Poly-L-lysine Functionalization of Single-Walled Carbon Nanotubes

Yuanjian Zhang,[†,‡] Jun Li,[†] Yanfei Shen,[‡] Meijia Wang,[‡] and Jinghong Li*[,†,‡]

Department of Chemistry, Tsinghua University, Beijing 100084, People's Republic of China, and State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China

Received: July 1, 2004; In Final Form: July 20, 2004

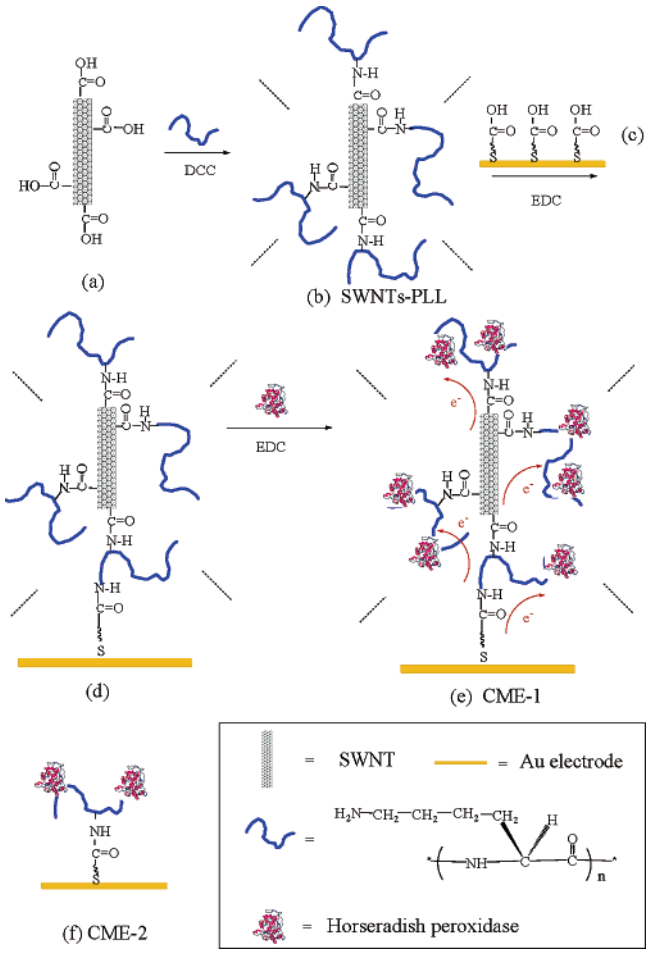
Single-walled carbon nanotubes (SWNTs) were covalently functionalized with biocompatible poly-L-lysine, which is useful in promoting cell adhesion. SWNTs played an important role as connectors to assemble these active amino groups of poly-L-lysine, which provided a relative "friendly" and "soft" environment for further derivation, such as attaching bioactive molecules. As an application example, by further linking peroxidase, an amplified biosensing toward H_2O_2 concerning this assembly was investigated.

Introduction

Single-walled carbon nanotubes (SWNTs), one kind of truly molecular entities, are of significant interest due to their unique properties and potential applications.[1,2] Considerable attempts have been made by derivatization of the tube ends and sides.[3] For instance, covalent[4] and noncovalent attachment[5] to SWNT sides with various molecules has been proved to be an effective way to bring numerous properties into SWNTs. Recently, SWNTs were considered to be useful tools for biological application by covalent attachment of bioactive molecules.[4a] In general, besides through physical absorption,[6] to effectively achieve this, a proper "linker" was needed between the SWNT sides and the bioactive molecule. Besides the use of diamine,[4c] Bianco and Prato reported a more effective way—organic functionalization of SWNTs—and further derivatized by coupling with amino acid and bioactive peptides.[4a]

To extend the application of SWNTs, we report here another facile pathway for functionalizing SWNTs directly with hydrophilic poly-L-lysine (PLL) as a linker through a covalent amide group. PLL has plentiful active amino groups and is useful in promoting cell adhesion. As the linker, PLL has several advantages, such as good biocompatibility, plentiful active amino groups, a flexible molecular backbone, and relatively good solubility in water. On one hand, some free amino groups on the PLL molecular chains cross-linked with the carboxyl groups of SWNTs and other residual free amino groups acted as a relative "friendly" and "soft" linker between the SWNTs and bioactive molecules; on the other hand, SWNTs played a role as connectors to assemble these poly amino chains (Scheme 1b). Therefore, the SWNT−PLL assembly with plentiful amino groups will open the way to a large number of opportunities, such as bioactive molecular attachment and the preparation of nanocomposites.[7] As a potential application, by further linking peroxidase, an amplified biosensing toward H_2O_2 concerning this SWNT−PLL assembly was investigated.

SCHEME 1. Illustration of the Process for Assembling SWNTs, Poly-L-lysine, and Horseradish Peroxidase[a]



[a] The whole cross-linked structure of the SWNT−PLL assembly was not shown for clarity.

Experimental Section

Materials. Single-walled carbon nanotubes were obtained from MER. Poly-L-lysine hydrobromide (M_w = 30 000−70 000), 3-mercaptopropionic acid (MPA, >99%), and dicyclohexylcarbodiimide (DCC, 99%) were obtained from Sigma. Horseradish peroxidase (HRP, E.C. 1.11.1.7, 250 U mg^{-1}) and 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were obtained from BBI. Hydrogen peroxide solution

* Corresponding author. Phone: 86-431-5262243. Fax: 86-431-5690851. E-mail: jhli@mail.tsinghua.edu.cn.
† Tsinghua University.
‡ Chinese Academy of Sciences.

10.1021/jp0471094 CCC: $27.50
Published on Web 09/01/2004

(30 wt %) was purchased from Beijing Chemical Reagent (Beijing, China), and a fresh solution of H_2O_2 was prepared daily. All other reagents were of analytical grade and were used as received.

Instruments. Fourier transform infrared (FTIR) spectroscopy was conducted with an FTS135 infrared spectrometer (Bio-Rad, U.S.A.). FTIR transmission spectra of the samples were obtained on the CaF_2 substrate containing the interesting materials in the solid state. Energy-dispersive X-ray microanalysis (EDS) was measured on a 2000XMS instrument. Resonance raman spectra were measured on a Raman Infinity spectrophotometer (France) at a resolution of 4 cm^{-1}. The 488 nm line with a power of 50 mW from an argon ion laser was used as the excitation source. Electrochemical measurements were carried out in a conventional three-electrode electrochemical cell. Typically, a bulk gold disk electrode was polished carefully with 1.0, 0.3, and 0.05 μm alumina slurries to a mirror finish and was cyclic-potential-polished in 0.5 M sulfuric acid. The working electrode was a gold electrode ($d = 3$ mm) and a modified gold electrode, the auxiliary electrode was a platinum wire, and the reference electrode was a Ag/AgCl (saturated KCl) electrode. Cyclic voltammetry measurements were performed with a CHI 832 electrochemical instrument (CHI Inc., U.S.A.). Electrolyte solutions were purged with high-purity nitrogen prior to and blanketed with nitrogen during electrochemical experiments. UV−visible spectra were measured by using a UV-360 spectrometer (Shimadzu, Japan).

Method for Preparing the SWNT−PLL Assembly. Before use, SWNTs were purified and shortened by suspending the as-received SWNTs with the aid of ultrasonic agitation for 1 h in a 3:1 v/v mixture of concentrated sulfuric and nitric acid. The ends and sidewalls of SWNTs should bear oxidized carbon sites such as carboxylic acids (Scheme 1a). Generally, the SWNT−PLL assembly (Scheme 1b) was prepared by vigorously stirring a solution of 1 mg of the SWNTs, 6 mg of PLL, and 2 mg of DCC in 6 mL of DMF at 50 °C for 24 h. After that, the SWNT−PLL assembly was collected by a polycarbonate membrane with 200 nm pores, thoroughly washed with alcohol and water, and dried in a vacuum,[5] to remove any unreacted PLL and DCC.

Method for Preparing the SWNT−PLL Modified Electrode. As an application example, this SWNT−PLL assembly was successfully further conjugated with horseradish peroxidase and used to construct a chemically modified electrode (CME) toward biosensing H_2O_2. The process of the preparation of the CME with SWNT−PLL is illustrated in Scheme 1c−e. Typically, the polished bulk gold electrode was immersed in 0.02 M MPA phosphate buffer (0.05 M, pH 7.4) for 24 h at room temperature (Scheme 1c). Then, it was immersed in phosphate buffer containing SWNT−PLL (0.1 mg/mL) and EDC (0.02 M) for 48 h with slight stirring. After that, the Au/MPA/SWNT−PLL electrode (Scheme 1d) was immersed in phosphate buffer containing HRP (1 mg/mL) and EDC (0.02 M) at 4 °C for 48 h. Thus, a Au/MPA/SWNT−PLL/HRP electrode (CME-1, Scheme 1e) was obtained. In each step of the assembly, the electrode was thoroughly rinsed with distilled water to remove the excess of the physical absorption. The reference Au/MPA/PLL/HRP electrode without SWNTs (CME-2, Scheme 1f) was prepared in the same way except that PLL was used instead of SWNT−PLL.

Results and Discussion

SWNT−PLL Assembly. The covalent attachment was first confirmed by Raman spectra (Figure 1). Both spectra of SWNTs

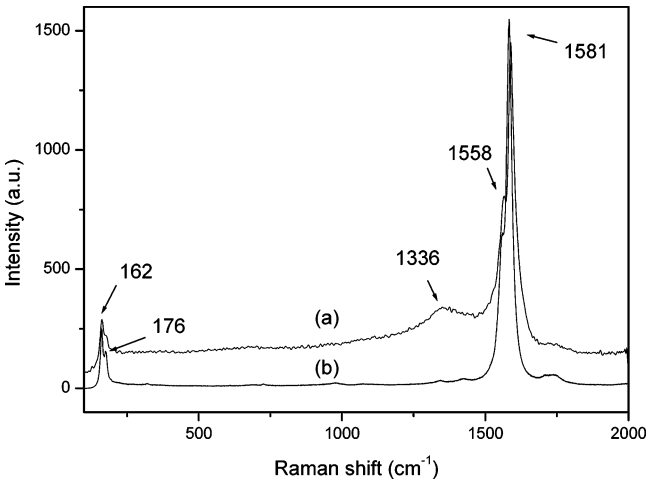


Figure 1. Raman spectra of the SWNT−PLL assembly (a) and shortened SWNTs (b).

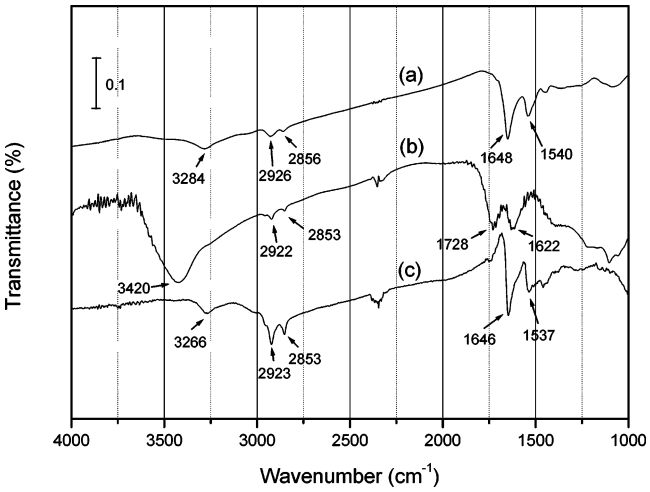


Figure 2. FTIR spectra of the SWNT−PLL assembly (a), shortened SWNTs (b), and PLL (c).

(Figure 1a) and SWNT−PLL (Figure 1b) contained characteristic peaks at 1558 and 1581 cm^{-1} (tangential modes) and at 1336 cm^{-1} (disorder mode). As the disorder mode is the diagnostic of disruptions in the hexagonal framework of the SWNTs, the fact that the relative intensity of this mode increased provided direct evidence of the covalent modification of SWNTs.[3a]

Figure 2 shows the FTIR spectra of SWNT−PLL (Figure 2a), shortened SWNTs (Figure 2b), and PLL (Figure 2c). From Figure 2c, it can be seen that the vibration of amide I appeared at ∼1646 cm^{-1}, that of amide II appeared at ∼1537 cm^{-1}, and that of free amino groups ($-NH_3^+$) appeared at ∼3266 cm^{-1}.[8] The FTIR spectrum of SWNTs (Figure 2b) displayed a peak at ∼1728 cm^{-1}, which could be assigned to the C═O stretch mode of carboxylic acid. Besides, the peaks at 1622 and 3420 cm^{-1} could be assigned to the stretch mode of trace water in shortened SWNTs. The covalent attachment of PLL to SWNTs could be proved by the changes in the FTIR spectrum (Figure 2a) of the resulting SWNT−PLL assembly. The C═O stretch mode of SWNTs shifted from 1728 to 1648 cm^{-1}, which indicated that the carboxylic acid groups of SWNTs were almost reacted completely with the amino groups of PLL and turned into amide groups. The vibration of $-NH_3^+$ also existed in the spectrum of the SWNT−PLL assembly, which indicated the existence of active amino groups in the SWNT−PLL assembly. From

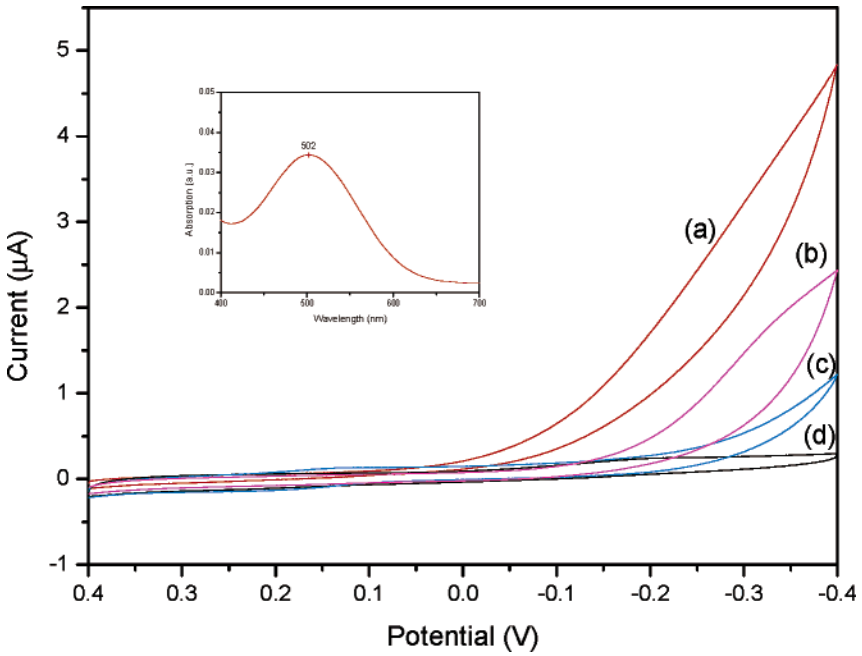


Figure 3. Cyclic voltammograms of CME-1 (a), CME-2 (b), the Au/MPA/SWNT−PLL electrode (c) in phosphate buffer (0.05 M, pH 7.4) containing 2.5 mM H_2O_2, and CME-1 (d) in phosphate buffer. Scan rate: 10 mV/s. Inset: UV−vis absorption spectrum of the Trinder reaction by CME-1.

the EDS results (Supporting Information Figure S1), the molar ratio of active amino groups to carbon atoms of SWNTs was calculated as ~1:2. It was relatively higher when compared with literature results[4a] and was reasonable if taking the molecular weight of PLL (30 000−70 000) into account.

Therefore, PLL was successfully modified on the SWNTs. The carboxylic acid groups of shortened SWNTs were fully covalently anchored with the PLL chain, and there were still a large quantity of residual free amino groups ($-NH_3^+$) on the SWNT−PLL assembly, which could provide a number of opportunities to attach bioactive molecules. From another point of view, SWNTs acted as connectors to assemble these active amino groups.

Potential Application Based on the SWNT−PLL Assembly. To apply this SWNT−PLL assembly in the potential field, as an example, an attempt was made to conjugate it with horseradish peroxidase[9] and used it to construct a chemically modified electrode toward biosensing H_2O_2. The successful conjugation was also proved by this electrochemical method.

The assembly process of CME-1 was monitored by the $K_3Fe(CN)_6$ electrochemical probe, for the electron transfer between the electroactive species and the electrode must occur by tunneling either through the barrier of the CME or through the defects in the barrier.[10] Supporting Information Figure S2 shows the cyclic voltammograms (CVs) of the SWNT−PLL modified electrode at the end of each assembly step in a 2 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl as the supporting electrolyte (scan rate: 100 mV/s). A well-defined CV, characteristic of a diffusion-limited redox process, was observed at the bare gold electrode (Supporting Information Figure S2a). After MPA was assembled on gold, an obvious decrease in the peak currents and an increased difference between the anodic and cathodic potentials (ΔE_p) were observed (Supporting Information Figure S2b), which was due to the fact that the monolayer of MPA hindered the electron transfer between $Fe(CN)_6^{3-}$ and the electrode. When SWNT−PLL was modified on the MPA monolayer, the remarkable increase in the peak currents (Supporting Information Figure S2c) was due to the positive charged free amino groups in neutral solution which attracted negative charged $Fe(CN)_6^{3-}$ and thus increased the electron transfer by tunneling. The good charge-transfer property of SWNTs was also expected to contribute to this increase. Since the isoelectric point of HRP is ~8.9 and it was also positively changed in neutral solution,[10] the CV after assembly of HRP slightly changed in this step (Supporting Information Figure S2d).

The successful modification of HRP on CME-1 and the larger loading of HRP compared to the reference electrode (CME-2) were confirmed by CVs of biosensing H_2O_2[9] in Figure 3 (assuming the enzyme activity in two electrodes was the same[10,12c]). From the distinctive reductive current difference between parts a and c of Figure 3, it could be seen that the catalytic ability toward H_2O_2 of CME-1 was mainly due to the HRP. The reductive current at CME-1 was much larger than that at CME-2, for instance, ~3.6-fold at the potential of −0.2 V. It indicated the larger HRP loading of CME-1, which reflected the greater quantity of free amino groups on CME-1. The immobilization of HRP on CME-1 was also confirmed by a standard procedure.[11] CME-1 was immersed into 2.0 mL of a vigorously stirred solution of 12 mM 4-aminophenazone, 85 mM phenol, and 1 mM H_2O_2. After 5 min, a red color was observed and the absorption was measured (Figure 3, inset). The absorption peak at 502 nm indicated that the HRP on CME-1 still had a similar physiological function for catalyzing the reduction of H_2O_2.

The biosensing upon successive addition of aliquot H_2O_2 was also observed (Supporting Information Figure S3). As the H_2O_2 was added into the stirring buffer solution, CME-1 responded rapidly to the substrate. The linear range of H_2O_2 was from 5.0 μM to 10.0 mM ($R = 0.999$; $n = 20$).

CME-1 was successfully prepared, the plentiful active amino groups of the SWNT−PLL assembly were expected to act as friendly and soft linkers to effectively conjugate HRP, and SWNTs were expected to promote the charge transport. The specific structure of the SWNT−PLL assembly played the major role in this amplified biosensing process. The use of carbon

nanotubes as the modifier in connection with another electrode surface has been well developed mainly due to their ability of promoting electron transport and their stable chemical properties.[1] The early works focused on the assembly by the casting method,[6] and the covalent attachment provided the more effective connection.[12] Understanding the assembly of carbon nanotubes at the molecular level is greatly needed.[13]

Conclusion

In summary, a new and versatile functionalized SWNT assembly was described, by using covalent modification with poly-L-lysine (PLL), which was useful in promoting cell adhesion. The SWNT−PLL assembly exhibited a large quantity of free active amino groups, and SWNTs played an important role as connectors to assemble these amino groups, which provided a number of opportunities for further related friendly and soft attaching, such as bioactive molecules and nanocomposites. As a potential application, HRP was successfully immobilized on the SWNT−PLL assembly and the chemically modified electrode of this structure exhibited amplified biosensing toward H_2O_2.

Acknowledgment. This work was financially supported by the National Natural Science Foundation of China (no. 20125513).

Supporting Information Available: Figures showing EDS of the SWNT−PLL assembly, CVs, and the amperometric response at CME-1 to the successive addition of H_2O_2. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Ajayan, P. M. *Chem. Rev.* **1999**, *99*, 1787. (b) Fukushima, A.; Kosaka, A.; Ishimura, Y.; Yamamoto, T.; Takigawa, T.; Ishii, N.; Aida, T. *Science* **2003**, *300*, 2072.

(2) Special Issue on Carbon Nanotubes. *Acc. Chem. Res*. **2002**, *35*, 997−1113.

(3) (a) Dyke, C. A.; Tour, J. M. *Chem.—Eur. J*. **2004**, *10*, 812. (b) Chen, J.; Rao, A. M.; Lyuksyutov, S.; Itkis, M. E.; Hamon, M. A.; Hu, H.; Cohn, R. W.; Eklund, P. C.; Colbert, D. T.; Smalley, R. E.; Haddon, R. C. *J. Phys. Chem. B* **2001**, *105*, 2525.

(4) (a) Bianco, A.; Prato, M. *Adv. Mater*. **2003**, *15*, 1765. (b) Shiral Fernando, K. A.; Lin, Y.; Sun, Y.-P. *Langmuir* **2004**, *20*, 4777. (c) Baker, S. E.; Cai, W.; Lasseter, T. L.; Weidkamp, K. P.; Hamers, R. J. *Nano Lett*. **2002**, *2*, 1413.

(5) Chen, J.; Liu, H.; Weimer, W. A.; Halls, M. D.; Waldeck, D. H.; Walker, G. C. *J. Am. Chem. Soc.* **2002**, *124*, 9034.

(6) (a) Yamamoto, K.; Shi, G.; Zhou, T.; Xu, F.; Xu, J.; Kato, T.; Jin, J.-Y.; Jin L. *Analyst* **2003**, 128, 249. (b) Musameh, M.; Wang, J.; Merkoci, A.; Lin, Y. *Electrochem. Commun.* **2002**, *4*, 743. (c) Wang, J.; Li, M.; Shi, Z.; Li, N.; Gu, Z. *Anal. Chem*. **2002**, *74*, 1993.

(7) (a) Calvert, P. *Nature* **1999**, *399*, 210. (b) Murthy, V. S.; Cha, J. N.; Stucky, G. D.; Wong, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 5292.

(8) (a) Muller, M.; Buchet, R.; Fringeli, U. P. *J. Phys. Chem*. **1996**, *100*, 10810. (b) Ke, Y.; Dong H. *Handbook of Analytic Chemistry: Spectroscopy Analysis*; Chemical Industry Press: Beijing, China, 1998.

(9) Hrapovic, S.; Liu, Y.; Male, K. B.; Luong, J. H. T. *Anal. Chem*. **2004**, *76*, 1083.

(10) Jia, J.; Wang, B.; Wu, A.; Cheng, G.; Li, Z.; Dong, S. *Anal. Chem*. **2002**, *74*, 2217.

(11) Schwartz, M. K. *Handbook of Enzymatic Methods of Analysis*; Dekker: New York, 1976.

(12) (a) Diao, P.; Liu, Z.; Wu, B.; Nan, X.; Zhang, J.; Wei, Z. *ChemPhysChem* **2002**, *10*, 898. (b) Patolsky, F.; Weizmann, Y.; Willner, I. *Angew. Chem., Int. Ed*. **2004**, *43*, 2113. (c) Chattopadhyay, D.; Galeska, I.; Papadimitrakopoulos, F. *J. Am. Chem. Soc.* **2001**, *123*, 9451. (d) Williams, K. A.; Veenhuizen, P. T. M.; de la Torre, B. G.; Eritja, R.; Dekker, C. *Nature* **2002**, *420*, 761.

(13) Ulman, A. *Chem. Rev*. **1996**, *96*, 1533.

Poly-L-lysine Functionalization of Single-Walled Carbon Nanotubes

Yuanjian Zhang, Jun Li, Yanfei Shen, Meijia Wang, Jinghong Li*

Supplementary information

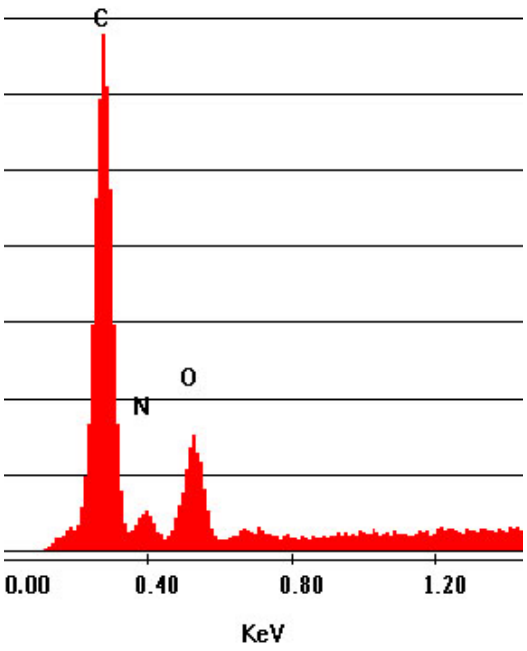


Figure S1 EDS of SWNTs-PLL assembly.

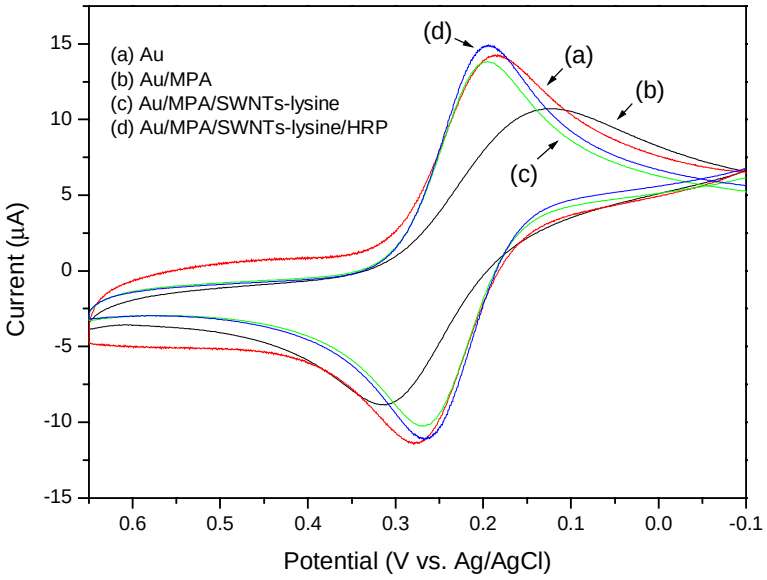


Figure S2 Cyclic voltammograms of 2 mM $K_3Fe(CN)_6$ at bare Au (a), Au/MPA (b), Au/MPA/SWNTs-PLL (c) and Au/MPA/SWNTs-PLL/HRP (d) electrode in solution containing 0.1 M KCl as supporting electrolyte. Scan rate: 100 mV/s.

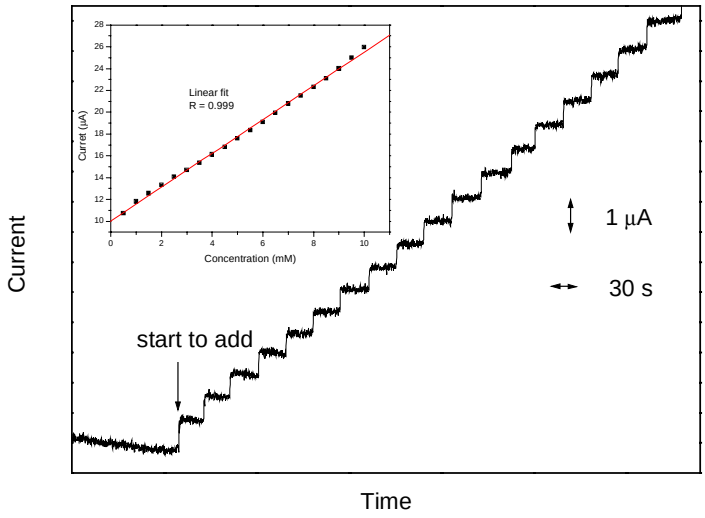


Figure S3 Amperometric response at the CME-1 to successive addition of 5.0×10^{-4} M H_2O_2. Inserted is the calibration plot. Apllied potential: 0.20 V (vs. Ag/AgCl), stirring rate: 300 rpm, supporting electrolyte: phosphate buffer.