RESEARCH LETTER

Development of a whole-cell biosensor by cell surface display of a gold-binding polypeptide on the gold surface

Tae Jung Park[1,2], Shun Zheng[1,2], Yeon Jae Kang[2] & Sang Yup Lee[1,2,3]

[1]BioProcess Engineering Research Center, Center for Systems and Synthetic Biotechnology, Institute for the BioCentury, KAIST, Daejeon, Korea; [2]Department of Chemical and Biomolecular Engineering (BK21 Program), KAIST, Daejeon, Korea; and [3]Department of Bio and Brain Engineering, Department of Biological Sciences, Bioinformatics Research Center, KAIST, Daejeon, Korea

Correspondence: Sang Yup Lee, Department of Chemical and Biomolecular Engineering (BK21 Program), KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305–701, Korea. Tel.: +82 42 350 3930; fax: +82 42 350 8800; e-mail: leesy@kaist.ac.kr

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Abstract

Cell surface display was used as a strategy to display the gold-binding polypeptide (GBP) fusion protein on the surface of *Escherichia coli*, and consequently to immobilize the cells on the gold surface. The DNA encoding the GBP was fused to the truncated *fadL* gene and was expressed by the *tac* promoter. For the display of the core streptavidin (cSA) of *Streptomyces avidinii*, the *cSA* gene was fused to the truncated *oprF* gene. After the dual display of FadL–GBP and OprF–cSA on the surface of *E. coli*, binding of cells on the gold surface and the interaction of OprF–cSA with the biotin–horseradish peroxidase (HRP) were studied by surface plasmon resonance (SPR) analysis. Cells displaying the FadL–GBP fusion protein could be immobilized on the SPR sensor chip as shown by the SPR angle shift of 0.5°, which was stably bound at least for 60 h with a washing solution. When the FadL–GBP and OprF–cSA fusion proteins were displayed on the same cell surface, the former was used to immobilize the cells on the gold surface and the latter was used for the interaction studies with the biotin–HRP, which demonstrates that the strategy should be useful for developing whole-cell biosensor chips.

Introduction

Biosensors are becoming more and more important in a wide range of applications requiring real-time analysis of specific binding phenomena including antigen–antibody, protein–protein and small molecule–protein interactions (Karlsson, 1994; Wohlhueter *et al.*, 1994). A whole-cell biosensor allows the detection of various chemicals that induce changes in cellular signaling, regulatory and metabolic networks, which cannot be achieved using one or several proteins. A whole-cell biosensor can also be used to study the effects of various stimuli on cellular physiological changes. Functional and stable immobilization of cells on a desired surface is one of the most critical factors in making a whole-cell biosensor. Currently, there are three representative methods for cell immobilization: adsorption, covalent attachment and entrapment (Collings & Caruso, 1997). The main advantage of direct adsorption is that it is a simple method that can be performed under mild conditions. However, it requires a long incubation time and is sensitive to changes in temperature, pH and ionic strength (Wang & Mulchandani, 2001). Covalent attachment is often achieved by activating the solid surface with a cross-linking agent and then attaching the cells through surface amine groups. However, it often results in the loss of cellular activity and viability due to the use of a cross-linking agent (Barie & Rapp, 2001). Entrapment has been used for many years, but has an inherent problem of mass transfer limitation. Therefore, a new method was needed for efficient, stable and specific immobilization of cells onto the solid surface (Pilkington *et al.*, 1998).

Gold-binding polypeptides (GBPs) were originally developed by Brown (1997), who showed that GBPs preferentially bind gold over chromium, demonstrating substrate specificity. More than 50 sequences of inorganic-binding polypeptides were identified, and the peptide having the amino acid sequence of MHGKTQATSGTIQS was studied (Brown, 1997; Sarikaya *et al.*, 2004). Interestingly, the GBP sequence does not contain cysteine, which is known to form a covalent bond with gold (Brown, 1997); the adsorption kinetics of GBP on the gold surface was reported using surface plasmon resonance (SPR) spectroscopy (Tamerler

et al., 2006). Whereas many proteins and self-assembled monolayers bind to the gold surface via thiol linkage (Zhang *et al.*, 1996; Tarek *et al.*, 1999), the nature of GBP binding is thought to be different. The GBP sequences are rich in serine and threonine, and thus these polar side-chains seem to interact with the gold surface. There are several advantages of using GBP as a gold-binding platform. For example, there is no need to introduce a hydrogel layer to provide a hydrophilic environment and it is possible to modify with different recognition molecules that can be attached to the carboxyl and hydroxyl groups present in the GBP (Woodbury *et al.*, 1998). Furthermore, proteins and peptides can be fused to the GBP for their immobilization on the gold surface (Whaley *et al.*, 2000; Park *et al.*, 2006). This approach was successfully used for studying antigen–antibody and protein–protein interactions (Park *et al.*, 2006).

SPR biosensors exploit electromagnetic waves to examine interactions between the biomolecules immobilized on the SPR chip surface and the analyte of interest (Homola, 2003). It allows real-time label-free detection of nanomolar concentrations of proteins (Rebecca *et al.*, 2002; Dotsenko *et al.*, 2003). Because of these excellent capabilities of monitoring optical changes at interfaces between a thin metal film and a dielectric fluid, the SPR technique has been used in various applications including immunochemical detection of biomolecules, cells and chemicals, as well as sensing of gases (Caruso *et al.*, 1995; Hide *et al.*, 2002; Dotsenko *et al.*, 2003).

In this paper, we report the development of a novel strategy for the cell immobilization by using a cell surface display that allows the display of GBP on the surface of *Escherichia coli*. Cells displaying GBP were immobilized on the gold surface, which was examined by SPR. Using a dual-display system, we also show that protein–biomolecule interactions can be studied on the cell surface immobilized onto the gold surface.

Materials and methods

Materials and apparatus

Bacto tryptone and Bacto yeast extract were purchased from BD Biosciences (Franklin Lakes, NJ). Other chemicals and reagents were purchased from Sigma (St. Louis, MO), unless otherwise stated. All the bacterial strains and plasmids used in this study are listed in Table 1. The gold-thin-layered sensor chips (18 mm × 18 mm × 0.5 mm with block prism) and the SPR instrument were purchased from K-MAC (*SPRLAB*^{TM}, Daejeon, Korea). All oligonucleotides were synthesized at Genotech (Daejeon, Korea). PCR experiments were performed with a PCR Thermal Cycler (Bio-Rad, Hercules, CA) using a High Fidelity PCR System (New England Biolabs, Beverly, MA). The PCR primers used in this study are listed in Table 2. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs. The DNA sequences of all clones were confirmed by an automatic DNA sequencer (ABI Prism 377, Perkin Elmer Co., IL).

Cell growth and sample preparation

Escherichia coli XL1-Blue was used as a host strain for the cell surface display of FadL–GBP. For the dual display of FadL–GBP and OprF–cSA, the *E. coli* XL10-Gold strain was used as a host strain. Recombinant *E. coli* transformants were cultivated in 250-mL flasks containing 100 mL Luria–Bertani medium supplemented with 100 μg mL^{-1} of ampicillin and/or 30 μg mL^{-1} of chloramphenicol according to the characteristic of each plasmid at 37 °C in a shaking incubator at 200 r.p.m. Cell growth was monitored by measuring $A_{600\,nm}$ ($OD_{600\,nm}$; DU® 650 Spectrophotometer, Beckman, Fullerton, CA). At an $OD_{600\,nm}$ of 0.6, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 1 mM to induce the gene expression from

Table 1. Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	References or sources
E. coli strains		
XL1-Blue	*recA1*, *endA1*, *gyrA96*, *thi*, *hsdR17*, *suppE44*, *relA1*, I^-, lac^-, F′[*proAB lacI*^{q} *lacZ*Δ*M15* Tn*10* (Tet)^r]	Stratagene Cloning Systems (La Jolla, CA)
XL10-Gold	Tet^r Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-l recA1 gyrA96 relA1 lac* Hte [F′*proAB lacI*^{q}*Z*Δ*M15* Tn*10*(Tet^r)*Amy Cam*^{r}]	Stratagene Cloning Systems
Plasmids		
pTrc99A	4.2 kb, Ap^r; *trc* promoter	GE Healthcare (Piscataway, NJ)
pACYC184	Tet^r, Cam^r, p15A ori, 4.2 kb	New England Biolabs
pTGE	Ap^r, pTrc99A derivative, *trc* promoter, GBP::EGFP, 5.0 kb	Park *et al.* (2006)
pTac99A	Ap^r, pTrc99A derivative, *tac* promoter, pBR322 ori, 5.7 kb	Park & Lee (2003)
pTacFadLGBP-1	Ap^r, pTac99A derivative, *fadL*, GBP, 6.9 kb	This study
pTac$OprF_{164}$cSA	Ap^r, pTac99A derivative, *oprF*, cSA, 6.6 kb	This study
pACYCtac$OprF_{164}$cSA	Cam^r, pACYC184 derivative, *tac* promoter, p15A ori, *oprF*, cSA, 5.1 kb	This study

Ap, ampicillin; Cam, chloramphenicol; Tet, tetracycline; r, resistance; ori, origin of replication.

Table 2. Primer sequences for PCR amplification in this study

Gene target	Template	Primer	Sequence (5′ → 3′)*
fadL	*E. coli* W3110 genomic DNA	*fadL*-F†	CAGCGAATTC**ATG**GTCATGAGCCAGAAAACC
		fadL-R	TGCATGCTAGCTCTAGAACGATTCTGTGCAGGAA
GBP-1	pTGE	GBP-1-F	AATCGTTCTAGAGCTAGCATGCACGGCAAAACCC
		GBP-1-R	GACAAGCTTACTAGTATTAAGACTGAATGGTACC
$oprF_{164}$	*P. aeruginosa* PA01 genomic DNA	*oprF*-F†	ACAGAATTC**ATG**AAACTGAAGAACACCTT
		oprF-R	TATTCTAGATTTCGAACCACCGAAGTT
cSA	*S. avidinii* genomic DNA	*cSA*-F	TGAATTCGGCATCACCGGCACCTGG
		cSA-R	GCCAAGCTTATTACGGCTTCACCTT
tac	pTacOprF$_{164}$cSA	*tac*-F	AAGGATCCGCAAATATTCTGAAATGA

*The restriction enzyme sites are indicated by underlines.
†The sequence for start codon is shown in bold.

the *tac* promoter. After further cultivation for 6 h, cells were harvested by centrifugation at 10 000 ***g*** for 10 min. The cell pellet was washed with phosphate-buffered saline (PBS, pH 7.4) solution, and resuspended in PBS solution at desired concentrations. Cell numbers were counted using a hemacytometer (Superior, Marienfeld, Germany) on the slide glass under an optical microscope (TE2000, Nikon, Tokyo, Japan).

SPR analysis

Before the SPR experiments, the sensor chip was dipped into the piranha solution [a 3 : 1 mixture of concentrated H_2SO_4 and 30% (v/v) hydrogen peroxide (H_2O_2)] for 5 min, immersed in 10 mM glycine–HCl solution for 30 min and washed with PBS solution. After flow-drying of the SPR sensor chip using nitrogen gas, PBS solution was flowed over the sensor chip surface for 3 h to equilibrate the chip. The sample of 300 μL was loaded at the flow rate of 30 μL min^{-1}. The SPR experiments were carried out at 25 °C using the repeated angle mode and the fixed angle mode as shown in supplementary data.

Activity assay of horseradish peroxidase (HRP) on the surface of cells

The enzyme activity was assayed by the colorimetric method (Liu *et al.*, 2006). First, cells displaying only GBP, and both GBP and cSA were incubated with biotin–HRP at 30 °C for 30 min to achieve full binding, followed by washing and reharvest. The experiment for HRP assay is as follows: a reaction mixture containing 10 mM phenol, 0.2 mM H_2O_2 and 2.4 mM 4-aminoantipyrin in a total volume of 3.0 mL was incubated at 25 °C for 4–5 min. All reagents were dissolved in PBS buffer. The reaction was then started by adding the outer membrane fraction of cells harvested from 0.1×10^7 CFU mL^{-1}, and the initial increase in absorbance was monitored at a wavelength of 510 nm for 1 min. Under such conditions, HRP activity was represented as the $A_{510\,nm}$ per cell densities ($OD_{600\,nm}$) that was for the enzyme consuming 1 μM of H_2O_2 per minute under the assay conditions.

Preparation and analysis of outer membrane proteins in *E. coli* cells

Proteins in the whole-cell lysates and membrane fraction were analyzed by 12% (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Outer membrane proteins were prepared as in the previous report (Lee *et al.*, 2005).

Results and discussion

Construction of plasmids for cell surface display of GBP and cSA

For the cell surface display of GBP, we used the truncated FadL as an anchoring motif (Lee *et al.*, 2004). The DNA fragment encoding the truncated *fadL* gene (GenBank accession no. 946820) encoding the first 384 amino acids from the N-terminus was obtained by PCR amplification with the primers *fadL*-F and *fadL*-R using the genomic DNA of *E. coli* W3110 (ATCC 27325) as a template. Likewise, the DNA fragment encoding the GBP (three repeats) and a stop codon was obtained by PCR with the primers GBP-1-F and GBP-1-R using the plasmid pTGE (Park *et al.*, 2006) as a template. Then, the *fadL*–GBP fusion gene was obtained by overlapping PCR amplification using the primers *fadL*-F and GBP-1-R, and subsequently cloned into the EcoRI and HindIII restriction sites of pTac99A (Park & Lee, 2003) with the ColE1 origin of replication to make pTacFadLGBP-1 (Supporting Information, Fig. S1a).

For the dual expression and display of cSA to study protein–biomolecule interaction, the truncated OprF was used as a different surface-anchoring motif (Lee *et al.*, 2005). The genomic DNA of *Pseudomonas aeruginosa* PAO1 (ATCC 47085D) was used as a template for PCR amplification of the truncated *oprF* gene (GenBank accession no. 878442) encoding 188 amino acids downstream from the N-terminus

using the primers *oprF*-F and *oprF*-R. The genomic DNA of *Streptomyces avidinii* ATCC 27419 was used as a template for PCR amplification of the *cSA* gene using the primers *cSA*-F and *cSA*-R. The $oprF_{164}$-*cSA* fusion gene was obtained by overlapping PCR amplification using the primers *oprF*-F and *cSA*-R, and cloned into the EcoRI and HindIII sites of pTac99A to make pTac$OprF_{164}$cSA. To construct a dual-display system, it is necessary to use a different origin of replication for plasmid compatibility. We used the pBR322 origin of replication for the display of FadL–GBP fusion protein, and consequently decided to use the p15A origin of replication for the display of the OprF–cSA fusion protein. The *tac* promoter-*oprF*-*cSA* fusion gene was amplified by PCR with the primers *tac*-F and *cSA*-R using pTac$OprF_{164}$cSA as a template. The PCR product was cloned into the BamHI and HindIII sites of pACYC184 to make pACYCtac$OprF_{164}$cSA (Fig. S1b). Even though FadL- and OprF-anchoring motifs previously developed in our lab were used in this study, other surface-anchoring motifs may be similarly used.

The schematic diagram for the immobilization using the dual-display system by the truncated FadL motif for the display of GBP and the truncated OprF motif for the display of cSA is shown in Fig. 1. The displayed GBP could immobilize *E. coli* cells onto the gold surface and the displayed cSA could interact with biotin–HRP. This specific immobilization onto the gold surface and streptavidin–biotin interaction can be detected as an angle shift on the gold chip surface by SPR analysis.

Immobilization of recombinant cells displaying GBP on the gold chip surface

An SPR spectrometer equipped with a gold-thin-layered sensor chip was used to examine the immobilization of *E. coli* XL1-Blue harboring pTacFadLGBP-1 onto the gold surface. *Escherichia coli* XL1-Blue harboring pACYCtac$OprF_{164}$cSA and XL1-Blue cells without the plasmid were used as negative controls. In all cases, the cell concentrations were fixed at 1×10^7 CFU mL^{-1} diluted in PBS buffer. At first, PBS buffer was flowed for 20 min to establish an initial steady baseline, and then cells of XL1-Blue harboring pTacFadLGBP-1, XL1-Blue harboring pACYCtac$OprF_{164}$cSA or XL1-Blue without the plasmid were injected and flowed for 25 min. After introducing the cells, PBS buffer was flowed again over the gold surface to wash away unbound cells for 1 h. Figure 2a shows the SPR profiles of the repetitive angle mode observed with three strains, XL1-Blue with and without pTacFadLGBP-1, and that harboring pACYCtac$OprF_{164}$cSA. In the case of XL1-Blue cells harboring pTacFadLGBP-1, the SPR angle shifted by 0.5° from 51.4° to 51.9° due to the cell immobilization even after the washing step. On the other hand, no angle shift and nonspecific adsorption were observed for the XL1-Blue cells without pTacFadLGBP-1 and XL1-Blue harboring pACYCtac$OprF_{164}$cSA, indicating that they were washed away by the final washing with the PBS buffer. From these results, we can suggest that cells displaying GBP on the cell surface can be specifically and efficiently immobilized on the gold chip.

To determine the effect of cell concentration, Fig. 2b shows the changes in the SPR angle shifts using the repetitive angle mode when three different concentrations of cells harboring pTacFadLGBP-1 were flowed over the gold sensor chip surface; three different cell concentrations of 1×10^5, 1×10^6 and 1×10^7 CFU mL^{-1} were examined. The SPR angle shifts observed at these cell concentrations were $0.049 \pm 0.007°$, $0.248 \pm 0.033°$ and $0.507 \pm 0.043°$, respectively, which showed the increase of angle shift according to the increase of cell concentration. We decided to use 1×10^7 CFU mL^{-1} for further experiments as it gave the largest angle shift. To confirm the cell immobilization on the SPR chip surface, immobilized cells were stained using 50 μL

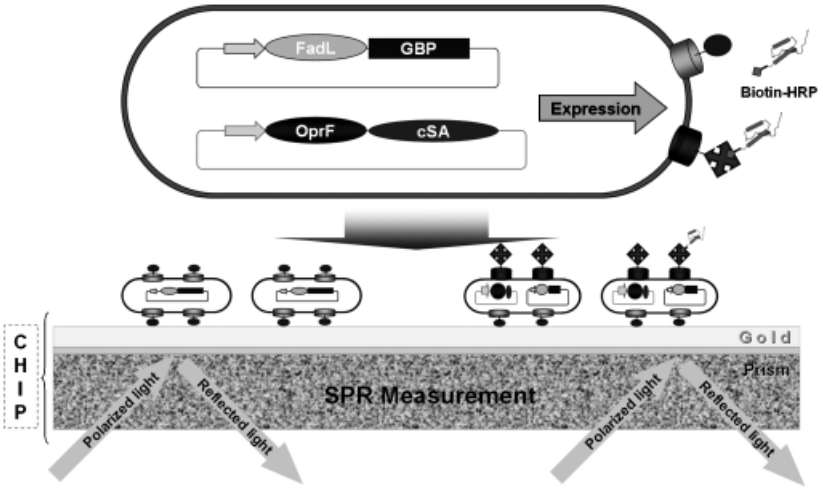

Fig. 1. Schematic diagram for the display of FadL–GBP and OprF–cSA fusion proteins on the surface of *Escherichia coli* for SPR analysis. The truncated FadL protein was used as a surface-anchoring motif for the display of GBP. Similarly, the truncated OprF protein was used for the display of cSA. XL1-Blue (pTacFadLGBP-1) was used for basic gold-binding studies, while XL10-Gold (pTacFadLGBP-1, pACYCtac$OprF_{164}$cSA) was used for the gold binding as well as the biomolecular interaction studies. All binding and interaction studies were performed by SPR analysis.

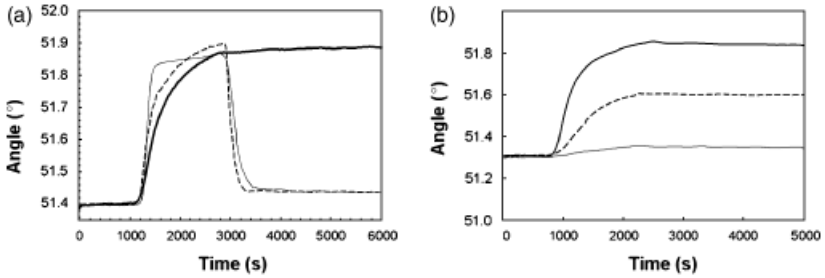

Fig. 2. SPR analysis of *Escherichia coli* XL1-Blue displaying GBP on the gold chip surface. (a) The graph showing the difference in SPR angle shifts observed with *E. coli* XL1-Blue harboring pTacFadLGBP-1 (bold line), XL1-Blue harboring pACYCtacOprF$_{164}$cSA (dashed line) and XL1-Blue without the plasmid (thin line). (b) The graph showing the effect of cell concentration on SPR angle shift. The curves represent the SPR results obtained by flowing XL1-Blue (pTacFadLGBP-1) cells at concentrations of 1×10^5 CFU mL^{-1} (thin line), 1×10^6 CFU mL^{-1} (dashed line) and 1×10^7 CFU mL^{-1} (bold line). The SPR angle shifts were $0.049 \pm 0.007^\circ$, $0.248 \pm 0.033^\circ$ and $0.507 \pm 0.043^\circ$, respectively.

of crystal violet solution and observed under an optical microscope. As shown in Fig. S2, although some elongated *E. coli* cells were observed, more cells were obviously bound to the gold surface as the cell concentration flowed over the gold chip increased.

Stability of immobilized cells on the gold sensor chip

For the binding assays on a sensor chip, cells need to be stably attached to the surface. In order to examine the binding stability of immobilized cells (*E. coli* XL1-Blue cells harboring pTacFadLGBP-1) on the gold surface, PBS solution was flowed for a relatively long time as a washing step. This was performed as follows: first, PBS buffer was flowed on the gold surface for about 15 min to provide a stable baseline. Sequentially, 300 μL of cells displaying GBP, at a concentration of 1.3×10^7 CFU mL^{-1}, were injected for 10 min. Finally, the PBS buffer was flowed again for 60 h. As can be seen in Fig. 3, the SPR angle was shifted by 0.62° from 52.16° to 52.78° due to the cell binding to the gold chip surface. During the entire washing period, the SPR angle did not decrease by $> 0.1^\circ$, suggesting that cells immobilized on the gold chip were stably bound. Thus, the use of GBP displaying cells can be a potential platform for the development of a whole-cell biosensor chip.

Development of a dual-display system as a platform for a biosensor chip

As *E. coli* cells displaying GBP could be successfully immobilized on the gold chip, we next examined the possibility of displaying another protein that can interact with other biomolecules. As a proof-of-concept for example, the cell surface display of cSA, followed by its interaction with biotin, was examined. Because we still need to have GBP displayed on the cell surface for the immobilization of cells on the gold chip, a dual-display system was necessary. Therefore, we used another surface-anchoring motif, OprF,

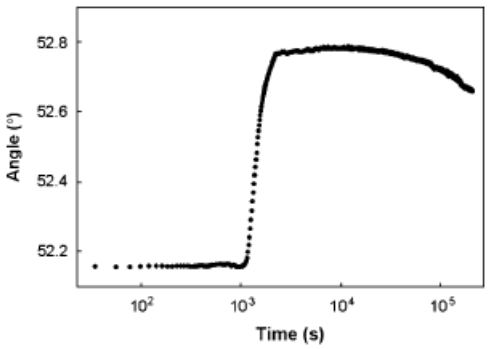

Fig. 3. Stability of immobilized cells on the SPR gold chip. Immobilization of *Escherichia coli* XL1-Blue (pTacFadLGBP-1) cells on the gold chip resulted in increase of 0.55° in the SPR angle from 52.16° to 52.71°, which was decreased by not more than 0.05° after a 60-h washing with a buffer solution.

from *P. aeruginosa* we had developed previously (Lee *et al.*, 2005). Two plasmids, pTacFadLGBP-1 and pACYCtacOprF$_{164}$cSA, were transformed into *E. coli* XL10-Gold and XL1-Blue strains, respectively. Because the *E. coli* XL1-Blue strain could display either GBP or cSA, but not both stably, *E. coli* XL10-Gold strain was used for dual display of GBP and cSA (data not shown). *Escherichia coli* XL10-Gold cells (1.3×10^7 CFU mL^{-1}) displaying both GBP and cSA were flowed over the gold chip surface for their immobilization via GBP displayed on the cell surface. Then, biotin–HRP was flowed to examine the interaction with cSA displayed on the cell surface. SPR measurements using the fixed angle mode at the most sensitive angle point clearly showed the successful immobilization of cells displaying two different proteins, GBP and cSA, and interaction with biotin–HRP (Fig. 4).

Upon immobilization of *E. coli* XL10-Gold cells displaying both GBP and cSA, the SPR reflectance changed by 9.84% from 0.86% to 10.70%, which is not much different from that observed with *E. coli* XL10-Gold cells displaying only GBP (Fig. 4). These results suggest that the dual display of GBP and cSA did not affect considerably the binding of

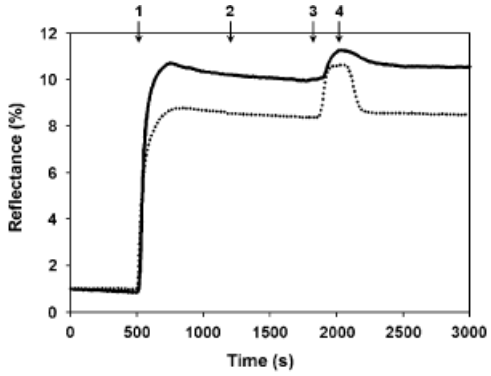

Fig. 4. SPR analysis of a dual-display system of GBP and cSA. The SPR results showing the immobilization of *Escherichia coli* XL10-Gold cells displaying only GBP (dotted line) and both GBP and cSA (solid line), and their subsequent reaction with biotin–HRP. To generate a steady baseline, PBS buffer was flowed for 10 min. At point 1, cells displaying only GBP or both GBP and cSA were flowed over the gold chip surface. At point 2, PBS solution was flowed to wash away nonimmobilized cells. At point 3, biotin–HRP (0.1 mg mL^{-1}) solution was flowed to interact with displayed cSA. At point 4, PBS solution was flowed for 10 min to wash away unbound biotin–HRP.

cells on the gold chip. When biotin–HRP was flowed over the gold chip with immobilized cells, followed by washing, little SPR angle change was observed for the cells displaying only GBP while a 0.62% increase in the SPR angle from 9.95% to 10.57% was observed for the cells displaying both GBP and cSA. This clearly indicates the biotin–HRP binding to the cells displaying cSA on the gold chip. This result was also supported by a colorimetric assay of HRP on the surface of *E. coli* cells and SDS-PAGE of outer membrane fractions (Fig. S3). From this result, we confirmed that the protein composition of the outer membrane fraction in a dual-display system displaying both GBP and cSA was different from the case of *E. coli* cells displaying GBP only.

Many surface-anchoring motifs can be used for the display of proteins and peptides (Lee *et al.*, 2003), and here we used two surface anchoring motifs: FadL and OprF. This will confer much more flexibility in developing the most suitable display system as the efficiency of cell surface display may vary depending on the protein to be displayed as well (Lee *et al.*, 2003). Furthermore, an SPR sensing of *E. coli* cells, which have a diameter of about 1 μm, was successful. This means our study was within the interrogation region of the SPR laser signal (Homola, 2003). Also, more than two proteins can be displayed on the cell surface by extending the strategy described here, which will allow the development of a multifunctional cell biosensor chip.

In summary, we showed that cells displaying GBP on their surface can be efficiently immobilized on the gold chip. Furthermore, using the dual-display strategy, we were able to demonstrate that protein–biomolecule interaction studies can be performed on the SPR gold chip. Therefore, the strategy of the GBP display on the cell surface and the protein/peptide of interest indeed has a potential to be used as a platform for developing a multifunctional cell biosensor system. Various proteins and peptides can be displayed for detecting their interactions with other biomolecules, allowing various applications. One such interesting application would be antibody library screening using antigen-displaying cells immobilized on a gold chip.

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Authors' contribution

T.J.P. and S.Z. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Construction of (a) pTacFadLGBP-1 and (b) pACYCtacOprF$_{164}$cSA used in this study.
Fig. S2. Optical microscopy of GBP-displaying *Escherichia coli* cells immobilized on the SPR chip after Gram staining.
Fig. S3. HRP assay and SDS-PAGE of outer membrane fractions in *Escherichia coli* cells.

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