# Supplementary Information

## Catalyst-free thiazolidine formation chemistry enables the facile construction of peptide/protein-cell conjugates (PCCs) at physiological pH

Xiangquan Liu,[a] Youyu Wang, [a] Bangce Ye *[a,b] and Xiaobao Bi *[a]

a. Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, China. E-mail: xbbi@zjut.edu.cn; yebangce@zjut.edu.cn

b. Lab of Biosystem and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, Shanghai, 200237, China. E-mail: bcye@ecust.edu.cn

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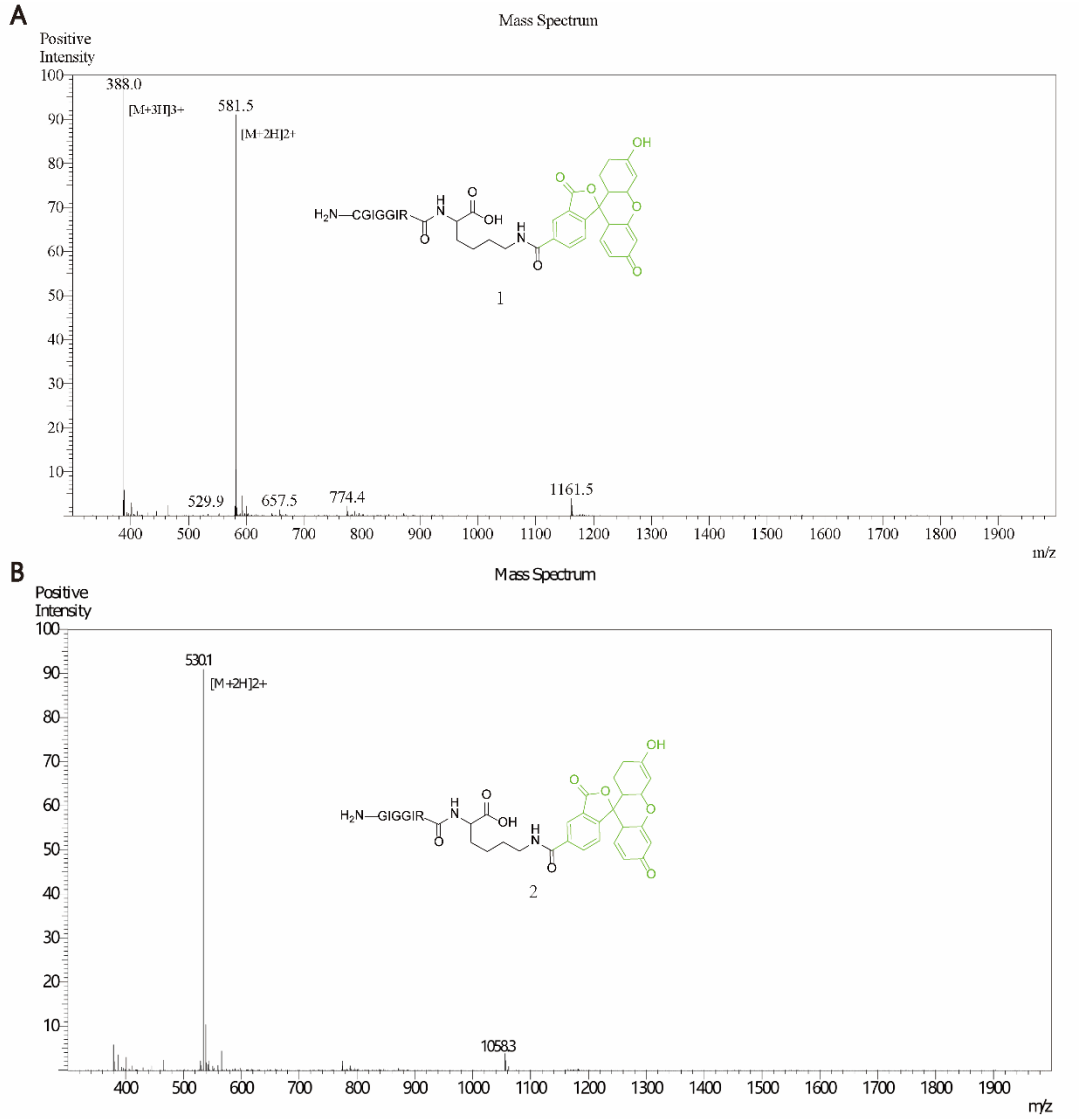


**Figure S1. Mass analysis of probe 1 and 2.** (A) Mass analysis of **1** with expected MW: 1161.28 Da, observed MW: 1161.0 Da. (B) Mass analysis of **2** with expected MW: 1058.27 Da, observed MW: 1058.2 Da.

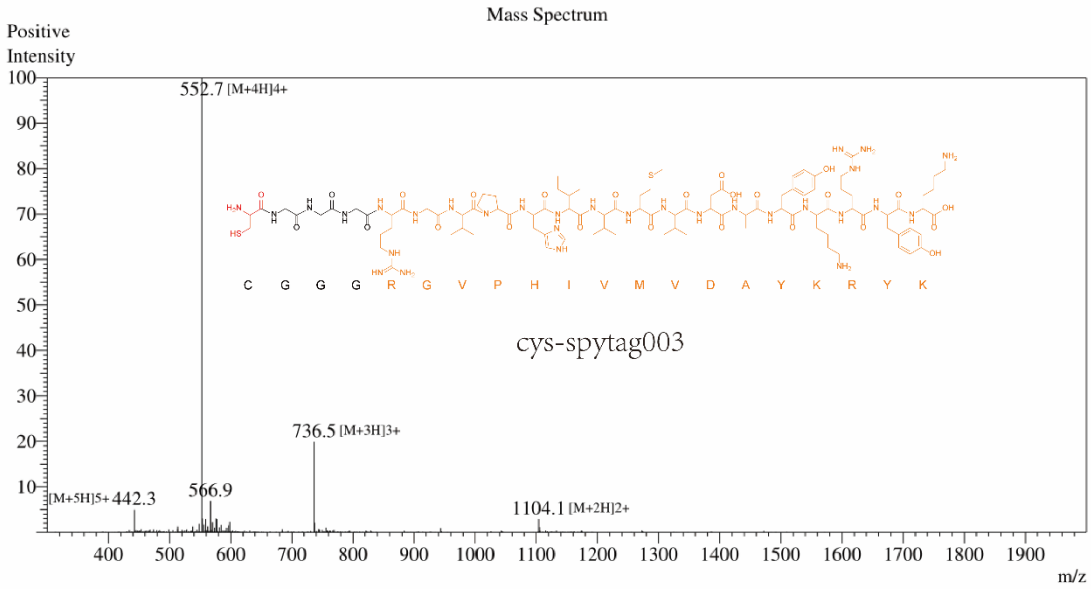


**Figure S2. Mass analysis of peptide 3.** Mass analysis of peptide **3** with expected MW: 2206.60 Da, observed MW: 2206.8 Da.

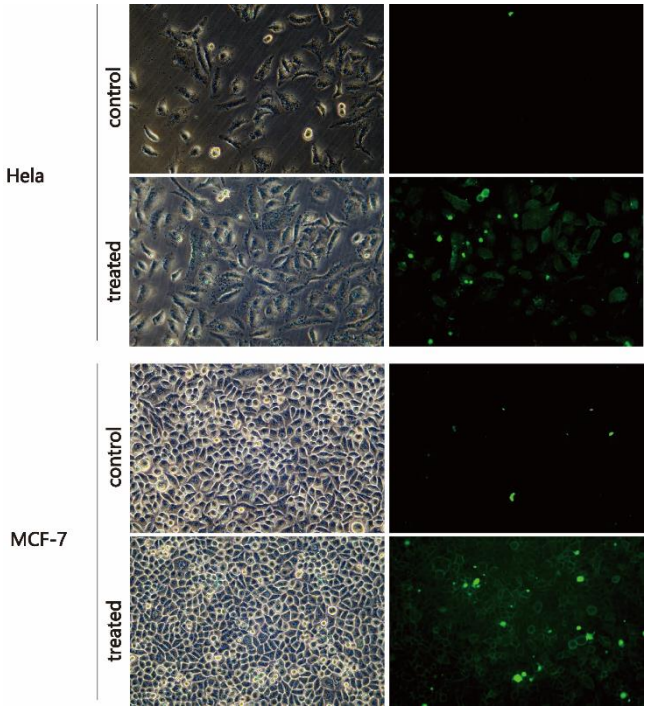


**Figure S3. Fluorescent microscopy images of fluorescently labeled Hela and MCF-7 cells using SpyCASE.** Cells (Hela, MCF-7) were first treated with 1 mM sodium periodate for 5 mins at RT and then co-incubated with 20 μM **3** in PBS (pH 7.4) for 1 h to immobilize SpyTag003 into the cell surface. Meanwhile, cells without periodate treatment were also incubated with **3** at the same conditions, serving as counterpart controls. After SpyTag003 conjugation and washing, the cells were re-grown in normal DMEM supplemented with 10 μM **4** for sfGFP labeling via SpyCatcher-SpyTag chemistry. Results clearly showed that only oxidized cells could be successfully labeled, suggesting that **3** cannot be attached if there are no aldehyde groups at the cell surfaces.

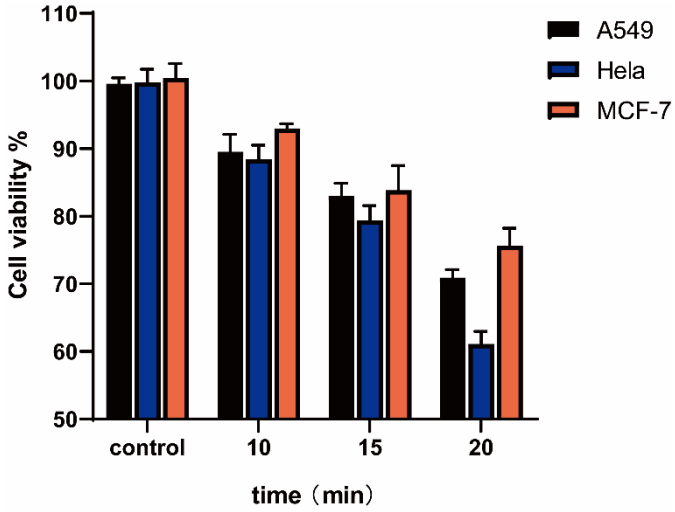


**Figure S4. Time-course study of the effects of Pd on cell viability.** Cells (A549, Hela, MCF-7, $1 \times 10^5$ cells) were treated with 5 μM $[PdCl(allyl)]_2$ in PBS (pH 7.4) supplemented with 5 μM GSH at 4 ℃ for 10, 15, 20 min, respectively. Treated cells were washed 3 times using PBS buffer, then resuspended in phenol-red-free MEM-α supplemented with both 10% FBS and 10% CCK-8 and incubated for 1 h at 37 °C. The optical density at 450 nm wavelength was then detected using a microplate reader (BioTek Instrument, Winooski, VT, USA).

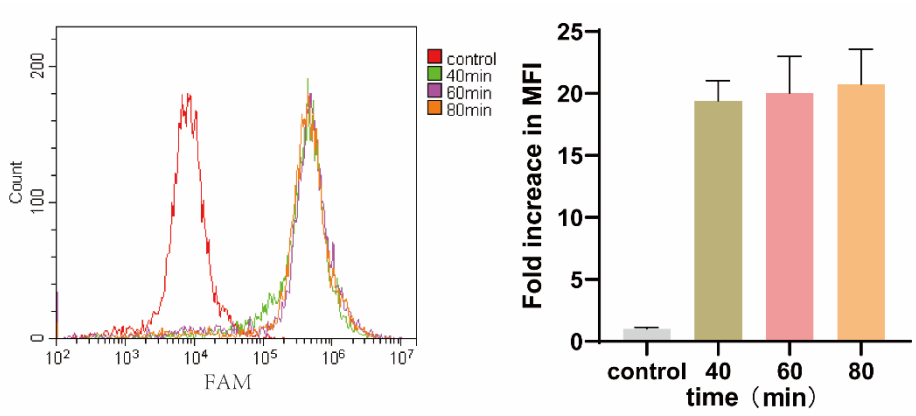


**Figure S5. Time-course study of the fluorescence intensity of fluorescently labeled NK-92MI analyzed by flow cytometry.** Periodate-treated NK-92MI cells were labeled with 15 μM **1** at RT for 40, 60, 80 min, respectively. Non-treated cells were used as the control.

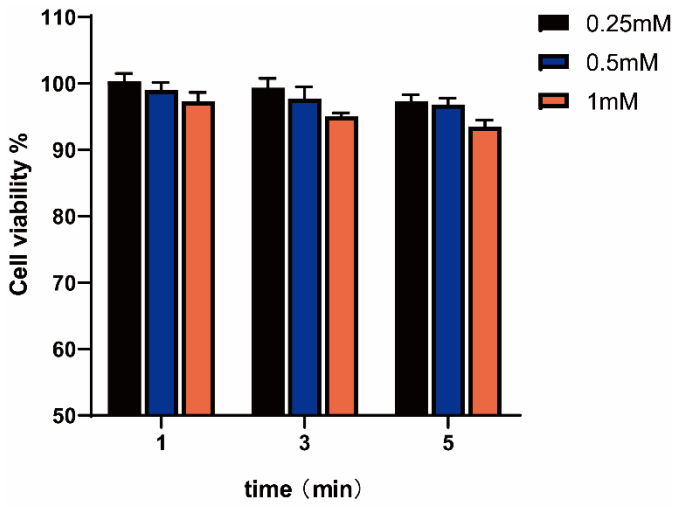


**Figure S6. The effects of periodate oxidation on NK-92MI cell viability.** NK-92MI cells ($1 \times 10^5$ cells) were treated with different concentrations of periodate (0, 0.25, 0.5, 1 mM) for different time (1, 3, 5 min). Treated NK-92MI cells were washed 3 times using PBS buffer, then resuspended in phenol-red-free MEM-α supplemented with both 10% FBS and 10% CCK-8 and incubated for 1 h at 37 °C. The optical density at 450 nm wavelength was then detected using a microplate reader (BioTek Instrument, Winooski, VT, USA).

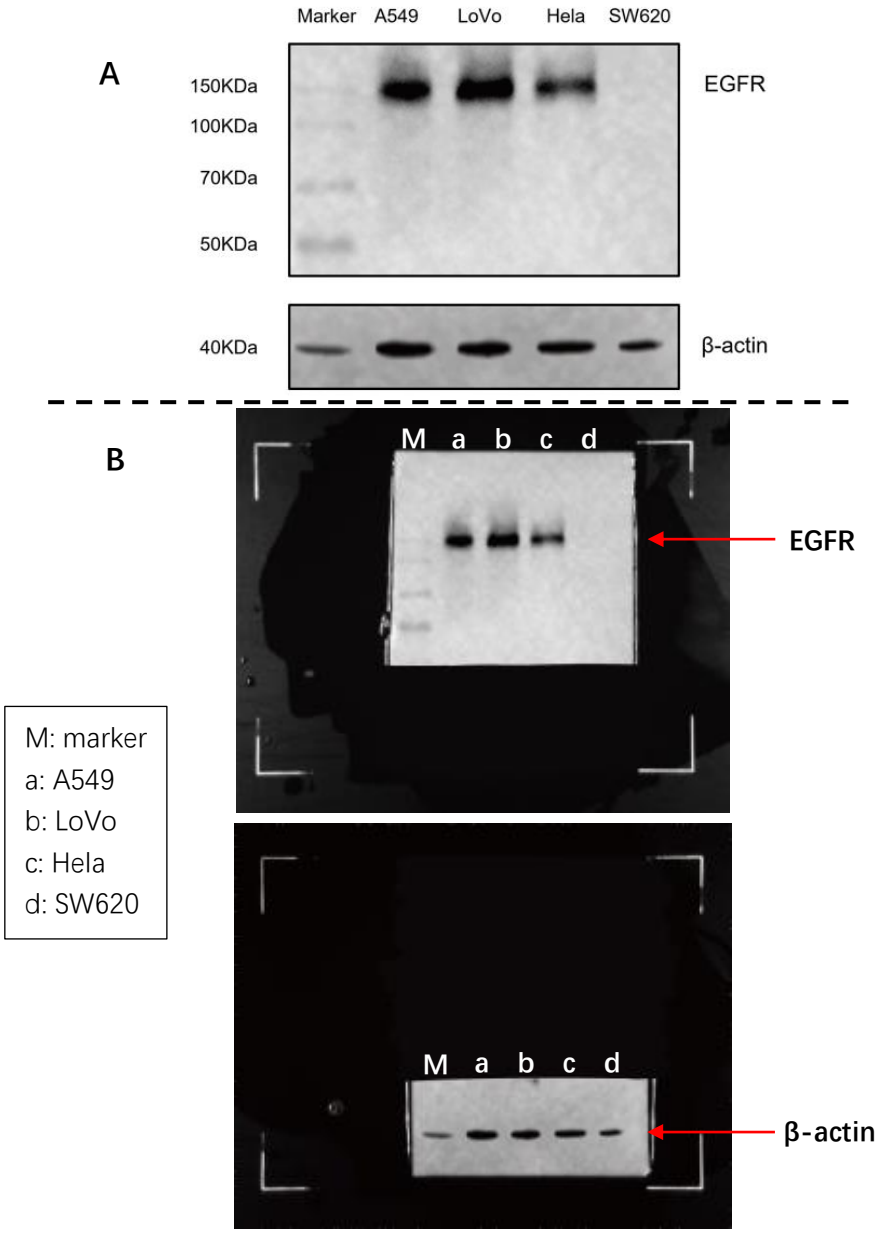


**Figure S7. Western blot analysis of EGFR expression level in different cancer cells.** (A) The processed image. (B) Raw data. Cancer cells collected in 1.5 mL tube were lysed in boiling water bath for 15 min after being suspended in an SDS-PAGE loading buffer. After BCA assay, samples were carried out for SDS-PAGE gel separation and then transferred to PVDF membrane. Since β-actin was used as the internal standard for quantification in this assay, PDVF membrane was cut into two pieces and needed to be incubated separately with the two different antibodies. The PVDF membrane after blocking by 5% skimmed milk was exposed into anti-EGFR antibody (1:1000) or β-actin antibody (1:1000) at 4 °C overnight for detection of EFGR and β-actin, respectively. Then HRP-conjugated goat anti-rabbit IgG (1:1000) antibodies was added for incubation at rt. for 1 h. After washing three times with TBST, the Chemical Scope series (Clinx Science Instruments Co., Ltd, China) was used to capture the membrane which was treated with an ECL kit. In panel a, the image was cropped from the original scan of blots and labeled with MW makers to localize the band corresponding to the detected EGFR and β-actin. The expected of molecular weight of EGFR is 170 KDa and that of β-actin is 41 KDa. In panel b, the unprocessed scans of original blots were also provided for the illustration of results.

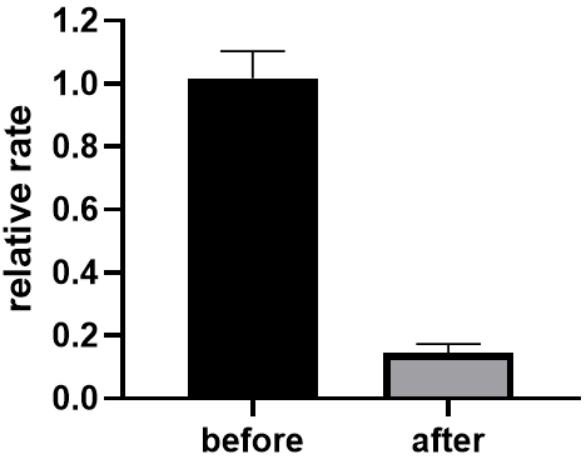


**Figure S8. Relative ratios of the number of adherent THP-1 cells before and after Pd treatment.** The number of cells before and after Pd cleavage was calculated using the "Image J" software. The data showed, after Pd treatment, that about 85% of the attached cells were separated from the interacting A549 cells.

**Table S1. Primers used for this study.**

| Primer | Sequence (5‘-3') |
|---|---|
| SC3-G F1 | AACTTTAAGAAGGAGATATACCATGGATGTCGTACTACCATCACCATCACC |
| SC3-G R1 | TTTCATGCTGCTACCACTGGATCCGCTACCACTGGATCCAGTATGAGC |
| SC3-G F2 | CAGTGGTAGCGGATCCAGTGGTAGCAGCATGAAAAAAGTTAGCAAAGGTGAAG |
| SC3-G R2 | GCATTATGCGGCCGCAAGCTTTTACACGTGATTGCTGCCTTTATACAGTT |
| SC3-M F1 | AACTTTAAGAAGGAGATATACCATGGATGTCGTACTACCATCACCATCACC |
| SC3-M R1 | CTTGCTCACGCTGCTACCACTGGATCCGCTACCACTGGATCCAGTATGAGC |
| SC3-M F2 | CAGTGGTAGCGGATCCAGTGGTAGCAGCGTGAGCAAGGGCGAGGACGAC |
| SC3-M R2 | GCATTATGCGGCCGCAAGCTTTTACTTGTACAGCTCGTCCATGCCGC |

**Gene sequence of SpyCatcher003-sfGFP fusion protein (SpyCatcher003 highlighted with yellow; sfGFP highlighted with green):**

ATGTCGTACTACCATCACCATCACCATCACGATTACGACATCCCAACGACCGAAAA

CCTGTATTTTCAGGGCGCCATGGTAACCACCTTATCAGGTTTATCAGGTGAGCAAG

GTCCGTCCGGTGATATGACAACTGAAGAAGATAGTGCTACCCATATTAAATTCTCA

AAACGTGATGAGGACGGCCGTGAGTTAGCTGGTGCAACTATGGAGTTGCGTGATT

CATCTGGTAAAACTATTAGTACATGGATTTCAGATGGACATGTGAAGGATTTCTACC

TGTATCCAGGAAAATATACATTTGTCGAAACCGCAGCACCAGACGGTTATGAGGTA

GCAACTCCAATTGAATTTACAGTTAATGAGGACGGTCAGGTTACTGTAGATGGTGA

AGCAACTGAAGGTGACGCTCATACTGGATCCAGTGGTAGCGGATCCAGTGGTAGC

AGCATGAAAAAAGTTAGCAAAGGTGAAGAACTGTTTACCGGCGTTGTGCCGATTCT

GGTGGAACTGGATGGTGATGTGAATGGCCATAAATTTAGCGTTCGTGGCGAAGGC

GAAGGTGATGCGACCAACGGTAAACTGACCCTGAAATTTATTTGCACCACCGGTAA

ACTGCCGGTTCCGTGGCCGACCCTGGTGACCACCCTGACCTATGGCGTTCAGTGCT

TTAGCCGCTATCCGGATCATATGAAACGCCATGATTTCTTTAAAAGCGCGATGCCG

GAAGGCTATGTGCAGGAACGTACCATTAGCTTCAAAGATGATGGCACCTATAAAAC

CCGTGCGGAAGTTAAATTTGAAGGCGATACCCTGGTGAACCGCATTGAACTGAAA

GGTATTGATTTTAAAGAAGATGGCAACATTCTGGGTCATAAACTGGAATATAATTTC

AACAGCCATAATGTGTATATTACCGCCGATAAACAGAAAAATGGCATCAAAGCGA

ACTTTAAAATCCGTCACAACGTGGAAGATGGTAGCGTGCAGCTGGCGGATCATTAT

CAGCAGAATACCCCGATTGGTGATGGCCCGGTGCTGCTGCCGGATAATCATTATCT

GAGCACCCAGAGCGTTCTGAGCAAAGATCCGAATGAAAAACGTGATCATATGGTG

CTGCTGGAATTTGTTACCGCCGCGGGCATTACCCACGGTATGGATGAACTGTATAA

AGGCAGCAATCACGTG

**Amino acid sequence of SpyCatcher003-sfGFP:**

MSYYHHHHHHDYDIPTTENLYFQGAMVTTLSGLSGEQGPSGDMTTEEDSATHIKFSKRDEDGRELAGATMELRDSS
GKTISTWISDGHVKDFYLYPGKYTFVETAAPDGYEVATPIEFTVNEDGQVTVDGEATEGDAHTGSSGSGSSGSSMKKV
SKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKR
HDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKN
GIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSKDPNEKRDHMVLLEFVTAAGITHGMDE
LYKGSNHV*

**Gene sequence of SpyCatcher003-mCherry fusion protein (SpyCatcher003 marked with yellow; mCherry highlighted with red):**

ATGTCGTACTACCATCACCATCACCATCACGATTACGACATCCCAACGACCGAAAA
CCTGTATTTTCAGGGCGCCATGGTAACCACCTTATCAGGTTTATCAGGTGAGCAAG
GTCCGTCCGGTGATATGACAACTGAAGAAGATAGTGCTACCCATATTAAATTCTCA
AAACGTGATGAGGACGGCCGTGAGTTAGCTGGTGCAACTATGGAGTTGCGTGATT
CATCTGGTAAAACTATTAGTACATGGATTTCAGATGGACATGTGAAGGATTTCTACC
TGTATCCAGGAAAATATACATTTGTCGAAACCGCAGCACCAGACGGTTATGAGGTA
GCAACTCCAATTGAATTTACAGTTAATGAGGACGGTCAGGTTACTGTAGATGGTGA
AGCAACTGAAGGTGACGCTCATACTGGATCCAGTGGTAGCGGATCCAGTGGTAGC
AGCGTGAGCAAGGGCGAGGACGACAACATGGCCATCATCAAGGAGTTCATGCGCT
TCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCG
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AGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGC
TCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTT
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GACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAG

CTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGG

GCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCG

AGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCCGAGGTCA

AGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAA

CATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC

GAGCGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAG

**Amino acid sequence of SpyCatcher003-mCherry:**

MSYYHHHHHHDYDIPTTENLYFQGAMVTTLSGLSGEQGPSGDMTTEEDSATHIKFSKRDEDGRELAGATMELRDSS
GKTISTWISDGHVKDFYLYPGKYTFVETAAPDGYEVATPIEFTVNEDGQVTVDGEATEGDAHTGSSGSGSSGSSVSKG
EDDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHP
ADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMY
PEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDEL
YK*