

## *Electronic Supplementary Information (ESI)*

# **Anchoring foreign substances on live cell surface using Sortase A specific binding peptide**

**Kyoungsook Park,<sup>‡a</sup> Juyeon Jung,<sup>‡a</sup> Jieun Son,<sup>a</sup> So Hyun Kim,<sup>b</sup> and Bong Hyun Chung<sup>\*a</sup>**

<sup>a</sup>BioNanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology, NanoBioengineering Major, University of Science and Technology, 125 Gwahangro, Yuseong-gu, Daejeon 305-806 Republic of Korea

<sup>b</sup>Department of Life Science, University of Seoul, 163 Siripdaero, Dongdaemun-gu, Seoul 130-743 Republic of Korea.

### ***Experimental Details***

**Construction of plasmids:** DNA polymerase (Takara Bio Inc., Japan) was used to perform PCR, and all PCR-amplified sequences were verified by DNA sequencing. *Escherichia coli* strain DH5 $\alpha$  was used as a host for subcloning, and *E. coli* BL21 (DE 3) (Novagen, USA) was used for gene expression. The *E. coli* strains were grown in LB medium at 37 °C, and 50 mg/ml of ampicillin was added for the plasmid-harboring strains. pET21a (Novagen, USA) was used as a vector for subcloning and expression. Restriction enzymes and modifying enzymes were obtained from Roche Applied Science (Germany) and used in accordance with the supplier's recommendations. DNA was prepared using a QIAEX II gel extraction kit (Qiagen, Germany).

The expression plasmids for HA-tagged SrtA $\Delta_{59}$  and HA-tagged opt-SrtA $\Delta_{59}$  were constructed as follows: The gene encoding SrtA $\Delta_{59}$  (corresponding to amino acids 60–206) was obtained by PCR from genomic DNA of *Staphylococcus aureus* using the primers (5'-GGAATTCATGCAAGCTAAACCTCAAATTCC-3') and (5'-CCGCTCGAGTTTGA CTTC TGTAGCTAC-3'). We optimized the codon usage of SrtA $\Delta_{59}$  using the codon optimization program developed by Bioneer (Korea) and KAIST (Korea Advanced Institute of Science and Technology). After the synthesis of opt-SrtA $\Delta_{59}$  (Bioneer, Korea), a second sequence was generated in the same manner using the primers (5'-GGAATTCATGCAGGCTAAACC TCAGATC-3') and (5'-CCGCTCGAGCTTCACCTCGGTGGCGACG-3'). The two PCR products were digested with *EcoRI/XhoI* and cloned into the modified pcDNA3 vector (Invitrogen, USA), and a hemagglutinin (HA) epitope was inserted at the C-terminus of the

multi-cloning sites to generate the constructs SrtA $\Delta$ <sub>59</sub>-HA and opt-SrtA $\Delta$ <sub>59</sub>-HA for protein expression in cultured cells.

The expression plasmids for 6x His-tagged opt-SrtA $\Delta$ <sub>59</sub>, 6x His-tagged EGFP-LPETG5, and 6x His-tagged EGFP-LPETA5 were constructed as follows: opt-SrtA $\Delta$ <sub>59</sub> was obtained through PCR using the primers (5'-GGAATTCATGCAGGCTAAACCTCAGATCC-3') and (5'-CCGCTCGAGCTTCACCTCGGTGGCGACG-3') and was subcloned into the *EcoRI/XhoI* sites of pET-21a (Novagen, USA). EGFP was obtained through PCR using the primers (5'-GGAATTCATGGTGAGCAAGGGCGAG-3') and (5'-CCGCTCGAGTCCTCCACCACCAGTTTCCGGCAGAGACTTGTACAGCTCGTCCATGC-3') for LPETG5 and (5'-CCGCTCGAGTGCTGCAGCAGCAGCAGTTTCCGGCAGAGACTTGTACAGCTCGTCCATGC-3') for LPETA5 and was subcloned into the *EcoRI/XhoI* sites of pET-21a.

The surface expression plasmid for opt-SrtA $\Delta$ <sub>59</sub>-TM was constructed as follows: Opt-SrtA $\Delta$ <sub>59</sub> was obtained through PCR using the primers (5'-CGGGATCCATGCAGGCTAAACCTCAGATCC-3') and (5'-CCGCTCGAGCTTCACCTCGGTGGCGACG-3') and was subcloned into the *BglII/SalI* sites of the pDisplay plasmid (Invitrogen, USA).

**Expression and purification of 6x His-tagged opt-SrtA $\Delta$ <sub>59</sub>, 6x His-tagged EGFP-LPETG5, and 6x His-tagged EGFP-LPETA5:** The plasmids were transfected into *E. coli* BL21 (DE3). The transfected cells were grown in LB medium with ampicillin (100  $\mu$ g/ml) to an OD<sub>600</sub> value of 0.6-0.8, at which time expression of the protein was induced by the addition of isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) (Gibco-BRL, USA) to a final concentration of 1 mM. After growth for an additional 16 h at 25 °C, the cells were harvested by centrifugation. The cell pellets were resuspended in binding buffer (50 mM Tris pH 7.0, 500 mM NaCl) and lysed by sonication. Then, 6x His-tagged opt-SrtA $\Delta$ <sub>59</sub>, 6x His-tagged EGFP-LPETG5, and 6x His-tagged EGFP-LPETA5 were purified from the soluble fraction of the lysate by IDA Excellose affinity chromatography (Bioprogen, Korea) according to the manufacturer's protocol and dialyzed with phosphate-buffered saline (PBS, pH 7.4). Protein concentrations were determined by the Bradford method using bovine serum albumin as a standard.

**Protein conjugation activity of opt-SrtA $\Delta$ <sub>59</sub> *in vitro*:** We mixed opt-SrtA $\Delta$ <sub>59</sub> (10  $\mu$ g) and EGFP-LPETG5 (10  $\mu$ g) with 5mM CaCl<sub>2</sub> in 1x PBS at 37 °C for 4 h. Afterward, the sample mixtures were resolved on 12 % SDS-PAGE gels stained with Coomassie brilliant blue R-250. We used EGFP-LPETA5 (10  $\mu$ g) as a negative control.

**Cell culture and plasmid transfection:** Human cervical carcinoma (HeLa) cells

were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10 % fetal bovine serum at 37 °C under 5 % CO<sub>2</sub>. Plasmid transfection was performed using Lipofectamine<sup>TM</sup> 2000 reagent (Invitrogen, USA) according to the manufacturer's protocol.

For western blotting analysis to detect protein expression levels, HeLa cells were transiently transfected with SrtAΔ<sub>59</sub> or opt-SrtAΔ<sub>59</sub>. Twenty-four hours after transfection, the cells were collected and lysed in 150 mM NaCl, 1 % NP-40, 0.5 % deoxycholic acid, 0.1 % SDS in 50 mM Tris-HCl (pH 8) with 0.5 ml protease inhibitor (Roche Applied Science, Germany). We measured the protein concentrations of cell lysates using Bradford reagent (Bio-Rad, USA) and resolved 50 μg of protein by 12 % SDS-PAGE with a molecular weight standard. After transferring the resolved proteins onto nitrocellulose membranes, we visualized the anti-HA antibody (Santa Cruz Biotechnology, USA) with a secondary antibody HRP conjugate (Thermo Scientific Inc., USA) and ECL reagent (Thermo Scientific Inc., USA).

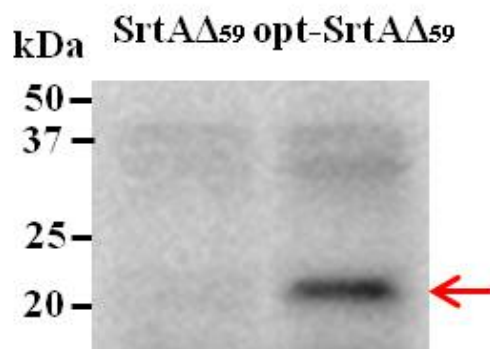
**Immunofluorescence staining (Cell-surface staining):** Twenty-four hours after transfection, we fixed cells with 4 % paraformaldehyde for 10 min, washed them with 1x PBS, and blocked them with 5 % goat serum (Vector Lab., USA) in TPBS for 1 h at 37 °C. We washed and incubated the nonpermeabilized cells with Alexa Fluor 647-labeled anti-HA antibody (Cell signaling, USA) at 4 °C for 24 h. After washing with 1x PBS, we mounted the cells on microscope slides using fluorescent mounting medium with DAPI (Vector Lab., USA) and visualized the cells with confocal fluorescence microscopy.

**Cell surface labeling via opt-SrtAΔ<sub>59</sub>-TM.** HeLa cells were transiently transfected with opt-SrtAΔ<sub>59</sub>-TM and incubated for 24 h. A site-specific labeling reaction was performed by incubating the cells with 10 μM EGFP-LPETG5 or 10 μM carboxytetramethylrhodamine (TAMRA)-LPETG5 at 37 °C for various lengths of time, after which the cells were washed with 1x PBS. As a negative control, cells transiently expressing opt-SrtAΔ<sub>59</sub>-TM were incubated with 10 μM EGFP-LPETA5 or 10 μM TAMRA-LPETA5 at 37 °C for 1 h. The cells were observed with a laser scanning microscope (LSM) 510 META confocal microscope system (Carl Zeiss, Germany) using a c-Apochromat 40x1.2 w objective. Images were taken using an excitation/emission band pass filter (or long pass filter) set at 405/420-480 nm, 488/505-550 nm, and 543/560 nm for DAPI, EGFP, and TAMRA, respectively. TAMRA-LPETG5 and TAMRA-LPETA5 were purchased from Pepton (Korea) and dissolved in DMSO.

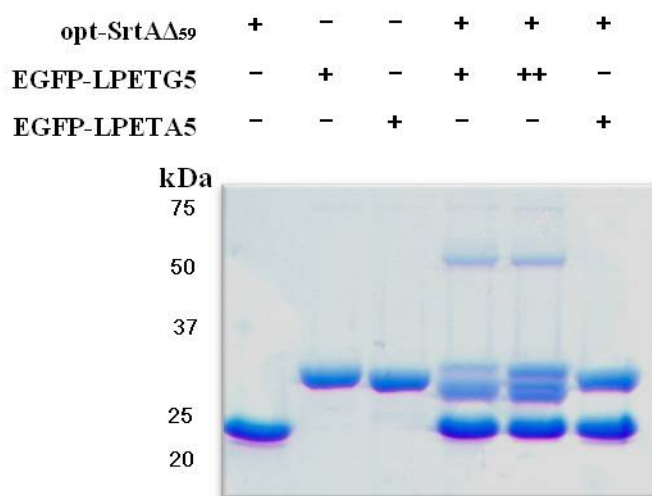
For the western blotting analysis, HeLa cells transiently expressing opt-SrtA $\Delta_{59}$ -TM were incubated with 10  $\mu$ M EGFP-LPETG5 or 10  $\mu$ M EGFP-LPETA5. Twenty-four hours after incubation, we collected the cells and lysed them in 150 mM NaCl, 1 % NP-40, 0.5 % deoxycholic acid, 0.1 % SDS in 50 mM Tris-HCl (pH 8) with 0.5 ml protease inhibitor (Roche Applied Science, Germany). We measured the protein concentrations of the cell lysates using Bradford reagent (Bio-Rad, USA) and resolved 100  $\mu$ g of protein using 15 % SDS-PAGE with a molecular weight standard. After transferring the proteins onto nitrocellulose membranes, we visualized the anti-Myc antibody (Cell signaling, USA) using a secondary antibody HRP conjugate (Thermo Scientific Inc., USA) and ECL reagent (Thermo Scientific Inc., USA).

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1   ATGCAGGCTAAACCTCAGAT CCCGAAGGAC AAGTCTAAGG TGGCCGGCTA
51  CATCGAGATT CCCGACGCTG ACATCAAGGA ACCAGTGTAC CCAGGACCCG
101 CGACCCCTGAACAGCTTAAT CGCGGTGTGA GCTTCGCAGA GGAGAACGAG
151 AGCCTGGACG ATCAAACAT TTCAATCGCC GGGCACACTT TTATTGACAG
201 ACCTAATTAC CAGTTTACAAACCTGAAGGC AGCCAAGAAG GGGTCCATGG
251 TGTACTTCAAGGTTGGCAAC GAGACCCGGA AGTATAAGAT GACGAGTATA
301 CGGGACGTCAAGCCCACAGA CGTAGGAGTC CTGGACGAGC AGAAAGGCAA
351 GGACAAGCAG CTGACACTCA TCACTTGC GA CGACTATAAT GAAAAGACCG
401 GCGTTTGGGA AAAGAGGAAG ATCTTCGTCG CCACCGAGGT GAAGTGA
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**Fig. S1** DNA sequence of codon-optimized SrtA $\Delta_{59}$ .



**Fig. S2** Western blotting analysis of the expression levels of SrtA $\Delta$ <sub>59</sub> and opt-SrtA $\Delta$ <sub>59</sub> using anti- HA antibody.



**Fig. S3** The specific anchoring activity of opt-SrtA $\Delta$ <sub>59</sub> was detected by incubating a mixture of purified opt-SrtA $\Delta$ <sub>59</sub> (10  $\mu$ g) with EGFP-LPETG5 (10  $\mu$ g) or EGFP-LPETA5 (10  $\mu$ g) at 37 °C for 4 h. Proteins were visualized by Coomassie Blue staining.