# pH-Controllable Bioelectrocatalysis Based on "On−Off" Switching Redox Property of Electroactive Probes for Spin-Assembled Layer-by-Layer Films Containing Branched Poly(ethyleneimine)

**Shaoling Song and Naifei Hu***

*Department of Chemistry, Beijing Normal University, Beijing 100875, People's Republic of China*

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Weak polybase branched poly(ethyleneimine) (BPEI) and strong polyacid poly(styrenesulfonate) (PSS) were assembled into BPEI/{PSS/BPEI}$_n$ layer-by-layer (LBL) films on electrodes by electrostatic interaction between them with spin-coating approach. The cyclic voltammetric (CV) response of ferrocenedicarboxylic acid (Fc(COOH)$_2$) at BPEI/{PSS/BPEI}$_n$ film electrodes was very sensitive to the pH of the testing solutions. At pH 4.0, the probe showed a well-defined CV peak pair with relatively large peak currents for the films, while, at pH 7.0, the CV response was significantly depressed. By switching the film electrodes in buffers between pH 4.0 and 7.0, the CV peak currents changed periodically between a relatively high value at the "on" state and a very low value at the "off" state, indicating that the pH-sensitive "on−off" switching function of the films toward the probe is reversible. A series of comparative experiments indicates that the electrostatic interaction between the films and the probe plays a predominant role in deciding the pH-sensitive behavior of the films. This pH-dependent property of the films could be used to control or modulate the bioelectrocatalysis of glucose by glucose oxidase (GOD) with Fc(COOH)$_2$ as the mediator by changing the surrounding pH. This "smart" bioelectrocatalytic film system may establish a foundation for fabricating novel pH-controllable electrochemical biosensors.

## Introduction

Bioelectrocatalysis based on the direct or mediated electrochemistry of enzymes is an important foundation for construction of electrochemical biosensors and other biodevices and has aroused great interest among researchers in recent years.[1–3] In this aspect, the controllable or tunable bioelectrocatalysis is of significance since the reversible activation and deactivation of bioelectrocatalysis by external stimuli may enable its application in bioelectronic devices and provide the basis for information storage, data processing, and signal amplification, as well as biosensor devices.[4–6] For example, Katz and co-workers reported that poly(4-vinylpyridine) brush functionalized with Os-complex redox units could be modified onto indium tin oxide (ITO) electrodes, creating a pH-controlled electrochemical system.[7] At pH 4.0, the redox polymer film at the swollen state demonstrated the reversible electrochemical process, while, at pH > 6.0, the polymer became electrochemically inactive due to its structure change with the shrunken state. This pH-sensitive property of the films was used to tune the bioelectrocatalytic oxidation of glucose catalyzed by glucose oxidase (GOD) and mediated by the Os-complex units of the films through changing external solution pH. However, the controllable bioelectrocatalysis has been reported by only a few groups,[4–13] is still a challenging task, and needs further development.

Recently, increasing attention and interest have been placed on the development of stimuli-responsive thin films, and different external stimuli, including temperature, light, pH, ionic strength and potential, have been used to control the film response.[14,15] Among various approaches of fabricating thin films on solid substrates, layer-by-layer (LBL) assembly demonstrates obvious advantages in its precise control of film thickness at a nanometer level and its extremely simple procedure in assembly with high versatility.[16,17] Stimuli-responsive LBL films,[15,18] including the pH-sensitive LBL films,[19–22] have also been reported. The pH-controllable LBL films are usually assembled with weak polyelectrolytes because the ionization degree of weak polyelectrolytes varies with surrounding pH. For example, Rubner et al. reported that the LBL films made of poly(allylamine hydrochloride) (PAH) and poly(strenesulfonate) (PSS) showed discontinuous swelling/deswelling transitions in response to a change of the environmental pH.[23] Advincula and co-workers assembled LBL films of benzophenone-modified poly(acrylic acid) (PAA-BP) and PAH (PAH-BP) on the electrode surface and stabilized the films by photo-cross-linking.[24] Under certain conditions, {PAA-BP/PAH-BP}$_n$ LBL films demonstrated pH-sensitive permselectivity toward different redox ions. However, almost no work has been reported in application of pH-sensitive LBL polyelectrolyte films to controlling the bioelectrocatalytic process.

Branched poly(ethyleneimine) (BPEI) is a water-soluble and synthetic polymer. It contains three different types of amino groups: primary, secondary, and tertiary amines. The ratio of primary-to-secondary-to-tertiary amino groups in BPEI is 1:2:1.[25–27] Since BPEI is a weak polybase, the protonation degree of BPEI can be adjusted by varying the external pH.[26,28–30] BPEI was also used to assemble pH-responsive LBL films with other polyelectrolytes. For example, Gao et al. prepared pH-responsive mircrocapsules assembled by cross-linked LBL films of BPEI and PAA.[19] These microcapsules showed reversible pH-dependent permeability to 2000 kDa FITC-dextran (FITC = fluorescein isothiocyanate). They were permeable to FITC-

* To whom correspondence should be addressed. Tel.: (+86) 10-5880-5498. Fax: (+86) 10-5880-2075. E-mail: hunaifei@bnu.edu.cn.

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dextran or at the open state when the external pH was below 4.0 and impermeable to the probe or at the close state at pH above 6.0.

In the present work, BPEI and PSS were selected as the building blocks to assemble BPEI/{PSS/BPEI}$_n$ LBL films by spin-coating method. The assembly of BPEI/PSS multilayer films with "solution-dipping" method has been reported previously by Elzbieciak et al.[31] Compared with the traditional and relatively time-consuming solution-dipping method, the spin-coating technique is much more time-saving in LBL assembly. With the spin-coating technique, the deposition of a single polyelectrolyte layer usually takes only about 10−15 s, and the assembly of whole LBL films may be completed in only a few minutes.[32,33] In our previous study, different types of polycation/polyanion LBL films were successfully assembled onto different solid surfaces with spin-coating method.[34] In this work, the BPEI/{PSS/BPEI}$_n$ films assembled on pyrolytic graphite (PG) electrodes demonstrated a pH-sensitive "on−off" property toward electroactive probe ferrocenedicarboxylic acid (Fc(COOH)$_2$). At pH 4.0, the films were permeable toward the probe and the cyclic voltammetric (CV) response of the probe was quite large, while, at pH 7.0, the films became impermeable to the probe and the CV response of the probe was greatly depressed. The factors influencing the reversible on−off switching behavior of BPEI/{PSS/BPEI}$_n$ films toward Fc(COOH)$_2$ were investigated. This pH-sensitive switching property of the films was further used to control or modulate the electrocatalytic oxidation of glucose by GOD with Fc(COOH)$_2$ as the mediator. The mechanism of the pH-dependent permeability of the films toward the probe was explored by a series of comparative experiments, and believed to be mainly attributed to the electrostatic interaction between the films and the probe. This work provided a novel example to combine pH-sensitive permeability of LBL films with bioelectrocatalysis and established a foundation for constructing the pH-controllable biosensors. The better understanding of the essence of interactions involved in this pH-sensitive "smart" model interface may also open a general way to develop the new kind of controllable electrochemical biosensors based on enzymatic electrocatalysis.

## Experimental Section

**1. Reagents.** Poly(diallyldimethylammonium) (PDDA; 20%; MW, 200−350 kDa), poly(styrenesulfonate) (PSS; MW, 70 kDa), glucose oxidase (GOD; E.C. 1.1.3.4; type VII; 192000 units g$^{-1}$), 1,1′-ferrocenedicarboxylic acid (Fc(COOH)$_2$), and 3-mercapto-1-propanesulfonic acid (MPS) were purchased from Sigma-Aldrich. Branched poly(ethyleneimine) (BPEI; 30%; MW, 70 kDa) and hexaammineruthenium(III) chloride (Ru(NH$_3$)$_6$Cl$_3$) were purchased from Alfa Aesar. Poly(methacrylic acid, sodium salt) (PMA; MW, 75.1 kDa) was obtained from Fluka. Potassium ferricyanide (K$_3$Fe(CN)$_6$) was obtained from the Beijing Chemical Engineering Plant. Hydroquinone was from Tianjin Bodi Chemical Engineering. Other chemicals were of reagent grade. Britton−Robinson buffers at pH 3.0−10.0 contained 0.1 M NaCl, and the pH was adjusted to the desired value with dilute HCl or NaOH solutions. Solutions were prepared with water purified twice successively by ion exchange and distillation.

**2. Films Assembly.** For electrochemical study, basal plane pyrolytic graphite (PG; Advanced Ceramics; geometric area, 0.16 cm$^2$) disks were used as working electrodes. Prior to coating, PG electrodes were abraded with 320-grit metallographic sandpaper while being flushed with water. The electrodes were then ultrasonicated in water for 30 s and dried in air. For the spin-coating LBL assembly, the electrode was fixed onto the rotor of KW-4A spin-coater (Siyouyen Electronics Technology) with the electrode surface toward upside. The assembly of typical BPEI/{PSS/BPEI}$_n$ LBL films was performed as follows: (1) 10 $\mu$L of 1 mg mL$^{-1}$ BPEI aqueous solutions at pH 10.0 were cast onto the PG substrate to completely cover the electrode surface followed by spinning at the speed of 500 rpm for 5 s and then 4000 rpm for 15 s; (2) 10 $\mu$L of water were cast onto the PG/BPEI surface followed by spinning with the same procedure as in step 1 to wash the surface; (3) step 1 was repeated but with 10 $\mu$L of 1 mg mL$^{-1}$ PSS aqueous solutions at pH 3.0; (4) step 2 was repeated for washing; (5) steps 1 and 2 were repeated. After these five steps, the BPEI/{PSS/BPEI}$_1$ films were assembled on PG surface. The cycle of the steps from 3 to 5 was then repeated until the desired number of bilayers ($n$) was reached, forming BPEI/{PSS/BPEI}$_n$ LBL films. BPEI/{PMA/BPEI}$_n$ and PDDA/{PSS/PDDA}$_n$ LBL films were assembled with the same way.

For UV−vis spectroscopic study, the quartz slides were cleaned with piranha solution (3:7 volume ratio of 30% $H_2O_2$ and concentrated $H_2SO_4$ [*Caution*! The piranha solution is highly corrosive and should be handled with extreme care, and only a small volume should be prepared at any time]) for 30 min. After being successively ultrasonicated in ethanol and water, the slides were dried in air, and the surface became negatively charged. The BPEI/{PSS/BPEI}$_n$ LBL films were then assembled on the quartz surface with the same way as on PG electrodes.

For contact angle and scanning electron microscopy (SEM) studies, quartz crystal microbalance (QCM) gold electrodes were used as the substrates for film assembly. After being cleaned with piranha solution for 15 min and then successively ultrasonicated in ethanol and water, the Au electrodes were immersed in MPS solution for 24 h to chemisorb MPS as a precursor layer, making the Au surface become negatively charged. The subsequent assembly of BPEI/{PSS/BPEI}$_n$ LBL films on the surface of Au/MPS was the same as on the PG electrodes.

**3. Apparatus and Procedures.** A CHI 660A electrochemical workstation (CH Instruments) was used for all electrochemical measurements. A traditional three-electrode system was used with a saturated calomel electrode (SCE) as the reference, a platinum wire as the counter, and the PG disk with films as the working electrode. In electrochemical studies, buffers containing electroactive probes in the cell were purged with high-purity nitrogen for at least 15 min prior to measurements, and a nitrogen environment was then maintained during the whole experiment.

UV−vis spectra were collected with a Cintra 10e UV−visible spectrometer (GBC) with a scan rate of 500 nm min$^{-1}$.

Water contact angles were measured through the sessile drop method[35] with an OCA 20 optical contact angle measuring system (DataPhysics Instruments, GmbH). Measurements were taken for each layer of BPEI/{PSS/BPEI}$_n$ multilayers assembled on the Au/MPS surface.

SEM was performed using an S-4800 scanning electron microscope (Hitachi) with an acceleration voltage of 3 kV. Before the SEM imaging, the surface of samples was coated by thin platinum films with an E-1045 ion sputter (Hitachi).

All experiments were performed at ambient temperature (20 ± 3 °C).

## Results

**1. Assembly of BPEI/{PSS/BPEI}$_n$ LBL Films.** At pH 10.0, partial amino groups of BPEI are positively charged,[36] while PSS is always negatively charged. Thus, BPEI at pH 10.0 and

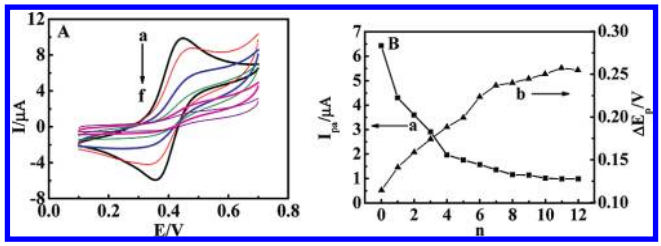


**Figure 1.** (A) CVs of 0.2 mM $Fc(COOH)_2$ at 0.1 V $s^{-1}$ in pH 7 buffers for (a) bare PG electrode and BPEI/{PSS/BPEI}$_n$ films with different numbers of bilayers ($n$): (b) 0, (c) 1, (d) 3, (e) 5, and (f) 10. (B) Dependence of CV oxidation peak current ($I_{pa}$) and peak separation ($\Delta E_p$) of $Fc(COOH)_2$ on $n$ for BPEI/{PSS/BPEI}$_n$ films.

PSS at pH 3.0 could be assembled into BPEI/{PSS/BPEI}$_n$ LBL films mainly by electrostatic interaction between them. The growth of BPEI/{PSS/BPEI}$_n$ films assembled by spin-coating approach on quartz slides was monitored or confirmed by UV−vis spectroscopy. The characteristic absorption peak at 226 nm for PSS[32,37] increased roughly in a linear mode with the number of bilayers ($n$) from $n = 2$ (Supporting Information Figure S1), suggesting the successful assembly of the LBL films in a regular way. The linear fitting equation can be expressed as absorbance $= 0.0194n + 0.103$ with correlation coefficient of 0.972.

Water contact angle approach was also used to characterize the LBL assembly of BPEI/{PSS/BPEI}$_n$ films on MPS/Au surface (Supporting Information Figure S2). From the third assembly step, the contact angle of the films with BPEI as the outermost layer was always larger than that when the following PSS layer was adsorbed, suggesting that the BPEI layer is more hydrophobic than the following PSS layer. The contact angle systematically switched between a relatively large value for the BPEI outermost layer and a smaller value for the PSS top layer, indicating that the BPEI/{PSS/BPEI}$_n$ LBL films are successfully assembled with a relatively good layer order.

The assembly of BPEI/{PSS/BPEI}$_n$ LBL films on PG surface was confirmed by CV with $Fc(COOH)_2$ as the electroactive probe (Figure 1). At bare PG electrode, $Fc(COOH)_2$ in pH 7.0 buffers displayed a well-defined and nearly reversible CV oxidation−reduction peak pair at about 0.41 V. When BPEI/{PSS/BPEI}$_n$ multilayers were fabricated on the PG surface, the CV response of $Fc(COOH)_2$ was severely suppressed, accompanied by the increase of peak separation ($\Delta E_p = E_{pa} - E_{pc}$), indicating that a barrier was formed on the PG electrode surface and the probe was hindered from reaching the PG electrodes and then exchanging electrons with the underlying electrodes. The oxidation peak current ($I_{pa}$) of $Fc(COOH)_2$ decreased with the number of bilayers ($n$) for the films and the $\Delta E_p$ value increased with $n$, all suggesting that the films are successfully fabricated on PG surface.

The stability of the BPEI/{PSS/BPEI}$_{10}$ films was examined by CV with $Ru(NH_3)_6^{3+}$ as the electroactive probe. For the study of short-term stability, 50 CV cycles were continuously performed, and the peak currents of the probe remained nearly the same. For the study of long-term stability, the films were stored in pH 7.0 blank buffers for most of the storage time and placed in pH 7.0 solutions containing $Ru(NH_3)_6^{3+}$ for CV testing periodically. After 6 days of storage, the CV peak potentials maintained the same position, and the reduction peak current decreased by about 20% of their initial value. All these results indicate that the BPEI/{PSS/BPEI}$_n$ films are quite stable.

**2. pH-Sensitive CV Behavior of $Fc(COOH)_2$ at BPEI/{PSS/BPEI}$_{10}$ Film Electrodes.** The CV response of $Fc(COOH)_2$ at BPEI/{PSS/BPEI}$_{10}$ film electrodes with $n = 10$ was very sensitive to the pH of $Fc(COOH)_2$ solutions, and the effect of pH on $I_{pa}$ and $\Delta E_p$ of $Fc(COOH)_2$ for the films is shown in Figure 2. While $Fc(COOH)_2$ showed relatively large $I_{pa}$ and quite small $\Delta E_p$ in the range of pH 4.0−6.0, the $I_{pa}$ decreased drastically between pH 7.0 and 9.0, accompanied by the great increase of $\Delta E_p$. From pH 4.0 to 9.0, the $I_{pa}$ experienced a dramatic decrease. This pH-sensitive CV behavior of $Fc(COOH)_2$ should not be attributed to the property of the probe itself since the CV behavior of $Fc(COOH)_2$ at bare PG electrodes was pH-independent.

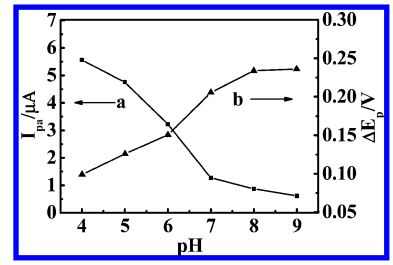


**Figure 2.** Influence of solution pH on (a) CV oxidation peak current ($I_{pa}$) and (b) peak separation ($\Delta E_p$) of 0.2 mM $Fc(COOH)_2$ at 0.1 V $s^{-1}$ for BPEI/{PSS/BPEI}$_{10}$ films.

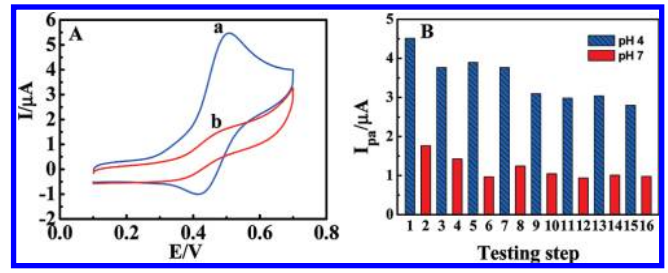


**Figure 3.** (A) CVs of 0.2 mM $Fc(COOH)_2$ at 0.1 V $s^{-1}$ for BPEI/{PSS/BPEI}$_{10}$ films in buffers at pH (a) 4.0 and (b) 7.0. (B) Dependence of CV oxidation peak current ($I_{pa}$) of $Fc(COOH)_2$ at 0.1 V $s^{-1}$ on solution pH switched between pH 4.0 (blue bars) and 7.0 (red bars) for the same BPEI/{PSS/BPEI}$_{10}$ films.

The pH-dependent CV behavior of $Fc(COOH)_2$ for BPEI/{PSS/BPEI}$_{10}$ films could be used to study the on−off switching property of the films, and two typical pH values, pH 4.0 and 7.0, were selected in the present study according to Figure 2. In pH 4.0 buffers, $Fc(COOH)_2$ showed a nearly reversible CV peak pair with relatively large peak currents for the films (Figure 3A, curve a), while, in pH 7.0 buffers, the CV response was significantly depressed and even could hardly be observed (Figure 3A, curve b). The dependence of CV of $Fc(COOH)_2$ on buffer pH for the films was quite reversible. By switching the film electrode in buffers between pH 4.0 and 7.0, the $I_{pa}$ changed periodically between a relatively high value at pH 4.0 and a very low value at pH 7.0 (Figure 3B), suggesting that the CV response of $Fc(COOH)_2$ for BPEI/{PSS/BPEI}$_{10}$ films can be switched by environmental pH, and the films are at the "on" state at pH 4.0 and at the "off" state at pH 7.0.

**3. Influencing Factors.** Different factors influencing the on−off behavior of BPEI/{PSS/BPEI}$_n$ films toward $Fc(COOH)_2$ were investigated by CV, including the number of bilayers ($n$) of the films, the outermost constituent, and the assembly pH.

The number of bilayers or the thickness of BPEI/{PSS/BPEI}$_n$ films showed considerable influence on the CV on−off property of the films toward $Fc(COOH)_2$. At pH 4.0, the CV oxidation peak current ($I_{pa}$) of the probe showed essentially no change with $n \geq 1$, suggesting that, at this pH, the permeability of the films toward the probe does not become poorer when the films become thicker (Supporting Information Figure S3). However,

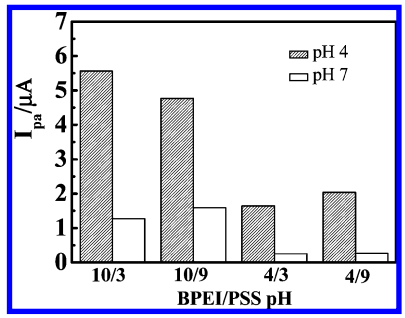


**Figure 4.** CV oxidation peak currents ($I_{pa}$) for BPEI/{PSS/BPEI}$_{10}$ films constructed at different assembly pH in testing solutions containing 0.2 mM Fc(COOH)$_2$ at pH 4.0 and 7.0, respectively.

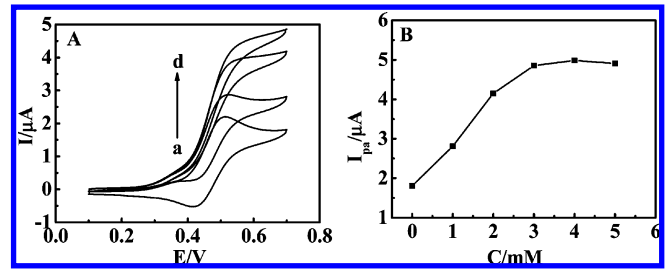


**Figure 5.** (A) CVs of BPEI/{PSS/BPEI}$_{10}$ films at 10 mV s$^{-1}$ in pH 4.0 buffers containing 0.2 mM Fc(COOH)$_2$, 1.0 mg mL$^{-1}$ GOD, and glucose with (a) 0, (b) 1.0, (c) 2.0, and (d) 3.0 mM. (B) Dependence of CV oxidation peak current ($I_{pa}$) on concentration of glucose for BPEI/{PSS/BPEI}$_{10}$ films in pH 4.0 buffers.

in pH 7.0 solutions, the $I_{pa}$ value decreased dramatically with $n$ from 1 to 12, and the CV response became very small when $n \geq 10$. Considering that the CV signal of the probe for BPEI/{PSS/BPEI}$_n$ films with $n < 10$ was not small enough at pH 7.0, and the films with $n = 10$ demonstrated a larger difference in the $I_{pa}$ value between pH 4.0 and 7.0, the films with $n = 10$ were usually used in the present work.

The outermost layer of PSS/BPEI multilayer films also had great influence on the CV behavior of Fc(COOH)$_2$ (Supporting Information Figure S4A). At pH 4.0, Fc(COOH)$_2$ demonstrated quite large CV peak heights for BPEI/{PSS/BPEI}$_{10}$ films with BPEI as the outermost layer. However, when an additional layer of PSS was assembled on the film surface, the CV response of the probe for BPEI/{PSS/BPEI}$_{10}$/PSS films was suppressed drastically. Similar results were also observed in pH 7.0 solutions. While both BPEI/{PSS/BPEI}$_n$ and BPEI/{PSS/BPEI}$_n$/PSS films demonstrated on−off switching behavior toward Fc(COOH)$_2$ between pH 4.0 and 7.0, the difference of $I_{pa}$ between pH 4.0 and 7.0 for the films with BPEI as the outermost layer was much larger than that of the films with PSS as the last surface layer, regardless of $n$ (Figure S4B). The interpenetration of neighboring layers is a common phenomenon in LBL assembly,[16,38] but, for the PSS/BPEI multilayer system, the different property of the probe for the films with different outermost layers under the same condition, as well as the results of contact angles (Figure S2), suggests that the interpenetration is not complete and quite limited at least for the top layer. At the same pH, the CV signal of Fc(COOH)$_2$ for the films with BPEI as the outermost layer was always much higher than that with PSS as the top layer. This is most probably because the negatively charged Fc(COOH)$_2$ will be attracted by positively charged BPEI surface layer but repulsed by negatively charged PSS outermost layer. Thus, the electrostatic interaction between the probe and the films should play an important role in influencing the CV response of the probe. Considering that the films with BPEI as the outermost layer demonstrated more obvious pH-sensitive CV behavior toward the probe, the BPEI/{PSS/BPEI}$_{10}$ films were usually used in our present study.

The effect of assembly pH for BPEI/{PSS/BPEI}$_{10}$ films on the pH-sensitive switching property of the films toward Fc(COOH)$_2$ was also investigated. Four different assembly pH conditions were chosen, designated as BPEI/PSS pH 10/3, 10/9, 4/3, and 4/9. For example, BPEI/PSS pH 10/3 means that the BPEI adsorbate solution at pH 10.0 and PSS assembly solution at pH 3.0 were used to fabricate BPEI/{PSS/BPEI}$_{10}$ films. CVs of the films assembled at different pH were performed in pH 4.0 and 7.0 buffers containing Fc(COOH)$_2$, respectively. All four types of BPEI/{PSS/BPEI}$_{10}$ films demonstrated the on−off behavior toward the probe between pH 4.0 and 7.0 to some extent (Figure 4). Among them, the pH-dependent CV behaviors of the probe for the films assembled at BPEI/PSS pH 10/3 and 10/9 were very similar, indicating that the assembly pH of PSS has no substantial influence on the switching property of the films toward the probe. This conclusion is also supported by the similar CV responses of the films assembled at BPEI/PSS pH 4/3 and 4/9. However, the CV behaviors of the probe for the films assembled at BPEI/PSS pH 10/3 and 4/3 were quite different. For example, in pH 4.0 testing solutions, the CV oxidation peak current of Fc(COOH)$_2$ ($I_{pa}$) for the films assembled at BPEI/PSS pH 10/3 was much larger than that at BPEI/PSS pH 4/3. Similar difference was also observed between the films assembled at BPEI/PSS pH 10/9 and 4/9. These results suggest that the assembly pH of BPEI has a significant effect on the pH-sensitive behavior of the films. Considering that the BPEI/{PSS/BPEI}$_{10}$ films assembled at BPEI/PSS pH 10/3 showed a higher $I_{pa}$ value at pH 4.0 and better switching behavior than the films assembled under other pH conditions, the BPEI/PSS pH 10/3 was selected as the assembly pH in this work.

**4. pH-Controllable Bioelectrocatalysis.** The pH-responsive switching property of BPEI/{PSS/BPEI}$_{10}$ films toward Fc(COOH)$_2$ could be used to control or modulate the bioelectrocatalytic oxidation of glucose by GOD enzyme. When glucose and GOD were added into the Fc(COOH)$_2$ solution at pH 4.0, in comparison with the system in the absence of glucose, the CV oxidation peak at about 0.47 V at BPEI/{PSS/BPEI}$_{10}$ film electrodes increased dramatically, accompanied by the decrease or even disappearance of the reduction peak (Figure 5A). The oxidation peak current ($I_{pa}$) increased initially with the concentration of glucose and then tended to level off (Figure 5B). All these results are characteristic of electrochemical oxidation of glucose catalyzed by GOD and mediated by Fc(COOH)$_2$, and the mechanism can be expressed by the following equations.[39,40]

$$\text{GOD(FAD)} + \text{glucose} \rightarrow \text{GOD(FADH}_2\text{)} + \text{gluconolactone}$$

$$\text{GOD(FADH}_2\text{)} + 2\text{Fc(COOH)}_2\text{(ox.)} \rightarrow \text{GOD(FAD)} + 2\text{Fc(COOH)}_2\text{(red.)}$$

$$\text{Fc(COOH)}_2\text{(red.)} - \text{e}^- \rightleftharpoons \text{Fc(COOH)}_2\text{(ox.)} \quad \text{at electrode}$$

where GOD(FAD) and GOD(FADH$_2$) represent oxidized and reduced forms of glucose oxidase, respectively.

However, when BPEI/{PSS/BPEI}$_{10}$ films were placed in pH 7.0 buffers containing the same amount of Fc(COOH)$_2$, glucose, and GOD, the electrocatalytic response became quite small or

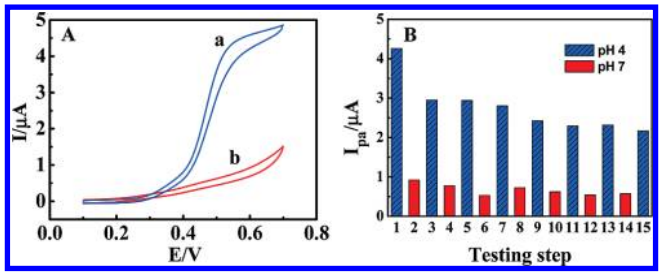


**Figure 6.** (A) CVs of BPEI/{PSS/BPEI}$_{10}$ films at 10 mV s$^{-1}$ in solutions containing 0.2 mM Fc(COOH)$_2$, 3.0 mM glucose, and 1.0 mg mL$^{-1}$ GOD at pH (a) 4.0 and (b) 7.0. (B) Dependence of CV catalytic oxidation peak current ($I_{pa}$) on solution pH switched between pH 4.0 (blue bars) and 7.0 (red bars) for the same BPEI/{PSS/BPEI}$_{10}$ film electrode.

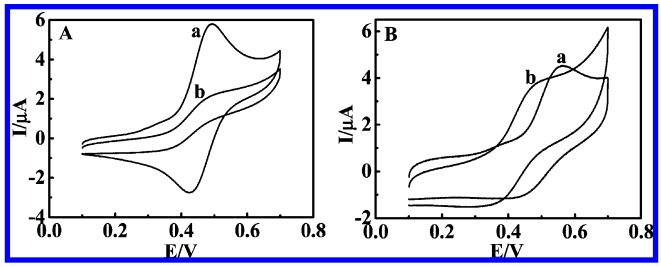


**Figure 7.** CVs of 0.2 mM Fc(COOH)$_2$ at 0.1 V s$^{-1}$ for (A) BPEI/{PMA/BPEI}$_{10}$ and (B) PDDA/{PSS/PDDA}$_{10}$ films in buffers at pH (a) 4.0 and (b) 7.0.

even could hardly be observed (Figure 6A, curve b). This is because the films become off for the probe at pH 7.0, resulting in the interruption of the catalytic cycles. Therefore, the bioelectrocatalysis of glucose by GOD can be controlled by the different permeability of BPEI/{PSS/BPEI}$_{10}$ films toward Fc(COOH)$_2$ at different pH. The electrocatalysis is on at pH 4.0 and off at pH 7.0 (Figure 6A). If $I_{pa4}$ and $I_{pa7}$ were defined as the oxidation peak current at pH 4.0 and 7.0, respectively, the $I_{pa4}/I_{pa7}$ ratio could be amplified by the electrocatalysis. For the same BPEI/{PSS/BPEI}$_{10}$ films in Fc(COOH)$_2$ solutions, the $I_{pa4}/I_{pa7}$ ratio was only 1.90 in the absence of GOD and glucose, while, in the presence of GOD and glucose, the ratio increased to 4.61. Moreover, the pH-sensitive on−off bioelectrocatalysis for the BPEI/{PSS/BPEI}$_{10}$ films could be repeated for at least several cycles by switching the same films in the Fc(COOH)$_2$ + GOD + glucose solutions between pH 4.0 and 7.0, although the $I_{pa}$ at pH 4.0 showed a small decreasing trend with the number of cycles (Figure 6B).

**5. Comparative Study with Other LBL Films.** BPEI is a weak polybase, and its ionization or protonation is pH-dependent, while PSS is a strong polyacid, and its ionization is pH-independent. Thus, the pH-sensitive behavior of BPEI/{PSS/BPEI}$_n$ films toward Fc(COOH)$_2$ should be attributed to the BPEI component but not PSS constituent. To further investigate which component of BPEI/{PSS/BPEI}$_n$ films, BPEI or PSS, played a predominant role in deciding the pH-sensitive behavior of the films toward the probe, the other two LBL films, BPEI/{PMA/BPEI}$_{10}$ and PDDA/{PSS/PDDA}$_{10}$ films were assembled on PG electrode surface with the same spin-coating LBL technique, and their CV behaviors toward Fc(COOH)$_2$ at different pH were compared with that of BPEI/{PSS/BPEI}$_{10}$ films. In BPEI/{PMA/BPEI}$_{10}$ films, PMA was used as the negatively charged constituent to replace the PSS component in BPEI/{PSS/BPEI}$_{10}$ films. As seen from Figure 7A, the BPEI/{PMA/BPEI}$_{10}$ films also showed pH-sensitive on−off switching function toward Fc(COOH)$_2$, and the films were at the on state at pH 4.0 and at the off state at pH 7.0. In PDDA/{PSS/PDDA}$_{10}$ films, the strong polybase PDDA was used as the positively charged component to replace the BPEI constitute of the BPEI/{PSS/BPEI}$_{10}$ films. However, the PDDA/{PSS/PDDA}$_{10}$ films showed no obvious difference in CV peak heights of Fc(COOH)$_2$ at pH 4.0 and 7.0 (Figure 7B). These results support the speculation that the BPEI component in the films plays a predominant role in deciding the pH-sensitive on−off behavior of the films toward Fc(COOH)$_2$.

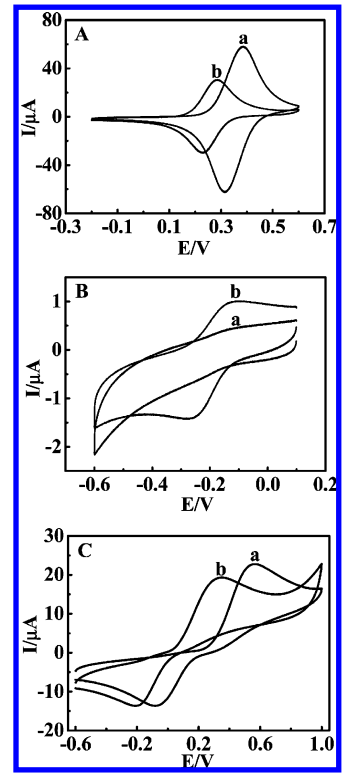


**Figure 8.** (A) CVs of 0.1 mM $Fe(CN)_6^{3-}$ at 0.1 V s$^{-1}$ for BPEI/{PSS/BPEI}$_{10}$ films in buffers at pH (a) 4.0 and (b) 7.0. (B) CVs of 0.2 mM $Ru(NH_3)_6^{3+}$ at 0.1 V s$^{-1}$ for BPEI/{PSS/BPEI}$_{10}$ films in buffers at pH (a) 4.0 and (b) 7.0. (C) CVs of 0.5 mM hydroquinone at 0.1 V s$^{-1}$ for BPEI/{PSS/BPEI}$_{10}$ films in buffers at pH (a) 4.0 and (b) 7.0.

**6. Comparative Study with Other Probes.** To understand the interaction between Fc(COOH)$_2$ and BPEI/{PSS/BPEI}$_{10}$ films, other electroactive probes, including $Fe(CN)_6^{3-}$, $Ru(NH_3)_6^{3+}$, and hydroquinone, were investigated by CV at BPEI/{PSS/BPEI}$_{10}$ film electrodes in different pH buffers, and their CV behaviors were compared with that of Fc(COOH)$_2$.

At pH 4.0, negatively charged $Fe(CN)_6^{3-}$ showed a nearly reversible CV peak pair at about 0.35 V at BPEI/{PSS/BPEI}$_{10}$ film electrodes (Figure 8A, curve a). At pH 7.0, the CV response greatly decreased, and the reduction−oxidation peak currents were about two times smaller than that at pH 4.0 (Figure 8, curve b). Thus, the films demonstrated the similar pH-sensitive on−off behavior toward $Fe(CN)_6^{3-}$ and Fc(COOH)$_2$. For both probes, the films became on at pH 4.0 and off at pH 7.0. For positively charged $Ru(NH_3)_6^{3+}$, however, the pH-dependent switching behavior of the films was completely opposite. At pH 7.0, $Ru(NH_3)_6^{3+}$ demonstrated a pair of CV quasi-reversible peaks at about −0.20 V with quite large peak heights (Figure 8B, curve b). At pH 4.0, this peak pair greatly depressed and could hardly be observed (Figure 8B, curve a). In control experiments with bare PG electrodes, both $Fe(CN)_6^{3-}$ and $Ru(NH_3)_6^{3+}$ in solution showed pH-independent CV behaviors, suggesting that the substantial difference of CV responses for these probes at different pH is related to the property of the films. Since the size of $Fe(CN)_6^{3-}$ (6.0 Å) and $Ru(NH_3)_6^{3+}$ (6.2

Å) is similar,[24] the different pH-sensitive switching behavior of the films toward these two probes should be attributed to the different types of charges that they carry rather than the pore size of the films, and the electrostatic interaction between the probe and the films should play a key role in deciding the direction of the on−off property.

To support the above speculation, hydroquinone was also used as the electroactive probe to test the CV property of the films at different pH. At both pH 4.0 and 7.0, hydroquinone is neutral and carries no charge[41] and showed a quasi-reversible oxidation−reduction peak pair at the film electrodes with similar peak heights but different peak positions (Figure 8C). The pH-dependent CV peak positions of hydroquinone originate from the property of the probe itself,[41–44] which was also confirmed by the control experiment with bare PG electrodes (Supporting Information Figure S5). The similar CV peak heights of hydroquinone at pH 4.0 and 7.0 at the film electrodes would thus be understandable since hydroquinone carries no charge, and there is no electrostatic interaction between the probe and the films.

$Fc(COOH)_2$ is a weak acid, but its two $pK_a$ values in aqueous solutions could not be found in literature. However, from its $pK_a$ values in ethanol−water[45,46] and THF−water solutions (THF = tetrahydrofuran),[47] and considering the influence of solvents on $pK_a$ values for other weak acids such as ferrocenecarboxylic acid (Fc(COOH)),[46,48–50] $Fc(COOH)_2$ would be partially ionized at pH 4.0 and completely ionized at pH 7.0 in its aqueous solutions. At BPEI/$\{$PSS/BPEI$\}_{10}$ film electrodes, the pH-sensitive CV behavior of $Fc(COOH)_2$ was similar to that of $Fe(CN)_6^{3-}$ but different from that of $Ru(NH_3)_6^{3+}$ and hydroquinone, also suggesting that $Fc(COOH)_2$ behaves like a negatively charged species at both pH 4.0 and 7.0.

## Discussion

There are two typical kinds of mechanism for pH-sensitive permeation behavior of polyelectrolyte films toward probes. One is mainly controlled by the structure change of films with environmental pH,[7,8,19,51,52] and another is controlled by the electrostatic interaction between probes and films, where the film charge is switched by external pH.[24,53–55] For example, the cross-linked BPEI/PAA multilayers belong to the first type, which showed a dramatic change in structure with pH.[19] At pH 10.0, the capsule wall made of the BPEI/PAA LBL films were impermeable toward 2000 kDa FITC-dextran. At pH 3.0, the capsule wall became open for permeation of FITC-dextran because of the reorganization of the wall structure. The structure difference of the BPEI/PAA films at pH 3.0 and 10.0 was obviously observed by SFM in the 1 $\mu$m scale. However, in the present work, the structure change for the BPEI/$\{$PSS/BPEI$\}_{10}$ films at pH 4.0 and 7.0 was not observed by SEM with similar magnification (Supporting Information Figure S6A,B). The similar surface morphology and roughness of the films at pH 4.0 and 7.0 indicate that the solution pH has no substantial influence on the structure of BPEI/$\{$PSS/BPEI$\}_{10}$ films at least with the present magnification, and the porosity of the films is not very sensitive to the solution pH. In addition, different outermost layers had great effect on the film morphology, and the BPEI/$\{$PSS/BPEI$\}_{10}$ and BPEI/$\{$PSS/BPEI$\}_{10}$/PSS films demonstrated obviously different surface morphology, which is in agreement with the results of CV (Figure S4) and water contact angle (Figure S2) measurements. However, no substantial difference was observed for the BPEI/$\{$PSS/BPEI$\}_{10}$/PSS films after being treated at different pH (Figure S6C,D). Thus, the pH-sensitive behavior of BPEI/$\{$PSS/BPEI$\}_{10}$ films toward $Fc(COOH)_2$ should be mainly attributed to the second mechanism and controlled by the electrostatic interaction between probes and films.

For BPEI/$\{$PSS/BPEI$\}_n$ films, it is the BPEI rather than PSS component that plays a key role in deciding the pH-sensitive behavior of the films. BPEI is a weak polybase with primary, secondary, and tertiary amino groups in its backbone and branches. Recently, Sammartano et al. reported that the protonation behavior of BPEI was very similar to that of low molecular weight tetramines with a series of $pK_a$ values of 2.50, 5.78, 8.17, and 9.38 in 0.11 M NaCl solutions.[56] Thus, the BPEI/$\{$PSS/BPEI$\}_n$ films assembled at BPEI/PSS pH 10/3 would contain a considerable amount of un-ionized or "free" amino groups, since most of the amino groups of BPEI would be unprotonated at pH 10.0 and these un-ionized amines would not form ionic pairing with the negatively charged groups of PSS in the assembly. When the films are immersed in pH 4.0 or 7.0 solutions, these "free" amines would be protonated, but the protonation degree is quite different between pH 4.0 and 7.0 considering that $pK_{a2}$ of BPEI is at 5.78.[56] This conclusion is consistent with that of other references, where the protonated nitrogen groups of BPEI were reported to account for about 70% at pH 4.0 and decrease to about 15−22% at pH 7.0.[28,30,57,58] Therefore, in pH 4.0 buffers, the highly positively charged BPEI/$\{$PSS/BPEI$\}_n$ films would have much stronger electrostatic attraction with negatively charged $Fc(COOH)_2$ in solution, making the probe to go through the films more easily and leading to the quite large CV response of the probe. At this low pH, the remaining $-SO_3^-$ groups of PSS in the films would tend to form ionic pairs with the free protonated amines of BPEI. In contrast, at pH 7.0, the much less positively charged films would not be in favor of the negatively charged probe entering the films, and some remaining $-SO_3^-$ groups of PSS in the films would be at the free state, and their repulsion effect on the negatively charged probe would become pronounced, thus resulting in the very small CV signal of $Fc(COOH)_2$. Therefore, the pH-sensitive behavior of BPEI/$\{$PSS/BPEI$\}_n$ films toward $Fc(COOH)_2$ is most probably attributed to the electrostatic interaction between the films and the probe. This conclusion is also supported by the CV results with different outermost layers (Figure S4) and different probes (Figure 8).

## Conclusions

BPEI/$\{$PSS/BPEI$\}_n$ LBL films assembled by spin-coating approach demonstrate pH-sensitive permeability toward negatively charged probe $Fc(COOH)_2$. It is the weak polybase BPEI but not strong polyacid PSS constituent in the films that plays a decisive role in the pH-dependent property of the films. The films assembled at BPEI/PSS pH 10/3 contain a considerable amount of free amino groups of BPEI, and these free amines are sensitive to surrounding pH and show different ionization degree at different pH, leading to the different charging state of the films. Mainly because of the electrostatic interaction between the films and $Fc(COOH)_2$, the CV response of the probe at the film electrodes show reversible on−off switching properties: the films are at the on state at pH 4.0 and at the off state at pH 7.0. The pH-sensitive permeability of the BPEI/$\{$PSS/BPEI$\}_n$ films can be used to control or modulate the electrochemical oxidation of glucose catalyzed by GOD and mediated by $Fc(COOH)_2$. This work provides a novel model to realize pH-controllable bioelectrocatalysis, and the understanding of the mechanism of pH-sensitive switching behavior of this model system may guide us to develop a new kind of electrochemical biosensors with stimuli-responsive property.

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**Supporting Information Available:** Figures showing UV−vis absorption spectra of BPEI/{PSS/BPEI}$_n$ films with different numbers of bilayers, contact angle measurements of BPEI/{PSS/BPEI}$_n$ films with assembly steps, influence of the number of bilayers of BPEI/{PSS/BPEI}$_n$ films on $I_{pa}$ of Fc(COOH)$_2$ in buffers at different pH, CVs of Fc(COOH)$_2$ for BPEI/{PSS/BPEI}$_{10}$ and BPEI/{PSS/BPEI}$_{10}$/PSS films at different pH, CVs of hydroquinone at bare PG electrodes at different pH, and SEM top views of BPEI/{PSS/BPEI}$_{10}$ and BPEI/{PSS/BPEI}$_{10}$/PSS films treated with different pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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