Published in final edited form as:

Conf Proc IEEE Eng Med Biol Soc. 2019 July ; 2019: 1163–1166. doi:10.1109/EMBC.2019.8856842.

A Single-chain Templated Polymer-based Target Receptor as a New Platform for Label-free Selective Electrochemical Sensing

Gaurab Dutta,

Department of Electrical and Computer Engineering, University of New Hampshire, Durham, NH 03824, USA.

Rongfang Yang,

Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA

Habib M. N. Ahmad,

Department of Electrical and Computer Engineering, University of New Hampshire, Durham, NH 03824, USA.

Bo Si,

Department of Electrical and Computer Engineering, University of New Hampshire, Durham, NH 03824, USA.

John Csoros,

Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA

Tianyu Ren,

Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA

Jeffrey M. Halpern,

Department of Chemical Engineering, University of New Hampshire, Durham, NH 03824, USA.

W. Rudolf Seitz,

Department of Chemistry, University of New Hampshire, Durham, NH 03824, USA

Edward Song [Member, IEEE]

Department of Electrical and Computer Engineering, University of New Hampshire, Durham, NH 03824, USA.

Abstract

We report a novel single-chain polymer-based chemical receptor that can be used for the label-free electrochemical detection of an analyte with high selectivity. The polymer was developed using poly-N-isopropylacrylamide (pNIPAM) as a backbone structure in addition to other functional monomers that are used to imprint the template molecule 4-nitrophenol. The polymer also contains a redox reporting monomers (ferrocene) which create a change in the electrochemical signal upon molecular recognition. We hypothesize that the analyte binding to the receptor causes the polymer conformation change from the extended to the collapsed phase. After anchoring the polymer-based receptors onto the surfaces of the gold electrode, when exposed to the analyte, the

changes in the electrochemical signals were observed which confirmed the selective target binding as well as the polymer conformation change as a result.

I. Introduction

Using electrochemical methods for the identification and quantification of chemical species provide many advantages such as low-cost implementation, fast readout, easy miniaturization and simple device architecture. In particular, voltammetric sensors utilize the target analyte's ability to be electrochemically oxidized or reduced and thereby generating an electron transfer between the analyte and the surface of the electrode – a chemical reaction can be transduced into an electrical current. Since voltammetric sensing relies on the analyte's redox activity, if other interfering chemicals with similar redox potentials are present in the sample solution, distinguishing the signal due to the analyte from that of the interfering species is difficult. Therefore, one of the key design parameters in developing an electrochemical sensor is optimizing the chemical selectivity.

To enhance the target selectivity in electrochemical sensing, several strategies have been suggested and investigated. Some examples include coating the electrode surface with permselective polymer film (such as Nafion) to selectively attract species based on charges [1], and immobilization of target capture units (such as antibodies and aptamers) on the surface of electrodes. Specifically, aptamer-based electrochemical sensing has seen much success in recent years due in part to its demonstration of continuous real-time monitoring of the analyte [2].

Aptamer-based electrochemical sensing is based on the premise that when the target binds to the aptamer, it undergoes conformation change which can generate electrochemically measurable signal through the use of a redox-active label attached to the terminus of the aptamer [3], [4]. The aptamer sequences are typically developed using an iterative and randomized screening process known as SELEX [5]. Indeed, many types of aptamers for a variety of target biomarkers and drugs have been developed. However, if a well-performing aptamer has not been identified for a particular analyte, the chemical selectivity in sensing may be compromised.

Another approach to enhance the chemical selectivity in electrochemical sensing is through the molecular templating method also known as molecular imprinting [6], [7]. Molecular templating is the technique where the polymer-based target receptor is synthesized in the presence of the templating molecule (i.e., the analyte) such that the polymer will capture the unique molecular features of the template thereby 'imprinting' the structure of the template into the polymer. This templated polymerization is done with the expectation that the polymer would exhibit preferential binding to the template molecule compared to other non-specific molecules that may be regarded as interfering species.

Traditionally, molecularly imprinted polymers were based on a highly crosslinked polymer matrix with the imprinted molecular cavities buried in the bulk of the material. However, in this framework, the analyte must penetrate through the polymer matrix in order to find the binding sites which lead to a slow rate of binding and release. Moreover, highly crosslinked

polymers tend to be rigid, further hindering the accessibility of the imprinted molecular cavities for the target analyte.

In this paper, we propose a new approach in developing templated polymer-based chemical receptors. Rather than a highly crosslinked polymer matrix, a single-chain templated polymer that behaves as an analyte receptor is proposed. With this platform, we hypothesize that (1) the templated linear polymer binds and releases with the template molecule with reasonably high binding affinity and short response time, and (2) upon selective target binding, the polymer undergoes a conformation change from an extended to a globular shape. This concept was partially motivated by the electrochemical aptamer-based sensing where the aptamer would change its conformation which can then be detected electrochemically. Here, we use a stimuli-responsive polymer, poly-N-isopropylacrylamide (pNIPAM) as a polymer backbone with the incorporation of functional monomers (for analyte recognition) and vinylferrocene as a redox-active label for the electrochemical transduction. Furthermore, the polymer enhances its binding affinity with the template molecule through the intrachain interactions including hydrophobic crosslinks between the N-isopropyl groups within the polymer chain as well as the acid-base crosslinks between the anionic and cationic functional monomers which were incorporated during the polymerization process [8]. The redox-tagged templated polymer exhibits selective detection of our model analyte 4-nitrophenol with reasonably good sensitivity and selectivity.

II. Experimental Methods

A. Polymer Synthesis

The templated polymers were prepared via reversible addition-fragmentation chain transfer (RAFT) polymerization. In brief, 7.4 mmol N-isopropylacrylamide (NIPAM), one mmol of methacrylic acid (MAA), 1.4 mmol of 4-vinyl pyridine (4-VP), and 0.2 mmol vinylferrocene (VF) were added in 100 mL dioxane solution for molecular assembly. The RAFT chain transfer agent used was 0.1 mmol of 2-(Dodecyl-thiocarbonothioylthio)-2-methyl-propanoic acid (DDMAT). The amount of template molecules 4-nitrophenol (4-NP) added for the polymerization was 0.1 mmol. The initiator was 0.05 mmol Azobisisobutyronitrile (AIBN). A freeze-pump-thaw process was carried out to extract the dissolved oxygen in the mixed solution followed by filling the flask with nitrogen to balance the pressure. Then the flask was heated to 70°C during polymerization. Once the polymerization is complete, the polymer solution was dialyzed against 50% THF to remove the templates and the unreacted species. Finally, the remaining polymer solution in the dialysis bag is dried out in a lyophilizer to obtain the polymer powder.

Figure 1 depicts the concept of molecular recognition using a single-chain templated polymer. The molecular orientation of the template 4-NP is 'imprinted' by the polymer chain during polymerization. When the template is removed from the polymer, the chain stretches out to a random coil. However, when the template rebinds to the receptor, the polymer conformation is returned to a globular or a collapsed state.

B. Sensor Electrode Preparation

The RAFT chain transfer agent attached at the end of the polymer was used to anchor the polymers to the electrode surface. Briefly, the obtained polymer powder and tris (2-carboxyethyl) phosphine (TCEP) were dissolved in deionized water, and the gold electrode was immersed in the mixture. TCEP is a reducing agent frequently used in ligand binding chemistry [9]. In the presence of TCEP, the RAFT agent residue at the end of the polymer chain converts to a thiol which can then be used to form a covalent link to the gold surface.

C. Electrochemical Characterization and Sensing Protocol

The electrochemical characterization and sensing were carried out in a MOPS ((3-(N-morpholino) propane sulfonic acid) buffer solution. The MOPS buffer contains NaOH in order to adjust the pH level to 7.0. The cyclic voltammetry (CV) was performed at a scan rate of 100 mV/s from -0.2 V to $+1.5$ V vs. Ag/AgCl reference. The differential pulse voltammetry (DPV) was performed with an amplitude of 250 mV with step voltage of 5 mV from -0.2 V to $+0.7$ V vs. Ag/AgCl in 1M NaClO₄ in deionized water with pH 7.0. The electrochemical experiments were carried out in a glass cell and a platinum wire was used as a counter electrode. All electrochemical experiments performed using Bio-Logic VSP dual channel potentiostat.

III. Results and Discussion

A. Electrochemical Characterization of the Templated Polymer without Vinylferrocene Redox Tag

The initial electrochemical analysis was performed through cyclic voltammetry (CV) of the electrode during various stages of the electrode modification from bare electrode to polymer attachment and exposure to 4-NP. After the templated polymer attachment, the electrode was exposed to 4-NP to allow them to bind to the polymer receptors, followed by rinsing the electrode to remove any unbound 4-NP molecules. Figure 2 shows the CV curves in MOPS buffer for the bare electrode (black), polymer attached electrode (red), and after 4-NP analyte were bound to the polymers (blue and green). The figure also shows the CV curve for the bare electrode with 4-NP added to the MOPS buffer (purple).

During the CV cycles, some radical species present in the MOPS buffer act as interferants during voltammetry [10], [11]. The peaks P1 and P2 in Figure 2 are attributed to the anionic (B) and cationic (A) radicals generated from the MOPS buffer during CV as shown below:

Also, the broad anodic peak P3 in the forward sweep (which has no cathodic counterpart on the subsequent backward sweep) can be attributed to the formation of the monolayer oxide (Au₂O₃) growth at the electrode surface [12] due to the presence of NaOH species in MOPS.

Upon polymer attachment and 4-NP binding, a gradual decrease in the peaks P1, P2, and P3 are observed. This peak decrease signifies a gradual reduction in the electroactive area of the gold surface due to the polymer attachment and collapse upon target binding. The presence of the polymers on the electrode surface restricts the diffusion of MOPS buffer radicals as well as the 4-NP binding. It is worth noting that the peaks P1, P2, and P3 are not due to the adsorption-based oxidation of 4-NP at the electrode. Instead, the peaks P4 and P5 correspond to the redox activity due to the bulk diffusion of 4-NP. The fact that only peaks P4 and P5 appear for the 4-NP present in MOPS (purple) indicates that the rate of reaction for 4-NP is much higher than MOP oxidation. The peak oxidation current for 4-NP (P4) is 31.4 μ A which is 4 to 10 times higher than the MOPS peak oxidation current (P1). The peak potential due to gold surface oxidation (P3) is higher than +1.0 V compared to 4-NP oxidation peak (P4) as summarized in Table 1.

The occurrence of the highly anodic peak (P3) at the polymer functionalized and 4-NP bound surface causes the generation of radical OH $\cdot$ or oxygen species that may initiate further oxidation of the bare electrode surface to gold oxide further hindering the diffusion-based charge transfer for detecting 4-NP. Therefore, another detection strategy is needed which does not oxidize the gold surface and yet provide the change in signal due to the conformational change in the polymer upon 4-NP binding. This can be achieved by introducing redox tags such as vinylferrocene into the polymer backbone.

B. Vinylferrocene-Tagged Templated Polymer Characterization

To enhance the redox signal from the vinylferrocene attached to the polymer, 1M NaClO₄ was used as a buffer [13]. Differential Pulse Voltammetry (DPV) was adopted to reduce the background noise and amplify the ferrocene signal for 4-NP detection. Figure 3(a) shows that upon binding of different concentrations of 4-NP, there is a gradual increase in signal which confirms the polymers collapse as they bind with 4-NP. When the polymers are in the extended conformation, the ferrocene tags are positioned away from the gold surface inhibiting the redox transfer and therefore resulting in smaller DPV signal. Upon binding of 4-NP, the ferrocene tags move near to the gold surface due to the polymer collapsing and results in enhanced charge transfer. The amplified signal confirms that the polymer is transforming from a coil to a globular form [13]. Furthermore, Figure 3(b) demonstrates chemical selectivity against an isomer 3-nitrophenol (3-NP) where minimal change in the DPV signal was observed.

C. Sensing Performances and Calibration Curve

The redox tagged sensor signal in Figure 4(a) shows an almost linear response upon analyte binding to the polymer-based receptors. The sensitivity slope calculation for 3-NP is almost zero which shows excellent selectivity against 3-NP. The sensitivity for 4-NP detection was calculated to be 460 nA/ μ M. Also, the peak potential (E_p) vs. concentration plot in Figure 4(b) shows a cathodic shift in E_p upon 4-NP binding which demonstrates enhanced charge transfer kinetics due to polymer conformation change. As the 4-NP concentration increases, the potential E_p decreases to about +0.08 V indicating that more ferrocene tags are in the close proximity to the electrode surface suggesting a greater polymer collapse into a globular formation.

IV. Conclusion

A single-chain pNIPAM-based templated polymer was developed that can selectively measure 4-nitrophenol. Such functionalized polymer tagged with ferrocene was able to show improved sensitivity and selectivity. Furthermore, upon target binding, the polymer undergoes conformation change from a random coil to a globular form which in turn enhances the electrochemical signal for the redox tagged functional polymers. The proposed sensing strategy further reduces the background noise, signals from other interfering species and oxygen evolution, rendering it an effective platform for the selective detection of chemicals.

Acknowledgment

This project was supported by the NIH COBRE Center of Integrated Biomedical and Bioengineering Research (CIBBR, P20 GM113131) through an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) and by the CoRE Pilot Research Partnership from the University of New Hampshire.

References

- [1]. Selvaraju T and Ramaraj R, "Electrochemically deposited nanostructured platinum on Nafion coated electrode for sensor applications," J. Electroanal. Chem, vol. 585, no. 2, pp. 290–300, 2005.
- [2]. Arroyo-Currás N, Somerson J, Vieira PA, Ploense KL, Kippin TE, and Plaxco KW, "Real-time measurement of small molecules directly in awake, ambulatory animals.," Proc. Natl. Acad. Sci. U. S. A, vol. 114, no. 4, pp. 645–650, 1 2017. [PubMed: 28069939]
- [3]. Fan C, Plaxco KW, and Heeger AJ, "Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA," Proc. Natl. Acad. Sci, vol. 100, no. 16, pp. 9134–9137, 2003. [PubMed: 12867594]
- [4]. White RJ, Phares N, Lubin AA, Xiao Y, and Plaxco KW, "Optimization of electrochemical aptamer-based sensors via optimization of probe packing density and surface chemistry," Langmuir, vol. 24, no. 18, pp. 10513–10518, 2008. [PubMed: 18690727]
- [5]. Keefe AD, Pai S, and Ellington A, "Aptamers as therapeutics," Nat. Rev. Drug Discov, vol. 9, no. 7, pp. 537–550, 2010. [PubMed: 20592747]
- [6]. Uzun L and Turner APF, "Molecularly-imprinted polymer sensors: Realising their potential," Biosens. Bioelectron, vol. 76, pp. 131–144, 2016. [PubMed: 26189406]
- [7]. Piletsky SA, V Piletska E, Bossi A, Karim K, Lowe P, and Turner AP, "Substitution of antibodies and receptors with molecularly imprinted polymers in enzyme-linked and fluorescent assays," Biosens. Bioelectron, vol. 16, no. 9–12, pp. 701–707, 12 2001. [PubMed: 11679247]
- [8]. Grenier CJ et al., "Rapid, high affinity binding by a fluorescein templated copolymer combining covalent, hydrophobic, and acid–base noncovalent crosslinks," Sensors (Switzerland), vol. 18, no. 5, 2018.
- [9]. Yigit MV, Mazumdar D, Kim HK, Lee JH, Odintsov B, and Lu Y, "Smart 'turn-on' magnetic resonance contrast agents based on aptamer-functionalized superparamagnetic iron oxide nanoparticles," ChemBioChem, vol. 8, no. 14, pp. 1675–1678, 9 2007. [PubMed: 17696177]
- [10]. Sankar M et al., "Buffers for the Physiological pH Range: Thermodynamic Constants of 3-(N-Morpholino)propanesulfonic Acid from 5 to 50 °C," J. Chem. Eng. Data, vol. 42, no. 13, pp. 41–44, 3 1997.
- [11]. Ai L and Jiang J, "Catalytic reduction of 4-nitrophenol by silver nanoparticles stabilized on environmentally benign macroscopic biopolymer hydrogel," Bioresour. Technol, vol. 132, pp. 347–377, 3 2013.
- [12]. Burke LD and Nugent PF, "The electrochemistry of gold: I. The redox behaviour of the metal in aqueous media," Gold Bull, vol. 30, no. 2, pp. 43–53, 6 1997.

- [13]. Steentjes T, Jonkheijm P, and Huskens J, "Electron Transfer Processes in Ferrocene-Modified Poly(ethylene glycol) Monolayers on Electrodes," *Langmuir*, vol. 33, no. 43, pp. 11878–11883, 10 2017. [PubMed: 28977744]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.
The working principle of a single-chain templated polymer.

Figure 2.

(a) CV of the 4-NP sensor during each stage of electrode modification. The arrows indicate the direction of the forward and reverse sweep of CV. (b) A magnified view of the peaks P1 and P2. A gradual decrease in MOPS oxidation and reduction upon polymer deposition and 4-NP binding is observed.

Figure 3.

(a) DPV after functionalized polymer attachment and exposure to 4-NP. (b) DPV after exposure to varying concentrations of 3-NP. The electrolyte is 1M NaClO_4 with pH.7

Figure 4.

(a) The calibration curves (peak current vs. concentration) for the target analyte (4-NP) and non-specific interfering species (3-NP) and (b) a shift in the peak potential vs. concentration indicating a change in the polymer conformation as a result of 4-NP binding.

TABLE 1.

THE CYCLIC VOLTAMMETRY (CV) CHARACTERISTICS OF THE POLYMER-MODIFIED ELECTRODE.

Table 2	MOPS oxidation		MOPS reduction		Base Oxidation	
Samples	Peak P1		Peak P2		Peak P3	
	E ₁ (V)	I ₁ (μA)	E ₂ (V)	I ₂ (μA)	E ₃ (V)	I ₃ (μA)
Bare Gold	0.43	7.8	0.37	−54.8	1.13	89.1
Polymer deposited Gold	0.48	9.2	0.38	−56.1	1.11	87.3
100μM 4-NP (Polymer)	0.44	5.9	0.37	−52.9	1.09	80.2
200μM 4-NP (Polymer)	0.44	4.2	0.39	−37.2	1.05	71.6
	4-NP oxidation		4-NP reduction			
	Peak P4		Peak P5			
	Potential	Current	Potential	Current		
Bare Gold	1.03 V	31.4 μA	0.07V	−22.7 μA		