Supporting Information

Vitamin E-facilitated Carbon Monoxide Pro-drug Nanomedicine for Efficient Light-Responsive Combination Cancer Therapy

Yaw Opoku-Damoah [1], Run Zhang [1,], Hang T. Ta [1,2,3], and Zhi Ping Xu[1, *]*

[1] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia.

[2] School of Environment and Science, Griffith University, Brisbane, QLD 4111, Australia

Queensland Micro and Nanotechnology Centre, Griffith University, Brisbane, QLD 4111, Australia

Corresponding author:

Tel: +61 7 334 63809; E-mail: gordonxu@uq.edu.au; r.zhang@uq.edu.au.

Figures

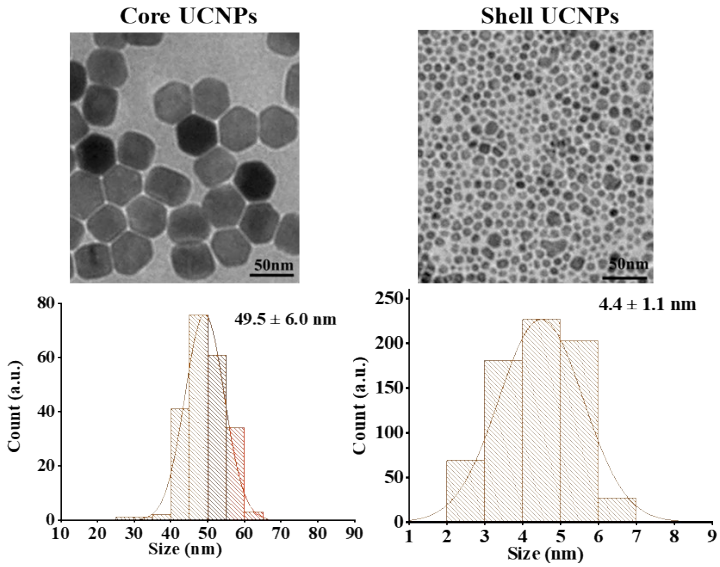

Figure S1. TEM imaging showing the morphology and particle size distribution of (A) NaYF4: Yb, Tm core (B) NaYF4: Nd shell UCNPs.

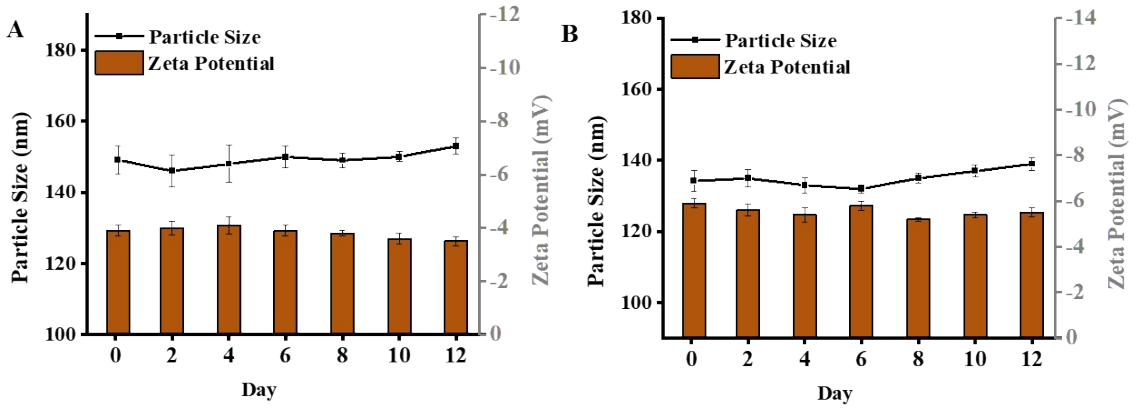

Figure S2. Particle Size and Zeta Potential Variation for (A) LUCTF and (B) LUTF in DMEM mimicking biological fluids. Samples tested every 2 days.

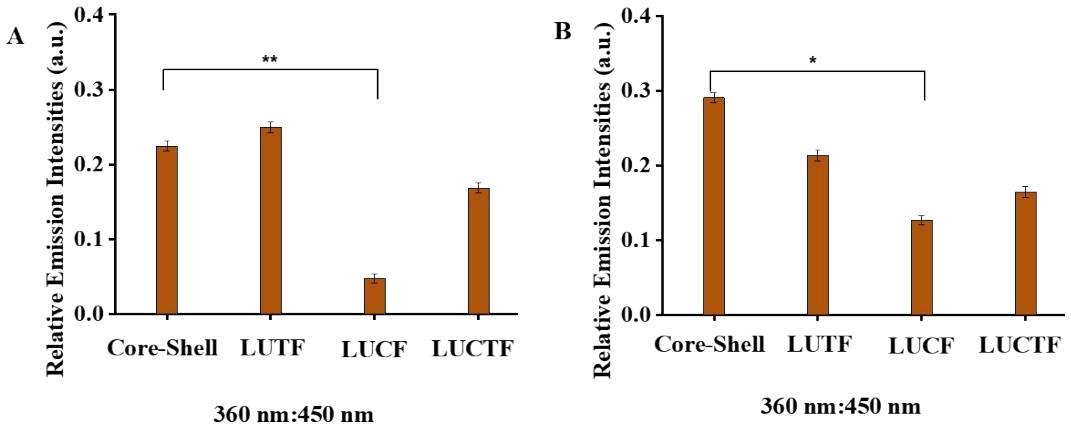

Figure S3. Ratio of highest peak to lower peak at different stages of drug formulation indicating the overlapping of CO absorbance at 360 nm for **(A)** 980 nm PL luminescence. **(B)** 808 nm PL luminescence.

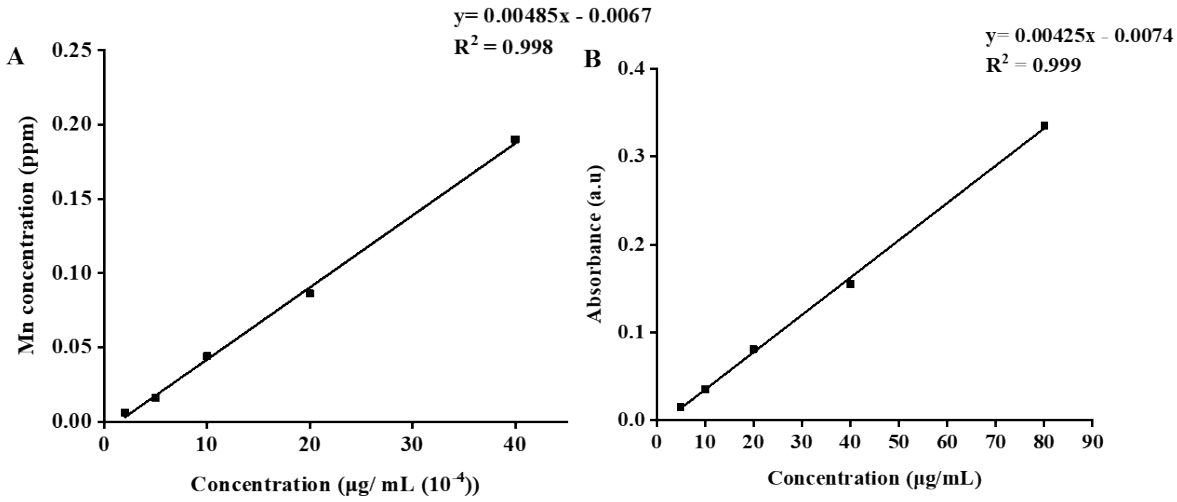

Figure S4. (A) ICP-OES standard measurement for CORM in HEPES Buffer. For LUCTF and LUCF the standard curve was used for the determination of CORM drug loading (**B)** UV Vis standard measurement for α-TOS in DMSO (λ max =283 nm). For LUCTF and LUTF the standard curve was used for the determination of α-TOS drug loading.

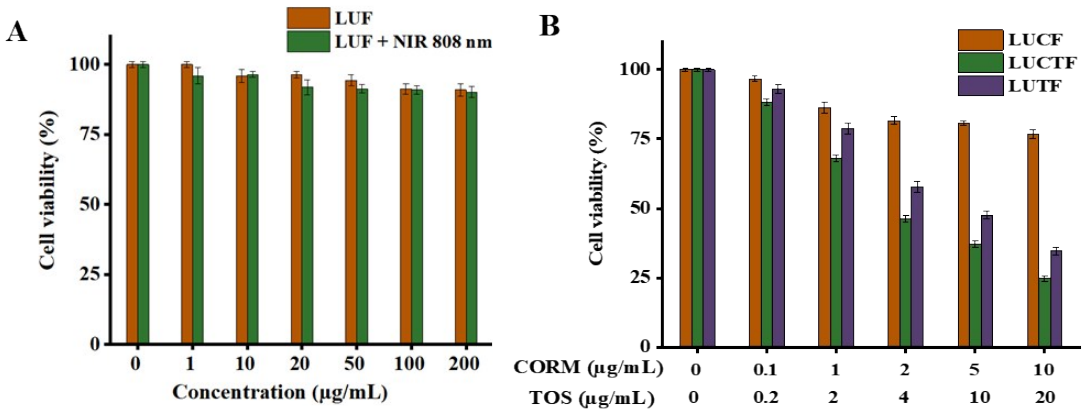

Figure S5. Cell viability assay of (A) LUF and (B) LUCF, LUF, LUCTF in HCT 116 cells without light irradiation and with 808 nm light irradiation after 3 h of nanoparticle incubation followed by incubation for a total of 48 h.

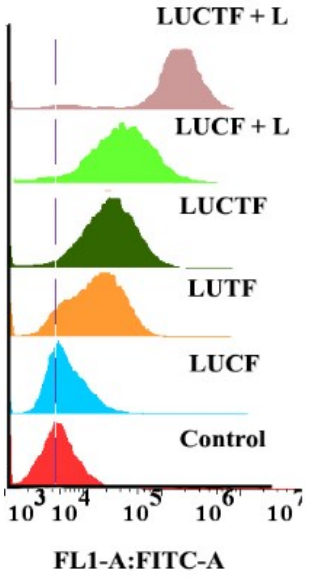

Figure S6. HCT 116 cells fluorescent signal of DCF at λex= 488 nm and λem= 525 nm in flow cytometry for intracellular ROS detection.

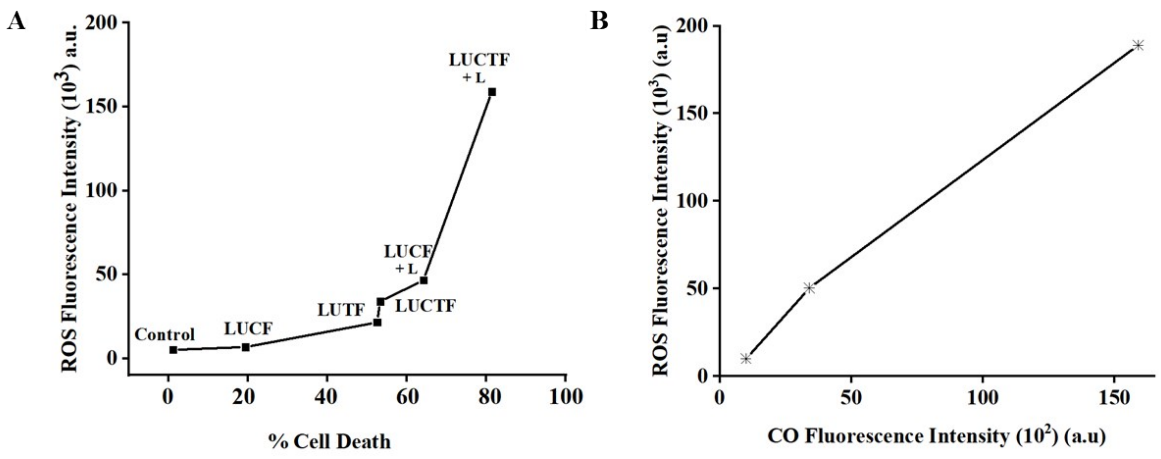

Figure S7. A. Graph of percentage cell death against ROS fluorescent measured at 10 µg/mL **B.** Graph of intracellular CO fluorescent measurement with respect to ROS fluorescent measurement after cell treatment

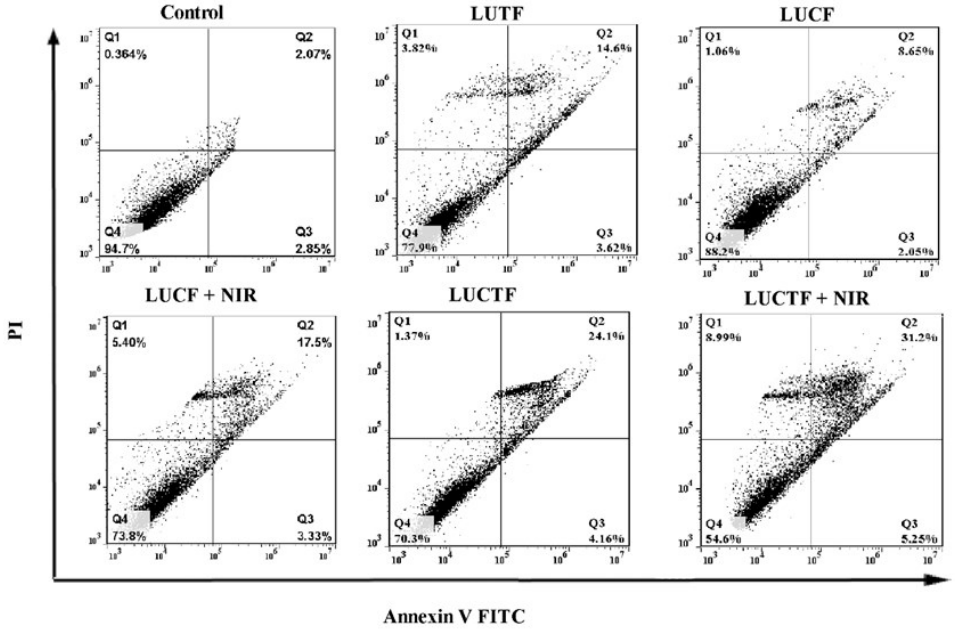

Figure S8. PI-Annexin V FITC flow cytometry analysis of cell distribution for early/late apoptosis and necrosis on HCT116 cells after typical treatment with various nanoparticles.

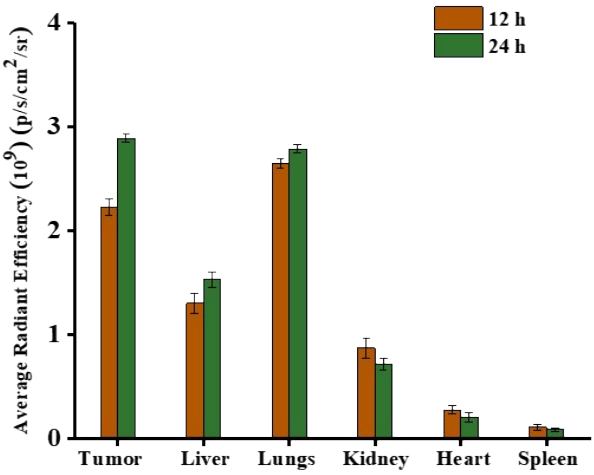

Figure S9. Average signals of radiant efficiency of IR780 in various organs and tumors after 12 and 24 h of iv injection.

Tables

Table S1. Drug loading and encapsulation efficiencies of optimized nanoformulations. LUCTF Theoretical Drug loading for α-TOS = 12.5 % and CORM = 6.25 %. LUFT Theoretical Drug loading for α-TOS = 13.33 %

Samples	α-TOS	TPGS	Yield (%)	Drug loading (wt %)	α-TOS Encapsulation Efficiency (%)
Lip/UCNPs/FA/α-TOS (LUFT)-(1:0)	1.0	0	59.3	9.10	73.6
Lip/UCNPs/FA/α-TOS (LUFT)-(1:1)	0.5	0.5	69.6	9.98	74.9

Table S2. Combination Index values for formulations after MTT assay.

Concentration (CORM/ α-TOS) (μg/mL)	CI (With NIR light)	CI (Without NIR light)
0.1/0.2	1.22	1.02
1/2	1.29	1.00
2/4	1.31	1.02
5/10	1.22	1.04
10/20	1.25	1.08
Average	**1.26**	**1.04**

$$Combination\ Index\ (CI) = \frac{Survival\ \%\ (LUTF) \times\ Survival\ \%(LUCF)}{Survival\ \%\ (LUCFT)}$$

Average CI = ∑ CI/n

CI < 0.8 : asynergy; 0.8-1.2: additive; 1.2-1.4: mild synergy; 1.4-1.6: moderate synergy; >1.6: strong synergy [1-3]

Table S3. IC_{50} values of all formulations (Concentration of CORM indicated in bold font)

Nanoformulation	IC_{50} (**α-TOS**, μg/mL)	IC_{50} (**CORM** μg/ml)
LUTF	10.6	--
LUCF	**--**	**143.9**
LUCTF	4.80	**2.40**
LUCF + NIR	**--**	**5.11**
LUCTF + NIR	2.08	**1.04**

Experimental

Materials

Lanthanides chloride hexahydrate ($TmCl_3 \cdot 6H_2O$, $NdCl_3 \cdot 6H_2O$, $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$), ammonium fluoride (NH_4F), oleic acid (99% purity), oleylamine, and 1-octadecene were obtained from Sigma Aldrich. Sodium hydroxide (NaOH) was obtained from Chem-Supply. Cholesterol, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol)-2000 (DSPE-PEG) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (18:1 Liss Rhod PE), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate (polyethyleneglycol)-2000 (DSPE-PEG-FA) were obtained from Avanti Polar Lipids, USA. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco, USA. DCFH-DA ROS assay kit was purchased from Promokine. Annexin V-FITC cell apoptosis detection kit and JC-1 mitochondria assay kit were purchased from Invitrogen (by Thermofisher Scientific). α-TOS and all other chemicals used were obtained from Merck KgaA (Darmstadt, Germany) and were of HPLC or analytical grade.

Synthesis, optimization and characterisation of core-shell upconversion nanoparticles (UCNPs)

Core UCNPs (NaYF4:Yb,Tm) were first synthesized using the thermal decomposition method. Under nitrogen gas atmosphere, YCl3.6H2O (0.795 mmol), YbCl3.6H2O (0.2 mmol) and TmCl3.6H2O (0.005 mmol) in a three-neck round bottom flask were dissolved with 6 mL of oleic acid (OA) and 15 mL of 1-octadecene (ODE) at 150 ºC for 60 min. Upon cooling to room temperature, 10 mL of methanol solution containing 0.148 g of ammonium fluoride (NH4F) and 0.1 g of sodium hydroxide (NaOH) was added. The mixture was stirred for another 60 min at room temperature, and then slowly heated to 120 ºC for 30 min to get rid of methanol. The temperature was then rapidly increased to 310 ºC for 90 min. . After cooling down to room temperature, 10 mL of ethanol was added to precipitate the UCNPs. The formed UCNPs were then washed with methanol, ethanol and cyclohexane three times. The synthesized UCNPs were dispersed in 10 mL of cyclohexane and stored at 4 ºC for subsequent use.

The shell nanocrystal seeds (α-NaYF4:Nd) (2 mmol) were prepared with the same thermal decomposition procedure. Typically, YCl3.6H2O (1.4 mmol) and NdCl3.6H2O (0.6 mmol) were magnetically dissolved in 12 mL of OA, 6 mL of oleylamine (OM) and 20 mL of ODE at 150 ºC for 60 min. After cooling down to room temperature, methanol solution (10 mL) containing 0.296 g NH4F and 0.2 g NaOH was added and the slurry was stirred at room temperature for another 30 min. Then, the reaction mixture was heated to 120 ºC for 30 min to remove methanol, followed by heating to 290 ºC for 30 min to produce α-NaYF4:Nd. The resultant α-NaYF4:Nd seeds obtained were dispersed in cyclohexane (2.0 mmol in 10 mL).

The core-shell nanoparticles (NaYF4:Yb,Tm@NaYF4:Nd) were synthesized by coating NaYF4:Yb,Tm cores with α-NaYF4:Nd nanocrystal seeds as follows: NaYF4:Yb,Tm (2 ml/0.2 mmol) cores stocked in cyclohexane were magnetically mixed with OM (1 mL), OA

(5 mL) and ODE (8 mL). α-NaYF4:Nd shells to be used for the coating were processed by replacing cyclohexane with 1.5 ml of OM, 7 ml of OA and 11.5 ml of ODE by heating to 110 ºC for 30 min under nitrogen gas. Upon heating the core nanoparticles to 303 ºC, the NaYF4:Yb,Tm core was quickly injected with 0.3 mL of α-NaYF4:Nd nanocrystal seeds using a syringe, followed by the addition of 0.2 mL every 10 min until the desired core: shell mass ratio (1:0.25, 1:0.5, 1:0.75 or 1:1) was achieved. After the last injection, the mixture was kept at 303 °C for 30 min to obtain the final core-shell NaYF4:Yb,Tm@NaYF4:Nd UCNPs. The precipitate was collected, washed and re-dispersed in cyclohexane using the same procedure for synthesizing core UCNPs.

Characterization of UCNPs

The photoluminescence (PL) of various UCNPs were determined using 1 mL cyclohexane solution containing 5 mg of UCNPs in a quartz cuvette in a spectrometer with the excitation wavelength of 980 and 808 nm at 1 W/cm^2. The phase purity of the lyophilized core-shell UCNPs was confirmed by powder X-ray diffraction (Bruker D8 Advance MKII XRD) using Cu Ka radiation (λ= 1.5406 Å) and compared to known standards. The size and morphology of UCNPs and lipid-coated UCNPs were imaged in a Hitachi HT7700A transmission electron microscope (TEM) operated at 80 kV. The lipid-coated UCNPs were stained with 1% phototungsten acid (PTA) to observe the lipid coating on the particle surface in TEM images

Optimization of lipid nanoformulations

For the formulation optimization, 1.5 mg of lipids (DOPC, DOPA, Cholesterol, DSPE-PEG and DSPE-PEG-FA) with lipid ratio of 40:40:10:5:5 were dissolved in 3 mL of chloroform. UCNPs (5 mg), CORM (0.5 mg) and α-TOS (1 mg) were also dissolved in the same chloroform mixture. Two more lipid formulations were produced by replacing α-TOS (1 mg) with α-TOS (0.5 mg) and TPGS (0.5 mg) as well as TPGS (1 mg). The lipid mixture was stirred for 4 h before the organic liquid was eliminated under pressure with a rotary

evaporator at 40 ºC. After getting rid of the organic solvent, the thin film obtained was left to dry overnight in a desiccator to ensure complete drying of the contents. The thin film was slowly dissolved with HEPES buffer (pH 7.4, 10 mmol) under constant stirring at 40 ºC for 4 h. The resultant mixture was sonicated for 10 min and centrifuged at 20000 g to obtain the lipid encapsulated UCNP, CORM and α-TOS formulation. The final formulation was obtained by dissolving the pellets in HEPES buffer to obtain a 1mg/mL LUCTF formulation.

References

1. A. Ito, M. Fujioka, T. Yoshida, K. Wakamatsu, S. Ito, T. Yamashita, K. Jimbow and H. Honda, 2007, **98**, 424-430.
2. J. Liu, L. Sun, L. Li, R. Zhang and Z. P. Xu, *ACS Applied Materials & Interfaces*, 2021, **13**, 7115-7126.
3. B. Li, J. Tang, W. Chen, G. Hao, N. Kurniawan, Z. Gu and Z. P. Xu, *Biomaterials*, 2018, **177**, 40-51.