Electronic Supplementary Information for: "Immobilization of trypsin on water-soluble endrimer-modified carbon nanotubes for on-plate proteolysis combined with MALDI-MS analysis"

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Experimental section.

1 Materials

Multi-walled carbon nanotubes (CNTs) was kindly provided by Shenzhen Nanotech Port Co. Ltd. 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98+%) , N-hydroxysulfosuccinimide (sulfo-NHS) were from Shanghai Medpep Co., Ltd. TPCK-treated trypsin, Myoglobin (Myo), Cytochrome c (Cyt-c), Bovine serum albumin (BSA), and sodium cyanoborohydride ($NaCNBH_3$) were purchased from Sigma Chemical (St. Louis, MO, USA). Lysozyme (Lys) was from Boguang biotech. Company (Shanghai,China). Trifluoroacetic acid (TFA) was purchased from Merck (Darmstadt, Germany); Acetonitrile (CH_3CN) was of HPLC grade from Fisher Scientific (Fairlawn, NJ). Water was purified using a Milli-Q system (Millipore, Molsheim, France). Glycine, glutaraldehyde (GA, 25% w/v aqueous solution), ammonium acetate (NH_4OAc), calcium chloride ($CaCl_2$), manganese chloride

($MnCl_2$) and methanol (CH_3OH) were of analytical grade and were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Unless noted otherwise, other chemicals and analytically pure reagents were used as received.

2 Preparation of dendrimer modified carbon nanotubes

The chemical modification of CNTs was first treated with concentrated HNO_3 and H_2SO_4 (3:1, v/v). Upon refluxing in the above mixed acid reagent for 4 h, most of the carboxylic acid groups are found at the end or side wall of CNTs. Dendrimers with a trimesyl core were synthesized as following: In brief, EDC (100 mg) and NHS (100 mg) were introduced into an aqueous suspension of carboxylated CNTs (50 mL, 0.1 mg mL^{-1}), then a methanol solution (1.5 mL) of dendrimers (G4.0) (0.2 g) with a trimesyl core was added slowly into the above suspension. Afterwards, the mixture was stirred vigorously for 2 h to drive the reaction to completion. The final product dendrimer-modified carbon nanotubes (dCNTs) were obtained by purifying with a dialyzer (molecular weight cutoff, 30000) using double distilled water to remove the excess dendrimers.

3 Immobilization of Trypsin onto dendrimer-modified carbon nanotubes

A total of 0.5 mg of the dCNTs were suspended in coupling buffer (CB: 50 mM NH_4OAc, pH 8.3, 1 mM $CaCl_2$, and 1 mM $MnCl_2$, 200 μL) in a 1.5-mL Eppendorf tube. After washing twice with the coupling buffer, the dCNTs were retained by 10 min centrifugation at 16400 rmp. The amine group of the dendrimer was activated at

room temperature under gentle rotation for 1.5 h by excess glutaraldehyde (5%, 200 μL) solution in CB (pH~7.0). The dCNTs were then retained by centrifugation, and the solution was removed, followed by four washes each in CB (200 μL). TPCK-treated trypsin (0.4 mg) was dissolved in CB (200 μL) and the dCNTs were incubated with the protein solution for 3 h under rotation and for another 0.5 h in the same solution with the addition of $NaCNBH_3$ (1.0%). After removal of the TPCK-treated trypsin solution, the dCNTs were incubated for 1 h with glycine (0.75%) and $NaCNBH_3$ (1.0%) in CB (200 μL). Finally the dCNTs were washed four times in 200 μL of CB. The trypsin linked dCNTs were dissolved in H_2O (50 μL) and stored at -20℃ as the stock solution.

4 On-Plate Digestion of Proteins

The sample solution of each protein was digested on the spot of the MALDI plate. Before digestion, the solutions of protein samples were prepared in NH_4HCO_3 buffer solution (25 mM, pH 8.1). Stock solution of trypsin-linked dCNTs was 10 times diluted in the NH_4HCO_3 buffer solution to form a homogeneous suspension in water close to a solution phase. Each diluted protein solution (0.5 μL) was deposited on the spots of a MALDI plate, after that, the diluted trypsin-linked dCNTs solution (0.5 μL) was spotted, then, aspirated and dispensed 5 to 10 cycles with pipette tip for simple mixture. The MALDI plate was placed in a home-built humidity chamber, made from a plastic box with wet paper tissues in the bottom and a tight-fitting lid, and incubated

at 37℃. For comparison, protein samples in NH_4HCO_3 buffer (25 mM, pH 8.1) were digested in-tube with trypsin at 37℃ for 12 h.

5 MALDI-TOFMS Process

All the mass spectra were taken from a 4700 Proteomics Analyzer of MALDI-TOF/TOF-MS (Applied Biosystems, Framingham, MA, USA). The instrument was operated at an accelerating voltage of 20 kV. A 200 Hz pulsed Nd:YAG laser (355 nm) was used for MALDI. Proteolytic peptides of standard myoglobin with known molecular masses were used for calibration. All spectra were taken from signal averaging of 1000 laser shots. The laser intensity was kept properly constant for all of the samples.

Fig. S1 Structure diagram of the dendrimers (G4.0).

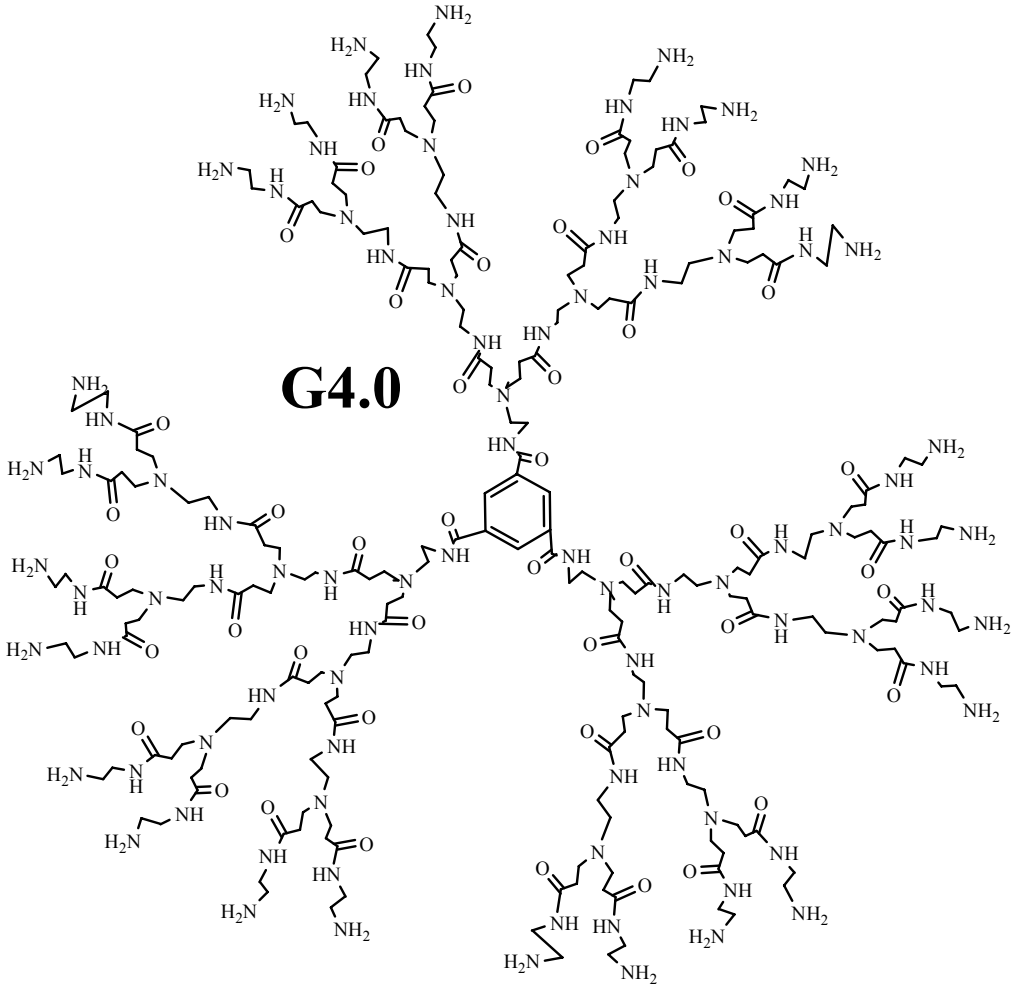
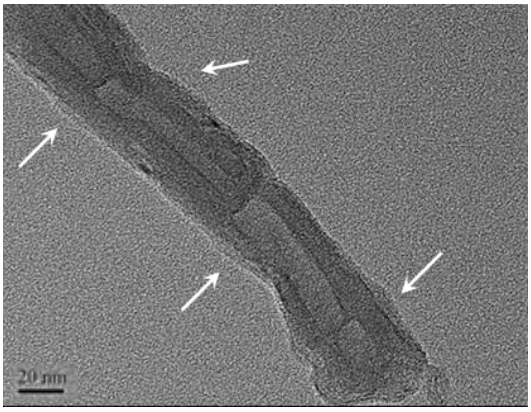
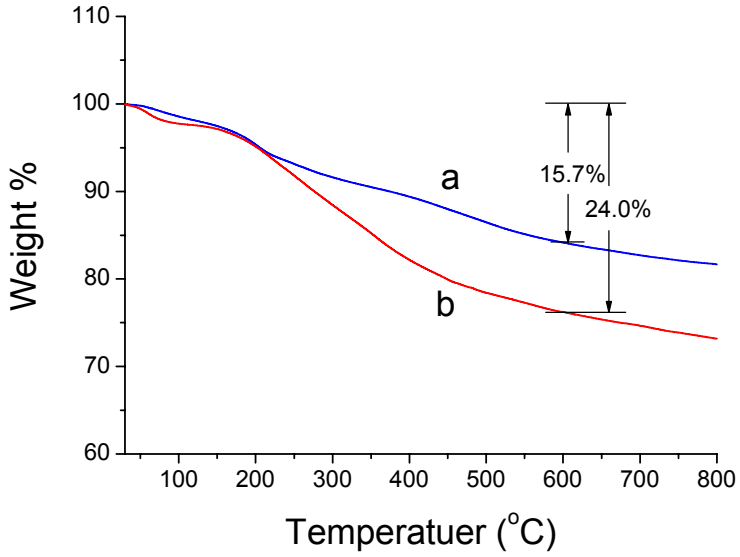


Fig. S2 The TEM images of dCNTs.



It can be clearly seen from the TEM image that the surface of CNTs is covered with layers of dendrimers (G4.0). On average, a layer of dendrimers with a thickness of 3-6 nm is observed.

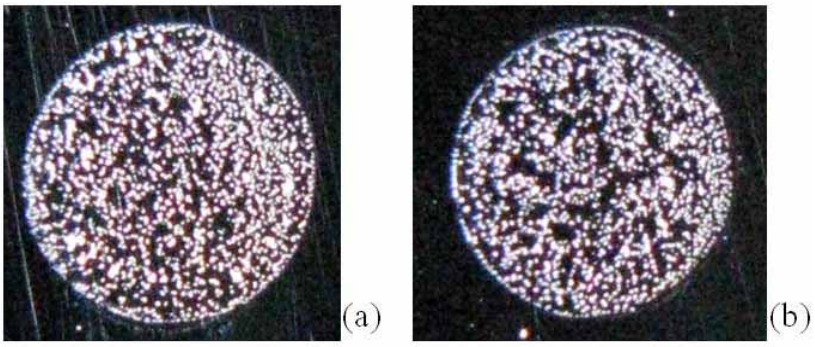
Fig. S3 TGA weight loss curves of (a) acid-treated CNTs and (b) dCNTs.



The content of dendrimers in dCNTs was estimated by TGA method. The acid-treated CNTs showed about 15.7% weight loss at 600 ^{o}C, demonstrating that the thermally unstable functional groups might be formed on the CNTs during acid treatment. The as-prepared dCNTs presented about 24.0% weight loss in the same temperature range due to the decomposition of dendrimers and other functional groups on CNTs. Comparing the weight loss curves of dCNTs with that of acid-treated CNTs, it can be deduced that the grafting amount of dendrimers is about 8.3%.

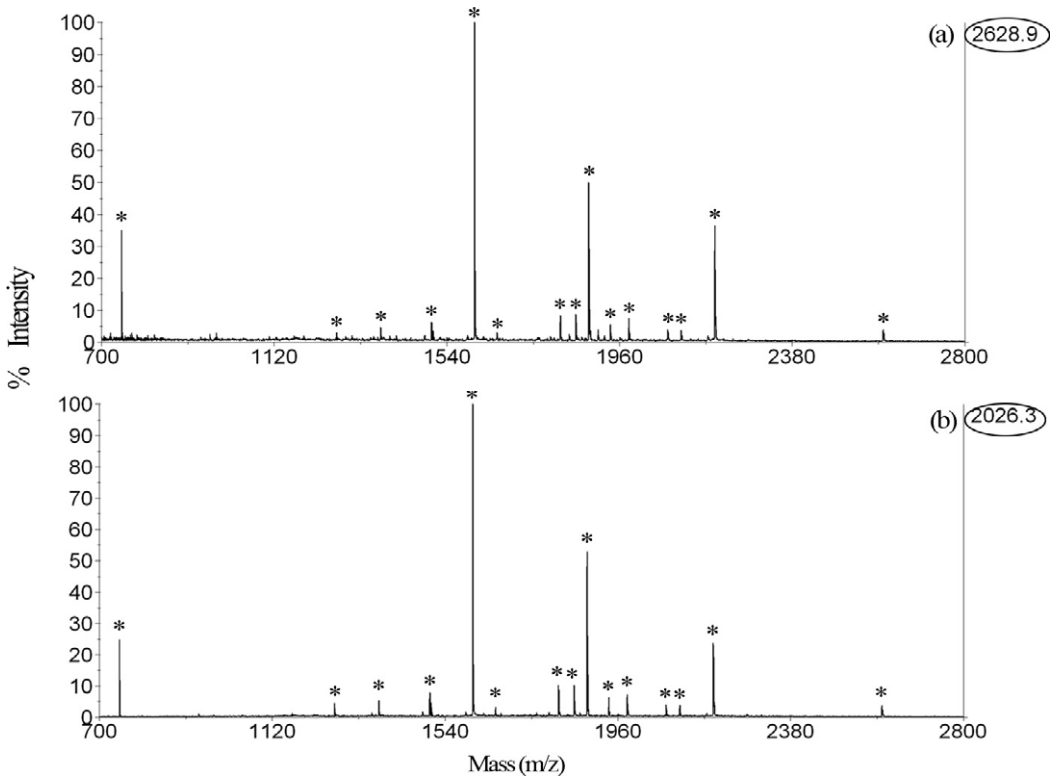
TGA conditions:
Thermal gravimetric analyses (TGA) were carried out with a SDT Q600 (TA instruments, USA) under nitrogen (30 ml·min^{-1}) at a heating rate of 10 ^{o}C/min.

Fig. S4 Digital photographs of prepared sample spots (a) Myo digests with dCNTs; (b) Myo digests without the dCNTs. On each spot, 0.5 μL Myo sample solution and 0.5 μL matrix solution CHCA were spotted and mixed together on the spot with pipette.



As can be seen from the picture, the analyte and the matrix formed homogeneously microcrystals with the existence of dCNTs. It may on account of the good solubility of the dCNTs.

Fig. S5 MALDI-TOF mass spectra of the digests of 10 ng μL^{-1}Myo digests (a) with and (b) without dCNTs. On each spot, 0.5 μL Myo sample solution and 0.5 μL matrix solution CHCA were spotted and mixed together on the spot with pipette. Peptides of Myo digests were marked with "*".



Mass spectra were taken from two spots with and without dCNTs corresponding to Figure S5 (a) and (b), signal intensity from the two spectra seemed no big differences. The mass spectra illustrates the existence of dCNTs do not interfere with the MS analysis.

Table S1 Identified Peptides of Cyt-c, Lys and MYO by 15-min On-Plate digestion with trypsin-linked dCNTs and 12-h In-Tube digestion with common used trypsin followed by MALDI-TOFMS analysis.

Cytochrome c (AC P00004)			**Lysozyme (AC P00698)**			**Myoglobin(AC P68082)**		
	[a]SC 84%			**SC 74%**			**SC 66%**	
MW	**On-Plate[b]**	**In-Tube[c]**	**MW**	**On-Plate**	**In-Tube**	**MW**	**On- Plate**	**In-Tube**
1168.57	+[d]	+	874.42	+	+	748.43	+	+
1296.71	+	+	936.38	+	+	941.47	+	+
1433.77	+	+	992.50	+	+	1086.56	+	-
1478.82	+	+	1030.52	+	+	1271.66	+	+
1561.87	+	+	1045.54	+	+	1360.76	+	-
1606.91	+	+	1268.61	+	+	1378.84	+	+
1633.82	+	+	1276.65	+	-	1506.94	+	+
1712.82	-[e]	+	1428.65	+	+	1606.85	+	+
1735.01	+	+	1474.76	+	-	1651.92	+	-
1761.92	+	+	1579.86	+	-	1661.85	-	+
1804.01	-	+	1675.80	+	+	1853.96	+	+
2081.19	+	+	1753.83	+	+	1885.02	-	+
2209.28	+	+	1803.90	+	+	1937.01	+	+
						1982.05	+	+
						2283.21	+	-

[a]SC: Sequence Coverage obtained with on-plate digestion method. [b]On-Plate digestion with trypsin-linked dCNTs. [c]In-Tube digestion with common used trypsin in solution (trypsin/substrate ratio, 1:40). [d]The detected peptides are labeled with "+". [e]The undetected peptides are labeled with "-".

Fig. S6 MALDI-TOF mass spectra of the digests of BSA (150 fmol μL^{-1}) in 25 mM NH_4HCO_3 buffer solution (pH 8.1) obtained by using 12-h in-tube digestion with different trypsin to protein ratio (a) 10:1; (b) 1:1 and (c) 1:10. For the ease of operation, the total sample volume for in-tube digestion was 50 μL, after digestion, 0.5 μL of the samples was used for MS analysis. All matched peptides were marked with "*", trypsin autolysis peaks are marked with "T".