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SUPPLEMENTARY INFORMATION

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Purification of protein therapeutics via high-affinity supramolecular host–guest interactions

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General Procedures

All the reagents and solvents employed were commercially available and used as supplied without further purification. FT-IR measurements were performed with Cary 660 FT-IR spectrometer. Fluorescence measurements were performed on a RF-5301PC spectrofluorometer (Shimadzu). UV-Vis measurements were performed on Agilent Cary 5000 UV-Vis-NIR Spectrophotometer or Eppendorf BioPhotometer D30. Freeze-dried Epoxy-Sepharose 6B beads (Epoxy beads) purchased commercially from G. E. Healthcare was washed and swelled with deionized and filtered water (Milli-Q water) before performing coupling reaction. Protein A-agarose beads were purchased from Roche. The epoxy group density is about 19-40 μmol ml^{-1} (70 – 140 nmol mg^{-1}) and 1.0 g dry powder gives about 3.5 ml final volume of medium. The genes of Herceptin used for recombinant protein generation was synthesized by Bionics. The genes of IFN-α and GCSF used for recombinant protein generation was synthesized by Genscript. Anti-histidine tag (mouse) antibody, anti-mouse IgG horseradish peroxidase conjugate antibody, Goat anti-Human IgG (Gamma chain) Cross-Adsorbed Secondary Antibody, HRP and Goat anti-Human IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 (2^{nd} Abs-Alexa555) were purchased from Thermofisher. Human BD Fc Block™ were purchased from BD Biosciences. Human B7-1/CD80 Alexa Fluor® 647-conjugated antibody and Human CD14 Alexa Fluor® 594-conjugated antibody were purchased from R&D Systems. SDS-PAGE was performed in a Mini-PROTEAN Tetra cell electrophoresis chamber (Bio-Rad). Western blot transfer was performed in a mini-trans blot cell (Bio-Rad). The enhanced chemiluminescence (ECL) kit was purchased from Bio-Rad. Gels and Western blot membrane images were documented by ImageQuant LAS500 (GE). Fluorescence images were collected with a LEICA DMI6000B. Flow cytometry was performed with a LSR Foretessa 5 Laser I (BD Biosciences). Centrifugal filtration was performed using a Merck™ Amicon™ Ultra Centrifugal Filter UNIT (0.5 ml, MWCO: 10,000). Human embryonic kidney cell (HEK293) and SK-BR-3 were obtained from American Type Culture Collection (ATCC) and were maintained according to the standard ATCC protocol. THP-1 cell was kindly supported from Prof. Y. T. Chang. HEK 293 Host cell protein (HCP) ELISA kit was purchased from Cygnus Technologies. FcA-BSA, AdA-BSA GGG-AdA, CB[7]-HRP and Sortase A were obtained by following the previous reports.[38,43,44]

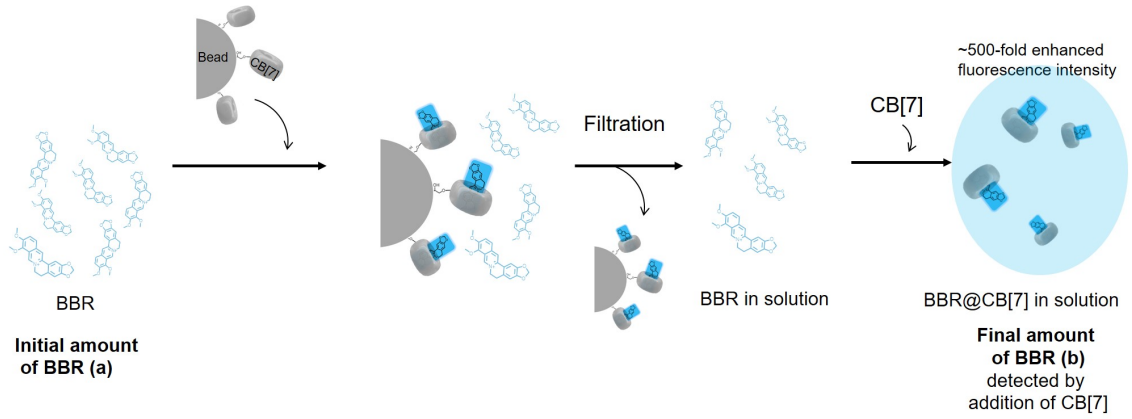
Methods

Fluorescence imaging of CB[7]-beads

CB[7]-beads (10 μl, swollen form) were washed with deionized water and added berberine (BBR, 250 μl, 10 μM). After 10 min, the beads were washed with water and analysed under a fluorescence microscope (λ_{ex} = 340-380 nm) (Supplementary Fig. S1e).

Determination of the number of CB[7] attached on agarose beads

Lyophilized CB[7]-beads (10 mg) and native beads (Epoxy-beads, 10 mg) were added to BBR (1 ml, 10 μM), separately. After shaking in a rotatory mixer (diameter = 20 cm, rpm = 5) at 25 °C for 48 h, the swelled beads were separated by filtration and washed five times with water to remove unbound BBR. The filtrate was mixed with the washed solutions, and the final volume was adjusted to 50 ml using water. A known volume from this solution (1 ml) was mixed with same volume of CB[7] (1 ml, 50 μM), measured its fluorescence emission intensity (FEI) (λ_{ex} = 345 nm, λ_{em} = 490 nm) and the concentration of remaining BBR in solution was obtained from FEI vs. [BBR] in 50 μM CB[7] calibration curve (Supplementary Fig. S1g,h). The experiments were repeated for five times to obtain the average values with standard deviation. BBR (# of mole) interacting with the beads were obtained by subtracting the final mole of BBR (b) from the initial mole of BBR (a) (see the below schematic drawing). Corrections for the non-specific interaction was performed based on the concentration of BBR interacting with native beads (1.7 ± 0.2 nmol mg^{-1}) and comparing with that with CB[7]-beads (2.8 ± 0.1 nmol mg^{-1}) (see Supplementary Fig. S1h). The average value for the number of CB[7] on the beads was calculated to be 1.1 ± 0.1 nmol mg^{-1}.



Isolation of FcA-BSA and AdA-BSA by CB[7]-beads

HEK293T cells were grown to 90% confluency on a 100 mm dish in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin / streptomycin in a humidified 5% CO_2 atmosphere at 37 °C. 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 phosphate buffer saline (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 (10-20 μg) and FcA-BSA[38] (3-10 μg) were mixed in 400 μl of RIPA buffer and incubated with CB[7]-beads (20 μl, swollen form) in end-over-end rotation for 2 h at 4 °C. After washing the CB[7]-beads with 1.0 ml of RIPA buffer for three times, the washed beads were incubated with 2 × SDS sample buffer (20 μl) containing *N*-(1-adamantyl)ethylenediamine (5 mM) followed by heating at 95 °C for 5 min. The incubated solution was collected and analysed by SDS-PAGE with coomassie brilliant blue staining and silver staining (Supplementary Fig. S3).

Thermal and chemical stability of CB[7]-beads

CB[7]-beads (20 μl, swollen form) were suspended in 0.5 ml each of water, radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA, 50 mM Tris-HCl, pH 7.5), phosphate-buffered saline (PBS) (1.37 M NaCl, 27 mM KCl, 100 mM Na_2HPO_4 and 18 mM KH_2PO_4, pH 7.4), SDS sample buffer (100 mM Tris-HCl (pH 6.8), 4% (w/v) SDS (sodium dodecyl sulfate; electrophoresis grade), 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 mM DTT (dithiothreitol)) at 95 °C for 5 and 30 min and, tested for its capability for the isolation of FcA-BSA from cell lysate/FcA-BSA mixture, as described above (Supplementary Fig. S4).

pH stability of CB[7]-beads

CB[7]-beads (20 μl, swollen form) were suspended in 0.5 ml of water with different pH conditions (pH 2, 4, 7, 10 and 13) at 4 °C for 12 h, and tested for its capacity to isolation FcA-BSA from cell lysate/FcA-BSA mixture, as described above. The pH of water was adjusted by 0.1 - 1.0 M HCl and NaOH (Supplementary Fig. S5).

Shelf-life of CB[7]-beads

CB[7]-beads (20 μl, swollen form) from the stock of CB[7]-beads (5 mL, stored in water at 25 °C) were tested for its FcA-BSA purification ability, after a week, a month, three months and a year of storage, as described above (Supplementary Fig. S6).

Gene construction and gene synthesis

The recombined genes of Herceptin were cloned into pcDNA 3.1 between EcoRI-HindIII restriction sites (Genscript, USA). The details of the individual sequence are described in Supplementary Table

S1. The recombinant genes of Herceptin (heavy chain and light chain) were cloned into pcDNA 3.1 vector between NheI-XbaI restriction sites (Bionics, Korea). Cleavage site by TEV protease, Sortase A recognition site and 6X His tag were sequentially linked to heavy chain of Herceptin. Each site was inserted for cleavage of the labelled AdA on Herceptin, AdA-labelling on Herceptin and protein purification using affinity chromatography. The recombined genes of IFN-α (or GCSF) were cloned into pET 28a vector between NcoI-HindIII restriction sites (Genscript, USA). The details of the individual sequence are also described in Supplementary Table S1. At C-terminal of IFN-α (or GCSF), cleavage site by TEV protease, Sortase A recognition site and 6 × His tag were sequentially linked with a spacer 'GG' to reduce the steric hindrance for the enzyme reaction. The inserted sites have same purpose as we described, previously.

Preparation of Herceptin in media

The plasmid DNA encoding Herceptin was transfected into host HEK293 (suspension cell) by following the instruction manual supplied from Thermofisher. The suspension cells were grown up to ~ 3.0 x 10^6 in 100 ml of Expi293™ Expression medium under a humidified 5% CO_2 atmosphere at 37 °C. The cells were transfected with the plasmid (100 μg) using ExpiFectamine 293 transfection kit. After 5 days, the medium was collected by centrifugation (5,000 × g, 15 min). The supernatant was used for investigation of protein purification.

Preparation of IFN-α in *E.coli* cell lysate

The plasmid DNA encoding IFN-α was transformed into *Escherichia coli* (*E. coli*) BL21 (DE3). The transformed bacterial cells were spread onto a luria-bertani (LB) agar plate containing kanamycin (50 μg ml^{-1}) 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 IFN-α was induced at an OD600 of ~0.8 by adding a solution of isopropyl *β*-D-1-thiogalactopyranoside (IPTG, 0.5 M, 300 μl) in deionized water at 25 °C overnight. The bacterial cells containing media were centrifuged at 6,000 rpm to provide a cell pellet. The pellet was resuspended using KPO_4 buffer (20 ml, 50 mM KPO_4, 300 mM NaCl, pH 7.5) with protease inhibitors (1 × protease inhibitor cocktail dissolved in water). A solution of lysozyme (50 mg ml^{-1}) in water (20 μl) was added to the resuspended solution of bacterial cells and incubated in an ice bath for 30 min. The bacterial cells were lysed by using a tip-sonicator and the cell debris was removed by centrifugation at 12,000 rpm for 30 min at 4 °C. Successful preparation of target proteins (IFN-α) was confirmed by western blot with anti-histidine (Supplementary Fig. S14). The soluble portion of the lysate was used for investigation of protein purification.

Preparation of GCSF in *E.coli* cell lysate

The plasmid DNA encoding GCSF was transformed into *Escherichia coli* Rosetta-gami™ 2(DE3)pLysS. The transformed bacterial cells were spread onto a luria-bertani (LB) agar plate containing kanamycin (50 μg ml^{-1}) 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 GCSF was induced at an OD600 of ~0.8 by adding a solution of isopropyl *β*-D-1-thiogalactopyranoside (IPTG, 0.5 M, 300 μl) in deionized water at 25 °C overnight. The bacterial cells containing media were centrifuged at 6,000 rpm to provide a cell pellet. The pellet was resuspended using KPO_4 buffer (20 ml, 50 mM KPO_4, 300 mM NaCl, pH 7.5) with protease inhibitors (1 × protease inhibitor cocktail dissolved in water). A solution of lysozyme (50 mg ml^{-1}) in water (20 μl) was added to the resuspended solution of bacterial cells and incubated in an ice bath for 30 min. The bacterial cells were lysed by using a tip-sonicator and the cell debris was removed by centrifugation at 12,000 rpm for 30 min at 4 °C. Successful preparation of target proteins (GCSF) was confirmed by Supra-blotting with CB[7]-HRP (anti-AdA) after AdA-tag conjugation using Sortase A (Supplementary Fig. S17). The soluble portion of the lysate was used for investigation of protein purification.

Site-specific conjugation of AdA on proteins by enzymatic reaction with Sortase A
A medium containing Herceptin or a lysate of cells expressing IFN-α or GCSF were incubated with Sortase A (5 mM, 5 μl) and GGG-AdA (100 mM, 5 μl) at 37 °C. After the incubation, the remaining GGG-AdA in the product was removed by centrifugal filtration (MWCO 10,000; 14,000 × g, 5 min, 3 times).

Investigation of solubility of AdA-Herceptin and Herceptin
UV-Vis spectra of Herceptin (50 mg ml^{-1}) and AdA-Herceptin (50 mg ml^{-1}) in Tris-HCl buffer (10 mM, pH 8) were measured (Supplementary Fig. S13). Aggregation Index (AI) values for the proteins were calculated based on absorbance at 280 nm and 350 nm following the equation below.[49]
Aggregation Index (AI) = (OD 350) / (OD 280 – OD 350) × 100
Where OD 350 represents the absorbance at 350 nm and OD280 – OD350 is difference in the absorbance at 280 and 350 nm, respectively.

Cell culture
SK-BR-3 cells were cultured at 37 °C in a 5% CO_2 humidified incubator with Dulbecco's modified Eagle's media (DMEM, Hyclone) containing 10% (v/v) fetal bovine serum (FBS, Hyclone) and 1% (v/v) penicillin and streptomycin. THP-1 cells were cultured with RPMI 1680 containing 10% (v/v) FBS and 2-mercaptoethanol (0.05 mM) and 1% (v/v) penicillin and streptomycin at the same conditions as above.

Investigation of targeting ability of AdA-Herceptin to human epidermal growth factor receptor 2 measured by flow cytometry
SK-BR-3 cells (1×10^6) were transferred to a tube in DMEM media (1 ml) containing 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin streptomycin. To the cells, the primary antibody (2 μg of Herceptin or AdA-Herceptin in 1 ml of DMEM media) was added. After 30 min of incubation in a humidified 5% CO_2 atmosphere at 37 °C, the cells were settled down under 1,200 rpm for 2 min centrifugation, washed with PBS (1 ml) and treated with the 2nd Abs-Alexa555 (2 μg, in 1 ml of DMEM media). After 30 min of incubation in a humidified 5% CO_2 atmosphere at 37 °C, the cells were washed with PBS, resuspended in 200 μl of PBS and subjected for flow cytometry (detection of Alexa555 fluorescent signal upon excitation at 488 nm) (Supplementary Fig. S11).

Cleavage of AdA tag from AdA-IFN-α using TEV protease
Purified AdA-IFN-α (1 μg in 20 μl of 10 mM Tris-HCl, pH = 8) was incubated with TEV protease (2 μl, 6 IU μl^{-1}, Genscript) in 37 °C for 1 h. The concentrated solution by centrifugal filtration (MWCO: 10,000, 14,000 × g for 15 min for three times) was analysed by Ponceau S staining and Supra-blotting (anti-AdA) with CB[7]-HRP (Supplementary Fig. S18).

Investigation of Sortase A reaction efficiency using western blotting assay
The AdA conjugation using Sortase A was performed with the Herceptin having LEPTG (120 ng) for 0 min, 60 min, 120 min and 150 min as above. The product at each time point was analysed by western blots detecting His_6 tag, AdA tag and human-IgG (Supplementary Fig. S15). The protein having His_6-tag was diluted with different dilution factors (1, 1/2, 1/4, 1/10, 1/16) and analysed by western blot with anti-His_6 tag Ab. The resulting intensities of the bands in the western blot were used as references to quantify the progress of Sortase A reaction.

Immune response of AdA-labelled protein drugs
THP-1 cells (monocytes, 5×10^5 cells per well) were differentiated to macrophages (M0) in RPMI media (0.5 ml) containing 10% (v/v fetal bovine serum (FBS) and 1% (v/v) penicillin and streptomycin with Phorbol 12-myristate 13-acetate (PMA; 150 nM) at 24 wells plate. The differentiated cells (macrophage; M0) were incubated in the RPMI 1640 media (0.5 ml; negative control), in the RPMI 1640 media (0.5 ml) with lipopolysaccharide (LPS, 1 μg ml^{-1}; positive control),

non-treated (negative control), commercial Herceptin (10 μg ml^{-1}) or AdA-Herceptin (10 μg ml^{-1}) at a humidified 5% CO_2 atmosphere at 37 °C overnight. After the incubation with each materials, the cells were washed with PBS (0.5 ml) and incubated with Human BD Fc Block™ (2.5 μg in PBS containing 1% FBS) at room temperature for 30 min. The blocked cells were washed with PBS (0.5 ml) and incubated with Human B7-1/CD80 Alexa Fluor® 647-conjugated antibody and Human CD14 Alexa Fluor® 594-conjugated antibody (1 μg each antibody in PBS containing 1% FBS). After 30 min incubation at room temperature, the cells were collected and transferred to 5 ml tube followed by washing with PBS (2 ml) twice. The washed cells were resuspended in 400 ul of PBS and subjected for flow cytometry. Three independent experiments were performed under the same condition (Supplementary Fig. S12).

Measurement of host cell proteins (HCPs) in the products recovered from both beads

The protein concentrations in the products recovered from both beads were matched to each other. The amount of host cell proteins in the samples was measured by HEK 293 HCP ELISA kit by following the instruction (Cygnus technologies). Three independent experiments were performed under the same condition (Supplementary Fig. S10b).

Investigation of effect of denaturant to stability and recovery-ability of CB[7]-beads

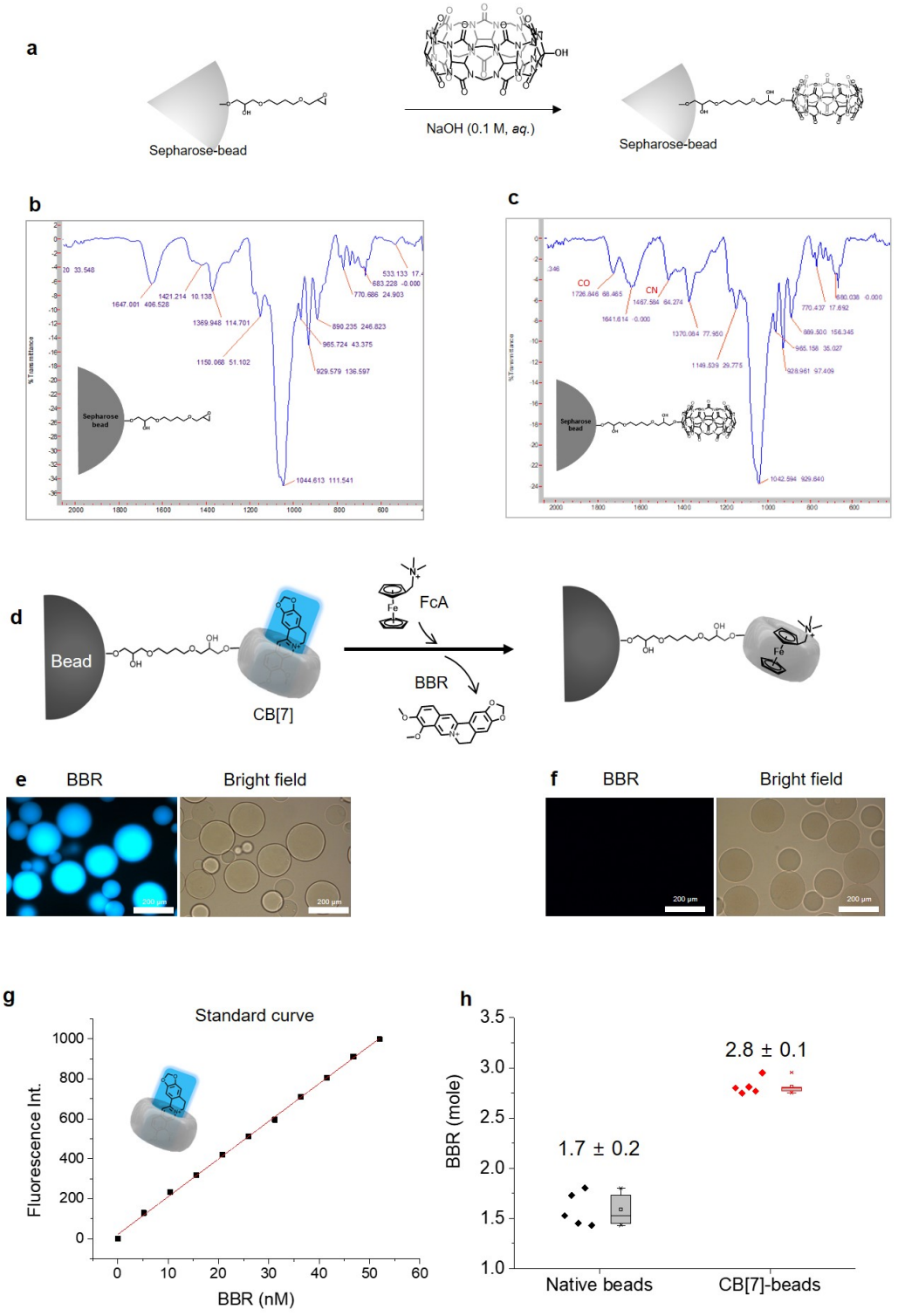
The regeneration of CB[7]-bead (1 ml, swollen form) was performed by following the procedure above; however, the buffer solutions used for bead washing step was replaced by Urea containing solution (1 M urea in the Tris-HCl buffer solution). The products from each cycle were analysed by Pierce™ Silver Stain Kit (Thermo Fisher Scientific) (Supplementary Fig. S16)

Supplementary Table S1. Gene sequence of the biopharmaceuticals

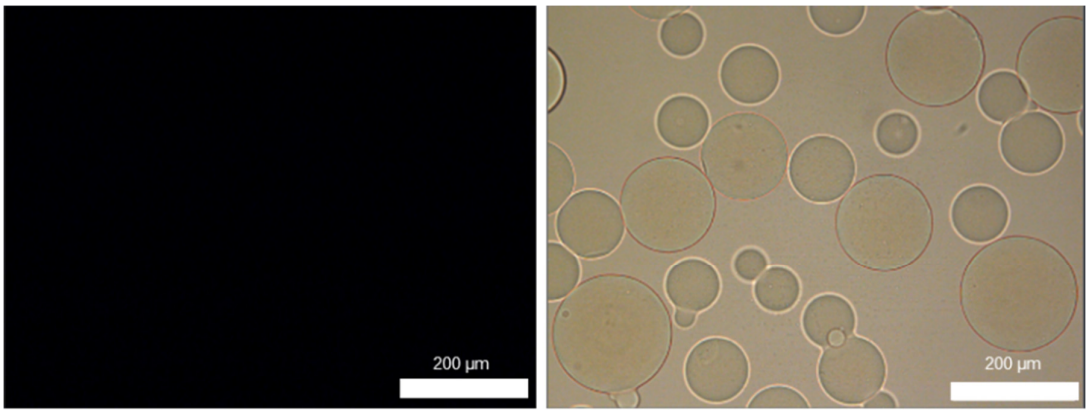
Herceptin in pcDNA 3.1 Heavy chain (Hc) Theoretical MW: 51587 Da EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTR YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGENLYFQGSLPETGGHHHHHH Light chain (Lc) Theoretical MW: 23443 Da DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS RFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
IFN-α in pET 28a Theoretical MW: 21842 Da CDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQEEFGNQFQKAETIPVLHEMI QQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQGVGVTETPLMKEDSILAVR KYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKEGGENLYFQGGGLPET GGGSHHHHHH
GCSF in pET 28a Theoretical MW: 21290 Da TPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAP LSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQ MEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQPGGEN LYFQGLPETGGGSHHHHHH

Supplementary Table S2 | Comparison of the recovered amount of mAb using 1 ml of the beads packed in a column tube. Note that the amount of the protein purified by the Protein A-beads in this study is different from that provided from the commercial source of Protein A-beads (Roche). The values cannot be directly compared, however, because (1) the nature and concentrations of the target proteins in samples, and (2) the process conditions for protein purification (such as incubation time and eluting rates) used in this study are quite different from those at an industrial level.

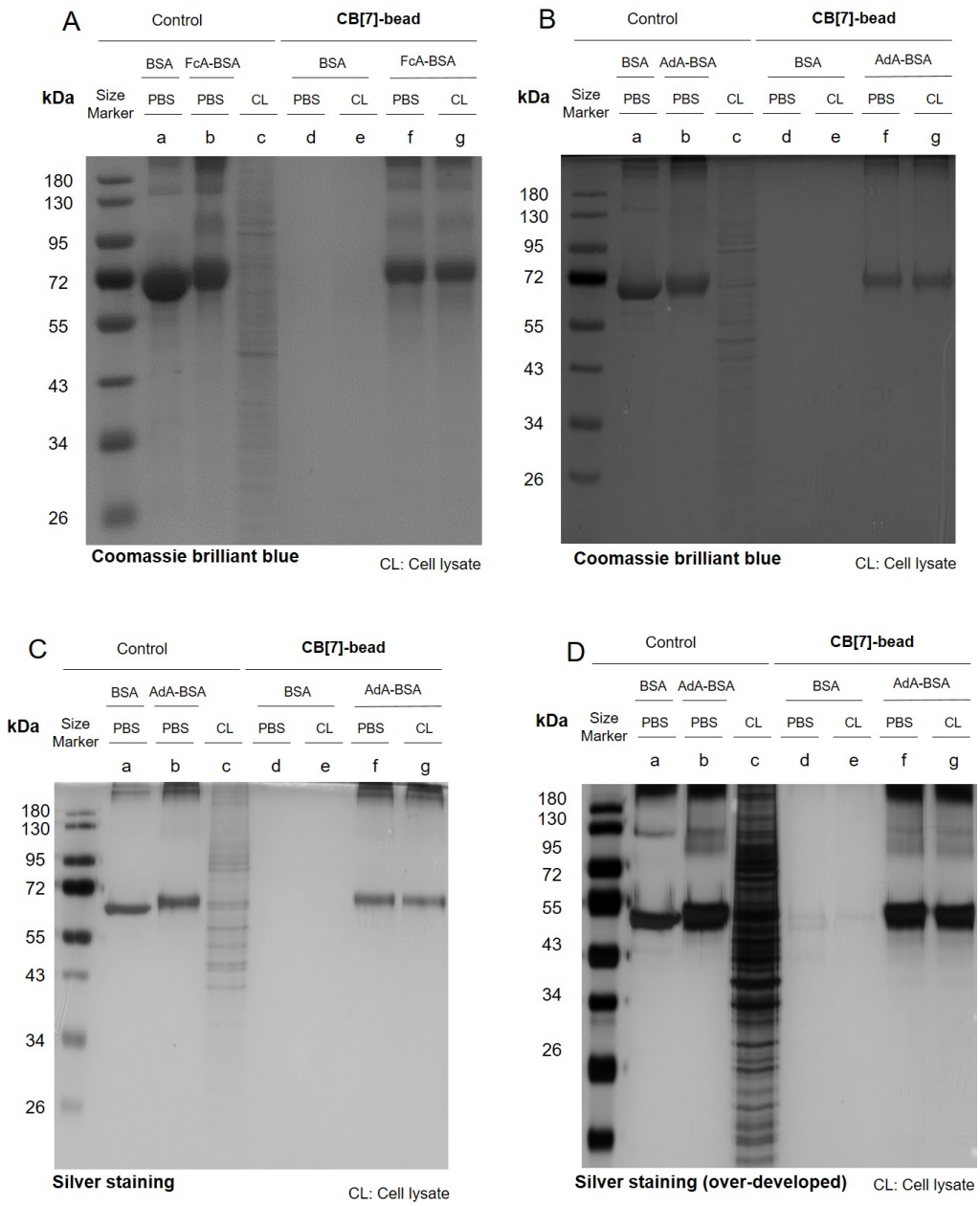
Material to recover mAb	**Recovered mAb** (μg)		
	Cell batch #1	Cell batch #2	Cell batch #3
CB[7]-beads	9	10	40
Protein A-beads	3	3	15
Comparison of mAb recovery-ability (**CB[7]-beads** Vs. **Protein A-beads**)			
CB[7]-beads / Protein A-beads (fold)	3.0	3.3	2.7
Average ± S.D.		3.0 ± 0.3	



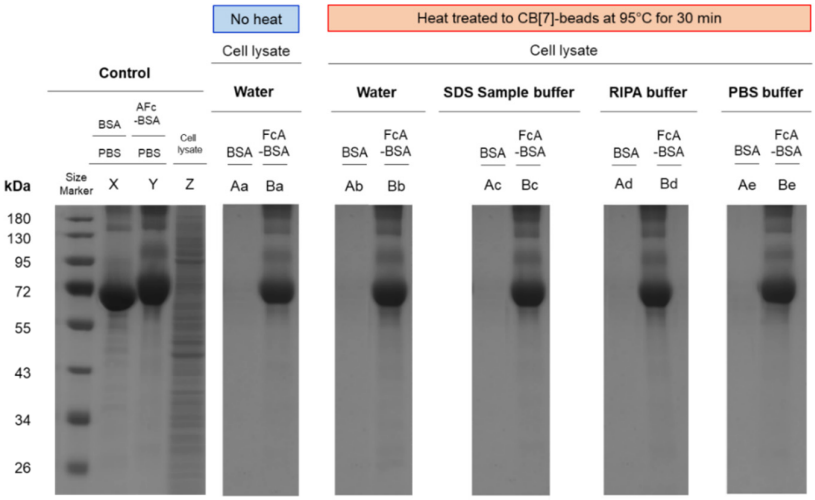
Supplementary Fig. S1 | Synthesis and characterization of CB[7]-beads
a) Synthetic scheme for CB[7]-beads, FT-IR spectra of epoxy-functionalized agarose beads b) before and c) after $(HO)_1$CB[7] conjugation, d) schematic illustration of an assay to confirm accessibility of the cavity of CB[7] conjugated on the beads, fluorescence and optical microscopy images of BBR complexed CB[7]-beads e) before and f) after treatment of FcA, g) standard curve for the measurement of BBR included in CB[7] in water and h) mole of BBR interacting to the beads (native beads: 1.7 ± 0.2 nmol mg^{-1}, CB[7]-beads: 2.8 ± 0.1 nmol mg^{-1}, $n = 5$; see the experimental procedure above for the detail)



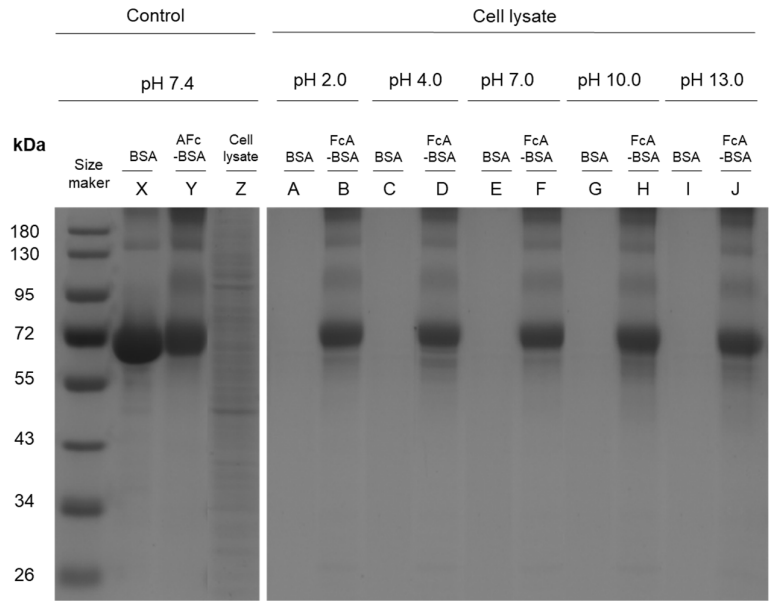
Supplementary Fig. S2 | Fluorescence (FL) and bright field (BF) images of Epoxy-beads with treatment of BBR



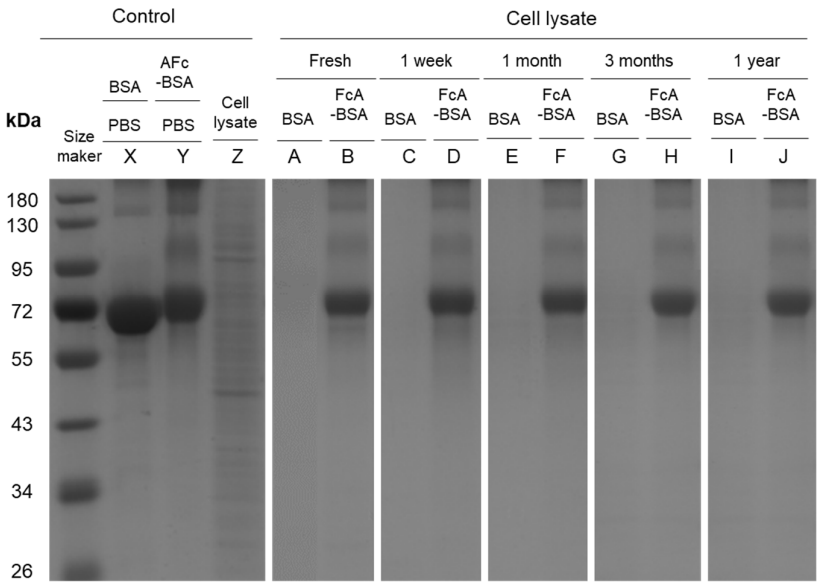
Supplementary Fig. S3 | Affinity purification of FcA-BSA (A) and AdA-BSA (B, C and D) using CB[7]-beads. Affinity capturing and recovery of FcA-BSA (A) and AdA-BSA (B, C and D) using CB[7]-beads; SDS-PAGE gel of native BSA in PBS (a), FcA(or AdA)-BSA in PBS (b) and cell lysate (c), and captured proteins recovered from the CB[7]-beads incubated in native BSA and FcA(or AdA)-BSA in PBS (d and f, respectively) and in cell lysate (e and g, respectively).The proteins were detected by Coomassie brilliant blue staining (A and B) and silver staining (C). Protein impurities were detected in the result of over-developed silver staining (D).



Supplementary Fig. S4 | Thermal and chemical stability test for CB[7]-beads; condition Aa, Ba: CB[7]-beads in water without heat treatment and condition Ab-e, Bb-e: CB[7]-beads in water (Ab, Bb), SDS sample buffer (Ac, Bc), RIPA buffer (Ad, Bd) and PBS buffer (Ae, Be) with heat treatment (95°C, 30 min). SDS-PAGE gel of native BSA in PBS (X), FcA-BSA in PBS (Y) and cell lysate (Z) and captured proteins recovered from CB[7]-beads incubated in native BSA in cell lysate (Aa-Ae), FcA-BSA in cell lysate (Ba-Be) (the proteins were stained with Coomassie brilliant blue).

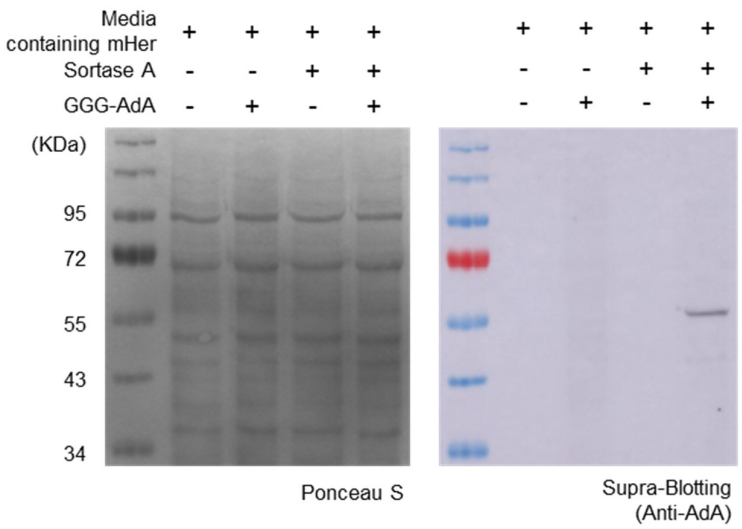


Supplementary Fig. S5 | Stability test for CB[7]-beads incubated at different pH in water for 12 h (pH was adjusted by adding HCl or NaOH); SDS-PAGE gel of native BSA in PBS (X), FcA-BSA in PBS (Y) and cell lysate (Z) and captured proteins recovered from the CB[7]-beads incubated in native BSA in cell lysate (A,C,E,G and I), FcA-BSA in cell lysate (B,D,F,H and J) (the proteins were stained with Coomassie brilliant blue)

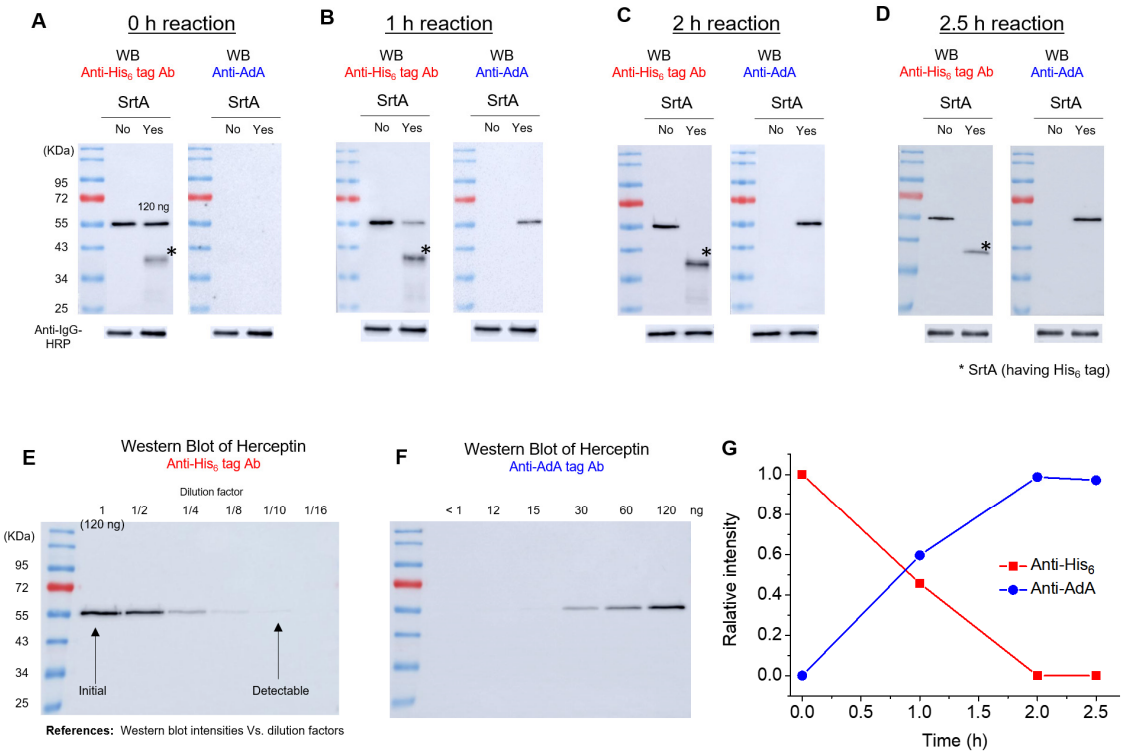


Supplementary Fig. S6 | Shelf-life test for CB[7]-beads kept at RT in water; SDS-PAGE gel of native BSA in PBS (X), FcA-BSA in PBS (Y) and cell lysate (Z) and captured proteins recovered from the CB[7]-beads incubated in native BSA in cell lysate (A,C,E,G and I), FcA-BSA in cell lysate (B,D,F,H and J) (the proteins were stained with Coomassie brilliant blue).

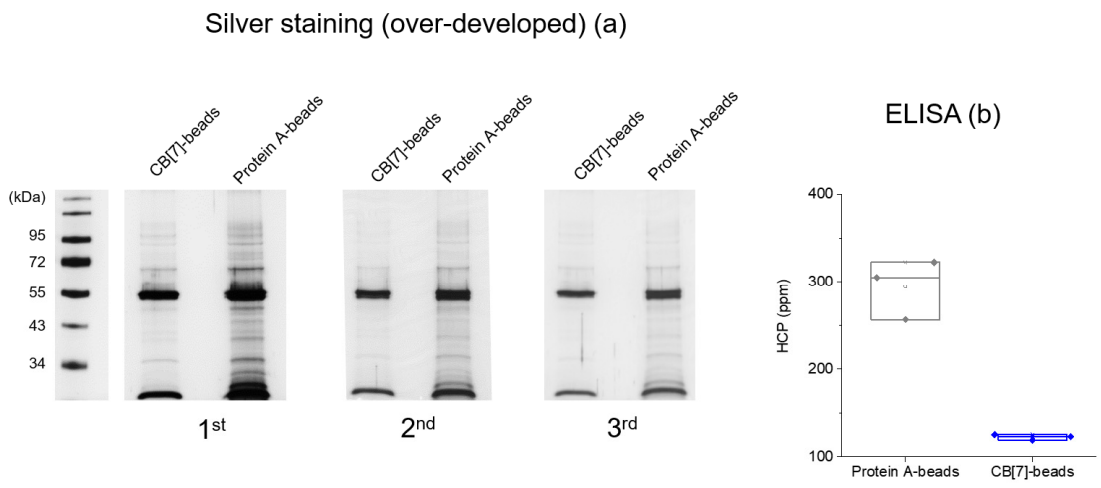
Supplementary Fig. S7 | Preparation of Herceptin from HEK293 (suspension cell). Medium used for culturing the transfected HEK293 cell was visualized by Ponceau S staining and western blot with anti-human IgG-HRP.



Supplementary Fig. S8 | Characterization of AdA-Herceptin. Conjugation of AdA moiety to Herceptin was confirmed by Supra-blot (anti-AdA).

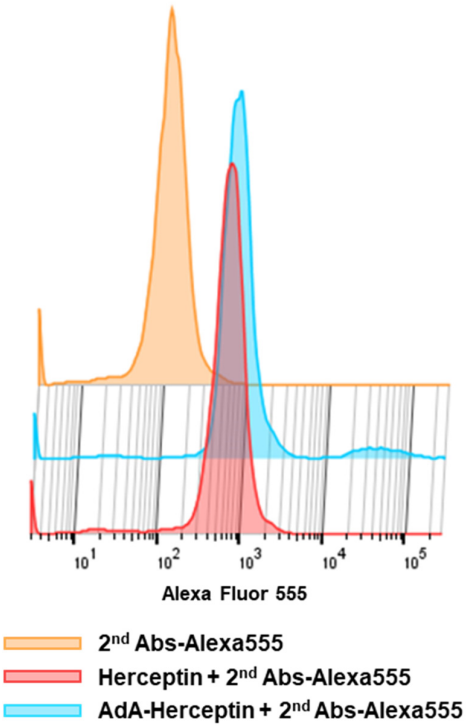


Supplementary Fig. S9 | Sortase A reaction with Herceptin and GGG-AdA. Western blot results of Herceptin (having His_6 tag, 120 ng) at 0, 1, 2, 2.5 h of Sortase A reaction with anti-His_6 Tag and anti-AdA (Supra-blot) (A-D). The western blot results of different concentrations of LPETG-Herceptin and AdA-Herceptin with anti-His_6 tag (E) and anti-AdA (F), respectively. Relative intensity changes of the blotting results for anti-His_6 tag and anti-AdA as a function of time (G). The reaction was started by addition of Sortase A and GGG-AdA to LPETG-Herceptin.

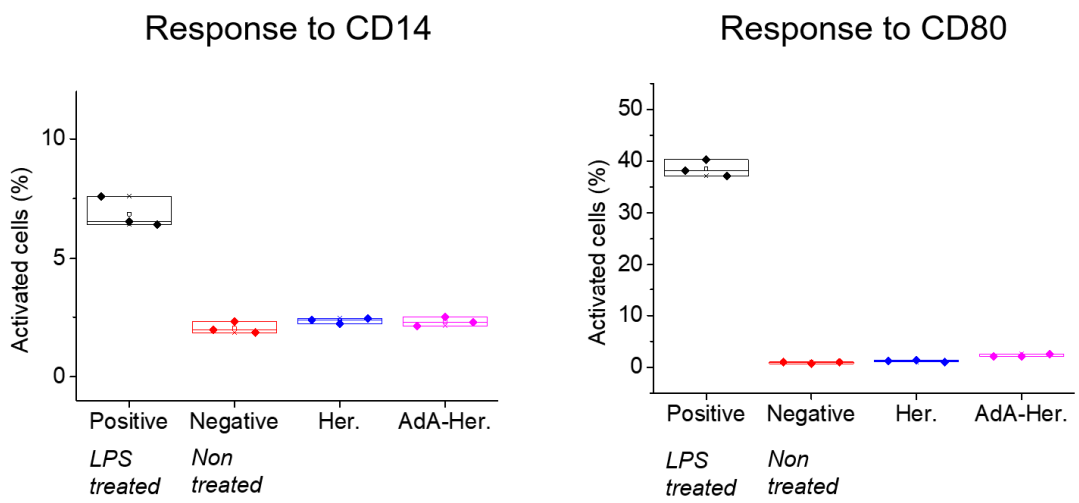


	CB[7]-beads	Protein A-beads
Target Protein (%)	85 ± 3%	57 ± 5%

Supplementary Fig. S10 | Comparison of protein impurities in recovered products. (a) Silver staining of the recovered product from CB[7]-beads and Protein A-beads (n = 3) See above for the calculation of the target protein percentage. (b) ELISA with polyclonal antibodies to detect host cell protein impurities in the product recovered from CB[7]-beads and Protein A-beads (n = 3). HCP values from Protein A-beads and CB[7]-beads are 294.6 ± 34.3 and 122.6 ± 3.3 ppm, respectively.

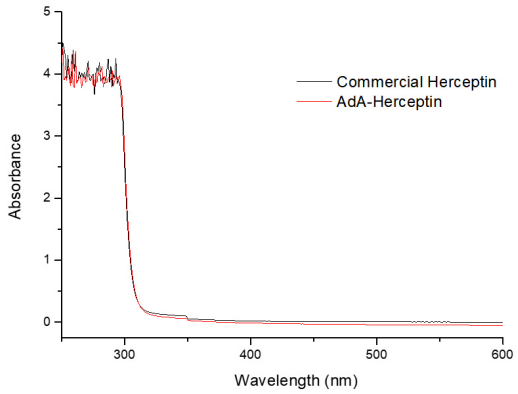


Supplementary Fig. S11 | Investigation of biological activity of AdA-Herceptin compared to Herceptin to SK-BR-3 breast cancer cells (1×10^4 cells) by flow cytometry



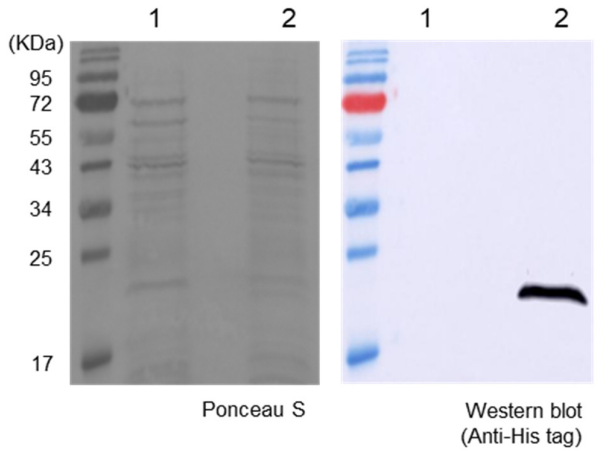
LPS: lipopolysaccharide

Supplementary Fig. S12 | Innate immune response to AdA-Herceptin. Flow cytometry of human macrophages treated with Herceptin and AdA-Herceptin to detect human CD14 Alexa Fluor® 594 antibody and human CD80 Alexa Fluor® 647 antibody. The cells treated with LPS and non-treated were used as positive and negative controls, respectively. Each data indicates the average value for the fluorescent intensity of 1×10^4 cells. The values of activated cells (%) for CD14 for positive and negative controls, Herceptin and AdA-Herceptin are 6.9 ± 0.6%, 2.0 ± 0.2%, 2.5 ± 0.1% and 2.3 ± 0.2%, respectively, and those for CD80 are 38.5 ± 1.0, 0.9 ± 0.1, 1.2 ± 0.2 and 2.3 ± 0.1, respectively (n = 3). See above for the experimental procedure.



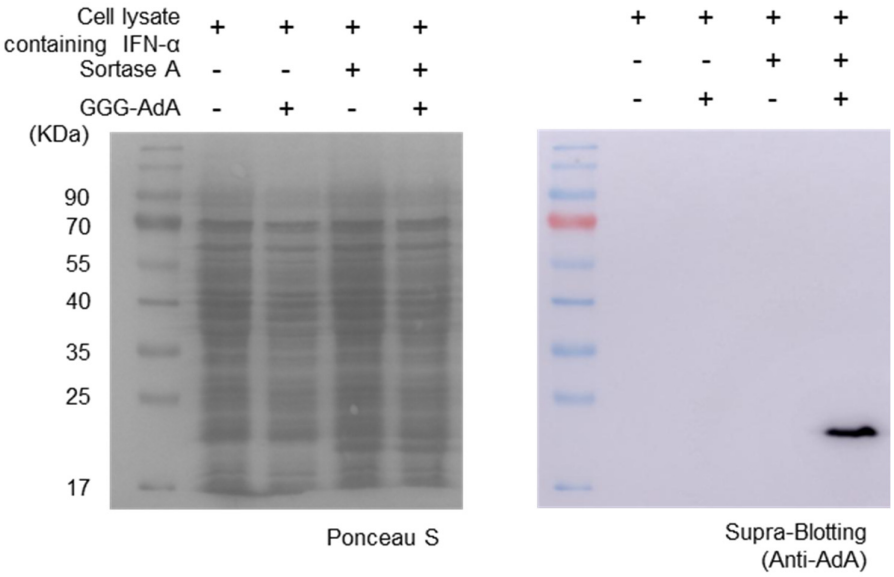
	Herceptin	AdA-Herceptin
Aggregation Index (%)	1%	1%

Supplementary Fig. S13 | UV spectra of AdA-Herceptin and Herceptin and their AI values (See above for the AI value calculation).

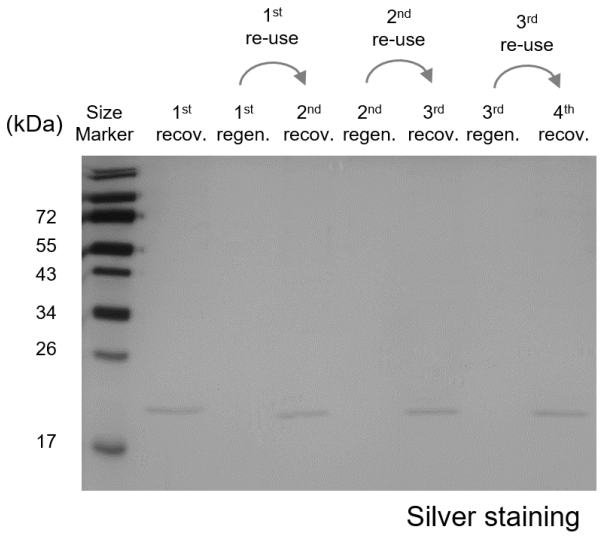


1: Cell lysate from *E.coli*
2: Cell lysate from IFN-α expressed *E.coli*

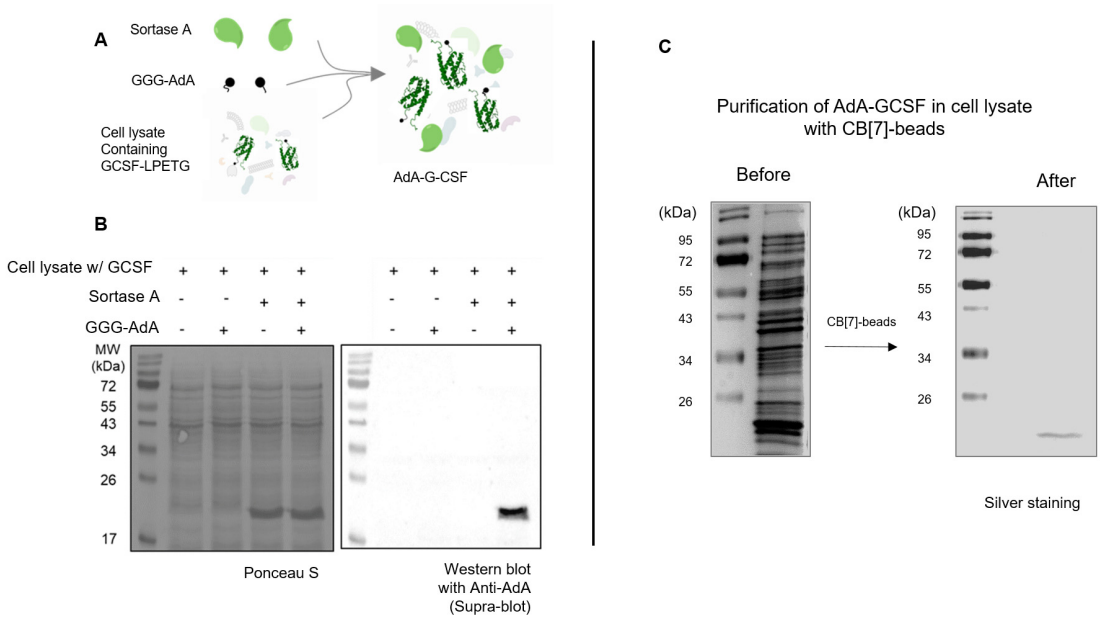
Supplementary Fig. S14 | Characterization of IFN-α expressed from *E.coli* BL21 (DE3). The cell lysate of the transformed cell was visualized by Ponceau S staining and western blot with anti-his-tag antibody (mouse) and anti-mouse antibody-HRP. (Dimerized IFN-α appears at 43 kDa[46]).



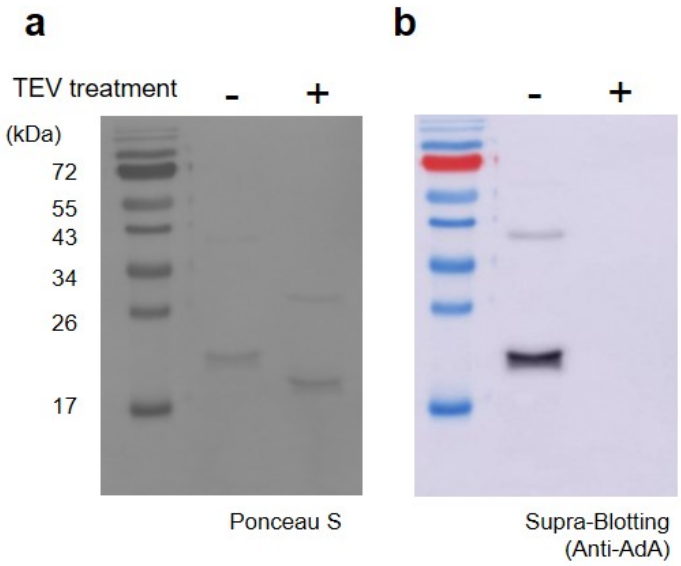
Supplementary Fig. S15 | Sortase A mediated AdA-labelling to IFN-α and analysis of proteins by Ponceau S staining and Supra-blot (Anti-AdA).



Supplementary Fig. S16 | Silver staining of AdA-IFN-α recovered with regenerated CB[7]-beads under the conditions with urea (1 M).



Supplementary Fig. S17 | Purification of AdA-GCSF. A) Schematic illustration of Sortase A reaction for AdA- GCSF, B) Ponceau S staining and western blot with anti-AdA (Supra-blot) of the resulting product and C) silver staining of the product before and after purification of CB[7]-beads.



Supplementary Fig. S18 | Cleavage of AdA-tag from AdA-IFN-α by TEV protease. Proteins detected by a) Ponceau S staining and b) Supra-blot (anti-AdA).