Supporting Information

Covalent and Site-Specific Immobilization of Antibody using Photoactivatable Antibody Fc-Binding Proteins Expressed in *Escherichia coli*

Yeolin Lee[†§], Jiyun Jeong[†§], Gabi Lee[†], Jeong Hee Moon[‡], and Myung Kyu Lee[*†§]

[†]Hazards Monitoring Bionano Research Center, [‡]Functional Genomics Research Center, Korea Research Institute of Bioscience and Biotechnology, and [§]Department of Nanobiotechnology, University of Science and Technology, 125 Gwahak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea

Corresponding Author

Email: mklee@kribb.re.kr; Tel: +82-42 8604169; Fax: +82-42-8798594

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Composition of M9VC minimal media.

48 mM Na_2HPO_4, 22 mM KH_2PO_4, 9 mM NaCl, and 19 mM NH_4Cl, 0.4% glucose, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.05 mM $MnCl_2$, 0.1 μM $FeCl_3$, 1 mg/ml thiamine, 0.2 mg/ml nicotinamide, 0.2 mg/ml folic acid, 0.2 mg/ml choline chloride, 0.02 mg/ml riboflavin and 0.75 mg/ml CMS-Met (mixture of 19 amino acids except methionine)

Table S1. PCR DNA primers for construction of plasmids encoding mutated FcBPs

Primers	DNA Sequences (5' to 3')	Remarks
PE1	TTCCCC**TCTAGA**[a]AATAATTTTGTTTAAC	From pET28a(+) vector sequence-forward
PE2	GCGC**GGATCC**[a]GCGCGGCACCAGGCCGC	For 3 Met deletion of pET28a(+)-reverse
PE3	TTATGCTAGTTATTGCTCAGCGGTGGCAG	From pET28a(+) vector sequence-reverse
PG1	ATA**GGATCC**[a]*TGCTGCGGCGGG*[b]ACAACTTACAAACTTGTTATT	C3PG cloning with CCGG-forward
PG2	GCGC**CTCGAG**[a]*TTA*[c]TTCAGTTACCGTAAAGGTC	C3PG cloning-reverse
PG3	AAAGCCTTCAAAATG[d]TACGCTAACGAGAACGG	Q32M mutation-forward
PG4	GTTAGCGTACAT[d]TTTGAAGGCTTTTTCTGCAGTTTCTGC	Q32M mutation-reverse
PG5	GACAACGGTGTTATG[d]GGTGTTTGGACTTATGATG	D40M mutation-forward
PG6	CCAAACACCCAT[d]AACACCGTTGTCGTTAGCG	D40M mutation-reverse
PG7	GCTAACGACCGC[d]GGTGTTATGGGTGTTTGG	N37R mutation- forward
PG8	CATAACACCGCG[d]GTCGTTAGCGTACATTTT	N37R mutation- reverse
PG9	GCTATG[d]GACCGC[d]GGTGTTATGGGTGTTTGG	N35M, N37R mutations- forward
PG10	CATAACACCGCG[d]GTCCAT[d]AGCGTACATTTTGAAGGC	N35M, N37R mutations- reverse
PL1	GAAGTG**ATCGAT**[a]GCGTCTGAATTA	From linker sequence- forward
PL2	TAATTCAGACGC**ATCGAT**[a]CACTTC	From linker sequence-reverse

[a] the bold bases indicate the restriction enzyme sites.
[b] the italicized and underlined bases indicate a DNA sequence encoding the inserted residues.
[c] the italicized bases indicate a stop codon.
[d] the underlined bases indicate the DNA sequences encoding the mutated residues as indicated in remarks.

Table S2. PCR DNA primers for construction of plasmids encoding mutated MRS proteins

Primers	Sequences (5' to 3')	Remarks
PB1	ATGCCATAGCATTTTTATCCA	From pBAD/*Myc* vector sequence
PM1	AATT**CCATGG**[a]CTCAAGTCGCGAA	MRS cloning-forward
PM2	CAGC**GGTACC**[a]*TTA*[b]TTCTTTAGAGGCTTCCACC	MRS cloning-reverse
PM3	GGG[c]CCGTACGCTAACGGCTCAATC	L13G mutation-forward
PM4	GATTGAGCCGTTAGCGTACGGCCC[c]TGCGCACGTCACCAG	L13G mutation-reverse
PM5	ACCGATTGGCTTC[c]ATGGGTTCTTTCAAGAATCTGTGCGA	Y260F mutation-forward
PM6	AAGAACCCATGAA[c]GCCAATCGGTGCGTCCAGC	Y260F mutation-reverse
PM7	GATATTGTTTACTTCCTG[c]AGCCTGTTCTGGCCTGC	H301L mutation-forward
PM8	CAGAACAGGCTCAG[c]GAAGTAAACAATATCTTTACC	H301L mutation-reverse
PM9	GACGTGCGGCTCG[c]CCGTACGCTAACGGCTCAATC	A12G, L13S mutations-forward
PM10	ACGGCGAGCC[c]GCACGTCACCAGAATTTTCTTCGC	A12G, L13S mutations-reverse
PM11	GGTAAAGATGTT[c]GTTTACTTCCTGAGCCTGTTCTGGCC	I297V mutation-forward
PM12	GGCTCAGGAAGTAAACAAC[c]ATCTTTACCGATGAAGTGGTACAG	I297V mutation-reverse

[a] the bold bases indicate the restriction enzyme sites.
[b] the italicized bases indicate a stop codon.
[c] the underlined bases indicate the DNA sequences encoding the mutated residues as indicated in remarks.

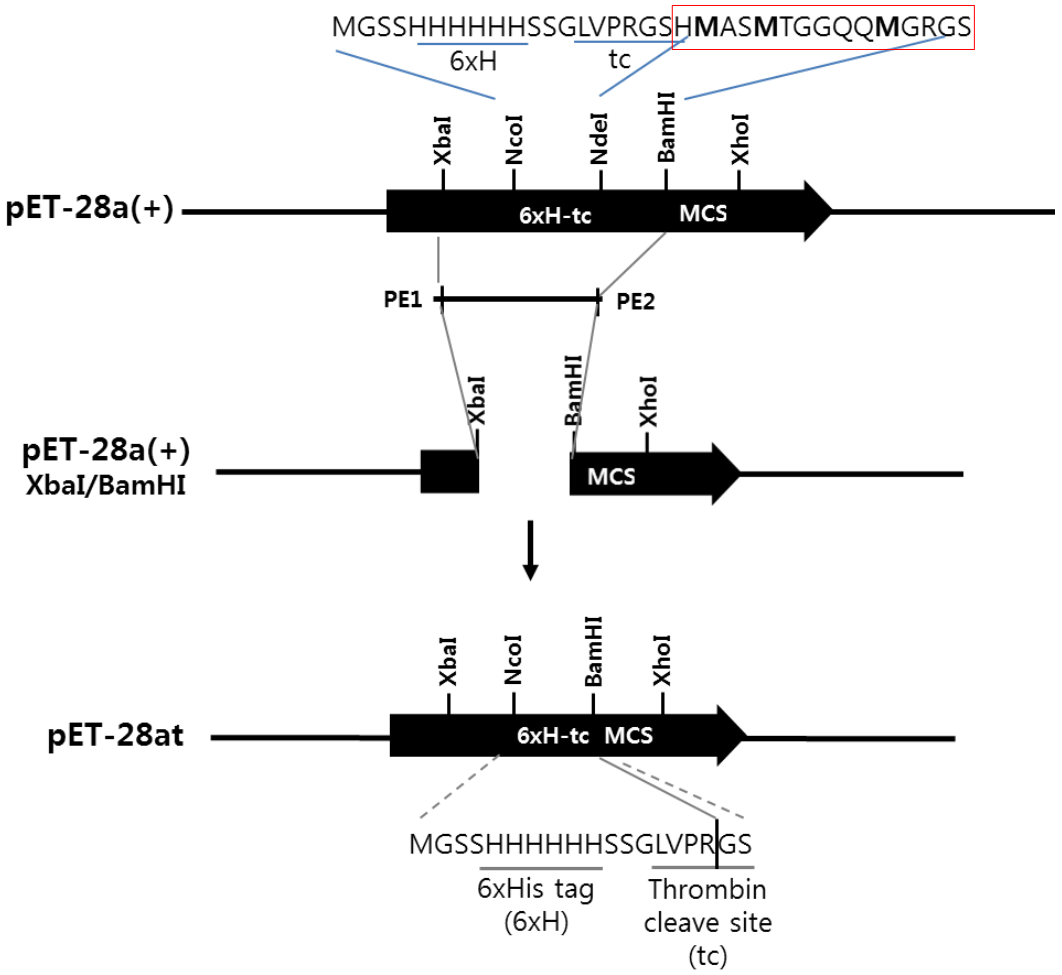
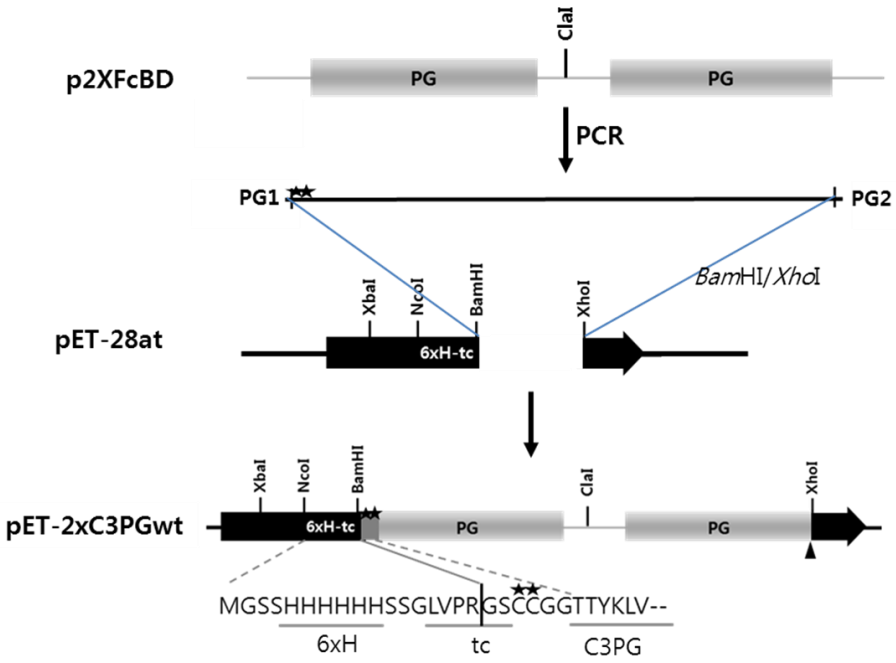


Figure S1. Construction scheme of pET-28at vector. A PCR DNA amplified using forward and reverse primers, PE1 and PE2 (Table S1), respectively, was cloned into the *Xba*I/*Bam*HI sites of the pET-28a(+) vector. The resulting pET-28at vector has a coding sequence with removal of 15 residues (red box) including three Met residues between the *Nde*I and *Bam*HI sites of the pET-28a(+) vector as indicated. The 6xHis motif (6xH), thrombin cleavage site (tc) and multicloning site (MCS) are indicated.



32 35 37 40

C3PG (PG) sequence: TTYKLVINGKTLKGETTTKAVDAETAEKAFKQYANDNGVDGVWTYDDATKTFTVTE
ClaI Insert sequence: KPEVIDASGLTPAV

Figure S2. Construction scheme of the pET-2xC3PGwt plasmid with two C3 domains of protein G. The PCR DNA with two C3 domains was amplified using PG1 and PG2 primers against p2xFcBP,[13] separated by agarose gel electrophoresis, and then purified using DNA gel extraction kit (Promega Co.). The purified DNA was cloned into the *Bam*HI/*Xho*I sites of pET-28at vector (Figure S1). The marks (★★ and ▲) indicate the introduced coding sequence for two consecutive Cys residues and the stop codon, respectively. The corresponding amino acid sequences for the wild type C3 domain of protein G (PG) and the *Cla*I insert are indicated below. The numbered residues indicate the mutated amino acids in this study.

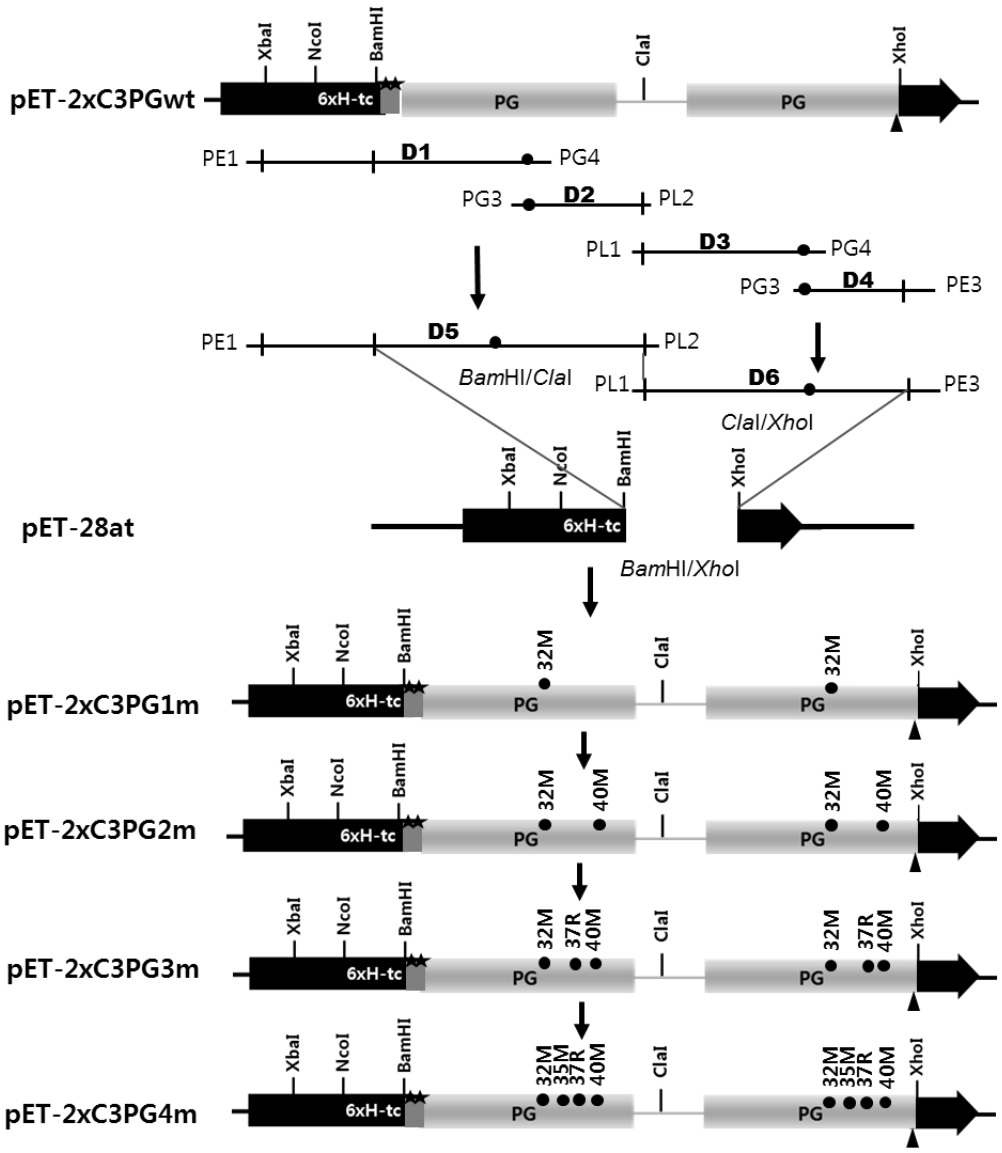


Figure S3. Construction scheme of the pET-2xC3PG mutant plasmids with two C3 domains of protein G (2xC3PG). The scheme shows the construction method of pET-2xC3PG1m. Four DNAs (**D1, D2, D3 and D4**) were first PCR-amplified using the primer sets, PE1/PG4, PG3/PL2, PL1/PG4 and PG3/PE3 (Table S1), respectively, against pET-2xC3PGwt. The **D5** and **D6** DNAs were second amplified by PCR using the primer sets, PE1/PL2 and PL1/PE3, against the PCR DNA mixtures, **D1**+**D2** and **D3**+**D4**, respectively. The **D5** and **D6** DNAs were separated by agarose gel electrophoresis, and purified using agarose gel extraction kit. The purified **D5** and **D6** DNAs were digested by enzyme sets, *Bam*HI/*Cla*I and *Cla*I/*Xho*I, respectively, and the digested DNAs were simultaneously cloned into the *Bam*HI/*Xho*I sites of the pET-28at vector. The other 2xC3PG mutant plasmids were sequentially constructed by the same strategy using the corresponding primers in Table S1. The marks (•, ★★ and ▲) indicate the mutated residues in the C3 domain of protein G (PG), the introduced residues coding two consecutive Cys and the stop codon, respectively.

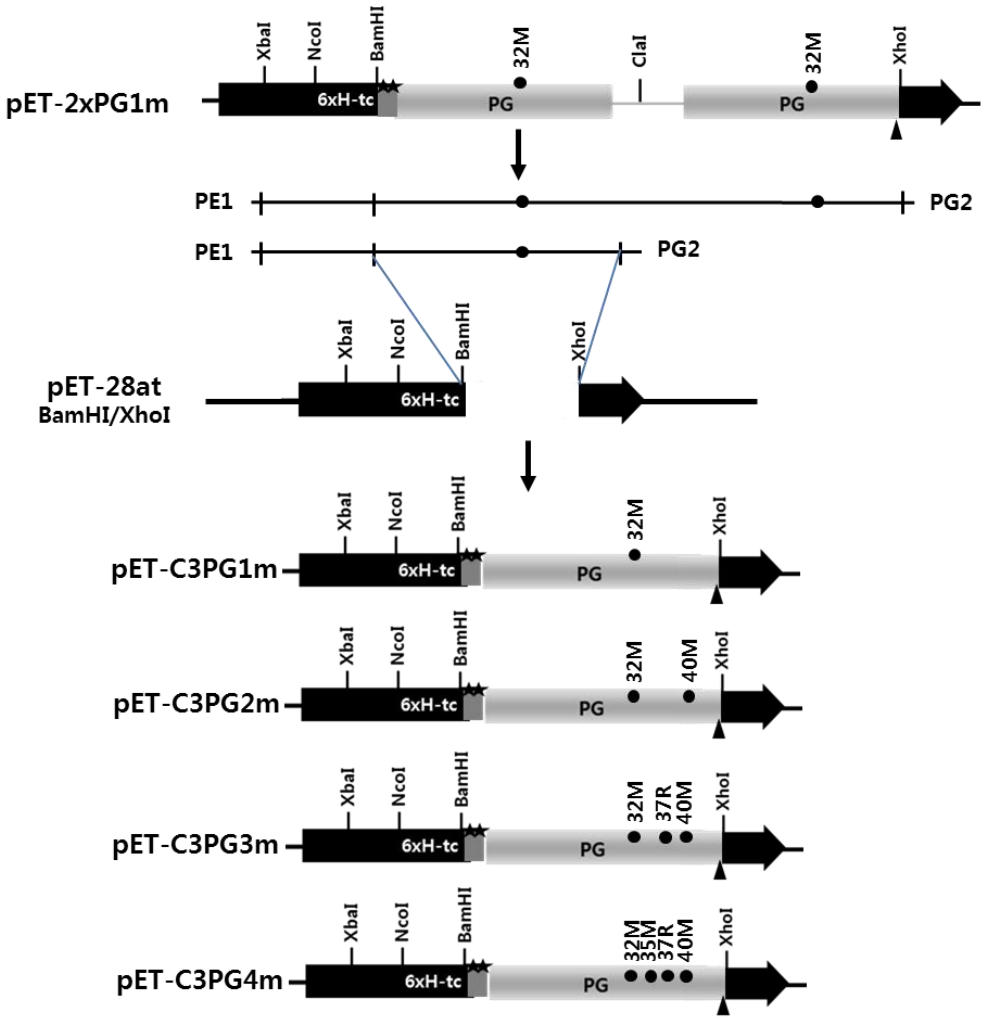


Figure S4. Construction scheme of the pET-C3PG mutant plasmids with a single C3 domain of protein G. The scheme shows the construction method of pET-C3PG1m. The DNA containing a single C3 domain was PCR-amplified using the primers, PE1 and PG2 against the pET-2xC3PG1m mutant plasmid, separated by agarose gel electrophoresis, and purified using the DNA gel extraction kit. The purified DNA was digested with *Bam*HI and *Xho*I and cloned into the *Bam*HI/*Xho*I sites of the pET-28at vector. The other C3PG mutant plasmids were constructed by the same method against the corresponding pET-2xC3PG mutant plasmids. The marks (•, ⋆⋆ and ▲) indicate the mutant residues in the C3 domain of protein G (PG), the introduced residues coding two consecutive Cys and the stop codon, respectively.

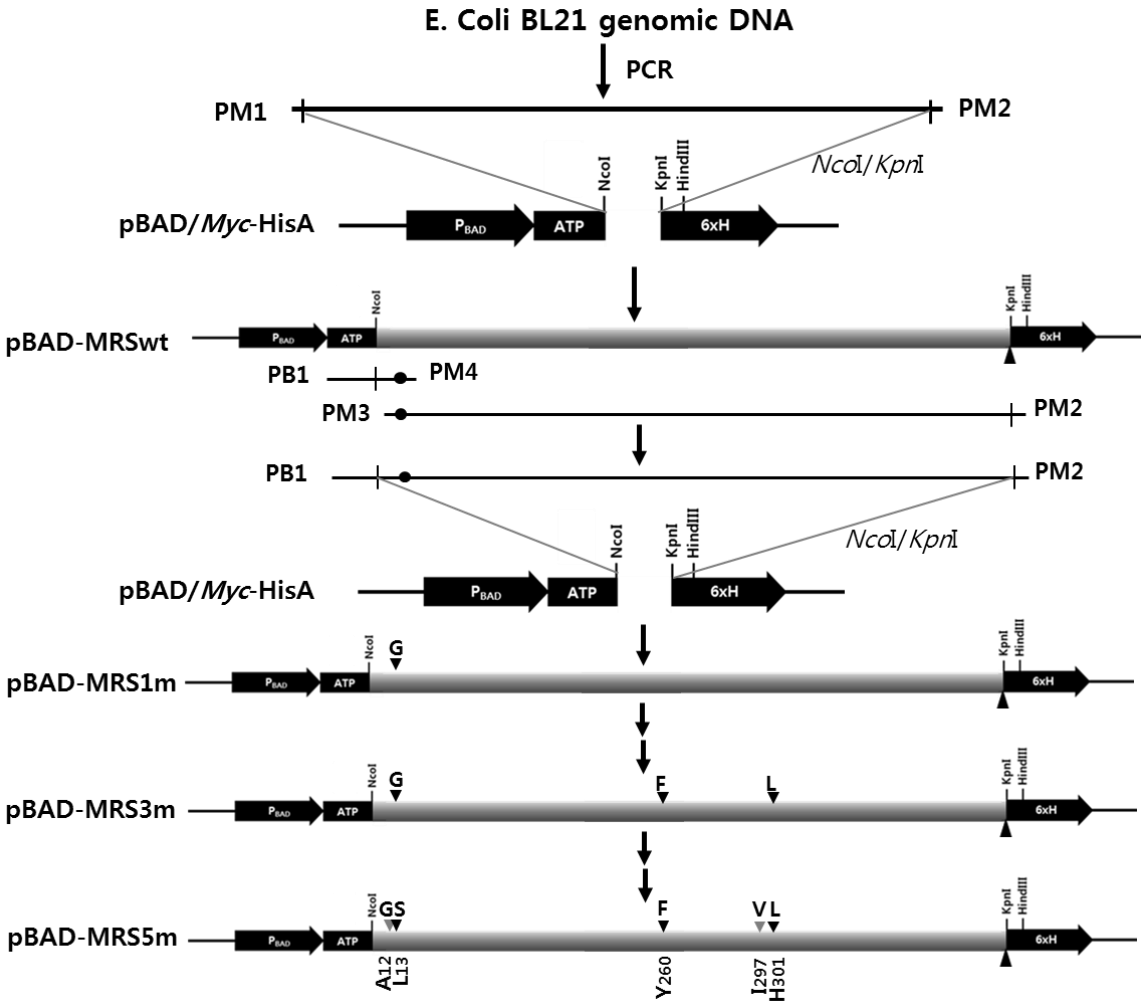


Figure S5. Construction scheme of the pBAD-MRS mutant plasmids. The DNA encoding the truncated wild type MRS sequence 1-548 (MRSwt) was amplified by PCR using the primers, PM1 and PM2 (Table S2), against the *E. coli* BL21 genomic DNA. The PCR amplified DNA was separated by agarose gel electrophoresis, and purified using the DNA gel extraction kit. The purified DNA was digested with *Nco*I and *Kpn*I and cloned into the *Nco*I/*Kpn*I sites of the pBAD/*Myc*-HisA vector. The resulting pBAD-MRSwt plasmid was used for construction of the pBAD-MRS mutant plasmids. The below scheme shows the construction method of pBAD-MRS1m. Two different DNAs were first amplified by PCR using the primer sets, PB1/PG4 and PM3/PM2 (Table S2), against pBAD-MRSwt. The DNA coding the L13G mutated MRS gene was obtained by the sencond PCR using the primers, PB1 and PM2, against the mixture of two PCR DNAs. The PCR DNA with L13G mutation was separated by agarose gel electrophoresis, and purified using the DNA gel extraction kit. The purified DNA was digested with *Nco*I and *Kpn*I and cloned into the *Nco*I/*Kpn*I sites pBAD/*Myc*-HisA vector. The other mutant plasmids were sequentially constructed by the same strategy using the primers in Table S2. The marks indicate the corresponding amino acid residues mutated at positions 13, 260 and 301 (▼) and at positions 12 and 297 (▼), and the stop codon (▲).

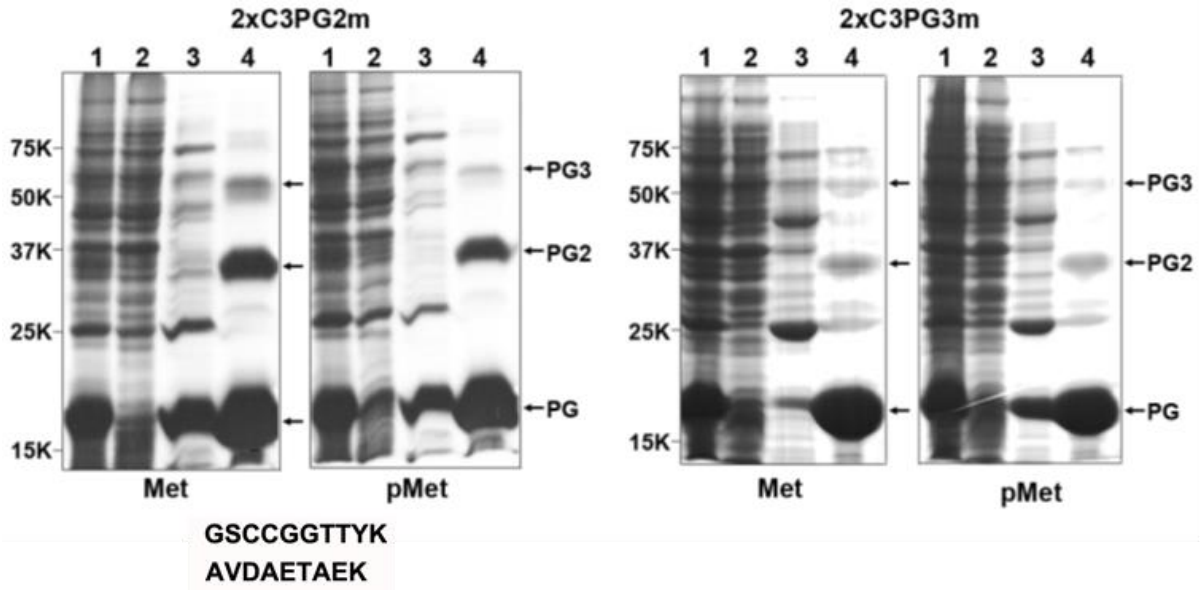


Figure S6. Purification of FcBPs using Ni-NTA-agarose and SDS-PAGE profiles. The *E. coli* cells containing the FcBPs expressed in Met- or pM- supplemented media were suspended in 1-2 ml of cell lysis buffer (20 mM Tris-HCl, 300 mM sodium chloride, 10% glycerol, 2 mM 2-mercaptoethanol, pH 7.5) per 100 ml culture. The cells were disrupted by sonication, and then the insoluble debris was removed by centrifugation for 10 min at 16,000×g. The supernatant containing recombinant proteins was directly loaded on Ni-NTA-agarose column (1 ml packed volume) equilibrated with buffer A (20 mM Tris-HCl, 300 mM sodium chloride). The column was sequentially washed with 3 ml of buffer A and 3 ml of 60 mM imidazole in buffer A, and the bound proteins were eluted using 3 ml of 150 mM imidazole in buffer A. The protein profiles of the fractions were analyzed by reducing SDS-PAGE. The indicated lanes represent the fractions of supernatant after sonication (1), unbound fraction on Ni-NTA-agarose (2), 60 mM imidazole elution (3), and 150 mM imidazole elution (4). The arrows indicate monomer and oligomer-like FcBP bands. The identical peptide sequences indicated below were commonly detected from in-gel trypsin digested monomer (PG) and dimer-like (PG2) bands of 2xC3PG2m by ESI-MS/MS analysis.

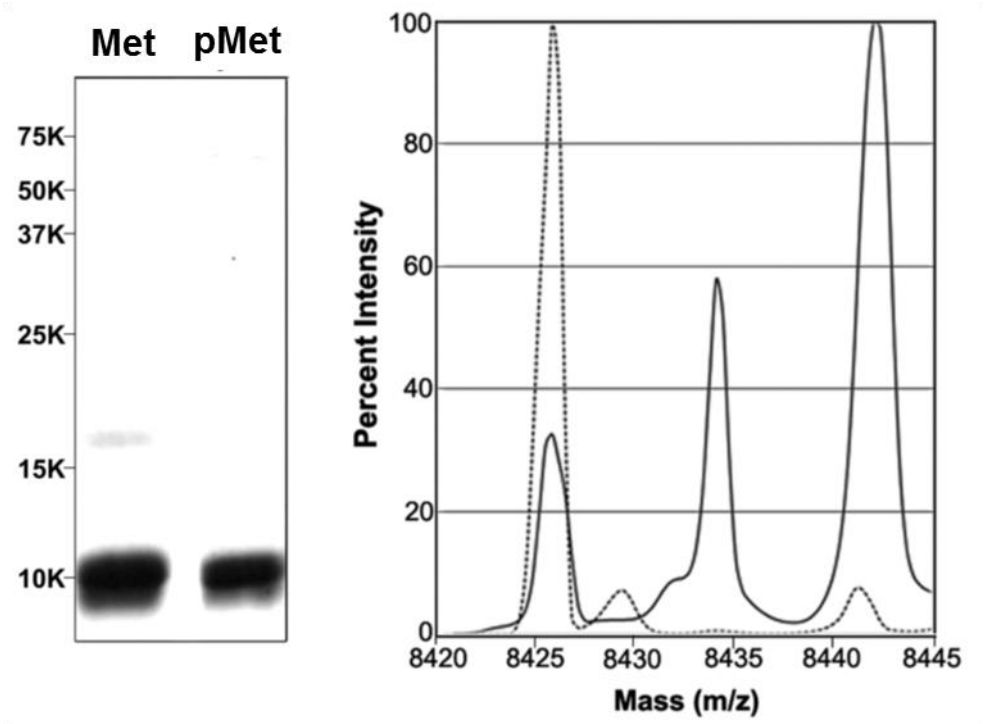


Figure S7. ESI-MS mass profiles of purified FcBPs. The C3PG3m proteins were expressed in the M9VC minimal media supplemented with either Met or pMet and purified using Ni-NTA agarose chromatography as shown in Figure S6. The purified proteins were analyzed by reducing SDS-PAGE. The purified proteins were desalted using a C18 reverse phase column, and their masses were analyzed by ESI-MS. The dashed and solid lines indicate the mass peaks of the purified C3PG3m proteins expressed in the presence of Met and pMet, respectively.

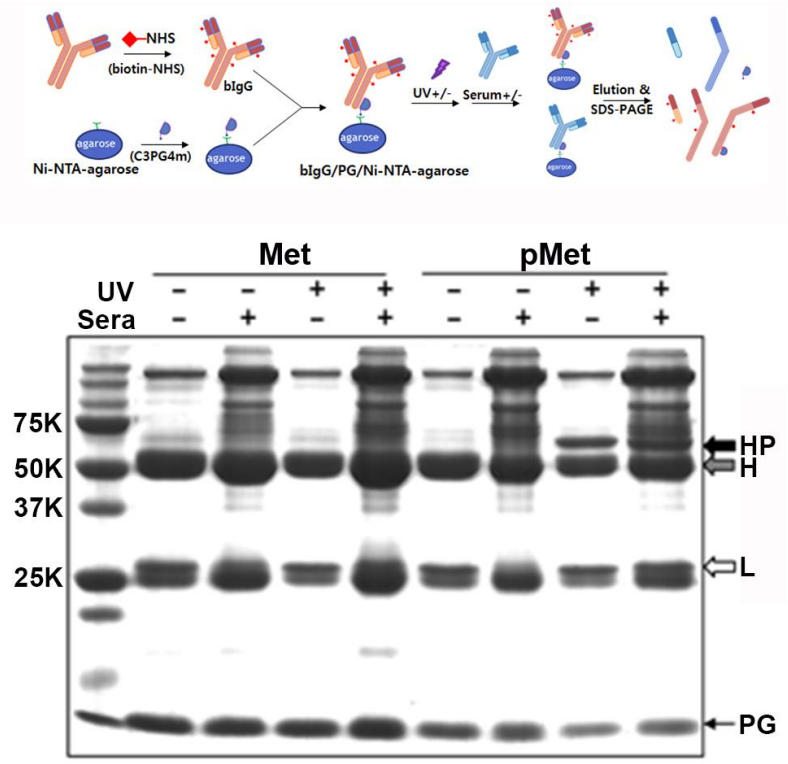


Figure S8. SDS-PAGE profiles of FcBP-bound proteins. The C3PG4m proteins with Met or pM bound on the Ni-NTA agarose beads were mixed with biotinylated antibodies (bAbs), and the mixtures were either incubated in the presence (+) or absence (-) of UV irradiation for 30 min. The resulting beads were further incubated either in human sera (+) or 2% BSA in PBS (-) for 1 h as indicated. After washing the beads with TBST, FcBP-bound proteins were selectively eluted by treatment of 150 mM imidazole and subjected to reducing SDS-PAGE. The protein bands were stained using coomassie brilliant blue R250. Heavy chain (H) and light chain (L), FcBP (PG) and photo-cross-linked heavy chain (HP) are indicated.

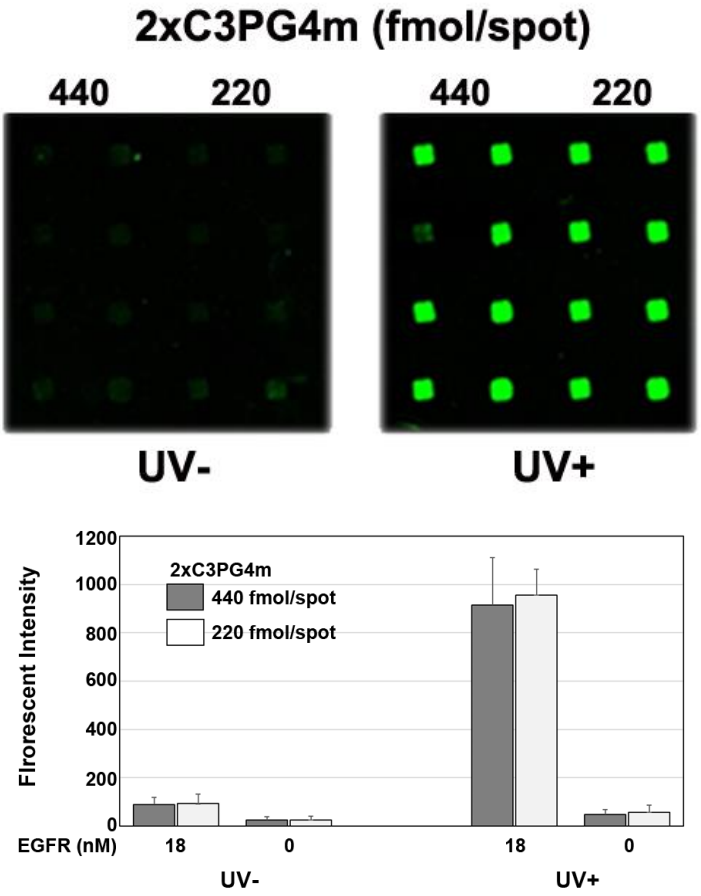


Figure S9. Comparison of detection sensitivity of EGFR proteins in human sera using EGFR-hmAb arrays depending on UV irradiation. The 2xC3PG4m proteins were spotted on the maleimide activated slide, and hmAbs were then incubated on the slide in the presence or absence of UV irradiation (UV+ or UV-, respectively). The spotted amounts of 2xC3PG4m proteins are indicated. Fluorescence images are obtained by incubation of the mixture of 18 nM EGFR and fluorescein-labeled anti-EGFR rat antibody in human sera. Fluorescence images were monitored using a slide scanner at 488 nm excitation and 510-560 nm emission wave length. The below graph shows fluorescent intensities (A.U.) obtained at 18 and 0 nM EGFR concentrations. No UV irradiated Ab array (UV-) shows much less detection sensitive for EGFR proteins in human sera than the UV irradiated one (UV+).