Electronic Supporting Information for

ε-Polylysine-Based Thermo-Responsive Adsorbents for Immunoglobulin Adsorption-Desorption under Mild Conditions

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Experimental Section

General. Reagents were purchased from TCI, Aldrich, or Wako. A 10wt% aqueous *ε*-polylysine (EPL) solution was purchased from Ichimaru Pharcos. ELISA kit (human tPA) and related reagents were purchased from AssayPro. Dialysis membranes were purchased from Spectrum Labs. 96-Well plates with carboxylated surfaces were purchased from Sumitomo Bakelite. All the reactions were carried out under ambient conditions otherwise noted. All NMR spectra were recorded on JEOL ECA-500, and reported in parts per million (ppm, δ scale) from 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid (TMSP) sodium salt for ^{1}H NMR (δ 0 ppm, D_2O).

Typical procedure for *N*-valeryl-*ε*-polylysine derivatives. A mixture of *N*-hydroxysuccinimide (NHS) (0.55 g, 4.7 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (0.61 g, 3.2 mmol) was added DFM (10 mL) and water (5 mL). Immediately after all the solids were dissolved, valeric acid (0.42 mL, 3.8 mmol) was added and the reaction mixture was stirred for 3 h to complete NHS esterification. A 10wt% aqueous solution of EPL **1** (5.0 mL, ~4.0 mmol as $–NH_2$) was added to the reaction mixture to proceed amidation and the resulting mixture was stirred for 3 h. Dialysis over deionized water (MWCO 2000), removal of precipitation by filtration, and freeze-drying gave EPL derivative **2** as a white solid (0.42 g). The incorporation ratio of valeryl groups was calculated from the peak area ratio of the terminal -C$\underline{H}_3$ signal of valeryl group (0.90 ppm) and the mixed -C$\underline{H}_2$- signals of EPL backbone and valeryl group (1.2–1.9 ppm).

Typical procedure for MEP introduction to EPL derivatives. A solution of 4-pyridineethanethiol hydrochloride (0.33 g, 1.9 mmol) in DMF/H_2O (2.5 mL/2.5 mL) was added divinylsulfone (0.17 mL, 1.7 mmol) and triethylamine (22 μL, 0.16 mmol) and stirred for 3 h under inert atmosphere. A 10wt% aqueous solution of EPL **1** (5.0 mL. ~ 4.0 mmol as $–NH_2$) was added and the reaction mixture was stirred for overnight to give a pale yellow suspension. Dialysis over deionized water (MWCO 2000), removal of precipitation by filtration, and freeze-drying gave EPL/MEP derivative **1m** as a pale yellow solid (0.31 g). The incorporation ratio of MEP groups was calculated from the peak area ratio of the -$SO_2C\underline{H}_2CH_2S$- signal (3.84 ppm) and the -C$\underline{H}_2$- signals (1.2–1.9 ppm).

Immobilization of EPL derivatives. A 96-well plate with carboxylated surface was treated with a 1wt% solution of EDC·HCl in pH5.8 phosphate buffer saline (PBS) for 2 h at 37 °C. The wells were washed with pH5.8 PBS, solutions of EPL derivatives in pH5.8 PBS were added, and the 96-well plate was kept at 37 °C for 2 h to proceed amidation. The wells were washed with pH5.8 PBS, pH7.4 PBS, and deionized water (see also Fig. S4).

Evaluation of temperature-dependent affinity toward IgG. The polymer-immobilized surfaces were treated with excess amount of biotinylated IgG for 30 min at 4 °C to allow adsorption of IgG to MEP moieties. The wells were washed with cold pH7.4 PBS and then the adsorbed IgG

was allowed to desorb to pH7.4 PBS at 4 °C or 37 °C for 30 min. The supernatants were removed and the wells were washed with pH7.4 PBS (4 °C or 37 °C). The residual biotinylated IgG on the well surface was allowed to bind streptavidin-peroxidase conjugate (SP-conjugate) and the amount of biotinylated IgG was determined by a conventional colorimetric analysis using 3,3',5,5'-tetramethylbenzene (TMB) as a chromogen. Standard curve was obtained on a Protein A surface. In order to minimize the IgG desorption from EPL/MEP derivatives during coloration, all the ELISA-like protocols were carried out at 4 °C (see also Fig. S5).

VT-^{1}HNMR Study of EPL/MEP Derivatives

The thermal conformational changes in EPL derivatives were studied using VT-^{1}H NMR in D_2O under diluted conditions (Figure S1).[1] The chemical shift of the α-C<u>H</u> in the valerylated lysine unit, as well as the 2- and 3-pyridyl positions in the MEP group, were found to be sensitive to temperature, while other signals were scarcely dependent on temperature.

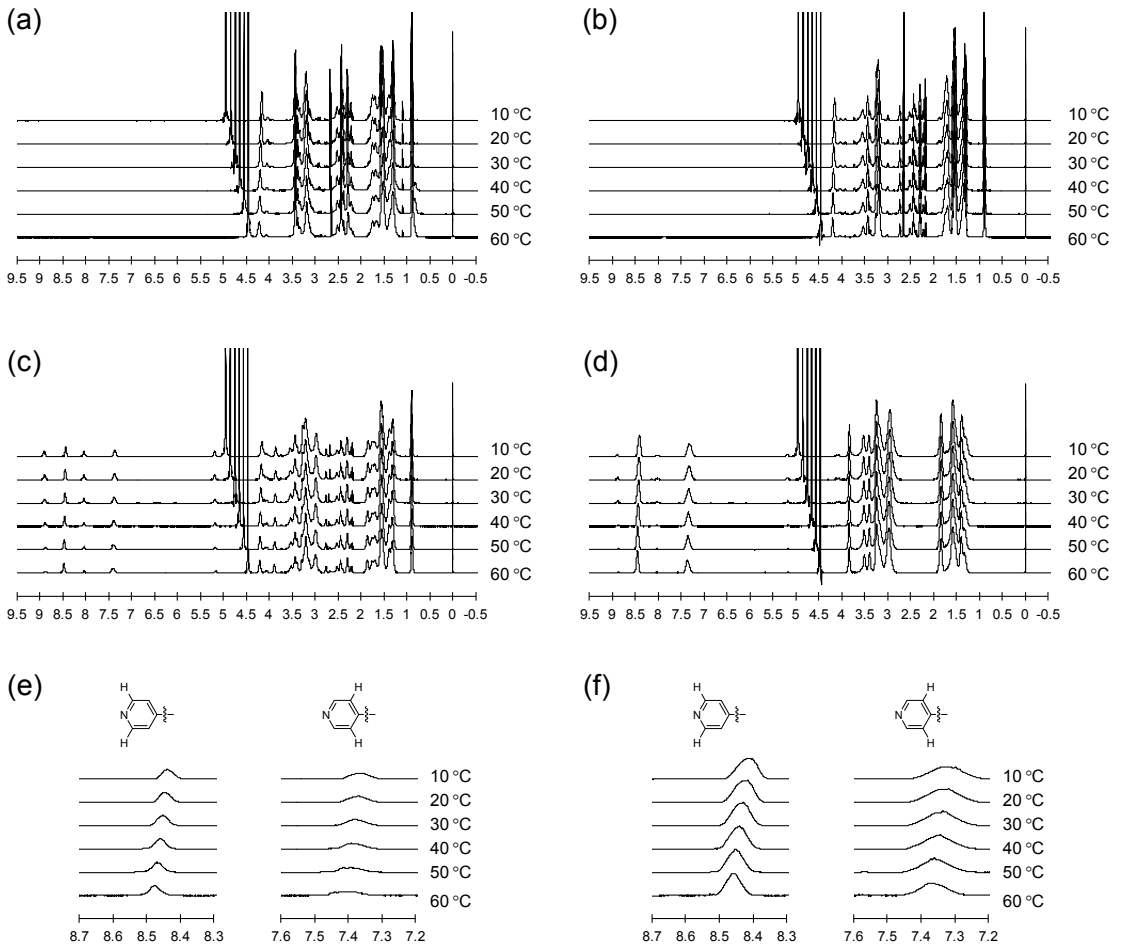

Fig. S1 Representative temperature-dependent ^{1}HNMR charts of EPL derivative **3** (a), EPL derivative **4** (b), EPL/MEP derivative **4m** (c) and (e), and EPL/MEP derivative **1m** (d) and (f).

[1] The solubility of MEP/EPL derivative **3m** to water was low when heating and ^{1}HNMR study could not be performed.

Investigations on the Correlations between VT-^{1}HNMR and Hydrophobicity

ClogP value[2] was used as an indicator for hydrophobicity of EPL derivatives. Instead of modeling the whole polymer, the average values of the repeating unit motifs were employed for simplicity (Fig. S2). Since the VT-^{1}HNMR measurements and ELISA-like measurements were both performed at neutral pH, a protonated form was chosen as the representative state for unreacted lysine unit and non-protonated forms were chosen for valerylated and MEP lysine units. Note that the ClogP values do not contain the information on the type of hydrophobicity (aliphatic or aromatic), the average ClogP values do not precisely reflect the alkyl-alkyl or π-π interactions. Moreover, the structural distributions in functionalization of EPL is quite wide. However, because the obtained correlation is quite reasonable, the average ClogP value is considered to be a good indicator within the investigated molecular structures.

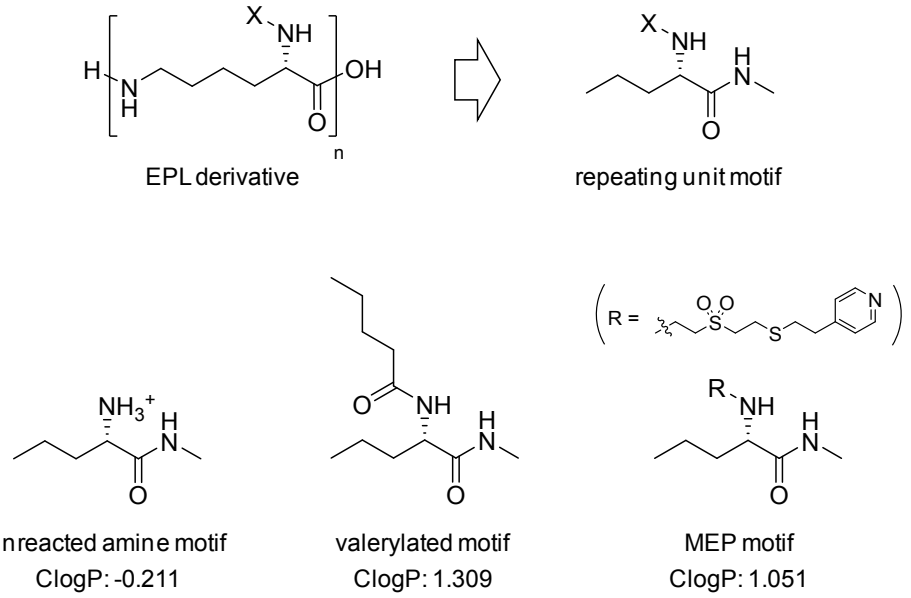

Fig. S2 Structural motifs and calculated ClogP values for the repeating units in EPL/MEP derivatives.

[2] ClogP values were calculated by the ClogP calculation algorithm and fragment database embedded in ChemBioDraw Ultra 14.

Besides the α-CH signals of the valerylated lysine units, pyridyl CH signals also show downfield shifts in EPL/MEP derivatives **1m**, **4m**, and **5m**, which are presumably contributed from changes in conformation and hydration states. However, they do not correlate to the average ClogP values (Fig. S3). We suspect the relatively small downfield shifts of EPL/MEP derivative **4m** and **5m** is because of the steric hindrance from valeryl groups to weaken the π-π interactions between MEP groups.

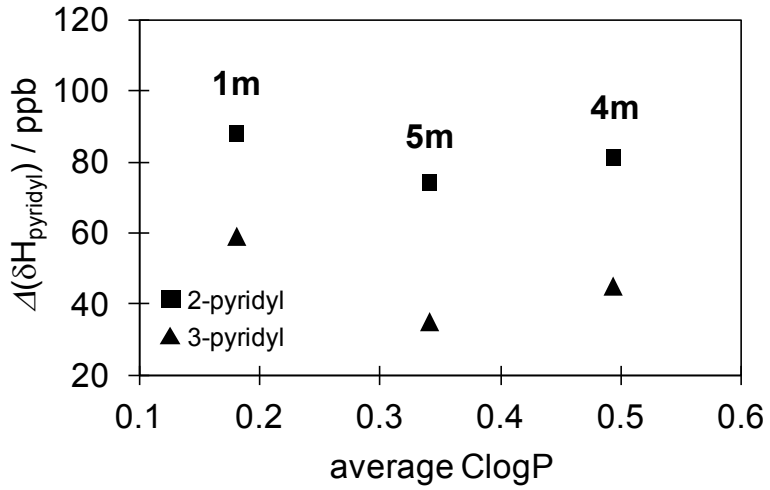

Fig. S3 Average ClogP versus VT-^{1}HNMR downfield shifts at the 2-pyridyl position of MEP groups.

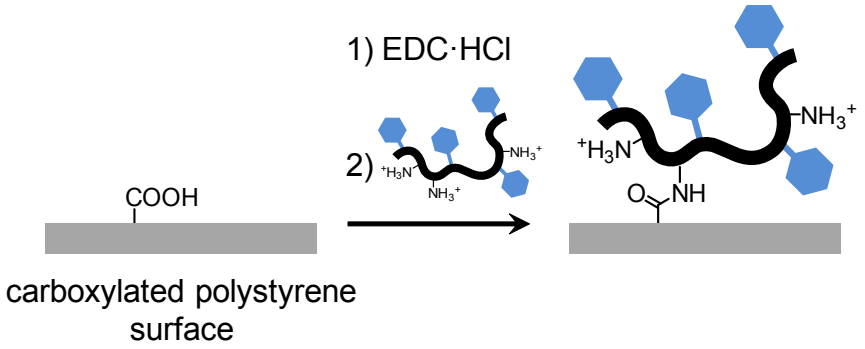

Fig. S4 Schematic illustration of surface immobilization of EPL/MEP derivatives to carboxylated surfaces. EPL/MEP derivatives were immobilized to carboxylated 96-well microplates through a two-step procedure of activation of carboxylic acid and amidation.

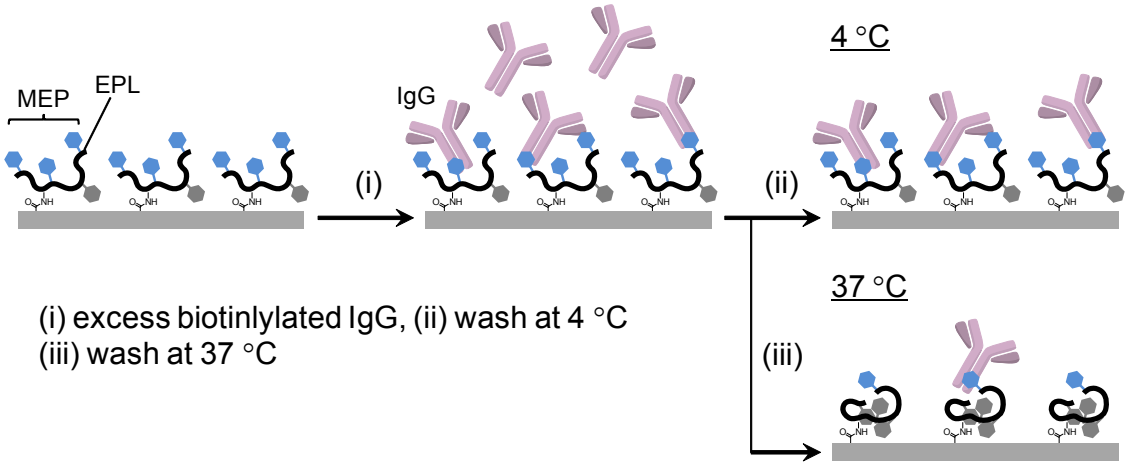

Fig. S5 Schematic illustration of temperature-dependent IgG adsorption measurements. Started from the same IgG-adsorbed states, the surfaces were washed at 4 °C or 37 °C and then the residual amounts of IgG were compared.

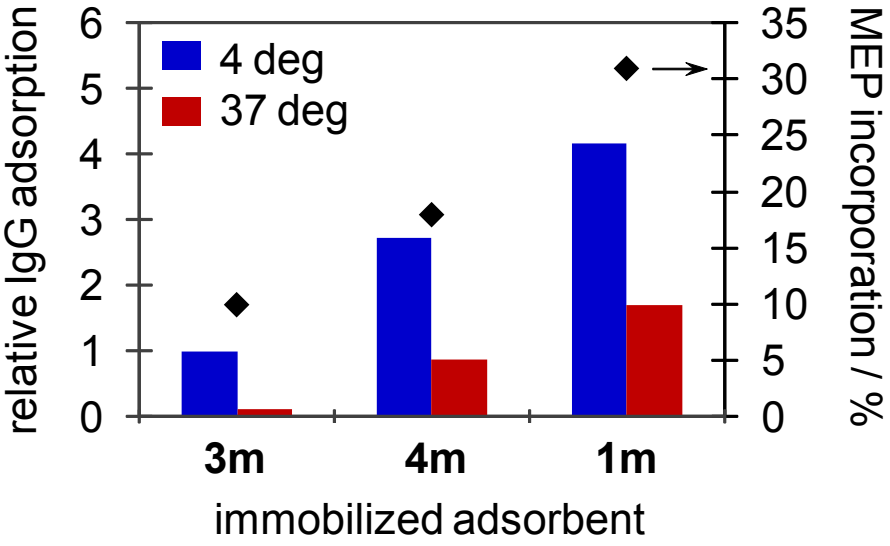

Fig. S6 Relative IgG adsorption of EPL/MEP derivatives **3m**, **4m**, and **1m** immobilized under 0.01wt% condition. The amounts of IgG adsorption were normalized by the average number of residual amine groups in each derivative and plotted as relative values against the IgG adsorption of EPL/MEP derivative **3m** at 4 °C.