



Label-free detection of cupric ions and histidine-tagged proteins using single poly(pyrrole)-NTA chelator conducting polymer nanotube chemiresistive sensor

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ABSTRACT

Novel chemical and biological sensors based on a single poly(pyrrole)-NTA chelator nanotube for sensitive, selective, rapid and real-time detection of histidine-tagged protein and cupric ions are reported. NTA groups on the nanotube surface provided a simple mechanism for metal ion sensing via the high-affinity interaction between NTA and the subsequent detection of histidine-tagged protein through the coordination with metal chelated nanotube. Poly(pyrrole)-NTA chelator nanotubes of 190 nm outside diameter, 35 nm wall thickness and 30 μm long were synthesized by electrochemical polymerization of pyrrole-NTA inside a 200 nm diameter alumina template and assembled as a chemoresistive device by bottom-up contact geometry on a pair of parallel gold electrodes with a gap distance of 3 μm . The chemoresistive sensors based on single poly(pyrrole)-NTA chelator nanotube exhibited detection as low as one-hundredth attomolar (0.6 ppt) cupric ions and 1 ng/ml of penta-histidine tagged syntaxin protein.

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1. Introduction

The use of chemical and biological sensors based on one-dimensional (1D) nanostructures for medical and environmental monitoring applications is the focus of intensive research. Sensors based on 1D nanostructured materials, namely carbon nanotubes, nanowires of semiconductors such as silicon, zinc oxide, indium oxide, etc. and conducting polymer nanowires have been demonstrated (Patolsky et al., 2006; Wanekaya et al., 2006; Kolmakov and Moskovits, 2004). Conducting polymers such as polyaniline and polypyrrole, because of their ability to directly convert binding/adsorption event into an electrical signal, easily chemical tunable electrical conductivity from metallic to insulating, environmental stability, biocompatibility and most importantly facile controlled processing down to nanoscale dimensions by environmentally benign processes of (electro)chemical polymerization and electrospinning, have emerged as promising materials for the development of high density sensor arrays (Ramanathan et al., 2005; Wanekaya et al., 2006, 2007).

The addition of a series of histidines tail to the N- or C-terminal of a cloned gene product of interest is one of the most common methods used by microbiologist for their purification and detec-

tion. These His-tagged fusion proteins bind to the Ni^{2+} ions chelated to the capture agent nitrilotriacetic acid (NTA) bound to a support matrix and are then eluted in the presence of excess competing chelator imidazole for easy purification. The detection of these proteins, however, requires an additional antibody against the His-tag epitope that has a label for analysis by optical and electrochemical transducers (Darain et al., 2004).

Recently, we reported the synthesis of a nitrilotriacetic acid (NTA) derivative of pyrrole (PY-NTA) and its electropolymerization and application for facile and reversible immobilization of histidine-tagged proteins to the metal ion complexed to the NTA group for fabrication of biosensors (Haddour et al., 2005). In this communication, we report for the first time, a simple, rapid and label-free detection of his-tagged protein using poly(pyrrole)-NTA (PPY-NTA) nanotubes. PPY-NTA nanotubes were synthesized by template directed electropolymerization of PY-NTA and then applied as a single PPY-NTA nanotube chemiresistive sensor for detection and quantification of histidine-tagged syntaxin. As a secondary application, we also demonstrated the detection of Cu^{2+} ions using the single PPY-NTA nanotube chemiresistive sensor.

2. Materials and methods

2.1. Reagents

Pyrrole, nitrilotriacetic acid, triethanol amine (TEA), dimethyl formamide (DMF), succinimidyl ester of 1-(11-undecanoic acid)

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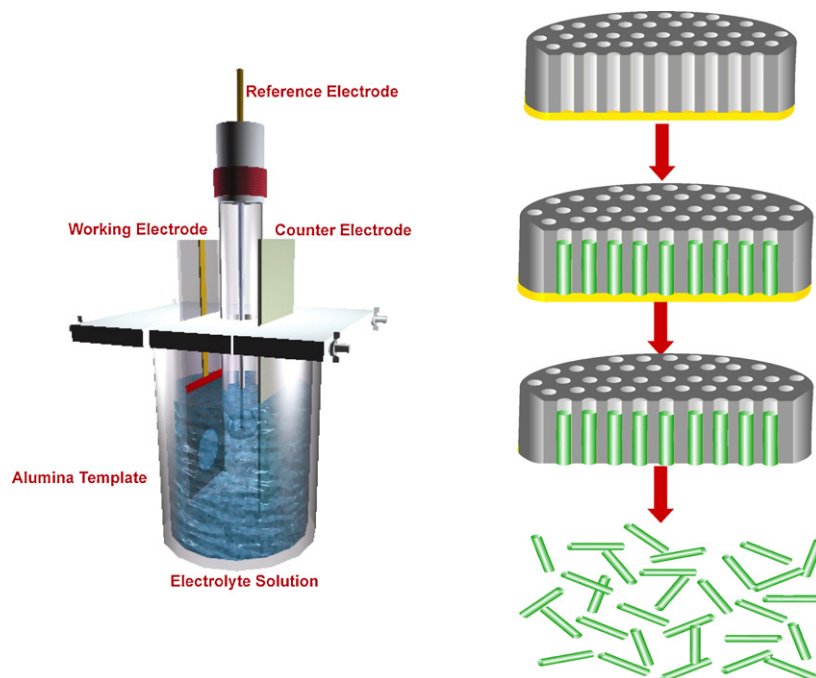


Fig. 1. (a) Experimental setup for electrodeposition of PPY-NTA nanotubes. (b) Protocol for the nanotube fabrication.

pyrrole, sodium hydroxide, ethylenediamine tetraacetic acid, hydrochloric acid, acetonitrile, phosphoric acid, perchloric acid, bovine serum albumin and lithium perchlorate were purchased from Sigma–Aldrich. Alumina membranes of 200 nm nominal pore size and 50 μm thickness were purchased from Whatman International Ltd. (Maidstone, England). Microstop was obtained from Pyramid Plastics Inc. His₅-Syntaxin was provided by Prof. Vladimir Parpura (University of California, Riverside, USA). All reagents were analytical grade and solutions were prepared in double distilled deionized water.

2.2. Pyrrole-NTA synthesis

Pyrrole-NTA monomer was synthesized as reported previously (Haddour et al., 2005). In brief, a solution of nitrilotriacetic acid (200 mg, 0.76 mmol) in a mixture of DMF (1 mL), TEA (500 μL), NaOH (91 mg, 2.28 mmol), and water (300 mL) was added to a solution of succinimidyl ester of 1-(11-undecanoic acid)pyrrole (265 g, 0.76 mmol) in DMF (2 mL). The solution was stirred for 24 h at 60 °C and concentrated under vacuum. The crude powder was dissolved in water (3 mL), and 1N hydrochloric acid was added dropwise. The resulting precipitate was filtered and washed with diethyl ether and dried to obtain PY-NTA as a white powder.

2.3. Nanotube fabrication

Nanotubes were fabricated by template directed electrochemical deposition at ambient conditions using nanoporous alumina templates. A 200 nm thick Au seed layer was sputtered on an alumina template using the Emitech K550 tabletop sputter. The template was fixed to a glass support, with the seed layer facing down, using a double sided conductive copper tape. The entire sample, except the middle of the template, was masked with a dielectric material. The nanotubes were grown potentiostatically within the template pores using a multichannel EG&G PAR VMP2 potentiogalvanostat. A three-electrode glass-cell with the Au seed layer serving as anode and a platinum mesh as cathode

and 10 mM Ag/Ag⁺ in acetonitrile as a reference electrode respectively, was used for this purpose (Fig. 1).

Based on the electrochemical behavior of the PY-NTA (Haddour et al., 2005) electro-oxidative polymerization was carried out for 30 min at a constant potential of 0.9 V vs. Ag/Ag⁺ from a solution of 5 mM PY-NTA monomers dissolved in acetonitrile acidified with 0.1 M HClO₄ containing 0.1 M LiClO₄. All solutions were purged with nitrogen prior to use and the electrochemical cell was protected from light to prevent the monomer oxidation in solution. After electropolymerization the Au seed layer was dissolved in KI/I₂ etchant (Mura et al., 2003) followed by dissolution of the alumina template using 30 wt% H₃PO₄ to release the nanotubes, washed several times with nanopure water and finally suspended in isopropanol.

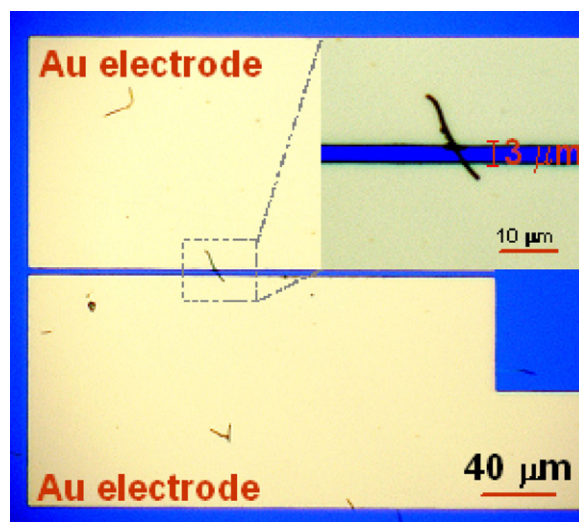


Fig. 2. An optical image of a typical bottom-contacted single PPY-NTA nanotube device. Inset: higher resolution image of a nanotube bridging across the gold electrodes.

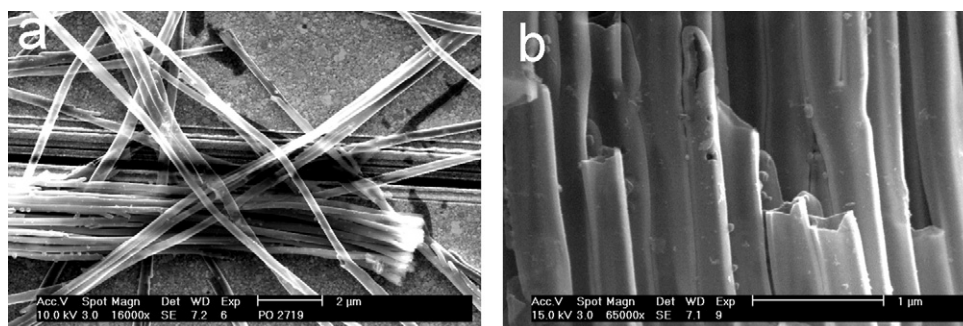


Fig. 3. (a) SEM image of PPY-NTA nanotubes following the removal of gold seed layer and alumina template. Scale bar: (a) 2 μm and (b) 1 μm .

2.4. Single PPY-NTA nanowires sensor device construction

A bottom-up contact geometry was employed for electrical connection of individual nanotubes on a pair of parallel gold electrodes on SiO_2/Si substrate with a gap distance of 3 μm (Fig. 2). Single PPY-NTA nanotube sensors were fabricated by drop casting a very dilute suspension of the nanotubes in isopropanol onto pre-cleaned electrodes followed by drying under vacuum at 40 $^\circ\text{C}$ for 24 h for improved adhesion and contact of nanotubes with the electrodes.

3. Results and discussion

3.1. Synthesis and characterization of PPY-NTA nanotubes

PPY-NTA nanotubes were synthesized by template-directed electrochemical polymerization at ambient conditions using nanoporous alumina templates according to the protocol provided in Section 2. SEM images of suspended PPY-NTA nanotubes showed primarily well-dispersed (with a few bundles) and well-defined continuous nanotubes with openings at the ends (Fig. 3a). The average tube length was $\sim 30 \mu\text{m}$, with a few individual tubes of up to 40 μm length. Additionally, the featureless surface of nanotubes (Fig. 3b) indicated the complete wetting of the pore walls of the template by the monomer. This is attributed to the NTA facilitated wetting of the electrolyte to the template pores and the slow diffusion of electroactive species leading to a preferential deposition along the walls of the template pores to form nanotubes (Martin, 1996; Steinhart et al., 2002).

Fig. 4a shows the TEM images of PPY-NTA nanotubes. The nanotubes were smooth with no defects along the entire length.

The variation in contrast confirmed the tubular structure of fairly uniform diameter along the entire length with an apparent wall thickness of $\sim 35 \text{ nm}$. The average outside diameter of the tubes was $\sim 190 \text{ nm}$ which was in close agreement with the 200 nm diameter template pores.

To establish the potential utility of nanotubes as sensors for histidine-tagged proteins and metal ions, chelation of Cu^{2+} ions to the PPY-NTA nanotubes was conducted. Nanotubes were incubated in sodium acetate and acetic acid buffer solution of pH 4.5 containing 1 mM CuCl_2 at room temperature for 30 min, washed several times with nanopure water to remove the excess un-chelated Cu^{2+} ions and dried. Energy dispersive X-ray spectroscopy (EDS) line scan analysis (Fig. 4b) showed the presence of a Cu peak in the EDS confirming the chelation of Cu^{2+} ions with the polymerized NTA groups constituting the nanotubes. The EDS line profiling across the bundle of nanotubes confirmed the presence of Cu^{2+} ions within the nanotubes. Further, a comparison of pre- and post- Cu^{2+} ions chelation SEM images showed no change in the nanotube morphology.

3.2. Single PPY-NTA nanotube chemoresistive sensor

A bottom-up contact geometry was used to form the single PPY-NTA nanotube chemoresistive device. Investigation by a digital optical microscope (Hirox KH-3000, Japan) after the delivery and drying of a dilute suspension of PPY-NTA nanotubes in isopropanol confirmed that a single nanotube was bridging across the gold electrodes (Fig. 2). Furthermore, the symmetric S shaped I - V curves confirmed the electrical connection between the nanotube and electrodes. The resistance (R), calculated at -0.5 V from the I - V curves, after device fabrication (dry) and in a pool of nanopure

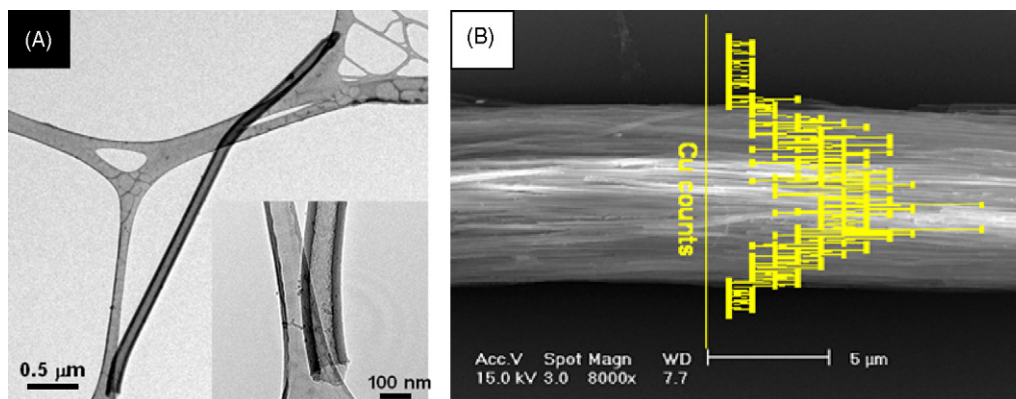


Fig. 4. (A) Bright field TEM image of single PPY-NTA nanotube. Inset: higher resolution TEM image. (B) SEM image and EDS line scan for Cu on bundle of Cu^{2+} chelated PPY-NTA nanotubes.

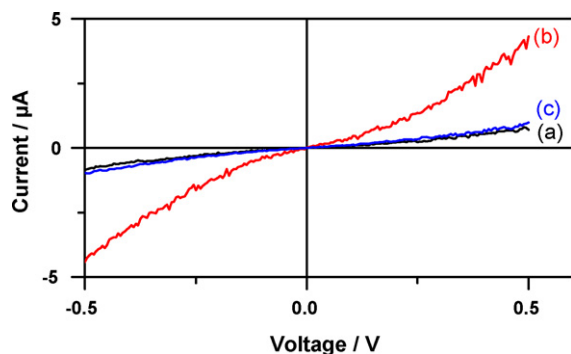


Fig. 5. I - V profile of single PPY-NTA nanotube (a) before contacting and (b) after contacting with 10^{-6} M Cu^{2+} and (c) after treatment with 1 mM EDTA.

water (wet) (R_0) were ~ 700 k Ω and ~ 722 k Ω , respectively (data not shown).

The single PPY-NTA nanotube device was applied for the detection and quantification of biomolecules by detecting pentahistidine (His_5)-tagged protein via coordination with the Cu^{2+} chelated nanotube. Fig. 5 (trace a) shows the I - V characteristics of PPY-NTA nanotube covered with a $5 \mu\text{l}$ water drop. Replacing the water with solution of 10^{-6} M CuCl_2 resulted in an expected upward shift of the I - V curve (trace b). It is clear that the presence of Cu^{2+} ions induce a resistance decrease and hence reinforces the global conductivity of the nanotubes.

To demonstrate the detection of His-tagged proteins, we followed the dynamic changes in the resistance of the PPY-NTA- Cu^{2+} nanotube at source-drain potential of -500 mV to different concentrations of His₅-Syntaxin protein. As shown in Fig. 6A, upon addition of 1 ng/ml of His₅-Syntaxin the resistance of the PPY-NTA nanotube increased rapidly and reached a plateau in less than 1 min. The resistance increased further when a higher concentration (10 ng/ml) of protein was added. However, no appreciable signal was observed when the concentration was increased beyond 100 ng/ml, indicating the saturation of binding sites for the protein molecules. The inset table in Fig. 6A gives the values of percentage change of resistance for the three tested concentrations. The sensor response was reproducible as evidenced by similar results with different sensors (data not shown).

Selectivity of the PPY-NTA- Cu^{2+} nanotube sensor was evaluated by challenging the nanosensor to bovine serum albumin (BSA). The sensor exhibited excellent selectivity for His₅-Syntaxin as evident by no response of the sensor to up to 10 mg/mL of BSA (Fig. 6B), a 10,000-fold higher concentration than the lowest His₅-Syntaxin tested earlier.

Reuse for multiple analysis is a desirable feature in a sensor. The restoration of the I - V curve to the original (Fig. 5, trace a) upon treatment of the Cu^{2+} chelated nanotube with 1 mM EDTA (Fig. 5, trace c) illustrates a facile method for regeneration of the sensor. Three successful cycles of binding to Cu^{2+} and regeneration by stripping with EDTA was achieved without a noticeable decrease in the response to metal ions (data not shown).

The single PPY-NTA nanotube sensor was also evaluated for the detection of Cu^{2+} ions. As shown in Fig. 7 the normalized change in resistance ($[(R_0 - R)/R_0]$) of the PPY-NTA nanotube-based sensor (calculated at -0.5 V) was a linear function of $\log [\text{Cu}^{2+}]$ over a wide dynamic range and the nanosensor was able to measure as low as 10^{-14} M (0.6 ppt) Cu^{2+} . This detection limit is substantially lower compared to commercially available thin film and a recently reported metal-binding peptide modified conducting polymer nanogap sensor (Zhang et al., 2004). The PPY-NTA nanotube sensor demonstrated high selectivity for Cu^{2+} as confirmed

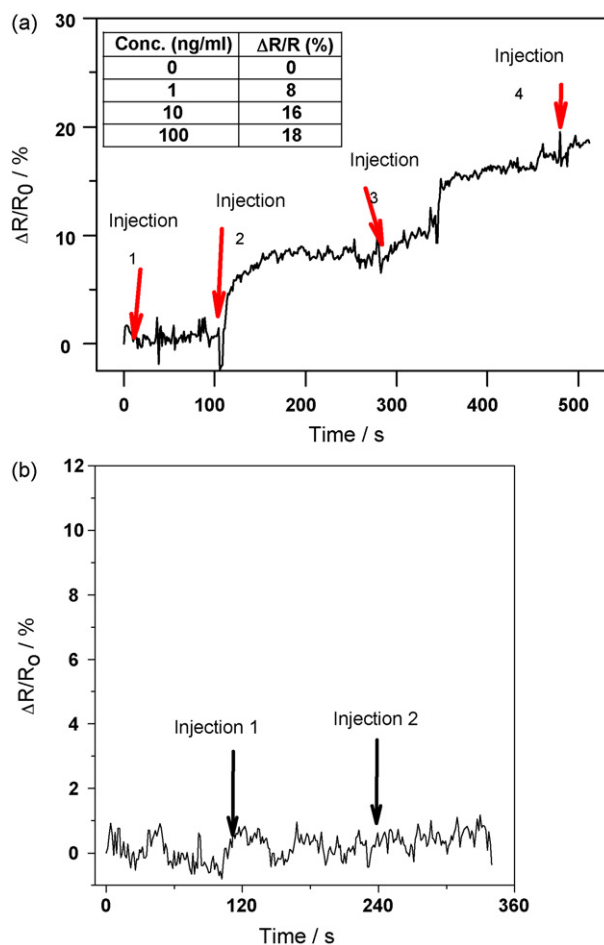


Fig. 6. (a) Real time detection of His₅-syntaxin with PPY-NTA single nanotube saturated with Cu^{2+} ions, E_{appl} : -0.5 V, $5 \mu\text{l}$ injections of: (1) water, (2) 1 ng/mL, (3) 10 ng/mL and (4) 100 ng/mL. (b) Selectivity of PPY-NTA single nanotube device to bovine serum albumin. E_{appl} : -0.5 V, injections: (1) 1 $\mu\text{g/mL}$ and (2) 10 $\mu\text{g/mL}$.

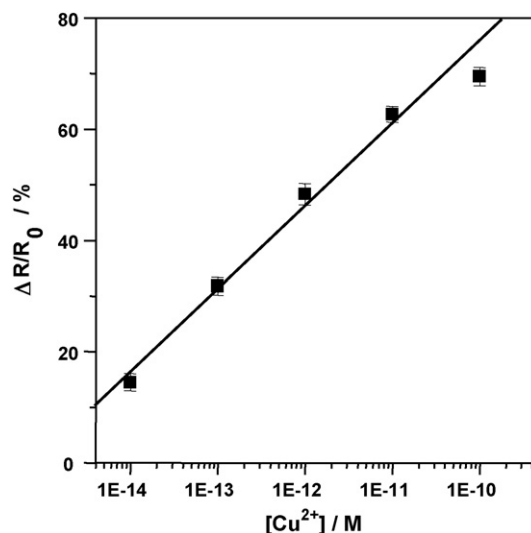


Fig. 7. Nanotube resistance change as a function of Cu^{2+} ions concentration. Error bar represents ± 1 S.D. for three replicates.

by no reduction of the signal for Cu^{2+} (10^{-14} M) in the presence of alkali metal ions such as Na^+ (0.1 M NaCl). It should be noted that the sensor would not be selective against transition metal ions such as Ni^{2+} , Cd^{2+} , etc. that chelate NTA.

4. Conclusions

In conclusion, novel chemiresistive chemical and biological sensors based on single PPY-NTA nanotube for sensitive, selective, rapid and real-time detection of histidine-tagged proteins and copper ions was demonstrated. The direct conversion of an ionic binding event into an electrical signal and the use of a nanoscale sensing element are the unique features of the device. The ng/ml and sub-attomolar sensitivity respectively, for His₅-Syntaxin and Cu^{2+} , detection achieved by a simple chemoresistive measurement can be enhanced by controlling the oxidation state of PPY by simple control of the gate potential using a liquid-ion gate and/or the nanotube diameter (Wanekaya et al., 2007; White et al., 1984; Kittlesen et al., 1984). Further, the nanotube can be easily functionalized with biorecognition element such as antibodies, binding proteins, etc., through the interaction of metal ion chelated to NTA and the His-tag of a biorecognition molecule to fabricate nanobiosensors.

Acknowledgments

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