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Critical Study of the Recognition between C-Reactive Protein and Surface-Immobilized Phosphorylcholine by Quartz Crystal Microbalance with Dissipation

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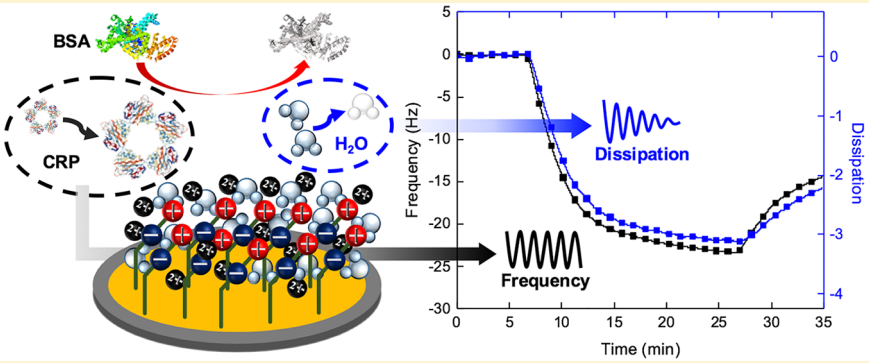
Supporting Information



ABSTRACT: C-reactive protein (CRP), a biomarker for cardiovascular disease, has been reported to have a strong affinity to zwitterionic phosphorylcholine (PC) groups in the presence of calcium ions. In addition, PC-immobilized surfaces have been used as a nonfouling coating to prevent nonspecific protein binding. By appropriately using the features of PC-immobilized surfaces, including specific recognition to CRP and nonfouling surface, it is reasonable to create an antibody-free biosensor for the specific capture of CRP. In this study, PC-functionalized 3,4-ethylenedioxythiophene (EDOT) monomers were used to prepare PC-immobilized surfaces. The density of PC groups on the surface can be fine-tuned by changing the composition of the monomer solutions for the electropolymerization. The density of PC group was confirmed by X-ray photoelectron spectroscopy (XPS). The specific interaction of CRP with PC groups was monitored by using a quartz crystal microbalance with dissipation (QCM-D). The amount of protein binding could be estimated by the reduction in frequency readout. Through the QCM-D measurement, we revealed the nonfouling property and the specific CRP capture from our PC-immobilized surfaces. Notably, the dissipation energy also dropped during the binding process between CRP and PC, indicating the release of water molecules from the PC groups during CRP adsorption. We anticipate that surface-bound water molecules are mainly released from areas near the immobilized PC groups. Based on Hofmeister series, we further examined the influence of ions by introducing four different anions including both kosmotrope (order maker) and chaotrope (disorder maker) into the buffer for the CRP binding test. The results showed that the concentration and the type of anions play an important role in CRP binding. The present fundamental study reveals deep insights into the recognition between CRP and surface-immobilized PC groups, which can facilitate the development of CRP sensing platforms.

INTRODUCTION

The human C-reactive protein (CRP) is a common biomarker for acute inflammation,[1] tissue damage,[2] and cardiovascular disease.[3−5] CRP can help the immune system recognize dead or dying cells, as well as certain bacteria through binding the phosphorylcholine (PC) headgroup of lipids in the cell membrane.[6] CRP usually binds PC on an apoptotic cell to trigger the preliminary stage of the classic complement pathway and then initiates the phagocytosis of macrophages to digest the apoptotic cell.[7] The concentration of CRP in normal human serum is less than 10 mg/L, but the average value increases slightly with age.[8] Typically, the concentration of CRP increases to 10−40 mg/L because of medium inflammation or viral infections. When the concentration increases to within 40−200 mg/L, an active inflammation and bacterial infection are diagnosed.[9] Severe bacterial infections and burns will drive the level of CRP above 200 mg/L.[9] CRP concentration is also used

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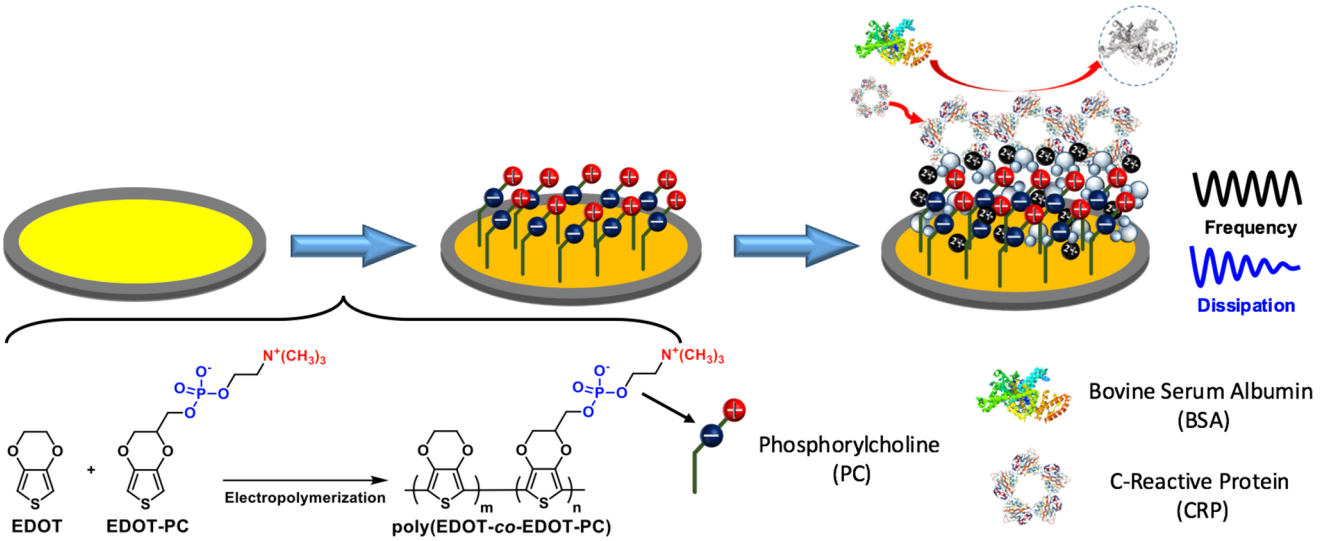
Scheme 1. Poly(EDOT-*co*-EDOT-PC) Thin Films Coated on a QCM-D Quartz by Electropolymerization[a]



[a]The frequency and dissipation readouts were measured by QCM-D to investigate the CRP recognition events on poly(EDOT-PC).

as an index to evaluate the risk of cardiovascular disease, and this is because cardiovascular disease induces myocardial infarction, which has been closely linked to CRP levels.[10,11] Overall, CRP is a useful biomarker in the identification of inflammation as well as the risks related to inflammation-related diseases.

The nonfouling coating is important to the biomedical engineering. It is generally recognized that prevention of biofouling could reduce the inflammatory responses, such as tissue fibrosis, thrombosis coagulation, and infection.[12] The PC functional group is part of the surface of plasma membranes. This zwitterion functional group is the headgroup of phospholipids on the plasma membranes and serves as a ligand to CRP while giving plasma membranes a hydrophilic property to avoid protein adsorption.[13−15] Previous studies have demonstrated that surface immobilized with poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) through a surface-initiated atom transfer radical polymerization method delivered low friction coefficient[16,17] and nonfouling surface.[18−20] A study also showed that 2-methacryloyloxyethyl phosphorylcholine (MPC) polymeric nanoparticles bound to CRP antibodies can specifically detect CRP with a high dynamic range from 0.01 to 10 mg/dL in serum-free CRP solution.[21] More recent research showed that CRP has excellent specific binding to materials functionalized with PC groups when immersed in calcium-containing buffer solutions.[22,23] Based on these conclusions, it is reasonable to develop an antibody-free CRP biosensing platform by simply using PC-immobilized substrates in the presence of Ca^{2+} ions.[24−29] Moreover, this platform should provide nonfouling properties to prevent nonspecific binding of other proteins, thus allowing specific detection of CRP.

More recently, bioelectronics approaches based on conducting polymers have received increasing attention because of their superior performance in several aspects compared with traditional ones.[30,31] Conducting polymers, such as poly(3,4-ethylenedioxythiophene) (PEDOT) and polypyrrole (PPy),[32] have stable electrical conductivity in aqueous buffers.[33,34] Their mechanical properties also match well with human body tissues.[31] In addition, PEDOT and its derivatives have a very low cytotoxicity level and display no inflammatory response in both *in vitro* and *in vivo* experiments, which makes them excellent candidates for implanted bioelectronics devices.[34,35] Recently, functionalized PEDOT has been applied to various biomedical applications,[36] such as biosensing,[37] cell capturing,[38] and nonfouling surfaces.[39] Zhu et al. first developed PC-functionalized PEDOT films and used such films to prevent nonspecific binding.[40] Goda et al. fabricated an electrochemical differential pulse voltammetry (DPV) biosensor for CRP detection using PC-functionalized PEDOT thin films.[41]

In this study, we adapted methods similar to previously published approaches[41] to immobilize PC groups on PEDOT thin films through electropolymerization, as shown in Scheme 1. The recognition of CRP and immobilized PC groups was monitored using a quartz crystal microbalance with dissipation (QCM-D).[42] Subsequently, we compared the differences between the specific and nonspecific binding of proteins on the PEDOT films. By illustrating both the frequency and dissipation readouts during specific recognition compared with nonspecific adsorption events, we provide more insight into the recognition mechanism. Furthermore, because Ca^{2+} ions play a key role in the recognition between CRP and PC, we investigated how the ions in solution influence the recognition process. A Hofmeister series has been used to describe the specific salt effect to determine protein stability, folding, aggregation, and precipitation.[43] It is recognized that the salts dissolve into the solution to form the cations and anions, which affects the interactions between water molecules and leads to a change in surface tension, hydration structure, and viscosity to alter the protein behavior in solution. In this study, we also evaluated the influence of ions and their concentrations on CRP-PC recognition by comparing binding efficiencies in the presence of different anions including SO_4^{2-}, Cl^-, Br^-, and I^- ions. Based on the Hofmeister series, the SO_4^{2-} ions belong to the kosmotropes (order maker) and I^- ions belong to chaotropes (disorder maker). We expect that our study provides more insight into the recognition event between CRP and immobilized PC groups and benefits the development of biosensing systems for CRP using immobilized PC-based platforms.

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MATERIALS AND METHODS

Materials. Hydroxymethyl EDOT (EDOT−OH), bovine serum albumin (BSA), and calcium chloride were purchased from Sigma-Aldrich. EDOT was purchased from Tokyo Chemical Industry (Tokyo, Japan). Dr. Hsiao-hua Yu from Academia Sinica provided PC-functionalized EDOT (EDOT-PC) as a gift. It was synthesized by following previously published procedures.[40] The recombinant human C-reactive protein was purchased from Wako Pure Chemicals (Tokyo, Japan). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), tetrabutylammonium perchlorate, dioctyl sulfosuccinate, sodium salt (DSS), and dodecyl sulfate, sodium salt (SDS) were purchased from ACROS. Lithium perchlorate and acetonitrile were purchased from Alfa Aesar. Sodium chloride was purchased from Fisher Chemical. All chemicals were used directly without further purification.

Electropolymerization of PEDOT Films. Electrochemical experiments were carried out with an Autolab PGSTAT128N potentiostat (Metrohm, The Netherlands) using a three-electrode setup. A Pt electrode was used as the counter electrode. A Ag/AgCl with saturated KCl electrode was used as the reference electrode for aqueous solutions, whereas a Ag/Ag^+ electrode was used as the reference electrode for nonaqueous solutions. The QCM chip was the working electrode. A 10 mM EDOT−OH solution was prepared by dissolving EDOT−OH in deionized (DI) water in the presence of 100 mM $LiClO_4$. A 10 mM EDOT/EDOT-PC monomer solution was prepared by dissolving monomers in CH_3CN in the presence of 100 mM $LiClO_4$ and 50 mM DSS. A poly(EDOT−OH) thin layer was deposited by applying a cyclic potential from −0.6 to 1.1 V (vs Ag/AgCl) at a scan rate of 100 mV/s at 15 °C to improve the adhesion between PEDOT and Au substrates.[44] Poly(EDOT-PC) and its copolymers were then deposited on poly(EDOT-OH) by applying a cyclic potential from −0.6 to 1.2 V (vs Ag/Ag^+).

Characterization of PEDOT Morphology and Surface Properties. Scanning electron microscopy (SEM) images were recorded on a JSM 7800F (Jeol, Japan) instrument at 4×10^{-6} Torr, with an accelerating voltage of 5 kV and a working distance of 10 mm. Samples were coated with a thin layer of Pt before observation. Surface morphology and roughness measurements were obtained by BioAFM Resolve (Bruker, United States) microscope operating with SCANASYST-AIR tips from Bruker (spring constant = 0.4 N m^{-1}, frequency = 70 kHz) in peak force mode. Samples were immersed in HEPES buffer overnight before atomic force microscopy (AFM) experiments, and a scanning area of 5 μm × 5 μm with a scan rate of 0.5 Hz was used for roughness measurements. Static contact angles were measured using a Model 100 SB contact angle goniometer (Sindatek, Taiwan) at room temperature. Apprximately1 μL of DI water was used for contact angle measurements. X-ray photoelectron spectroscope (XPS) was performed by an ESCA system with a twin anode X-ray gun of 15 kV.

Protein Binding and Ion Adsorption Studies. The protein binding and ion adsorption were monitored by a QCM-D E1 (Biolin Scientific, Västra Frölunda, Sweden) system. The polymer thin films were coated on the surface of a QSX 301 QCM chip by electropolymerization before being placed into the Q-sense flow module. All measurements were performed at 25 °C. The flow rate was maintained at 75 μL/min using an Ismatec ISM829B pump. In this study, all QCM-D tests were repeated for at least three times to ensure the accuracy of our data and confirm the results were highly reproducible. The binding test was performed in HEPES buffer. Protein solutions at concentrations between 4 μg/mL to 1 mg/mL were used to assess protein binding. When protein adsorbs on the surface of QCM chip, the shifts in frequency and dissipation could be recorded directly. The QCM data was recorded at five overtones (n = 1, 3, 5, 7, 9) which showed different at dissipation and normalized frequency ($\Delta f = \Delta f_n/n$). In this study, we mainly presented the third overtone (n = 3), because the first overtone is too sensitive, such that any vibration could interfere with the balance. The fifth to ninth overtone provides comparable information. Usually, for the rigid coated surface, all the QCM-D frequency readouts can be converted to a mass change using the Sauerbrey equation, $\Delta f = -C \times \Delta m$, which implies a proportional relationship between frequency change and mass change. The constant, C, is related to the density, resonant frequency, active crystal area, and shear modulus of the quartz crystal.[45] However, for the soft coating surface (polymer film in liquids), the oscillation frequency would decay due to the soft film. This phenomenon of energy dissipation would cause error of evaluating mass change. Therefore, in this study, we estimated the amount of protein binding by illustrating the reduction in frequency readout. The energy dissipation or damping of the quartz oscillation was defined as $D = E''/(2\pi E')$, where E'' is the energy dissipated during one oscillation cycle and E' is the total energy stored in the oscillator. Higher dissipation indicates faster energy losses during the oscillation. It usually occurs when large or soft molecules adsorbed on the quartz, which leads to a higher impedance to maintain the oscillations. Lower dissipation indicates less energy losses. It may occur when the hydration left the coating surface and make the surface rigid.

RESULTS AND DISCUSSION

Surface Properties of PC-Functionalized PEDOT Films. We first coated PEDOT films on Au chips for QCM-D measurement by directly applying electropolymerization. The surface density of PC groups can be altered simply by changing the composition of the monomer solutions. In this study, five monomer solution compositions were tested: 0%, 25%, 50%, 75%, and 100% EDOT-PC. Moreover, in order to identify the PC groups on the poly(EDOT-*co*-EDOT-PC) film, we introduced X-ray photoelectron spectroscope (XPS) to conform the chemical compositions of the surface films in this study. The immobilized PC group was confirmed by XPS, as shown in Figure S1 in Supporting Information. We used SEM and AFM to observe the surface morphology of the polymer films. The wettability was evaluated through water contact angle measurement. The SEM images, AFM scans, and water contact angles are provided in Figure S2 in Supporting Information. The root-mean-square surface roughness (R_q) estimated from AFM images and water contact angles are summarized in Figure 1. The R_q of all polymer films was less than 10 nm over a 5 × 5 μm^2 area, indicating highly smooth surfaces. Therefore, the water contact angle was mainly driven by the functional groups present on the surface. When the EDOT-PC feed ratio increased, the contact angle decreased, indicating more hydrophilic surfaces due to the increasing

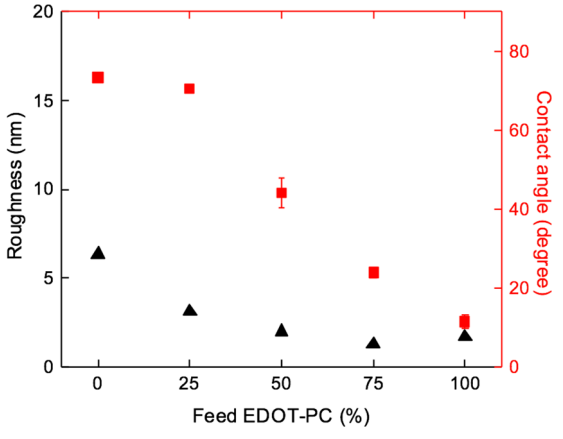

Figure 1. Roughness (black triangle) and water contact angle (red square) of poly(EDOT-*co*-EDOT-PC) films at 0%, 25%, 50%, 75%, and 100% EDOT-PC feed ratio. The roughness of the films at different EDOT-PC feed ratio was calculated directly from AFM images.

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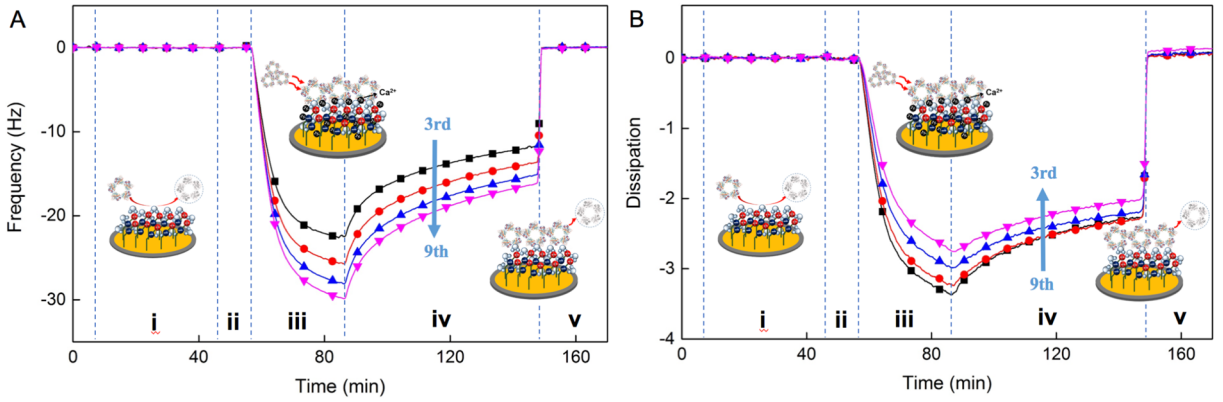

Figure 2. Comparison of the (A) frequency and (B) dissipation readout of CRP binding on poly(EDOT-PC) films in HEPES buffer with and without Ca^{2+} ions. The mobile phase in the QCM-D chamber was as follows: (i) HEPES buffer containing 4 μg/mL CRP without Ca^{2+} ions; (ii) HEPES buffer containing 1 mM Ca^{2+} ions; (iii) HEPES buffer containing 4 μg/mL CRP and 1 mM Ca^{2+} ions; (iv) HEPES buffer containing 1 mM Ca^{2+} ions; and (v) HEPES buffer without Ca^{2+} ions.

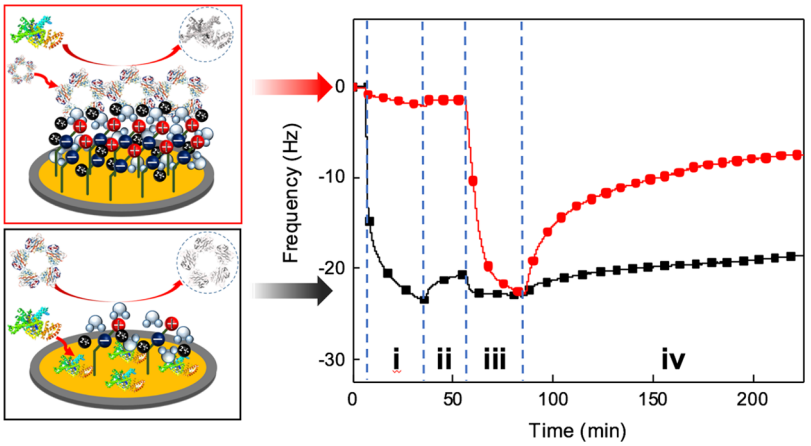

Figure 3. QCM showed that different EDOT-PC feed ratios, 25% (black line) and 100% (red line), provide different ratios of nonspecific to specific binding. The QCM chamber flow solution was changed for (i) 1 mg/mL BSA in HEPES buffer, to (ii) blank HEPES buffer, to (iii) 4 μg/mL CRP in HEPES buffer, and to (iv) blank HEPES buffer. EDOT-PC feed ratios 25% (black line) show an obvious BSA binding from (i) to (ii). EDOT-PC feed ratios 100% (red line) show little BSA binding from (i) to (ii), indicating a nonfouling surface, and specific CRP binding from (iii) to (iv).

density of surface PC groups. Pure poly(EDOT-PC) films provided the most hydrophilic surfaces. This observation is consistent with previous publications.

CRP Binding on PC-Functionalized PEDOT Films. We first examined CRP binding with and without Ca^{2+} ions in buffer solution. A QCM chip coated with poly(EDOT-PC) was used for this binding test, as illustrated in Figure 2. The QCM-D frequency and dissipation readout of third to ninth overtones initially reached an equilibrium value under the flow of buffer solution without Ca^{2+} ions and CRP as the mobile phase. In stage i, we subsequently shifted the mobile phase to a buffer solution dissolving 4 μg/mL CRP. No frequency drop was observed, indicating the absence of considerable surface fouling and the lack of CRP adsorption onto the QCM chip. In stage ii, we rinsed the chamber with pure buffer solutions for 10 min, then shifted the mobile phase to a buffer solution containing 1 mM Ca^{2+} ions. In stage iii, we changed to a solution containing both 1 mM Ca^{2+} ions and 4 μg/mL CRP as the mobile phase. A significant frequency drop (~22 Hz) indicated strong binding of CRP on poly(EDOT-PC). In stage iv, after rinsing with buffer solution, most of the CRP remained attached to the surface. In stage v, we rinsed the chamber with buffer without Ca^{2+} as the mobile phase. CRP left the PC-immobilized surface immediately and the frequency return to the initial state before CRP binding in 3 min. These results clearly illustrate the necessity of Ca^{2+} ions to induce recognition between CRP and immobilized PC groups. For CRP-PC recognition, each protomer in human CRP has a calcium binding pocket in the PC binding domain and the interaction of CRP with two calcium ions play a major role in the PC recognition.[46] Moreover, it is interesting to note that the frequency and the dissipation readout dropped spontaneously. Usually, the dissipation would increase as the frequency decrease as protein binding on the QCM chip. More details regarding this phenomenon are provided in the following section.

We also demonstrated the adsorption of BSA and CRP on poly(EDOT-*co*-EDOT-PC) of 25% EDOT-PC feed ratio compared with poly(EDOT-PC). BSA is usually used for test nonfouling property of surfaces. The frequency readout of third overtone presented in Figure 3 and the statistical analysis is summarized in Figure 4. In this study, poly(EDOT-PC) showed minimum adsorption of BSA, indicating excellent nonfouling properties. Compared with poly(EDOT-PC), poly(EDOT-*co*-EDOT-PC) of 25% EDOT-PC feed ratio showed stronger BSA binding, indicating that the density of the PC groups is too low to prevent nonspecific binding.

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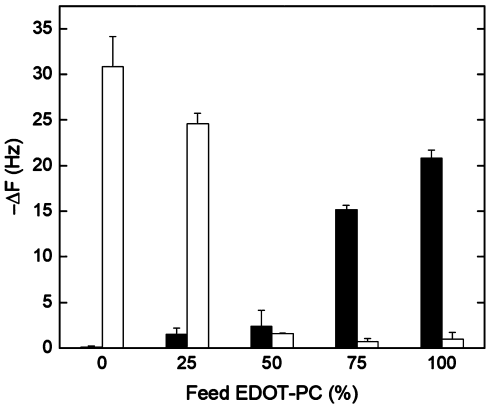

Figure 4. Changing of frequency readout of nonspecific BSA binding (white) and specific CRP binding (black) measured on films prepared at different EDOT-PC feed ratios of 0%, 25%, 50%, 75%, and 100%. As the EDOT-PC feed ratio increased, BSA binding decreased while CRP binding increased. At EDOT-PC feed ratios of 75% and 100%, the surface could efficiently prevent BSA binding and specifically capture CRP.

Moreover, after adsorption of BSA on these copolymer films, only a few CRP adsorption events were detected with QCM-D. However, pure poly(EDOT-PC) showed strong CRP binding. We also tested poly(EDOT-*co*-EDOT-PC) of different compositions, involving 0%, 50%, and 75% EDOT-PC feed ratio; the results are summarized in Figure 4. In general, CRP binding increases as the feed ratio of EDOT-PC increases, whereas the binding of BSA is prevented. Notably, although poly(EDOT-*co*-EDOT-PC) of 50% EDOT-PC feed ratio could successfully prevent most of the nonspecific adsorption of BSA, CRP appeared to not significantly interact with the surface. This result illustrates that BSA blocking is not the main issue to prevent CRP binding and indicates that a minimum density of PC groups is required to induce efficient recognition between CRP and immobilized PC groups. The dissipation readout of third overtone is provided in Figure S3 for reference.

As mentioned in Figure 2, we observed an unusual dissipation readout during the binding of CRP on immobilized PC. The third overtone of frequency and dissipation in Figure 2 was solely shown in Figure 5A. During the binding of 4 μg/mL CRP, the dissipation readout dropped spontaneously with the frequency readout. In Figure 5B,C,D, we illustrate both the frequency and dissipation readouts during the binding of 1 mg/mL BSA, lysozyme (LYZ), and fibrinogen (FNG) on immobilized PC, respectively. The frequency drop caused by nonspecific adsorption is summarized in Figure S4 in Supporting Information. Different from the CRP binding, all dissipation readouts increased during the nonspecific binding of BSA, LYZ, and FNG. The dissipation represents the decay rate of quartz oscillations. Higher dissipation indicates faster energy losses during the oscillation. It usually occurs when large or soft molecules adsorbed on the quartz lead to a higher impedance to maintain the quartz oscillations. The adsorption of BSA, LYZ, and FNG on QCM chips presented a similar tendency, which was observed on PPy.[47] In addition, we presented the QCM-D measurement which is CRP adsorption on Au surface in Figure S5. CRP adsorption caused the frequency readout decreasing about 15 Hz and the dissipation show slightly change. However, the dissipation decreased when CRP was adsorbed on the immobilized PC groups. The dissipation change due to CRP binding is significantly different, shown in Figure 5E, from what is usually observed when the proteins are nonspecifically adsorbed on PEDOT films. Previous studies have shown that the reduction of the dissipation might be

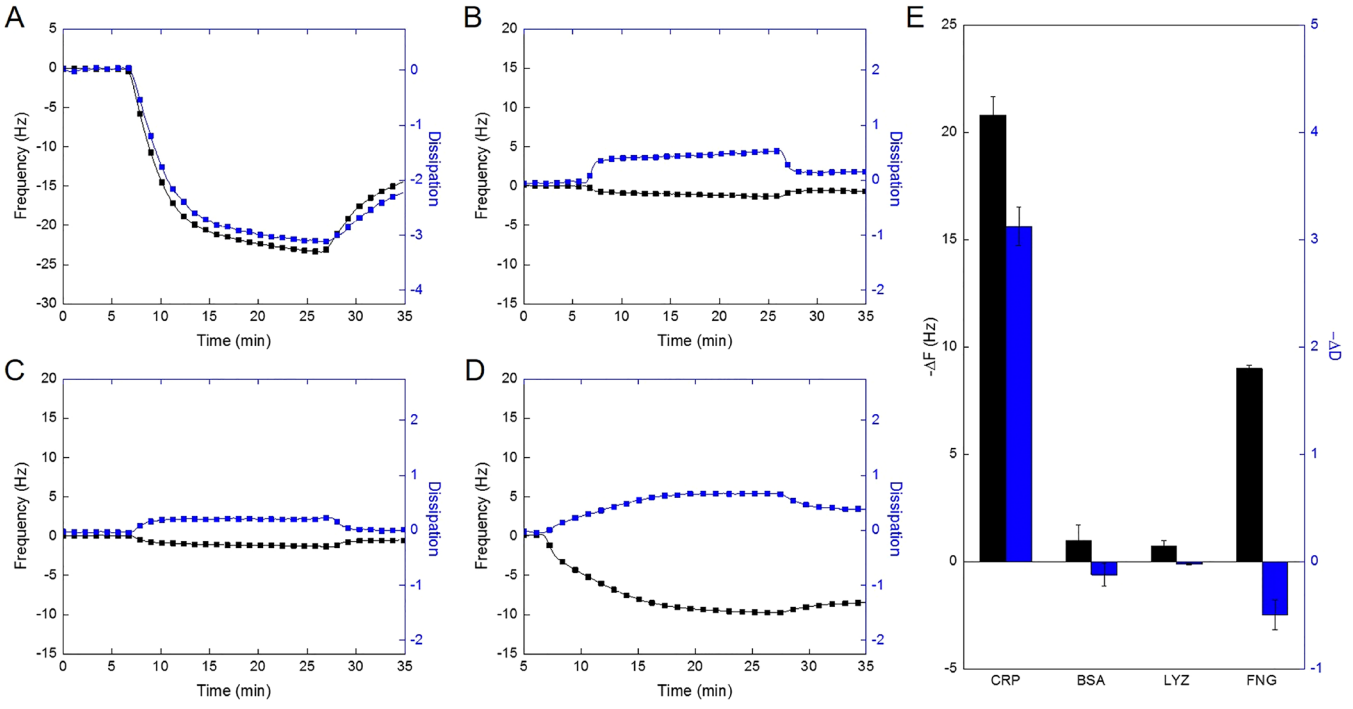

Figure 5. Frequency and dissipation readouts measured by QCM-D showing the results from (A) 4 μg/mL CRP adsorbed on poly(EDOT-PC), which is different from nonspecific protein of (B) 1 mg/mL BSA, (C) 1 mg/mL LYZ, and (D) 1 mg/mL FNG adsorbed on poly(EDOT-PC). (E) Summarized statistic results of the frequency and dissipation change. When CRP adsorbed on poly(EDOT-PC), the frequency and dissipation readout decreased simultaneously. Nonspecific protein binding shows decreasing frequencies but increasing dissipation.

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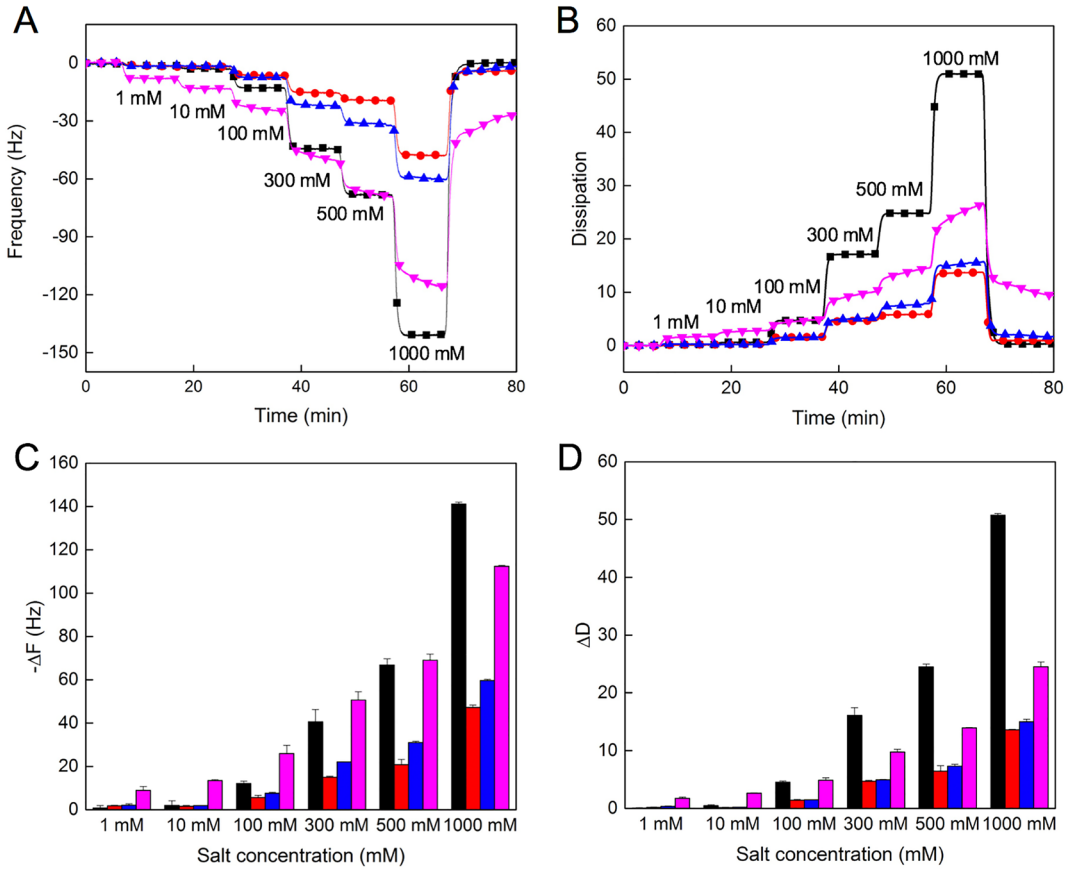

Figure 6. Relationship between (A) frequency versus time, (B) dissipation versus time measured by QCM-D in DI water in the presence of Na_2SO_4 (black), NaCl (red), NaBr (blue), and NaI (pink). (C) Statistical data of (A) and (D) the statistical data of (B).

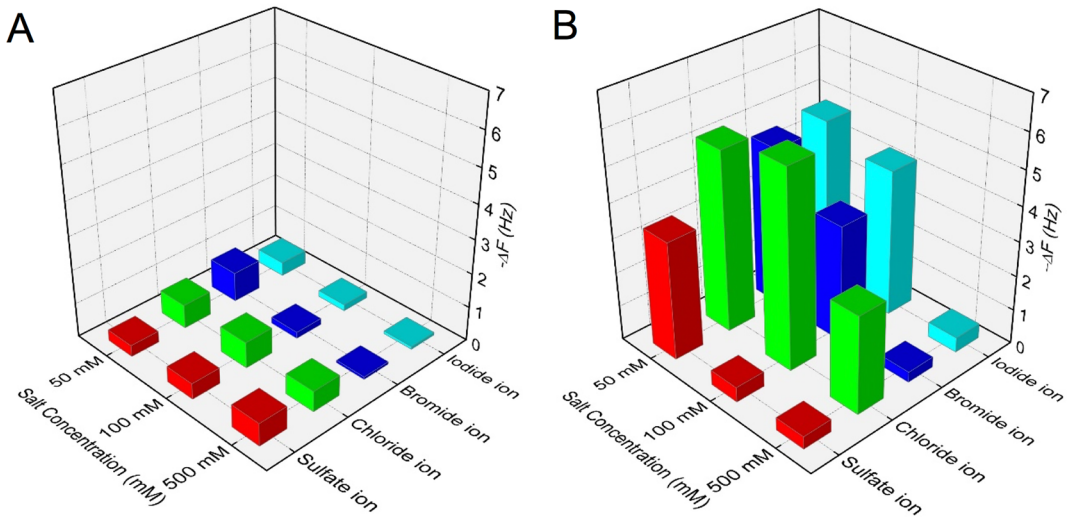

Figure 7. Changing of frequency readout of protein binding on poly(EDOT-PC) in HEPES buffer containing different anions (SO_4^{2-}, Cl^-, I^-, Br^-) at various concentrations (50, 100, 500 mM): (A) Minimum BSA binding indicating nonfouling properties from poly(EDOT-PC) substrates; (B) CRP binding depending on the anions and their concentrations. The protein concentration of BSA and CRP solution are 1 mg/mL and 1 μg/mL.

caused by the removal of water molecules.[48] Therefore, we believe this is mainly due to the release of water molecules in the vicinity of the PC groups when CRP binds to the groups. PC groups initially capture a thin layer of water molecules on the surface to prevent nonspecific adsorption,[12] which leads to a high dissipation from the surfaces. The adsorption of CRP released the water molecules from the surface, leading to a lower dissipation. Further research is required to fully understand the mechanism of water release upon CRP binding.

Ion Effect on CRP Binding to Immobilized PC. We selected four anions, namely, SO_4^{2-}, Cl^-, Br^-, and I^- ions, to investigate how ions influence the binding of CRP to immobilized PC groups. Based on the Hofmeister series, the SO_4^{2-} ions belong to the kosmotropes (order maker) and I^-

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ions belong to chaotropes (disorder maker). We first tested how poly(EDOT-PC) films responded to these ions by simply monitoring the frequency and dissipation readouts when these ions were introduced into the QCM-D system at different concentrations (Figure 6). In general, these ions were adsorbed on the poly(EDOT-PC) films, and the adsorption was proportional to the concentration of ions in solutions (Figure 6A). The adsorption of these ions is reversible except for I^- ions. When the ions were adsorbed on the poly(EDOT-PC) films, the dissipation also increased, indicating that the adsorbed ions on surfaces made the poly(EDOT-PC) films dissipate energy easily during oscillations, as illustrated in Figure 6B. A previous study demonstrated that the adsorption of SO_4^{2-} ions on PC-based polymer brushes induced lower dissipation,[48] but this was not observed in our platform. We believe that the differences originate from different surface properties between immobilized PC groups and PC-based polymer brushes. In addition, the statistical data for Figure 6A,B are shown in Figure 6C,D, respectively.

The ion effects on both the nonspecific adsorption of BSA and the specific recognition of CRPs on immobilized PC groups are summarized in Figure 7 and Table 1. Four anion solutions were prepared in HEPES buffer at three different concentrations (50, 100, 500 mM). The protein concentration of BSA and CRP solution are 1 mg/mL and 1 μg/mL. As shown in Figure 7A, poly(EDOT-PC) showed minimal BSA adsorption under most conditions, indicating preserved non-fouling properties. However, specific CRP adsorption showed significant variations, indicating that both the type and the concentration of the anions were critical for the recognition (Figure 7b). In general, the specific binding of CRPs decreased as the salt concentration increased. This could be mainly due to the interference of anions adsorbed on poly(EDOT-PC), as shown in Figure 6. For SO_4^{2-} ions, the binding was suppressed for SO_4^{2-} concentrations higher than 100 mM. We believe that this is mainly due to the formation of $CaSO_4$, which has a solubility product (K_{sp}) of 4.93×10^{-5}. Because the buffer contained 1 mM Ca^{2+} ions, $CaSO_4$ might precipitate at a SO_4^{2-} concentration exceeding 50 mM. Upon $CaSO_4$ formation, the concentration of Ca^{2+} decreased in solutions and led to less CRP adsorption. However, Br^- and I^- suppressed CRP binding more efficiently as the concentration increased, compared with Cl^-. Based on the Hofmeister series,[49] Br^- and I^- ions could cause protein denaturation, which leads to a weaker CRP binding to immobilized PC groups. This could provide an explanation as to why Br^- and I^- suppressed CRP binding more efficiently than did Cl^-.

Table 1. Frequency Drop (n = 3) (Hz) Caused by CRP and BSA Adsorption on PC-Immobilized Surface in the Presence of Different Salt and Salt Concentration (± standard deviation)

CRP	sulfate ion	chloride ion	bromide ion	iodide ion
50 mM	3.43 ± 0.54	5.26 ± 0.39	4.64 ± 0.71	4.78 ± 0.62
100 mM	0.42 ± 0.11	5.74 ± 0.68	3.40 ± 0.23	4.25 ± 0.68
500 mM	0.36 ± 0.09	2.83 ± 0.62	0.30 ± 0.05	0.43 ± 0.09
BSA	sulfate ion	chloride ion	bromide ion	iodide ion
50 mM	0.34 ± 0.06	0.71 ± 0.10	0.87 ± 0.15	0.41 ± 0.08
100 mM	0.47 ± 0.10	0.76 ± 0.08	0.18 ± 0.03	0.18 ± 0.05
500 mM	0.66 ± 0.15	0.60 ± 0.04	0.07 ± 0.02	0.08 ± 0.03

CONCLUSIONS

In summary, we successfully used QCM-D to monitor the binding between CRPs and immobilized PC groups and provide insight into the recognition event. The immobilized PC groups were created by directly electropolymerizing EDOT-PC monomers to form poly(EDOT-PC) thin films on QCM chips. When buffer solutions contained Ca^{2+}, CRP could adsorb on the substrate. The density of PC groups is critical for the binding efficiency. Furthermore, the poly(EDOT-PC) not only prevents the nonspecific binding of BSA, LYZ, and FNG but also specifically captures CRP without CRP antibody. Notably, the dissipation also dropped during the binding process. We believe that this is mainly due to the release of water molecules in the vicinity of PC groups during CRP adsorption. We also examined the influence of ions on the binding process by introducing four different anions into the CRP binding buffer. Based on the data of QCM-D measurement, we observed a decrease in the amount of CRP binding on the PC-immobilized surface while the concentration of anions increased. Two potential mechanisms could be used to explain this phenomenon. First, the ions adsorbed on the poly(EDOT-PC) films could interfere with the recognition between CRPs and immobilized PC groups. On the other hand, the anions in buffer might change the conformation of protein and restrict the CRP binding on PC-immobilized surfaces. Especially, the SO_4^{2-} ions affected the CRP binding at even low concentration because the formation of $CaSO_4$ reduced the concentration of Ca^{2+} ions, which leads to low CRP binding. We believe that the present work provides important insights to understand the recognition process between CRPs and surface-immobilized PC groups for the development of antibody-free CRP biosensors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b02724.

SEM and AFM images showing surfaces of PEDOT, poly(EDOT-PC), and copolymer thin films; the cross section of poly(EDOT-PC) thin film monitored by AFM; and the comparison of protein binding between the poly(EDOT-PC) and PEDOT thin films (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

EDOT, 3,4-ethylenedioxythiophene; PPy, polypyrrole; PC, phosphorylcholine; QCM-D, quartz crystal microbalance with dissipation; CRP, C-reactive protein; BSA, bovine serum albumin; LYZ, lysozyme; FNG, fibrinogen; SEM, scanning electron microscope; AFM, atomic force microscope

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