Stepwise preparation of a polymer comprising protein building blocks on a solid support for immunosensing platform

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Abstract

In immnosensing, immobilization of the antibody on the sensing platform significantly influences the performance of the sensor. Herein, we propose a novel antibody-immobilization method based on a protein-polymer chain containing multiple copies of an antibody-binding protein, the Z-domain. In our approach, the Z-domain-containing polymer is prepared on the surface of the sensing platform with a biotinylation reaction from the archaeon Sulfolobus tokodaii. Biotinylation from S. tokodaii has a unique property by which biotin protein ligase (BPL) forms an extremely stable complex with its biotinylated substrate protein (BCCP). Here, we employed two types of engineered proteins: one was the fusion protein of BCCP with the Z-domain (BZB), in which BCCP was genetically attached to the N- and C-termini of the Z-domain; the other was a BPL dimer prepared by connecting two BPL molecules with a cross-linking reagent. We applied these two engineered proteins alternately onto the BPL-modified solid support of the surface plasmon resonance sensor chip, and succeeded in growing polymer chains comprising multiple units of BZB and the BPL dimer. The antibody-binding capability of the Z-domain-containing polymer thus prepared is adjustable by controlling the number of cycles of protein addition and the surface density of the polymer on the solid support.

**Keywords:** Immunosensor; Antibody-binding protein; Z-domain; Surface plasmon resonance; Biotinylation; Protein interaction.
Introduction

Immunosensing is a versatile analytical method based on the specific binding interaction between antibody and antigen, and is exploited in various fields such as life sciences, medical diagnosis, food chemistry, and environmental monitoring. In immunosensing, antibodies are generally immobilized on the solid support of a sensing platforms; since this antibody immobilization exerts a significant influence on the sensitivity of the systems, various antibody-immobilization techniques have been developed so far. One of the factors governing the sensitivity of immunosensors is the surface density of the antibodies immobilized on the solid supports; the surface density of the antibodies must be high enough for detecting low-abundance analytes in samples.

Recently, to enhance the surface density of the antibody on the solid support, immunosensing platforms based on polymer brushes have been reported, where the polymer brushes are prepared on the solid support via the surface-initiated radical reactions and the antibodies are then attached to those polymers. These approaches are very promising because various functional groups can be incorporated into the polymers, which impart hydrophilicity suppressing non-specific protein adsorption and provide reactive sites for immobilizing the antibody. However, in these approaches, it is hard to prepare polymers with precisely defined structures; thus, they are not suitable for designing sensing platforms at the molecular level. Precise design of sensing platforms at the molecular level is crucial to the fine optimization and reproducibility of the sensing systems.

Another important factor in antibody-immobilization is the orientation of the antibodies on the solid supports. Antibodies can be immobilized on the supports via physical absorption or covalent coupling with their amino groups; however, in these cases, orientation of the antibodies on the supports cannot be controlled, thereby, resulting in the limited sensitivity of these
systems. Therefore, various site-directed antibody-immobilization techniques have been developed so far; in particular, many methods have been proposed with antibody-binding proteins such as Protein A and Protein G from bacteria. These proteins specifically bind to the Fc portion of antibodies from many mammalian species. Thus, the antibodies captured by these antibody-binding proteins on the supports retain their antigen-binding capability, enabling highly sensitive detection compared with systems in which the orientation of the antibodies is not controlled.

In this paper, we demonstrate a method for preparing a polymer containing multiple copies of an antibody-binding protein on a solid support, aiming for construction of an immunosensing platform with high antibody-loading capacity that can be designed at the molecular level (Fig. 1A). Here, we selected the mutated antibody-binding domain of Protein A, the Z-domain, as the antibody-binding protein, and exploited the biotinylation reaction from the archaeon *Sulfolobus tokodaii* to prepare the Z-domain-containing polymer. In biotinylation, biotin protein ligase (BPL) mediates the attachment of biotin to the specific lysine residue of biotin carboxyl carrier protein (BCCP) in a two-step reaction:

\[
\text{Biotin} + \text{ATP} \rightarrow \text{Biotinyl-AMP} + \text{PPI} \quad (1)
\]
\[
\text{Biotinyl-AMP} + \text{BCCP} \rightarrow \text{Biotinylated BCCP} + \text{AMP} \quad (2)
\]

In the first step, biotinyl-AMP is produced by the reaction of biotin with ATP; in the second step, the biotin moiety of biotinyl-AMP is transferred to BCCP to produce biotinylated BCCP. Biotinylation from *S. tokodaii* has a unique property by which BPL forms an extremely stable complex with its product, biotinylated BCCP, under physiological conditions. In a previous work, we succeeded in oriented immobilization of the antibody using fusion proteins of BCCP with the Z-domain, which were captured on the BPL-modified solid support.
Herein, we designed two types of engineered proteins to prepare the Z-domain-containing polymer on the solid support. One was the fusion protein of BCCP with the Z-domain (BZB), in which BCCP was genetically attached to the N- and C-termini of the Z-domain (Fig. 1B). The other engineered protein was a BPL dimer, prepared by connecting two BPL molecules with a cross-linking reagent (Fig. 1C). These two engineered proteins were alternately applied onto the BPL-modified solid support; through the biotinylation reaction upon each addition of these proteins, we envisaged that a polymer chain containing multiple copies of the Z-domain molecule could be prepared in a stepwise manner (Fig. 1A). In this case, by adjusting the number of protein addition cycles, degree of the polymerization is expected to be accurately controlled, thereby allowing for the precision design of an immunosensing platform at the molecular level. In this work, we exploited the gold substrate of the surface plasmon resonance (SPR) sensor chip as the solid support, and assessed our strategy by evaluating the binding level of proteins based on the change in the SPR response.

Experimental

Materials

The oligonucleotides used for PCR amplification were custom-synthesized by Gene Design (Osaka, Japan). The vectors pEZZ 18, pTAKN-2, and pET21a were obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK), BioDynamics Laboratory (Tokyo, Japan), and Novagen (Madison, WI, USA), respectively. NiꞏBind resin and phosphorylated cellulose (P-cellulose) were obtained from Novagen and GE Healthcare, respectively. Butyl Toyopearl and PD-10 column were obtained from Tosoh (Tokyo, Japan) and GE Healthcare, respectively. 1,6-bis(maleimido)hexane was purchased from Tokyo Chemical Industry (Tokyo, Japan).
Anti-green fluorescent protein (GFP) rabbit antibody was purchased from Rockland (Gilbertsville, PA, USA). The SIA Kit Au used in the SPR measurements was purchased from GE Healthcare.

Construction of the expression plasmid for BZB

The expression plasmid for BZB in *E. coli* cells was constructed as follows. The DNA sequence coding for the Z-domain was PCR-amplified with pEZZ 18 as a template using the following primers: 5'-GGATCCGGTGCGGAAGTAGACAACAAATTCAA-3' and 5'-GAGCTCGCCACCTCTTTCTGCGGCACCTTGAGCATC-3' (restriction enzyme sites are underlined and the extra codons for glycine residues are shown in bold). The amplified fragment was TA cloned into a pTAKN-2 vector; following sequence analysis, the DNA fragment for the Z-domain was cut out from the vector by digestion with BamHI/SacI. The obtained fragment was inserted into pET-SH2(LL)BCCP digested with BamHI/SacI to give a plasmid, pET-SH2-Z-BCCP; pET-SH2(LL)BCCP is an expression plasmid constructed in our previous work by inserting the DNA fragments coding for the human SH2 domain and *S. tokodaii* BCCP into the NdeI/BamHI and SacI/XhoI sites of a pET21a vector, respectively.

Likewise, the DNA fragment coding for *S. tokodaii* BCCP was cut out from pET-BCCP(LL)SH2 by digestion with NdeI/BamHI; pET-BCCP(LL)SH2 is an expression plasmid constructed in our previous work by inserting the DNA fragments coding for *S. tokodaii* BCCP and human SH2 domain into the NdeI/BamHI and SacI/XhoI sites of a pET21a vector, respectively. The obtained DNA fragment was inserted into pET-SH2-Z-BCCP digested with NdeI/BamHI to yield an expression plasmid for BZB, pET-BZB.

Preparation of BZB

The fusion protein BZB was isolated from *E. coli* cells as follows. *E. coli* BL21(DE3)
cells harboring pET-BZB were grown at 37°C in a Luria-Bertani broth supplemented with 50 μg mL⁻¹ ampicillin. Following addition of 1 mM isopropyl β-D-thiogalactoside, the cells were further cultured for 8 h; subsequently, the cells were collected by centrifugation and then disrupted by sonication. After centrifugation, the supernatant was subjected to Ni²⁺ affinity chromatography, and the desired fusion protein was further purified by cation-exchange chromatography on P-cellulose to yield a nearly homogeneous protein (Fig. S1). Typically, 10 mg of the purified protein was obtained from a 0.5-L culture. The integrity of the substrate activity of BZB for biotinylation was confirmed by MALDI-TOF mass spectrometry (MS) analysis of the biotinylation reaction with BPL (Fig. S2).

**Preparation of BPL dimer**

The BPL dimer was prepared by connecting the cysteine residues of two BPL molecules with a cross-linking reagent, 1,6-bis(maleimido)hexane. Here, a mutated BPL, BPL-Cys1, which has a single cysteine residue, was used for the reaction. First, the cysteine residue of BPL-Cys1 was reduced by incubation with 20 mM DTT in HEPES (pH 7.5) containing 5 mM EDTA at 37°C for 90 min. After that, the buffer was replaced with PBS (pH 7.4) containing 5 mM EDTA by gel-filtration chromatography on a PD-10 column. To 1.1 mL of a fraction containing 100 nmol of BPL-Cys1, 40 nmol of 1,6-bismaleimide hexane in 50 μL of N,N-dimethylformamide was added; then the mixture was incubated at 25°C for 16 h. Subsequently, the reaction mixture was subjected to hydrophobic chromatography on butyl Toyopearl to yield a nearly homogeneous product (Fig. S3). The integrity of the biotinylation activity of the BPL dimer was confirmed by MS analysis of the reaction with BCCP as a substrate (Fig. S4).
SPR measurement

SPR measurements were conducted on Biacore J (GE Healthcare) with a sensor chip prepared with SIA Kit Au. All measurements were conducted at 25°C. Prior to measurements, the gold surface of the sensor chip was cleaned with piranha solution (3:1 mixture of sulfuric acid and 30% hydrogen peroxide) and then rinsed with distilled water. The mutated BPL, BPL-Cys2,26 was immobilized on the cleaned sensor chip; BPL-Cys2 carries two cysteine residues which could be involved in binding to the gold surface of the sensor chip. Then, the Z-domain-containing polymers were prepared on the BPL-modified sensor chip with BZB and the BPL dimer. For evaluation of the antibody-binding capability, the anti-GFP antibody was injected over the sensor chip modified with the Z-domain-containing polymer. Detailed experimental conditions are described in the legends of the figures.

Results and Discussion

Design of the antibody-capturing platform

In this work, we utilized two engineered proteins, BZB and the BPL dimer, to prepare Z-domain-containing polymers on solid supports. BZB was prepared by isolation from *E. coli* cells over-expressing its protein. In this fusion protein, the BCCP moiety is attached to the N- and C-termini of the Z-domain (Fig. 1B). Here, the N- and C-termini of Z-domain reside on opposite sides of each other, while those of BCCP reside on the same side (Fig. 1B). Thus, the most plausible overall conformation of BZB is the linear form. On the other hand, the BPL dimer was prepared by connecting the cysteine residues of two molecules of the mutated BPL, BPL-Cys1,26 with a bismaleimide reagent (Fig. 1C); BPL-Cys1 contains a single cysteine residue which resides on the opposite side of the binding interface with BCCP (Fig. 1D).
Considering these structural characteristics of the proteins, the polymer formed by the reaction of BZB and the BPL dimer could adopt a linear conformation.

Here, the polymerization is started from the biotinylation reaction in the mutated BPL, BPL-Cys2,\textsuperscript{26} immobilized on the solid support; BPL-Cys2 carries two cysteine residues, both of which are located in the opposite side of the binding interface with BCCP (Fig. 1D). In our previous work, it was suggested that BPL-Cys2 binds to the surface of the gold substrate via the thiol groups of its cysteine residues.\textsuperscript{26} Accordingly, it is likely that the polymer chain extends from BPL-Cys2 in the direction perpendicular to the surface of the support, as depicted in Fig. 1A.

*Preparation of the Z-domain-containing polymer chain on the SPR sensor chip*

To assess the feasibility of our strategy, we successively applied the proteins onto the gold surface of the SRP sensor chip and measured the change in the SPR response. First, BPL-Cys2 dissolved in HBS was injected over the bare gold substrate of the sensor chip. Figure 2A shows a typical binding curve of BPL-Cys2 obtained by monitoring the SPR signal. The surface density of BPL-Cys2 estimated from the SPR signal observed 10 min after the buffer wash was 5.45 ± 0.33 pmol cm\textsuperscript{-2} under the present conditions.

Next, preparation of the protein-polymer chain was conducted on the BPL-modified sensor chip with HBS-T (HBS containing Tween 20) as a running buffer (Fig. 2B). First, HBS-TB (HBS-T containing biotin, ATP, and MgCl\textsubscript{2}) was injected to produce the biotinyl-AMP at the active site of the BPL-Cys2 immobilized on the sensor chip. Following a wash with HBS-T, BZB was injected; then the sensor chip was again washed with HBS-T. Next, the BPL dimer dissolved in HBS-TB was injected, and after washing with HBS-T, BZB was again injected; a set of the addition of the BPL dimer and BZB was referred to as one cycle, and this cycle was repeated three times in total.
Table 1 summarizes the binding levels of BZB and the BPL dimer observed after buffer washing at each step of protein addition. The surface density of BZB observed in its initial addition was 3.10 ± 0.27 pmol cm\(^{-2}\); this value was comparable with that of BPL-Cys2 immobilized on the sensor chip, suggesting that considerable portions of BPL-Cys2 molecules on the sensor chip participated in the reaction with BZB.

On the other hand, the binding level of the BPL dimer was significantly lower than that of BZB obtained by its initial addition. This indicates that only some of the BZB molecules captured by BPL-Cys2 participated in the reaction with the BPL dimer. This may be attributed to the steric hindrance between protein molecules and ununiformity of the gold surface of the sensor chip. However, the binding of the BPL dimer was kept at virtually a constant level in each cycle of the protein addition, suggesting that polymer chains composed of the protein-building units grew on the surface of the sensor chip.

Incidentally, comparing the binding levels of the BPL dimer and BZB in each cycle, the values of BZB were found to be higher than those of the BPL dimer. This may be explained by the fact that the active sites of BPL-Cys2 on the surface of the sensor chip were not saturated by BZB on its initial addition and the remaining sites were occupied by the subsequent addition of BZB; in fact, the binding level of BZB was gradually decreased as the number of the cycles was increased.

Capture of the antibody on the SPR sensor chip

In previous works using polymer brushes as an antibody-binding support, it was demonstrated that the antibody-binding capability could be enhanced by controlling the surface density of the polymer because a highly packed configuration of the polymers would hamper access of the molecules to the space between the polymer chains due to steric hindrance.\(^{28,29}\) Thus, we attempted to control the surface density of the protein-polymer chain grown on the
surface of the sensor chip. Here, the active sites of some of the immobilized BPL-Cys2 molecules were blocked by a BCCP molecule not carrying the Z-domain unit (hereafter, we simply call this protein BCCP); following the treatment of BPL-Cys2 with HBS-TB, BCCP was injected along with BZB (Fig. 3). In this case, the active sites of some of the immobilized BPL-Cys2 molecules could be occupied by BCCP, resulting in the reduction of the number of active sites participating in the reaction with BZB, which in turn the decrease of the surface density of the polymer chain (Fig. 3A).

Thus, we injected the desired proteins on the BPL-Cys2-modified sensor chip and monitored the SPR response (Fig. 3B). Following activation with HBS-TB, the mixture of BZB and BCCP was injected on the sensor chip. Here, molar ratio of BCCP to BZB was set to 3:1 to ensure the large space between the polymer chains to be grown. Subsequently, the cycle of the addition of the BPL dimer and BZB was repeated three times. The SPR signal was increased upon addition of the BPL dimer and BZB; however, the binding levels of the two proteins were clearly lower than those observed in the system without the BCCP treatment (Figs. 2B and 3B, Table 1).

To evaluate the antibody-binding capability, the antibody was injected over the surfaces of the sensor chips modified with Z-domain-containing polymers prepared under both conditions (with and without BCCP treatment) (Fig. 4). The SPR signal in the system with BCCP treatment was more than two times higher than that without BCCP treatment; the binding level of the antibody observed 14 min after the buffer wash in both systems were as follows: BCCP treatment, 370 RU, 0.25 pmol cm\(^{-2}\); no BCCP treatment, 156 RU, 0.10 pmol cm\(^{-2}\). From these results, the antibody-binding capability was found to be enhanced by treatment of the sensor surface with BCCP, demonstrating that control of surface density is an important factor for constructing the antibody-binding platform. This fact also supports the suggestion that a protein-polymer chain was indeed formed on the solid substrate through our strategy.
Next, to assess the effect of the length of the polymer chain on the antibody-binding capability, we increased the number of cycles of the BPL dimer and BZB addition. Figure 5 shows the SPR response obtained by repeating the cycles of addition of two proteins four times following the initial addition of the mixture of BZB and BCCP. The binding levels of the two proteins are summarized in Table 1. The binding of the BPL dimer was kept at a constant level in all cycles, showing extension of the polymer chain in all cycles. The antibody-binding capability of the system with four cycles of addition was compared with that of the system with three cycles. As shown in Figure 6, the antibody-binding level was enhanced by increasing the cycles of the protein addition; the binding levels observed 15 min after buffer washing were as follows: four cycles, 633 RU, 0.42 pmol cm\(^{-2}\); three cycles, 490 RU, 0.33 pmol cm\(^{-2}\). This result demonstrates that the antibody-binding capability is adjustable by controlling the number of cycles of protein addition.

As a preliminary experiment, an antigen was applied onto the antibody-immobilized sensor chip prepared under the same conditions as those in Fig. 6 with three cycles of addition. As a result, the binding response was clearly increased on the addition of the antigen, and its binding level (surface density) was comparable with that of the antibody (Fig. S5), suggesting that considerable amount of the immobilized antibodies could participate in binding to the antigen. These results indicate that our system could be exploited as a method for constructing precisely controlled architectures for immunosensing.

**Conclusions**

In summary, we have proposed a novel method for preparing a polymer containing multiple copies of an antibody-binding protein, the Z-domain, on a solid support using the
unique biotinylation reaction from the archaeon *S. tokodaii*. In our approach, two engineered proteins, BZB and the BPL dimer, were successively applied onto the BPL-modified solid support, and through biotinylation, a polymer chain containing multiple copies of the Z-domain was prepared in a controlled manner. In contrast with the methods using synthetic polymers as the antibody-capturing scaffold, our method enables design of the scaffold at the molecular level, which is crucial to the sensitivity and reproducibility of the immunosensors. Although further investigation and optimization are necessary for exploring our system as a highly sensitive immunosensing platform, our system could provide a useful approach for improving the performance of immunosensors. In addition, our method can be applied to the *in situ* preparation of protein polymer chains containing certain desired proteins. Thus, our method could be exploited as a useful approach for preparing various protein-based biosensors and biomaterials.

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**Supporting Information**

Figures S1-S5 are provided. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
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Table 1  Binding levels\(^{a}\) of BZB and the BPL dimer on the surface of the SPR sensor chip

| Initial addition | 3 cycles-addition without BCCP-treatment | 3 cycles-addition with BCCP-treatment | 4 cycles-addition with BCCP-treatment |
|------------------|------------------------------------------|--------------------------------------|---------------------------------------|
|                  | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) |
|                  | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) | Change in SPR response/ RU               | Surface density\(^{b}\)/ pmol cm\(^{-2}\) |
| Initial addition | BZB                                       | 718 ± 62                              | 3.10 ± 0.27                            |                                       |                                       |                                       |
| 1st cycle        | BPL dimer                                 | 190 ± 14                              | 0.36 ± 0.03                            | 137 ± 11                              | 0.26 ± 0.02                            | 131 ± 12                              | 0.24 ± 0.02                            |
|                  | BZB                                       | 194 ± 15                              | 0.84 ± 0.07                            | 139 ± 15                              | 0.60 ± 0.07                            | 134 ± 14                              | 0.58 ± 0.06                            |
| 2nd cycle        | BPL dimer                                 | 181 ± 14                              | 0.34 ± 0.03                            | 136 ± 11                              | 0.25 ± 0.02                            | 132 ± 13                              | 0.25 ± 0.03                            |
|                  | BZB                                       | 129 ± 11                              | 0.55 ± 0.05                            | 113 ± 11                              | 0.49 ± 0.05                            | 106 ± 11                              | 0.46 ± 0.05                            |
| 3rd cycle        | BPL dimer                                 | 177 ± 13                              | 0.33 ± 0.02                            | 126 ± 13                              | 0.23 ± 0.02                            | 123 ± 10                              | 0.23 ± 0.02                            |
|                  | BZB                                       | 112 ± 11                              | 0.48 ± 0.05                            | 101 ± 7                               | 0.43 ± 0.03                            | 92 ± 9                                | 0.40 ± 0.04                            |
| 4th cycle        | BPL dimer                                 |                                       |                                       | 118 ± 11                              | 0.22 ± 0.02                            |                                       |                                       |
|                  | BZB                                       |                                       |                                       | 81 ± 7                                | 0.35 ± 0.03                            |                                       |                                       |

\(^{a}\) Binding levels 10 min after the buffer wash following addition of each protein are shown. The values shown are means and standard deviations determined from three independent experiments.

\(^{b}\) The values were calculated from the change in the SPR response. A change of SPR response of 1 RU corresponds to a weight change of 100 pg cm\(^{-2}\).
**Figure Captions**

Fig. 1  Preparation of Z-domain-containing polymer on a solid support using the *S. tokodaii* biotinylation reaction. (A) Schematic illustration of the strategy. (B) A domain structure of the fusion protein BZB (upper) and three dimensional structures of BCCP and the Z-domain showing the N- and C-termini of those protein with arrow heads (lower). The lysine residue of BCCP to be biotinylated is shown as a space filling model. (C) Preparation of the BPL dimer. The cysteine residues of two molecules of BPL-Cys1 were cross-linked with 1,6-bis(maleimido)hexane. (D) Model structures of BPL-Cys1 and BPL-Cys2 complexed with BCCP. The cysteine residues of the BPLs, the lysine residue of BCCP to be biotinylated, and biotin are shown as space-filling models.

Fig. 2  Immobilization of BPL-Cys2 (A) and the stepwise preparation of Z-domain-containing polymer (B) on the SPR sensor chip. (A) Following equilibration with HBS (10 mM HEPES, pH 7.5, 150 mM NaCl), 2 µM BPL-Cys2 was injected over the gold surface of the sensor chip for 15 min. Then, the sensor chip was washed with HBS. The flow rate was 15 µL min⁻¹. (B) Following equilibration of the BPL-modified sensor chip with HBS-T (HBS containing 0.005% Tween20), HBS-TB (HBS-T containing 1 µM biotin, 10 µM ATP, and 1 mM MgCl₂) was injected over the sensor chip for 6 min; then the sensor chip was washed with HBS-T for 4 min. After that, 0.5 µM BZB was injected for 10 min, and the sensor chip was washed with HBS-T for 10 min. Subsequently, 0.5 µM BPL dimer dissolved in HBS-TB was injected for 10 min followed by a 10-min wash with HBS-T; then 0.5 µM BZB was again injected for 10 min followed by a 10-min wash with HBS-T. The cycle of the addition of the BPL dimer and BZB followed by the buffer wash was repeated three times in total. The flow rate was 30 µL min⁻¹.
Fig. 3  Control of the surface density of the polymer chains on the SPR sensor chip. (A) Schematic illustration of the strategy. The active sites of some of the immobilized BPL-Cys2 molecules were blocked by BCCP molecules not carrying the Z-domain unit by injecting it along with BZB. Subsequently, the BPL dimer and BZB were alternately applied onto the sensor chip. (B) The stepwise preparation of Z-domain-containing polymer. Following activation of BPL-Cys2 with HBS-TB on the sensor chip, a mixture of 0.5 μM BZB and 1.5 μM BCCP was injected over the sensor chip for 10 min followed by a 10 min-wash with HBS-T. Subsequently, the addition of the BPL dimer and BZB was repeated three times in total under the same conditions as those in Fig. 2B. The flow rate was 30 μL min\(^{-1}\).

Fig. 4  Immobilization of an antibody on the SPR sensor chip modified with Z-domain-containing polymer. The Z-domain-containing polymers were prepared on the sensor chips under the same conditions as those in Fig. 2B (without BCCP treatment) or Fig. 3B (with BCCP treatment). Following equilibration with HBS-T, 10 nM anti-GFP antibody was injected for 6 min over those sensor chips prepared under the conditions without BCCP treatment (blue line) and with BCCP treatment (red line). Then, the sensor chips were washed with HBS-T. The flow rate was 30 μL min\(^{-1}\).

Fig. 5  The stepwise preparation of Z-domain-containing polymer on the SPR sensor chip. Following activation of BPL-Cys2 with HBS-TB on the sensor chip, a mixture of 0.5 μM BZB and 1.5 μM BCCP was injected over the sensor chip for 10 min followed by a 10-min wash with HBS-T. Subsequently, the addition of the BPL dimer and BZB was repeated four times in total (four cycles of addition) under the same conditions as those in Fig. 2B. The flow rate was 30 μL min\(^{-1}\).
Fig. 6  Investigation on the effect of the length of the polymer chain on the antibody-binding capability. The Z-domain-containing polymers were prepared on the sensor chips under the same conditions as those in Fig. 3B (three cycles of addition) or Fig. 5 (four cycles of addition). Following equilibration with HBS-T, 10 nM anti-GFP antibody was injected for 10 min over those sensor chips prepared under the conditions based on three cycles (blue line) and four cycles (red line). Then, the sensor chips were washed with HBS-T. The flow rate was 30 μL min⁻¹.
Fig. 1 Preparation of Z-domain-containing polymer on a solid support using the *S. tokodaii* biotinylation reaction. (A) Schematic illustration of the strategy. (B) A domain structure of the fusion protein BZB (upper) and three dimensional structures of BCCP and the Z-domain showing the N- and C-termini of those protein with arrow heads (lower). The lysine residue of BCCP to be biotinylated is shown as a space filling model. (C) Preparation of the BPL dimer. The cysteine residues of two molecules of BPL-Cys1 were cross-linked with 1,6-bis(maleimido)hexane. (D) Model structures of BPL-Cys1 and BPL-Cys2 complexed with BCCP. The cysteine residues of the BPLs, the lysine residue of BCCP to be biotinylated, and biotin are shown as space-filling models.
Fig. 2 Immobilization of BPL-Cys2 (A) and the stepwise preparation of Z-domain-containing polymer (B) on the SPR sensor chip. (A) Following equilibration with HBS (10 mM HEPES, pH 7.5, 150 mM NaCl), 2 μM BPL-Cys2 was injected over the gold surface of the sensor chip for 15 min. Then, the sensor chip was washed with HBS. The flow rate was 15 μL min\(^{-1}\). (B) Following equilibration of the BPL-modified sensor chip with HBS-T (HBS containing 0.005% Tween20), HBS-TB (HBS-T containing 1 μM biotin, 10 μM ATP, and 1 mM MgCl\(_2\)) was injected over the sensor chip for 6 min; then the sensor chip was washed with HBS-T for 4 min. After that, 0.5 μM BZB was injected for 10 min, and the sensor chip was washed with HBS-T for 10 min. Subsequently, 0.5 μM BPL dimer dissolved in HBS-TB was injected for 10 min followed by a 10-min wash with HBS-T; then 0.5 μM BZB was again injected for 10 min followed by a 10-min wash with HBS-T. The cycle of the addition of the BPL dimer and BZB followed by the buffer wash was repeated three times in total. The flow rate was 30 μL min\(^{-1}\).
Fig. 3  Control of the surface density of the polymer chains on the SPR sensor chip. (A) Schematic illustration of the strategy. The active sites of some of the immobilized BPL-Cys2 molecules were blocked by BCCP molecules not carrying the Z-domain unit by injecting it along with BZB. Subsequently, the BPL dimer and BZB were alternately applied onto the sensor chip. (B) The stepwise preparation of Z-domain-containing polymer. Following activation of BPL-Cys2 with HBS-TB on the sensor chip, a mixture of 0.5 μM BZB and 1.5 μM BCCP was injected over the sensor chip for 10 min followed by a 10 min-wash with HBS-T. Subsequently, the addition of the BPL dimer and BZB was repeated three times in total under the same conditions as those in Fig. 2B. The flow rate was 30 μL min⁻¹.
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