

## **Supporting Information**

### **(-)/(+)-Sparteine Induced Chirally-active Carbon Nanoparticles for Enantioselective Separation of Racemic Mixtures**

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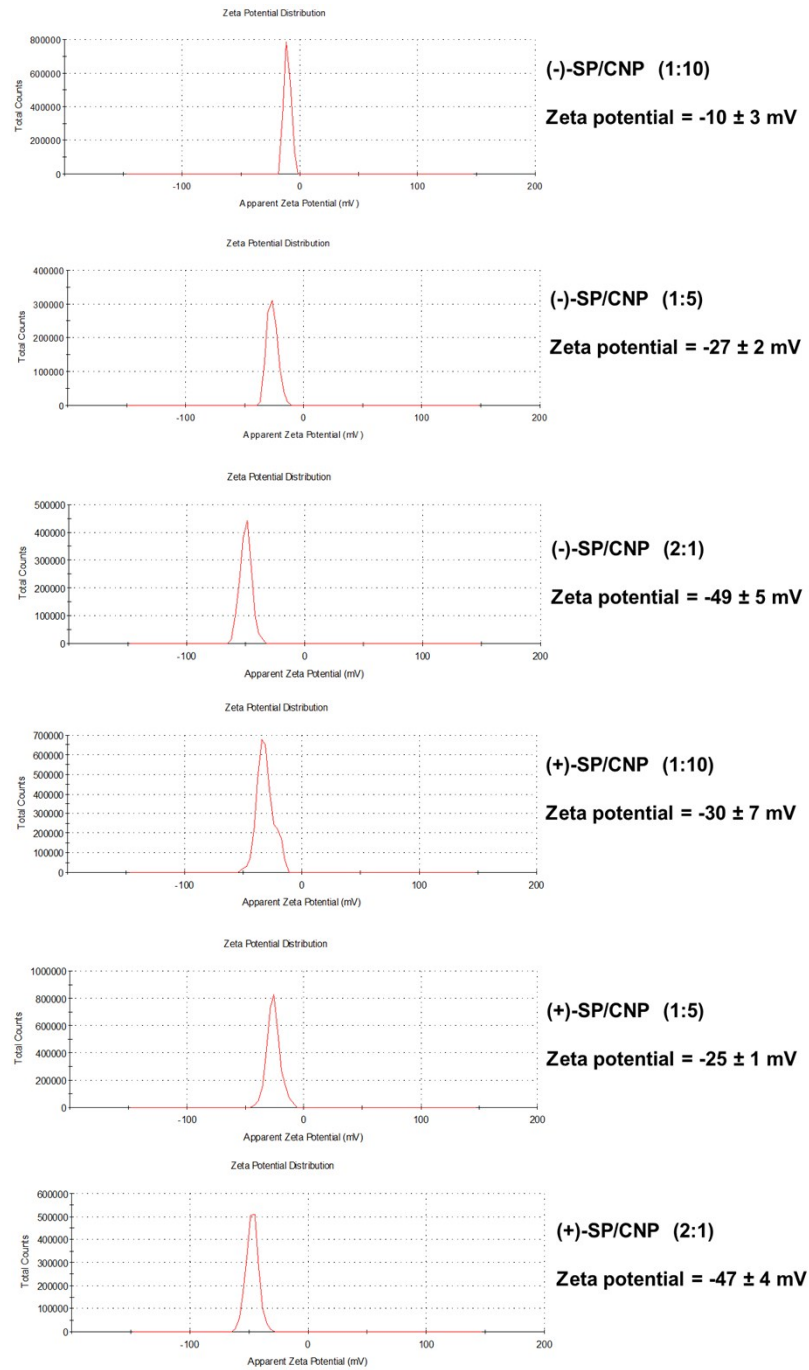
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**Table S1.** Physico-chemical characteristics of aqueous suspensions of various CCNPs<sup>a</sup>

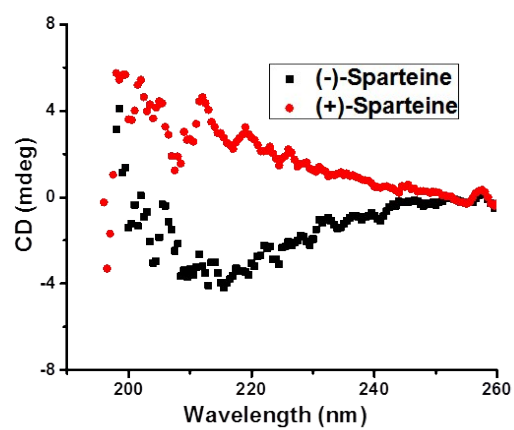
Nanoparticle formulation	(-)-or (+)- Sparteine:Sucrose ratio (w/w)	DLS (nm)	Zeta Potential (mV)	Observed optical rotation (degrees)
CNP	0:1	50 ± 7	-23 ± 4	-
(-)-SP/CNP	1:10	21 ± 5	-10 ± 3	-0.080 ± 0.003
	1:5	70 ± 10	-27 ± 2	-0.129 ± 0.005
	2:1	164 ± 8	-49 ± 5	-0.210 ± 0.006
(+) -SP/CNP	1:10	24 ± 5	-30 ± 7	+0.108 ± 0.005
	1:5	77 ± 5	-25 ± 1	+0.136 ± 0.005
	2:1	155 ± 8	-47 ± 4	+0.229 ± 0.009

<sup>a</sup>[CCNPs] = 0.5mg/mL

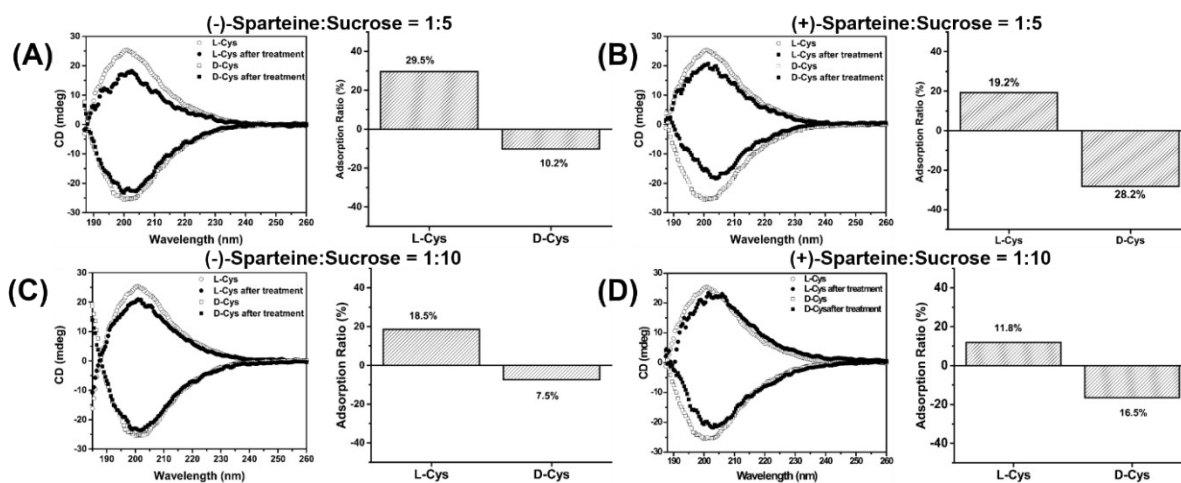


**Fig. S1.** Zeta potential plots of various

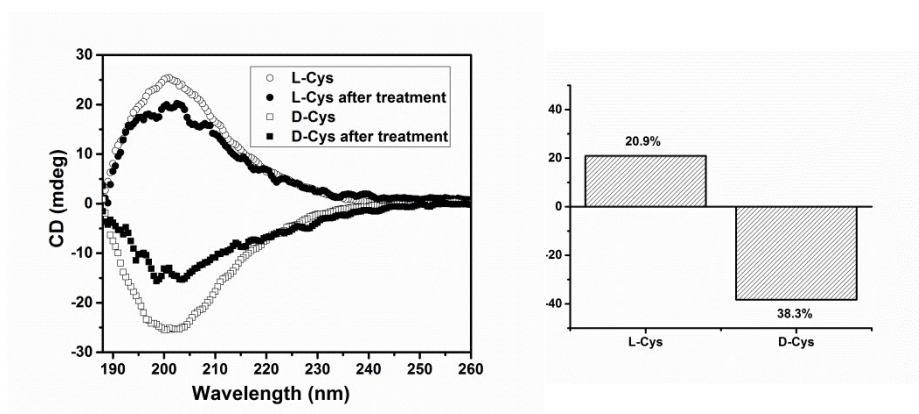
chiral carbon nanoparticle (CCNP) suspensions in aqueous medium.



**Fig. S2.** Circular dichroism of (-)-sparteine and (+)-sparteine in water.



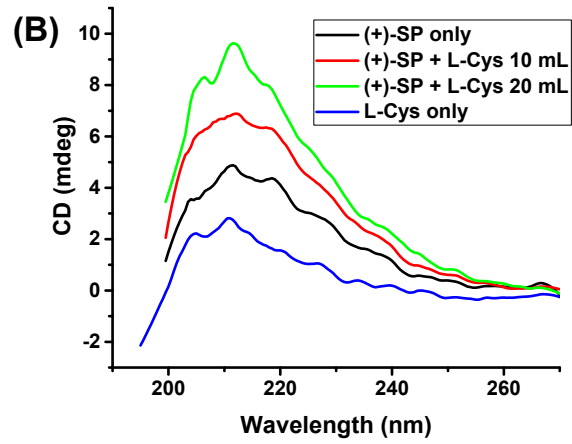
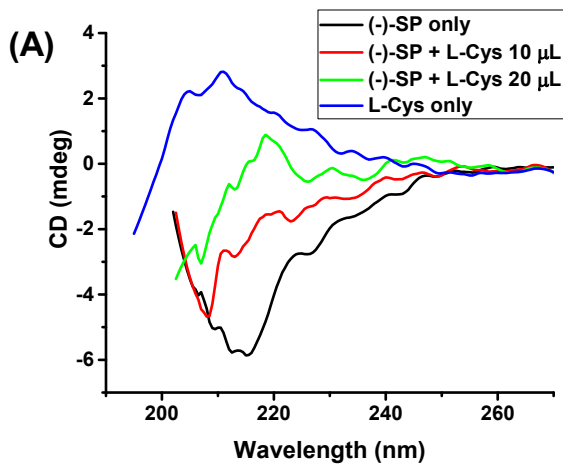
**Fig. S3.** Circular dichroism spectra and adsorption ratio of L-/D-cysteine by (-)-SP/CNP and (+)-SP/CNP. Absorbance of L- and D- cysteine solution (circles and squares, respectively) by (-)-SP/CNP (A and C) and (+)-SP/CNP (B and D). Blank symbols correspond to signals from the solution of 2 mM concentration while filled symbols correspond to signals of the spectra after treatment with (-)-SP/CNP or (+)-SP/CNP. Chiral CNPs were prepared from (-)- or (+)-sparteine:sugar = 1:5 and 1:10. Right: bar chart of the adsorption ratio for both the chiral CNPs.



**Fig. S4.** Circular dichroism spectra and adsorption ratio of L- and D-cysteine solution (circles and squares, respectively) by (+)-SP/CNP prepared from (-)-sparteine:sugar = 10:1. Blank symbols correspond to signals from the original amino acid solution of 2 mM concentration while filled symbols correspond to signals of the solution after treatment with (+)-SP/CNP. Right: bar chart of the adsorption ratio for both the chiral CNPs.

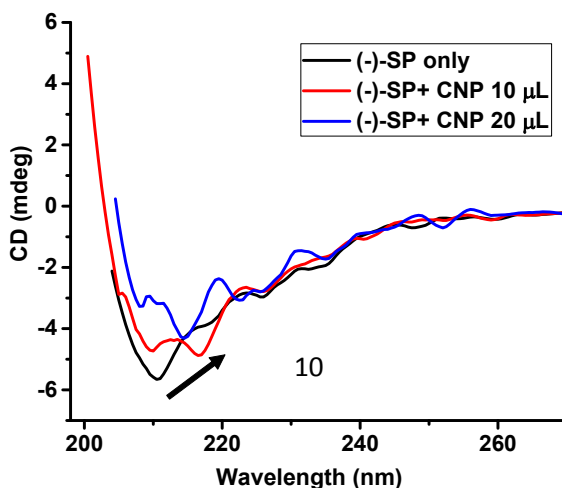






**Figure S5.** Circular dichroism of (-) Sparteine only (**A**) and (+)-Sparteine only (**B**) upon its interaction with incremental addition of L-cysteine. Concentration of (-)-/(+)-SP is 3 mM and the L-Cys stock is 5 mM. The samples were incubated for 1 h before the CD spectrum was recorded.

The selectivity could be due to the preferential chiral binding of L-cysteine or L-proline with the chiral surfaces of (-)-SP/CNP than that of (+)-SP/CNP. Similarly, D-cysteine and D-proline bind preferentially to (+)-SP/CNP. In order to probe this effect, we studied the effect on the CD spectra of (-)-sparteine upon incremental additions of L-Cysteine (**Figure S5**). We found that the peak at 215 nm for (-)-sparteine shifted to 208-209 nm after treatment with L-Cys (Fig S5A) whereas the peak of (+)-sparteine shifts from 211 nm to 212 nm, a very insignificant shift upon treatment with L-Cys demonstrating that L-cysteine has significantly higher chiral interaction with (-)-sparteine than (+)-sparteine.



**Figure S6.** Circular dichroism spectra of (-)-sparteine and its interaction with CNPs with incremental addition of CNPs (4 mg/mL stock). The samples were incubated for 1 h before the CD spectrum was recorded.

The interaction between the carbon nanoparticles and (-)-/(+)-sparteine is purely physical and electrostatic in nature, presumably arising from the tertiary amines of sparteine and surface abundant carboxylic acids groups in CNPs.<sup>7b,e,f</sup> In order to get more insight, we studied the CD of (-)-sparteine alone and incremental addition of CNPs to (-)-sparteine. We observed that the CD peak of (-)-sparteine shifted towards higher wavelengths upon interaction with CNP.

## **Materials and methods.**

Sucrose, L-cysteine, D-cysteine, L-proline and D-proline were purchased from Sigma-Aldrich (USA). (-)-Sparteine and (+)-sparteine were purchased from TCI chemicals and AK Scientific (USA) respectively.

### **Preparation of (-)-SP/CNP and (+)-SP/CNP.<sup>1</sup>**

Sucrose (1g) and (-)-sparteine or (+)-sparteine (appropriate amount, such that the final ratios of sparteine:sugar are 1:10, 1:5 and 2:1) were dissolved in 5 mL of nanopure water (0.2  $\mu$ M, 18 M $\Omega$  ·cm). Then the aqueous solution was subjected to microwave irradiation in a domestic microwave oven for 10 min. Microwave power was set at 1200 W with an output power of 50%. The resultant dark brown mass was resuspended in 5 mL nanopure water followed by probe sonication (Q700TM, Qsonica Sonicators, CT, USA) (Amp:1, On: 2 sec, Off: 1 sec). The suspension was passed through a syringe filter with a 0.2  $\mu$ m pore size and then subjected to dialysis (10000 KDa cutoff). Other ratios of sugar:sparteine (for eg 20:1 and 30:1) were also explored but resulted in poor CNP solubility in water possibly due to inadequate surface passivation of the carbon core by the chiral ligand.

### **Characterization Methods.**

#### **Dynamic Light Scattering and Zeta Potential Measurements**

The hydrodynamic diameters and zeta potential of CNPs were determined by a Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK). Scattered light was collected at a fixed angle of 90°. A photomultiplier aperture of 400 mm was used, and the incident laser power was adjusted to obtain a photon counting rate between 200 and 300 kcps. Only measurements for which the measured and calculated baselines of the intensity autocorrelation function agreed to within  $\pm 0.1\%$  were used to calculate nanoparticle

hydrodynamic diameter values. The averaged hydrodynamic diameter was obtained from the peak values of the number distribution for five successive measurements for each sample. For zeta potential measurements, the data were acquired in the phase analysis light scattering (PALS) mode following solution equilibration at 25 °C. Calculation of  $\zeta$  from the measured nanoparticle electrophoretic mobility ( $\mu$ ) employed the Smoluchowski equation:  $\mu = \varepsilon\zeta/\eta$ , where  $\varepsilon$  and  $\eta$  are the dielectric constant and the absolute viscosity of the medium, respectively. Measurements of  $\zeta$  were reproducible to within  $\pm 5$  mV of the mean value given by 20 determinations of 10 data accumulations.

### **UV-Vis and Fluorescence Measurements**

Ultraviolet-visible (UV-Vis) absorbance of CNPs was recorded via GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Scientific, MA, USA). The emission spectra of fluorescence measurements were obtained through NanoDrop 3300 Fluorospectrometer (Thermo Scientific, MA, USA). The excitation maximum was set to 360 nm and the wavelength covered an emission range between 400 and 750 nm.

### **Fourier transform infrared spectroscopy (FT-IR) measurements**

For Fourier transform infrared spectroscopy (FT-IR) measurements, the aqueous suspensions of the CNPs were drop-casted and dried onto MirrIR IR-reflective glass slides (Kevley Technologies, Chesterland, Ohio, USA). The measurements were performed using a Nicolet Nexus 670 FTI-IR (MRL facility, UIUC). For each measurement  $100 \times 100 \mu\text{m}$  images were collected at  $1 \text{ cm}^{-1}$  spectral resolution with 64 scans per pixel and a  $25 \times 25 \mu\text{m}$  pixel size and individual spectra were corrected for atmospheric contributions.

### **Raman Measurements**

All Raman measurements were taken on a Nanophoton Raman instrument (MRL facility, UIUC) with a 532 nm wavelength laser for one min at 0.2% laser power using a 20 x objective. For each spectrum a grating ( $600 \text{ l mm}^{-1}$ ) scan was taken over the range of  $120\text{--}2700 \text{ cm}^{-1}$ . An average of 20 spectra were recorded and averaged per sample.

### **<sup>1</sup>H-NMR Measurements**

<sup>1</sup>H-NMR measurements were carried out on Varian VXR 500 (Varian, Inc., Palo Alto, CA) spectrometer operating at 500 MHz equipped with 5mm Nalorac QUAD probe. Chemical shifts were reported in ppm and referenced to the solvent proton impurities. D<sub>2</sub>O was used as the deuterated solvent for all samples. A total of 256 acquisitions were made and were averaged out. The data was processed and analyzed with MestRenova™ 8.1 software (Mestrelab Research SL; Santiago de Compostela, Spain).

### **Transmission Electron Microscopy Measurements (TEM)**

The samples for transmission electron microscopy (TEM) were prepared by placing a 10  $\mu\text{L}$  aliquot of diluted stock of CNPs suspension on 200-mesh Quantifoil® holey carbon grids (Structure Probe, Inc., PA, USA) for 1 min. The excess fluid was removed by a filter paper followed by drying of the sample. TEM images were recorded on JEOL 2010 LaB6 and JEOL 2100 Cryo (JEOL Ltd., Tokyo, Japan) operating at 200 kV.

### **Atomic Force Microscopy Measurements (AFM)**

For AFM, 5 $\mu$ L of the CCNP sample was placed on clean glass and allowed to stabilize for 5 min. filter paper was used to remove liquid, and the sample was further dehydrated using vacuum chamber. AFM images were obtained using an Asylum Cypher (Asylum Research, Santa Barbara, CA, USA) with tapping mode. The surface of the glass was scanned in air using a Tap300Al-G AFM probe (Budget Sensors, Sofia, Bulgaria) at a set point of 0.63 V, a 1 Hz scan rate, and a drive frequency of 750 kHz.

### **Circular Dichroism Measurements (CD)**

Circular dichroism measurements of the aqueous suspensions were carried out in a 2 mm quartz cuvette with a Jasco 710 CD spectrometer (Mary's Court Easton, MD, USA) at room temperature. For each sample, three measurements, each consisting of 5 sub runs were performed and the average spectrum is obtained from the software provided by the manufacturer.

### **Optical Rotation by Polarimetry**

Optical rotation measurements were performed on a Jasco P-2000 digital polarimeter using a quartz cell of 10 mm path length at a wavelength of 436 nm, and all measurements were made at room temperature. For each sample, three measurements, each consisting of 10 sub runs were performed and the average observed optical rotation value is calculated by the software provided by the manufacturer.

### **Reference.**

(1) Wu, L.; Luderer, M.; Yang, X.; Swain, C.; Zhang, H.; Nelson, K.; Stacy, A. J.; Shen, B.; Lanza, G. M.; Pan, D. *Theranostics*, **2013**, *3*, 677.