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Supplementary Material

CO_2-Responsive Spherical Polyelectrolyte Brush with Multi-Simulation for Reversible Protein Immobilization and Release

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Materials. 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (HMP) (IRGACURE 2959) was purchased from Ciba Specialty Chemicals Inc. Both of Methacryloyl chloride (MC) and 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 99%) were obtained from Aladdin Industrial Corporation and purified through basic aluminum oxide column before use. Bovine serum albumin (BSA) and 2-(N-morpholino) ethanesulfonic acid (MES) were purchased from J&K Chemical company and used as received. Sodium chloride (NaCl), Sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium dodecyl sulfate (SDS), potassium persulfate (KPS), acetone and styrene were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. N_2 and CO_2 (99.9%, purity) were purchased from Shanghai Hukang Industrial Gases Co. Ltd. Styrene was purified by reduced pressure distillation to remove the inhibitors, and stored in the refrigerator at 4 °C before use. Acetone was purified by distillation. Deionized water used in this work was purified by reverse osmosis and subsequent ion exchange (Millipore Milli-Q). Other reagents were used without further purification unless otherwise specified.

Synthesis and characterization of PS-PDMAEMA. Polystyrene-poly(2-(dimethylamino)ethyl methacrylate) (PS-PDMAEMA) brushes were synthesized by photoemulsion polymerization which details are following as: Firstly, the PS core emulsion with narrow size distribution was prepared by conventional emulsion polymerization under nitrogen protection. Secondly, photoinitiator 2-[p-(2-hydroxy-2-methylpropiophenone)] ethylene glycol methacrylate(HMEM) was added under starved conditions to generate a thin layer of HMEM on the PS core surface. Finally,

the PS core latex was diluted to 1 wt% and DMAEMA monomer (from 50 to 300 wt% weight percent of the amount of PS core) was added to the latex. The entire system was illuminated by use of UV/Vis radiation in a photo-reactor for 2 hours under nitrogen protection. The production PS-PDMAEMA brushes were purified by pure water until its conductivity was constant.

The effect of pH, ionic strength and temperature on the hydrodynamic diameter of PS-PDMAEMA. Figure S1a indicated that the diameter of PS-PDMEMA was more sensitive to pH than to temperature at the experiment range. Take for example, the diameter of PS-PDMEMA was changed from 236.7 nm to 112.8 nm by increasing pH from 6.0 to 9.7, while it moved from 236.7 nm to 140.8 nm as temperature changed from 25 °C to 70 °C. Compared with temperature, ionic strength hold a primary affection to the size of polymer particles as shown in Figure S1b. The hydrodynamic diameter of PS-PDMAEMA reduced about 123, 107 and 96 nm by increasing pH or ionic strength value of the solution or temperature respectively from line II, III and I in Figure S1.

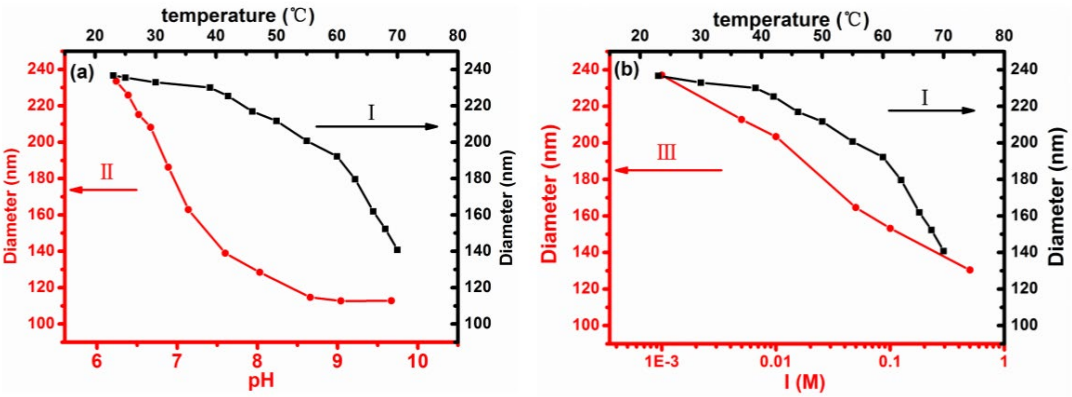

Figure S1. (a) Hydrodynamic diameter of PS-PDMAEMA as a function of temperature(I), pH(II), (b) temperature (I), and ionic strength (III) Condition: PS-PDMAEMA concentration: 0.012 mg/ml.

Initial BSA concentration on the adsorption process. The amount of BSA adsorbed per unit mass of PS-PDMAEMA (q) and separation efficiency of BSA (R) were calculated according to Equations (1) and (2):

$$q = V(C_0\text{-}C_t)/m \quad (1)$$

$$R = 100(C_0 - C_t)/C_0 \quad (2)$$

C_0 is the initial concentration of BSA solution (mg/mL) and C_t is the concentration of BSA solution after separation (mg/mL). While V (mL) is the volume of BSA solution and m (mg) represents the mass of PS- PDMAEMA.

Figure S2 suggest that the q value, which is the adsorption capacity of PS-PDMAEMA, is dependent on the initial BSA concentration before the quantity of protein exceeds the capability of the SPB. It also indicated that the PS-PDMAEMASPB is a highly effective adsorbent for the BSA protein.

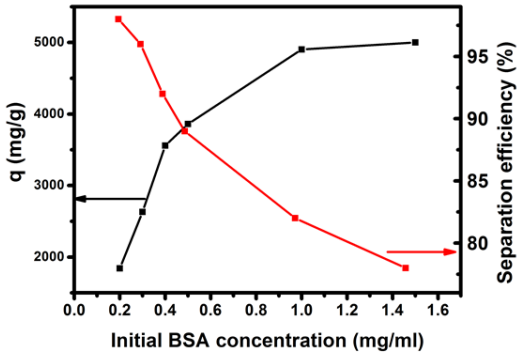

Figure S2. Effects of initial BSA concentration on the adsorption capacity of PS-PDMAEMA and the effects of initial BSA concentration on the separation efficiency.

Condition: PS-PDMAEMA concentration: 0.1mg/mL; temperature: 20ºC; stirring time: 24 h.

The effect of temperature on the BSA adsorption process. Figure S3 showed most of PDMAEMA chains were deprotonated at pH 9.0 at 25 °C, which induced BSA could not be adsorbed by polymer brush, only small amount of BSA move to the shell by Brownian movement. PDMAEMA chains shrinked with temperature increasing as mentioned above, which fixed the BSA in the shell and the turbidity increased slightly. Especially after 40 °C, hydrophobicity played an important role on the proteins adsorption process except the electrostatic interactions.[1] PS-PDMAEMA is hydrophobic and prone to adsorb BSA, which could make aggregation and the turbidity increased at higher temperature. However, the protein could be denaturated if the temperature raised too high which induced aggregation and sedimentation later.

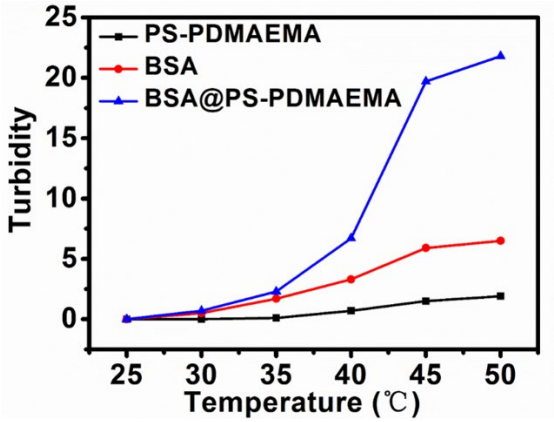

Figure S3. Turbidimetric titration for the mixture of PS-PDMAEMA and BSA as a function of temperature at 1 mM NaCl solution. Condition: PS-PDMAEMA (150 wt%) concentration: 0.006 mg/ml; BSA concentration: 0.030 mg/ml; pH: 9.5.

References

[1]. N. Welsch, A. Wittemann, M. Ballauff, *J Phys Chem B*, **2009**, 113, 16039. doi: 10.1021/jp907508w