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NOTE

Enhanced sugar chain detection by oriented immobilization of Fc-fused lectins

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ABSTRACT

We report a novel scaffold for clustering and oriented immobilization of human IgG1 Fc-fused lectins on biosensors without chemical modifications. This approach uses a bio-nanocapsule (BNC) displaying a tandem form of IgG Fc-binding Z domains derived from *Staphylococcus aureus* protein A (ZZ-BNC). Incorporating ZZ-BNC effectively increased both the sensitivity and sugar chain-binding capacity compared with the condition without ZZ-BNC.

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Sugar chain; Fc-fused lectin; biosensor; oriented immobilization; bio-nanocapsule

Sugar chains on the mammalian cell surface play important roles in many biological processes, including cancer progression, cell migration, fertilization, and host-pathogen interactions [1]. Through activation of signal transduction pathways, sugar chains interact specifically with a family of sugar-binding proteins called lectins, eliciting various cellular responses, such as cell growth, cell motility, and cell-cell adhesion [1]. Sugar chain detection is useful for early diagnosis of cancers and viral diseases, identification of pathogenic bacteria and toxins, and selection of stem cells. Lectin-based biosensors have been applied in various detection methods, such as surface plasmon resonance (SPR) [2], quartz crystal microbalance (QCM) [3], enzyme-linked immunosorbent assay (ELISA) [4], electrochemical impedance spectroscopy (EIS) [5], and microarrays [6].

To fabricate these lectin-based biosensors, lectins are immobilized with random orientation onto solid phases (*e.g.*, gold, glass, and graphene) by nonspecific interactions (*e.g.*, hydrophobic interaction and electrostatic interaction). Because most lectins show lower affinity to target sugar chains (K_d = 10^{-4}–10^{-7} M) compared with antigen-antibody interactions (K_d = 10^{-8}–10^{-12} M) [7], aligning the orientation of sugar-binding sites perpendicular to the solid phase is crucial to ensure effective binding of sugar chains. Conventionally, lectins are clustered and immobilized onto solid phases with crosslinkers [2,3,5–7] or self-assembled monolayers [8,9] using chemicals that react nonspecifically with the free NH_2 residues of lectins, resulting in random orientation and steric hindrance around the sugar chain-binding sites of immobilized lectins, as well as reducing the binding capacity of lectins and the sensitivity to sugar chains.

To achieve oriented immobilization of lectins onto the solid phase, two scaffolds have been reported using human IgG1 (hIgG1) Fc region-tag to align lectin molecules [10]. One scaffold uses boronic acid to crosslink with the solid phase through the reducing group of the sugar moiety in the Fc regions. This technique can achieve fully oriented immobilization of Fc-fused lectins; however, chemical modifications may denature Fc-fused lectins. The other scaffold uses *Streptococcus* sp. protein G (Fc-binding protein) to crosslink with the solid phase through the Fc regions. This technique is suitable only for partially oriented immobilization of Fc-fused lectins due to lack of possibility to control the orientation of protein G. These drawbacks show the need for developing new scaffolds that can achieve fully oriented immobilization of Fc-fused lectins without any chemical modification.

We have previously developed about 30-nm bio-nanocapsule (BNC) displaying a tandem form of IgG Fc-binding Z domains derived from *Staphylococcus aureus* protein A (ZZ-BNC) (Figure 1(a)) [11]. ZZ-BNC was efficiently synthesized in recombinant yeast, *Saccharomyces cerevisiae*, expressing ZZ-L protein [12,13]. Because each ZZ-BNC displays about 120 molecules of the ZZ domain on its surface, it could spontaneously tether the Fc regions of IgGs without chemical modifications while displaying all Fv regions outwardly for efficient antigen binding [14,15]. Moreover, ZZ-BNCs immobilized on a solid phase, namely ZZ-BNC-coated biosensors, could serve as a scaffold for IgGs (Figure 1(b), left panel) and Fc-fused receptors (Figure 1(b), center panel). This method has been shown to enhance both the sensitivity and binding capacity of antigens and ligands through clustering and oriented immobilization on biosensor surfaces [11,14,16]. In this study, for expanding the versatility of ZZ-BNCs, we used ZZ-BNC as a scaffold for clustering and oriented immobilization of Fc-fused lectins (Figure 1(b), right panel), and measured the sensitivity of sugar chain-binding using QCM (Twin-Q; As One Corp., Osaka, Japan).

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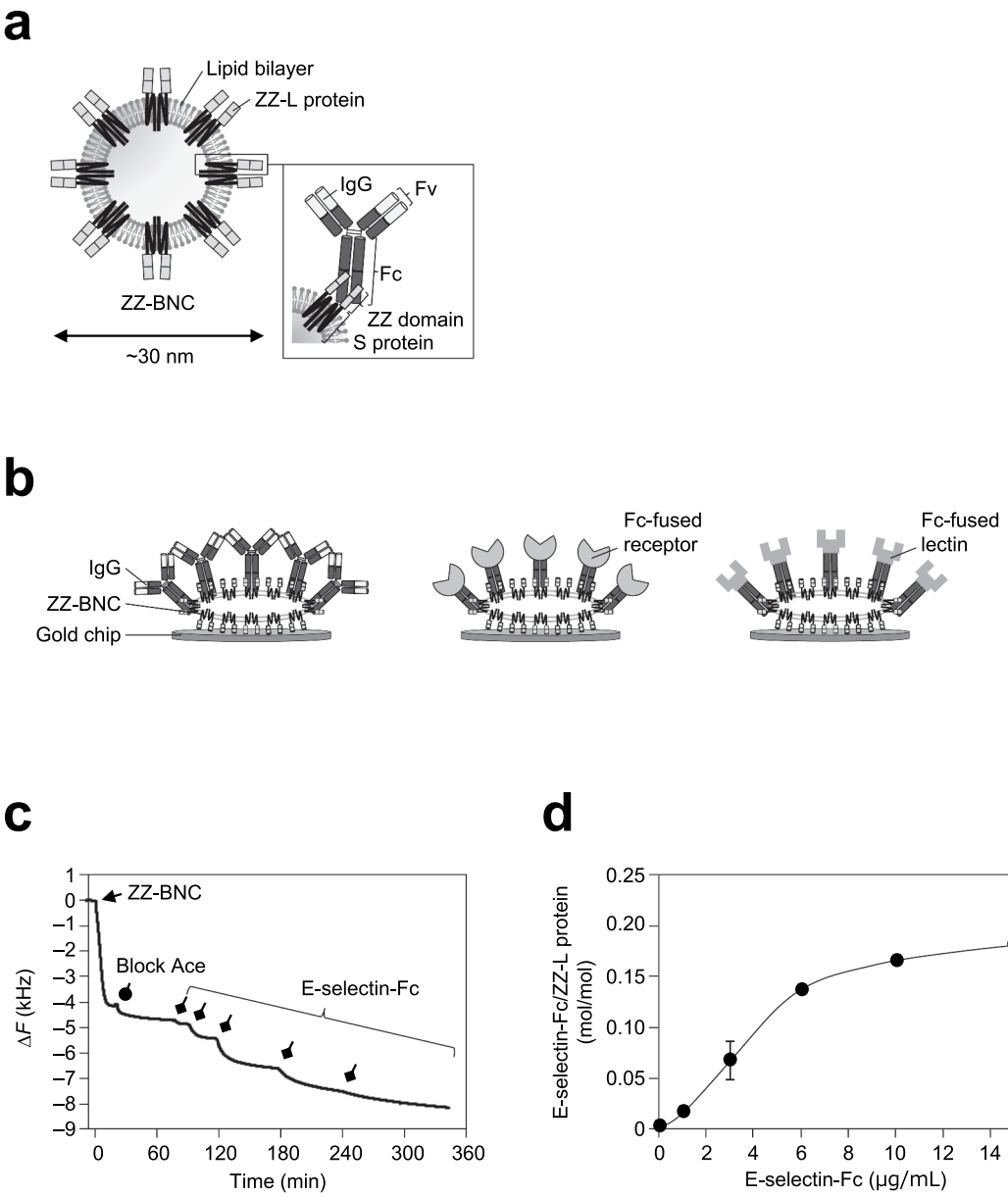

Figure 1. Structures and functions of ZZ-BNCs. (a) Capsular structures of ZZ-BNCs. One ZZ-BNC particle consists of ~120 ZZ-L proteins and a lipid bilayer. (b) Application of the ZZ-BNC-based scaffold for clustering and oriented immobilization of IgGs (left), Fc-fused receptors (center), and Fc-fused lectins (right). (c) Binding capacity of E-selectin-Fc to ZZ-BNC. The QCM sensor chip (9-mm diameter gold disk) was washed with piranha solution (1:3 (v/v) H_2O_2:H_2SO_4), rinsed with distilled water, and then treated with ZZ-BNCs (10 μg protein/mL, final concentration) in 500 μL of phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2PO_4, 2 mM KH_2PO_4, pH 7.4). After blocking with PBS containing 5% (w/v) Block Ace powder, E-selectin-Fc (0–15 μg/mL) was immobilized onto the gold surface. The amount of bound E-selectin-Fc was calculated based on the frequency changes (Δ*F*) in QCM and plotted against the concentrations of injected E-selectin-Fc. The protein–protein interaction was considered equilibrated when a stable frequency (less than ± 3 Hz) was observed for >1 min. The measuring bath was mixed at 600 rpm with a stirring tube and kept at 25°C. All measurements were repeated at least 3 times. A frequency change (Δ*F*) of 1 Hz corresponded to a weight change of 0.6 ng/cm^2, as determined by Sauerbrey equation [17]. (d) The molar ratio of dimeric E-selectin-Fc bound to monomeric ZZ-L protein was determined by QCM. Error bars, standard deviation (n = 3).

We determined the maximum IgG Fc-binding capacity of ZZ-L protein of ZZ-BNC using the C-terminal hIgG1 Fc (100–330 amino acids of γ1 chain)-fused form of the extracellular domain of human E-selectin (E-selectin-Fc) (R&D Systems, Inc., Minneapolis, MN, USA). After the gold surface of the QCM sensor chip was treated with an excess amount of ZZ-BNC (10 μg/mL as protein), 2475 ng/cm^2 of ZZ-BNCs was bound on the sensor chip as estimated from the initial frequency change (Δ*F*) of −4124 Hz (Figure 1(c)). The sensor chip was then treated with the blocking reagent Block Ace (DS Pharma Biomedical Co. Ltd., Osaka, Japan), followed by repeated injection of E-selectin-Fc (15 μg/mL, final concentration), resulting in adsorption of 2052 ng/cm^2 of E-selectin-Fc (corresponding to Δ*F* of −3420 Hz). The molar ratio – interpreted as the binding capacity – of dimeric E-selectin-Fc (172 kDa) bound to ZZ-L protein (48 kDa) was ~0.18 ± 0.01 (Figure 1(d)),

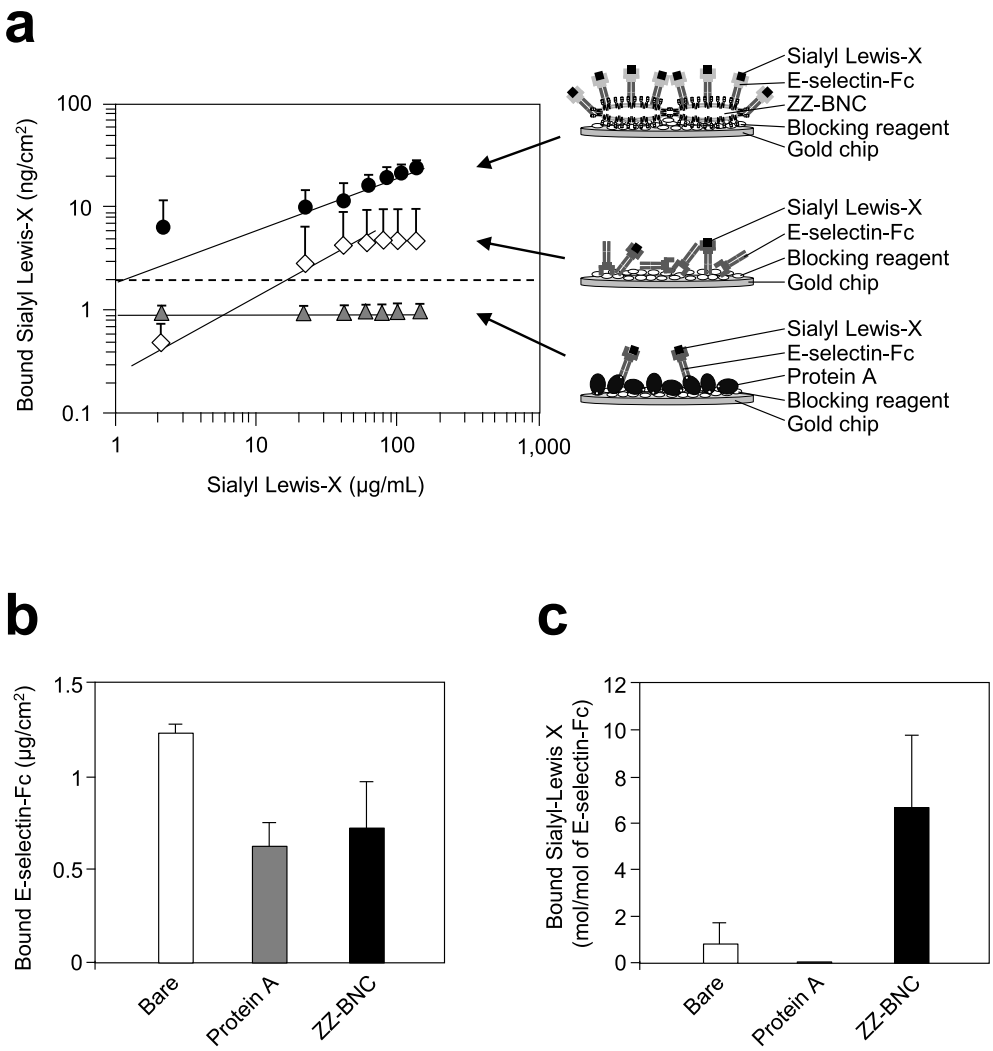

Figure 2. Sensitivity and sugar-binding capacity of QCM on three types of sensor chips. (a) Effects of the immobilization methods on the function of E-selectin-Fc. The QCM sensor chips were treated with protein A (10 μg/mL) or ZZ-BNCs (10 μg/mL), and then blocked with Block Ace (2 mg/mL). E-selectin-Fc (2 μg/mL) was immobilized onto bare, protein A-coated, and ZZ-BNC-coated chips, followed by addition of Sialyl-Lewis X (0–142.2 μg/mL). The amount of bound Sialyl-Lewis X was calculated based on the frequency changes in QCM and plotted against the concentrations of injected Sialyl-Lewis X. Diamonds, E-selectin-Fc-coated chip; triangles, E-selectin-Fc/protein A-coated chip; circles, E-selectin-Fc/ZZ-BNC-coated chip. The LOD of the QCM equipment (2 ng/cm^2) is indicated by dashed lines. Error bars, standard deviation (n = 3). Schematic representations of three types of chips are shown on the right. (b) Binding capacity of E-selectin-Fc in each immobilization method. White, bare; gray, protein A-coated; black, ZZ-BNC-coated chip. Error bars, standard deviation (n = 3). (c) Molar ratios of monomeric sugar chains bound to dimeric E-selectin-Fc. White, bare; gray, protein A-coated; black, ZZ-BNC-coated chip. Error bars, standard deviation (n = 3).

comparable to previous studies on hIgG1 (~0.30) and dimeric Fc-fused prolactin receptor (~0.21) [14,16]. Furthermore, since each ZZ-BNC particle is composed of ~120 molecules of ZZ-L protein embedded in a liposome [14,15], the molar ratio of dimeric E-selectin-Fc bound to ZZ-BNC was ~22.

Next, we examined the effect of E-selectin-Fc immobilization on the sensitivity and sugar chain-binding capacity of QCM with respect to three types of sensor chips, bare, protein A-coated, and ZZ-BNC-coated chip. To generate protein A-coated or ZZ-BNC-coated chip, bare sensor chip was treated with an excess amount of recombinant protein A (Sigma-Aldrich, St Louis, MO, USA; 10 μg/mL) or ZZ-BNC (10 μg/mL), followed by Block Ace. Then, bare, protein A-coated, and ZZ-BNC-coated chips (Figure 2(a), right panel) were treated with E-selectin-Fc (2 μg/mL) thoroughly to produce E-selectin-Fc-coated, E-selectin-Fc/protein A-coated, and E-selectin-Fc/ZZ-BNC-coated chips, respectively. All chips were then treated with 0–142.2 μg/mL of Sialyl-Lewis X (Toronto Research Chemicals, Ontario, Canada), a ligand of E-selectin. Based on the limit of detection (LOD) of QCM at a weight change of 2 ng/cm^2 (corresponding to ΔF of −3.3 Hz), the lowest concentrations of Sialyl-Lewis X necessary to give the LOD values were ~15.2, >142.2, and ~1.3 μg/mL for E-selectin-Fc-coated, E-selectin-Fc/protein A-coated, and E-selectin-Fc /ZZ-BNC-coated chips, respectively (Figure 2(a), left panel). The results showed ~12-fold and >109-fold increase in the sensitivity of E-selectin-Fc/ZZ-BNC-

coated chip compared to that of the E-selectin-Fc-coated and E-selectin-Fc/protein A-coated chip, respectively.

We then studied the sugar chain-binding capacity of these three chips using 142.2 μg/mL of Sialyl-Lewis X. We found that sugar chains bound more efficiently to the E-selectin-Fc/ZZ-BNC-coated chip than to the E-selectin-Fc-coated and E-selectin-Fc/protein A-coated chips. The amount of bound Sialyl-Lewis X was ~4.8, <2.0, and ~24.1 ng/cm^2 in E-selectin-Fc-coated, E-selectin-Fc/protein A-coated, and E-selectin-Fc/ZZ-BNC-coated chips, respectively (Figure 2(a), left panel), indicating ~5-fold and >12-fold increase in Sialyl-Lewis X-binding capacity of E-selectin-Fc-coated and E-selectin-Fc/protein A-coated chip with the ZZ-BNC scaffold. As demonstrated in IgGs and Fc-fused receptors on ZZ-BNC-coated chips [14,16], these data suggest that the ZZ-BNC-coated chip provides an environment for Fc-fused lectins to form ordered orientation, thereby the sugar-binding sites on ZZ-BNC-coated chip was more readily accessible to sugar chain than those on bare and protein A-coated chips. Consequently, ZZ-BNC-coated chip can increase both sensitivities and sugar chain-binding capacities of the lectins.

Finally, we calculated the amount of E-selectin-Fc immobilized onto each chip based on the weight changes of the QCM sensor chips. Among the three types of chips, the bare chip could immobilize about twice the amount of E-selectin-Fc than the protein A-coated and ZZ-BNC-coated chips (Figure 2(b)). Based on the amount of immobilized E-selectin-Fc calculated from this results, the molar ratios of sugar chains bound to dimeric E-selectin-Fc (Sialyl-Lewis X: E-selectin-Fc) on these three chips were calculated after the sensor chips were treated with Sialyl-Lewis X (142.2 μg/mL; 820 Da) (Figure 2(c)). We found the ratios of 7.52 for the ZZ-BNC-coated, 0.80 for the bare, and <0.01 for protein A-coated chip, indicating that ZZ-BNC allowed E-selectin-Fc to capture sugar chains more efficiently in an oriented immobilization manner. Moreover, it is plausible that ZZ-BNC facilitates the high-density deployment of sugar-binding sites on its surface, thereby increasing the avidity of clustered E-selectins. Meanwhile, since the orientation of protein A itself may not ordered on the gold surface, protein A-coated chip did not show the sugar-binding capacity.

In conclusion, we have demonstrated that ZZ-BNC is an ideal scaffold for clustering and oriented immobilization of Fc-fused lectins on a solid phase without chemical modifications, thereby improving the sensitivity and sugar chain-binding capacity of the QCM biosensor. Since ZZ-BNC-coated chips can be applicable to various biosensors [14,16], this method could be further applied to increase the sugar chain detection efficiency of other biosensors, such as SPR, ELISA, EIS, and microarrays. Thus, BNC-based scaffolds may enhance the detection sensitivity toward various target molecules, including antigens, ligands, sugars, DNA-binding proteins and enzymes.

Author contribution

M.I. and Y.Y. designed and performed the experiments, analyzed the data, and wrote the manuscript. T.N. gave helpful comments. S.K. designed and survived the experiments, analyzed the data, and wrote the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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