Supporting Information

Spectroscopic study of the loading of cationic porphyrins by carbon nanohorns as high capacity carriers of photoactive molecules to cells

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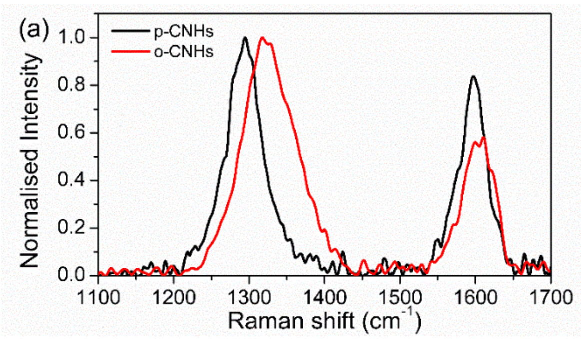

Fig. S1 (a) Raman spectra of solid samples of **p-CNH**, **o-CNH** (λ_{exc}= 785 nm).

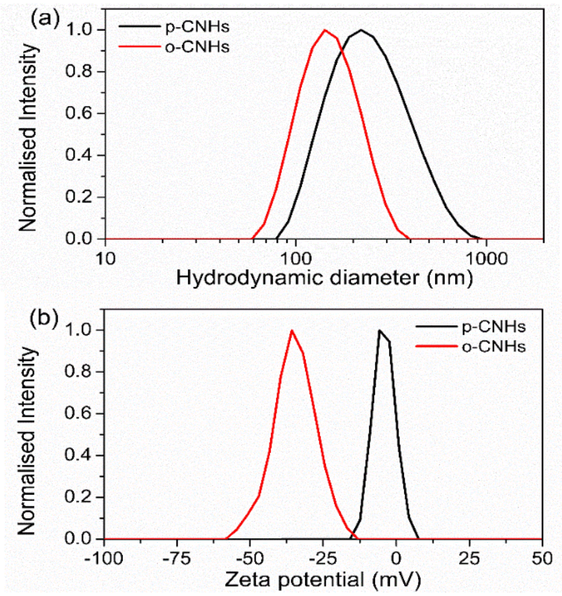

Fig. S2 (a) DLS measurements and (b) Zeta-potential measurements of **p-CNH** and **o-CNH** recorded in aqueous solution pH 7.

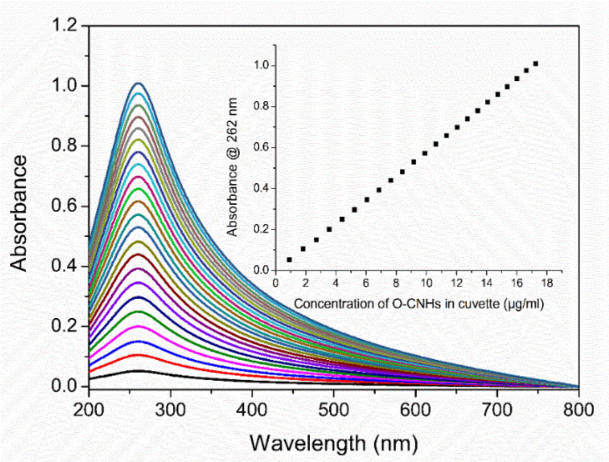

Fig. S3 UV-visible absorption spectra of **o-CNH** in water at pH 7, inset shows linear absorbance with concentration, (0-18 μg mL^{-1}), inset beer lambert plot.

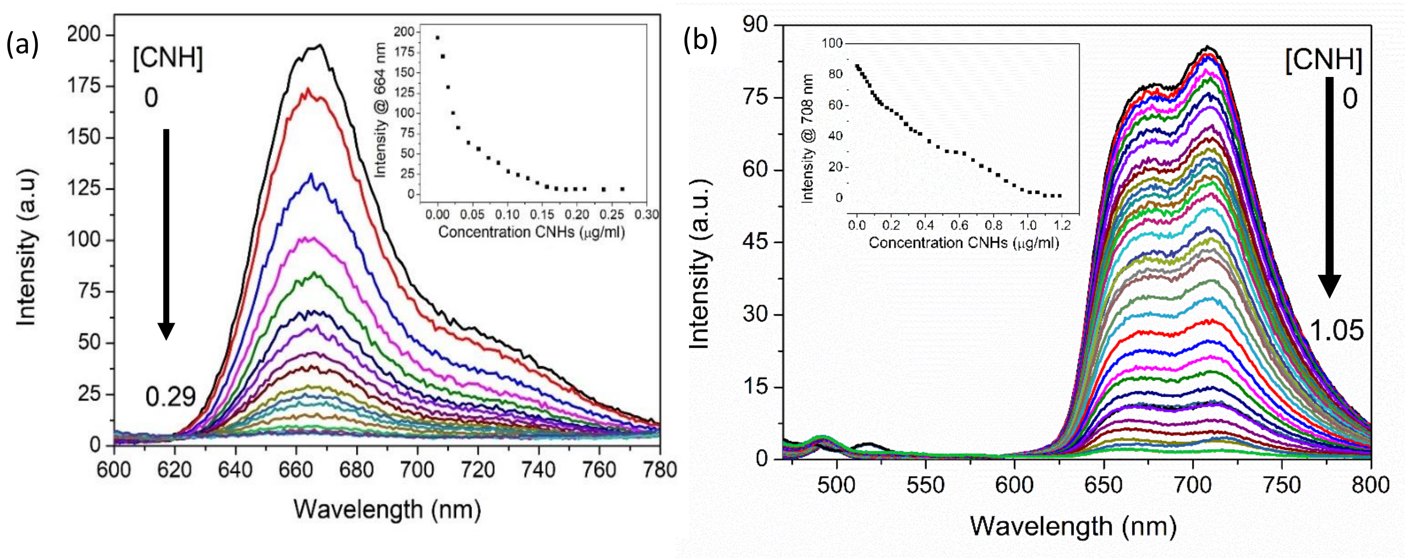

Fig. 4 Emission spectra of (a) 0.6 μM **Pt-TMPyP4** in the presence of increasing concentrations of **o-CNH** (0 – 0.29 μg mL^{-1}) λ_{ex}= 402 nm and (b) 0.4 μM **H_2-TMPyP4** in the presence of increasing concentrations of **o-CNH** (0 – 1.05 μg mL^{-1}) λ_{ex}= 422 nm, performed in in milliQ water.

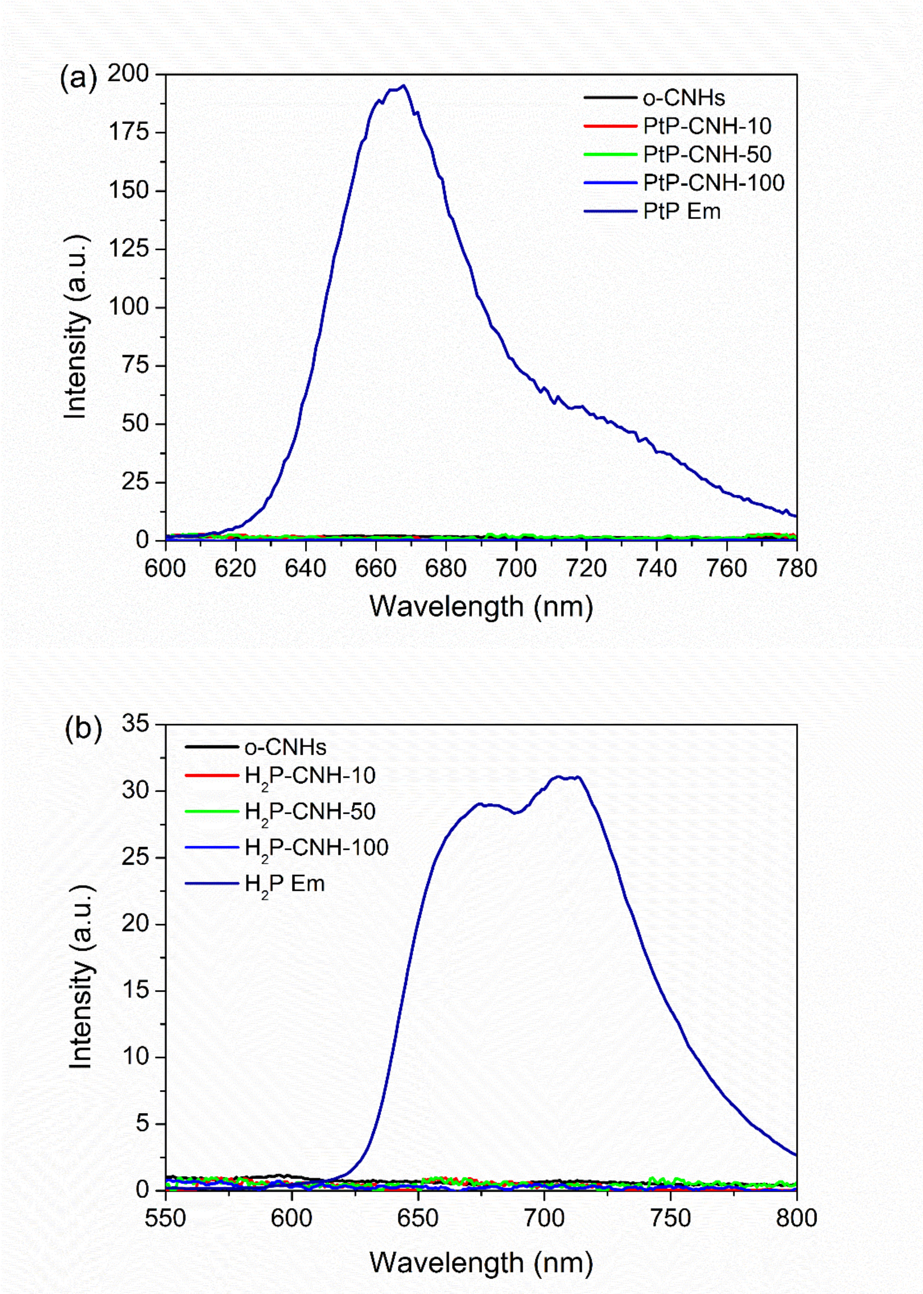

Fig. S5 Emission spectra of porphyrin prepared on a 100 µg scale. (a) **CNH-PtTMPyP4** hybrids with 100, 50 and 10 % loading of porphyrin and free **Pt-TMPyP4** λ_{ex} = 402 nm and (b) **CNH-H_2TM PyP4** hybrids with 100, 50 and 10 % loading of porphyrin free **H_2TMPyP4** λ_{ex} = 421 nm.

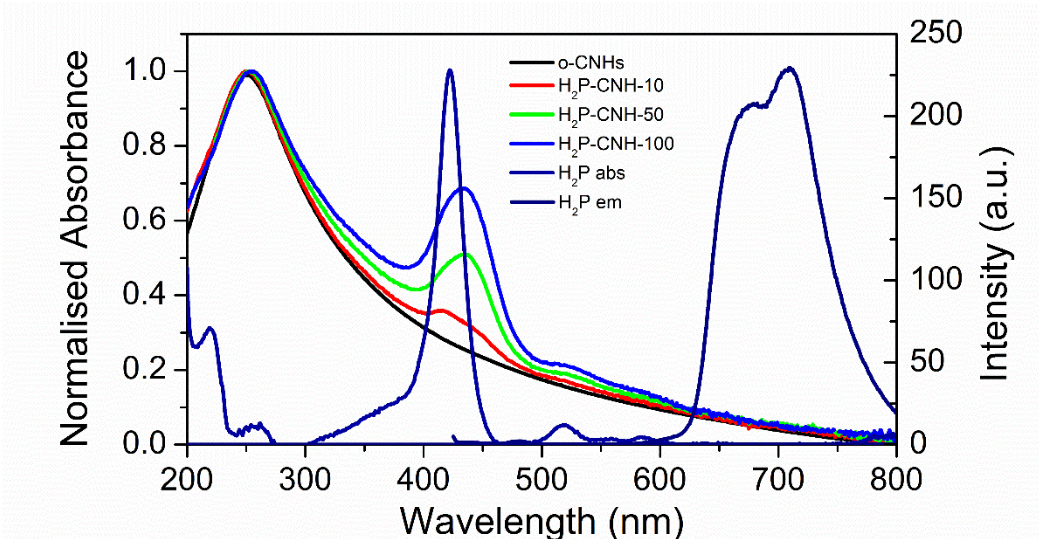

Fig. S6 Absorption spectra of **CNH-H_2TMPyP_4 hybrids** with 100, 50 and 10 % loading of porphyrin prepared on a 100 µg scale, corresponding emission spectra of PtP, λ_{ex} = 421 nm.

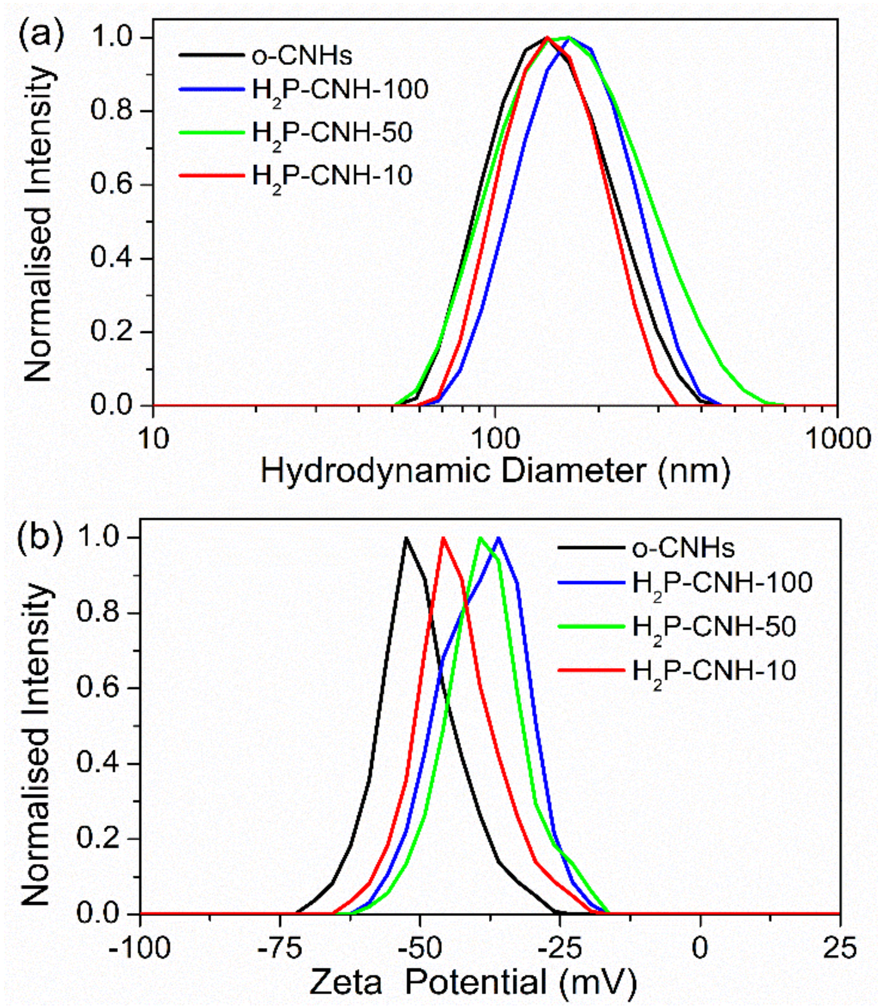

Fig. S7 (a) DLS measurements and (b) Zeta-potential measurements of **o-CNH** and **H_2P-CNH-10-100** hybrid samples recorded in aqueous solution pH 7.

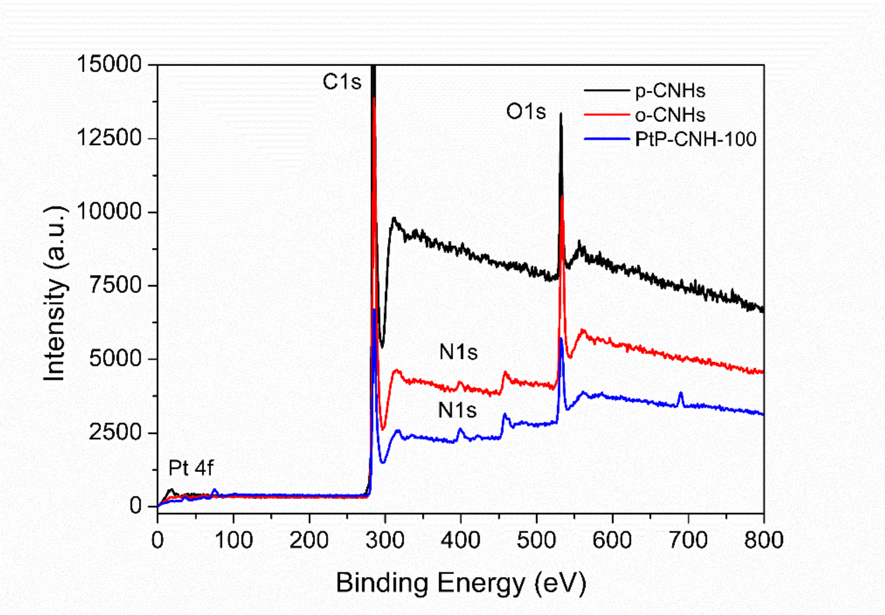

Fig. S8 (a) XPS spectra of **p-CNH**, **o-CNH** and **PtP-CNH-100**

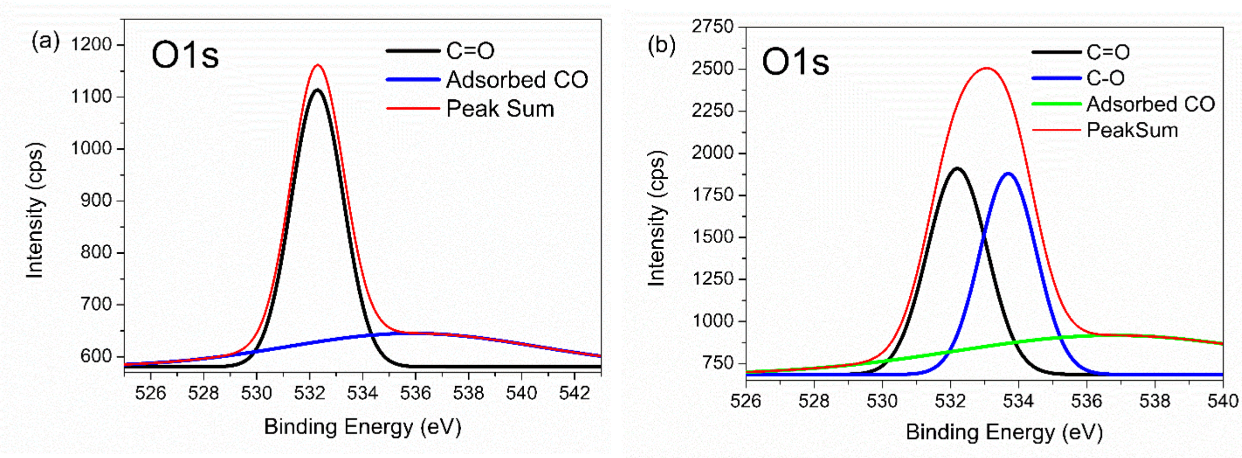

Fig. S9 O1s XPS spectra of (a) p-CNHs and (b) o-CNHs

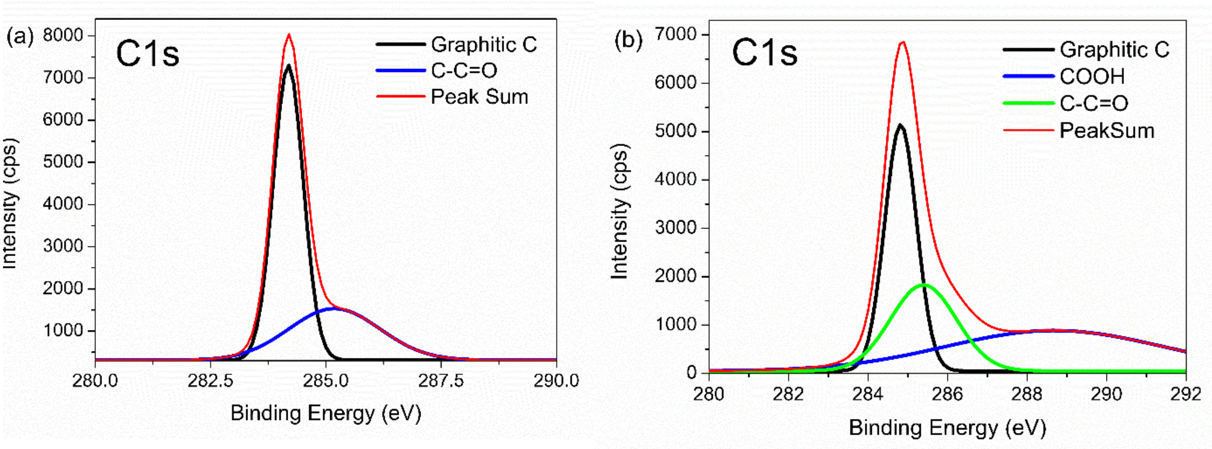

Fig. S10 C1s XPS spectra of (a) **p-CNH** and (b) **o-CNH**

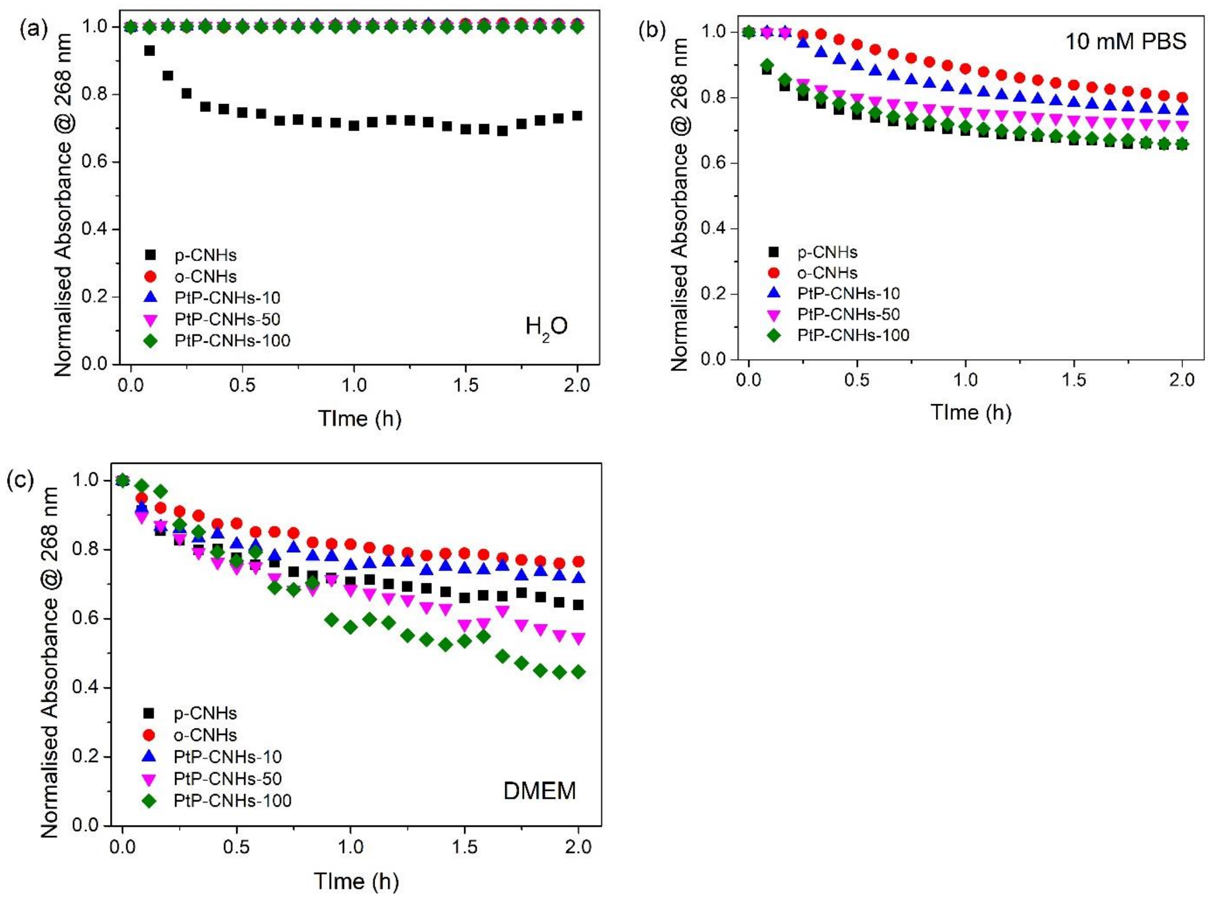

Fig. S11 Normalised change in absorbance of the **o-CNH** and **PtP-CNH-10-100** systems @ 268 nm recorded every 5 mins for 2 h. Obtained in (a) H_2O pH 7.2 (b) 10 mM PBS pH 7.4 and (c) DMEM pH 8.2, @ 22 ^oC.

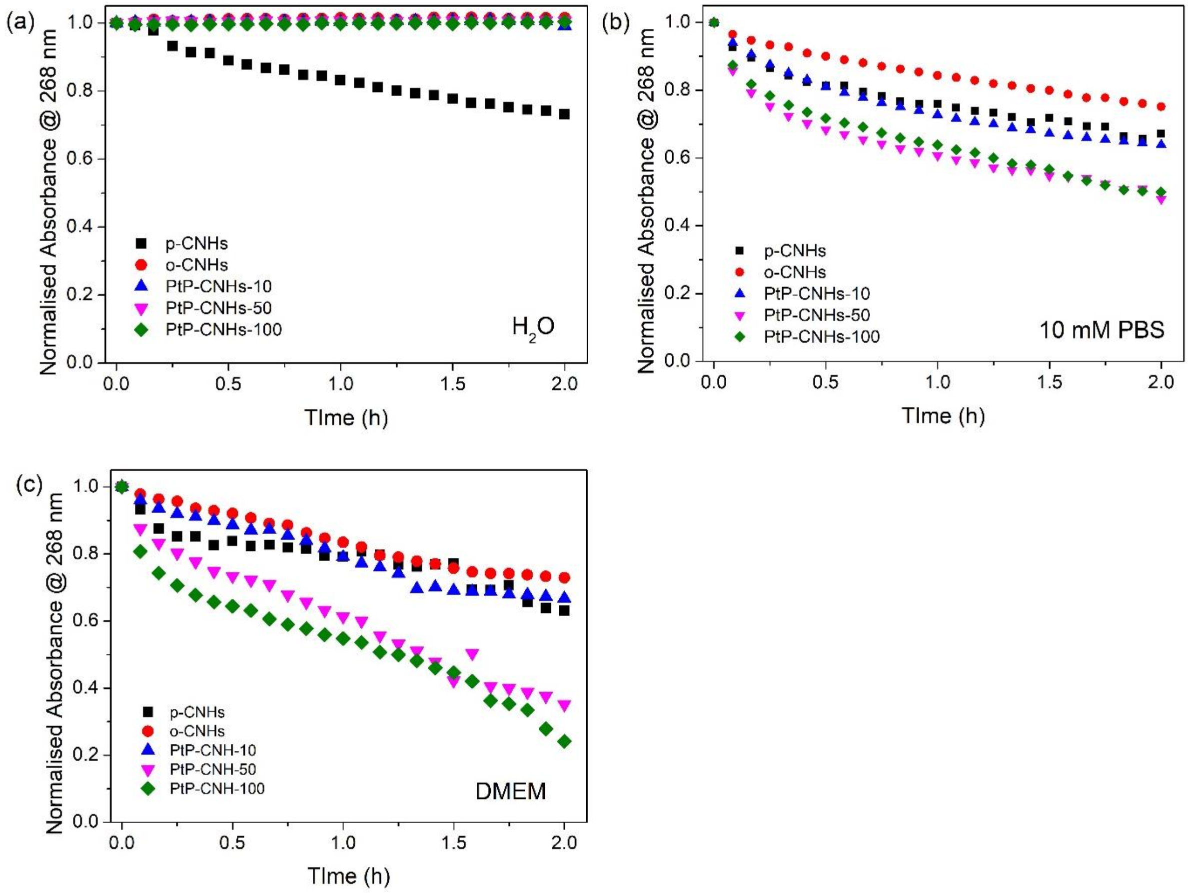

Fig. S12 Normalised change in absorbance of the **o-CNH** and **PtP-CNH-10-100** systems @ 268 nm recorded every 5 mins for 2 h. Obtained in (a) H_2O pH 7.2 (b) 10 mM PBS pH 7.4 and (c) DMEM pH 8.2, @ 37 ^oC.

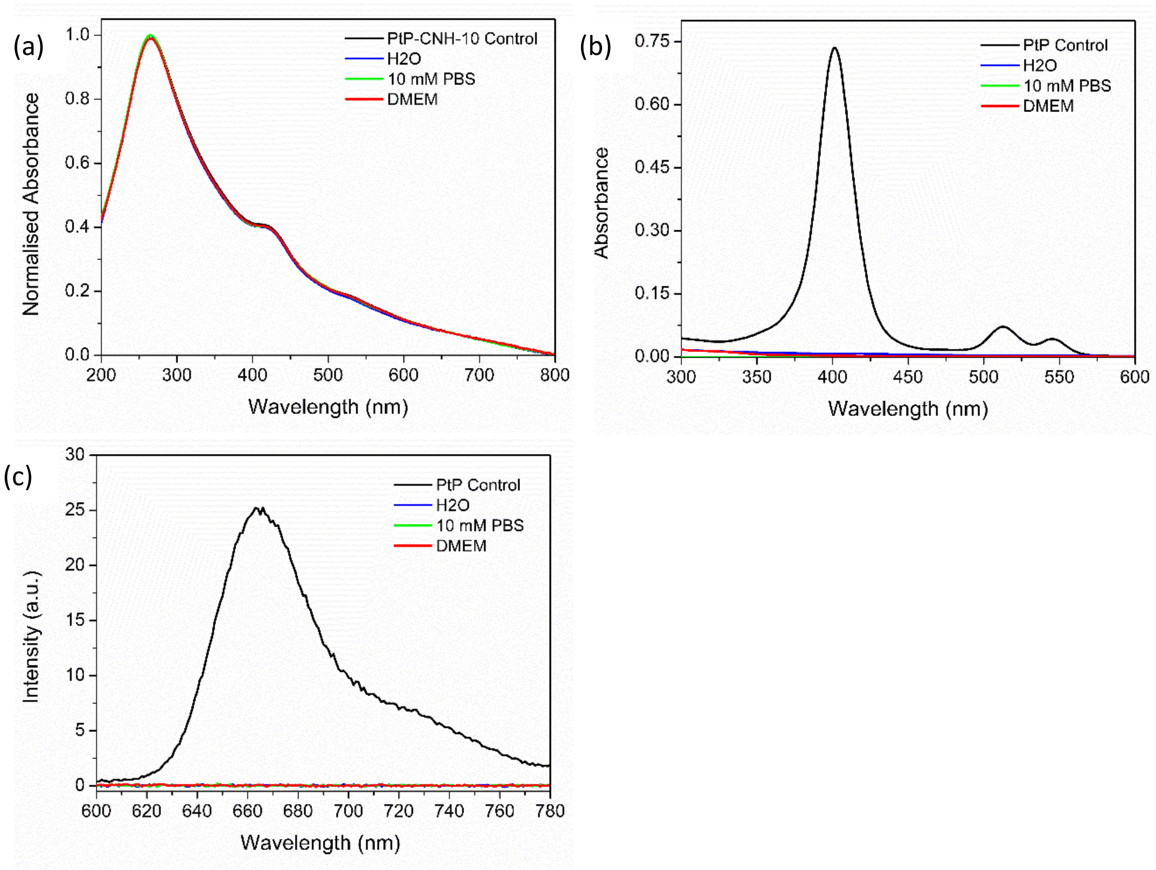

Fig. S13 (c) Absorption spectra of **PtP-CNH-10** hybrid prior and post incubation period. (b) Absorption spectra of **PtP-CNH-10** supernatant post 16 h incubation at 37 °C in H_2O, 10 mM PBS and cell media (c) corresponding emission spectra λ_{ex} = 402 nm.

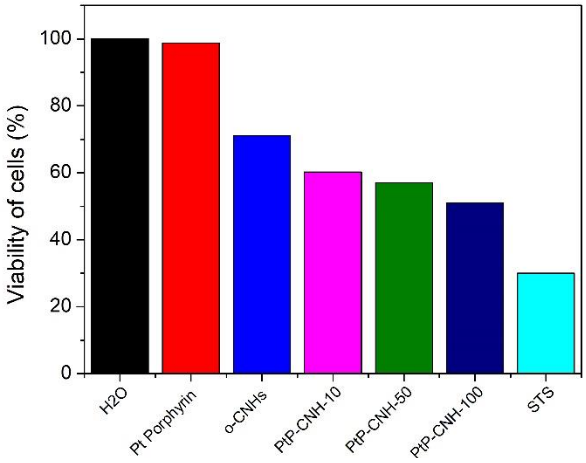

Fig. S14 CellTiter-Glo vell viability assay of HeLa cells incubated with water, **Pt-TMPyP4** (0.32 µM) **o-CNH** and **PtP-CNH-10-100** (10 µg mL^{-1}) and staurosporine (20 µM). In the absence of an excitation source.

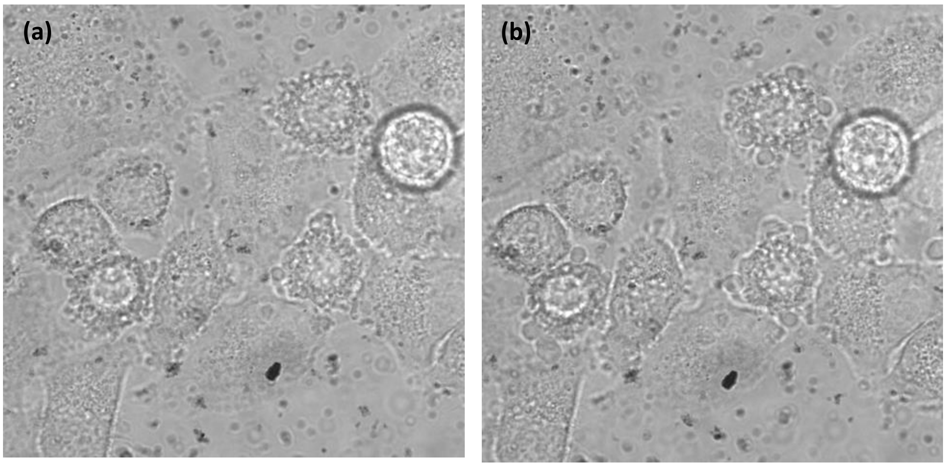

Fig. S15 Bright field microscopy of Hela cells in the presences of **PtP-CNH-10** (10 µg mL^{-1}) after (a) 15 sec exposure and (b) 30 sec exposure time to a CY3 channel excitation source.

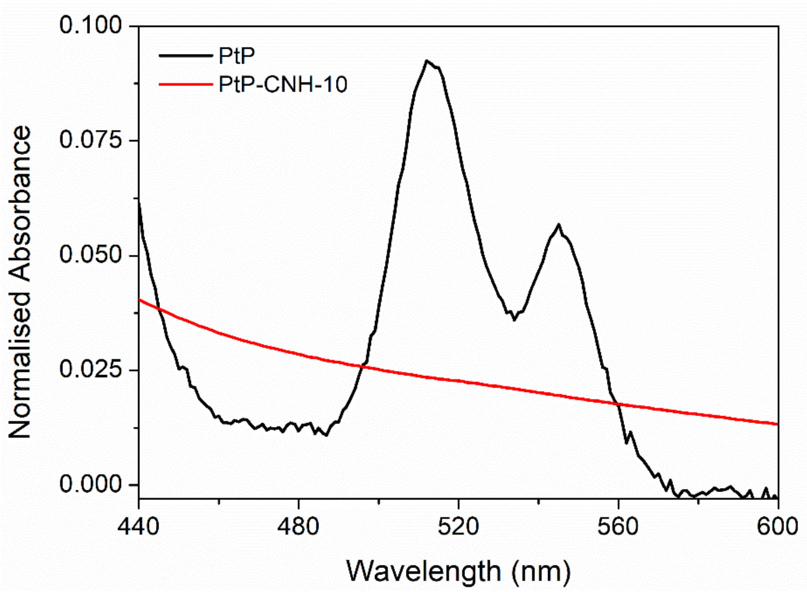

Fig. S16 Normalised absorption spectra of **Pt Porphyrin** and **PtP-CNH-10** hybrid showing Q band region. Molar extinction coefficient of **Pt porphyrin** @ 546 nm 9200 M^{-1} cm^{-1}.

Determining the moles of o-CNH used for Pt-TMPyP4 titration:

- Carbon atoms in core = 128,000[1]
- Carbon atoms on outer surface = 366,000
- Total atoms per aggregated bundle of o-CNHs = 494,000

For each bundle of **o-CNH**

$$\frac{0.840\ \mu\text{g (final mass)}}{5{,}928{,}000\left(\frac{\text{g}}{\text{mol}}\right)\text{CNH bundle}} = 1.41\text{x}10^{-13}\ \textbf{moles of bundles of o} - \textbf{CNH}$$

Determining the concentration of Pt-TMPyP4:

A = εCl
C = Concentration (mol/L)
A = Starting Absorbance (0.106)
ε = Extinction coefficient of free porphyrin (172,000 $dm^3mol^{-1}cm^{-1}$)
l = Path length (1 cm)

$$c = \frac{0.106}{(172{,}00)(1)} = 6.163\ x10^{-7}mol\ L^{-1}$$

Determining the loading ratio of Pt-TMPyP4 to o-CNH

Concentration of **Pt-TMPyP4**at the start =

Moles of porphyrin at the start = $(6.163\ x10^{-7}mol\ L^{-1})$(2.7 $x10^{-3}L$) = 1.664 $x10^{-9}$moles of **Pt-TMPyP4**.

$$\frac{1.664\ x10^{-9}\text{moles porphyrin}}{1.41\ x10^{-13}\ moles\ of\ bundles\ of\ CNH} = 11793$$

Loading ratio of Pt-TMPyP4 to o-CNH = 11800:1

Determining the loading efficiency by mass of Pt Porphyrin to o-CNH

Moles of porphyrin = 1.664 $x10^{-9}$moles
Mass of porphyrin = (1.664 $x10^{-9}$ moles)(1013.7 g mol^{-1}) = 1.69 $x10^{-6}$ g
Mass of o-CNHs = (0.840 μg)
Loading efficiency **Pt-TMPyP4**:CNH = $\frac{(1.69\ x10^{-6}\ \text{g})}{(0.840\ x10^{-6}\ \text{g})}(100) = \mathbf{201}\ \%$

Determining the moles of o-CNH used for free base H_2TMPyP_4 titration:

- Carbon atoms in core = 128,000
- Carbon atoms on outer surface = 366,000
- Total atoms per aggregated bundle of o-CNHs = 494,000

For each bundle of o-CNH

$$\frac{3.28\ \mu\text{g (final mass)}}{5{,}928{,}000\left(\frac{\text{g}}{\text{mol}}\right)\text{CNH bundle}} = 5.53\mathbf{x}10^{-13}\ \textbf{moles of bundles of CNH}$$

Determining the concentration of $H_2TMPyP4$:

A = εCl

C = Concentration (mol/L)

A = Starting Absorbance (0.101)

ε = Extinction coefficient of **$H_2TMPyP4$** (226,000 $dm^3mol^{-1}cm^{-1}$)

l = Path length (1 cm)

$$c = \frac{0.101}{(226{,}000)(1)} = 4.47\ x10^{-7}mol\ L^{-1}$$

Determining the loading ratio of $H_2TMPyP4$ to o-CNH

Concentration of **$H_2TMPyP4$** at the start =

Moles of **$H_2TMPyP4$** at the start = $(4.47\ x10^{-7}mol\ L^{-1})(2.7\ x10^{-3}L)$ = $1.21\ x10^{-9}$moles of **$H_2TMPyP4$**.

$$\frac{1.21\ x10^{-9}\text{moles porphyrin}}{5.53\ x10^{-13}\ moles\ of\ bundles\ of\ CNH} = 2190$$

Loading ratio of H_2TMPyP_4 to o-CNHs = 2190:1

Determining the loading efficiency by mass of $H_2TMPyP4$ to o-CNH

Moles of porphyrin =$1.21\ x10^{-9}$moles

Mass of porphyrin = $(1.21\ x10^{-9}\ mol)(820.6\ \text{g}\ mol^{-1})$ = $9.93\ x10^{-7}$ g

Mass of **o-CNH** = (3.28 μg)

Loading efficiency **$H_2TMPyP4$**:CNH = $\frac{(9.93\ x10^{-7}\ \text{g})}{(3.28\ x10^{-6}\ \text{g})}(100) = \mathbf{30}\ \%$

Table S1: Loading ratios of porphyrin molecules for CNH hybrids

	Moles CNH	Moles Porphyrin	Loading ratio (moles) Porphyrin:CNH	Loading Efficiency% (wgt) Porphyrin:CNH
PtP-CNH-10	$1.69\ x10^{-11}$	$2.37\ x10^{-8}$	1400	24
PtP-CNH-50	$1.69\ x10^{-11}$	$1.19\ x10^{-7}$	7040	120
PtP-CNH-100	$1.69\ x10^{-11}$	$2.37\ x10^{-7}$	14100	240
H_2P-CNH-10	$1.69\ x10^{-11}$	$4.95\ x10^{-9}$	293	4
H_2P-CNH-50	$1.69\ x10^{-11}$	$2.48\ x10^{-8}$	1470	20
H_2P-CNH-100	$1.69\ x10^{-11}$	$4.95\ x10^{-8}$	2930	40

Table S2. Loading efficiency summary of active pharmaceutical agents on carbon allotropes

Nano material	API	Loading Efficiency (wt:wt)%
Pegylated SWCNTs[2]	Mitoxantrone	52
Hyaluronic acid-derivatized SWCNTs[3]	hematoporphyrin monomethyl ether (HMME)	240
Pegylated SWCNTs[4]	Doxorubicin	400
Pegylated Graphene oxide[5]	7-ethyl-10-hydroxycamptothecin (SN-38)	10
Graphene Oxide[6]	Hypocrellin A (HA)	100
Pegylated Graphene oxide[7]	2-(1-Hexyloxyethyl)-2-devinyl pyropheophorbide-alpha (HPPH)	131
Graphene Oxide[8]	Polyphenol	185
Graphene Oxide[9]	Doxorubicin	>200

Table S3. Percentage retained in solution of **p-CNH**, o-**CNH** and **PtP-CNH-10-100** in H_2O, 10 mM PBS and DMEM @ 22 °C

Environment	p-CNH	o-CNHs	PtP-CNH-10	PtPCNH-50	PtP-CNH-100
H_2O	74 ± 5	100 ± 1	100 ± 2	100 ± 1	100 ± 1
PBS	65 ± 4	80 ± 3	74 ± 8	69 ± 5	64 ± 7
DMEM	64 ± 6	77 ± 5	70 ± 5	53 ± 7	46 ± 9

References

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