Supporting information

A membrane-based immunosensor enabling high antifouling performance and sensitive molecular recognition

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Modeling of membrane-based immunosensor

1st STEP

The antigen (Ag) was associated with the capture antibody (Ab_1) in this step. The dissociation of Ag from the complex of the antigen and capture antibody (Ab_1–Ag) also occurred in this step. Thus, this step is described as follows:

1. Association of Ag

$$\frac{\partial C_{Ag}}{\partial t} = -\vec{u}\frac{\partial C_{Ag}}{\partial x} - k_{on1}C_{Ag}C_{Ab1} + k_{off1}C_{Ab1-Ag} \#(1)$$

2. Dissociation of Ag

$$\frac{\partial C_{Ab1-Ag}}{\partial t} = k_{on1}C_{Ag}C_{Ab1} - k_{off1}C_{Ab1-Ag} \#(2)$$

Here, k_{on1} and k_{off1} are the association and dissociation rate constants, respectively, in the recognition of Ag.

2nd STEP

The association and dissociation between the detection antibody (Ab_2) and Ab_1–Ag mainly occurred in this step.

1. Association of Ab_2

$$\frac{\partial C_{Ab2}}{\partial t} = -\vec{u}\frac{\partial C_{Ab2}}{\partial x} - k_{on2}C_{Ab1-Ag}C_{Ab2} + k_{off1}C_{Ab1-Ag-Ab2} \#(3)$$

2. Dissociation of Ab_2

$$\frac{\partial C_{Ab1-Ag-Ab2}}{\partial t} = k_{on2}C_{Ab1-Ag}C_{Ab2} - k_{off2}C_{Ab1-Ag-Ab2} \#(4)$$

Here, k_{on2} and k_{off2} are the association and dissociation rate constants, respectively, in the reaction of the Ab_2 and Ab_1–Ag complex. In this step, the dissociation and reassociation of Ag captured in the 1st step occurred as follows:

3. Association of Ag

$$\frac{\partial C_{Ab1-Ag}}{\partial t} = k_{on1}C_{Ag}C_{Ab1} - k_{off1}C_{Ab1-Ag} \#(5)$$

4. Reassociation of Ag

$$\frac{\partial C_{Ag}}{\partial t} = -\vec{u}\frac{\partial C_{Ag}}{\partial x} - k_{on1}C_{Ag}C_{Ab1} + k_{off1}C_{Ab1-Ag} \#(6)$$

3rd STEP

Here, horseradish peroxidase (HRP)-labeled streptavidin was bound to biotin-labeled Ab_2. The avidin–biotin binding was assumed to not dissociate because the binding was strong (k_{off} / k_{on} = 10^{-15}).

4th STEP

The amount of enzyme in the membrane pore was estimated from the calculated amount of the sandwich complex, and the signal was calculated using the equation including the enzyme reaction. The substrate for the enzyme in the biotin-labeled Ab_2 (HRP) was oxidized. This reaction is described with the Michaelis–Menten equation. Thus,

$$\frac{\partial C_{sub(ox)}}{\partial t} = -\vec{u}\frac{\partial C_{sub(ox)}}{\partial x} - \frac{k_{cat}C_{enz}}{C_{sub} + K_m}C_{sub} \#(7)$$

Here, C_{sub} and $C_{sub(ox)}$ are the concentrations of the substrate (3,3′,5,5′- tetramethyl benzidine, TMB) and oxidized substrate, respectively. Here, the number of biotins bound to the detection antibody and the number of HRP bound to streptavidin were assumed to be 8 and 2, respectively, per molecule.

Table S1. Parameters used in the numerical modeling.

classification	parameter	symbol	unit	value	refs
membrane structure	membrane thickness	l	μm	22	[1]
	membrane area	A	cm^2	0.785	[1]
	porosity	φ		0.173	[1]
	pore radius	r_{pore}	μm	0.5	[1]
	density of capture antibody	d_{Ab1}	ng cm^{-2}	0–600	
molecular property	association rate constant of first step	k_{on1}	m^3 mol^{-1} s^{-1}	1.6×10^3	a
	dissociation rate constant of first step	k_{off1}	s^{-1}	4.3×10^{-4}	a
	association rate constant of first step	k_{on2}	m^3 mol^{-1} s^{-1}	7.4×10^2	a
	dissociation rate constant of second step	k_{off2}	s^{-1}	8.2×10^{-4}	a
	turnover number of TMB–HRP	k_{cat}	s^{-1}	4.0×10^2	[2]
	Michaelis constant of TMB–HRP	k_m	mM	0.434	[2]
	diffusion coefficient of antigen (IL-6)	D_{Ag}	m^2 s^{-1}	1×10^{-10}	b
	diffusion coefficient of detection antibody (anti-IL-6)	D_{Ab1}	m^2 s^{-1}	4×10^{-11}	[3]
operational condition	permeated IL-6 concentration	C_{Ag}	M	4.76×10^{-14} to 4.76×10^{-11}	
	permeated secondary anti-IL-6 concentration	C_{Ab2}	M	1.33×10^{-9}	[1]
	permeation time of first step	t_1	min	10	[1]
	permeation time of second step	t_2	min	30	[1]
	flow rate of first step	u_1	μl min^{-1}	50	[1]
	flow rate of second step	u_2	μl min^{-1}	50	[1]

a: These binding constants were measured using a Biacore T200.

b: The diffusion coefficient of Interleukin-6 (IL-6) was calculated from the molecular radius [4] using the Stokes–Einstein equation.

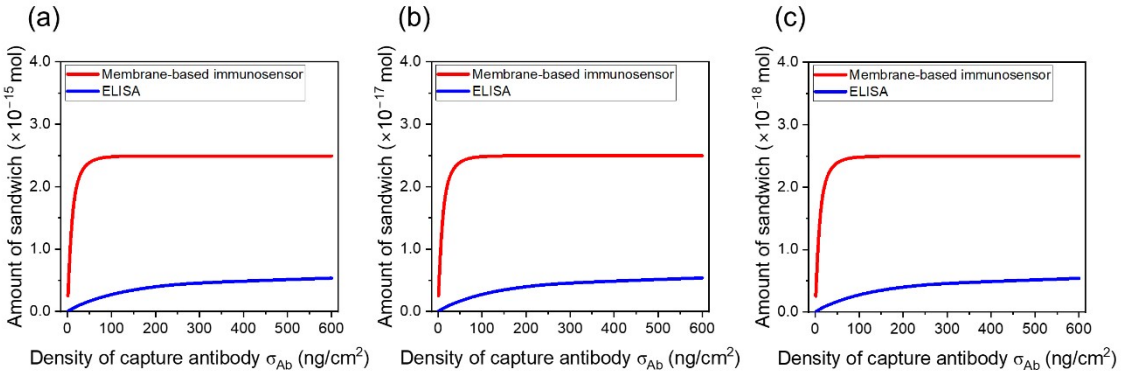

Figure S1. Estimated amount of sandwich complex in the detection test of IL-6 at the antigen concentrations of (a) 1000, (b) 10, and (c) 1 pg/mL for the enzyme-linked immunosorbent assay (ELISA) and membrane-based immunosensor.

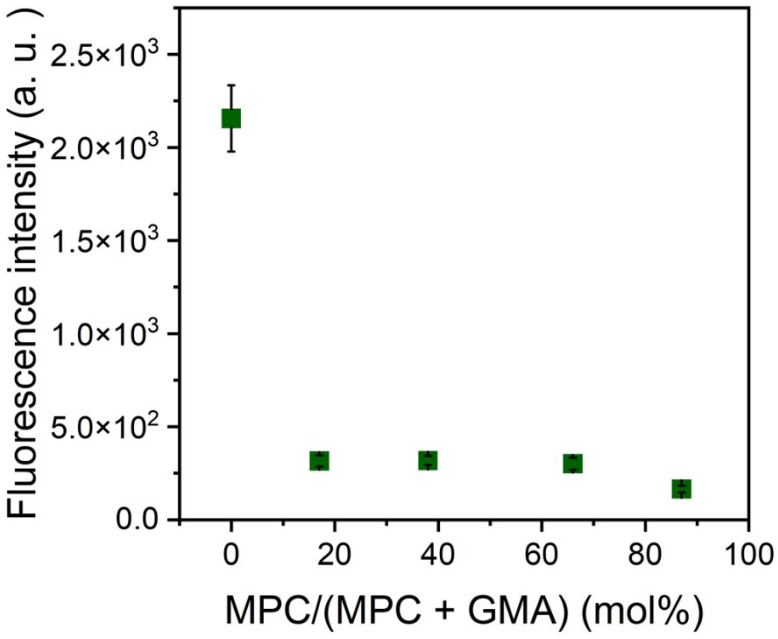

Figure S2. Fluorescence intensities of PET/PMPC$_x$-GMA$_y$ membrane pores after the permeation of the fluorescein isothiocyanate labeled-bovine serum albumin (FITC-BSA) (0.5 mL, 1.0 mg/mL) solution.

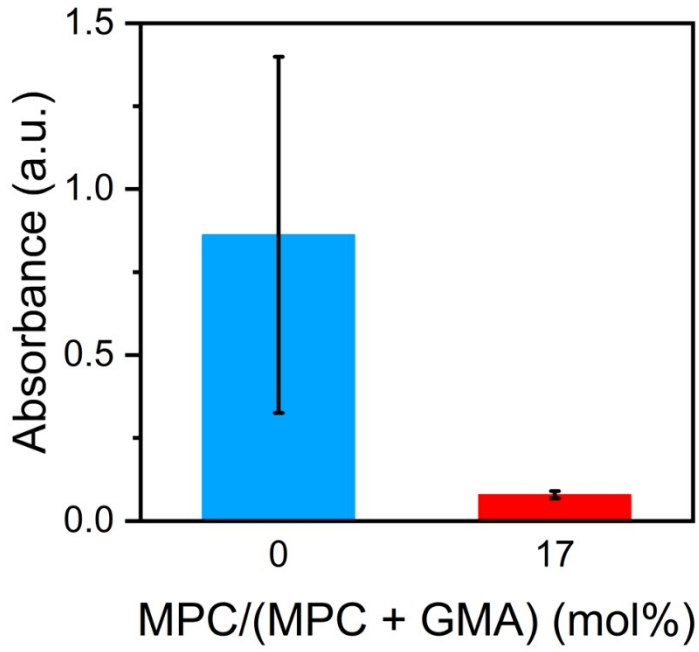

Figure S3. Background of artificial serum using membrane-based sensors with the composition of PET/PGMA and PET/PMPC$_{17}$-GMA$_{83}$.

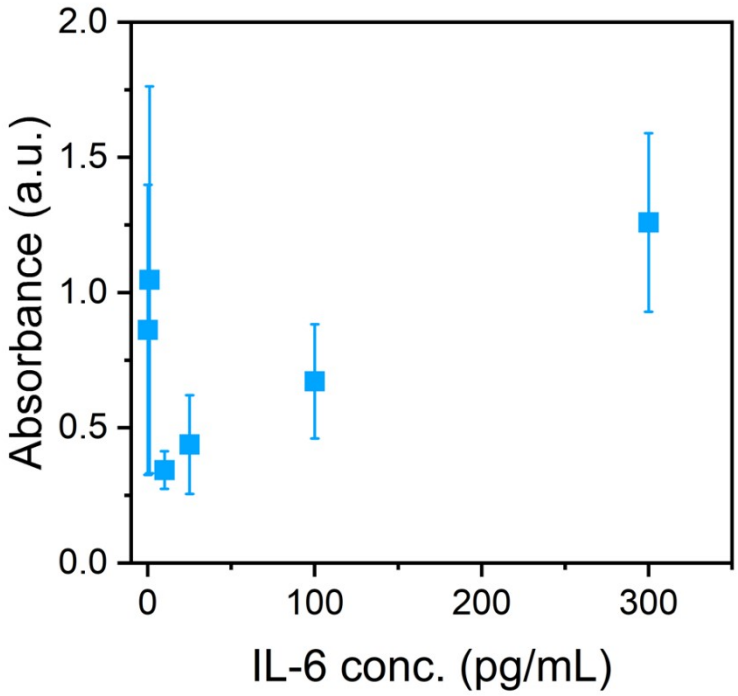

Figure S4. Calibration plots for IL-6 detection test in artificial serum using the PET/PGMA-based sensor.

- **Estimation of the amount of azide groups in the grafted copolymer**

According to our previous report,[5] the molecular weight of the grafted polymer in the membrane pores was 2×10^5 g/mol when the grafting ratio was less than 10%. In the case of MPC:GMA = 85:15, the degree of polymerization would be approximately 1000, and a single polymer chain includes 150 units of GMA. However, the maximum number of immobilized antibodies is limited to X units per polymer chain owing to steric hindrance between antibodies. Thus, the amount of the azide group was sufficient in the case of the copolymer with the composition of MPC:GMA = 85:15.

- **Quantification of antibody density in the pores**

To quantify the antibody density immobilized in the membrane-based sensors, an experiment was performed using an unmodified membrane substrate. The nonspecific adsorption of antibodies is due to the hydrophobic property of the substrate. However, the majority of the antibodies can be desorbed by solution permeation, and the adsorbed amount can be quantified. Thus, the relationship between the fluorescence intensity of the immobilized antibody and its actual amount can be estimated. The specific experimental procedure for calculating the antibody density in a membrane-based sensor is as follows.

(1) FITC-anti-BSA antibody buffer solutions with known antibody concentrations (1, 0.5, and 0.1 μg/mL) were prepared. The fluorescence intensity of the solutions was measured using a fluorescence spectrophotometer (Hitachi, F-2500) to generate a calibration curve (Figure S5a).
(2) PET substrates were immersed in an FITC-anti-BSA antibody buffer solution (100 μL) at the concentrations of 1.0, 0.5, 0.1, and 0.05 mg/mL at 4°C overnight. The immersed membrane was rinsed with deionized water, placed in a filter holder, and permeated with 6 mL/h of Wash buffer for 2 h. The collected permeate was measured using the fluorescence spectrophotometer, and the amount of antibody in the permeate was estimated from the calibration curve shown in Figure S5a.
(3) PET substrates were immersed in an FITC-anti-BSA antibody buffer solution (100 μL) at 1.0, 0.5, 0.1, and 0.05 mg/ml) at 4°C overnight. The membranes were rinsed with deionized water and cut using a microtome. Afterward, fluorescence images of the membrane cross-sections were obtained under a fluorescence microscope. The relationship between the fluorescence intensity and amount of antibody in (2) was summarized (Figure S5b).
(4) The FITC-anti-BSA antibody was immobilized on PET/PMPC$_x$-GMA$_y$, lightly washed with Wash buffer, and the membrane cross section was cut out using a microtome. The fluorescence intensity of the cross section was measured using a fluorescence microscope (Figure S5c). The density of the capture antibodies in the grafted polymer-grafted membrane of each copolymerization ratio was calculated using the calibration curve shown in Figure S5b. The

antibody density for PET/PMPC$_x$-GMA$_y$ (Figure 4f) was calculated from Figures S5b and S5c.

Table. S2 Relationship between the antibody immobilization ratio and the MPC/(MPC + GMA) ratio in the grafted polymer.

PET/PMPC$_x$-GMA$_y$	Immobilization ratio [mol%]
PET/PGMA	9.0
PET/PMPC$_{17}$-GMA$_{83}$	9.0
PET/PMPC$_{38}$-GMA$_{62}$	2.9
PET/PMPC$_{66}$-GMA$_{34}$	0.7
PET/PMPC$_{87}$-GMA$_{13}$	0.1

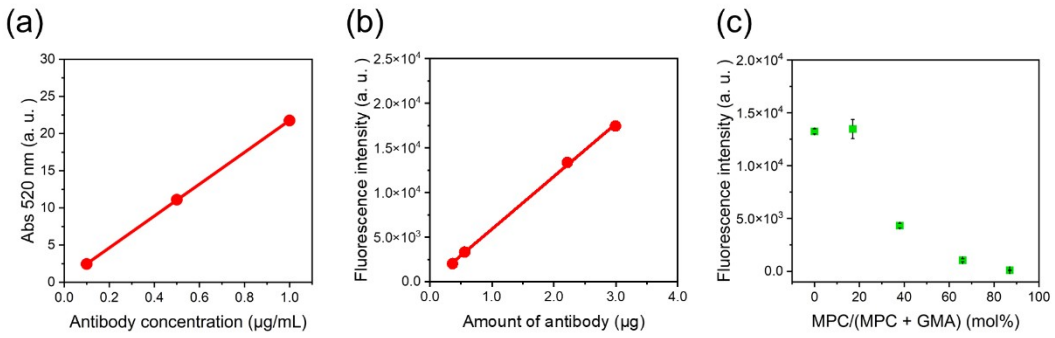

Figure S5. (a) Calibration curve of the fluorescence intensity against the concentration of the FITC-labeled anti-BSA. (b) Relationship between the fluorescence intensity measured using a fluorescence microscope on the vertical axis and the amount of FITC-anti-BSA antibody on the horizontal axis. (c) Fluorescence intensity measured using a fluorescence microscope on the vertical axis and PET/PMPC$_x$-GMA$_y$ on the horizontal axis.

References

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