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Megan L. O'Grad, and Kevin Kit Parker

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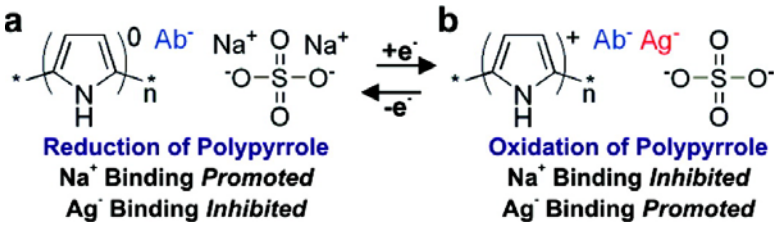

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Dynamic Control of Protein−Protein Interactions

Megan L. O'Grady and Kevin Kit Parker*

School of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts 02138

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The capability to selectively and reversibly control protein−protein interactions in antibody-doped polypyrrole (PPy) was accomplished by changing the voltage applied to the polymer. Polypyrrole was doped with sulfate polyanions and monoclonal anti-human fibronectin antibodies (αFN). The ability to toggle the binding and dissociation of fibronectin (FN) to αFN-doped polypyrrole was demonstrated. Staircase potential electrochemical impedance spectroscopy (SPEIS) was performed to characterize the impedance and charge transfer characteristics of the αFN-doped PPy as a function of applied voltage, frequency, and FN concentration. Impedance measurements indicated oxidation of αFN-doped PPy promoted selective binding of FN to αFN antibodies and reduction of the polymer films facilitated FN dissociation. Moreover, SPEIS measurements suggested that the apparent reversibility of antigen binding to antibody-doped PPy is not due to the suppression of hydrophobic binding forces between antibody and antigen. Instead, our data indicate that reversible antigen binding to antibody-doped PPy can be attributed to the minimization of charge in the polymer films during oxidation and reduction. Furthermore, αFN-doped PPy was utilized to collect real-time, dynamic measurements of varying FN concentrations in solution by repeatedly binding and releasing FN. Our data demonstrate that antibody-doped PPy represents an electrically controllable sensing platform which can be exploited to collect rapid, repeated measurements of protein concentrations with molecular specificity.

Introduction

Affinity sensors based on conductive polymer films have become an important class of bioanalytical tools, mainly due to their potential applications in diagnostics, DNA genotyping, and toxin detection. Polypyrrole (PPy) is one of the most extensively used conducting polymers utilized in biosensor technologies, namely due to its excellent environmental stability,[1] mechanical properties,[2] high conductivity,[3] biocompatibility,[4] and ease of preparation in nontoxic, aqueous solutions.[5] Recent studies[6,7] have indicated that the quality of the interface at which molecular recognition occurs is of extreme importance. Toward this end, there have been significant efforts to immobilize highly concentrated probe molecules such as DNA,[8−10] enzymes,[11,12] antibodies,[13−16] or small molecules[17,18] in a PPy matrix while retaining biological activity. Strategies to improve biomolecule immobilization include entrapment of probe molecules,[8−10,13,16,19] synthesis of new pyrrole compounds,[17,20] adsorption,[21,22] chemical grafting,[14,23−27] and attachment by affinity interactions.[28,29]

Although numerous studies have investigated PPy functionalization strategies, less attention has been focused on exploiting the electrochemical properties of PPy to control biomolecular interactions. Electrochemical sensors that reversibly mediate antibody−antigen interactions were first developed in the early 1990s[30−33] with the goal of dynamically controlling antibody−

* Corresponding author: 29 Oxford Street, Pierce Hall, Room 322A, Cambridge, MA 02138. Phone: (617) 495-2850. E-mail: kkparker@deas.harvard.edu.

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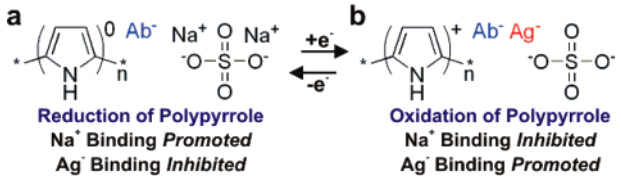

Figure 1. Oxidation and reduction of PPy doped with negatively charged antibodies (Ab^-) and sulfate polyions (SO_4^{2-}) in a physiological salt buffer. During reduction of PPy (a) negative charges in the polymer are neutralized via interactions with Na^+ ions. Conversely, during oxidation of PPy (b) binding of negatively charged antigens (Ag^-) is facilitated such that the polymer remains in a charge neutral state.

antigen binding affinities. These sensors were fabricated by depositing PPy in the presence of antibodies, such that the antibodies became entrapped in the polymer matrix. The repetition of the current traces over more than 10 injections of antigen indicated binding to the antibody-doped polypyrrole was reversible.[31] Based on this technology, PPy sensors that reversibly modulate biomolecule−receptor interactions have been developed for human serum albumin,[30] *p*-cresol−BSA conjugates,[32] thaumatin,[33] oligonucleotides,[34] rabbit IgG antigen,[35] and bovine serum albumin.[36]

Despite over a decade of development, the mechanisms underlying the apparent reversibility of antibody−antigen interactions in PPy remain to be fully elucidated. Previous studies have indicated that application of a positive potential to the PPy−electrolyte interface oxidizes the polymer and facilitates binding of negatively charged antigens to the antibodies entrapped in the PPy matrix. Conversely, application of a negative potential reduces the PPy and inhibits antibody−antigen interactions at the PPy−electrolyte interface.[35,37] Past studies[35] have attributed the apparent reversibility of antigen binding in antibody-doped PPy to the minimization of strong binding interactions, by quickly switching between binding and dissociation states at 200 ms. However, in previous reports, antibody−antigen interactions were not characterized as a function of applied potential, frequency, and antigen concentration.[30−36] Furthermore, the ability of antibody-doped PPy to mediate specific antibody−antigen interactions has yet to be demonstrated with positive and negative controls.[35−37]

We hypothesize that the antibody-doped PPy is able to selectively and reversibly modulate antibody−antigen interactions by maintaining a charge neutral state in the PPy film. PPy substrates designed to mediate antibody−antigen interactions utilize the ability of PPy to interact with counterions and biomolecules to minimize free charges in the polymer matrix.[31,35,38−40] In a physiological saline solution, application of a negative potential to PPy films causes Na^+ ions to neutralize negative charges present in the polymer (Figure 1a). Consequently, interactions between negatively charged antibodies and negatively charged antigens are inhibited during reduction since they impede the ability of the polymer to maintain a charge neutral state.[35,41−43] Conversely, during application of a positive potential to the PPy films (oxidation), the PPy films promote the addition of negative charges to approach a charge neutral state (Figure 1b). As a result of the addition of negative charges, negatively charged antibody−antigen interactions occur at the PPy surface during oxidation.[5,35,41−43] Antibodies entrapped in the PPy matrix act as anions, but because of their large size, they cannot move to balance PPy surface charges.[35] Previous studies of sulfate-doped PPy films indicate that Cl^- diffusion into the polymer is not a significant effect in NaCl solutions, and Cl^- will not displace the sulfate polyanions in the PPy.[41] Together, these studies suggest that antibody−antigen interactions can be reversibly modulated in PPy films by changing the voltage applied to the polymer.

Here we report the ability to selectively and reversibly mediate protein−protein interactions by exploiting the propensity of antibody-doped PPy to approach a charge neutral state during oxidation and reduction. This was accomplished by investigating antigen binding to antibody-doped PPy as a function of frequency, applied voltage, and antigen concentration, which have not been previously studied in combination. Impedance measurements suggest that the apparent reversibility of antibody-doped PPy is not due to the suppression of strong hydrophobic binding forces, as was formerly suggestd,[35] but rather due to the ability of PPy to approach a charge neutral state during polymer oxidation and reduction.

Experimental Section

Solution Preparation. Human fibronectin (FN) (BD Biosciences, Franklin Lakes, NJ) and anti-fibronectin (αFN) (Developmental Studies Hybridoma Bank, University of Iowa, IA) were used as the antigen and antibody of interest. The αFN antibody is directed against the flexible linker between the ninth and tenth type III repeat of human FN.[44−46] The isoelectric point (pI) of the antibody was verified using 2D electrophoresis with an immobilized pH gradient (IPG) strip (GE Healthcare, Piscataway, NJ). Results from multiple 2D gels indicated the light chain of the αFN antibody (molecular weight 25 kDa) had a pI of 4.9−5.9 and the heavy chain of the αFN antibody (molecular weight 50−75 kDa) had a pI of 5.7−7.0. Therefore, the αFN antibody is negatively charged in solutions with pH 7.40−7.50 since these solutions are above the pI of the αFN antibody heavy and light chains. Pyrrole monomer was purchased from Aldrich Chemical Co. (St. Louis, MO). BSA was purchased from Jackson ImmunoResearch (West Grove, PA). The FN, αFN, bovine serum albumin, and pyrrole were stored at 4 °C until use. Analytical reagent-grade Na_2SO_4, NaCl, KCl, $CaCl_2$, $MgCl_2$, NaH_2PO_4, HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], and glucose were acquired from Aldrich Chemical Co. (St. Louis, MO). A normal Tyrode's (NT) solution was prepared with (in mmol/L) 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 0.33 NaH_2PO_4, 5 HEPES, and 5 glucose. The pH of the NT solution was equilibrated to 7.40 at 37 °C with the addition of NaOH or HCl to remain consistent with previous cell and tissue electrophysiology studies. The pH of the NT solution was 7.49 at 19 °C after equilibration. All solutions were used without purification and were mixed in deionized (18 Ω/cm) water (Millipore, Billerica, MA) at 19 °C.

Apparatus. A Versatile Modular Potentiostat (Princeton Applied Research, Oak Ridge, TN) was used for electropolymerization, impedance, and potentiostatic measurements. The working electrode used for macroscale electrochemical experiments was a 0.25 mm diameter 99.95% gold wire (Alfa Aesar, Ward Hill, MA). The reference electrode was an Ag/AgCl saturated KCl electrode (Cypress Systems, Lawrence, KS) and a 1.0 mm diameter 99.997% platinum wire (Alfa Aesar, Ward Hill, MA) counter electrode. All applied voltages are given versus the Ag/AgCl reference electrode.

Electrode Fabrication. Gold wires were cleaned prior to use by washing with deionized (18 Ω/cm) water followed by sonication for

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2 min in deionized (18 Ω/cm) water. Pyrrole was electropolymerized galvanostatically on the gold wire to form polypyrrole (PPy) from a solution of 0.1 M pyrrole dissolved in 0.01 M Na_2SO_4 and was calibrated to pH 7.40 before the addition of antibodies. The pH measurements were carried out using a SympHony pH meter (VWR, West Chester, PA) after a two-point 7.00 and 10.00 pH calibration. To create αFN-doped PPy films, αFN was included in the electropolymerization solution at a concentration of 200 μg/mL for SPEIS experiments and 360 μg/mL for dose response experiments. Current densities between 1.25 and 2.5 mA/cm^2 for a surface area of ∼0.08 cm^2 were employed for up to 15 min versus Ag/AgCl to polymerize the PPy. Oxygen was not removed from the solution during polymerization. After electropolymerization the functionalized electrode was rinsed with deionized water to remove excess pyrrole monomer. Electrodes were conditioned in NT solution containing 1% BSA for more than 20 h at 4 °C prior to an experiment. The αFN concentrations in solution following electropolymerization were quantified using a SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA) following antibody staining with Bio-Rad Protein Assay concentrate (Bio-Rad, Hercules, CA). This step was performed to estimate the αFN concentration entrapped in the PPy.

Impedance Analysis. Staircase potential electrochemical impedance spectroscopy (SPEIS) measurements were conducted within the frequency range of 100 kHz to 100 mHz at a voltage amplitude of 20 mV (rms). At each frequency, impedance measurements were collected five times and the average impedance of those five measurements was recorded. Impedance spectra were recorded between −600 mV (vs Ag/AgCl) and +700 mV (vs Ag/AgCl) with a voltage step of 87−100 mV. Experiments were conducted in NT solution at 19 °C. For various experiments, 250 μg/mL FN or 250 μg/mL BSA were present in the NT solution. To determine the impedance response of αFN-doped PPy as a function of FN concentration, experiments were conducted in a NT solution which contained 0, 0.25, 2.5, 25 125, or 250 μg/mL FN. Impedance data were analyzed at +700 mV (vs Ag/AgCl) since SPEIS experiments indicated that FN binding was highly facilitated at this voltage.

Chronoamperometry. Chronoamperometry (constant voltage) was used to assess the PPy response to various FN concentrations. The αFN-doped PPy electrode was held at +650 mV (vs Ag/AgCl) for 5 s and subsequently held at −550 mV (vs Ag/AgCl) for 95 s. This procedure was repeated 2−3 times, and the PPy was subsequently held at +350 mV (vs Ag/AgCl) for 15 s to facilitate the relaxation of the electrochemical cell. These applied voltages were selected based on SPEIS results, as well as previous studies of antigen binding in PPy films.[35−37] Between successive measurements, the system was permitted to equilibrate for an extended period of time and perfused in NT. The current in the PPy was averaged over the last 200 ms of each 5 s oxidizing pulse. Fibronectin concentrations of 0, 0.025, 0.125, 0.25, 1.25, 2.5, 12.5, 25, 125, and 250 μg/mL in NT were examined.

Prediction of FN Concentration. To fit the current vs FN concentration data to a dose response curve, equilibrium binding was assumed using the Hill equation. This equation is commonly used in pharmaceutical drug efficacy assays, where the output represents the fractional response to addition of an agonist as compared to when no agonist is added. It is important to note that this mathematical formulation cannot predict the PPy current response from first principles, but rather characterizes relative changes in the PPy current response as a function of antigen concentration. Dose response fit using a Michaelis−Menten mathematical formulation is not optimal for this affinity sensor because we did not measure any kinetic parameters of antigen binding and dissociation. A quasi-steady-state assumption was made since only the last 200 ms of a 5 s pulse was examined, at which time the rate of FN−αFN complex formation should be nearly constant.[47] A four-parameter, sigmoidal dose−response function assuming one-site competition of a ligand−receptor binding[47] was used to fit the measured αFN−PPy current response,

$$F = F_{\min} + \frac{F_{\max} - F_{\min}}{1 + \left(\frac{X}{EC_{50}}\right)^{HillSlope}} \quad (1)$$

where F is the fraction αFN-doped PPy current response as compared to the PPy current response when all the binding sites are saturated (250 μg/mL of FN present in solution), F_{min} is the fractional response when all the binding sites are saturated (250 μg/mL of FN present in solution), F_{max} is the fractional response when no FN is present in solution, X is the FN concentration, EC_{50} is the FN concentration when the PPy current response is halfway between F_{min} and F_{max}, and HillSlope is the slope of the dose−response curve. The fractional values of the PPy current response were normalized to the current response when 250 μg/mL of FN was present in solution. This assumption was made since there was no significant change in the PPy current response when 125 and 250 μg/mL of FN were present in solution, indicating the αFN binding sites were saturated. The parameter F_{max} represents the fractional PPy current response when no FN was present in solution relative to the PPy current response when 250 μg/mL of FN was present in solution. The constants EC_{50} and HillSlope were approximated using a nonlinear least-squares fit using Matlab curve-fitting toolbox (MathWorks, Natick, MA).

Results and Discussion

Control of Protein Binding with PPy Electrodes. To test our hypothesis that antibody-doped PPy is able to selectively and reversibly modulate antibody−antigen interactions by maintaining a charge neutral state in the PPy film, we examined the ability of αFN-doped PPy films to bind FN in a NT solution. When FN was present in solution, a significant increase in the PPy impedance occurred at frequencies less than 100 Hz as a result of the slow adsorption of FN to αFN antibodies entrapped in the PPy matrix. Previous impedance spectroscopy studies have shown that antibody−antigen complex formation in antibody-doped PPy leads to significant increases in impedance at low frequencies,[36,37,48,49] although these studies did not address the apparent reversibility of antibody-doped PPy. As shown in Figure 2, impedance measurements indicated that FN adsorption increases the impedance of the polymer in a dose-dependent manner. This effect is particularly notable at frequencies greater than 2 Hz, when mass transfer effects due to protein adsorption are significant. Although diffusion of ions into the PPy also occurs over these slow time scales, the results shown in Figure 2 demonstrate that the increase in the PPy impedance due to ion diffusion is much smaller than the increase in PPy impedance due to FN adsorption. When no FN is present in solution (green line − 0 μg/mL), only ion transfer occurs at the polypyrrole−electrolyte interface. Yet the polymer impedance at 0.1−1.0 Hz is 1 order of magnitude higher when 2.5 μg/mL of FN is present in solution, and 2 orders of magnitude higher when 250 μg/mL of FN is present in solution. Therefore, the drastic increases in polymer impedance can be attributed to protein binding at the polymer surface.

Furthermore, FN binding increased the charge transfer resistance of the polymer and minimally altered its double layer capacitance (Figure S1, Supporting Information). Thus, increases in impedance during FN binding represented morphological changes in the polymer structure, rather than changes in ionic concentrations at the polymer surface. Grant et al.[36] and Sadik and Xu[50] demonstrated that in antibody-doped PPy films, the

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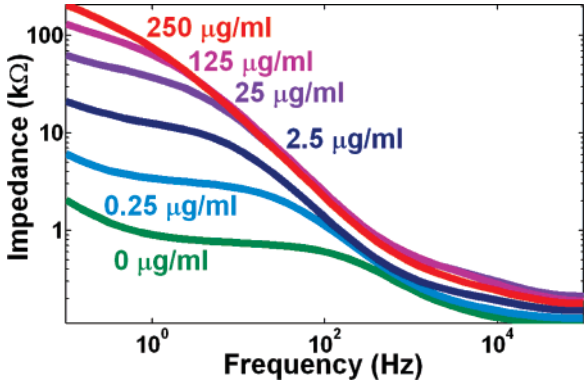

Figure 2. αFN-doped PPy impedance at +700 mV (vs Ag/AgCl) as a function of FN concentration. When FN−αFN binding is facilitated at +700 mV (vs Ag/AgCl), the impedance of the polymer is highly dependent on FN concentration at frequencies less than 2 Hz. When no FN is present in solution (green line, 0 μg/mL), only ion transfer occurs at the polypyrrole−electrolyte interface. The polymer impedance at 0.1−1.0 Hz is 2 orders of magnitude higher when 250 μg/mL of FN is present in solution. Therefore, the significant increases in polymer impedance are due to protein binding at the polymer surface.

PPy charge-transfer resistance increased as a function of the antigen concentration in solution, which is consistent with our results. These data demonstrate that the formation of FN−αFN complexes occurs over slow time scales (>500 ms) and increases the PPy impedance in a dose-dependent manner by hindering charge transport in the polymer films.

Reversible Protein Binding by Charge Minimization. Although electrochemical impedance spectroscopy (EIS) is a powerful technique to examine biomolecular and electrochemical interactions,[36,50−53] this technique generally requires that the dc voltage must be held constant while an impedance spectra is recorded. The unique ability of SPEIS to collect impedance spectra at multiple dc voltages provides the framework for comparing dc electrochemical data (voltammograms) with kinetic parameters (charge transfer resistance, double layer capacitance, rate constants) that can be measured with EIS. Consequently, SPEIS measurements are sensitive to the magnitude of dc voltage, the direction in which the voltage is varied during a potentiodynamic experiment, and the applied ac frequency. In particular, SPEIS is a valuable technique to distinguish between adsorption and desorption at an electrode surface[54−56] since SPEIS measurements are sensitive to the direction of applied voltage. Because the extent of electrochemical adsorption/desorption often depends on the direction of applied voltage,[35,55] and electrochemical adsorption kinetics are generally measured using EIS techniques,[51] SPEIS is particularly well-suited for electrochemical adsorption/desorption studies. However, this capability of SPEIS has yet to be exploited in investigating protein binding and release kinetics.

To demonstrate that αFN-doped PPy films undergo reversible oxidation and reduction in a physiological salt solution, current vs applied voltage data were collected during SPEIS experiments. Figure 3a displays the current−voltage characteristics of a typical αFN-doped PPy electrode in a NT solution, as well as NT solutions with 250 μg/mL FN and 250 μg/mL bovine serum albumin (BSA). Since BSA has the ability to nonspecifically bind to proteins and substrates, it was utilized to verify the molecular specificity of αFN-doped PPy. As shown in Figure 3a, application of negative potentials to αFN-doped PPy reduced the PPy films, while application of positive potentials oxidized the PPy films.

Although the PPy response displayed a noticeable hysteresis when the polymer was oxidized and subsequently reduced, the polymer response at −514 mV (vs Ag/AgCl) after oxidation closely resembled the response at −514 mV (vs Ag/AgCl) preceding oxidation. These data indicate that αFN-doped PPy films undergo reversible oxidation and reduction in a NT solution.

Binding of FN increased the impedance of the αFN-doped polypyrrole by approximately an order of magnitude at low frequencies where adsorption effects are dominant (Figure 3b). However, in solutions containing BSA only, no such increases in impedance were observed. This result demonstrated that the polymer did not facilitate BSA binding or adsorption. Similar experiments were conducted with a 25% fetal bovine serum (FBS) solution in NT which contained bovine FN. Nonspecific binding to the polymer was not observed in the 25% FBS/NT solution, further signifying that PPy doped with monoclonal human anti-FN antibodies permits binding of human FN with molecular specificity (Figure S2, Supporting Information). Moreover, human FN and BSA did not bind or adsorb to undoped polypyrrole films, demonstrating that the molecular specificity of the polymer films is critically dependent on the entrapment of αFN antibodies (Figure S3, Supporting Information).

The extent of FN binding could be controlled by changing the voltage applied to the αFN-doped PPy. Specifically, when FN was present in solution (Figure 3b), slight changes in applied voltages at which FN−αFN interactions occurred (+400−700 mV, vs Ag/AgCl) led to appreciable differences in polymer impedance at frequencies where mass transfer effects are significant. These differences in αFN-doped PPy impedance correspond to differences in FN adsorption (Figure 2). The increase in polymer impedance at low frequencies was not as significant in NT and NT with 250 μg/mL BSA, indicating that αFN-doped PPy was exchanging ions with these electrolytes via slow (>200 ms) diffusion and migration processes.

In addition, binding of FN to αFN-doped PPy is selective and reversible. The decreases in αFN-doped PPy impedance in the presence of FN (Figure 3c) can be attributed to FN dissociation from αFN. If FN remained permanently bound to the polymer, the impedance would not decrease significantly upon slight reduction of the polymer. Furthermore, when only ion transfer interactions occurred at the PPy/NT interface (Figure 3b), the total impedance of the PPy increased upon slight reduction (black arrow). Previous reports suggest that the increase in impedance is due to diffusion of Na^+ ions into the polymer matrix to neutralize any negative charges.[41−43] The decrease in PPy impedance when FN was present in solution is due to the release of negative charges from the PPy instead of the addition of positive (Na^+) charges to maintain a charge neutral state.[37,48,50] Measurements of the isoelectric point of the αFN antibody verified that the antibody is negatively charged in solutions with pH 7.40−7.50. Therefore, the decrease in αFN-immobilized PPy impedance when FN was present in solution (Figure 3b) can be attributed to the dissociation of negatively charged FN from αFN, as presented in Figure 1.

The αFN-doped PPy also did not undergo significant degradation when FN was bound and subsequently released, indicating oxidation and reduction of the polymer is a reversible process. Figure 3d displays a comparison of the PPy impedance

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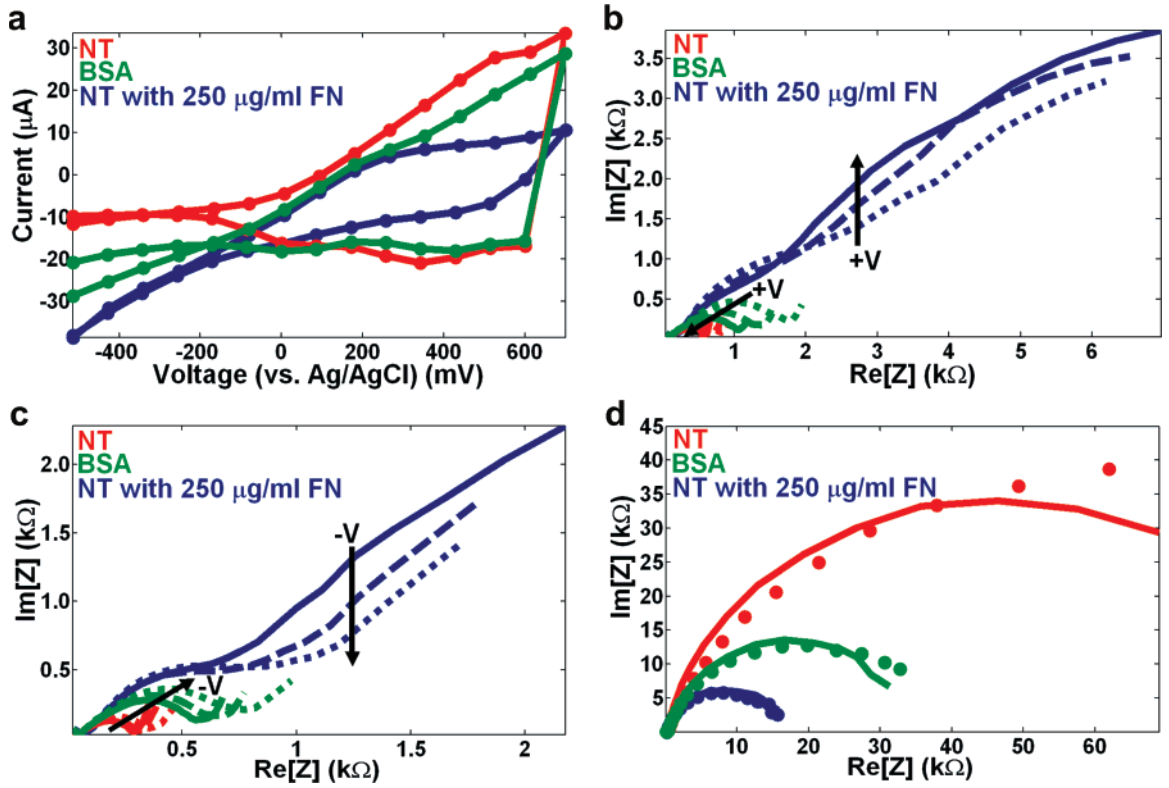

Figure 3. Reversible binding of FN to αFN-doped PPy. (a) Oxidation and reduction of αFN-doped PPy in a NT physiological buffer. (b,c) Nyquist plots display the PPy impedance for applied potentials greater than +400 mV during both the forward sweep (reduction to oxidation in b) and the backward sweep (oxidation to reduction in c). Data from the forward sweep (b) indicate that adsorption of FN increases the impedance of the PPy by approximately an order of magnitude (blue lines). Dotted lines in (b) indicate application of +527 mV vs Ag/AgCl, dashed lines indicate +613 mV vs Ag/AgCl, and solid lines indicate +700 mV vs Ag/AgCl. Panel (c) illustrates that αFN-doped PPy allows FN to bind in a reversible manner with molecular specificity. Dotted lines in (c) indicate application of +427 mV vs Ag/ AgCl, dashed lines indicate +514 mV vs Ag/AgCl, and solid lines indicate +600 mV vs Ag/AgCl. Finally, (d) is a comparison of the PPy impedance at −514 mV (vs Ag/ AgCl) before (lines) and after (circles) polymer oxidation, which demonstrates that oxidizing and subsequently reducing the polymer does not significantly affect the overall PPy impedance.

during reduction, both before (forward sweep, lines) and after (reverse sweep, circles) the polymer was oxidized. If considerable morphological changes and corrosion occurred within the polymer during these redox reactions and FN adsorption, we would expect noticeable differences in impedance before and after oxidation. However, the similarity in polymer impedance before and after oxidation attests to the reversibility of FN binding to αFN-doped PPy.

Sensor Applications: Dose Response of Antibody-Doped PPy. To test the dose response characteristics of αFN-doped PPy, the polymer was switched between oxidized and reduced states in the presence of varying FN concentrations. Oxidation of the αFN-doped PPy was performed for only 5 s to prevent overoxidation of the polymer, and the subsequent reduction of the polymer lasted 95 s such that the charge on the polymer would reach a quasi-steady state before re-oxidation. An example of a typical current response is shown in Figure 4, where application of the −550 mV (vs Ag/AgCl) pulses caused a sharp decrease in current, followed by return to a quasi-steady state after 15 s. The return to baseline current response in Figure 4 indicates the reversibility of the polymer, where only an average of 2.15 ± 1.22% (n = 12 redox cycles) difference was observed in the current response between successive oxidation cycles.

To determine the dose response of αFN-doped PPy, the current within the last 200 ms of the 5 s, +650 mV (vs Ag/AgCl) pulse was averaged (Figure 5a). Data of the PPy current response within the first 10 ms of polymer oxidation is included in the Supporting Information (Figure S4). The current was examined after 5 s of oxidation (red square in Figure 5a) since mass transfer effects

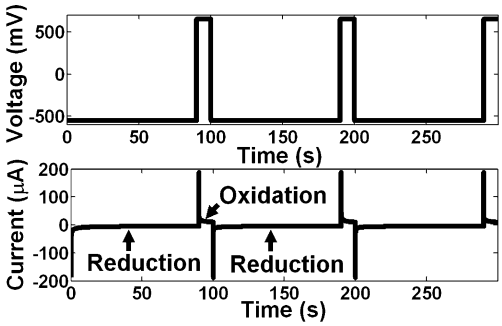

Figure 4. Typical current response of αFN-doped PPy during repeated oxidation and reduction in a NT solution.

due to FN adsorption should be predominant at this time scale, as indicated in Figures 2 and 3b. Consequently, only slow interactions were considered when constructing the dose−response curve, during which FN binding had enough time to occur. These data indicate that the charge transfer kinetics at the PPy−electrolyte interface (characterized in Figures 2 and 3) can be manipulated to control antibody−antigen binding in a selective and reversible manner. Moreover, the decrease in current response with increasing FN concentration (Figure 5a) is supported by the impedance results in Figure 2, where greater FN adsorption leads to greater increases in αFN-doped PPy impedance, and thus a decreased current response.

Finally, by measurement of the current response as a function of FN concentration, Figure 5b demonstrates that the antibody-doped PPy can be used as a rapid, re-useable immunosensor.

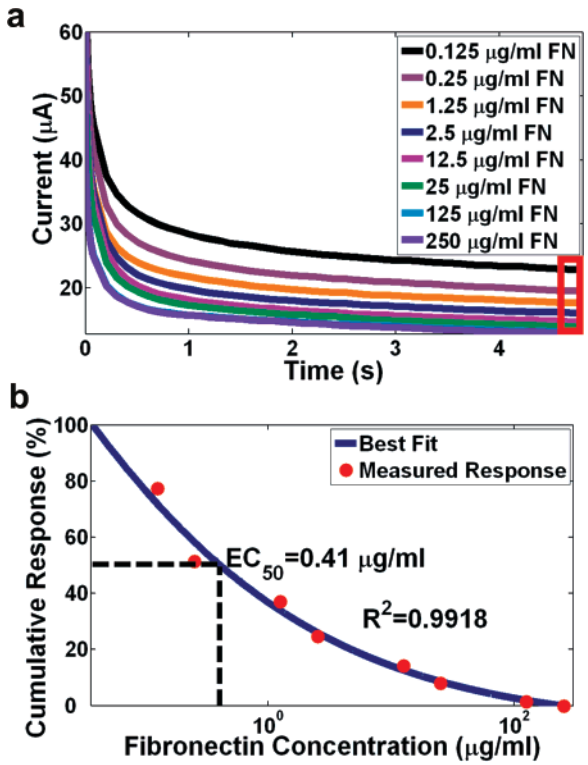

Figure 5. Response of αFN-doped PPy to various FN concentrations during oxidation (a). The rectangle indicates current values that were averaged to determine the PPy current response as a function of FN concentration. (b) Dose response of a αFN-doped PPy electrode. The data was fitted to a curve using a sigmoidal dose response equation, with an EC_{50} value of 0.41 μg/mL and a Hill coefficient of 0.33. These results indicate the αFN-doped PPy electrodes can be used to collect real-time, repeated measurements of FN concentrations in solution.

The fractional values of the PPy current response were normalized to the current response when 250 μg/mL of FN was present in solution since there was no significant change in the PPy current response when 125 and 250 μg/mL of FN were present in solution (Figure 5a). Sigmoidal fitting indicated the maximal sensitivity of the αFN-doped PPy to be 0.030 μg/mL, which was consistent with experimental observations of the PPy current response with 0.025 μg/mL of FN present in solution. No change in the PPy current response was observed when 0 and 0.025 μg/mL of FN was present in solution, indicating this low concentration of FN was not detected. The EC_{50} value indicates that the current response decreases to 50% of its maximal value when 0.41 μg/mL (0.9 μM) of FN is present in solution. This result suggests that the αFN-doped PPy can be used for real-time detection of protein concentrations between 300 nM and 500 μM.

Implications of Impedance Spectroscopy for Quantifying Protein−Protein Interactions. Previous studies[35] indicated that pulsing antibody-doped PPy between 400 and −200 mV (vs Ag/AgCl) would allow rabbit immunoglobulin (IgG) to reversibly bind to anti-rabbit IgG antibodies (αIgG). The extent of IgG−αIgG binding was modulated by applying positive (+400 mV vs Ag/AgCl) pulses for 200 ms to oxidize the PPy and facilitate IgG−αIgG binding. To explain the apparent reversibility of IgG binding to αIgG-doped PPy, Gooding et al.[35] suggest that the binding of an antigen with an antibody can be subdivided into primary and secondary reactions. The initial antibody−antigen recognition and binding are dominated by Coloumbic and van der Waals forces.[57,58] Although these electrostatic forces facilitate antibody−antigen interactions, their total energy constitutes a small fraction of the total binding energy. Secondary bonding forces, such as hydrogen bonding and hydrophobic forces, will contribute substantially to the final binding energy, but take much longer to establish.[57,59−61] The authors assert that by using 200 ms pulses, they are allowing the (primary) Coloumbic and van der Waals interactions to occur between IgG and αIgG, but not the secondary binding forces. By allowing only the primary IgG and αIgG interactions to occur, the authors hypothesize that the reversibility of the αIgG-doped PPy is due to the fact that the stronger secondary binding forces between IgG and αIgG are never present.

Our data, however, suggest that 200 ms is too short a time period for significant protein adsorption to occur in the PPy matrix. To detect notable changes in polymer impedance upon protein adsorption, particularly at high antigen concentrations, the time scale for adsorption to occur is 500 ms or more (Figure 2). At these low frequencies (2 Hz or less) the impedance of the PPy increased significantly, demonstrating that FN is binding to the αFN in the PPy. When FN is not binding to the PPy, no marked increases in impedance are observed at low frequencies (Figure 3b) since ion diffusion does not alter the polymer structure as significantly as antigen binding. In addition, FN can still bind and release from αFN-doped PPy when FN binding is facilitated for 5 s (Figures 3b and 3c), indicating that reversible protein adsorption can occur long after hydrophobic binding forces have been established. Increasing the hydrophobicity of the PPy with dodecylbenzene sulfonate (DBS), a hydrophobic surfactant, did not promote FN−αFN interactions (Figure S5, Supporting Information). Moreover, exchange of ions at the PPy−electrolyte interface occurred on much slower time scales when doped with DBS. This result can perhaps be attributed to the fact that the dislocation of π-bonds with a hydrophobic surfactant impeded the ability of the PPy to act as a polymer aggregate, leading to a loose array of pyrrole monomers that did not as readily promote ion exchange and protein−protein interactions. Overall, the results presented in this paper suggest that the reversibility of antigen binding is not due to the minimization of secondary antibody−antigen binding forces. Rather, the reversibility of antigen binding is due to the minimization of free charges in the PPy during oxidation and reduction. By interacting with Na^+ and FN at the PPy−electrolyte interface, the antibody-doped PPy is able to maintain charge neutrality by enhancing or impeding various electrochemical interactions.

The time scale of antigen dissociation may depend on the affinity constant of the antibody−antigen interaction, where a higher affinity constant leads to a longer dissociation time. If the affinity constant of the antibody−antigen interactions is low, mass transfer processes due to antigen binding and release will occur on a faster time scale, and vice versa for higher antibody−antigen affinity constants. However, we expect the same dose-dependent increase in polymer impedance as occurs with antigen binding (Figure 2 and Figure 3a) and a subsequent decrease in polymer impedance as the antigen is released (Figure 3b) such that only the time scale of the antibody−antigen interaction will vary with the affinity constant. Consequently, the same trends in polymer impedance should be observed for antibody−antigen interactions with different affinity constants, but the time scale of antigen binding and release should vary with affinity constant.

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It should be noted that although ion exchange processes barely alter the properties of sulfate-doped PPy films,[5] sulfate dopants have been shown to diffuse out of PPy following neutralization via Na^+ cations.[41,42] Although after the initial oxidation and reduction of the PPy the leakage of sulfate from the PPy should be minimal,[42] we observe a degradation in the PPy response after extended use (15−20 redox cycles), which may be attributed to diffusion of sulfate dopant ions into the electrolyte.

Conclusions

Our results demonstrate that antibody-doped PPy can be engineered to selectively and reversibly control protein−protein interactions. Impedance spectroscopy results demonstrated that oxidation of the αFN-doped PPy promoted selective FN binding to αFN antibodies and reduction of the polymer films facilitated FN release. Moreover, SPEIS measurements indicate that the apparent reversibility of antibody-doped polypyrrole is due to the minimization of charge in the polymer films during oxidation and reduction. These charge transport characteristics can be utilized to selectively and reversibly control FN−αFN interactions, as well as to dynamically detect FN concentrations in solution. Although the specific polymer chemistry utilized depends on the ligand−receptor interaction of interest, functionalized PPy films could be used to perform real-time, dynamic measurements of biomolecule concentrations for diagnostic or toxicology screening.

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Supporting Information Available: Additional information on the double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}) of αFN-doped PPy as a function of FN concentration (Figure S1) when FN−αFN interactions were facilitated (+700 mV vs Ag/AgCl); supplementary SPEIS measurements of a αFN-doped PPy films in NT, NT with 25% FBS (w/v), and NT with 250 μg/mL FN (Figure S2); additional SPEIS data collected for undoped PPy films in NT, NT with 250 μg/mL BSA, and NT with 250 μg/mL FN (Figure S3); the PPy current response during the first 10 ms of polymer oxidation is also included (Figure S4); impedance measurements of αFN-doped PPy above a hydrophobic dodecylbenzene sulfonate (DBS)-doped PPy layer (Figure S5). This information is available free of charge via the Internet at http://pubs.acs.org.

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