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Zinc Oxide-Coated Plasmonic Chip Modified with a Bispecific Antibody for Sensitive Detection of a Fluorescent Labeled-Antigen

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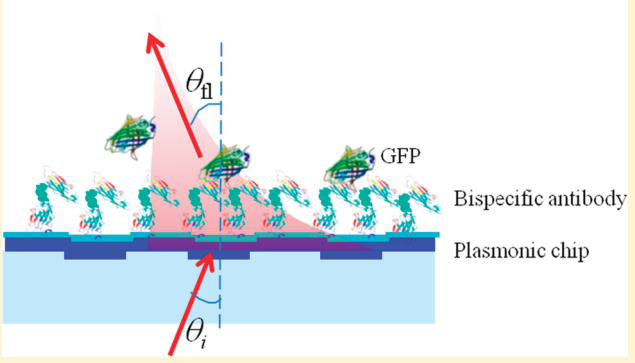
Supporting Information

ABSTRACT: A plasmonic biosensor chip of silver-coated PMMA grating with a zinc oxide (ZnO) overlayer is fabricated for surface plasmon field-enhanced fluorescence (SPF) detection of Cy5-labeled green fluorescent protein (GFP). A bispecific antibody (anti-GFP x anti-ZnO antibody) prepared in our lab is densely immobilized on the sensor chip for GFP detection. The sensitivity of the plasmonic biosensors is improved due to densely packed antibodies and ZnO-coating that suppresses nonspecific protein adsorption and fluorescent quenching. With the ZnO-coated plasmonic chip, Cy5-labeled GFP of 10 pM can be detected through SPF. This sensitivity is 100 higher compared with the normal fluorescent detection on a ZnO-coated glass slide.



The surface plasmon resonance (SPR) technique is a popular label-free tool used to detect biomolecules on substrates. However, the sensitivity of the reflectivity measured using the SPR method is not higher than that of fluorescence, as its limit of detection (LOD) is generally several nanomolar. Therefore, the surface plasmon field-enhanced fluorescence (SPF) method indicating the better LOD has attracted attention in the field of biosensing.[1−4] Grating-coupled SPR (GC-SPR)[5−8] is one of the propagated SPR forms that provides direct coupling between surface plasmon polaritons and the radiation modes possible; therefore, several advantages, i.e., its simple optical setup, simple operation, and prompt and highly sensitive detection are expected. Therefore, we have studied the grating-coupled surface plasmon field-enhanced fluorescence (GC-SPF). The plasmonic (metal-coated grating) structure had been improved for biosensing,[9−12] and the plasmonic chip was developed as a prototype of the biosensor to detect biotin−streptavidin interactions.[13,14]

In the present study, in order to quantitatively and sensitively detect an antigen−antibody interaction with a much lower affinity constant or larger dissociation rate compared with the biotin−streptavidin interaction with an extraordinarily high affinity above 10^{15} M^{-1} (though the value may be smaller than 10^{9} M^{-1} in the inhomogeneous surface), we used a recombinant antibody with bispecific affinity for an antigen protein, a specific inorganic material surface,[15−17] and a new type of zinc oxide (ZnO)-coated plasmonic chip.

Advances in molecular evolution technology has opened the way to identify several peptides with an affinity for inorganic materials,[18−21] which are promising for use in bottom-up fabrication procedures in the field of bionanotechnology.[21,22] Recently, we have identified an antibody with a high affinity for a specific inorganic material surface,[15−17] and the molecules are used as biointerface units for nanoscale quantitative biosensing and protein accumulation by designing bispecific antibodies.[15,17] Here, ZnO was selected as a surface material on the plasmonic chip, since the matter can suppress nonspecific adsorption of proteins on a sensor chip in comparison with metal surfaces. As a marker protein, green fluorescent protein (GFP) was selected. GFP has been used for a wide variety of different applications including visualization of protein−protein interactions, Ca^{2+} detection, and a reporter for protein folding. Sensitive detection of such an important protein is considered to contribute to the development of the above applications including fluorescence microscopic imaging. A bispecific recombinant antibody composed of two kinds of variable domains with affinity for GFP and for ZnO surfaces was densely bound to a plasmonic chip covered with a layer of ZnO.[15] Subsequently, the interaction with fluorescent-labeled GFP was monitored via GC-SPF including the reverse-coupling mode.[23−25] The enhanced fluorescence originated from the enhanced excitation field at a resonance angle and from the recoupling with surface plasmon polaritons (reverse-coupling mode); therefore, an enhancement factor

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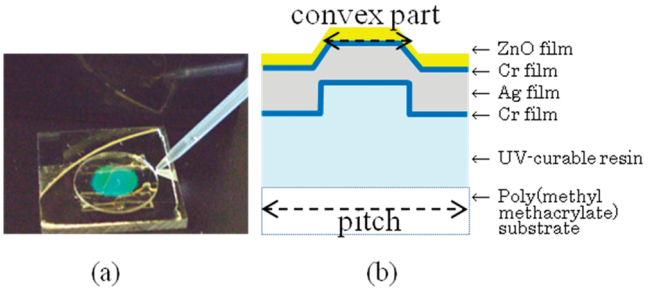

Figure 1. Plasmonic chip: (a) picture of the pouring of antigen solution onto the plasmonic chip using a pipet; the blue area corresponds to the surface of the grating structure. (b) Schematic representation of the layer structure of our plasmonic chip.

(EF) over 100× is expected. The EFs obtained in biosensing on our plasmonic chip is compared with that using the other enhanced fluorescence techniques[26,27] that are based on localized SPR, for instance, a gold nanoantenna array.[27] Further, the bispecific antibody is expected to be modified densely on the ZnO surface and can improve the LOD. Comparison of the LOD with that of the enzyme-linked immunosorbent assay (ELISA), which is one of the most popular biosensing techniques, is important. Rapid and sensitive detection of antigen−antibody interactions and the suppressing effect of nonspecific adsorption will be shown as the advantages of biodetection using a ZnO-coated plasmonic chip with bispecific antibody.

EXPERIMENTAL SECTION

Fabrication of Plasmonic Chip. Our plasmonic chip was fabricated using UV nanoimprint lithography (UV-NIL). A UV-curable resin (PAK-02-A, Toyo Gosei) was dropped onto a PMMA plate and was exposed to UV light from the rear of a mold for 30−40 s.[28] Thin silver layer covered with a ZnO overlayer (with an adhesion layer of Cr) was prepared on the grating substrates using an rf sputter setup (Rikensya, specially made) as shown in Figure 1a,b. Glass slides coated with a ZnO film (with an adhesion layer of Cr) were also prepared as references. The topography of the plasmonic chip was measured using scanning probe microscopy (Figure S-1 in the Supporting Information). The periodicity of the plasmonic chip, the groove depth, and the duty ratio (ratio of the convex part to the pitch, see Figure 1b) were 500 ± 10, 31 ± 3, and 0.68 ± 0.03 nm, respectively. To detect the most enhanced fluorescence under rear irradiation, the thickness of the silver film was controlled at 37 ± 3 nm. In addition, to suppress the quenching of fluorescence, the thickness of the ZnO film was 17 ± 2 nm.

Measurement of Angle Dependent Reflectivity and Fluorescence. For the reflectivity measurement, a He−Ne laser beam (wavelength, 632.8 nm) was used as an irradiation source. With the use of a θ-2θ goniometer, the light reflected by the substrate under irradiation from the rear panel of the chip was monitored against incident angles (θ_i) of 5−40° with a photodiode placed on another stage that rotated at 2θ (Figure S-2 in the Supporting Information). We found resonance angles at 14° and 23° (Figure S-3 in the Supporting Information), which were assigned to the coupling between the silver and ZnO films (top interface) and to the coupling between the UV-curable resin and the silver film (rear interface), respectively. The resonance angle depends on the thickness of the silver layer. The enhanced fluorescence peak

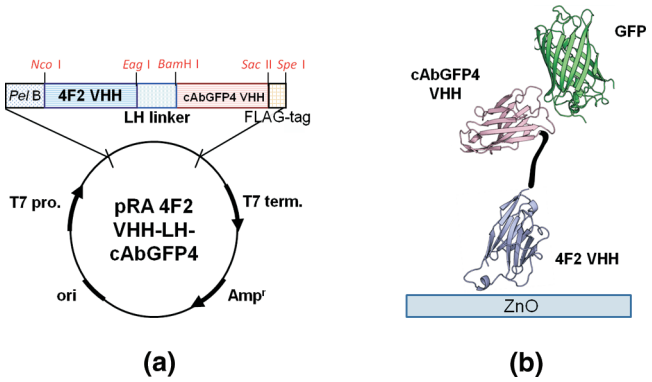

Figure 2. (a) Vector maps of the bispecific antibody 4F2-GFP4VHH. (b) Molecular configuration of the bispecific antibody.

of labeled GFP was in agreement with the resonance angle on the plasmonic chip (Figure S-3 in the Supporting Information).

Preparation of Samples. The reported single variable domain of the heavy chain of a heavy chain camel antibody (VHH) with affinity for ZnO and GFP (4F2[15] and cAbGFP4[29]) were used to construct a bispecific antibody with affinity for the ZnO surface and GFP as previously reported.[15] The recombinant bisopecifc antibodies where cAbGFP4VHH was fused at the C-terminus of 4F2 VHH via a llama IgG2 upper hinge-linker (EPKIPQPQPKPQPQPQPQPQPKPQPKPEP) was expressed in *E. coli* and purified as previously reported.[15] As a target, GFPs were labeled with Cy5 at a labeling ratio of 3.9 using a Cy5-labeling kit (GE Healthcare). The bispecific antibody was prepared at 1 μM in phosphate-buffered saline solution (10 mM phosphate (pH 7.5)/200 mM NaCl). GFP solutions were prepared at concentrations ranging from 5.2 μM to 10 pM. A total of 30 μL of GFP solution was injected using a pipet. For rinsing after a 10 min incubation, 40 μL of phosphate-buffered saline solution was injected. Detergent (Tween 20, 0.05%) was added to the Cy5-GFP and buffered saline solutions for rinsing and GFP sample dilution.

RESULTS AND DISCUSSION

Immobilization of a Plasmonic Chip with Bispecific Antibody Prepared. A bispecific antibody with affinity for GFP and for ZnO surfaces was prepared as a sensitive strategy of detection of GFP. Recently, we generated antibodies with high affinity for ZnO surfaces (dissociation constant, 9 nM), called 4F2, using the variable domain of the heavy chain (VHH) as a scaffold.[15] The 4F2 VHH can selectively adsorb only a ZnO surfaces with enthalpy-dependent interactions via hydrogen and coordinate bonding with the complementarity determining regions in VHH.[15] We also designed a bispecific recombinant antibody, in which an anti-GFP VHH (cAbGFP4VHH)[29] was fused to the C terminus of the anti-ZnO 4F2 VHH via a llama IgG2 upper hinge-linker (Figure 2).[15] The use of the bispecific VHH dimer resulted in the highly dense immobilization of GFP in a flow system.[15] A cover glass was attached to the top of chips using adhesive tapes (Figure 1a). The 4F2-GFP4VHH dimer (= bispecific antibody) solution was injected into the narrow space between the chip and the cover glass, and dimer binds to the ZnO layer on the plasmonic chip with high affinity; therefore, the density of the antibody prepared can be as high as eight molecules per 100 nm^2.[15] The amount of bound bispecific

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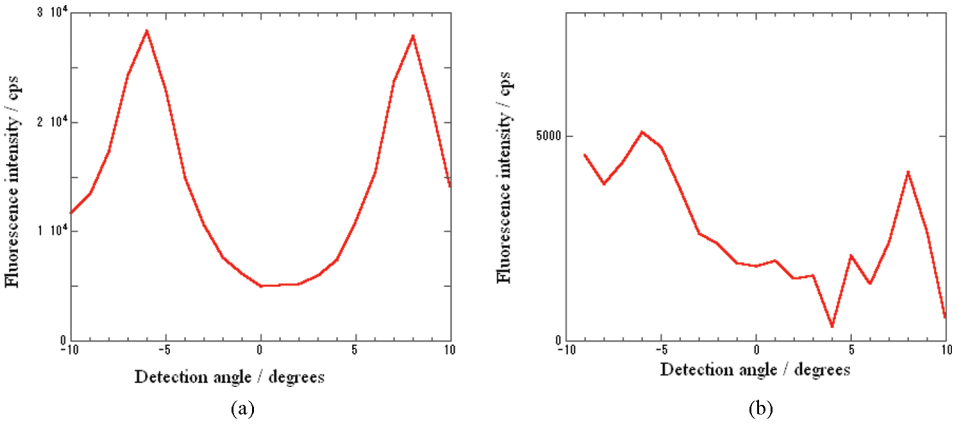

Figure 3. Fluorescence intensities measured against the detection angles for (a) 64 pM GFP solution and (b) 10 pM GFP solution.

antibody was also evaluated via prism-coupled SPR measurement, and the thickness of the dimer layer was evaluated at 5 nm. It corresponds to that the bispecific antibody bound to the ZnO surface forms a densely packed film, taking into account the molecular size expected from the molecular weight of the 4F2-GFP4VHH dimer (M_w = 31 562 g mol^{-1}).

Rapid and Highly Sensitive Detection of Antigens. A labeled-GFP solution was poured into the chip surface modified with bispecific antibodies. The emission was monitored with a photomultiplier against the detection angles (θ_{fl}) from −15° to +15° for the direction normal to the substrate under the incident angle fixed at the resonance angle. The excitation of fluorescent molecules under the irradiation at the surface plasmon resonance angle was expected to enhance fluorescence 20−30× due to the enhanced electric field. As a result of detection of angle-dependent fluorescence, double peaks were found at +7 ± 1° and −7 ± 1° for the direction normal to the substrate (Figure 3a,b), and they were considered as corresponding to the directional fluorescence enhanced by the reverse-coupling mode,[18−20] which means that the fluorescence was recoupled with plasma polaritons on the plasmonic chip. These reverse coupling angles were in agreement with the angles estimated from a dispersion relationship at a fluorescence wavelength of 670 nm. The peak intensities were 3.0 × 10^4 counts per second (cps) for a 64 pM GFP solution (Figure 3a); conversely, the fluorescence intensity measured at the direction normal to the substrate was 7 × 10^3 cps. The reverse-coupling mode enhances fluorescence intensity greater than 4 times; therefore, it is an effective and sensitive biodetection tool.

On the other hand, the LOD of the plasmonic chip was below 10 pM (Figure 3b). The double peaks assigned to the reverse-coupling mode were found at the same detection angles for both the 64 and 10 pM GFP solutions. The LOD is evaluated as 7 pM by the definition of 3σ (= standard deviation) divided by a slope around 10 pM (Figure 4). The LOD of 7 pM is consistent with the value evaluated by the noise level measured out of recoupled angles. The fluorescence intensities plotted in Figure 4 were obtained from the fluorescence peak measured against the detection angle of Cy5-GFP solutions as shown in Figure 3a. The GFP concentration was quantitatively evaluated in the range of 5 orders from 100 nM to 10 pM on the plasmonic chip. The LOD of the general SPR method, one of the typical techniques for biousers, is several nanomolar in the detection of antigen−antibody interactions. The sensitivity of fluorescence on a

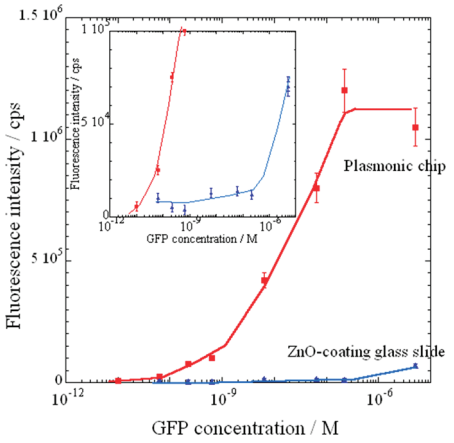

Figure 4. Fluorescence peak intensities measured against GFP concentration: on the plasmonic chips (red squares) and on the ZnO-coated glass slides (blue triangles). The inset is an enlargement figure.

plasmonic chip is found to be improved by a factor of 1000 compared with the SPR method.

In the ZnO-coated glass slides, the fluorescence values were evaluated as the mean values obtained at ±7°. GFP solutions with a concentration less than 1 μM exhibited a nondirectional fluorescence intensity less than 2.0× 10^4 cps, the standard error was ±2 × 10^3 cps (Figure 4), and the values were not quantitatively evaluated. Because the values are also included the fluorescence from labeled-GFP adsorbed nonspecifically to the cover glass, and in the low concentration of GFP, the contribution by a nonspecifid adsorption is considered to be large. The GFP concentration was quantitatively evaluated above 1 μM on the ZnO-coated glass slides. Therefore, our plasmonic chip improved the quantitative detection range and LOD compared with those obtained on the ZnO-coated glass slides. The EF in a 220 nM GFP solution was 100×, which was calculated from the fluorescence intensity on the plasmonic chip divided by the 1.2 × 10^4 cps value detected on the ZnO-coated glass slide at the same GFP concentration. Such a large EF obtained is due to including the enhancement of the excitation field under GC-plasmon coupling and the fluorescence enhancement by the recoupling between fluorescence and GC-surface plasmon polaritons. In biosensing using the other enhanced fluorescence techniques[26,27] based on localized SPR, for instance,

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Table 1. Ratio of the Amount of Nonspecific Adsorption to the Specific Binding of cy5-GFP on the Plasmonic Chips at Protein Concentrations of 100 nM and 100 pM

protein concentration	Cy5-GFP (%)	Cy5-BSA (%)	Cy5-lysozyme (%)	Cy5-GFP without bispecific antibody (%)
100 nM	100	17	<1	1
100 pM	100	0	0	0

a gold nanoantenna array,[27] the EFs of 40−70× were reported. Our plasmonic chip is superior to these approaches regarding the EF in biosensing and yields a wide detection range and a high sensitivity.

Our plasmonic chip with a high sensitivity and a wide quantitative detection range, from the point of view of the application for biousers, has a great potential as a clinical diagnostic chip if the suitable sandwich immunoassay including fluorescent labeled-second antibody could be selected. The use of a second antibody would make a distance from the silver surface far away compared with the assay system for a labeled-antigen used in this study. However, the difference in the distance could not influence the fluorescence enhancement because the decay of an enhanced electric field is several percent for around 5 nm corresponding to the size of a second antibody. Therefore, our plasmonic chip could be appropriate for application to the sandwich immunoassay.

Suppression of Nonspecific Adsorption. The suppression of nonspecific adsorption to achieve high sensitivity is important in biosensing. The ZnO surface was expected to suppress nonspecific adsorption.[21] As summarized in Table 1, the ratios of the amount of nonspecific adsorption to the specific binding of Cy5-GFP on the plasmonic chips were 17%, 1%, and <1% at 100 nM for Cy5-labeled bovine serum albumin (BSA), Cy5-labeled GFP without bispecific antibody, and Cy5-labeled lysozyme, respectively. The ratio of nonspecific adsorption to specific binding was reported as 0.5%−36% in the 1 nM marker detection due to the difference in surface chemistry.[30] Therefore, the ZnO-surface was considered to well suppress nonspecific adsorption without special chemical modification. At dilute protein concentrations of 100 pM, the fluorescence intensities corresponding to any nonspecific adsorption were not detectable.

On the other hand, the suppression of nonspecific adsorption was also examined for the ZnO-coated glass slides. As shown in Figure 4, the fluorescence intensity in the specific binding was less than 2×10^4 cps below 1 μM, but that in the nonspecific adsorption of GFP to ZnO surface without bispecific antibody was 2×10^4 cps in a 220 nM GFP solution. In ZnO-coated glass slides, the signal corresponding to a specific binding was too low; thus, we were unable to distinguish between nonspecific and specific signals less than 1 μM. The fluorescence intensity measured on the ZnO-coated glass slides includes contributions of nonspecific adsorption not only to a ZnO surface but also to a cover glass surface, differing from the situation on the plasmonic chip in which only the fluorescence from nonspecific adsorption to a ZnO surface is measured due to surface selectivity. The contribution of nonspecific adsorption to a cover glass is included in any measurements on the ZnO-coated glass slide modified with and without bispecific antibody. Therefore, the ratio of fluorescence intensity of Cy5-GFP for ZnO surface modified with antibody to that without antibody in the ZnO-coated glass is worse than that for ZnO-coated plasmonic chips. The ZnO-coated plasmonic chip has great potential as a biosensor chip, as the signal-to-noise ratio can be large, even when applied practically to human serum or whole blood.

CONCLUSIONS

The creation of a ZnO-coated plasmonic chip densely modified with a bispecific antibody allowed the suppression of nonspecific adsorption, which contributed to the enhancement of the detection sensitivity of antigen−antibody interactions. On the plasmonic chip that was simply fabricated using UV-nanoimprint lithography (NIL) and was coated with silver and ZnO films, we monitored the enhanced fluorescence of the antigen: (1) under irradiation from the rear panel at a resonance angle, i.e., excitation by an electric field enhanced under GC-plasmon coupling and (2) including a directional fluorescence, i.e., fluorescence enhanced by the recoupling between fluorescence and GC-surface plasmon polaritons. The fluorescence EF on the plasmonic chips was 100× compared with that obtained for the ZnO-coated glass slides; in addition, a 10 pM GFP solution was simply detected 10 min after incubation. The LOD of 7 pM evaluated was comparable with that obtained using the sensitive ELISA. With regards to the nonspecific adsorption, the ZnO-coated plasmonic chips used here suppressed nonspecific adsorption sufficiently and the surface selectivity in detection improved the signal-to-noise ratio. Further, the ZnO-coated plasmonic chip is expected to keep the LOD of marker proteins even under the concentrated albumin solution like human blood.

In the future, the use of a plasmonic chip with a smaller pitch of grating and a duty ratio close to 0.5 may enhance fluorescence intensity, i.e., it can improve the LOD less than 1 pM. In conclusion, our plasmonic chip has great potential as a clinical diagnostic chip if the suitable sandwich immunoassay including fluorescent labeled-second antibody could be selected, as it was a highly sensitive, rapid, and simple detection system.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Liebermann, T.; Knoll, W. *Colloids Surf. A* **2000**, *177*, 115–130.
(2) Yu, F.; Yao, D.; Knoll, W. *Anal. Chem.* **2003**, *75*, 2610–2617.
(3) Tawa, K.; Knoll, W. *Nucleic Acids Res.* **2004**, *32*, 2372–2377.
(4) Tawa, K.; Morigaki, K. *Biophys. J.* **2005**, *89*, 2750–2758.
(5) Raether, H. *Surface Plasmons on Smooth and Rough Surfaces and on Gratings*; Springer-Verlag: Heidelberg, Germany, 1988.

(6) Knoll, W. *Annu. Rev. Phys. Chem.* **1998**, *49*, 569–638.
(7) Popov, E.; Tsonev, L.; Maystre, D. *Appl. Opt.* **1994**, *33*, 5214–5219.
(8) Popov, E.; Bonod, N.; Enoch, S. *Opt. Express* **2007**, *15*, 4224–4237.
(9) Hori, H.; Tawa, K.; Tatsu, Y.; Kintaka, K.; Nishii, J. *Opt. Rev.* **2009**, *16*, 216–221.
(10) Cui, X. Q.; Tawa, K.; Hori, H.; Nishii, J. *Appl. Phys. Lett.* **2009**, *95*, 133117.
(11) Cui, X. Q.; Tawa, K.; Hori, H.; Nishii, J. *Adv. Funct. Mater.* **2010**, *20*, 546–553.
(12) Hori, H.; Tawa, K.; Kintaka, K.; Nishii, J.; Tatsu, Y. *J. Appl. Phys.* **2010**, *107*, 114702.
(13) T awa, K.; Hori, H.; Kintaka, K.; Kiyosue, K.; Tatsu, Y.; Nishii, J. *Opt. Express* **2008**, *16*, 9781–9790.
(14) Cui, X. Q.; Tawa, K.; Kintaka, H.; Nishii, J. *Adv. Funct. Mater.* **2010**, *20*, 945–950.
(15) Hattori, T.; Umetsu, M.; Nakanishi, T.; Togashi, T.; Yokoo, N.; Abe, H.; Ohara, S.; Adschiri, T.; Kumagai, I. *J. Biol. Chem.* **2010**, *285*, 7784–7793.
(16) Hattori, T.; Umetsu, M.; Nakanishi, T.; Tsumoto, K.; Ohara, S.; Abe, H.; Naito, M.; Asano, R.; Adschiri, T.; Kumagai, I. *Biochem. Biophys. Res. Commun.* **2008**, *365*, 751–757.
(17) Watanabe, H.; Nakanishi, T.; Umetsu, M.; Kumagai, I. *J. Biol. Chem.* **2008**, *283*, 36031–36038.
(18) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. E.; Belcher, A. M. *Nature* **2000**, *405*, 665–668.
(19) Sarikaya, M.; Tamerler, C.; Jen, A. K.-Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2*, 577–585.
(20) Umetsu, M.; Mizuta, M.; Tsumoto, K.; Ohara, S.; Tamaki, S.; Watanabe, H.; Kumagai, I.; Adschiri, T. *Adv. Mater.* **2005**, *17*, 2571–2575.
(21) Yokoo, N.; Togashi, T.; Umetsu, M.; Tsumoto, K.; Hattori, T.; Nakanishi, T.; Ohara, S.; Takami, S.; Naka, T.; Abe, H.; Kumagai, I.; Adschiri, T. *J. Phys. Chem. B* **2010**, *114*, 480–486.
(22) Woodbury, R. G.; Wendin, C.; Clendenning, J.; Melendez, J.; Elkind, J.; Bartholomew, D.; Brown, S.; Furlong, C. E. *Biosens. Bioelectron.* **1998**, *13*, 1117–1126.
(23) Malicka, J.; Jryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Anal. Chem.* **2003**, *75*, 6629–6633.
(24) Winter, G.; Barnes, W. L. *Appl. Phys. Lett.* **2006**, *88*, 051109.
(25) Aslan, K.; Geddes, C. D. *Anal. Chem.* **2009**, *81*, 6913–6922.
(26) Moal, E. L.; Lévêque-Fort, S.; Potier, M.-C.; Fort, E. *Nanotechnology* **2009**, *20*, 225502.
(27) Bakker, R. M.; Drachev, V. P.; Liu, Z.; Yuan, H.-K.; Pedersen, R. H.; Boltasseva, A.; Chen, J.; Irudayaraj, J.; Kildishev, A. V.; Shalaev, V. M. *New J. Phys.* **2008**, *10*, 125022.
(28) Tawa, K.; Yokota, Y.; Kintaka, K.; Nishii, N.; Nakaoki, T. *Sens. Actuators, B: Chem.* 2011, *157*, 703–709.
(29) Rothbauer, U.; Zolghadr, K.; Tillib, S.; Nowak, D.; Schermelleh, L.; Gahl, A.; Backmann, N.; Conrath, K.; Muyldermans, S.; Cardoso, M. C.; Leonhardt, H. *Nat. Methods* **2006**, *3*, 887–889.
(30) Anderson, A. S.; Dattelbaum, A. M.; Montaño, G. A.; Price, D. N.; Schmidt, J. G.; Martinez, J. S.; Grace, W. K.; Grace, K. M.; Swanson, B. I. *Langmuir* **2008**, *24*, 2240–2247.