# Spontaneous and specific chemical cross-linking in live cells to capture and identify protein interactions

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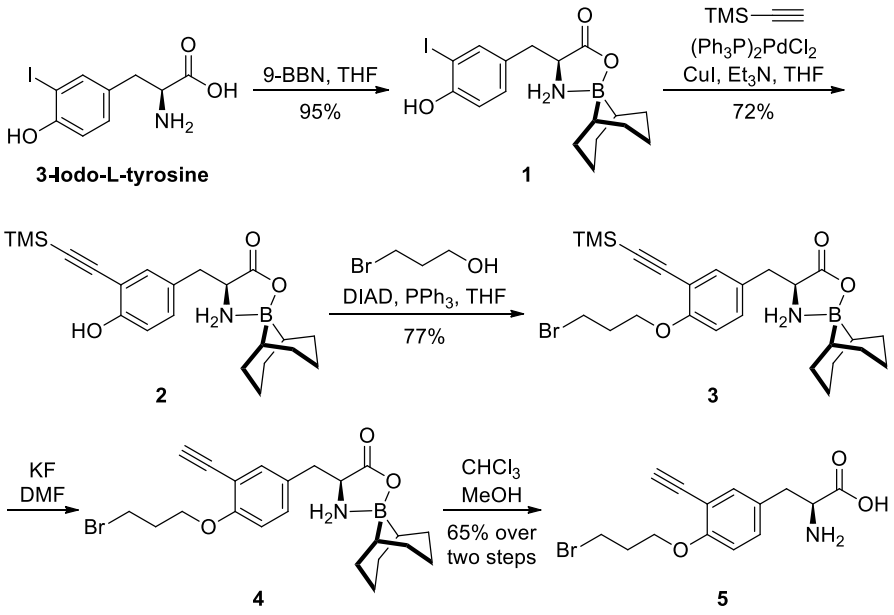
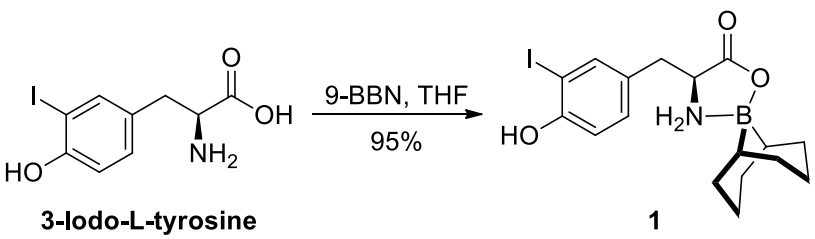
## Supplementary Methods

### Synthesis of Uaa EB3.

**General information**. All commercially available reagents were purchased from Sigma-Aldrich and used without further purification. Solvents (ACS grade) were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was dried by standard methods prior to use. Oxygen- and moisture-sensitive reactions were carried out under argon atmosphere. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm EMD Millipore TLC Silica Gel 60 $F_{254}$ using UV light for visualization and an ethanolic solution of phosphomolybdic acid under heat or powdered iodine for developing. Flash column chromatography was generally performed on silica gel (200-300 mesh). Yields refer to chromatographically homogeneous materials. High-resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI). The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian 400 spectrometer at 400 MHz and 100 MHz, respectively. The chemical shifts ($\delta$) were calibrated using residual undeuterated solvent as an internal reference and reported in ppm and coupling constants ($J$) in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet.

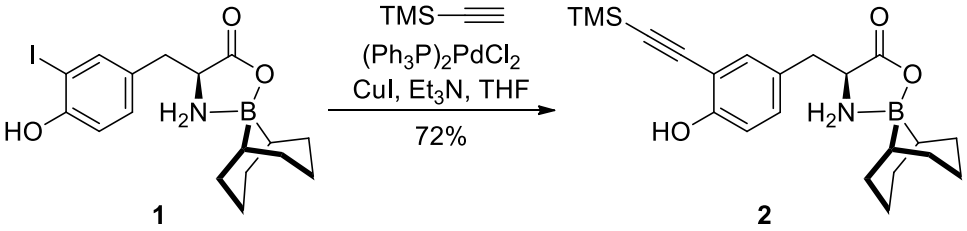



**Overall scheme for synthesis of Uaa EB3** (**5**). 3-Iodo-L-tyrosine was protected with 9-BBN to give compound **1** that was coupled with ethynyltrimethylsilane under the Sonogashira cross-coupling conditions to yield compound **2**. Compound **3** then was synthesized by Mitsunobu reaction, which gives the final Uaa **5** after deprotection of alkyne and release of free amino acid.



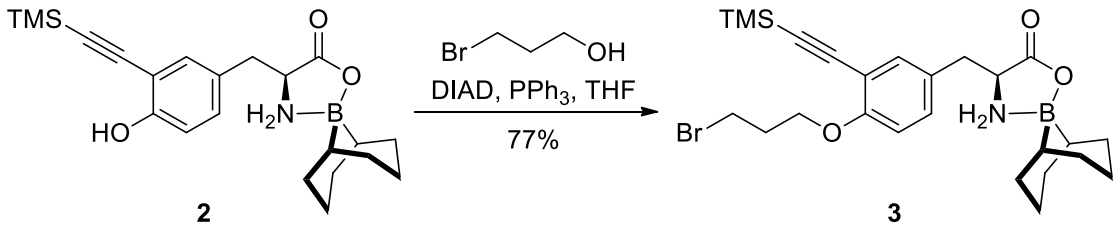

**3-Iodo-L-tyrosine** **1**

**Compound 1**. Compound 1 was synthesized from commercially available 3-iodo-L-tyrosine in 95% yield as a white powder by a reported protocol, and the spectral data of which were in good agreement with those reproted[1, 2]. $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.70 (d, $J$ = 1.6 Hz, 1H), 7.15 (dd, $J$ = 1.6, 8.0 Hz, 1H), 6.81 (d, $J$ = 8.0 Hz, 1H), 3.90 (dd, $J$ = 5.2, 7.6 Hz, 1H), 3.16 (dd, $J$ = 5.2, 14.8 Hz, 1H), 2.96 (dd, $J$ = 7.6, 14.8 Hz, 1H), 1.83-1.44 (m, 12H), 0.54 (s, 1H), 0.23 (s, 1H).




**1** **2**

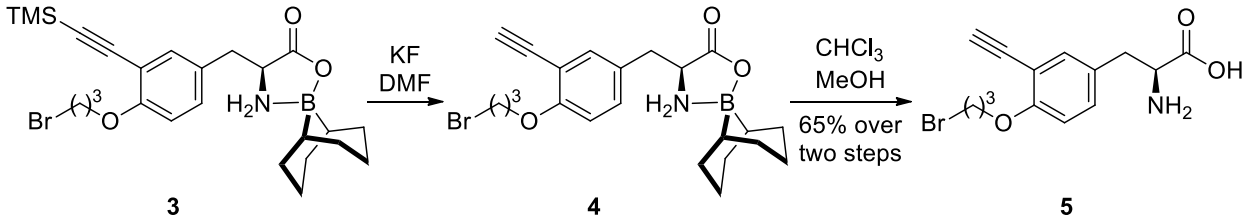
**Compound 2**. Compound 2 was synthesized from compound 1 in 72% yield as a light yellow solid by a reported protocol, and the spectral data of which were in good agreement with those reported[1, 2]. $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.31 (d, $J$ = 2.0 Hz, 1H), 7.14 (dd, $J$ = 2.0, 8.4 Hz, 1H), 6.82 (d, $J$ = 8.4 Hz, 1H), 6.41 (dd, $J$ = 8.0, 11.2 Hz, 1H), 5.14 (dd, $J$ = 8.0, 11.2 Hz, 1H), 3.91 (m, 1H), 3.16 (dd, $J$ = 5.2, 14.8 Hz, 1H), 2.97 (dd, $J$ = 8.0, 14.8 Hz, 1H), 1.86-1.43 (m, 12H), 0.55 (s, 1H), 0.28 (s, 1H), 0.23 (s, 9H).




**2** **3**

**Compound 3**. To a mixture of compound 2 (1.192 g, 3.0 mmol), 3-bromo-1-propanol (834 mg, 6.0 mmol), triphenylphosphine (1.574 g, 6.0 mmol) in 30 mL dry THF, diethyl azodicarboxylate (DIAD, 1.20 mL, 6.0 mmol) was added drop-wise with stirring at 0 $^o$C. The reaction was slowly warmed to room temperature and stirred for additional 2 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (hexanes/ethyl acetate, 50/50, v/v) to afford the compound 3 in 77% yield (1.201g) as a light yellow solid. $^1$H

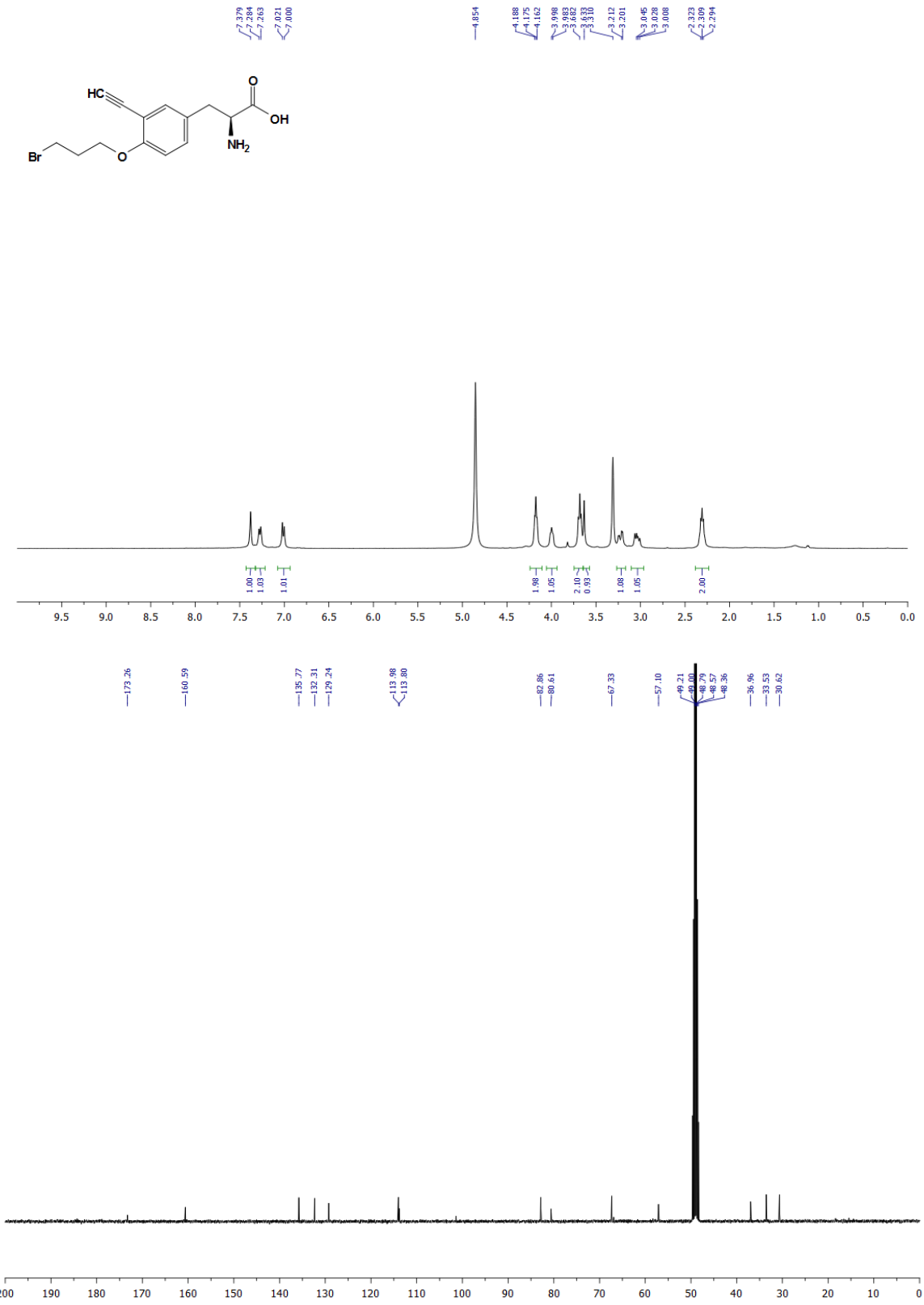
NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, $J$ = 2.0 Hz, 1H), 7.17 (dd, $J$ = 2.0, 8.4 Hz, 1H), 6.88 (d, $J$ = 8.4 Hz, 1H), 4.97 (t, $J$ = 9.2 Hz, 1H), 4.15 (t, $J$ = 5.6 Hz, 2H), 3.98 (p, $J$ = 4.4 Hz, 1H), 3.87 (dd, $J$ = 6.0, 10.8 Hz, 1H), 3.68 (t, $J$ = 6.4 Hz, 2H), 3.29 (dd, $J$ = 4.4, 14.8 Hz, 1H), 3.01 (dd, $J$ = 5.2, 14.8 Hz, 1H), 2.35 (p, $J$ = 6.0 Hz, 2H), 1.82-1.32 (m, 12H), 0.57 (s, 1H), 0.36 (s, 1H), 0.24 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.8, 159.4, 134.0, 130.7, 126.8, 114.0, 113.3, 100.2, 100.1, 66.1, 56.4, 35.5, 32.3, 31.6, 31.1, 29.7, 24.3, 23.7, 0.1; HRMS Calcd. for $C_{25}H_{38}BBrNO_3Si$ $[M+H]^+$ 518.1892, found 518.1885.



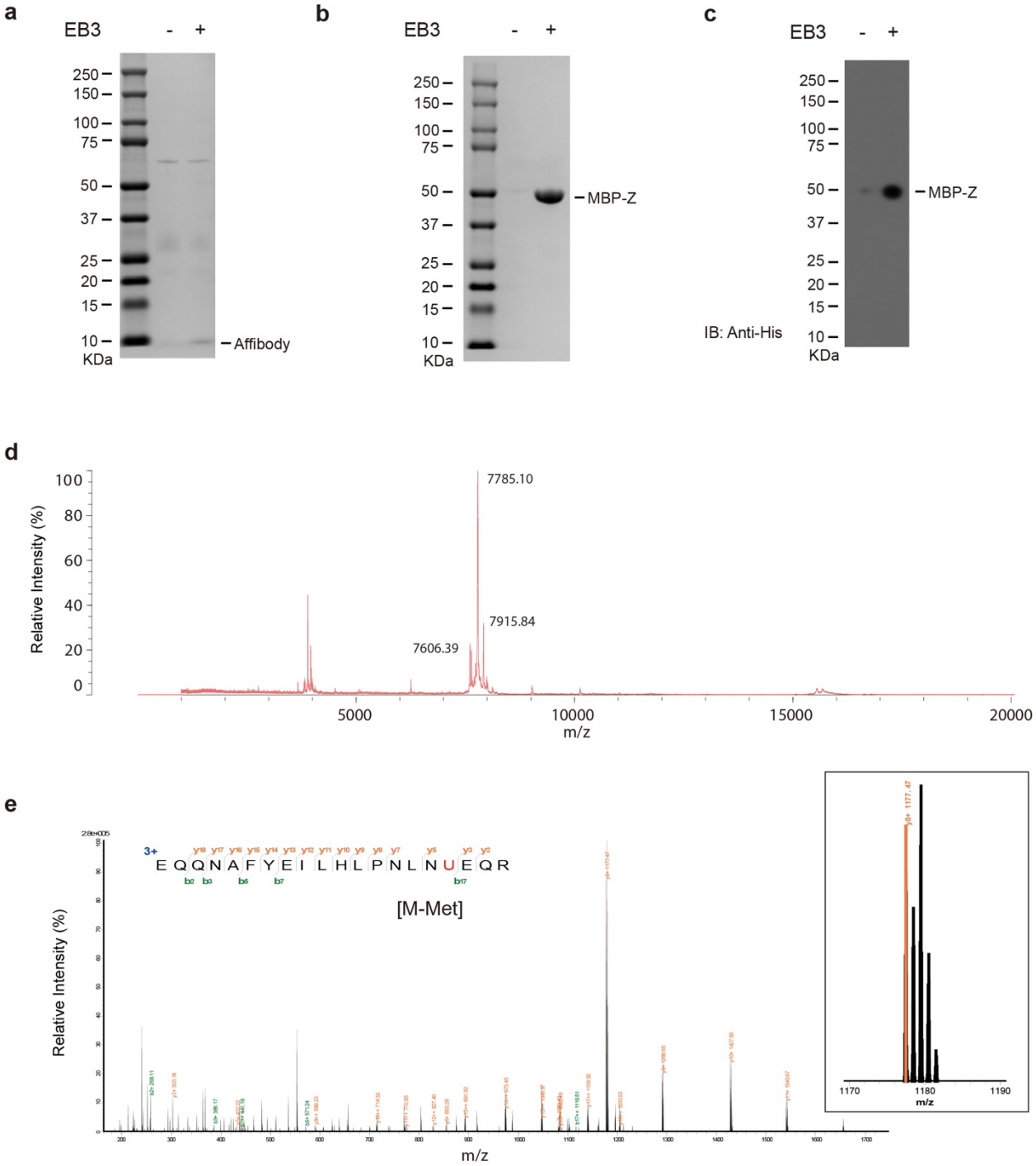

**3** **4** **5**

**Compound 4 and compound 5**. To a solution of compound 3 (1.201g, 2.32 mmol) in 8.0 mL wet DMF, KF (134.8 mg, 2.32 mmol) was added with stirring. The reaction was stirred for additional 2 h at ambient temperature, then was quenched with 1 M $KHSO_4$ (8.0 mL). The solution was extracted with EtOAc (3 × 30 mL). The combined EtOAc layers were washed with water (3 × 10 mL) and brine (10 mL) then dried over $Na_2SO_4$, filtered, and concentrated to provide crude compound 4 that was used as such in the next step.

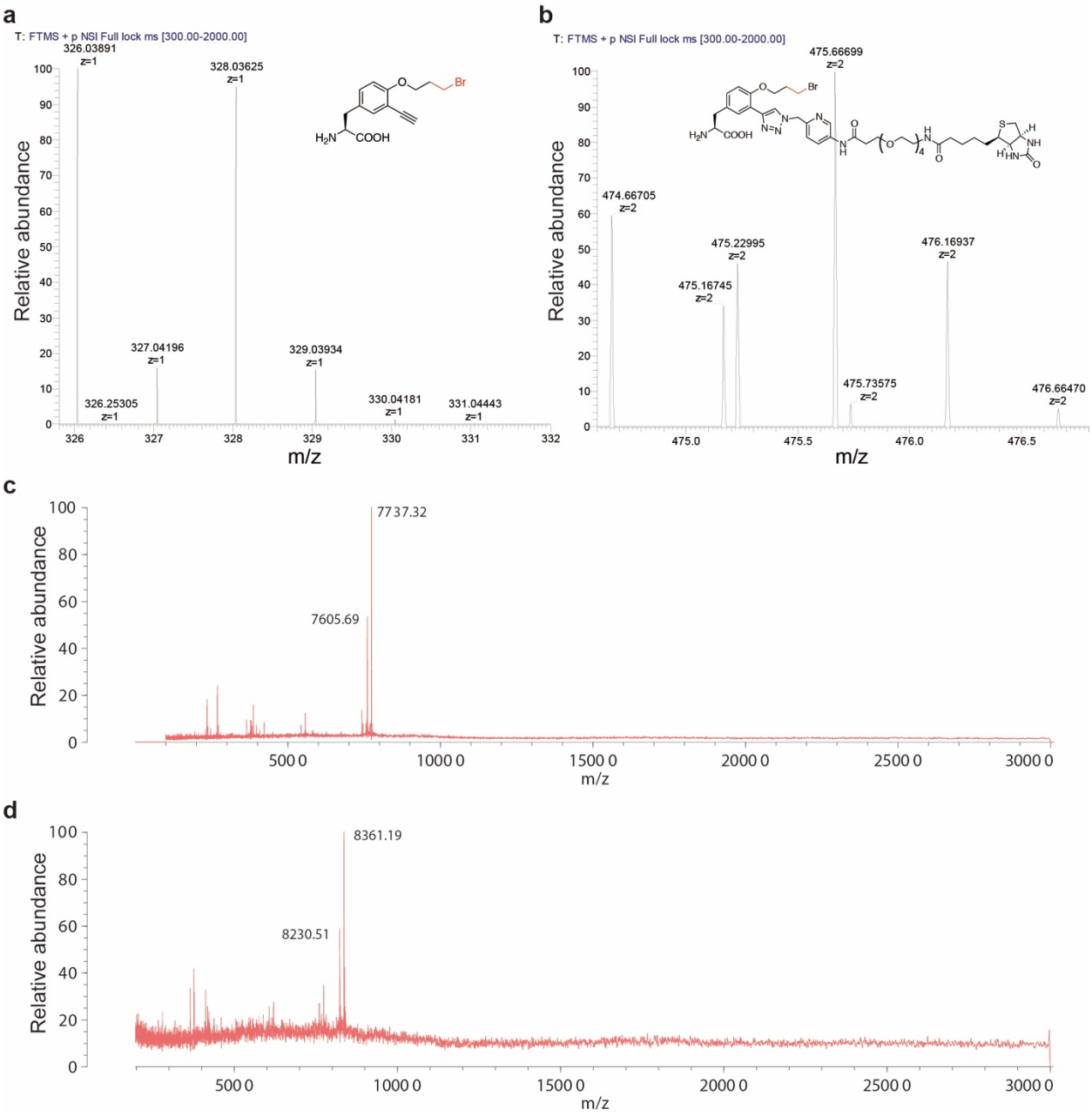
The above obtained compound 4 was dissolved in methanol (2 mL) and diluted with chloroform (20 mL). The solution was stirred at room temperature for 24 h. Hexanes (60 mL) was added to the solution, which led to precipitation of the compound 5. The crude product was washed with dichloromethane (2 × 3 mL) and diethyl ether (2 × 6 mL) and was dried in vacuo to afford final compound 5 in 65% yield over two steps (491.9 mg) as a white powder. $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.38 (d, $J$ = 2.4 Hz, 1H), 7.27 (dd, $J$ = 2.4, 8.4 Hz, 1H), 7.01 (d, $J$ = 8.4 Hz, 1H), 4.18 (t, $J$ = 5.2 Hz, 2H), 3.40 (t, $J$ = 5.6 Hz, 1H), 4.00 (t, $J$ = 5.6 Hz, 1H), 3.68 (t, $J$ = 6.0 Hz, 2H), 3.63 (s, 1H), 3.22 (dd, $J$ = 4.4, 14.4 Hz, 1H), 3.04 (dd, $J$ = 8.0, 14.4 Hz, 1H), 2.31 (p, $J$ = 6.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 173.3, 160.6, 135.8, 132.3, 129.2, 114.0, 113.8, 82.7, 80.6, 67.3, 57.1, 37.0, 33.5, 30.6; HRMS Calcd. for $C_{14}H_{17}BrNO_3$ $[M+H]^+$ 326.0386, found 326.0389.



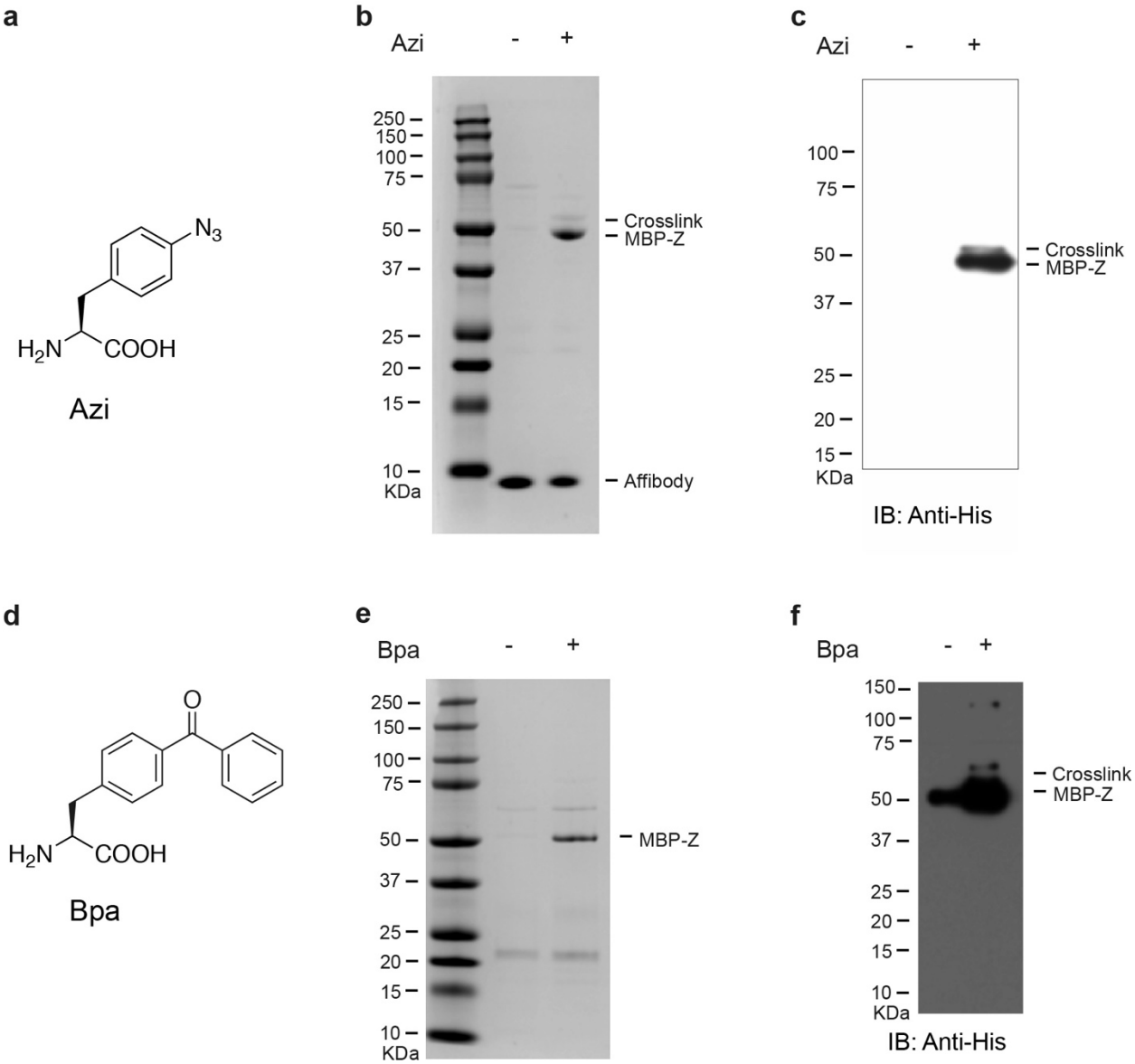

**Supplementary Figure 1. $^{1}$H NMR (400 MHz, top) and $^{13}$C NMR (100 MHz, bottom) spectra of UAA EB3.**



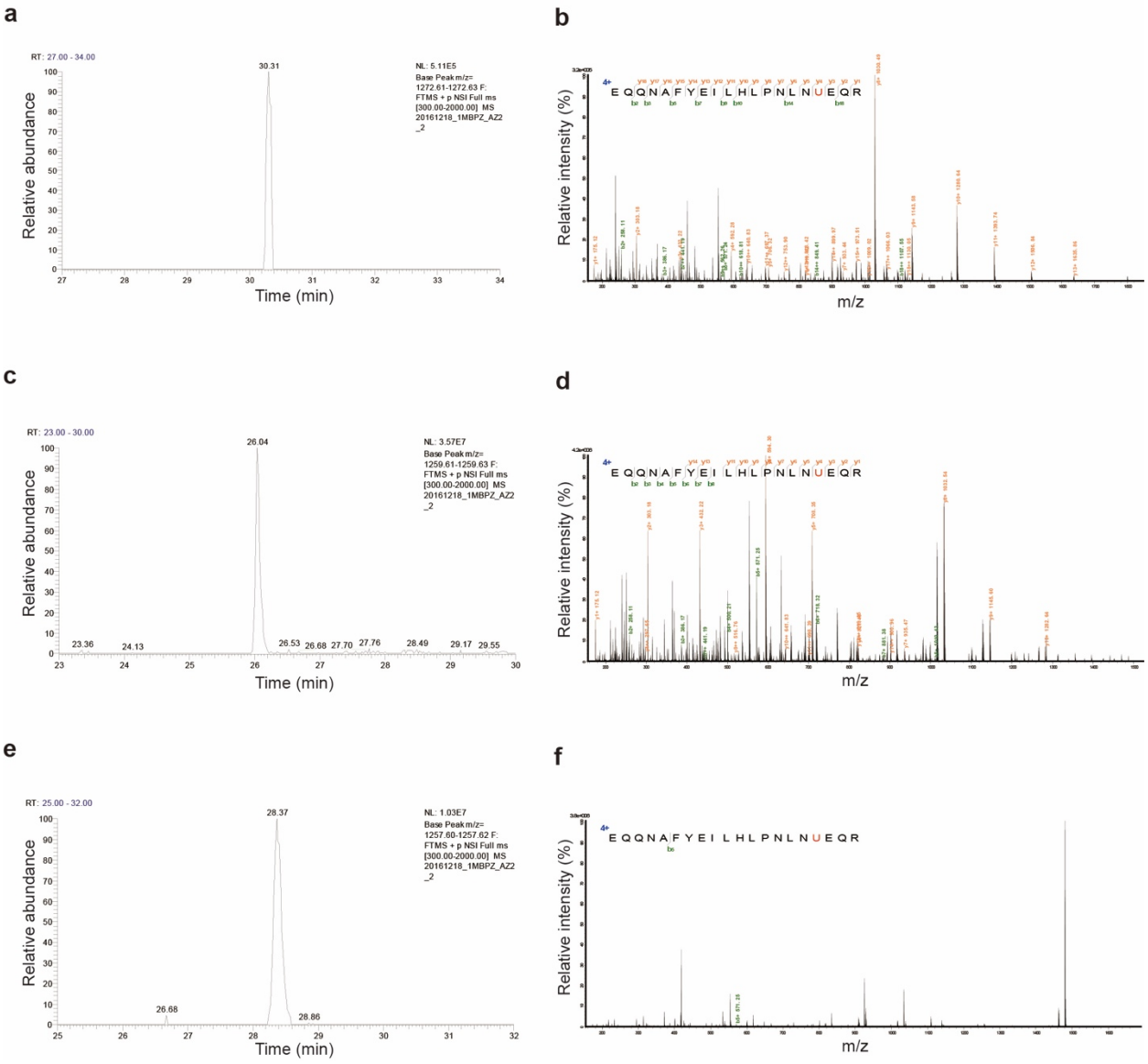

**Supplementary Figure 2. Genetic incorporation of EB3 into proteins in *E. coli*.** (**a**) SDS-PAGE gel analysis of affibody(36TAG) expression in *E. coli* in the absence and presence of EB3. (**b**) SDS-PAGE gel of expression of MBP-Z(24TAG) in *E. coli* in the absence and presence of EB3. (**c**) Western blot analysis of MBP-Z(24TAG) expression in *E. coli* in the absence and presence of EB3. (**d**) MALDI-TOF analysis of EB3-incorporated intact affibody. Expected mass: $[M]^+$ = 7913.91 Mono.; 7919.61 Avg. $[M\text{-}Met]^+$ = 7782.87 Mono.; 7788.42 Avg. (**e**) Tandem mass spectrum of EB3-incorporated MBP-Z peptide. U indicates EB3. The insert is the zoomed-in peak for y8 ion, which showed correct bromine isotope pattern. This distinct bromine isotope pattern is present in all other peaks for Uaa-containing ions, further confirming the incorporation of EB3.



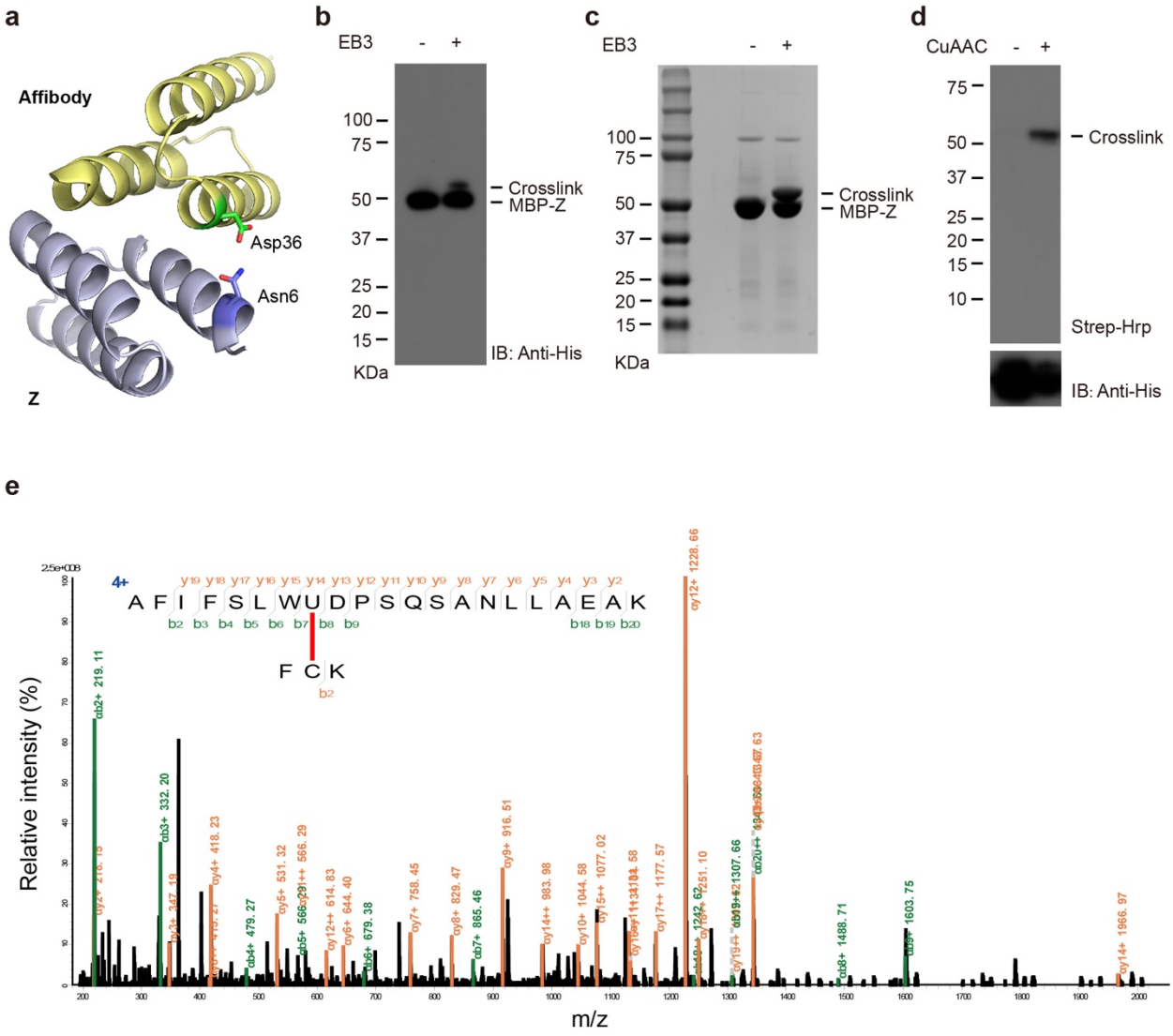

**Supplementary Figure 3. Labeling of biotin onto EB3 via CuAAC at the amino acid and protein level.** (**a**) High resolution mass spectrum of EB3 (Expected mass: $[M+H]^+$ = 326.03863 mono). (**b**) High resolution mass spectrum of biotin labeled EB3 (Expected mass: $[M+2H]^{2+}$ = 474.66808 mono) (**c**) MALDI-TOF spectrum of EB3 incorporated affibody (Expected mass: $[M+H]^+$ = 7731.43 Avg, $[M+H\text{-Met}]^+$ = 7600.24 Avg.) (**d**) MALDI-TOF spectrum of biotin labeled, EB3 incorporated affibody (Expected mass: $[M+H]^+$ = 8354.17 Avg, $[M+H\text{-Met}]^+$ = 8222.97 Avg.)



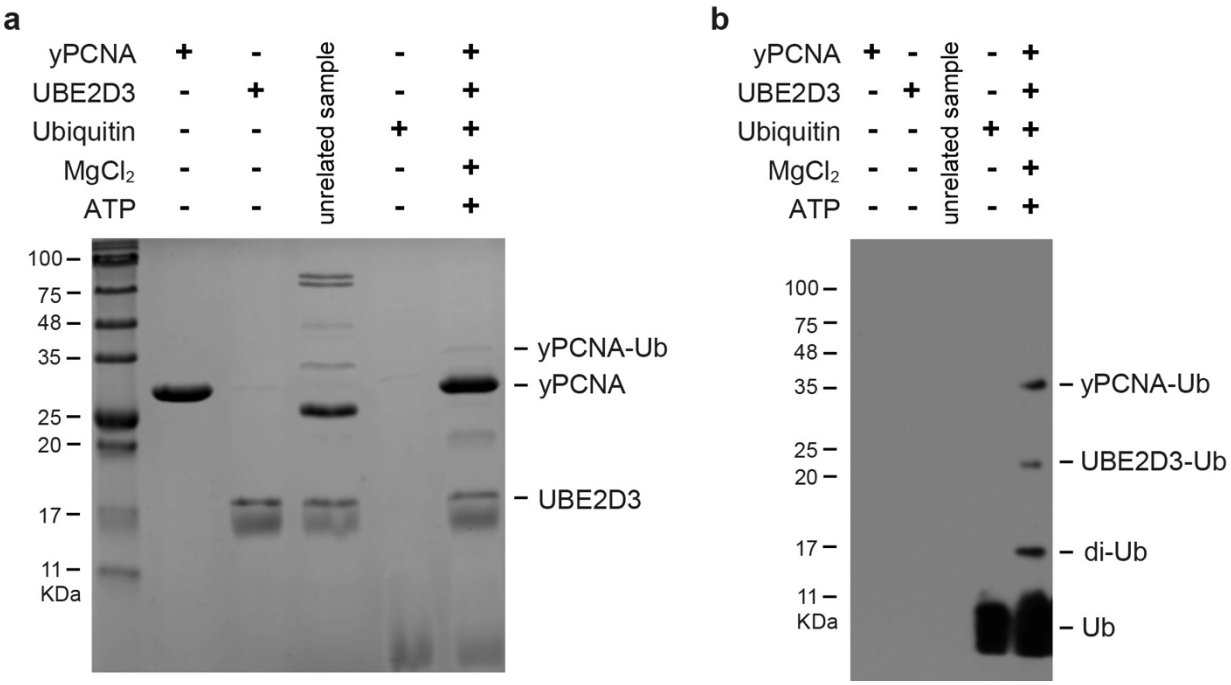

**Supplementary Figure 4. Photo-crosslinking of affibody to Z protein in *E. coli* via the genetically encoded Azi or Bpa.** (**a**) Structure of the photo-crosslinking Uaa Azi. (**b**) SDS-PAGE gel of His-tag purified proteins from cells expressing affibody(7C)_His6 and MBP-Z(24Azi)_His6 that had been photo-crosslinked by UV light. (**c**) Western blot analysis of cell lysate of cells expressing affibody(7C)_His6 and MBP-Z(24Azi)_His6 that had been photo-crosslinked by UV light. (**d**) Structure of the photo-crosslinking Uaa Bpa. (**e**) SDS-PAGE gel of His-tag purified proteins from cells expressing affibody(7C)_His6 and MBP-Z(24Bpa)_His6 that had been photo-crosslinked by UV light. (**f**) Western blot analysis of cell lysate of cells expressing affibody(7C)_His6 and MBP-Z(24Bpa)_His6 that had been photo-crosslinked by UV light.



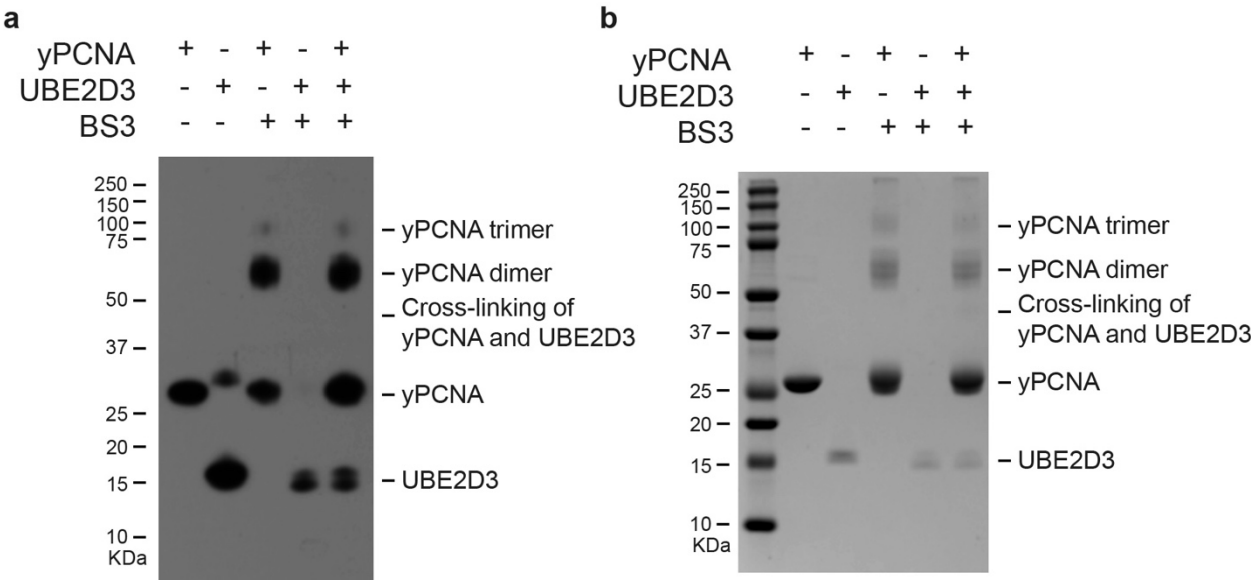

**Supplementary Figure 5. Products of Azi-incorporated peptide after photo-crosslinking.** (**a**) Extracted ion chromatography of Azi-incorporated peptides. (**b**) Tandem mass spectrum of Azi-incorporated peptides. (**c**) Extracted ion chromatography of Azi-incorporated peptides (-2N+2H). (**d**) Tandem mass spectrum of Azi-incorporated peptides (-2N+2H). (**e**) Extracted ion chromatography of Azi-incorporated peptides (-2N-2H). (**f**) Tandem mass spectrum of Azi-incorporated peptides (-2N-2H). U indicates the Azi incorporation site.



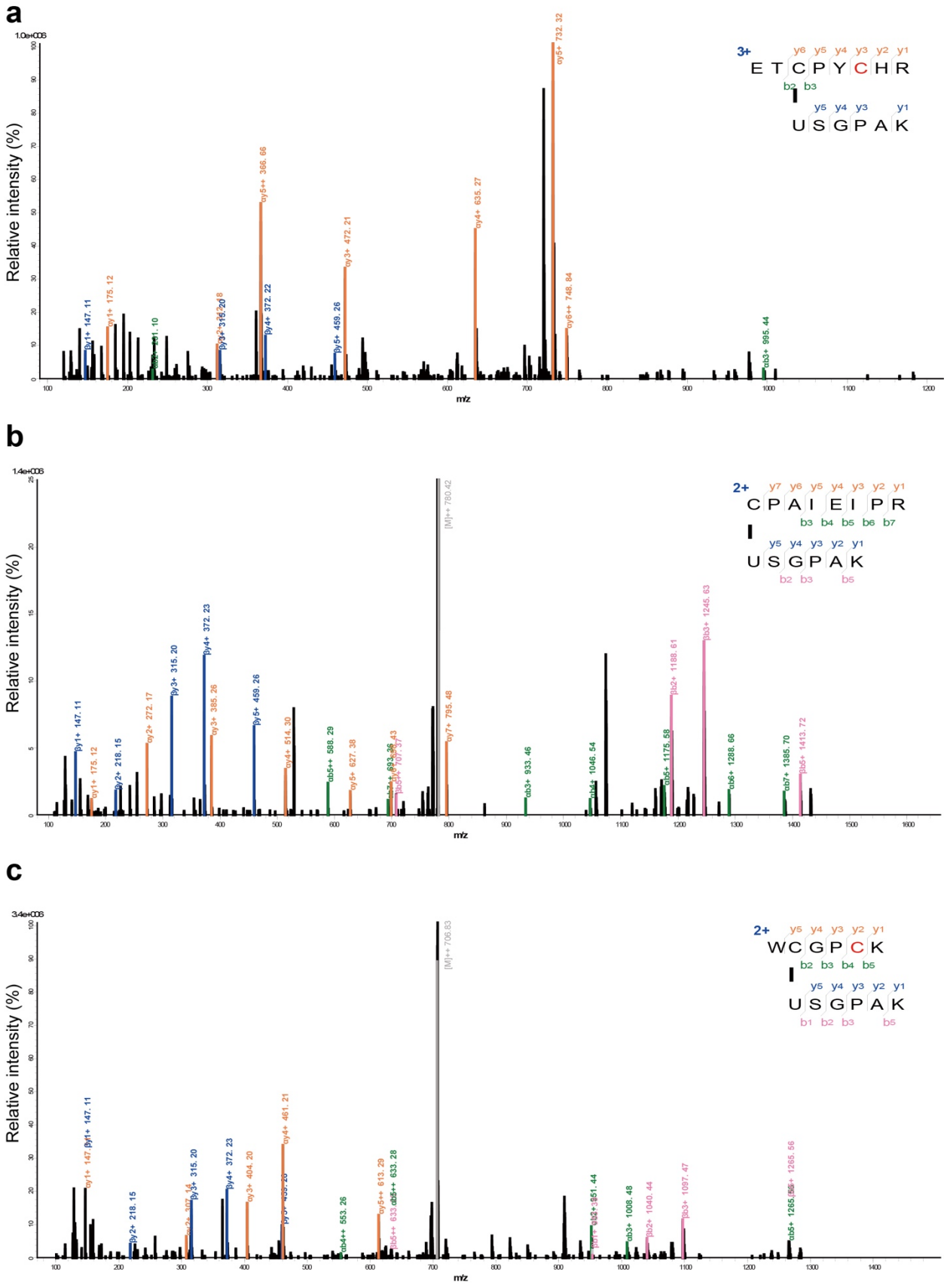

**Supplementary Figure 6. Validation of *in vivo* cross-linking of affibody(36EB3) with MBP-Z(6C) and biotin labeling of the crosslinked complex.** (**a**) Structure of the affibody-Z protein complex (PDB ID 1LP1), with two proximal sites Asp36 in the affibody and Asn6 in the Z protein highlighted. (**b**) Western blot of cell lysate of cells expressing affibody(36EB3) and MBP-Z(6C). (**c**) SDS-PAGE gel of His-tag purified proteins from cells expressing affibody(36EB3) and MBP-Z(6C). (**d**) Western blot of cross-linked affibody(36EB3) and MBP-Z(6C) before and after biotin labeling via CuAAC click reaction. (**e**) Mass spectrum of cross-linked peptide between affibody(36EB3) and MBP-Z(6C). The crosslink was clearly mapped to EB3 at site 36 of the affibody and the Cys6 of the Z protein. U represents EB3 in the peptide sequence.




**Supplementary Figure 7. *In vitro* ubiquitination assay showing that UBE2D3 ubiquitinates yPCNA.** (**a**) SDS-PAGE gel of *in vitro* ubiquitination assay. (**b**) Western blot analysis of *in vitro* ubiquitination assay. A Flag tag was appended at the N-terminus of Ubiquitin, and was detected with an anti-Flag antibody (Catalog number F9291, Sigma-Aldrich).




**Supplementary Figure 8. *In vitro* crosslinking of yPCNA with UBE2D3 using small molecule chemical cross-linker BS3 yielded no crosslinking.** (**a**) Western blot analysis of BS3 crosslinking yPCNA and UBE2D3. (**b**) SDS-PAGE gel of BS3 crosslinking of yPCNA and UBE2D3. No crosslinking band for yPCNA/UBE2D3 complex was detected in either SDS-PAGE or Western blot.


a
1.0e+006
Relative intensity (%)
m/z
3+
y6 y5 y4 y3 y2 y1
E T C P Y C H R
b2 b3
y5 y4 y3 y1
U S G P A K
y5+ 732.32
oy5++ 366.66
oy4+ 635.27
oy3+ 472.21
y4+ 372.22
y5+ 459.26
oy6++ 748.84
oy1+ 175.12
y1+ 147.11
ob3+ 995.44
b
1.4e+006
Relative intensity (%)
m/z
2+
y7 y6 y5 y4 y3 y2 y1
C P A I E I P R
b3 b4 b5 b6 b7
y5 y4 y3 y2 y1
U S G P A K
b2 b3 b5
[M]++ 780.42
y4+ 372.23
y3+ 315.20
y5+ 459.26
y1+ 147.11
y2+ 272.17
y2+ 218.15
y3+ 385.26
y4+ 514.30
y5+ 627.38
y7+ 795.48
ob5++ 588.29
ob3+ 933.46
ob4+ 1046.54
ob5+ 1175.58
ob2+ 1188.61
ob3+ 1245.63
ob6+ 1288.66
ob7+ 1385.70
ob5+ 1413.72
c
3.4e+006
Relative intensity (%)
m/z
2+
y5 y4 y3 y2 y1
W C G P C K
b2 b3 b4 b5
y5 y4 y3 y2 y1
U S G P A K
b1 b2 b3 b5
[M]++ 706.83
y1+ 147.11
y2+ 218.15
y3+ 315.20
y4+ 372.23
y3+ 404.20
y4+ 461.21
ob4++ 553.26
oy5++ 613.29
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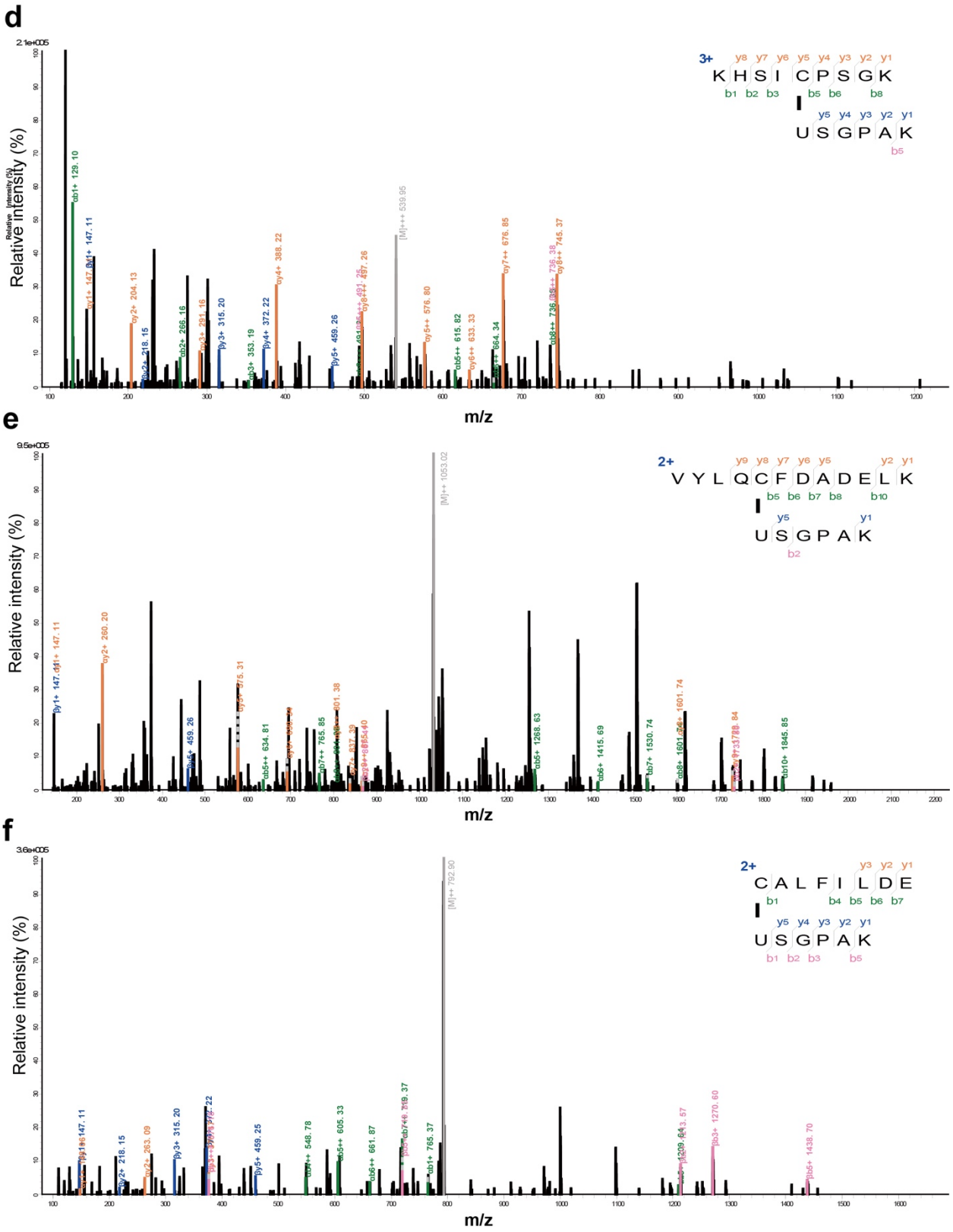
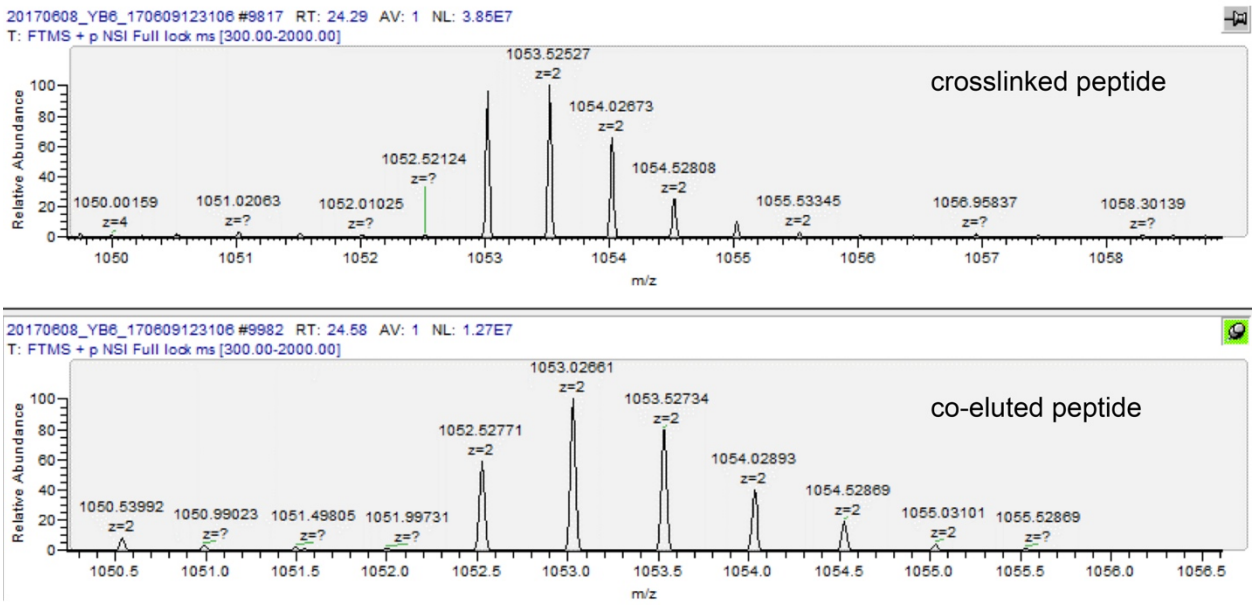
ob5+ 1265.56



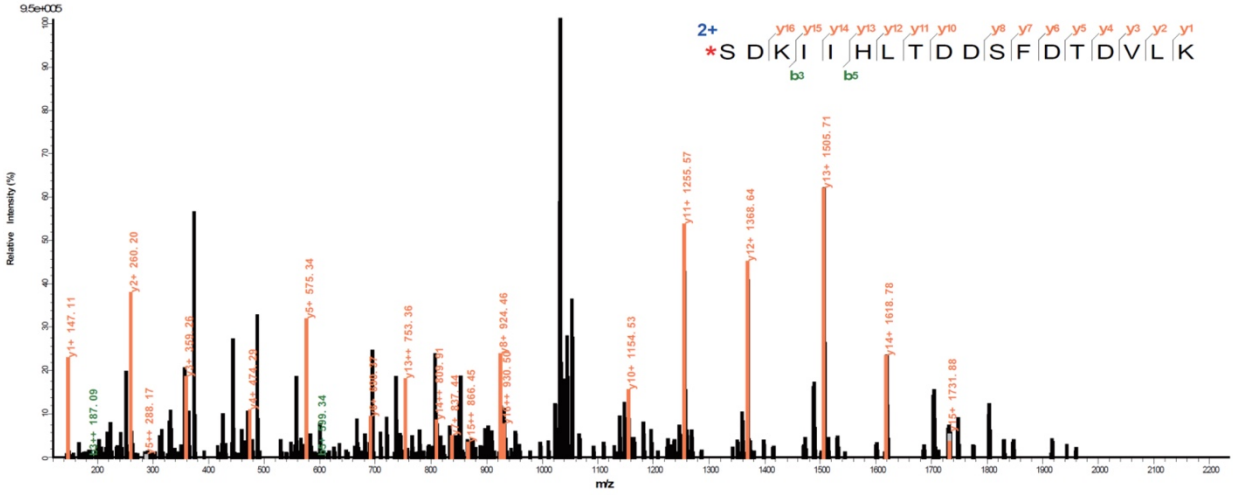

**Supplementary Figure 9. Mass spectrometric analysis of *in vivo* crosslinking of Trx1(U32-S33-A36) to endogenous proteins in *E. coli* cells.** Tandem mass spectra are shown for Trx1 crosslinked with GLRX3 (**a**), yccU (**b**), trxA (**c**), ppiC (**d**), glpQ (**e**), and dhaR (**f**). U represents

BprY in the peptide sequence. The monoisotopic peak of the precursor ion is labeled in grey. In **e**, the unlabeled major peaks came from a peptide acetyl-SDKIIHLTDDSFDTDVLK ($[M+2H]^{2+}$ = 1052.527), which was co-eluted with the crosslinked peptide (VYLQCFDADELK-USPGAK, $[M+2H]^{2+}$ = 1053.017) with similar retention time and very close m/z, as shown in Supplementary Figure 10.

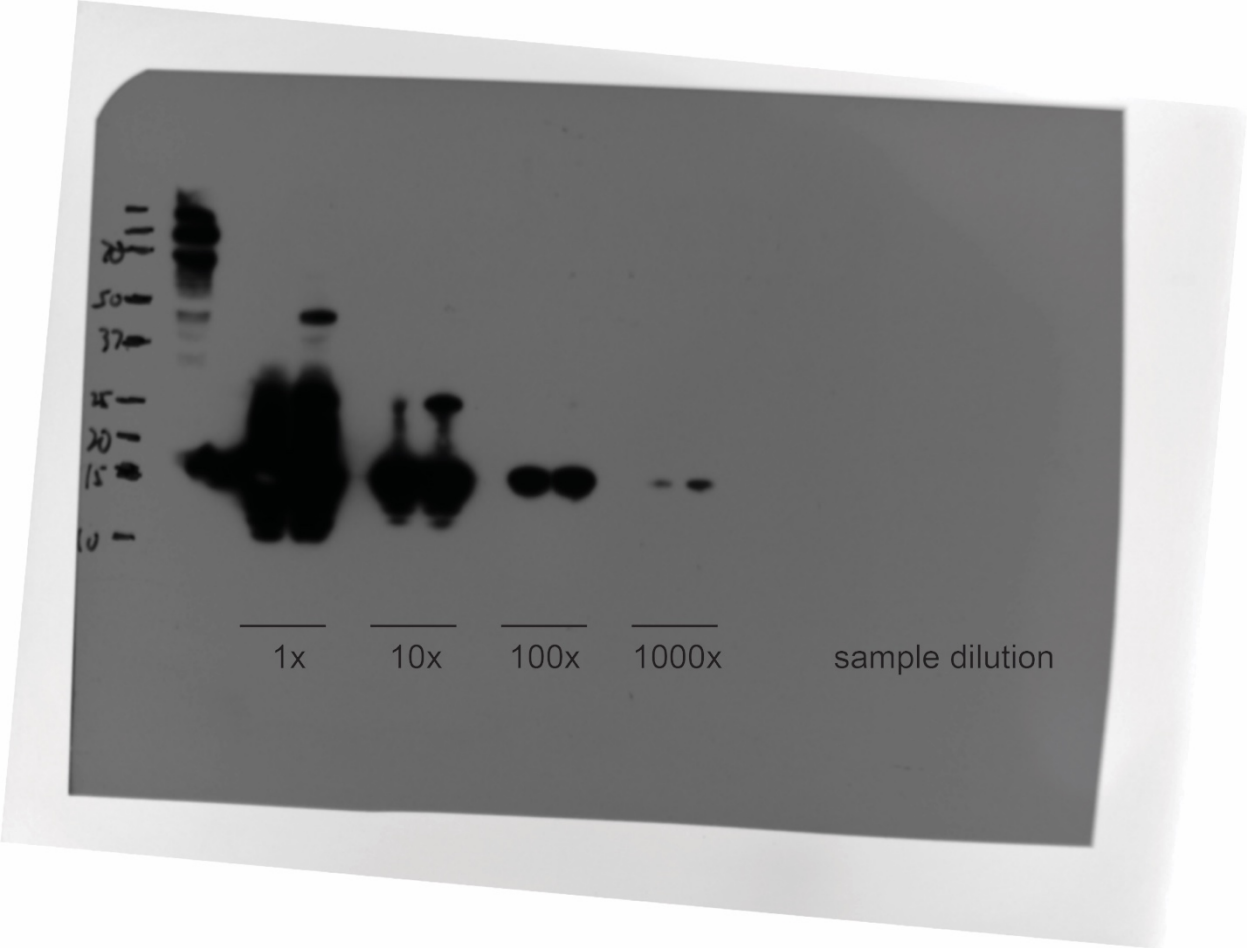
**a**




**b**




**Supplementary Figure 10. A peptide co-eluted with the crosslinked peptide in Supplementary Figure 9e.** (**a**) Precursor ion for the crosslinked peptide (VYLQCFDADELK-USPGAK, $[M+2H]^{2+}$ = 1053.017) and the co-eluted peptide (acetyl-SDKIIHLTDDSFDTDVLK, $[M+2H]^{2+}$ = 1052.527). (**b**) Tandem mass spectrum for the co-eluted peptide acetyl-SDKIIHLTDDSFDTDVLK.




**Supplementary Figure 11. Full Western blot of Figure 3c**.

**Supplementary Table 1. Thioredoxin-interacting proteins identified by GECX-MS.**

| Majority protein IDs | Fasta headers | #Peptides | Sequence coverage [%] | Mol. weight [kDa] | #Cys | Gene Name |
|---|---|---|---|---|---|---|
| A0A0A7L5L4 | L-glutamine:D-fructose-6-phosphate aminotransferase | 47 | 62.9 | 66.894 | 4 | glmS |
| A0A0A7L5N5 | Alkyl hydroperoxide reductase, F52a subunit, FAD/NAD(P)-binding | 25 | 45.9 | 56.176 | 6 | ahpF |
| A0A0A7KXP5 | Catalase HPII, heme d-containing | 24 | 36.3 | 84.162 | 2 | katE[3] |
| A0A0A7L6I2 | DNA-binding transcriptional dual regulator | 21 | 74.8 | 23.64 | 3 | crp |
| A0A0A7L310 | GTP cyclohydrolase I | 16 | 61.3 | 24.83 | 2 | folE |
| A0A0A7L6H5 | FKBP-type peptidyl prolyl cis-trans isomerase (Rotamase) | 15 | 59.7 | 20.853 | 6 | slyD[4] |
| A0A0A7L4R7 | Adenylosuccinate synthetase | 14 | 39.6 | 47.344 | 4 | purA[5] |
| A0A0A7L4A9 | Alpha-galactosidase, NAD(P)-binding | 13 | 35 | 50.657 | 12 | melA |
| A0A0A7KX67 | Uridylyltransferase | 13 | 15.6 | 102.39 | 9 | glnD |
| P39451 | Alcohol dehydrogenase, propanol-preferring | 13 | 49.1 | 35.379 | 9 | adhP[6] |
| A0A0A7KXZ0 | Fused ribonucleaseE: endoribonuclease/RNA-binding protein/RNA degradosome binding protein | 13 | 13.6 | 118.2 | 4 | rne |
| A0A0A7KYQ0 | 16S rRNA pseudouridine(516) synthase | 13 | 59.7 | 25.865 | 2 | rsuA |
| B8LFD6 | Beta-galactosidase | 12 | 13.2 | 116.48 | 16 | lacZ |
| A0A0A7L5T7 | Thioredoxin 1 | 12 | 73.4 | 11.806 | 2 | trxA[7] |

| | | | | | | |
|---|---|---|---|---|---|---|
| A0A0A7L4G9 | tRNA s(4)U8 sulfurtransferase | 11 | 20.5 | 54.972 | 5 | thiI |
| A0A0A7L661 | Protein chain elongation factor EF-Tu (Duplicate of tufA) | 11 | 34 | 43.313 | 3 | tufB[4] |
| A0A0A7L440 | Glycerol dehydrogenase, NAD | 10 | 30 | 38.712 | 7 | gldA |
| A0A0A7L0K3 | Fused histidinol-phosphatase/imidazoleglycerol-phosphate dehydratase | 10 | 31 | 40.278 | 6 | hisB |
| A0A0A7L1V2 | Succinylornithine transaminase, PLP-dependent | 10 | 24.1 | 43.665 | 6 | astC |
| A0A0A7KYL9 | Putative DNA-binding transcriptional regulator | 10 | 41 | 32.723 | 3 | yeiE |
| A0A0A7L9M9 | dTDP-4-deoxyrhamnose-3,5-epimerase | 10 | 50.8 | 21.27 | 3 | rfbC |
| A0A0A7L3M4 | Catalase-peroxidase HPI, heme b-containing | 10 | 14.6 | 80.023 | 1 | katG[7] |
| A0A0A7L1F0 | Fumarate hydratase (Fumarase A), aerobic Class I | 9 | 19.5 | 60.298 | 9 | fumA |
| A0A0A7KY21 | Uncharacterized protein | 9 | 47.2 | 21.226 | 4 | ycfP |
| A0A0A7LAG3 | DNA-binding transcriptional dual regulator of siderophore biosynthesis and transport | 9 | 57.4 | 16.795 | 4 | fur |
| A0A0A7KYZ0 | Fused UDP-L-Ara4N formyltransferase/UDP-GlcA C-4-decarboxylase | 8 | 10.9 | 74.288 | 11 | arnA |
| A0A0A7L235 | Iron-sulfur cluster assembly scaffold protein | 8 | 53.1 | 13.848 | 3 | iscU[8] |
| A0A0A7KWA5 | Fused acetaldehyde-CoA dehydrogenase/iron-dependent alcohol dehydrogenase/pyruvate-formate lyase deactivase | 7 | 8.6 | 96.126 | 9 | adhE |

| | | | | | | |
|---|---|---|---|---|---|---|
| A0A0A7L4R2 | DNA-binding transcriptional repressor, Ni-binding | 7 | 55.6 | 15.094 | 2 | nikR |
| A0A0A7L3S2 | Stationary phase protein, binds sigma 70 RNA polymerase subunit | 7 | 46.8 | 18.243 | 1 | rsd |
| A0A0A7L934 | Putative CoA-binding protein | 7 | 57.7 | 14.701 | 1 | yccU |
| A0A0A7L068 | CP4-6 prophage predicted dehydratase | 6 | 12.2 | 69.398 | 10 | yagF |
| A0A0A7L183 | Putative transcriptional regulator, PadR family | 6 | 29 | 23.401 | 8 | yqjI |
| A0A0A7L1Y3 | Dihydrodipicolinate synthase | 6 | 21.2 | 31.27 | 5 | dapA |
| A0A0A7L494 | Isocitrate lyase | 6 | 17.7 | 47.521 | 5 | aceA[9] |
| A0A0A7KXX8 | Pyruvate kinase II | 6 | 16 | 51.357 | 4 | pykA |
| A0A0A7L010 | Cysteine desulfurase (TRNA sulfurtransferase), PLP-dependent | 6 | 15.8 | 45.089 | 3 | iscS |
| A0A0A7L755 | Glutathione S-transferase homolog | 6 | 31.7 | 22.545 | 3 | yibF |
| A0A0A7L5S1 | Alkyl hydroperoxide reductase, C22 subunit | 6 | 42.2 | 20.761 | 2 | ahpC |
| A0A0A7L278 | 30S ribosomal subunit protein S4 | 6 | 21.4 | 23.469 | 1 | rpsD |
| A0A0A7L1Y9 | 30S ribosomal subunit protein S14 | 6 | 29.7 | 11.58 | 1 | rpsN |
| A0A0A7L8G9 | 2-oxoglutarate decarboxylase, thiamin-requiring | 5 | 5.6 | 105.06 | 10 | sucA |
| A0A0A7KYQ9 | D-tagatose 1,6-bisphosphate aldolase 2, subunit | 5 | 11.7 | 47.108 | 8 | gatZ |
| A0A0A7L058 | Thioredoxin 2 | 5 | 40.3 | 15.555 | 6 | trxC |

| | | | | | | |
|---|---|---|---|---|---|---|
| A0A0A7L6X8 | Thioredoxin reductase, FAD/NAD(P)-binding | 5 | 15.9 | 34.623 | 4 | trxB |
| A0A0A7L0S7 | Fructose-bisphosphate aldolase, class II | 5 | 20.9 | 39.147 | 4 | fbaA[10] |
| A0A0A7KYJ8 | D-tagatose 1,6-bisphosphate aldolase 2, catalytic subunit | 5 | 21.5 | 30.812 | 4 | gatY |
| A0A0A7L0P9 | Lipid hydroperoxide peroxidase | 5 | 44.6 | 17.835 | 3 | tpx |
| A0A0A7L4E2 | 50S ribosomal subunit protein L14 | 5 | 35.8 | 13.541 | 2 | rplN |
| A0A0A7L077 | Glutamate-cysteine ligase | 4 | 10 | 58.269 | 9 | gshA |
| A0A0A7KWQ6 | tRNA 2-thiocytidine biosynthesis protein | 4 | 14.8 | 35.561 | 8 | ttcA |
| A0A0A7L5K5 | DNA-binding transcriptional dual regulator | 4 | 11.3 | 33.384 | 5 | araC |
| A0A0A7KZG1 | NADH:ubiquinone oxidoreductase, chain E | 4 | 25.3 | 18.59 | 5 | nuoE[11] |
| A0A0A7L6B4 | Pyridoxal phosphate (PLP) phosphatase | 4 | 20.2 | 30.201 | 3 | ybhA |
| A0A0A7L6E7 | 50S ribosomal subunit protein L2 | 4 | 18.7 | 29.86 | 2 | rplB |
| A0A0A7L1S2 | Putative enzyme IIB component of PTS | 4 | 23.1 | 11.735 | 2 | fryB |
| A0A0A7L1V5 | 30S ribosomal subunit protein S13 | 4 | 31.4 | 13.099 | 1 | rpsM |
| A0A0A7L439 | Thiamin phosphate synthase | 4 | 31.2 | 15.656 | 1 | yjbQ |
| A0A0A7L1W1 | 50S ribosomal subunit protein L5 | 4 | 21.8 | 20.301 | 1 | rplE |
| A0A0A7L1V7 | D-arabinose 5-phosphate isomerase | 3 | 11.9 | 35.196 | 7 | kdsD |
| A0A0A7L4U8 | Phosphopentomutase | 3 | 9.3 | 44.369 | 6 | deoB |

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|---|---|---|---|---|---|---|
| A0A0A7L417 | 6-phosphofructokinase I | 3 | 11.2 | 34.842 | 6 | pfkA[12] |
| A0A0A7L0M7 | Colanic acid biosynthesis protein | 3 | 7.5 | 47.343 | 5 | wcaK |
| A0A0A7L707 | Methionine sulfoxide reductase A | 3 | 22.2 | 23.315 | 4 | msrA |
| A0A0A7L625 | Triosephosphate isomerase | 3 | 7.8 | 26.972 | 3 | tpiA |
| A0A0A7L222 | Glyceraldehyde-3-phosphate dehydrogenase A | 3 | 10.9 | 35.532 | 3 | gapA[13] |
| A0A0A7L2T8 | Glutaredoxin 3 | 3 | 27.7 | 9.1374 | 3 | grxC[4] |
| A0A0A7L3P2 | Sigma factor-binding protein, stimulates RNA polymerase holoenzyme formation | 3 | 13.5 | 15.655 | 3 | crl |
| A0A0A7KXQ8 | Free methionine-(R)-sulfoxide reductase | 3 | 19.4 | 18.121 | 3 | msrC |
| B8LFD8 | Galactoside O-acetyltransferase | 3 | 5.4 | 22.799 | 2 | lacA |
| A0A0A7L1X5 | 30S ribosomal subunit protein S11 | 3 | 22.5 | 13.845 | 2 | rpsK[13] |
| A0A0A7L5S4 | Peptidyl-prolyl cis-trans isomerase C (Rotamase C) | 3 | 47.3 | 10.232 | 2 | ppiC |
| A0A0A7L144 | Aminomethyltransferase, tetrahydrofolate-dependent, subunit (T protein) of glycine cleavage complex | 3 | 7.1 | 40.146 | 2 | gcvT |
| A0A0A7L3R4 | Primosome factor n (Replication factor Y) | 2 | 3.1 | 81.654 | 11 | priA |
| A0A0A7L866 | Lipoate synthase | 2 | 8.7 | 36.071 | 8 | lipA |
| A0A0A7KYS5 | DNA-binding transcriptional dual regulator, global regulator of anaerobic growth | 2 | 9.2 | 27.967 | 5 | fnr |
| A0A0A7L2E8 | 30S ribosomal subunit protein S12 | 2 | 12.1 | 13.71 | 4 | rpsL[14] |

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|---|---|---|---|---|---|---|
| A0A0A7L5W3 | Pyrroline-5-carboxylate reductase, NAD(P)-binding | 2 | 7.4 | 28.145 | 4 | proC |
| A0A0A7KYV6 | DNA-binding transcriptional regulator of rRNA transcription, DnaK suppressor protein | 2 | 11.9 | 17.528 | 4 | dksA |
| A0A0A7L338 | Protein export chaperone | 2 | 12.3 | 17.337 | 4 | secB |
| A0A0A7L2V1 | Galactitol-specific enzyme IIB component of PTS | 2 | 28.7 | 10.222 | 4 | gatB |
| A0A0A7L283 | Fe/S biogenesis protein possible scaffold/chaperone for damaged Fe/S proteins | 2 | 14.7 | 20.997 | 4 | nfuA |
| A0A0A7L3K0 | Putative cytoplasmic sugar-binding protein | 2 | 7.2 | 15.292 | 3 | rbsD |
| A0A0A7L1I8 | Glutaredoxin-4 | 2 | 21.7 | 12.879 | 3 | grxD |
| A0A0A7KX48 | Iron-sulfur cluster insertion protein | 2 | 18.4 | 12.1 | 3 | erpA |
| A0A0A7L0C6 | 2-keto-3-deoxy gluconate (KDG) aldolase CP4-6 prophage | 2 | 7.6 | 32.53 | 3 | yagE |
| A0A0A7L2B9 | 30S ribosomal subunit protein S17 | 2 | 20.2 | 9.7043 | 2 | rpsQ |
| A0A0A7L2X6 | Putative acyltransferase with acyl-CoA N-acyltransferase domain | 2 | 13 | 17.104 | 2 | yiaC |
| A0A0A7L368 | 50S ribosomal subunit protein L28 | 2 | 12.8 | 9.0064 | 1 | rpmB |
| A0A0A7L6A1 | 50S ribosomal subunit protein L17 | 2 | 8.7 | 14.364 | 1 | rplQ |
| A0A0A7L1H3 | 30S ribosomal subunit protein S21 | 2 | 14.1 | 8.4999 | 1 | rpsU |

MsrA, MsrC, Tpx, and AhpC are well-known substrate proteins of *E. coli* Trx1. Genes for proteins that are known to interact with Trx in other organisms are labeled with reference numbers, and the references are listed in Supplementary Reference below.

## Supplementary Reference


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