Supplementary Information

Artificial hydrolase based on carbon nanotubes conjugated with peptides

Qiang Zhang,[a] Xingxing He,[a] Ailing Han,[a] Qingxia Tu,[a] Guozhen Fang,[a] Jifeng Liu,*[a] Shuo Wang,*[a] and Haibo Li[b]

[a] Key Laboratory of Food Nutrition and Safety, Ministry of Education of China, Tianjin University of Science and Technology, Tianjin 300457, China.
Email:liujifeng111@gmail.com , s.wang@tust.edu.cn
[b] Department of Chemistry, Liaocheng University, Liaocheng 252059, China

Experiment

Construction of CNT-$(SHE/W)_{2:1}$-LKLKLKL.

500 μl freshly prepared mixture contained 0.3 M EDC and 0.6 M NHS were added to 1 mL CNT suspended (0.28 mg/mL) in Tris-HCl buffer (25 mM, pH 8.0). After vortex mixed for 1 h, 10.8 μL polypeptide of SHELKLKLKL (19 mM) and 5.4 μL WLKLKLKL (19 mM) were added into the CNT suspension and allowed to react at room temperature for 24 h. The solution was centrifuged three times to remove the suspension.

Construction of other CNT-peptides.

500 μL freshly prepared mixture contained 0.3 MEDC and 0.6 M NHS were added to CNT suspended (0.28 mg/mL) in Tris-HCl buffer (25mM, pH 8.0). After vortex mixed for 1 h, 16 μl polypeptide of SHELKLKLKL, LKLKLKLEHS, SHLKLKLKL, HELKLKLKL, SELKLKLKL, LKLKLKL or CSHESH (19 mM) was added into the CNT suspension and allowed to react at room temperature for 24 h. The solution was centrifuged three times to remove the suspension.

Kinetics Determinations.

Kinetic measurements were done by a UV-Vis spectrophotometer (Shanghai Jinghua Technology Instruments Co. Ltd.) monitoring absorbance of the product (p-nitrophenol) at 400 nm. PNPA stock solution of 83 mM was prepared in acetonitrile (the final acetonitrile content was 3% in all reaction mixtures). 100 μL of freshly prepared substrate solution, was added to 3.1 ml buffered (8.3 mM) the artificial enzyme solution at pH 8 (to a final artificial enzyme concentration of 3.5 μg/mL). The concentration of p-nitrophenol was obtained by standard curve. Kinetic parameters (K_{cat}, K_m) were obtained by the Michaelis–Menten equation

$$v_0 = v_{max}[S]/([S]+K_m) \qquad (1)$$

where v_0, v_{max}, [S], and K_m are initial rate, maximum initial rate of the enzyme catalytic reaction, substrate concentration, and Michaelis–Menten constant, respectively. pH profile studies were performed by monitoring absorbance of the p-nitrophenol at 400 nm at room temperature using a 1 cm quartz cuvette. 50 μL of CNT-peptides (to a final artificial enzyme concentration of 3.5 μg/mL) was added to 3.05 ml Tris-HCl buffer (pH 7.1–8.9, 8.3 mM) in a cuvette, followed by the addition of 100 μL PNPA in acetonitrile. Temperature profile studies were performed at different temperature using a 1 cm quartz cuvette. The formation of p-nitrophenol was monitored at 4 min. 50 μL of CNT-peptides (to a final artificial enzyme concentration of 3.5 μg/mL) was added to 3.05 ml Tris-HCl buffer(pH 8.0, 8.3 mM) in a cuvette, followed by the addition of 100 μL PNPA in acetonitrile. The formation of p-nitrophenol was monitored at reaction time of 4 min.

Quantification of peptides bound on CNT.

Rhodamine B labelled SHELKLKLKL and fluorescein isothiocyanate labelled WLKLKLKL were used in this study to calculate the number of peptides bound on CNT and ratio of SHELKLKLKL to WLKLKLKL. The number of peptide on CNT was calculated by performing using fluorescence measurements. The excitation wavelengths of the two dyes are 555, 494 nm, respectively. And the emission wavelengths of the two dyes are 580, 518 nm, respectively. The supernatant solutions were collected after EDC-NHS conjugation reaction and the unreacted peptides in the suspension can be quantified using the corresponding standard curve. And then the bound peptides can be quantified.

Circular Dichroism Spectroscopy.

Circular dichroism (CD) spectra were collected on a J-810 spectropolarimeter (JASCO Inc., Japan) at room temperature in Tris-HCl buffer (pH 8.0, 8.3 mM) at 0.5 mg/mL concentration of the SHELKLKLKL, WLKLKLKL, LKLKLKLEHS and CSHESH with a 1cm quartz cell. The spectra were recorded at a 0.1-nm interval from 190 to 250 nm.

Scanning Electron Microscope Characterization.

SEM imaging and Elemental mappings were obtained by Scanning Electron Microscope (Zeiss Merlin Compact, Germany) with an Oxford Energy-dispersive Spectrometer using a 5 kV electron beam. Suspension of CNT-peptides was dropped onto a Cu mesh and then attached onto the stage using conductive glue.

XPS Characterization.

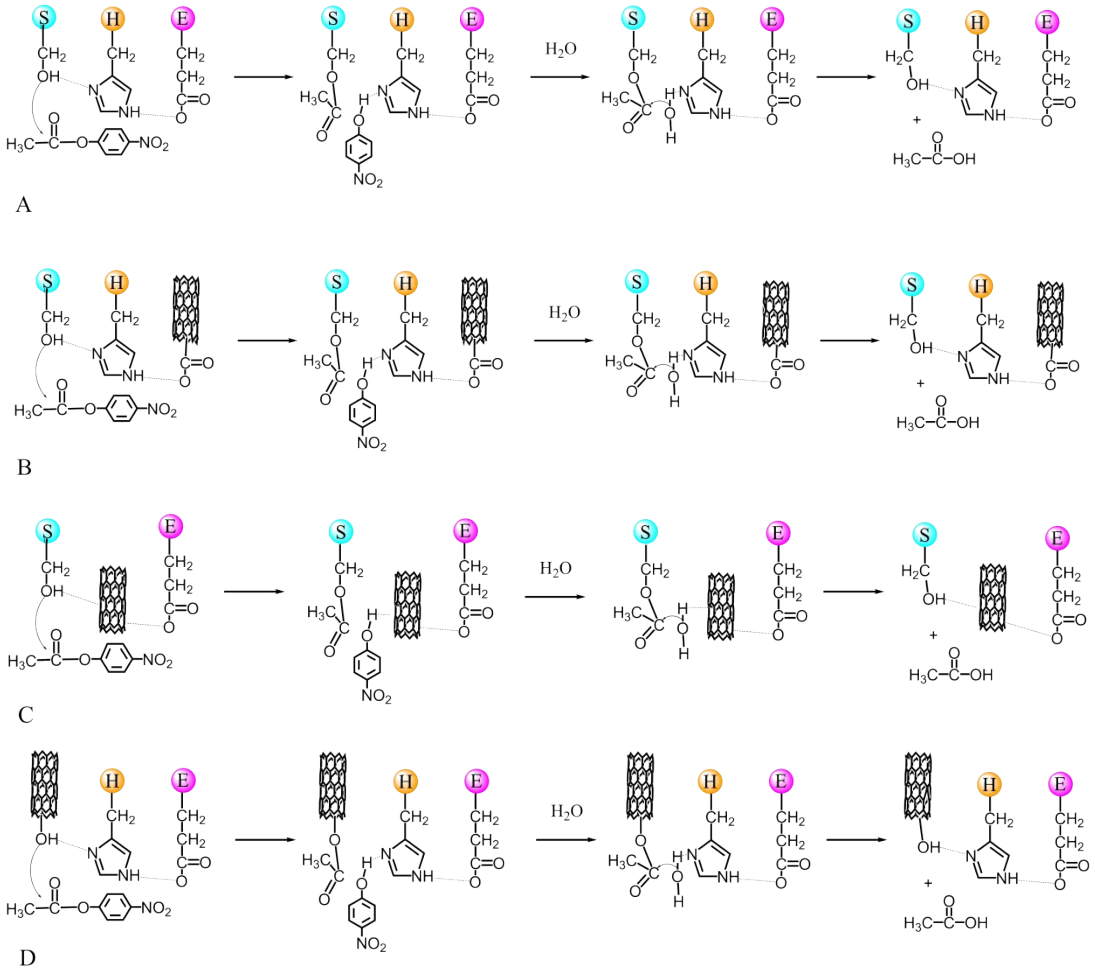
XPS measurement was tested on an ESCALAB-MKII spectrometer (VG Co., United Kingdom) with Al Ka X-ray

radiation as the X-ray source for excitation. Peak fit analysis was done using the program XPS PEAK version 4.1.

Fluorescence Spectroscopy Characterization.

Fluorescence measurements were performed on an F-2500 fluorescence spectrometer (Hitachi, Japan) connected with an external 980 nm diode laser (1 W, continuous wave with 1 m fiber, Beijing Viasho Technology Co.) as the excitation source with a 1cm quartz cell.

Supporting Schemes and Figures



Scheme SI-1. Proposed catalytic mechanism and proton-transfer process of (A) CNT-SHE-LKLKLKL; (B) CNT-SH-LKLKLKL; (C) CNT-SE-LKLKLKL; (D) CNT-HE-LKLKLKL. Carbon nanotube was proposed to participate into the proton transfer relay process.

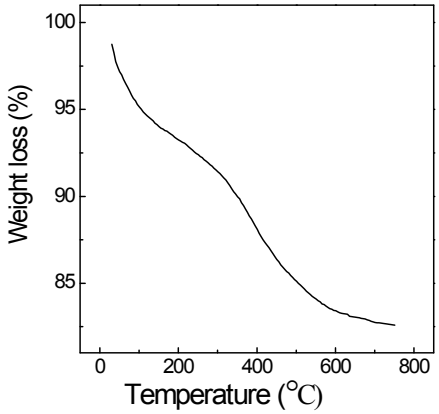


Figure SI-1. Thermogravimetric analysis data of CNT, with a temperature ramp rate of 10 °C/min. Sample were stored at 80°C for 24 h before experiment. CNT showed 12.5% weight loss within 100-600 °C and these may be due to the decomposition of –COOH or –OH or other oxygen-containing groups.

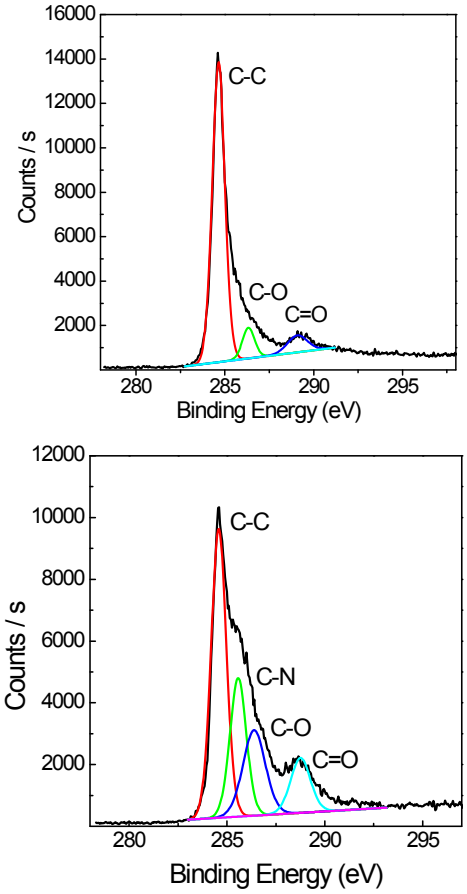


Figure SI-2. High resolution C 1s XPS spectra of CNT (left) and CNT-SHELKLKLKL (right). The peak at 284.6, 286.3, 289.0 eV attributed to C-C, C-O, C=C, respectively. And the peak at 285.6 in right attributed to C-N, indicating the exsting of peptide in the CNT-peptide congujation.

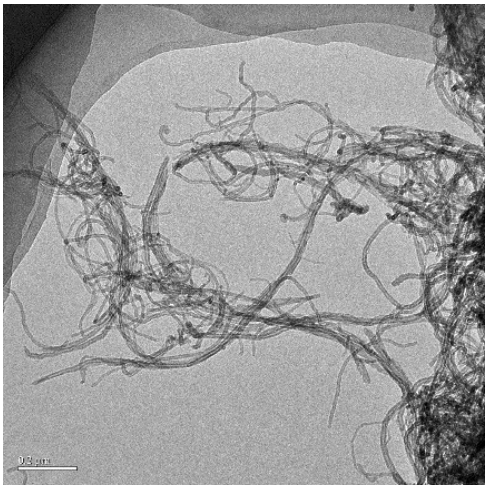


Figure SI-3. Characterization of CNT by TEM.

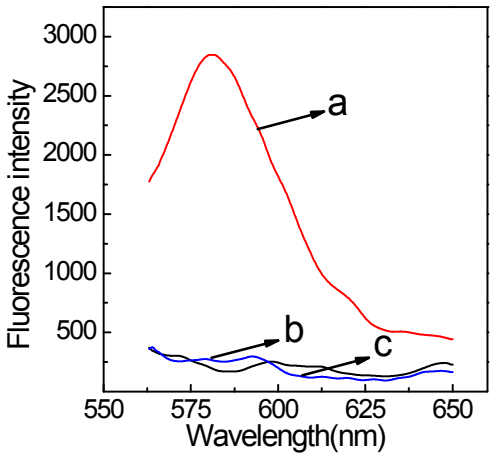
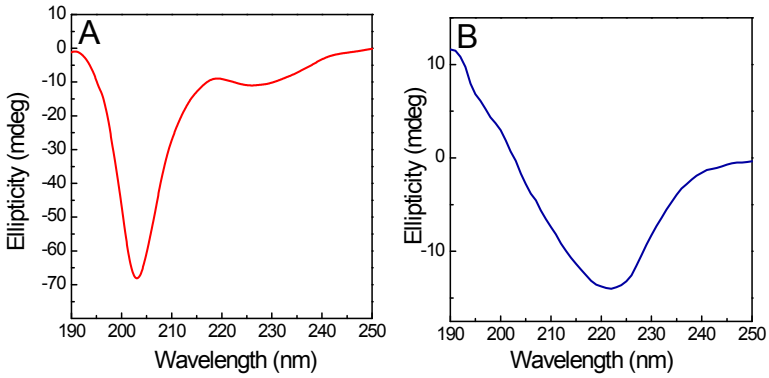


Fig. SI-4 Fluorescent characterization for CNT-SHE-LKLKLKL. The SHELKLKLKL was labelled by rhodamine B. (a) CNT-SHE-LKLKLKL, (b) mixture of CNT and SHELKLKLKL, (c) CNT.



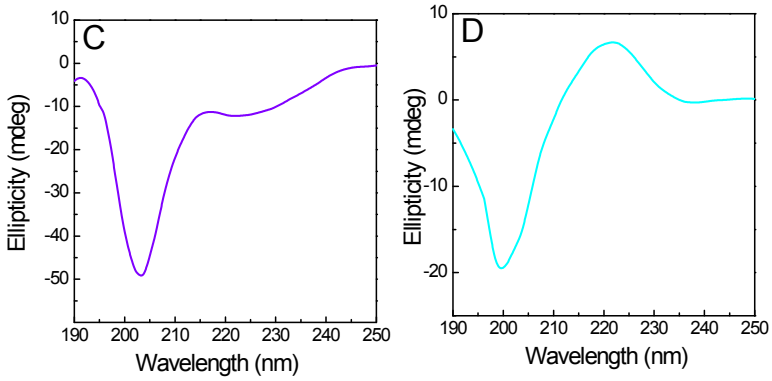


Figure SI-5. Characterization of the secondary structure of peptides by circular dichroism. (A) SHELKLKLKL. (B) WLKLKLKL. (C) LKLKLKLEHS. (D) CSHESH.

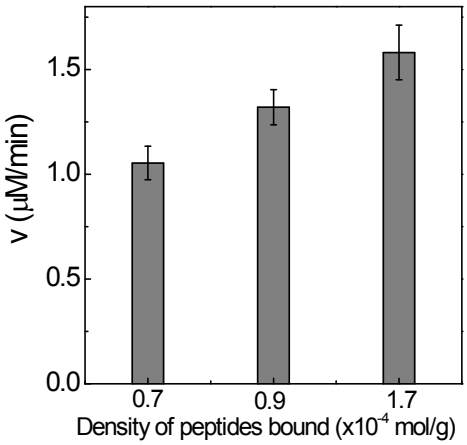


Figure SI-6. The dependence of reaction rates upon the density of bound peptides (a, 0.7, b, 0.9, c, 1.7×10^{-4} mol/g).

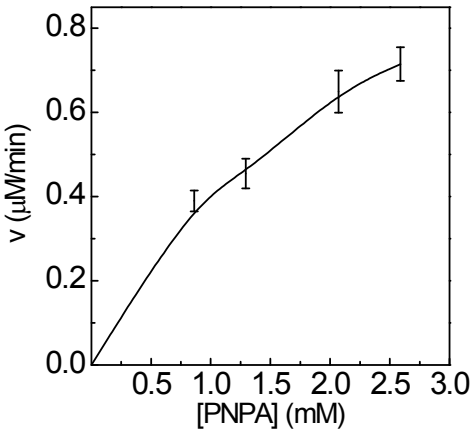


Figure SI-7. The Michaelis–Menten equation plot of PNPA hydrolysis catalyzed by (CNT-(SHE/W)$_{2:1}$-LKLKLKL.

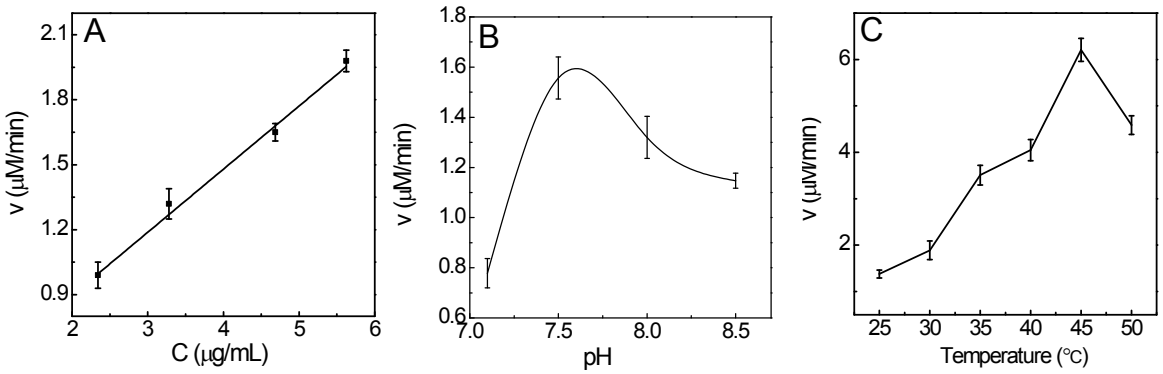


Figure SI-8. Functional characterization of the catalysts. (A) Dependence of PNPA hydrolysis activity catalysed by CNT-$(SHE/W)_{2:1}$-LKLKLKL on the concentration; (B) The pH profile of the catalytic activity of CNT-$(SHE/W)_{2:1}$-LKLKLKL; (C) The temperature profile of the catalytic activity of CNT-$(SHE/W)_{2:1}$-LKLKLKL.

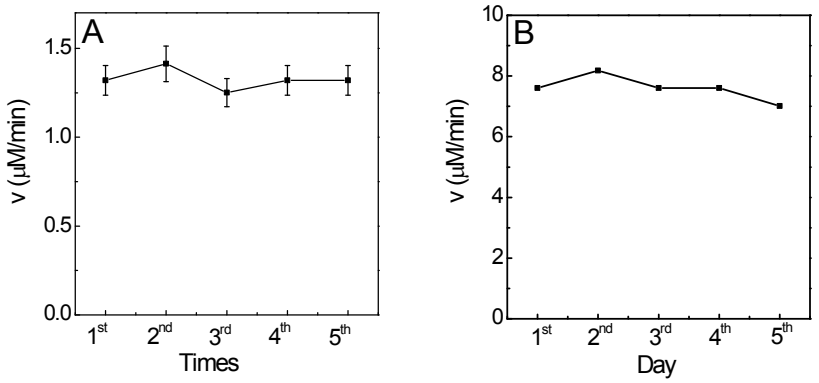


Figure SI-9. The renewable ability and durability of CNT-$(SHE/W)_{2:1}$-LKLKLKL catalyzed hydrolysis of PNPA. (A) 3.5 μg/mL and (B) 250 μg/mL of CNT-$(SHE/W)_{2:1}$-LKLKLKL.