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Recognition of Photoresponsive Polymer Targets by Protein Fused with *cis*-Form Azobenzene-binding Peptide

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The Trp–His–Thr–Leu–Pro–Asn–Ala sequence, which preferentially recognizes *cis*-form azobenzene groups of synthetic polymers, was fused to the N-terminus of green fluorescent protein. Quartz crystal microbalance measurements were used to investigate its absorption behavior on the polymer film surfaces. The results indicated that the peptide succeeded in immobilizing the fused protein onto the film surfaces containing *cis*-form azobenzene groups.

Immobilization of functional proteins onto solid surfaces is useful for biotechnological applications such as microchips, protein arrays, and biosensors.[1] Besides the reaction of chemical groups within proteins with reactive groups presented on the substrate surface, protein immobilization through protein site-specific interactions has been widely used to avoid protein denaturization. Material-binding peptide (MBP) is a novel and attractive tool for an affinity capture ligand, because of their large variety of biochemical and molecular characteristics.[2]

Recently, peptide libraries displayed on phage coat proteins have been employed for selections of MBP targeting on various inorganic and organic materials.[3] In our earlier study, a c16 peptide with the Trp–His–Thr–Leu–Pro–Asn–Ala sequence that binds to *cis*-form azobenzene groups was identified by phage display techniques against the film surface of azobenzene-containing copolymers.[4] The binding constant (K_a) for *cis*-form films ($K_a = 1.4 \times 10^6\,M^{-1}$) composed of polymer **1** (see below) was tenfold greater than that for *trans*-form films ($K_a = 1.3 \times 10^5\,M^{-1}$). To the best of our knowledge, the peptide is the first example that recognizes the isomerization of small photoresponsive molecules. Therefore, fusion of the peptide with other valuable molecules will extend potential applications of the peptide. Herein, we report the preparation and characterization of a protein mutant fused with the conformer-recognizing peptide. The peptide was successfully used as linkers to perform preferential adsorption of the fused protein onto polymer films composed of *cis*-form azobenzene groups.

The biochemistry and molecular biology of green fluorescent protein (GFP) have been widely investigated, and the extensions at both C- and N-termini are known not to disrupt the motif structure of the protein.[5] Therefore, GFP was selected as the model protein. Considering that the peptide was identified from phage clones, which display peptides fused to the N-terminus of the pIII coat proteins via a Gly–Gly–Gly–Ser linker, GFP with the c16 peptide fused to the N-terminus (c16-GFP) was designed (Figure 1a) and was expressed in *E. coli* cultures, similarly to a previous paper.[6] Quartz crystal microbalance (QCM) measurements were used to analyze protein adsorption.

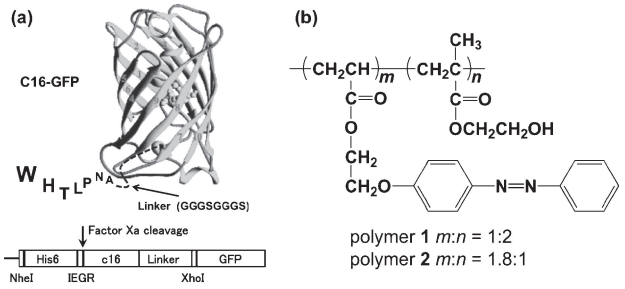

Figure 1. (a) A model of peptide c16 fused GFP (up) and the design of c16-GFP gene (down). The figure of GFP was taken from ref. 5. (b) The structure of polymer **1** and **2**.

Fusion proteins were expressed in *E. coli* with His-tagged and purified using HIS-Select™ Cartridge (from Sigma). c16-GFP was obtained after cleavage by Factor Xa at the C-terminus of recognition site IEGR to remove His-tag. The experimental details are summarized in the Supporting Information.[7] Poly[2-(4-phenylazophenoxy)ethyl acrylate-*co*-2-hydroxyethyl methacrylate]s (polyAzo-HEMA) with two different compositions were used in this study (Figure 1b). Films of polymer **1** ($M_n =$ 36000, $M_w/M_n = 1.69$, Azo:HEMA = 1:2) and **2** ($M_n = 14200$, $M_w/M_n = 1.40$, Azo:HEMA = 1.8:1) were prepared with thickness of approximately 40 nm by spin coating. The QCM chips (electrode area: 4.9 mm^2) coated with polymer films were maintained at 25 °C in 7 mL of PBS buffer (pH 7.4, 150 mM NaCl) with stirring, and the frequency changes were recorded continuously over time with a 27 MHz QCM (Affinix Q, Initiam) with a sensitivity of 30 pg Hz^{-1}.[8]

The addition of c16-GFP at a final concentration of 50 nM into PBS buffer induced almost no frequency changes for polymer **1** films under visible (vis) light. On the contrary, the frequencies decreased with time when irradiated with ultraviolet (UV) light (365 nm) for 4 min (Figure S2a[7]). Figure 2a shows the averages of frequency changes of wild-type GFP (wGFP, purchased from Abcam) and c16-GFP at 50 nM to polymer **1** films under vis and UV light. In the case of wGFP, detectable frequency changes were not observed for polymer **1** films both under vis and UV light (Figure S2b[7]). The nonspecific adsorption of GFP onto the polymer film surfaces seemed to be restrained at the present low concentration. On the other hand, the decreased frequency (−95 Hz) in bound c16-GFP to the films under UV light was obviously greater than that under vis light. This observation suggests that c16-GFP preferentially bound to the surface composed of *cis*-form azobenzene groups and that the c16 peptide is rarely affected by steric hindrance when fused

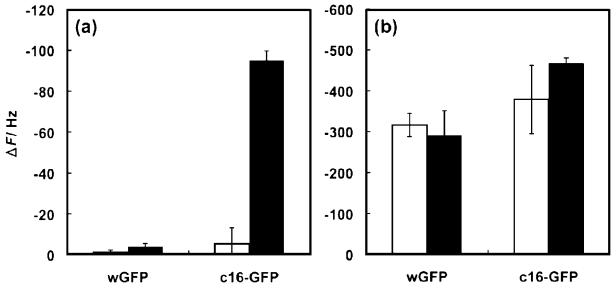

Figure 2. The average frequency changes of wGFP and c16-GFP at 50 nM for polymer (a) **1** and (b) **2** under vis (open) or UV (filled) light.

Table 1. Air in water contact angles (°) of spin-coated polymer **1** and **2** films under vis or UV light

	Polymer **1**		Polymer **2**	
	vis	UV	vis	UV
Mean value	141.5 ± 1.3	143.4 ± 1.5	131.2 ± 1.7	135.3 ± 0.7

into GFP. Considering the molecular dimension of wGFP (a cylinder-like structure with a diameter of about 3 nm and a length of about 4 nm),[5] the surface coverage of c16-GFP on polymer **1** films under UV light was estimated to be approximately 9–15%, which is dependent on binding direction of the distorted protein.

Next, the content of azobenzene groups was increased in polymer **2**, which induced almost −300 Hz changes of wGFP at 50 nM regardless of films under vis or UV light (Figure 2b). Although c16-GFP showed a larger binding amount to polymer **2** compared with wGFP, the nonspecific protein adsorption caused by wGFP increased background frequencies interfering with correct evaluation of c16-GFP. The air contact angles of polymer films were measured after incubation in water (Table 1). The contact angles for polymer **2** were smaller than that of polymer **1**, which indicated that the film surface of polymer **2** was more hydrophobic than that of polymer **1**, resulting in the increased amounts of azobenzene groups on polymer **2**. Therefore, the nonspecific adsorption of wGFP was considered due to the increased hydrophobicity of the surfaces.[1c,1f] The angles under UV light were larger than those under vis light for both polymers **1** and **2**, which is considered reasonable as *trans*-form azobenzene groups are more hydrophobic than *cis*-form.[9] The changes of contact angles induced by isomerization suggested that there were still azobenzene groups on the surfaces in water.

The frequency changes of c16-GFP for polymer **1** under UV light were plotted against five different concentrations, and a saturation curve was obtained (Figure 3). The curve was then fitted to acquire the K_a of c16-GFP, assuming Langmuir adsorption. The K_a was estimated to be $2.3 \times 10^7\,M^{-1}$, which was one order larger than that of the c16 peptide as aforementioned.[4b] The increased K_a was considered to be overall binding ability of the c16 peptide and GFP, although nonspecific adsorption of wGFP was observed slightly for polymer **1** at 50 nM (Figure 2a).

In conclusion, QCM measurements were used to investigate the binding ability of a GFP mutant fused with the Trp–His–Thr–Leu–Pro–Asn–Ala sequence for azobenzene-containing

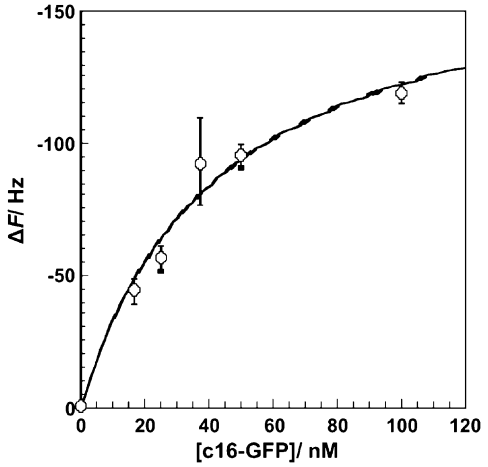

Figure 3. The adsorption isotherm of c16-GFP for polymer **1** under UV light. The K_a was estimated to be $2.3 \pm 0.7\,M^{-1}$, of which the R^2 was 0.98.

polymers. The ability of the peptide to recognize *cis*-form azobenzene groups was embodied when fused to GFP. The binding amount of the fused protein was controlled by light irradiation that induces isomerization of azobenzene groups. This research will provide a new strategy aiming at photo-responsive biological systems.

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