Electronic Supporting Information

Self-assembly and cell imaging behavours of Tripeptide-naphthalenediimide and interactions with carbon nano-materials

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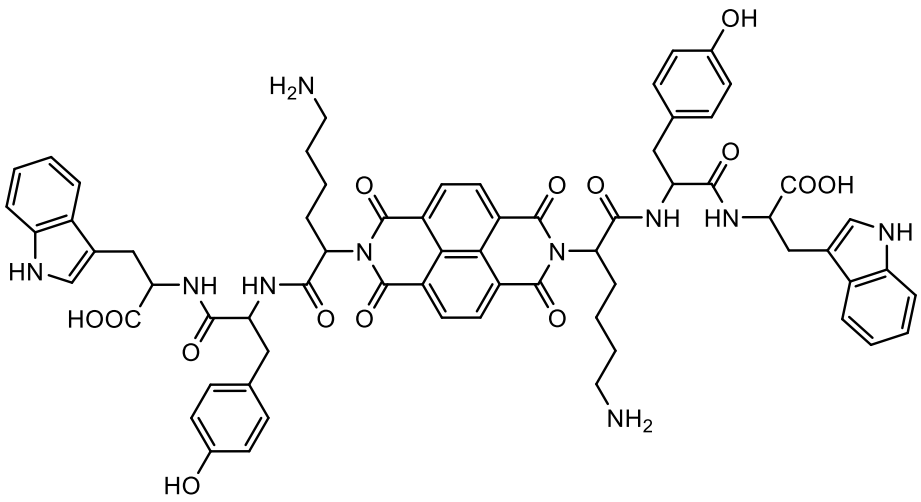
Organic Synthesis Details

General Considerations. Chemicals for solid phase peptide synthesis (O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU), Wang resin, Fmoc-amino acids) were obtained from Nanjing peptide Biotechnology Co., Ltd. All other chemicals were supplied by Energy Chemical or Aladdin and used as received.

General Synthesis of Tri-Peptides. All peptides were synthesized using standard solid phase 9-fluorenylmethoxycarbonyl (Fmoc) chemistry on a Wang resin preloaded with the Fmoc-protected leading amino acid. First the resin (the amino acid loading is 1 mmol) was soaked in 20 mL CH_2Cl_2 (DCM) to be swelled and washed with DMF.Fmoc deprotection was performed by mixing the resin in a piperidine/DMF (2:8) solution for 3 minutes (4x), then rinsing with DMF, MeOH, and DCM. For all standard amino acid couplings, 2 eq. (relative to the resin substitution) of Fmoc- protected amino acid was activated externally with 2 eq. of HBTU and 4 eq. of diisopropylethylamine (DIPEA) are dissolved in 15 mL of DCM. The activated Fmoc-protected amino acid was then added to a polypeptide solid phase synthesis tube containing the Wang resin and mixed for 1.5 hours (2x). The synthetic tube was rotated or pumped into nitrogen bubbling for sufficient reaction. The resin was then drained and rinsed with DMF, Et_2O and CH_2Cl_2 then allowed to dry. All coupling and deprotection steps were monitored by performing

a Kaiser test on a few resin beads which were removed from the synthesis tube after drying. When all amino acids were attached to the resin, washed with DMF, Et_2O and MeOH. Finally, it was dried and stored in -4 ℃ refrigerator. Cleavage from the resin and removal of side-chain protecting groups was accomplished by stirring the resin with trifluoroacetic acid (TFA), water, and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. The resin was removed by filtration and washed with the cleavage mixture. The filtrate volume was then reduced on a rotary evaporator fitted with a KOH (aq) trap and the peptide was precipitated by the addition of cold diethyl ether. Crude peptide was collected by one of two methods; filtered, rinsed with cold diethyl ether and eluted off the filter with ammonium hydroxide and lyophilized, or collected by centrifugation. The peptide was dried under high vacuum.

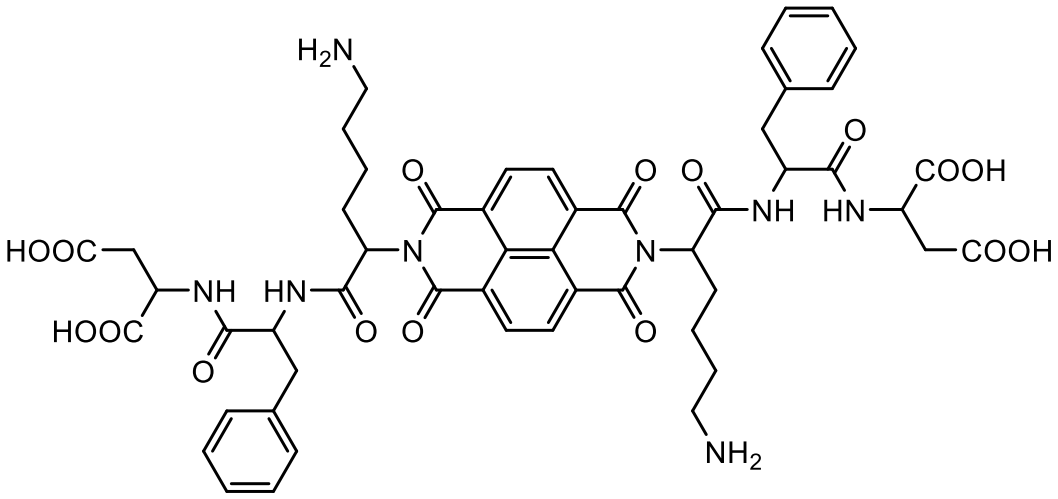
Synthesis of peptide 1: $[WYK]_2$-NDI



Peptide 1. 1,4,5,8-Naphthalenetetracarboxylic dianhydride (NDI). (0.0161 g, 0.06 mmol) was suspended in pyridine (6 mL) and added to Wang-WYK-Fmoc resin (0.2

mmol) which was deprotection with pipendine/DMF for 10 min (2x) in advance. This mixture was heated to 65 °C followed by the addition of diisopropyl ethyl amine (DIPEA, 0.523 mL, 3 mmol) and reaction was further heated to 135 °C and refluxed for 10 hours. A second portion of NDI (0.0107 g, 0.04 mmol) was added along with a second portion of pyridine (4 mL). Reaction was maintained at 135 °C and continued for an additional 10 hours. Resin was cooled to room temperature, filtered and washed with MeOH. The resin was then suspended in pyridine (10 mL), heated to 65 °C followed by the addition of DIPEA (0.523 mL, 3 mmol) and further heated to 135 °C for 6 hours. Resin was cooled to room temperature, filtered and washed with one cycle each of H_2O, CH_2Cl_2, isopropanol, MeOH, NMP, Et_2O and CH_3CN. The resin was cleaved by mixing with 20 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then reduced to 1-2 mL under reduced pressure. The concentrate was slowly added dropwise to 150 mL of cold ether and stirred. The solidwas collected by centrifugation or filtration. Dissolve the solid with ammonia water and lyophilized to yield the final light-red powder (35 mg, 0.0284 mmol, 28.43%). ^{1}H-NMR (d_6-DMSO, 400 MHz) δ: 10.86 (1H, s), 9.09 (1H, s), 8.65 (6H, s), 8.21 (3H, s), 7.55 (6H, d, *J*=7.9 Hz), 7.35 (1H, d, *J*=8.6 Hz), 7.21 (1H, s), 7.12-6.96 (2H, m), 6.79 (2H, d, *J*=8.1 Hz), 5.33 (2H, s), 4.45 (4H, s), 3.24-3.14 (4H, m), 2.71 (6H, s), 2.26 (1H, s), 2.07 (1H, s), 1.86 (2H, s), 1.51 (5H, s), 1.24 (4H, s), 1.02 (3H, dd, *J*=26.3, 6.6 Hz).

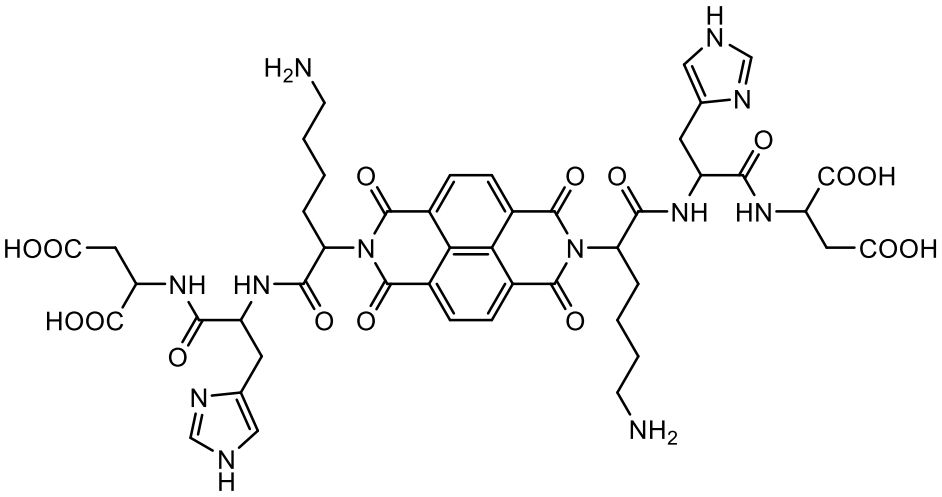
Synthesis of peptide 2: $[DFK]_2$-NDI



Peptide 2. NDI (0.0161 g, 0.06 mmol) was suspended in pyridine (6 mL) and added to Wang-DFK-Fmoc resin (0.2 mmol) which was deprotection with pipendine/DMF for 10 min (2x) in advance. This mixture was heated to 65 °C followed by the addition of diisopropyl ethyl amine (DIPEA, 0.523 mL, 3 mmol) and reaction was further heated to 135 °C and refluxed for 10 hours. A second portion of NDI (0.0107 g, 0.04 mmol) was added along with a second portion of pyridine (4 mL). Reaction was maintained at 135 °C and continued for an additional 10 hours. Resin was cooled to room temperature, filtered and washed with MeOH. The resin was then suspended in pyridine (10 mL), heated to 65 °C followed by the addition of DIPEA (0.523 mL, 3 mmol) and further heated to 135 °C for 6 hours. Resin was cooled to room temperature, filtered and washed with one cycle each of H_2O, CH_2Cl_2, isopropanol, MeOH, NMP, Et_2O and

CH_3CN. The resin was cleaved by mixing with 20 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then reduced to 1-2 mL under reduced pressure. The concentrate was slowly added dropwise to 150 mL of cold ether and stirred. The solidwas collected by centrifugation or filtration. Dissolve the solid with ammonia water and lyophilized to yield the final gray-white powder (36 mg, 0.0343 mmol, 34.32%). ^{1}H-NMR (d_6-DMSO, 400 MHz) δ: 8.74 (2H, s), 8.17 (1H, d, *J*=8.3 Hz), 7.65 (4H, s), 7.25 (5H, dd, *J*=20.9, 7.0 Hz), 5.33 (1H, s), 4.58-4.52 (1H, m), 4.35 (1H, s), 3.03 (1H, d, *J*=12.0 Hz), 2.90 (1H, s), 2.74 (3H, s), 2.56 (1H, d, *J*=4.6 Hz), 2.42 (2H, d, *J*=17.4 Hz), 2.24 (1H, s), 1.50 (2H, s), 1.27 (1H, d, *J*=6.3 Hz), 1.19 (2H, s).

Synthesis of peptide 3: $[DHK]_2$-NDI



Peptide 3. NDI (0.0161 g, 0.06 mmol) was suspended in pyridine (6 mL) and added to Wang-DHK-Fmoc resin (0.2 mmol) which was deprotection with pipendine/DMF for 10 min (2x) in advance. This mixture was heated to 65 °C followed by the addition of diisopropyl ethyl amine (DIPEA, 0.523 mL, 3 mmol) and reaction was further heated to 135 °C and refluxed for 10 hours. A second portion of NDI (0.0107 g, 0.04 mmol) was added along with a second portion of pyridine (4 mL). Reaction was maintained at 135 °C and continued for an additional 10 hours. Resin was cooled to room temperature, filtered and washed with MeOH. The resin was then suspended in pyridine (10 mL), heated to 65 °C followed by the addition of DIPEA (0.523 mL, 3 mmol) and further heated to 135 °C for 6 hours. Resin was cooled to room temperature, filtered and washed with one cycle each of H_2O, CH_2Cl_2, isopropanol, MeOH, NMP, Et_2O and CH_3CN. The resin was cleaved by mixing with 20 mL of a mixture of trifluoroacetic acid (TFA), water and triisopropylsilane (TIPS) (95:2.5:2.5) for 2 hours. Resin was filtered and washed with 5 mL of the cleavage mixture. The filtrate volume was then

reduced to 1-2 mL under reduced pressure. The concentrate was slowly added dropwise to 150 mL of cold ether and stirred. The solid was collected by centrifugation or filtration. Dissolve the solid with ammonia water and lyophilized to yield the final black-brown powder (31 mg, 0.0301 mmol, 30.13%). ^{1}H-NMR (d_6-DMSO, 400 MHz) δ: 14.33 (5H, s), 12.78 (5H, s), 8.95 (2H, s), 8.66 (4H, s), 8.43 (2H, d, *J*=8.2 Hz), 8.23 (2H, d, *J*=8.0 Hz), 7.71 (7H, s), 6.91 (2H, d, *J*=13.2 Hz), 5.45 (2H, dd, *J*=8.7 Hz), 4.76 (2H, s), 4.56 (2H, d, *J*=7.3 Hz), 3.54-3.36 (3H, m), 3.02 (2H, d, *J*=15.2 Hz), 2.76 (2H, dd, *J*=15.9, 10.5 Hz), 2.72 (9H, s), 2.64 (2H, dd, *J*=16.7, 6.6 Hz), 2.29 (2H, s), 2.08 (1H, s), 1.87 (3H, d, *J*=11.4 Hz), 1.54 (4H, s), 1.23 (2H, d, *J*=9.5 Hz), 1.08 (4H, dt, *J*=14.0, 7.0 Hz).

Characterization Details

HPLC. HPLC was performed on the Agilent1200 DAD HPLC system equipped with the InerSustain 5 μm C-18 (250 x 4.6 mm) column with a flow rate of 1 mL/min. Mobile phase A was water, mobile phase B was MeOH. The gradient was T = 0 min, B = 10%; T = 5 min, B = 10%; T = 30 min, B = 70%; T = 35 min, B = 70%; T = 40 min, B = 10%; T = 50 min, B = 10%.

UV-Vis. UV-Vis spectra were recorded using a UV2600 dual beam UV-Vis spectrophotometer. Samples were dissolved in acidic and alkaline solutions (0.05 wt%, 10 μL 1 M HCl into 3 mL and 10 μL 1 M NaOH into 3 mL). The sample pool is 5 mL quartz sample pool with wavelength scanning range of 190 nm~600 nm.

Circular Dichroism (CD). CD measurements were recorded at 20 °C using a Chirascan spectropolarimeter, the British applied light physics company. Samples were dissolved in acidic and alkaline solutions (0.5 wt%, 10 μL 1 M HCl into 3 mL and 10 μL 1 M NaOH into 3 mL). The sample pool was composed of 200 μL quartz sample pool with a scanning wavelength ranging from 200 nm to 400 nm.

Scanning Electron Microscope (SEM). The type of equipment used was ZEISS GEMINI 300 series field emission scanning electron microscope. Products were prepared for SEM as follows: Dissolved in acidic and alkaline solutions (0.5 wt%, 10 μL 1 M HCl into 3 mL and 10 μL 1 M NaOH into 3 mL). One or two drops of the solution were dropped onto the sample stage and the samples were frozen overnight at -10 °C. The samples were freeze-dried for 10 hours before scanning electron microscope.

HPLC Spectra

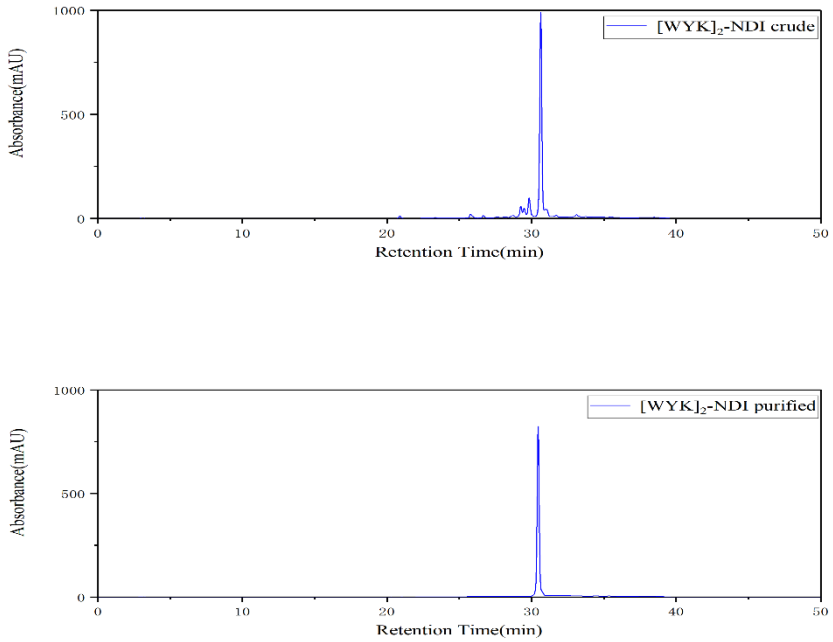


Fig. S1. HPLC of $[WYK]_2$-NDI.

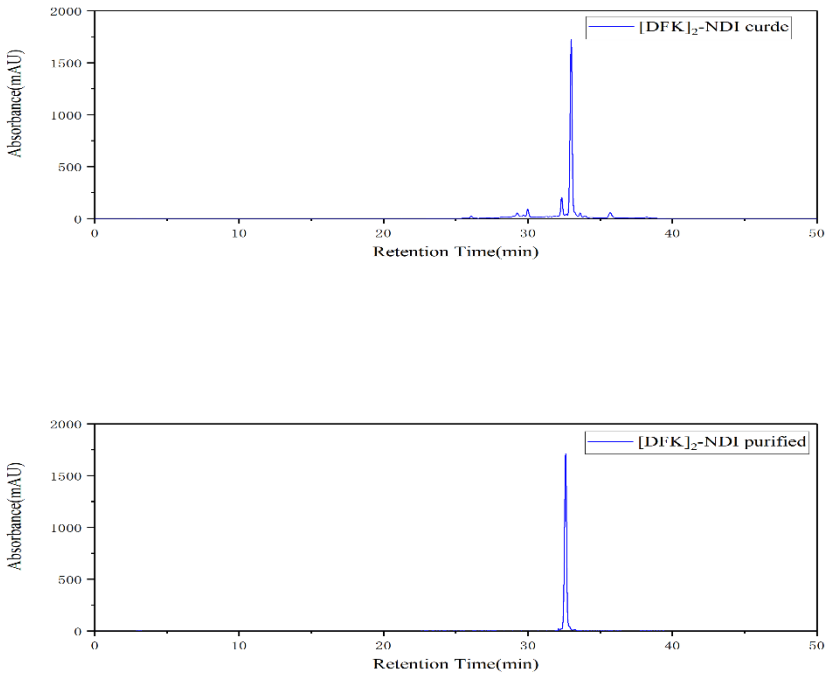


Fig. S2. HPLC of $[DFK]_2$-NDI.

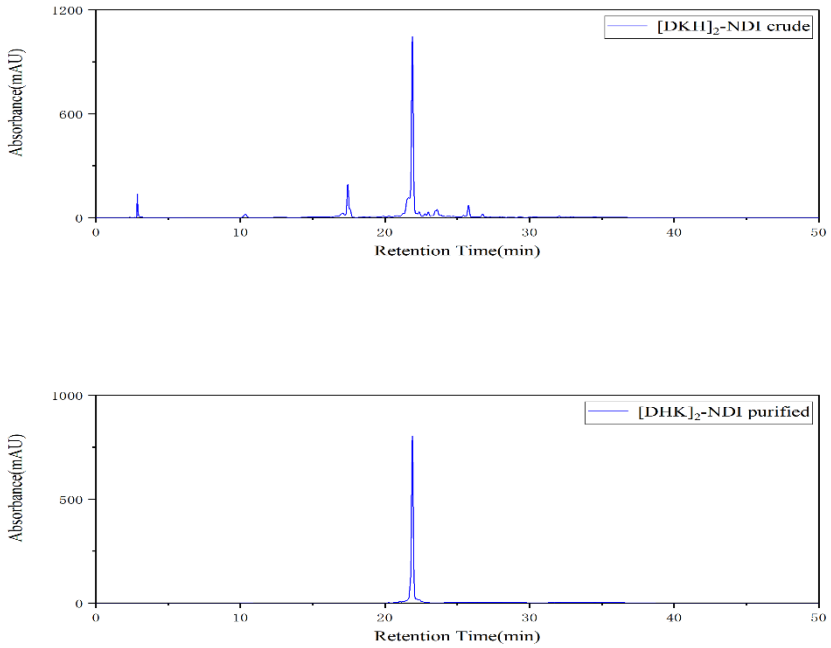


Fig. S3. HPLC of $[DHK]_2$-NDI.

NMR Spectra

$[WYK]_2$-NDI

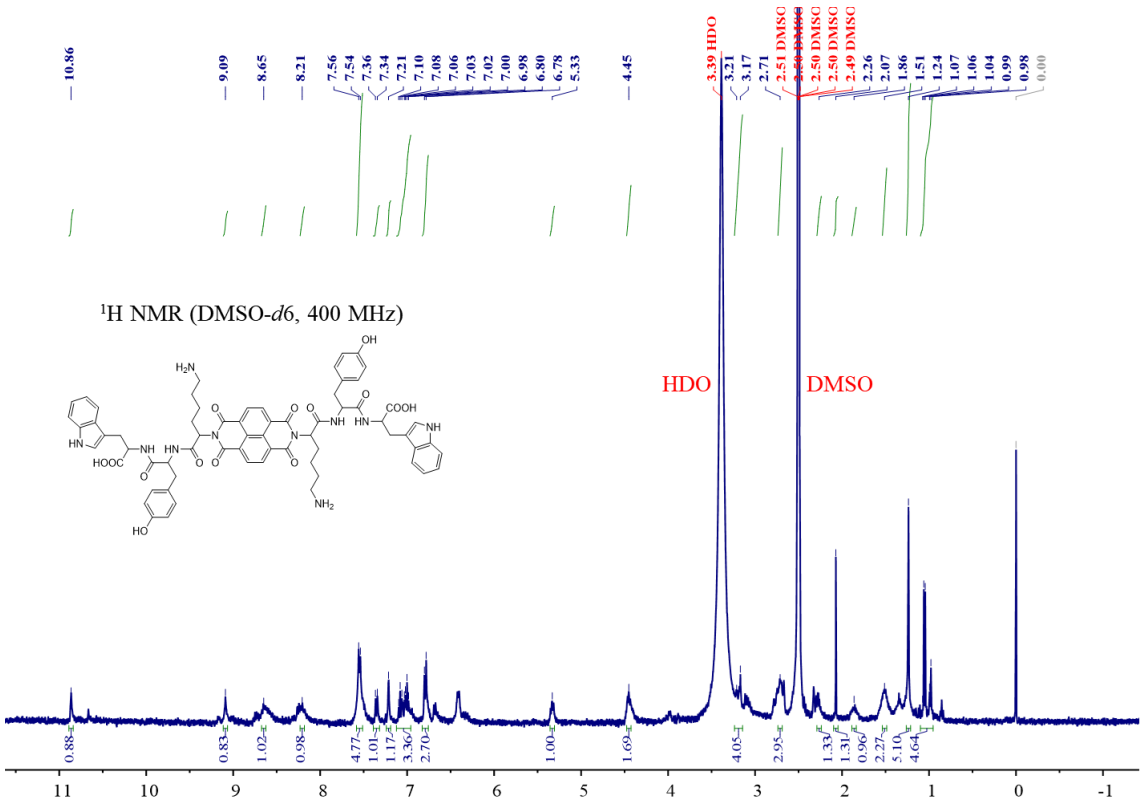


Fig. S4. [1]H NMR (d_6-DMOS) of [WYK]$_2$-NDI. A list of data is located on page S3.

[DFK]$_2$-NDI

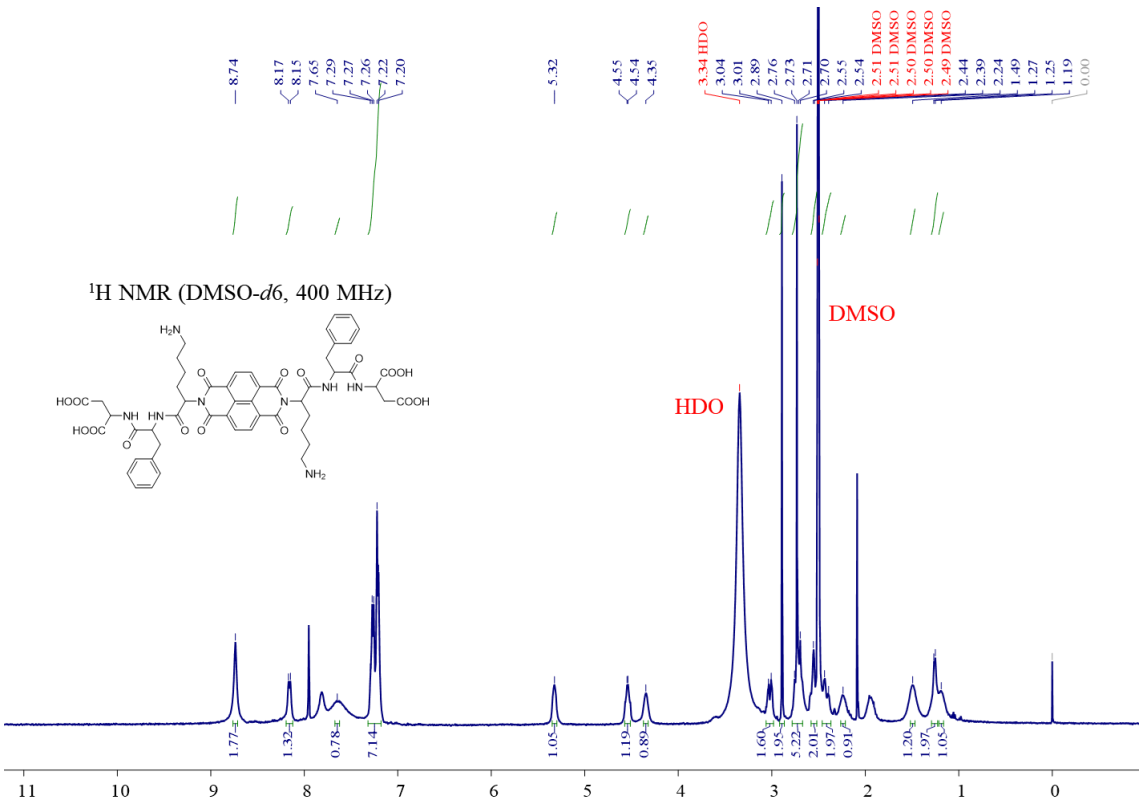


Fig. S5. ^{1}H NMR (d_6-DMOS) of [DFK]$_2$-NDI. A list of data is located on page S5.

[DHK]$_2$-NDI

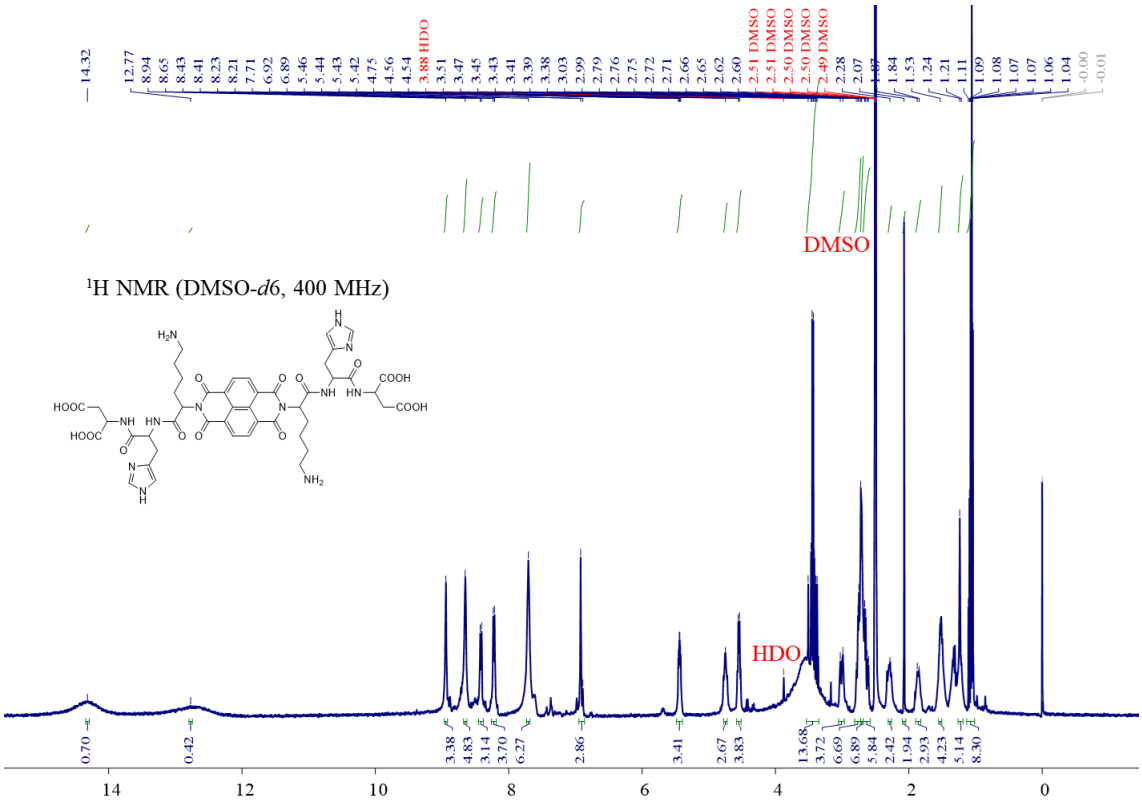


Fig. S6. ^{1}H NMR (d_6-DMOS) of [DHK]$_2$-NDI. A list of data is located on page S7.

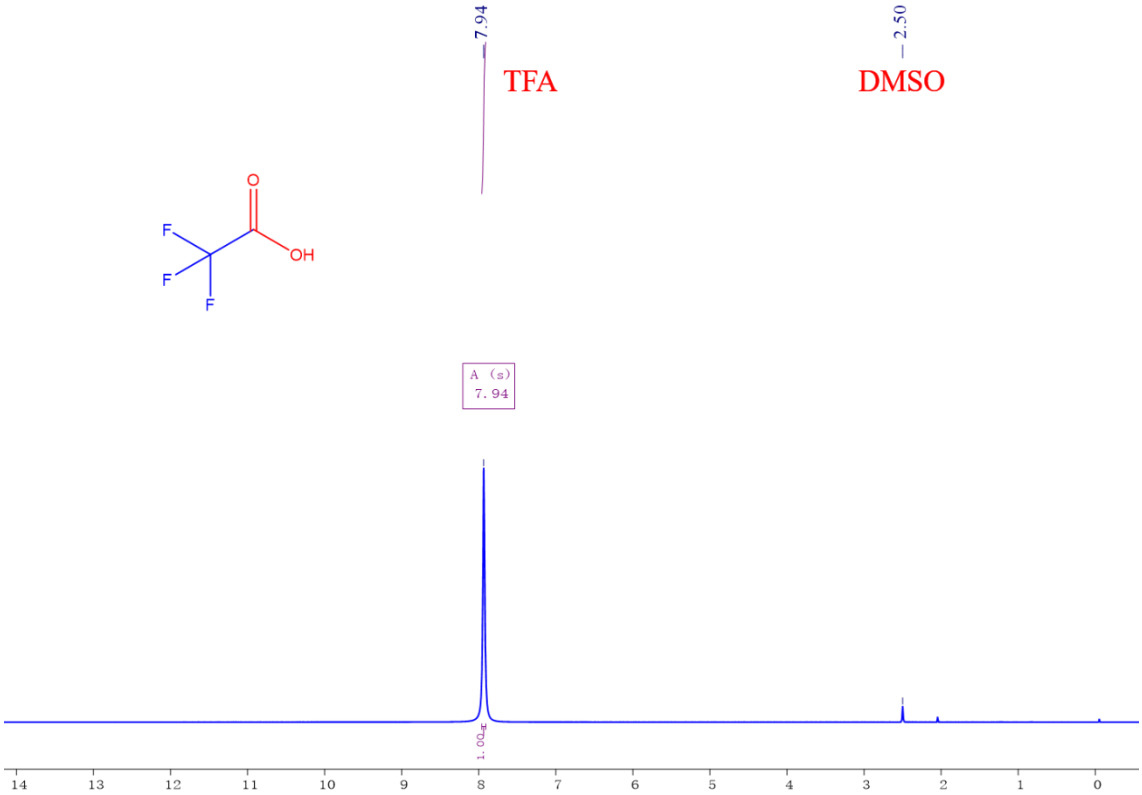


Fig. S7. ^{1}H NMR (d_6-DMOS) of TFA. (^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (s, 1H)).

SEM Imaging

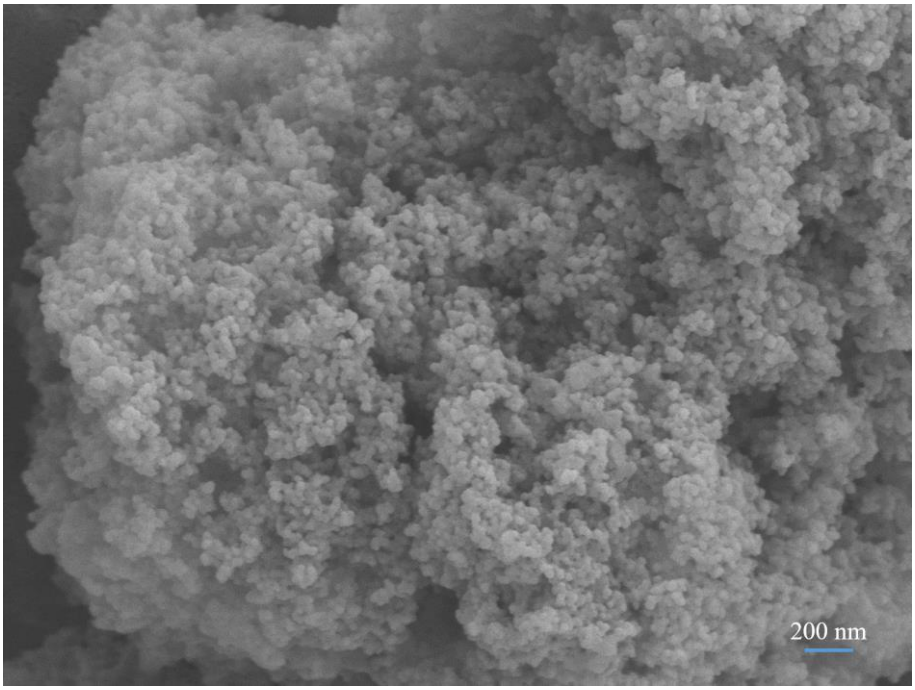


Fig. S8. SEM of $[WYK]_2$-NDI in acid solution (0.05 wt% in water, 10 μL 1 M HCl into 3 mL).

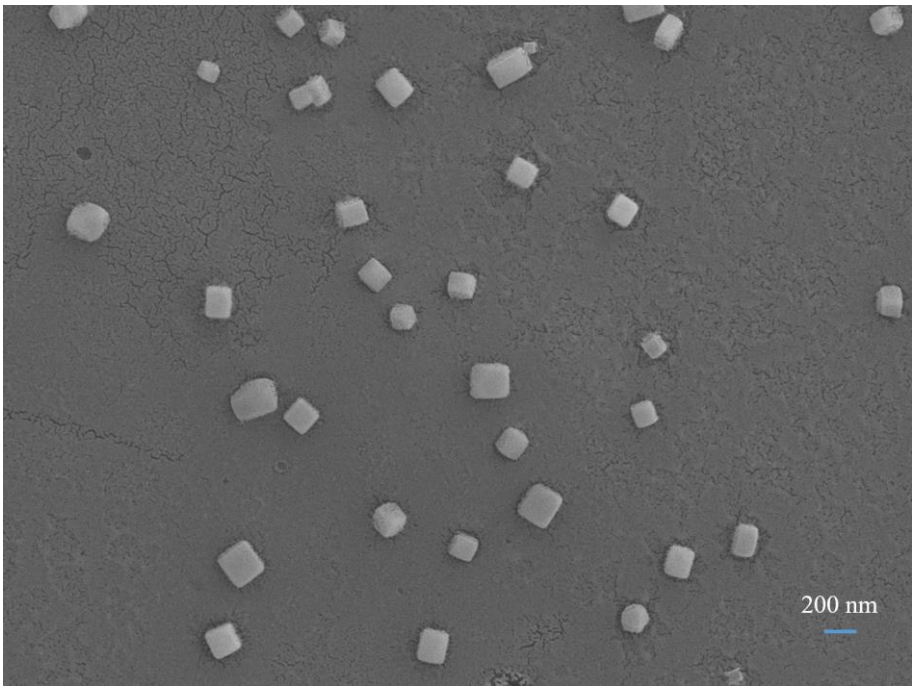


Fig. S9. SEM of $[DFK]_2$-NDI in acid solution (0.05 wt% in water, 10 μL 1 M HCl into 3 mL).

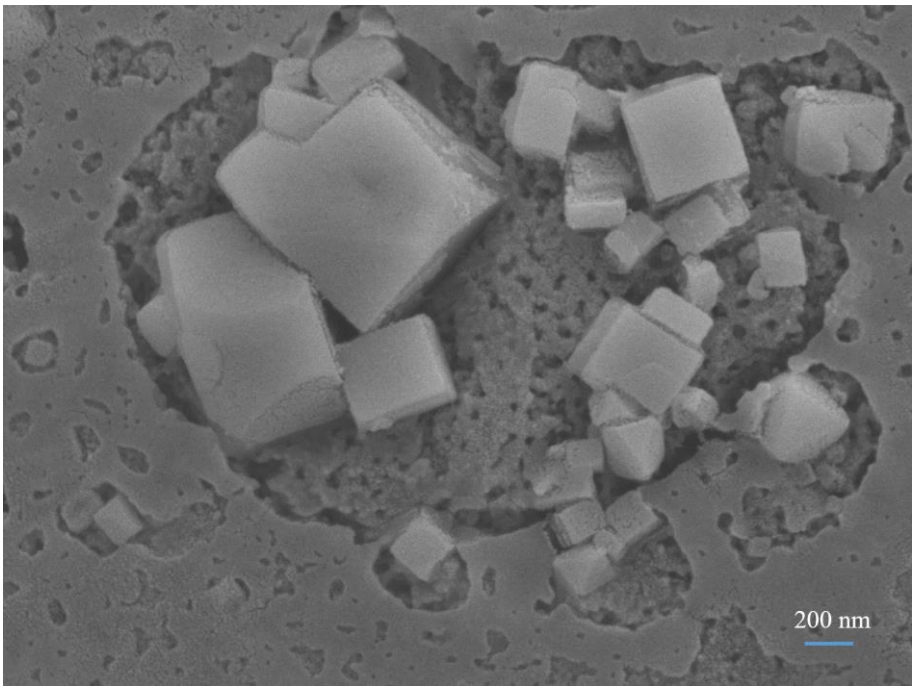


Fig. S10. SEM of $[DHK]_2$-NDI in acid solution (0.05 wt% in water, 10 μL 1 M HCl into 3 mL).

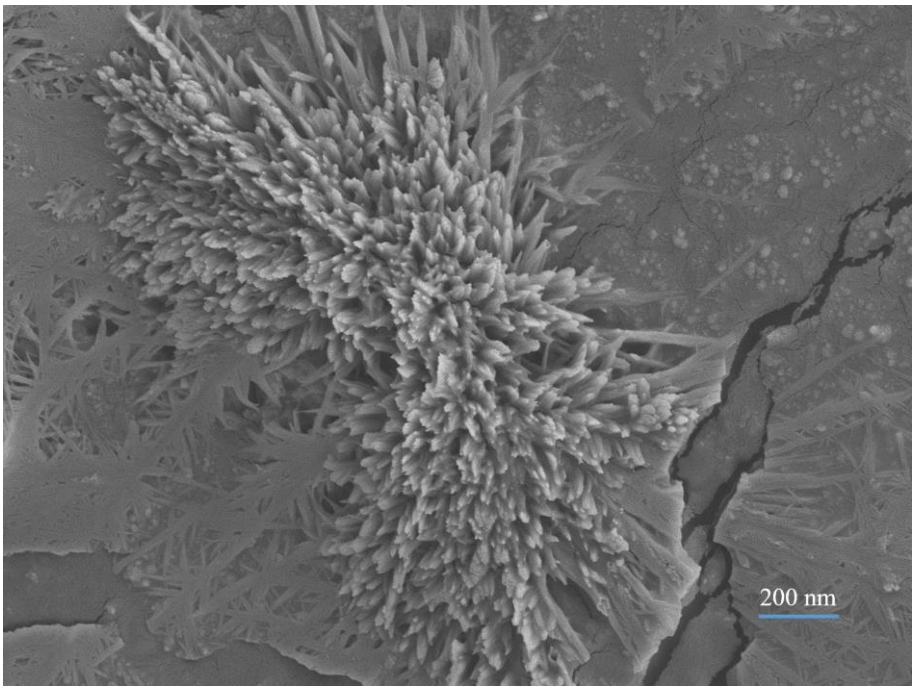


Fig. S11. SEM of $[WYK]_2$-NDI in basic solution (0.05 wt% in water, 10 μL 1 M NaOH into 3 mL).

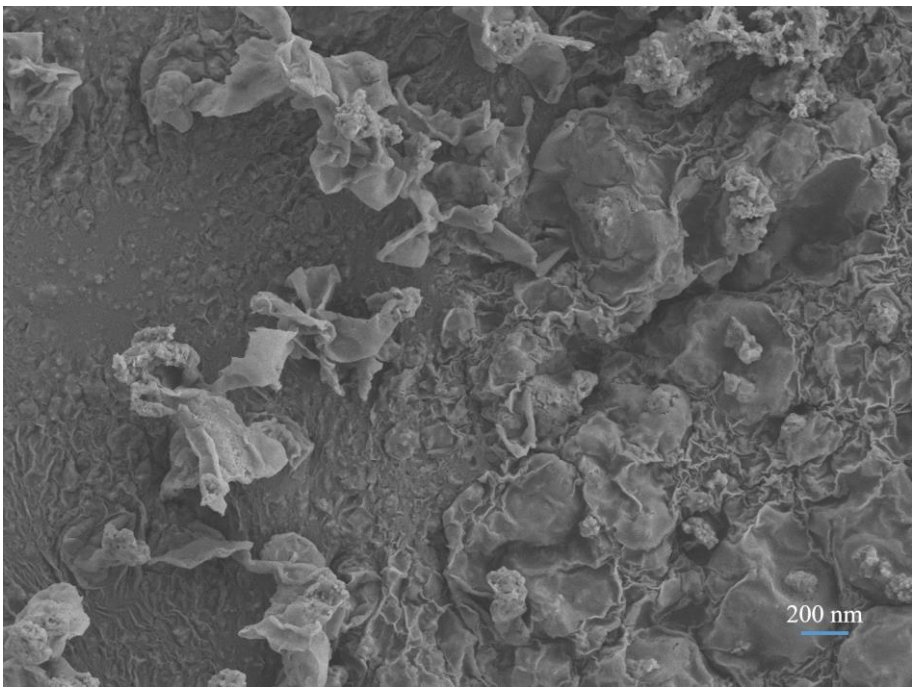


Fig. S12. SEM of $[DFK]_2$-NDI in basic solution (0.05 wt% in water, 10 μL 1 M NaOH

into 3 mL).

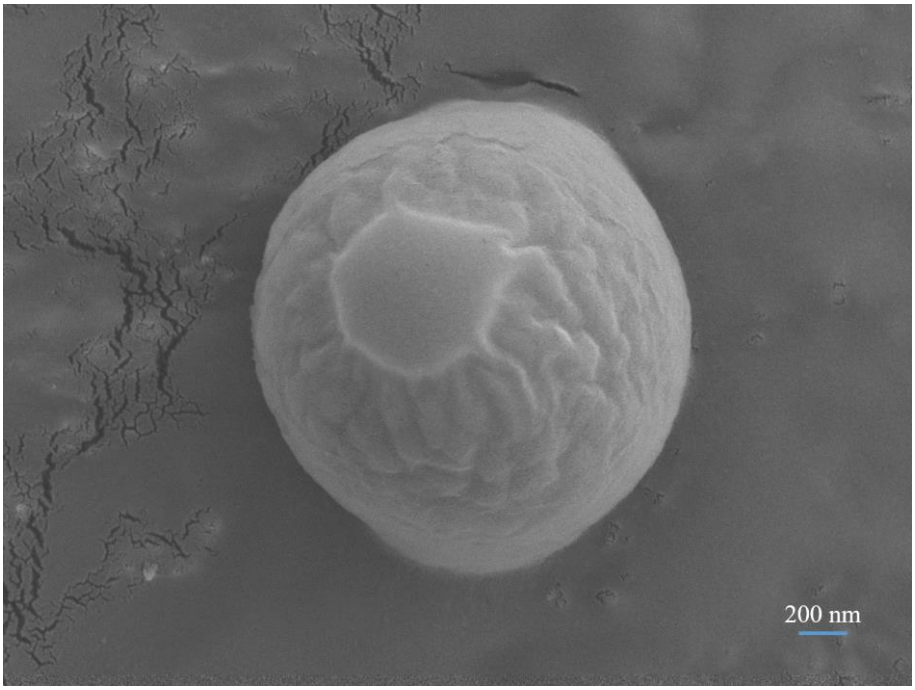


Fig. S13. SEM of $[DHK]_2$-NDI in basic solution (0.05 wt% in water, 10 μL 1 M NaOH into 3 mL).

The Combined With Carbon Materials

20 mg of NDI product was dissolved in 20 mL of chloroform, and 5 mg of EG (SWNT) was dispersed in 5 mL of absolute ethanol. After ultrasonic dispersion for 1 hour, the two solutions were mixed for 1 hour, and the ultrasonic process should be performed at a lower power as much as possible, or the ultrasonic time should be extended as appropriate. The resulting solution was suction filtered with a 0.22 μm organic filter and washed three times with a mixed solution of trichloromethane and absolute ethanol (volume ratio 4:1). The collected solids were vacuum dried at low temperature, which is the NDI@EG (NDI@SWNT) complex.

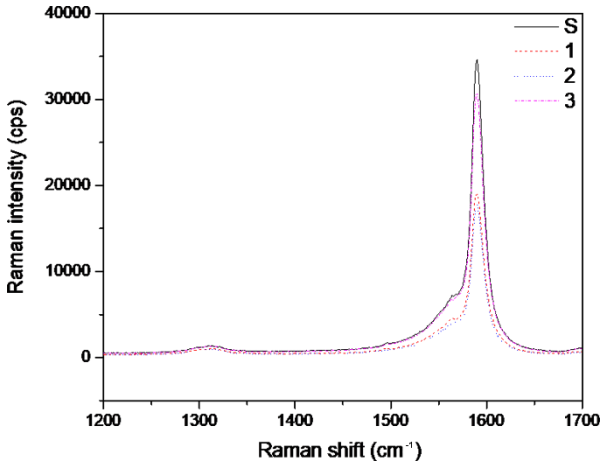


Fig. S14. Raman spectroscopy of purified SWNT and NDI-functionalized SWNT. Showing D and G bands for pure SWNT and product 1, 2, 3 ($CHCl_3$: EtOH 4:1, λ_{ex}= 532 nm).

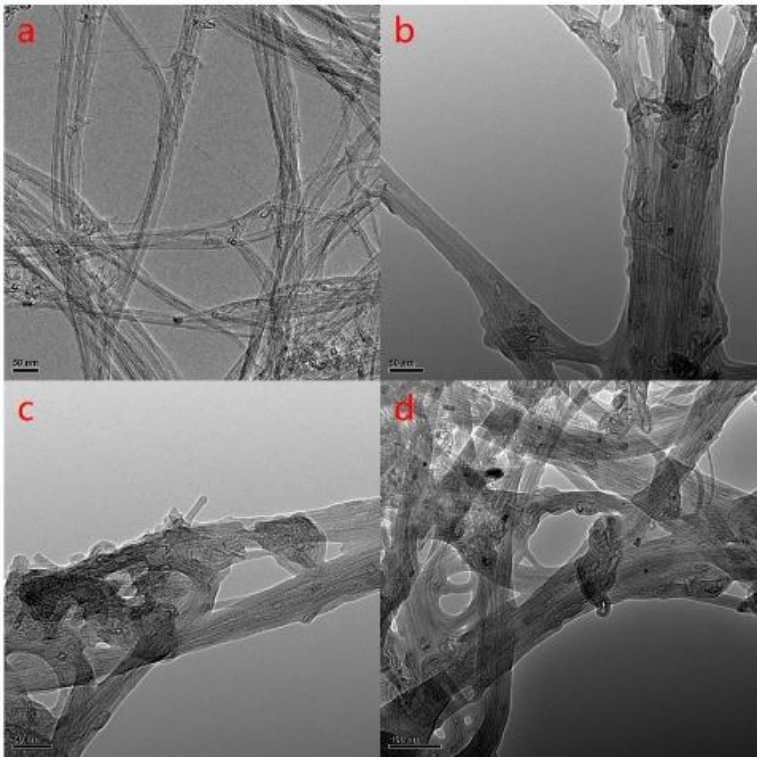


Fig. S15. (a) TEM images of pure SWNT distributed in $CHCl_3$: EtOH (4:1). (b) (c) (d) TEM images of NDI@SWNT for product 1, 2, 3 in $CHCl_3$: EtOH (4:1).

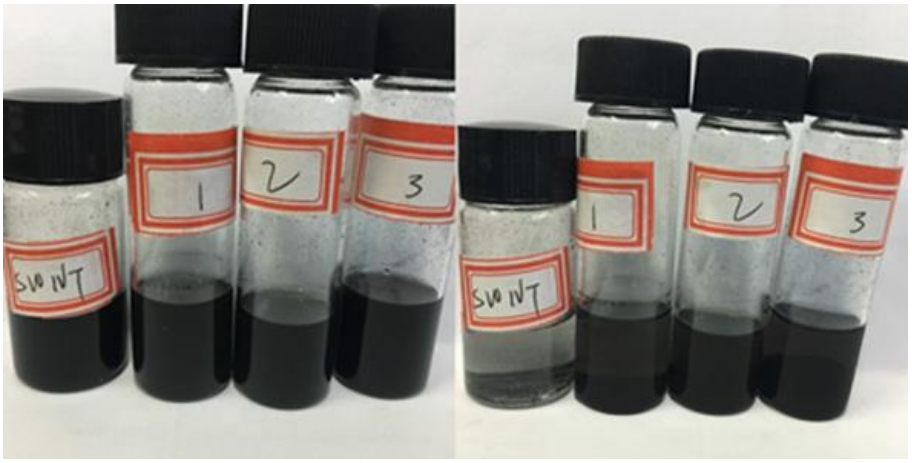


Fig. S16. Composites of three products and carbon nanotubes. Left) Ultrasonic

dispersion. Right) Place for a while. After a period of time, the dispersion of composite product 1, 2, 3 is better than that of pure carbon nanotubes.

Cell Fluorescence Imaging

Fluorescence Absorption and Fluorescence Emission Spectrum.

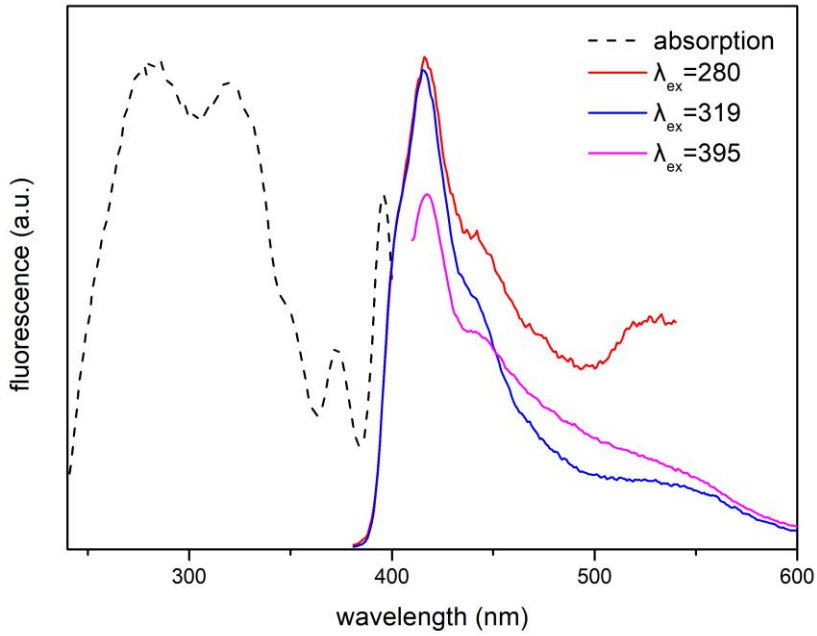


Fig. S17. Fluorescence absorption (dashed line) and fluorescence emission (solid line) spectra of $[DFK]_2$-NDI (0.05 wt% in acid solution).

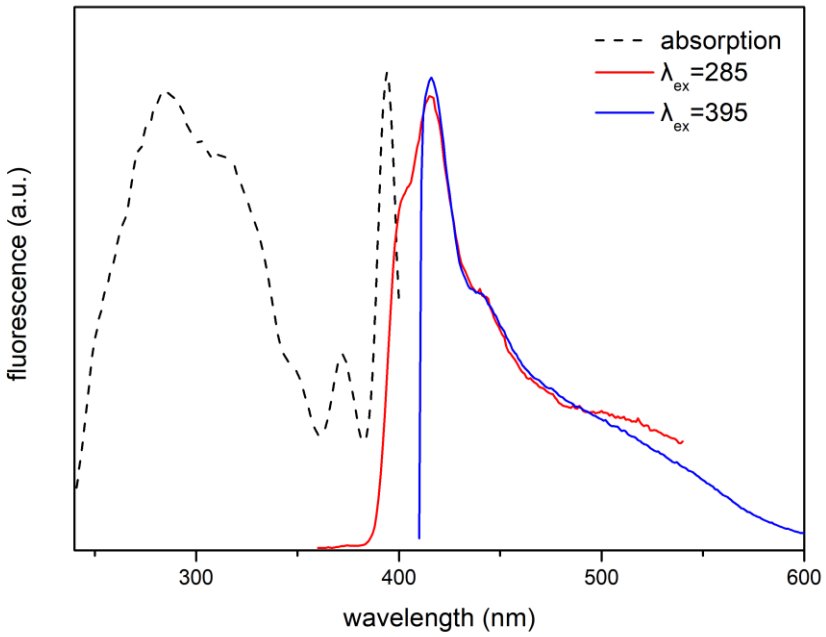


Fig. S18. Fluorescence absorption (dashed line) and fluorescence emission (solid line) spectra of $[DHK]_2$-NDI (0.05 wt% in acid solution).

PC-3 Cell Fluorescence Imaging. In general PC-3 cells were cultured at 37 ℃ in a humidified atmosphere of 5% CO_2 in air and split once confluence had been reached. Cells were cultured in EMEM medium (Eagle's Modified Essential Medium) with 15% foetal calf serum (FCS), 200 U mL^{-1} L-glutamine and 100 U mL^{-1} penicillin. The medium contained no fluorescent indicator dyes such as phenol red and was therefore suitable for use in fluorescent studies. Samples for live cells fluorescence imaging were prepared as described below. Surplus supernatant after culturing (containing dead cell matter and excess proteins) was discarded. The live adherent cells were then washed with two 5 mL aliquots of phosphate buffer saline solution to remove any remaining

medium containing FCS (since this contains protease inhibitors which inactivate trypsin, thus inhibiting the resuspension of the cells). To re-suspend the cells in solution, they were incubated in 3 mL of trypsin-EDTA (500 mg L^{-1} trypsin, 200 mg L^{-1} EDTA) solution for 5 min at 37 ℃. After trypsinising, fresh EMEM was added to the suspended cells to give a sufficient concentration of cells (ca. 50,000 cells/mL). The concentration of cells required varies between cell lines and is chosen to be optimal for achieving sufficient coverage and optimal imaging. Cells were maintained at 37 °C in a 5% CO_2 humidified atmosphere and grown to approximately 85% confluence before being split using 2.5% trypsin. For microscopy, cells were seeded onto chambered coverglass slides and incubated for 12 h to ensure adhesion. Prior to imaging, the solution was replaced with 1 mL fresh EMEM. Background autofluorescence was measured by imaging the cells in 1 mL of EMEM medium only. Final concentrations on cell plates used was 10 μM and the solution was DMSO.

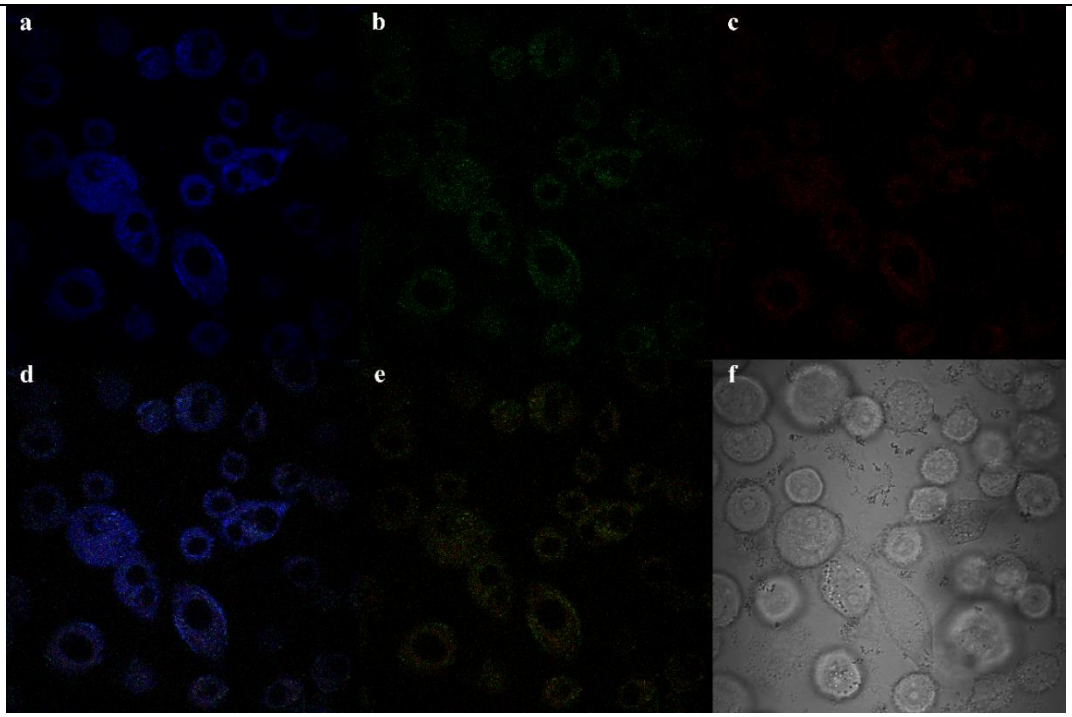


Fig. S19. Single-photon laser-scanning confocal microscopy of PC-3 cells incubated for 20 min at 37 ℃ with $[DHK]_2$-NDI (10 mM in 1:99% DMSO: serum-free medium). (a) blue channel, (b) green channel, (c) red channel, (d)(e) mix channel, (f) DIC channel.