Supplementary information

Novel anti-HER2 Peptide conjugated Theranostic Nanoliposome combining $NaYF_4$:Yb,Er nanoparticles for NIR-activated Bioimaging and Chemo-Photodynamic therapy against Breast Cancer

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Supporting Figures

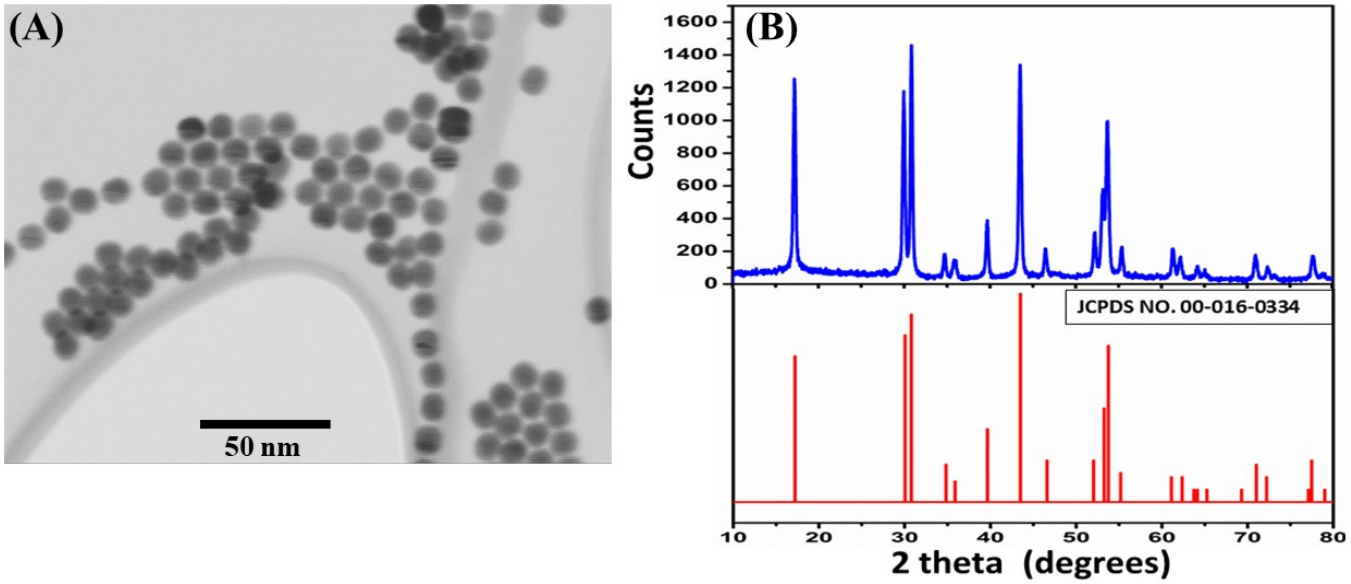

Figure S-1. (A) Scanning Transmission Electron Microscopy (STEM) images of the synthesized UCNPs with an average size of ~12 nm. (STEM images were obtained using a JEOL JSM-7800F microscope) **(B)** The XRD pattern of the UCNPs before surface functionalization, and comparison with the JCPDS NO. 00-016-0334 standard data

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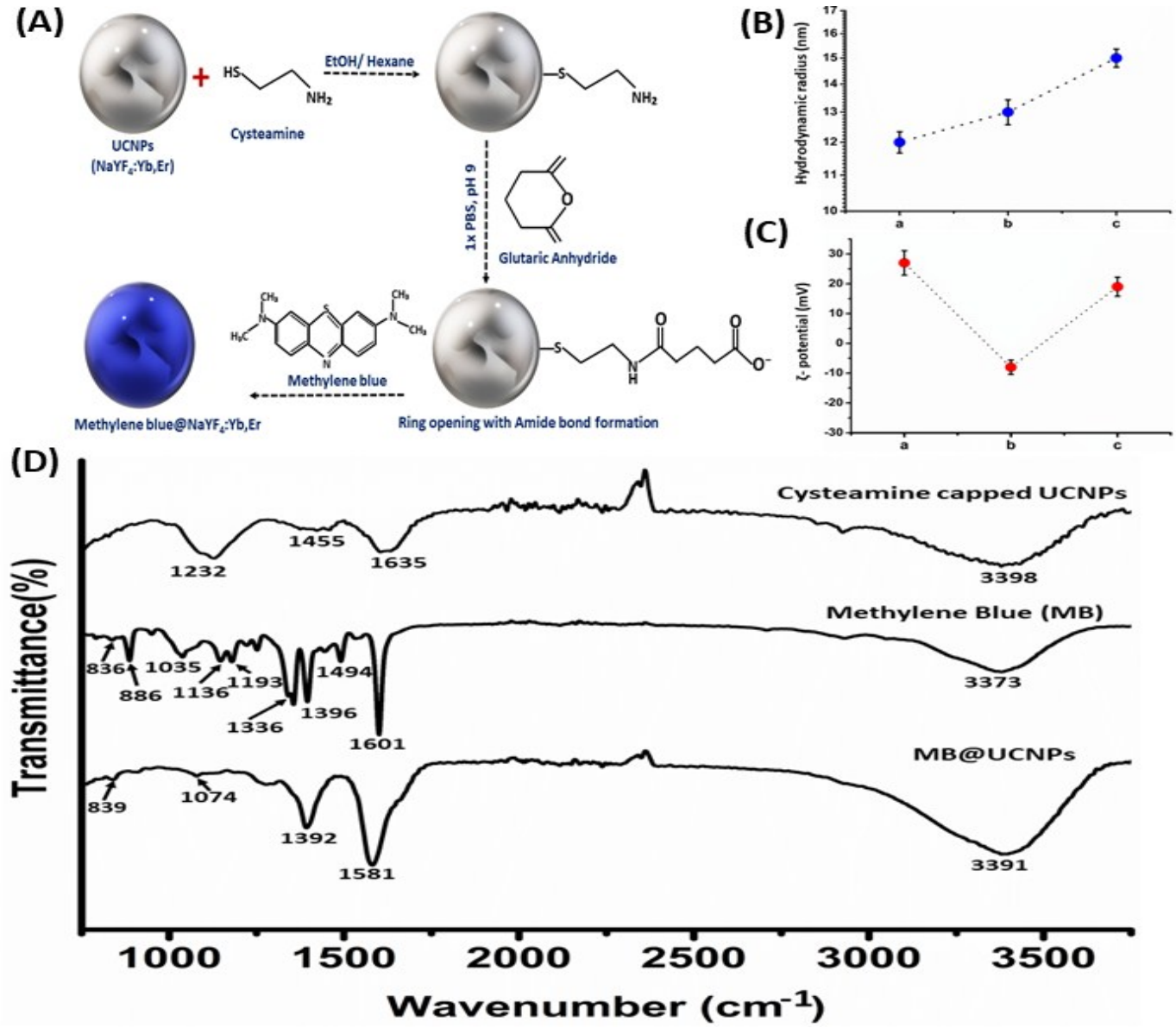

Figure S-2. (A) Schematic representation of the surface modifications of UCNPs with methylene Blue, **(B-C)** Estimated hydrodynamic size and zeta potential values of the UCNPs: in X axis **(a)** Cysteamine capped UCNPs, **(b)** carboxyl modified UCNPs, **(c)** methylene blue capped UCNPs (i.e., MB@UCNPs) and **(D)** FTIR spectra of the cysteamine capped UCNP and MB@UCNPs compared to only methylene blue as reference.

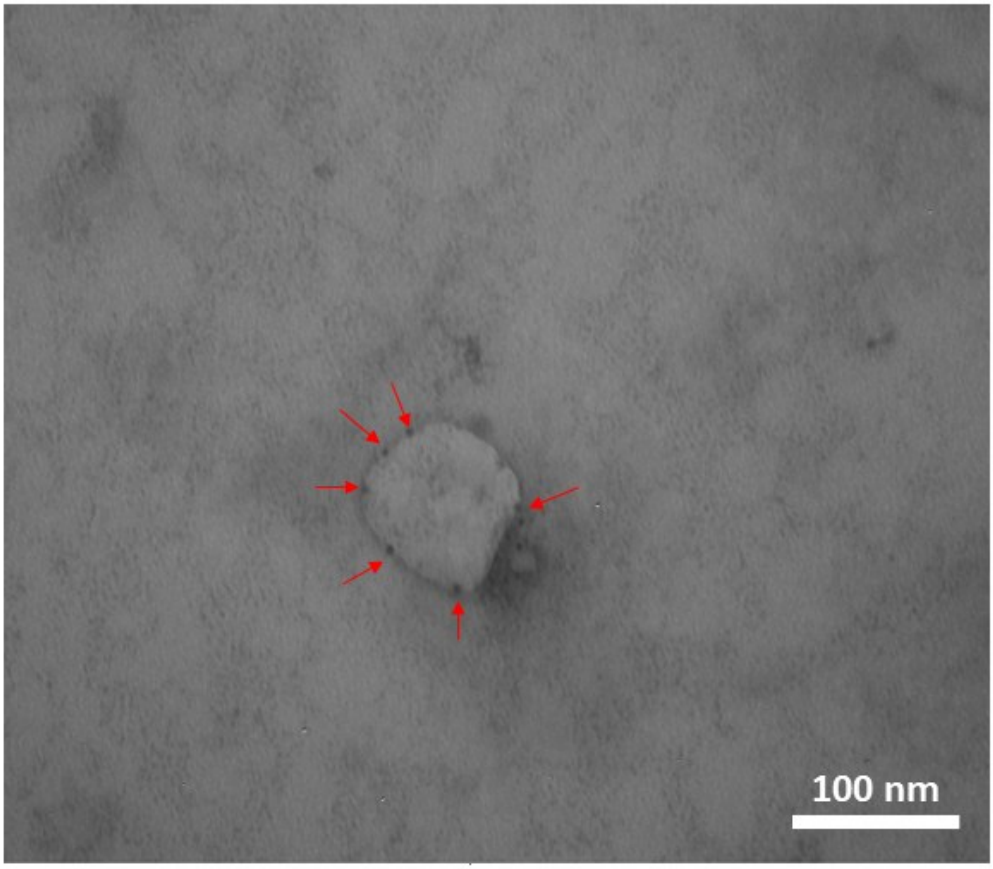

Figure S-3. TEM analysis of liposome with oleate@UCNPs. It was observed that the oleate (lipidic molecule) present on the UCNPs surface was having a strong interaction with the liposomal membrane as shown by red arrows. This interaction would apparently disturb the liposomal membrane and would result in leaking the DOX molecule loaded within it.

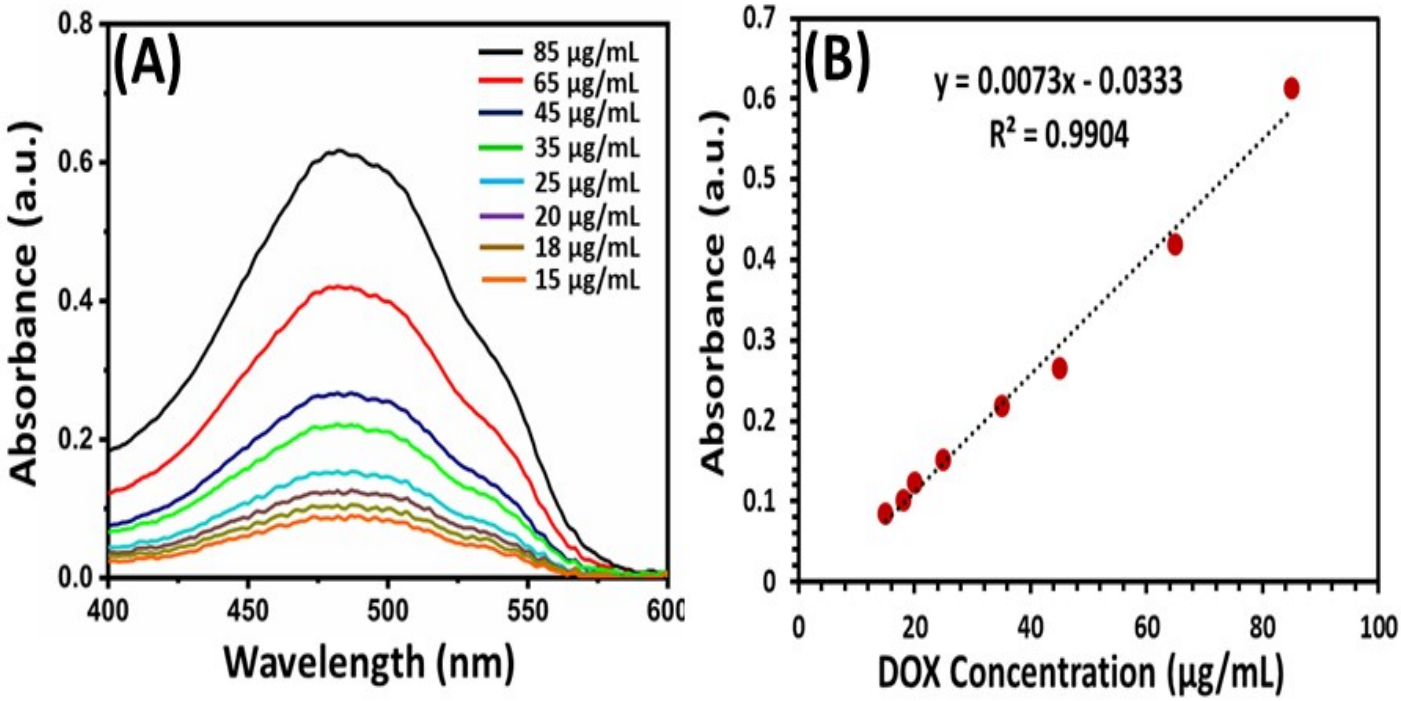

Figure S-4. (A) UV-vis absorbance spectra of varied concentration of DOX in 1xPBS (pH 7.5). **(B)** Standard calibration curve of DOX

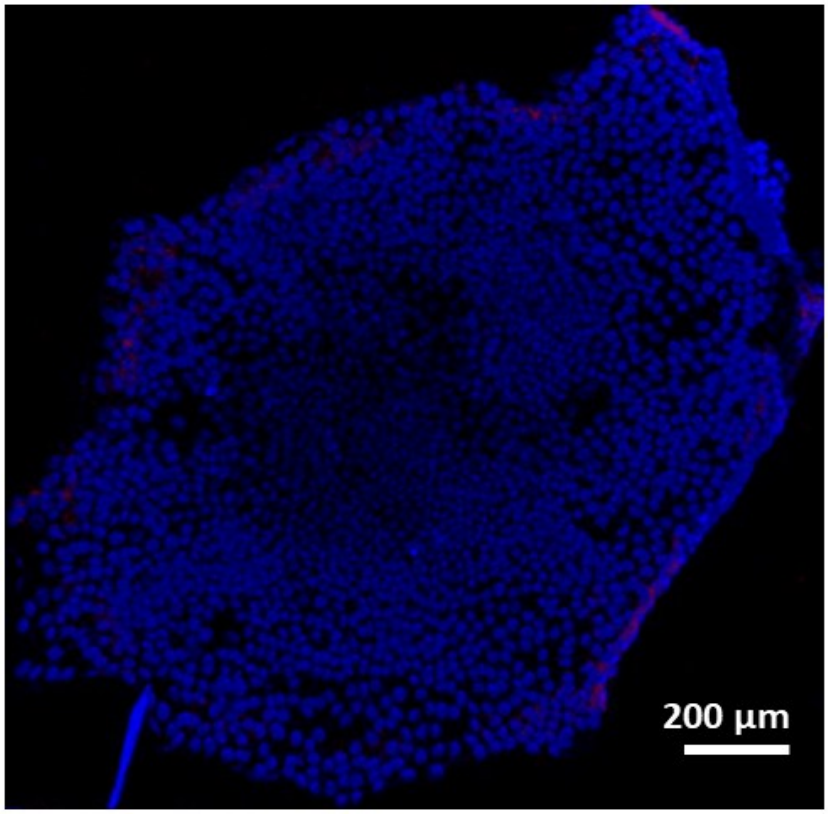

Figure S-5. Confocal laser scanning microscopy image of the tumor spheroid cryo-sectioned to 10 µm sections on the glass substrates after 24 h incubation with Group 1 nanoliposomes (without anti-HER2 peptides), followed by nuclei counterstaining with Hoechst (Blue) and upconverting fluorescence (in Red).

Estimation of Ligand Targeted Nanoliposomes (LTLs) Loading Efficiency

To understand the efficiency of the Loading within the hydrophilic core of LTLs used in the study, we estimated the number of DOX molecules and UCNPs.

- ***DOX Loading in Absence of UCNPs***
  The number of the DOX molecules in the different groups were estimated by considering its efficiency of loading in the absence of UCNPs while loading within the respective LTLs groups.

The total amount of DOX added for loading within LTLs: $0.000055 * 10^{-3}$mol

Loading efficiency in the absence of UCNPs: 87%

Total amount of DOX within LTLs in absence of UCNPs = $0.87 * 0.000055 * 10^{-3}$mol

= $0.00004785 * 10^{-3}$mol

With the use of the Avogadro constant, where 1 mol is $6.022*10^{23}$ particles. Hence, the number of DOX determined in the absence of UCNPs was:

Number of DOX in the absence of UCNPs = $0.00004785 * 10^{-3} * 6.022*10^{23}$

= $0.00028815* 10^{20}$ **or** $2.88*10^{16}$

- ***DOX Loading in the Presence of UCNPs***
  The number of the DOX molecules in the different groups were estimated by considering its efficiency of loading in the presence of UCNPs while loading within the respective LTLs groups.

The total amount of DOX added for loading within LTLs: $0.000055* 10^{-3}$mol

Loading efficiency in the presence of UCNPs: 74%

Total amount of DOX within LTLs in presence of UCNPs = $0.74*0.000055* 10^{-3}$mol

= $0.0000407 * 10^{-3}$mol

With the use of the Avogadro constant, where 1 mol is $6.022*10^{23}$ particles. Hence, the number of DOX determined in the presence of UCNPs was:

Number of DOX in the presence of UCNPs = $0.0000407 * 10^{-3} * 6.022*10^{23}$

= $0.0002830* 10^{20}$ **or** $2.830*10^{16}$

Theoretical Calculations for maximal number of UCNPs Loaded within LTLs

The amount of UCNPs loaded within the LTLs were estimated by calculating the mass (m) of one UCNPs and the amount of UCNPs added while preparing the LTLs. However, the m of one UCNPs can be calculated by using volume (V), which was observed to be ~12 nm by TEM and DLS analysis. And the density of the β-$NaYF_4$:Yb,Er upconversion nanoparticles (($\rho = 4.2\ g/cm^3$)[1] (JCPDS NO. 00-016-0334)

Mass of one UCNP: m(UCNP) $= \rho(UCNP) * V(UCNP)$

$$= \rho(UCNP) * \frac{4}{3} * \pi * (\frac{d(UCNP)}{2})^3$$

$$= 4.2 \frac{g}{cm^3} * \frac{4}{3} * 3.14 * (6 * 10^{-7} cm)^3$$

$$= 3.79 * 10^{-18}\ g$$

Total amount of UCNPs used in the fabrication of UCNPs: m (UCNPs) $= 5 * 10^{-3}\ g$

Estimated No. of UCNPs Loaded within LTLs

$$= \frac{5 * 10^{-3}}{3.79 * 10^{-18}}$$

$$= 18.95 * 10^{15}$$

The total amount of UCNPs loaded within 1 mM of the LTLs was estimated to be $18.95 * 10^{15}$ particles which was utilized for Bioimaging and as the source to Photoexcite the Methylene Blue for ROS generation after exciting with 975 nm NIR laser

Reference

1. Liu, H.; Xu, C. T.; Dumlupinar, G.; Jensen, O. B.; Andersen, P. E.; Andersson-Engels, S., Deep tissue optical imaging of upconverting nanoparticles enabled by exploiting higher intrinsic quantum yield through use of millisecond single pulse excitation with high peak power. *Nanoscale* **2013,** *5* (20), 10034-10040.