIP-EGFP)

Table S1. Sequences of parental tecton peptides.

p_1	E IAALKQE NQALEQK IAALKGY
p_2	E IAALKQK NKYLKQE IQQLE
p_3	K IQALQQK IKQLKQK IAALKGY
p_4	YGQ IAALEQE IAALEQE IAALE
p_5	E IAALEQQ NKYLKQE IAALKGK
p_6	K IKALKQE NAYLQQE IQALK

Table S2. List of the synthetic peptides and their molecular weights.

Name	Peptide sequence	Calculated	Found
CCK-1	*fl*-GGGK **X**AALKEK VAALKEK VAALKE	2828.3	2827.8
CCK-2	*fl*-GGGK VAA**X**KEK VAALKEK VAALKE	2814.3	2813.4
CCK-3	*fl*-GGK VAALKEK **X**AALKEK VAALKE	2771.3	2770.8
CCK-4	*fl*-GGK VAALKEK VAA**X**KEK VAALKE	2757.3	2756.3
CCK-5	*fl*-GGGK VAALKEK **X**AALKEK VAALKEK VAALKE	3567.8	3567.1
CCK-6	*fl*-GGGK VAALKEK VAA**X**KEK VAALKEK VAALKE	3553.7	3553.0
CCK-7	*fl*-GGK VAALKEK VAALKEK **X**AALKEK VAALKE	3510.7	3509.9
CCK-8	*fl*-GGK VAALKEK VAALKEK VAA**X**KEK VAALKE	3496.7	3495.9
CCE-1	*tmr*-GGGE **C**AALEKE VAALEKE VAALEK	2825.8	2824.7
CCE-1'	*tmr*-KSEESYE**C**AALEKEVAALEKEVAALEK	3379.1	3377.8
CCE-2	*tmr*-GGGE VAA**C**EKE VAALEKE VAALEK	2811.9	2810.7
CCE-3	*tmr*-GGE VAALEKE **C**AALEKE VAALEK	2768.8	2767.2
CCE-4	*tmr*-GGE VAALEKE VAA**C**EKE VAALEK	2755.8	2754.3
CCE-5	*tmr*-GGGE VAALEKE **C**AALEKE VAALEKE VAALEK	3566.3	3566.0
CCE-6	*tmr*-GGGE VAALEKE VAA**C**EKE VAALEKE VAALEK	3552.3	3551.9
CCE-7	*tmr*-GGE VAALEKE VAALEKE **C**AALEKE VAALEK	3509.3	3509.1
CCE-8	*tmr*-GGE VAALEKE VAALEKE VAA**C**EKE VAALEK	3495.3	3494.9
	tmr-GG **X**KEKVAALKEKNAALKEKVAALKE	3239.8	3237.9
	tmr-KSEESY**C**EKEVAALEKENAALEKEVAALEK	3750.3	3750.0
P1x	*tmr*-GG-EXAALKQENQALEQKIAALKGYK	3131.6	3130.5
P2c	*tmr*-GG-ECAALKQKNKYLKQEIQQLE	2930.0	2929.5
P3x	*fl*-GGKXQALQQKIKQLKQKIAALKGY	3059.4	3058.7
P4c	*fl*-GGQCAALEQEIAALEQEIAALE	2613.4	2635.2 ($+Na^+$)
P5c	*Cy5*-GG-ECAALEQQNKYLKQEIAALKGK	3226.7	3226.7
P6x	*Cy5*-GG-KXKALKQENAYLQQEIQALK	3157.2	3156.6
	tmr-GGG KXAALKEKVAALKEKVAALKE	2882.6	2881.8
CCK-1'-dimer	(*tmr*-GGKXAALKEKIAALKEKIAALKEGG)$_2$KGG	6160.4	6192.7 ($+Na^+$)

References:

(1) Wise, H. (2006) Lack of interaction between prostaglandin E2 receptor subtypes in regulating adenylyl cyclase activity in cultured rat dorsal root ganglion cells. *Eur. J. Pharmacol. 535*, 69-77.

(2) Ng, K. Y., Wong, W. H., and Wise, H. (2011) Glial cells isolated from dorsal root ganglia express prostaglandin E(2) (EP(4)) and prostacyclin (IP) receptors. *Eur. J. Pharmacol. 661*, 42-48.

(3) Lindhout, D.A., Litowski, J.R., Mercier, P., Hodges, R.S., and Sykes, B.D. (2004) NMR solution structure of a highly stable de novo heterodimeric coiled-coil. *Biopolymers 75*, 367-375.