Electronic Supplementary Information

Supra-blot: an accurate and reliable assay for detecting target proteins with a synthetic host molecule-enzyme hybrid

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General procedures

All the reagents and solvents employed were commercially available and used as supplied without further purification. The nuclear magnetic resonance (NMR) spectra were acquired at 298 K on a Bruker Advance 850 MHz. High-resolution mass (electrospray ionization, ESI) data were obtained with a SYNAPT G2 (Waters, U.K.) mass spectrometer at the Korea Basic Science Institute (Ochang, Korea). Mass (MS) analysis was performed using a LTQ-XL mass spectrometer (Thermo Fisher Scientific, Inc.) equipped with an electrospray ionization (ESI) source and Orbitrap Q-Exactive Plus (Thermo Fisher Scientific, Inc.) equipped with a quadrupole mass spectroscopy. For automatic measurement and data analysis for the mass spectroscopy, Xcalibur software was used (Thermo Fisher Scientific, Inc.). MALDI-TOF spectra were acquired on an Autoflex speed (Bruker). UV-Vis absorption was recorded by using an Agilent Cary 5000 UV-Vis Spectrophotometer. For protein analysis, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), western blotting and fluorescence gel imaging were obtained as images using ImageQuant Las500 instrument (GE healthcare) and G:BOX Chemi XRQ instrument (Syngene). Monoamine cucurbit[7]uril,[S1] AdA-FITC,[S2] AdA-TEG-NH_2,[S3] AdA-TEG-COOH,[S3] AdA-phenol[S3]and AdA-BG[S3] were synthesized by following the previous reports.

Preparation of Cucurbit[7]uril-conjugated horseradish peroxidase (CB[7]-HRP) (Fig. S1)

Aldehyde-functionalized horseradish peroxidase (Act-HRP, 200 μg, 4.7 nmol, EZ-Link™ Plus Activated Peroxidase Kit, Prod# 31489, Lot# RJ234706) was dissolved in milliQ-water (100 μL). A solution of monoamine cucurbit[7]uril (MA-CB[7], 180 μg, 140 nmol)[S1, S2] in dimethyl sulfoxide (DMSO, 120 μL) was mixed with the solution of Act-HRP and stirred for 1 h at RT. Then, the reaction mixture was treated with a solution of sodium cyanoborohydride (2 μL, 5 M) in water. After additional stirring for 1 h, the reaction was quenched with a solution of ethanolamine buffer (5 μL, 3 M, pH 9). After addtionally stirring for 15 min, the target protein was purified through centrifugal filteration (MWCO 10 kDa). The number of MA-CB[7] conjugated to HRP was confiremd to be *ca.* 2 by MALDI-TOF (See Fig. S3)

Confirmation of CB[7] conjugation to HRP (by fluorescence gel imaging)

All samples, MA-CB[7] (0.1 μg), native HRP (2 μg) and CB[7]-HRP (2 μg) with adamantane-conjugated fluorescein isothiocyante (AdA-FITC, 4 μg), were resolved by SDS-PAGE (12% gel). For the fluorescence gel imaging corresponding to AdA-FITC, the gel was imaged by ImageQuant Las500 with oragne filter (560LP). In addition, the gel was stainned with coomassie brilliant blue (CBB) staining solution for 6 h to visualize the target protein in the gel. (See Fig. S2)

Enzyme activity assay for CB[7]-HRP

A solution (50 μL) of *o*-dianisidine solution (1% wt/v) in methanol was added to PBS (0.01 M, 6 mL, pH 7) containing H_2O_2 (0.003% v/v). To the mixted solution (2.9 mL), HRP or CB[7]-HRP (0.1 μg) in PBS (100 μL, 0.01M, pH 7) was added. A solution of PBS buffer (100 μL) without HRP or CB[7]-HRP was served as control. The reactions were monitored by UV-VIS spectrophotometer at 460 nm (the same expreiemnts were repeated 5 times for HRP and CB[7]-HRP respectively). Enzyme activity was derived from the equation below.

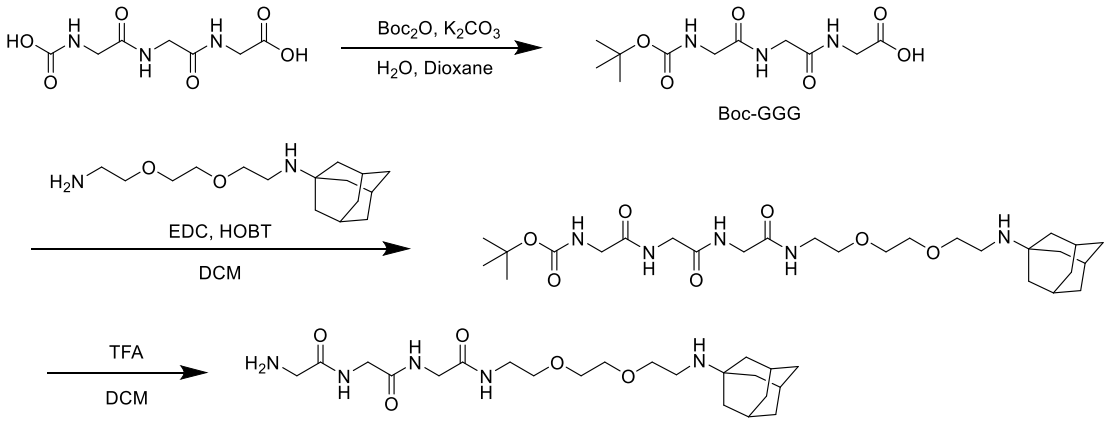
OD_{460}/min = ($OD_{460\text{-}HRP}$/3 min – $OD_{460\text{-}ctrl}$/3 min)/3 min.
mg enzyme / mL reaction mixture = [Enzyme dilution]/30

units/mg = (OD460/min) / ((11.3*mg enzyme)/ mL reaction mixture)

*11.3 is extinction coefficient.
One unit of activity is the amount of enzyme to decompose 1 μmole of peroxide / minute at 25 °C.

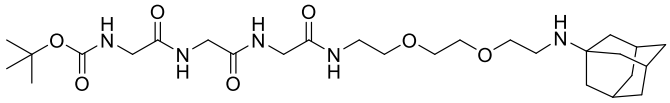
Preparation of AdA-conjugated BSA (AdA-BSA)
A solution of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 1.5 mg), *N*-hydroxysuccinimide (NHS, 1.0 mg) and AdA-TEG-COOH (2.6 mg) in dimethylformamide (DMF, 200 μL) was stirred for 1 h. To the reaction mixture, bovine serum albumin (BSA, 5 mg) in PBS (2 mL) was added. After additional stirring for 30 min at RT, the target protein was purified by centrifugal filtration (MWCO 30 kDa). Cross-linked AdA-BSA was removed by centrifugal filtration (MWCO 100 kDa), then the target protein in the filtrate was obtained by additional centrifugal filtration (MWCO 30 kDa). Successful conjuagtion (*ca.* 6 of AdA-TEG-COOH) was confiremd by MALDI-TOF (See Fig. S4)

Detection of AdA-conjugated BSA by CB[7]-HRP without or with cell lysate.
BSA and AdA-BSA (200 ng, respectively) without (or with) lysate of COS7 (10 μg) were resolved by SDS-PAGE (10% gel). All the samples were transfered onto the nitrocellulose (NC) membrane at 400 mA for 2 h. The membrane was stainned with a solution of Ponceau S (0.1% w/v) in 5% acetic acid in water (50 mL) for 1 min. The stained membrane was imaged by ImageQuant Las500 and destained by washing with tris-buffered saline with Tween 20 (0.1 % v/v) (TBST) for 5 min. The membrane treated with a soltuion of skimmed milk (5% w/v) in TBST (50 mL) was shaken for 1 h at RT for blocking. The blocked membrane was incubated with CB[7]-HRP diluted 1:2500 in a solution of BSA (5% w/v) in TBST (10 mL) on the shaker for 1 h and subsequently washed with TBST (20 mL) for 5 times (5 min per each). By treating with an enhanced chemiluminescence kit (Clarity™ Western ECL Substrate, Bio-Rad), the membrane was developed to show a chemiluminescence signal, which was imaged by ImageQuant Las500 instrument. (See Fig. S5)



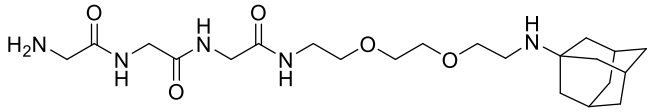
Scheme S1. Synthetsis of GGG-AdA

Synthesis of Boc-GGG-AdA



Boc-GGG (194 mg), EDC·HCl (154 mg) and 1-hydroxybenzotriazole (HOBt, 105 mg) in dichloromethane (DCM, 5 mL) were stirred at 0 °C for 15 min. To the reaction mixture, AdA-TEG-NH_2 (190 mg) in DCM (2 mL) was added dropwise. The reaction mixture was stirred for overnight in RT and dried under the reduced pressure. The product was purified by silica column chromatography (eluent, DCM:MeOH:NEt_3 = 90:9:1) to give a pale yellow oily comound (290 mg, 78%). ^{1}H NMR (850 MHz, CD_3OD): δ 3.92 (2H, s), 3.88 (2H, s), 3.77 (2H, s), 3.65-3.62 (6H, m), 3.59 (2H, t, J = 5.1 Hz), 3.59 (2H, t, J = 5.7 Hz), 2.85 (2H, t, J = 5.4 Hz), 2.11 (3H, s), 1.75-1.73 (9H, m), 1.69 (3H, d, J =11.0 Hz), 1.47 (9H, s). ^{13}C NMR: δ 170.8, 170.2 (2C), 157.4, 79.54, 69.92, 69.40, 68.99, 56.71, 44.00, 43.54, 42.41, 42.03, 41.05, 40.88, 39.2, 38.9, 36.1, 27.3. ESI-MS (m/z): $[M+H]^+$ calcd for $C_{27}H_{47}N_5O_7$, 554.36; found, 554.49.

Synthesis of GGG-AdA



Trifluoroacetic acid (TFA, 200 µL) was added to a soluton of Boc-GGG-AdA (25 mg) in DCM (800 µL) and stirred at 0 °C for 2 h. The reaction mixture was dried under the reduced pressure and washed with cold ether (-20 °C) to give the target product. The product was dissolved in deionized water and lypholized to give a white solid (20 mg, 88%). ^{1}H NMR (850 MHz, CD_3OD): δ 4.00 (2H, *s*), 3.89 (2H, *s*), 3.79 (2H, *s*), 3.75 (2H, *t*, *J* = 5.1 Hz) 3.68 (4H, *s*), 3.60 (2H, *t*, *J* = 5.5 Hz), 3.43 (2H, *t*, *J* = 5.1 Hz), 2.85 (2H, *t*, *J* = 5.4 Hz), 2.23 (3H, *s*), 1.95 (6H, *s*), 1.82 (3H, *d*, *J* =12.2 Hz), 1.75 (3H, *d*, *J* =12.1 Hz). ^{13}C NMR: δ 171.7, 170.5, 161.3, 70.01, 69.01, 66.07, 57.05, 42.18, 42.03, 40.19, 39.58, 38.79, 37.86, 35.15, 29.16, 29.13. HR-ESI-MS (m/z): $[M+H]^+$ calcd for $C_{22}H_{39}N_5O_5$, 454.3029; found, 454.3036.

Preparation of Sortase A (Srt A-His$_6$)

The plasmid DNA encoding Sortase A was transformed into *Escherichia coli (E. coli)* BL21 (DE3). The transformed bacteria cells were spread onto a luria-bertani (LB) agar plate containing kanamycin (50 µg/mL) to select the transformed bacteria colonies. After the selection of colonies, selected colony was inoculated into a LB media (10 mL) with kanamycin (500 µg) and incubated at 37 °C overnight. Expression of the recombinant Sortase A was induced at an OD_{600} of ~0.8 by adding a solution of isopropyl β-D-1-thiogalactopyranoside (IPTG, 0.5 M, 300 µL) in deionized and filtered water (Milli-Q water) at 25 °C overnight. The bacteria cell containing media was centrifuged at 6,000 rpm to provide cell pellet. The pellet was resuspended using Tris buffer (20 mL, 50 mM Tris-HCl, 150 mM NaCl, 2 mM $MgCl_2$, pH 7.5) with protease inhibitors (1X protease inhibitor cocktail dissolved in Milli-Q water). A solution of lysozyme (50 mg/mL) in water (20 µL) was added to the resuspended solution of bacateria cells and incubated in ice bath for 30 min. The bacteria cells were lysed by using tip-sonicator and the cell debris was removed by centrifugation at 12,000 rpm for 30 min at 4 °C. The soluble portion of lysate was incubated with nickel-nitriloacetic acid (Ni-NTA) resin in Tris buffer (10 mL) at 4 °C for 2 hours. The lysate incubated resins were washed with Tris buffer (50 mL) and Tris buffer (50 mL) with immidazole (50 mM) sequentially on a disposable gravity column. The proteins bound on the resins were eluted by using Tris buffer (35 mL) with imidazole (250 mM). The eluted solution was dialyzed by dialysis tubing (SnakeSkin^{TM} dialysis tube, ThermoFisher, MWCO 10 kDa) with Tris buffer. The target protein the target protein was purified by centrifugal filtration (Amicon Ultra, Millipore, MWCO 10 kDa). The target protein was characterized by MALDI-TOF. (Fig. S8)

Preparation of GFP-LPETG-His$_6$ and *E.coli* cell lysate

The same procedure was applied as above except using the plasmid DNA encoding for GFP-LPETG-His6 intead of Sortase A-His$_6$. PBS buffer was used for lysis, purification by using Ni-NTA resins and dialysis instead of Tris buffer. A solution of LB media containing GFP-LPETG expressing bacteria cells was centrifuged in 1.5 mL tube to collect the cells. The prepared cell pellet was washed with LB media (1 mL) and subsequently centrifuged to remove the remaining LB media. The cell pellet was re-suspended with Tris buffer (500 µL) and kept in an ice bath for 30 min. The cells were lysed by using tip-sonicator and centrifuged at 12,000 rpm for 30 min at 4 °C. The resulting supernatant was separated as cell lysate for the ligation reaction.

Sortase A based Ligation: bacteria cell lysate containing GFP-LPETG-His$_6$ with purified Sortase A^{S4}

A cell lysate (40 µL, 300 µg/mL) containing GFP-LPETG was incubated with GGG-TEG-AdA (1 µL, 10 mM) and Sortase A (0.44 µL, 22 µM in MilliQ water) at 37 °C for 18 h.

Detection of AdA-GFP expressed from *E. coli* using Supra-blot

A specific protein in bacterial cells, green fluorescent protein (GFP) with an additional LPETG peptide sequence (GFP-LPETG) expressed as a target protein in bacterial cells and labeled with GGG-AdA using Sortase A-mediated transpeptidation (Fig. S6) after lysis the bacterial cells. This approach enabled us to site-specifically conjugate GGG-containing AdA derivatives (GGG-AdA; Scheme S1) to the LPETG motif on GFP for production of GFP-LPETGGG-AdA (Fig. S6a). After the sortase A liagation as stated above, cellular proteins in lysate were well-resolved on the NC membrane as multiple bands following staining with Ponceau S (Fig. S6). Successful expression of GFP-LPETG in bacteria was confirmed by observation of a strong chemiluminescent band at a

size equal to that of the target protein (~29 kDa) following conventional western blotting with anti-GFP primary antibody and HRP-conjugated secondary antibody upon treatment with luminol and H_2O_2 (Fig. S6c). In addition, Supra-blot analysis of the NC membrane allowed us to observe a single chemiluminescence band among a number of cellular proteins in the lysate of GFP-LPETG-expressing bacteria only when incubated with Sortase A and GGG-AdA (lane 4 in Fig. S6b). The presence of the clear chemiluminescence signal indicates that CB[7]-HRP selectively detected the AdA-labeled protein expressed in bacteria via genetic engineering.

Mammalian cell culture and transfection of genes to the cells (SNAP-tag)

HEK293T cells were cultured using Dulbecco's modified Eagle's medium (DMEM, Hyclone) containing fetal bovine serum (FBS, Gibco, 10% v/v) and penicillin/streptomycin (Gibco, 1% v/v) in a humidified 5% CO_2 atmosphere at 37 °C. The cells were transfected with plasmid (4 μg) encoding a recombinant gene using Lipofectamine™ 2000 (Invitrogen, 40 μL).

SNAP-tag based histone H3 labeling with AdA-BG[S6]

After 24 h from the transfection of H3-SNAP-FLAG to HEK293T cells, the cells were harvested and centrifuged at 14,000 rpm for 10 min at 4 °C. After centrifugation, the cell pellets were resuspended with 1.0 mL PBS and lysed with sonication. The cell debris was cleared by centrifugation at 14,000 rpm for 10 min at 4 °C. The resulting supernatant (100 μL) was treated with AdA-BG[S3] (10 μM in DMSO) and DTT (5 mM in MilliQ water), then incubated for additional 30 min at 37 °C. The reaction mixture was treated with 5X SDS sample buffer (25 μL) and heated at 95 °C for 5 min. All samples were resolved by SDS-PAGE and transferred to a PVDF membrane. The membrane was stainned with a solution of Ponceau S (0.1% w/v) in 5% acetic acid in water (50 mL) for 1 min. The stained membrane was imaged by ImageQuant Las500. The stainned membrane was destained by washing with tris-buffered saline with Tween 20 (0.1 % v/v) (TBST) for 5 min and blocked with skimmed milk (5% w/v) in TBST (20 mL) on shaker for 30 min at RT. The blocked membrane was incubated with CB[7]-HRP diluted 1:2500 in a solution of skimmed milk (5% w/v) in TBST (10 mL) for 1 h and subsequently washed 5 times for 5 min each with TBST (20 mL). By treating the washed membrane with ECL substrate, the membrane was developed to show the chemiluminescence signal. The membrane with chemiluminescence signal was imaged by ImageQuant Las500 instrument. (Fig. S7)

Mammalian cell culturing and transfection (APEX2 enzymes)

HEK293 cells were cultured using DMEM (Hyclone) containing FBS (10% v/v) and penicillin/streptomycin (Gibco, 1% v/v) in a humidified 5% CO_2 atmosphere at 37 °C. The cells were transfected at 60–80% confluence using Turbofect (Thermofisher). Turbofect (6 μL) and APEX2 plasmid DNA (2 μg) were used per well.

AdA-phenol and Biotin-phenol labeling in live cells

After 18–24 h from transfection of APEX2 to HEK293 cells, the medium was changed to 1 mL of a fresh growth medium containing AdA-phenol (250 μM) or biotin-phenol (500 μM). The cells were incubated in a humidified 5% CO_2 atmosphere at 37 °C for 30 min. Then, hydrogen peroxide (H_2O_2, 110 μL, 10 mM diluted from 30% H_2O_2, H1009, Sigma Aldrich, St. Louis, MO, USA) was added to each well for a final concentration of 1 mM, and the plate was gently agitated for 1 min at RT. After quenching the reaction by washing with a solution of Dulbecco's Phosphate buffered

saline (DPBS, 2 mL) containing Trolox (5 mM), sodium azide (10 mM) and sodium ascorbate (10 mM) for 5 times, the cells were lysated for western blot (Fig. S11) and Supra-blot (Fig. 3) analysis.

Western blot analysis of AdA-phenol and Biotin-phenol labeling and expressed V5-tagged APEX2 (Fig. S9, S10, and S11)

After AdA-phenol or biotin-phenol labeling, the cells were harvested and lysed with RIPA lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate and 1% Triton X-100) containing 1X protease cocktail (Thermofisher, catalog no. 78438), sodium azide (10 mM), sodium ascorbate (10 mM) and Trolox (5 mM) for 10 min at 4 °C. The cell debris was removed from the lysates by centrifuging at 15,000 g for 10 min at 4 °C. The lysates were resolved by SDS-PAGE (10% gels) and transferred to NC membranes. The membranes were stained with a solution of Ponceau S (0.1% w/v) in 5% acetic acid in water (20 mL) for 10 min. The stained membranes were washed with TBST for 10 min. The membranes were treated with a solution of dialyzed BSA (2% w/v) in TBST (20 mL) at RT for 1 h.

Streptavidin-HRP or CB[7]-HRP: (Detection of BT- or AdA-labeled proteins)

The blocked membrane was incubated with SA-HRP (Thermo Scientific, #21126) diluted 1:10000 or CB[7]-HRP diluted 1:3000 in a solution of blocking buffer on the shaker for 1 h at RT and subsequently washed 4 times for 5 min each with blocking buffer. By treating the washed membrane with enhanced chemiluminescence kit (Clarity™ Western ECL Substrate, Bio-Rad), the membrane was developed to show a chemiluminescence signal. The membrane with chemiluminescence signal was imaged by G:BOX Chemi XRQ instrument (Syngene).

Conventional western blot: (Enzyme expression Level)

The blocked membrane was incubated with anti-V5 primary antibody (mouse, Invitrogen) diluted 1:10000 in a solution of blocking buffer on the shaker for 1-2 h at RT and subsequently washed 4 times for 5 min each with TBST. The washed membrane was incubated with HRP-conjugated anti-mouse secondary antibody (Bio-Rad) diluted 1:3000 in a solution blocking buffer on the shaker for 1 h at RT. The membranes were washed 4 times for 5 min each with TBST. By treating the washed membrane with enhanced chemiluminescence kit (Clarity™ Western ECL Substrate, Bio-Rad), the membrane was developed to show a chemiluminescence signal. The membrane with chemiluminescence signal was imaged by G:BOX Chemi XRQ instrument (Syngene).

Comprehensive description for correlation value equation

Line scans analysis of CB[7]-HRP and streptavidin-HRP based western blot images were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) after applying background subtraction in the software. Our formulation for the correlation values is based on a correlation function appearing in the signal process, where the autocorrelation function for a discrete signal $y(n)$ is defined as $C(m) = \sum_n y(n)y(n-m)$. We apply this to quantify the similarity of two barcodes by associating two digitized intensities, $I_i(n)$ and $I_j(n)$, to two discrete signals. Also $m = 0$, because all the intensities are given for the same sequence of mass density. Thus the correlation value was calculated by $\sum_{k=1}^{N} I_i(k)I_j(k)/N$. A normalization factor needs to be considered, because the overall intensity varies from one barcode to another. For this reason, we make the correlation value equal to one, when two barcodes are the same kind. Thereby we formulate the correlation value as following (or Equation 1).

$$C_{ij} = \frac{\frac{1}{N}\sum_{k=1}^{N} I_i(k) I_j(k)}{\sqrt{\frac{1}{N}\sum_{k=1}^{N} I_i(k)^2}\sqrt{\frac{1}{N}\sum_{k=1}^{N} I_j(k)^2}}$$

Supra-blot on *Caenorhabditis elegans* (*C. elegans*)

C. elegans (wild-type N2) was provided by Caenorhabditis Genetics Center (CGC), University of Minnesota, and maintained as required. Briefly, C. elegans was cultured on solid Nematode Growth Medium (NGM) plates and fed with a lawn of OP50 (a uracil auxotrophic Escherichia coli strain which grows slowly on NGM plates) at RT. 2,000 larvae at L3 stage were isolated and washed with M9 buffer (1.0 mL) three times. The isolated larvae were suspended in PBS (200 µL) and heated at 95 °C for 5 minutes followed by lysis using a probe sonicator. 5x SDS sample buffer (50 µL) was added to the lysate and heated at 95 °C for 5 minutes. The samples were analyzed with Supra-blot and western blot with CB[7]-HRP and SA-HRP, respectively (See Fig. S12).

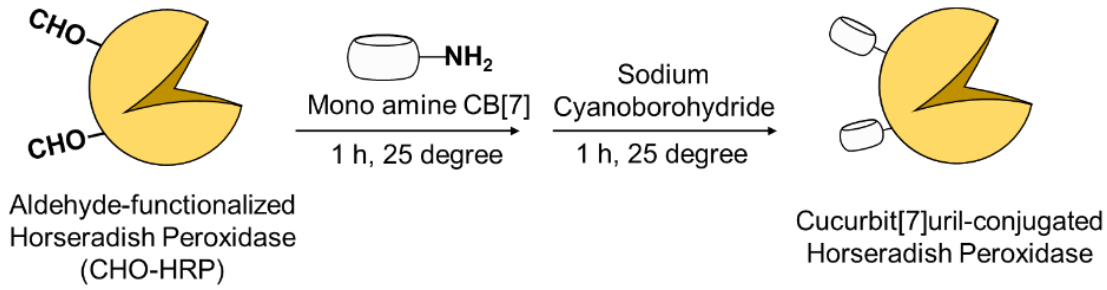


Fig. S1. Schematic illustration of preparation of CB[7]-HRP.

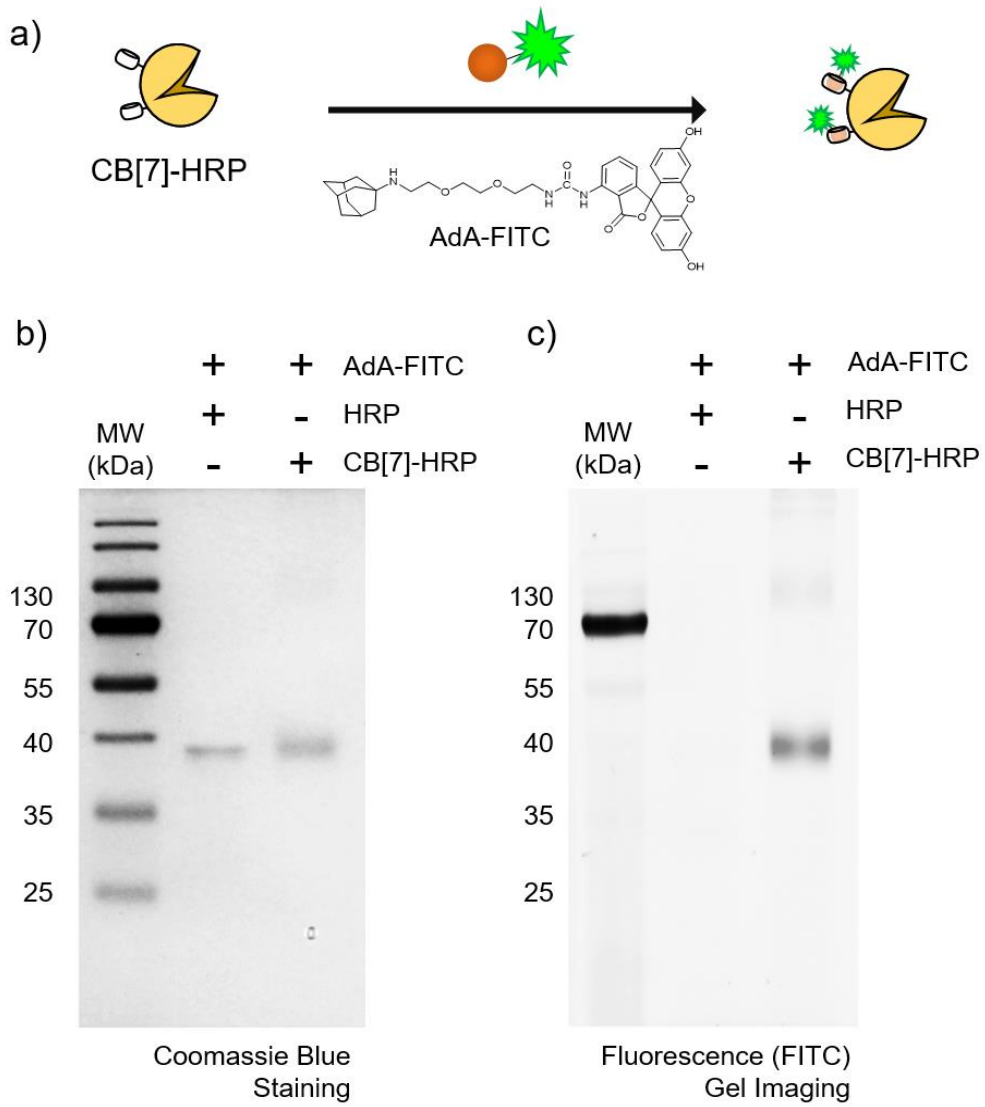


Fig. S2. a) Schematic illustration of ultrastable host-guest complex formation between AdA-FITC and CB[7]-HRP, and visualization of proteins by b) CBB staining and c) AdA-FITC fluorescence signal detection.

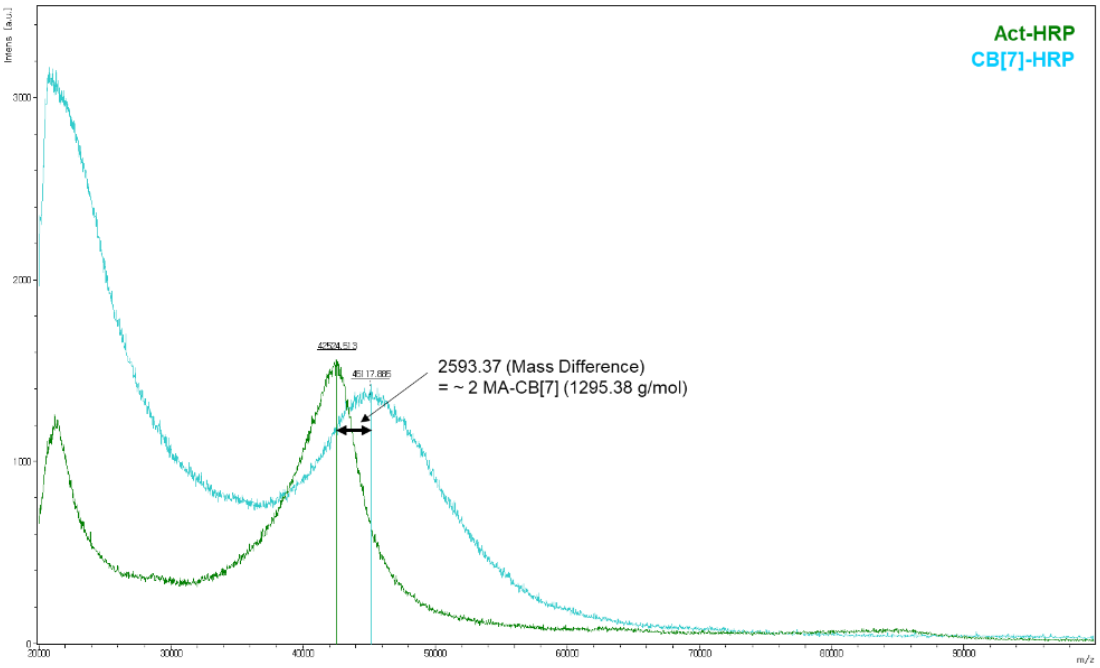


Fig. S3. MALDI-TOF MS spectra of CB[7]-HRP and Act-HRP.

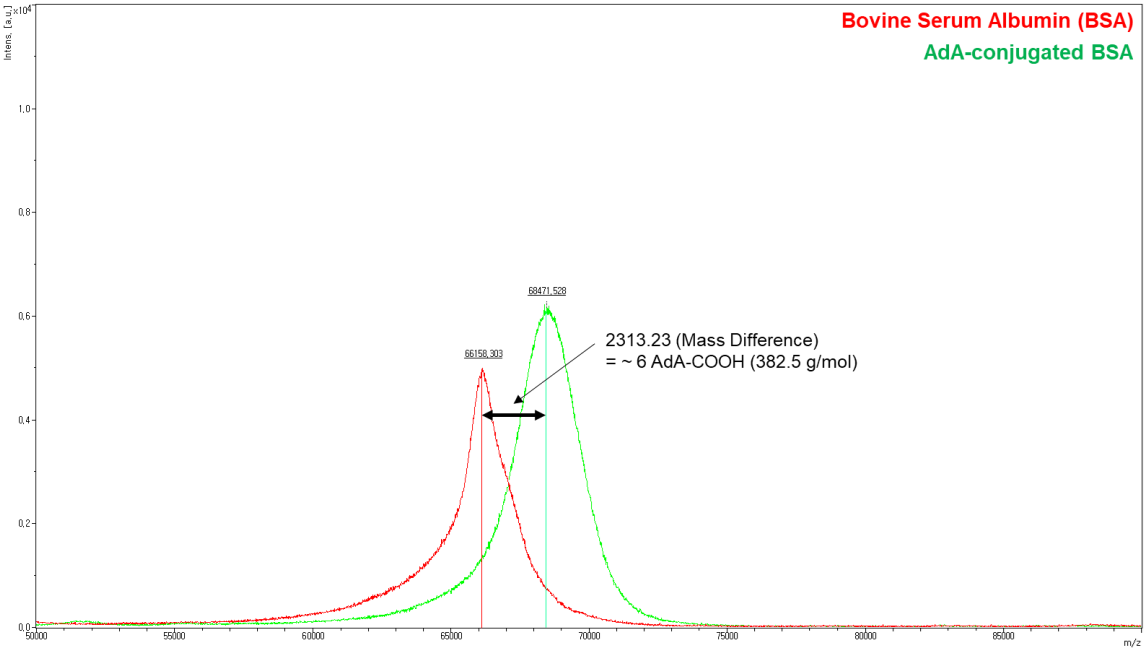


Fig. S4. Characterization of BSA and AdA-conjugated BSA to confirm the number of AdA ligands conjugated to the BSA by MALDI-TOF.

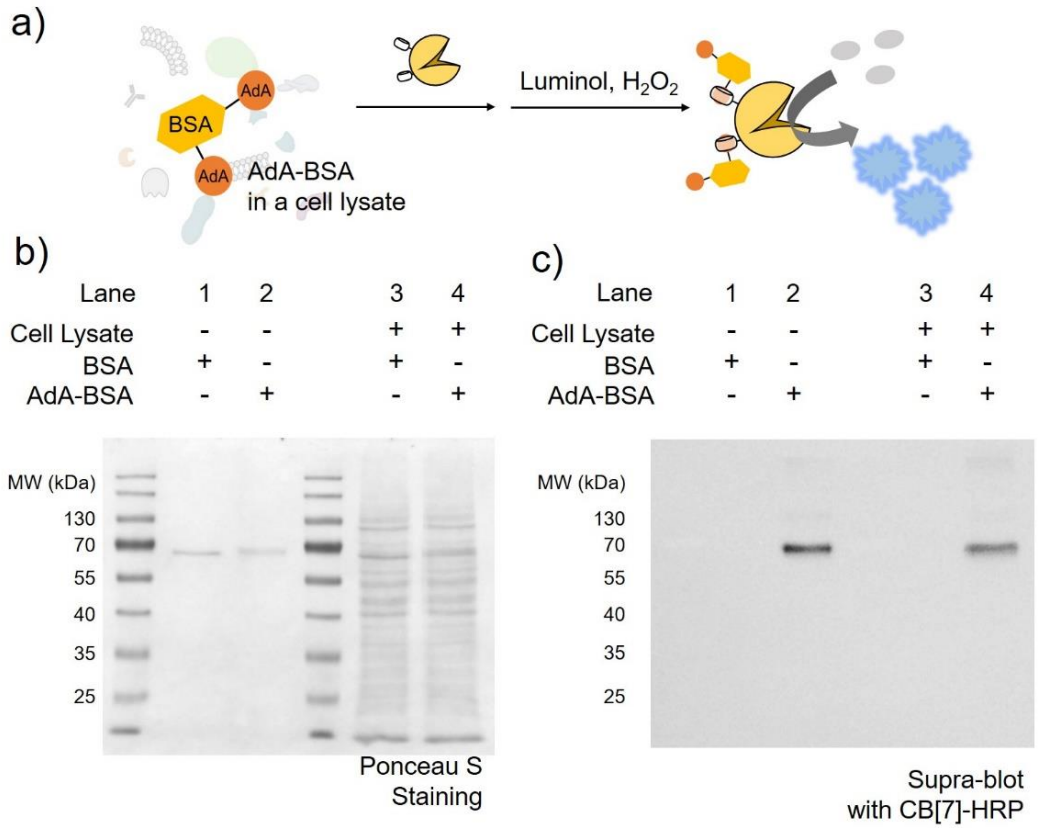


Fig. S5. a) Schematic illustration of AdA-BSA detection in a cell lysate. b) Detection of proteins by Ponceau S staining and c) Supra-blotting using CB[7]-HRP.

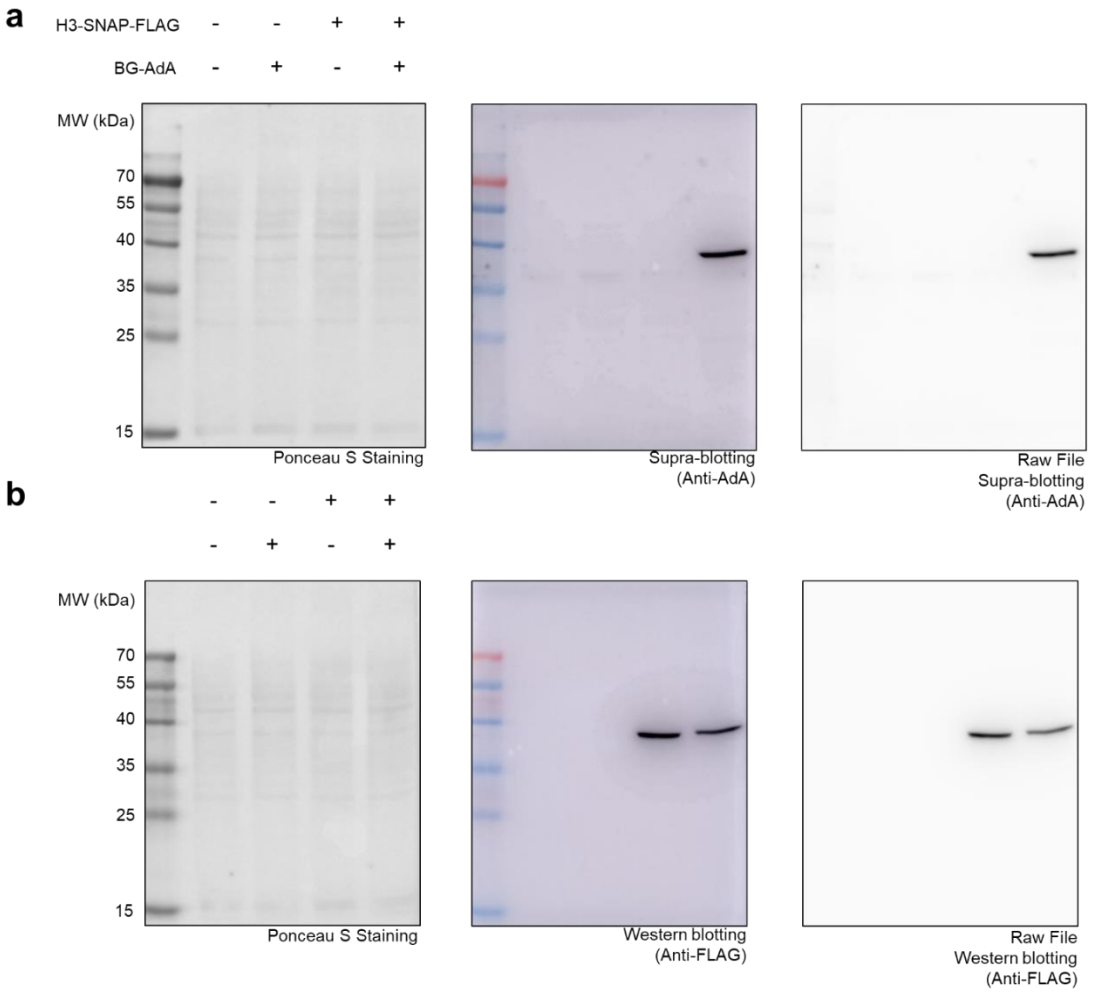


Fig. S6. SNAP-tag based BG-AdA labeling to H3-SNAP-FLAG and analysis of proteins by Ponceau S staining and (a) Supra blotting using CB[7]-HRP, (b) western blotting using anti-FLAG antibody.

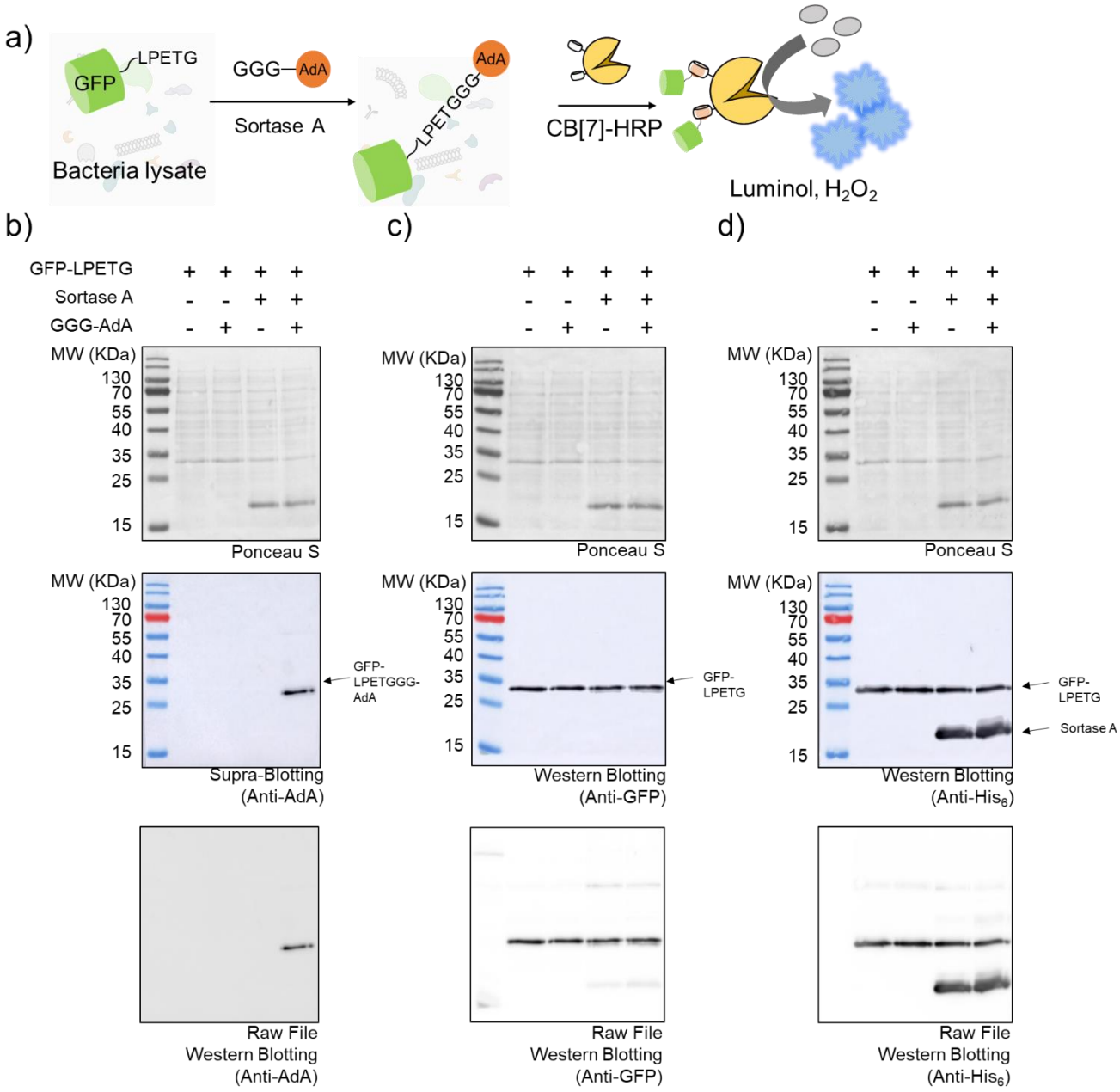


Fig. S7. a) Schematic illustration of AdA labelling of GFP using sortagging. b) Proteins detected by Supra-blot with CB[7]-HRP, c) western blot with anti-GFP antibody and d) western blot with anti-His6 antibody

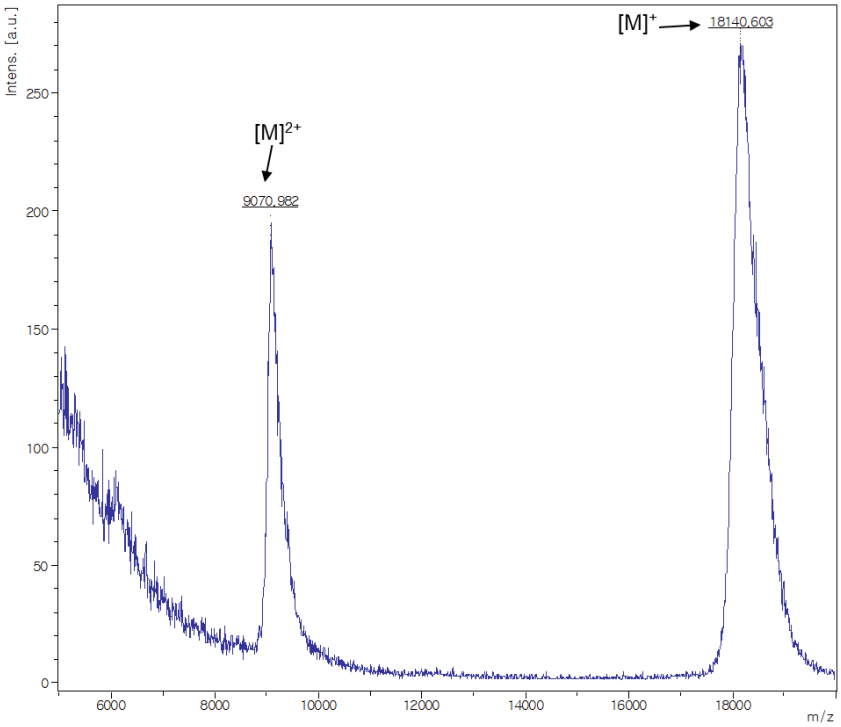


Fig. S8. Characterization of purified Sortase A-His_6 by MALDI-TOF.

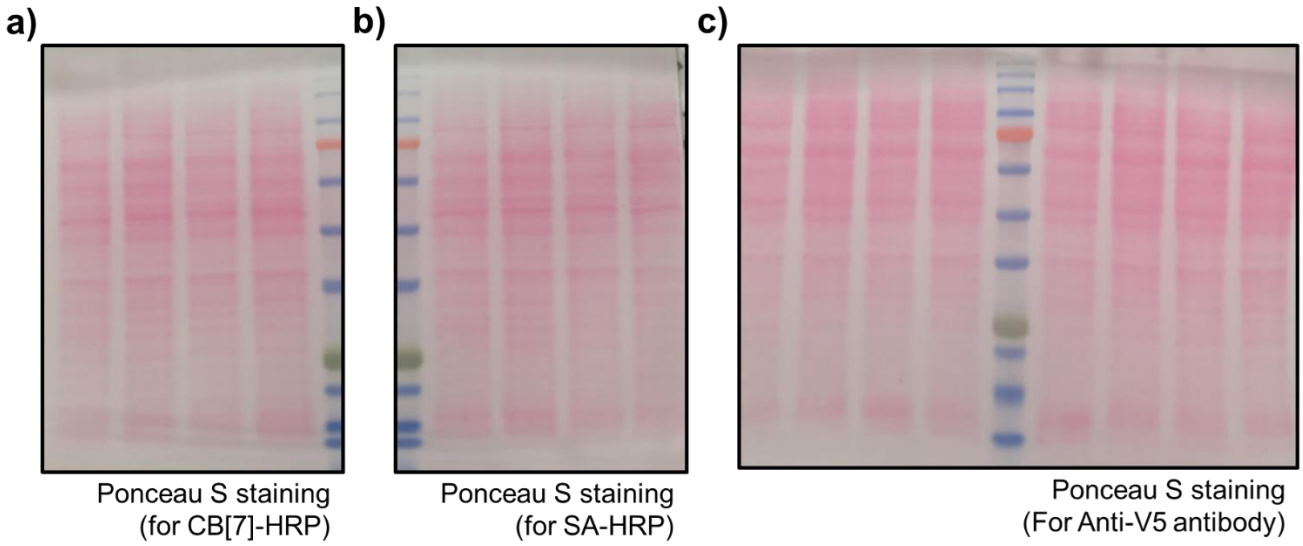


Fig. S9. Ponceau S stained NC membranes used for a) western blotting with CB[7]-HRP in Fig. 3, b) SA-HRP in Fig. S11b, and c) anti-V5 antibody in Fig. S10

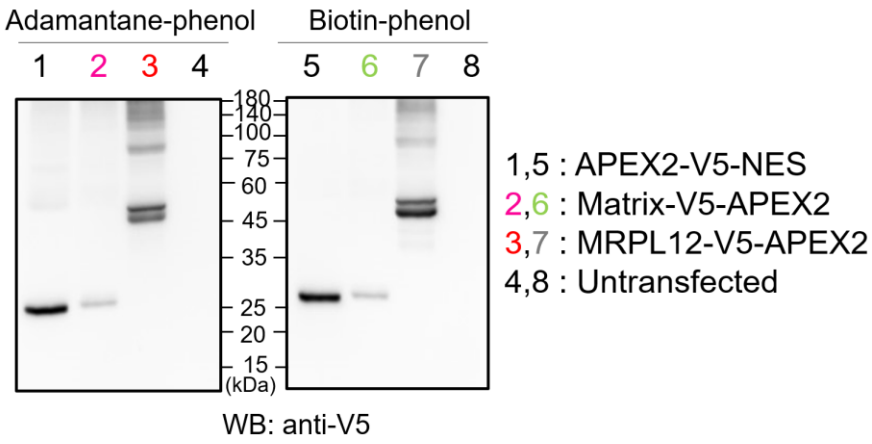


Fig. S10. Western blotting with Anti-V5 antibody to confirm the expression of V5-tagged APEX2 enzyme.

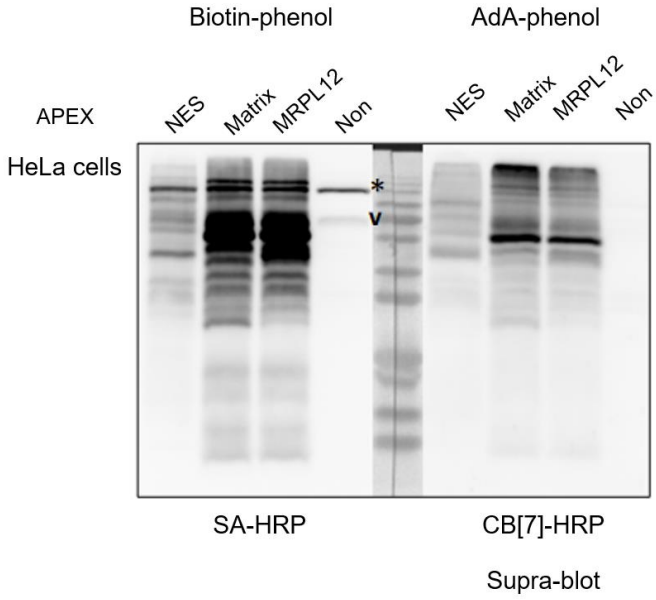


Fig. S11. Detection of biotin-phenol and AdA-phenol labeled proteins with SA-HRP and CB[7]-HRP (Supra-blot), respectively. (*, V indicate endogenously biotinylated proteins detected by SA-HRP), Cell line: HeLa cells

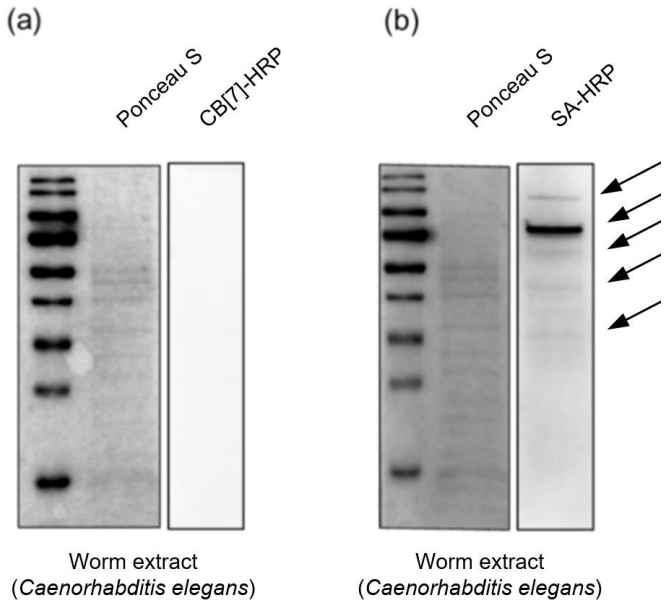


Fig. S12. Ponceau S staining, (a) Supra-blotting with CB[7]-HRP and (b) western blotting with SA-HRP using the lysate of worm (*C. elegans*). (The arrows indicate endogenously biotinylated proteins detected by SA-HRP)

Table S1. List of the plasmid DNA used in this study.

Name	Features	Promoter/Vector	Details
H3-SNAP-FLAG	AgeI-Histone H3-SNAP-FLAG-Stop-Not1	CMV/pcDNA3.1 myc/his	
Sortase A	NdeI-SrtA-6xHis-XhoI	T7/pET30b	(addgene #51141)
GFP-LPETG	NcoI-GFP LPETG-6xHis-XhoI	T7/pET29	(addgene #71754)
Mito-V5-APEX2 (unprocessed: 32 kDa, processed: 29 kDa)	NotI-Mito-BamHI-V5-APEX2-Stop-XhoI	CMV/ pcDNA3	Mito-: MLATRVFSLVGKRAISTSVCV RAH (matrix targeting sequence, Fornuskova et al., 2010)
V5-APEX2-NES* (30 kDa)	NotI-V5-APEX2-NES-Stop-XhoI	CMV/ pcDNA3	NES: LQLPPLERLTLD (nuclear exclusion signal)*S5
MRPL12-V5-APEX2-AP (unprocessed: 52 kDa, processed: 47kDa)	HindIII-MRPL12-BamHI-NheI-V5-APEX2-AP-Stop-XhoI	CMV/ pCDNA5	MRPL12 (NM_002949.3)

Protein processed size during translocation was obtained by programs: Mitoprot (http://ihg.gsf.de/ihg/mitoprot.html or Mitoprot software is available by: ftp://ftp.biologie.ens.fr/pub/molbio) and SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/) .

*The imaging pattern of these constructs was previously characterized in Ref. S5.

References

S1. M. Li. A. Lee. K. L. Kim, J. Murray, A. Shrinidhi, G. Sung, K. M. Park, K. Kim, *Angew. Chem. Int. Ed.* 2018, *57*, 2120-2125.

S2. K. M. Park, K. Baek, Y. H. Ko, A. Shrinidhi, J. Murray, W. H. Jang, K. H. Kim, J. –S. Lee, J. Yoo, S. Kim, K. Kim, *Angew. Chem. Int. Ed.* 2018, *57*, 3132-3136.

S3. K. L. Kim, G. Sung, J. Sim, J. Murray, M. Li, A, Lee, A. Shrinidhi, K. M. Park, K. Kim, K. *Nature Commun.* 2018, *9*, 1712

S4. M. Li, Y. Tao, Y. Shu, J. R. LaRochelle, A. Steinauer, D. Thompson, A. Schepartz, Z. Y. Chen, D. R. Liu, *J. Am. Chem. Soc.* 2015, *137*, 14084-14093.

S5. S. Y. Lee, M. G. Kang, J. S. Park, G. Lee, Y. Y. Ting, H. –W, Rhee, *Cell Rep*. 2016, *15*, 1837-1847.

S6. N. B. Cole, *Curr. Protoc. Protein Sci.* 2013, *73*, 30.1.1-30.1.16