Supporting Information

Synergetic Tumor Probes for Facilitating Therapeutic Delivery by combined-functionalized Peptide Ligands

Xiangqian Jia[b#], Mingmei Guo[a#], Qiuju Han[c#], Yuwei Tian[a], Yafei Yuan[a], Zihua Wang[d], Yixia Qian[d], Weizhi Wang[a*]

[a.] *School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing 100081, PR China;*

[b.] *School of Pharmaceutical Sciences, MOE Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 10084, China;*

[c.] *College of Pharmacy, Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, China;*

[d.] *CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, PR China.*

Contents

Experimental details

1. Materials

1,2-distearoyl-sn-glycero-3-phos-phoethanolamine-N-[poly(ethylene glycol)-2000]-N-hydroxy-succinimidyl (NHS-PEG_{2000}-DSPE) was purchased from Nanosoft Biotechnology LLC (USA). Soybean phosphatidylcholine (SPC, AR) and cholesterol (CHO, AR) were purchased from A.V.T. Pharmaceutical Co., Ltd. HP2 peptide (YDLKEPEH) and TAT (GRKKRRQRRRPPQ) were synthesized by ourselves. Doxorubicin hydrochloride (DOX) was supplied by Hisun Pharmaceutical Co. Ltd (Zhejiang, China). 9-Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids and 2-(1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethylur onium hexafluoro-phosphate (HBTU) were purchased from GL Biochem (China). Wang Resin was from Rapp Polymere (Germany). Trifluoroacetic acid (TFA) and other reagents were all of analytical grade and used without further purification.

The human breast cancer cell line SKBR3 and Human Embryonic Kidney 293T were from American Type Culture Collection (ATCC; Manassas, VA). Cell culture medium and fetal bovine serum were from WisentInc (Multicell, WisentInc, St. Bruno, Quebec, Canada). Culture dishes and plates were from Corning (Corning, New York, USA). SKBR3 cells were maintained in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin solution. 293T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum and 1% penicillin solution. All cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 ºC.

2. Synthesis process of the photo labile OBOC peptide library towards HER2

The general sequence of the library is $X_1X_2X_3X_4X_5X_6X_7X_8$GM. X_1 represents any of V, N, Y, A, and K residues at N-terminal to form hydrophobic or hydrophilic interactions with HER2. X_2 represents H, S, W, L, or D. X_3 represents R, Q, F, L, or D. X_4 represents K, S, F, L, or D. X_5 represents T, S, V, or E. X_6 represents P, F, Y, or W. X_7 represents L, N, or E. X_8 represents H, R, or K residues because basic amino acid residues at the C-terminal were reported to improve ligands interaction with HER2 protein. In the library, the sequence on each bead was randomly distributed so that the complexity of the peptide library was $5\times5\times5\times5\times4\times4\times3\times3= 90000$ and the redundancy of the library was eight. As a result, peptide screening was carried out from 7×10^5 candidate beads. Solid phase peptide synthesis (SPPS) with Fmoc strategy was employed for the OBOC library synthesis. Tentagel Resin (loading 0.35 mmol/g) was used as the solid phase support. Scheme S1 shows the synthesis process. All the synthesis process was carried out in dehydrous DMF. During the coupling step, HBTU (4 mmol) and Fmoc-amino acid (4 mmol) reagent was dissolved in 0.4 mol/L NMM (4 mL) and the coupling time was 30 min. In the deprotection step, 20% (v/v) piperidine was used to remove the Fmoc group and the deprotection time was 10 min. During the OBOC library synthesis, solid support beads were split equally in each cycle and different amino acids were added. It means that amino acid coupling process was carried out in the "split" step while the deprotection process was carried out in "pool" step. After elongation, cleavage reagent (92.5% (v/v) TFA, 2.5% (v/v) water, 2.5% (v/v) EDT and 2.5% (v/v) Tips) was introduced into the vessel to cleave the SP (side chain protecting group) of each residue (2 h). All the above experiments were carried out in the solid phase peptide synthesis vessels with sieves in it. For the *in situ* chemical cleavage in the microwells, 30 mg/mL cyanogen bromide (BrCN) solution was used overnight. NMM is the abbreviation for N-Methyl morpholine. TFA is the abbreviation for Trifluoroacetic acid.

3. Synthesis of peptide HP2 and TAT

All peptides were synthesized by solid-phase methods using a standard Fmoc-Chemistry. Wang Resin (Rapp Polymere, Germany) is used as the solid phase support. The deprotection of Fmoc group on N-terminal was used piperidine (20% v/v) in anhydrous DMF. Qualitative Fmoc deprotection was confirmed by a ninhydrin test (ninhydrin, phenol, VC 1:1:1 v/v). Amino acid activation was achieved by NMM (0.4 M) and HBTU (the same mole of amino acid) in anhydrous DMF. Cleavage from the resin and deprotection of the amino acid side chains were performed by reaction with the mixture of TFA (95% v/v), H_2O (2.5% v/v) and TIPS (2.5%, v/v) for 30 min in ice bath and then at room temperature for another 3 h. After separated from the resin, the mixture above was vacuum rotary evaporated to remove the TFA. The crude peptides were then precipitated in cold anhydrous diethyl ether, collected by centrifuge and dried under vacuum. Finally, the peptides were purified by preparative reversed-phase high performance liquid chromatography (HPLC) (Figure S1a, b) on a preparative reversed-phase Inertsil C18 HPLC column (ODS-3, 5 μm, 20 × 250 mm). A linear gradient of acetonitrile/water with 0.1% TFA respectively from 5%/95% to 70%/30% in 25 min， then 70%/30% to 90%/10% in 2min and in this flow continue 1min ,next return to 5%/95% tilled for 2 min was used as the mobile phase. The separation was performed with a flow rate of 10 mL/min and the monitoring wavelength was 220 nm using a UV detector (Waters 2535Q). The purified peptide was determined by MALDI-TOF mass spectrometry (Bruker Daltonics, Germany). MALDI-TOF-MS analysis was performed on a Bruker ULTRAFLEXTREME mass spectrometer equipped with a nitrogen laser (wavelength 337nm, laser pulse uration 3ns) with provide laser pulse energy between 0 and 100 μJ per pulse. The mass spectra were typically recorded at an accelerating voltage of 19 kV, a reflection voltage of 20 kV, and with laser pulse energy of 60 μJ. Each mass spectrum was acquired as an average of 500 laser shots.

4. The molecular docking of HP2 towards HER2 protein

The 3D structure of HER2 was obtained from the SWISS-MODEL. The configuration of HP2 was first optimized using *PEP-FOLD3* and docking into HER2 using *ZDOCK 3.0.2*. The docking results showed that HP2 binding to the 379-516 site of HER2 through several interactions. The H atom in Tyr1 (HP2) and O in Glu379 (HER2) exhibited hydrogen bonding. The imidazole in His8 (HP2) and Cys506 (HER2) exhibited hydrogen bonding. The O in Pro6 (HP2) and H in Glu516 (HER2) showed hydrogen bonding, similar to O in Glu7 (HP2) and H in Cys509 (HER2) as well as O in His8 (HP2) and H in Arg437 (HER2). Glu7 (HP2) and Leu513 (HER2) exhibited Van der Waals' force. These effects synergistically led to specific HP2-HER2 recognition with a small K_D.

5. Surface Plasmon Resonance Imaging (SPRi) for Detection of the Affinity Peptide towards HER2.

SPRi analysis was performed on a Plexera PlexArray HT system (Plexera LLC, Bothell, WA) using a bare gold SPRi chip (Nanocapture gold chips, with a gold layer of 47.5 nm thickness). The purified peptide was printed onto the gold chip surface by the thiol group of the cystein residue. The printed chip was then incubated in 4 °C overnight in a humid box. The SPRi chip was washed and blocked using 5% (m/v) nonfat milk in PBS overnight before use. The SPRi analysis procedure followed the following cycle of injections: running buffer (PBST, baseline stabilization); sample (five concentrations of the protein, binding); running buffer (PBST, washing); and 0.5% (v/v) H_3PO_4 in deionized water (regeneration). HER2 protein was diluted with PBST to concentrations of 10 μg/mL, 5 μg/mL, 2.5 μg/mL, 1.25 μg/mL, and 0.625 μg/mL. Real-time binding signals were recorded and analyzed by PlexArray HT software. Dissociation constants between the peptide ligands and control

proteins were also determined by SPRi under the same experiment condition. Human Serum Albumin was chosen to check the non-specific absorption.

6. Peptide Ligands as Probes for Imaging Cancer Cells *In Vitro*.

The human breast cancer cell line SKBR3 were cultured in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin solution at 37 °C containing 5% CO_2. The human embryonic kidney cell lines 293T were cultured in Hyclone DMEM/high glucose with 10% fetal bovine serum and 1% penicillin solution at 37 °C containing 5% CO_2 . For SKBR3 and 293T cells, approximately 1×10^5 mL^{-1} cells were seeded into culture dishes and cultured overnight for cell adherence. FITC-labeled peptide (HP2) was dissolved in cold PBS at a concentration of 5.0×10^{-5}M. The cells were incubated with FITC-labeled peptide solution (200 μL, with Hoechst 33342 (1 mM))in the dark for 30 min at 4 °C. Finally, the cells were washed three times with cold PBS. Confocal fluorescence imaging was performed on an Olympus FV1000-IX81 confocal laser scanning microscope. A FV5-LAMAR 488 nm laser was the excitation source for FITC throughout the experiment, and emission was collected between 520 and 620 nm. Hoechst 33342 was excited at 50 mW; Ex, 405 nm; Em, 472 nm. The objective lens used for imaging was a UPLSAPO 100 × oil-immersion objective (Olympus).

7. Synthesis of HP2-PEG_{2000}-DSPE and TAT-PEG_{2000}-DSPE.

Activated NHS-PEG_{2000}-DSPE was used to conjugate HP2 and TAT. Briefly, HP2 (or TAT) and NHS-PEG_{2000}-DSPE (molar ratio 1:1.5) were dissolved in newly distilled DMF, and pH 8.0 was adjusted by N, N-Diisopropylethylamine (DIPEA). After 48 h, the coupling reaction was achieved near completely by HPLC monitoring of the conjugation between peptide and NHS-PEG_{2000}-DSPE. The peak of peptide was decreased along the time. The reaction mixture were purified by dialysis with a molecular weight cut-off of 3,500 Da, then lyophilized. The product was analyzed by MALDI-TOF-MS using a Microflex LRF System spectrometer (Bruker Daltonics, USA).

8. Preparation and Characterization of liposomal probes

Different kinds of liposomal probes (HT-LS^{DOX} (HP2 and TAT functionalized liposomes loading DOX), mono-functionalized liposomes T-LS^{DOX} (TAT functionalized liposomes loading DOX), H-LS^{DOX} (HP2 functionalized liposomes loading DOX) and blank liposome (LS^{DOX})) were prepared by thin film dispersion method. They were prepared by mixing of soy phospholipids (SPC), cholesterol (CHO), targeting compound and DOX (LS^{DOX} (SPC: CHO: DOX 4: 1: 1 w/w/w/w), T-LS^{DOX} (SPC: CHO: TAT-PEG_{2000}-DSPE: DOX 4: 1: 1: 1 w/w/w/w), H-LS^{DOX} (SPC: CHO: HP2-PEG_{2000}-DSPE: DOX 4: 1: 1: 1 w/w/w/w) and HT-LS^{DOX} (SPC: CHO: HP2-PEG_{2000}-DSPE: TAT-PEG_{2000}-DSPE: DOX 8: 2: 1: 1:2 w/w/w/w)) in 6 mL dichloromethane/methanol (v/v 2:1). The solvent was removed by vacuum rotary evaporation to form a dry drug-containing lipid film. The dried film was hydrated with phosphate-buffered saline (PBS; pH7.4) at 60 °C for 15 min, then sonicated for 15 min with a bath type sonicator at 60 °C. Finally, the liposome suspension was eluted by 220nm hyperfiltration membrane. The morphology of LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} were determined using a Tecnai G220 STWIN transmission electron microscope (TEM, Philips, Netherlands) with 200 kV acceleration voltage. Liposomes were pre-stained with 1% uranyl acetate. Put 8 μL liposome suspension onto carbon membrane support copper mesh for 1 min, and then move away the residual liposome suspension. When the membrane was dried, 6 μL negative staining solution uranyl acetate was added to the membrane for 5 min. Following, remove the negative staining solution, and dry the membrane in the air over night. Finally, the samples were observed by TEM. Particle size and zeta potential were determined by dynamic light scattering (DLS) using a Zetasizer 5000 (Malvern Instruments, Malvern, Worcestershire, U.K.).

9. Encapsulation efficiency detection of the liposomal probes

In our study, DOX was dissolved in methanol at 100 μg/mL concentration. The standard curve was measured with serial dilutions: 20, 10, 5, 2.5, 1.25, 0.625 μg/mL. Fluorescence intensities of the standard DOX solutions were measured at excitation wavelength of 488 nm and emission wavelength of 560-590 nm. It showed a linear standard curve with R^2 of 0.9921 (Figure S3a). The liposomal probes encapsulation efficiency were determined from the amount of entrapped drugs using the centrifugation technique. Briefly, total doxorubicin was determined after having dissolved and un-loaded DOX was centrifuged by ultrafiltration centrifugal tube at 50000 rpm for 20 min. The free DOX amount was determined in the outer tube. Next, the loading efficiency of DOX in liposomes was also measured using a following method. The amount of DOX loaded in each liposome was calculated by measuring the fluorescence intensity of the collected liposomes. According to formula (1):

$$EE\%=(W_{encapsulation} / W_{total})\times 100\% \quad (1)$$

The encapsulation efficiency of LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} were calculated as about 79.35%, 80.36%, 80.48% and 81.26%. The DOX loading efficiency of LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} were calculated as about 7.94%, 8.04%, 8.05% and 8.13% calculated by formula (2):

$$LE\%=(W_{encapsulation} / W_{liposomes})\times 100\%. \quad (2)$$

$W_{encapsulation}$, W_{total} and $W_{liposomes}$ represent the amount of DOX in liposomes, the total amount of DOX in the system and the total amount of liposomes, respectively.

10. The characterization of all the liposomal probes.

HP2 and TAT (Figure S1a-d) were conjugated with an amphiphilic molecular 1,2-distearoyl-sn-glycero-3-phos-phoethanolamine-N-[poly(ethylene glycol)-2000]-N-hydroxy-succinimidyl (NHS-PEG_{2000}-DSPE), respectively. The reaction process was monitored by HPLC (Figure S2a and b) and MALDI-TOF-MS. It showed that the peaks of HP2-PEG_{2000}-DSPE (from m/z 3100 to m/z 4130) and TAT-PEG_{2000}-DSPE (from m/z 3100 to m/z 4819) were both right-shifted, which indicated that the successful conjugation (Figure 2 a-c). In order to prepare a nano delivery system, drugs were encapsulated into the liposomal probe. Herein, DOX (doxorubicin) was introduced. DOX is widely-used as a chemotherapy drug which shows the anti-cancer effect by damaging the DNA structure. DOX could also be used as a signal molecule for its fluorescence-emission wavelength in the 560-590 nm window. The liposomal probes were prepared by thin film dispersion method. The size and zeta potential were measured by dynamic light scattering (DLS) (Figure 2d-g). The average diameter of synergetic liposomes HT-LS^{DOX} (HP2 and TAT functionalized liposomes loading DOX), mono-functionalized liposomes T-LS^{DOX} (TAT functionalized liposomes loading DOX), H-LS^{DOX} (HP2 functionalized liposomes loading DOX) and blank liposome (LS^{DOX}) were 154.7 nm, 157.6 nm, 150.3 nm and 140.2 nm and the zeta potential were around -13.8 mV, -14.0 mV, -13.6 mV and -12.8 mV, respectively. The results indicated that the diameters were increased after peptide functionalization. And all the functionalized liposomal probes were in a stable status. The morphologies of these liposomal probes were observed by transmission electron microscope (TEM). It is suggested that all of them were nanospherical in shape with stable dispersion. Furthermore, the DOX encapsulation efficiency was also calculated as 81.26% of HT-LS^{DOX}, 80.36% of T-LS^{DOX}, 80.48% of H-LS^{DOX} and 79.35% of LS^{DOX} (Figure S3a). The release rates of the probes were also verified. As shown in Figure S3b, the release rates of blank liposomal probes were more rapid than functionalized probes, which suggested a sustained-release effect of functionalized liposomal

probes. We suspect that a steric protection effect endowed by peptides may minimize the premature leakage of liposomes and lead to the timed release.

11. *In vitro* release study

To investigate the DOX release, the required quantity of DOX-loaded liposomes was transferred into a dialysis bag (MWCO 3,500). 1 mL of DOX solution (DOX-sol), LS-DOX solution, LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} solution were placed into the dialysis bags. Then the dialysis bags were introduced into the *in vitro* release medium containing 40 mL PBS buffer solution with 0.5% Tween-80 (200 μL), and were then gently shaken in a water bath at a constant temperature (37 °C). In all cases, the sink conditions were maintained by replacing 200 μL of the release medium with fresh medium at defined time intervals. To estimate the amount of drug release, the fluorescence intensity of drug in the release medium at each sampling point was measured by infinite M200 microplate reader (Tecan, Durham, USA). As shown in Figure S3b, after 60 h of dialysis in PBST. All of the release rates of DOX from loaded liposomes were much slower than that of DOX-sol. This could be an indication that DOX was steadily incorporated into the lipid core. A steric protection effect endowed by HP2 and TAT on the surface of liposomes may minimize the drug premature leakage and lead to slower release.

12. Confocal fluorescence imaging of cancer cells

The human breast cancer cell line SKBR3 were cultured in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin solution at 37 °C containing 5% CO_2. The human embryonic kidney cell lines 293T were cultured in Hyclone DMEM/high glucose with 10% fetal bovine serum and 1% penicillin solution at 37 °C containing 5% CO_2. For SKBR3 and 293T cells, approximately 1×10^5 mL^{-1} cells were seeded into culture dishes and cultured overnight for the adhesion of cells. Both the cells were incubated with LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} (15 μg/mL, 200 μL) as well as Hoechst 33342 (10 μg/mL, 200 μL) for 15 min at 37 °C. Finally, the cells were washed three times with cold PBS. Confocal fluorescence imaging was performed on an Olympus FV1000-IX81 confocal laser scanning microscope. A FV5-LAMAR 488 nm laser was the excitation source for DOX throughout the experiment, and emission was collected between 560-590 nm. Hoechst 33342 was excited at 50 mW, Ex: 405 nm, Em: 472 nm. The objective lens used for imaging was a UPLSAPO 63×oil-immersion objective (Olympus).

13. Uptake by breast cancer cells at different time

Cellular uptake of LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} at different time was investigated by qualitative confocal. SKBR3 cells were seeded into 35mm microscope dishes. LS-DOX, TAT-LS-DOX, HP2-LS-DOX and HP2/TAT-LS-DOX (15 μg/mL, 200 μL) as well as Hoechst 33342 (10 μg/ mL) were incubated with the cells at 37°C for 10, 15, 30, 60 and 120 min at 37°C. Cells were washed with PBS and observed by confocal laser scanning microscope.

14. Flow cytometry detection for cellular uptake of all liposomal probes

The cellular uptake of all liposomal probes were studied by flow cytometry analysis. Typically, SKBR3 cells were seeded into six-well plates at a density of 5×10^5cells/well and cultured at 37°C for 24 h, respectively. Prior to the experiment, cells were washed twice with PBS (pH 7.4) to remove the remnant growth medium, and then incubated in serum-free medium containing various DOX formulations at the final DOX concentration of 20 μg/mL. After 1 h incubation, the cells were washed three times with cold PBS and then re-suspended in 500 mL of PBS. The DOX fluorescence intensity was measured by a flow cytometer (Becton Dickinson FACS Calibur, Mountain View, USA).

15. Binding specificity of HT-LS^{DOX} towards breast cancer cells

To investigate cell binding specificity of HT-LS^{DOX} towards SKBR3, the competition experiment was carried out. After SKBR3 cells were treated with the anti-HER2 antibody (the recommended dilution of 1:20) for 1 h, the cells were incubated with Hoechst 33342 (10 μg/mL, 200 μL) and HT-LS^{DOX} (15 μg/mL, 200 μL) for 15min at 37 °C. Finally, the cells were washed three times with cold PBS. Confocal fluorescence imaging was performed on an Olympus FV1000-IX81 confocal laser scanning microscope (CLSM). The binding and drug delivery by HT-LS^{DOX} was dramatically decreased to SKBR3 cells.

Furthermore, we evaluated the significant effect of HP2. SKBR3 and 293T cells were seeded into 35mm microscope dishes. T-LS^{DOX} and HT-LS^{DOX} (15 μg/mL, 200 μL) as well as Hoechst 33342 (10 μg/ mL) were incubated with the cells at 37°C for 10, 15, 30, 60 and 120 min at 37°C. Cells were washed with PBS and observed by confocal laser scanning microscope. As shown in Figure S7a-j, The DOX delivery efficiency of T-LS^{DOX} showed negligible differences between SKBR3 and 293T cells. However, the delivery efficiency of HT-LS^{DOX} towards the two cells were totally different (Figure S7k-t). Through the synergetic delivery effect, HT-LS^{DOX} could enter the SKBR3 in an efficient way. 2 h later, a large portion of DOX has been delivered into the cytoplasm (Figure S7t). The enhanced effect was negligible for 293T. So, HT-LS^{DOX} had high delivery efficiency towards SKBR3 cells because the specific recognition element HP2. However, it is reported that TAT is non-specific cell penetrating peptide. It has a region consisting of two lysine residues and six arginine residues. Their cationic charges facilitate interaction with the normally negatively charged cell membrane and trigger permeabilization of the cell membrane. So, the delivery effect of T-LS^{DOX} is receptor-independent.

16. Inhibition ratio of cells studies

SKBR3 cells were seeded at 5 ×10^3 cells per well in 96-well plate, pre-incubated for 24 h, then incubated with LS^{DOX}, T-LS^{DOX}, H-LS^{DOX}, HT-LS^{DOX} and free DOX for 24 h at doxorubicin concentrations ranging from 0.001 to 100 μg/mL (200 μL). The other SKBR3 cells were seeded at a density of 5×10^3cells per well in flat bottom 96 well plates and incubated overnight. Thereafter, LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} and free DOX (100 μg/mL, 200 μL) were added in the wells of the plates and then exposed for 2, 4, 6, 8, 10 and 12 h. After that, removing the medium and adding fresh basic medium (200 μL) continue to incubation for12h at 37 °C in a 5% CO_2 incubator. The medium was replaced with 100 μL 0.5 mg/mL MTT and after 3 h the MTT solution was replaced with 150 μL DMSO solution. The absorbance was measured at 570 nm with a reference wavelength of 630 nm using an Infinite M200 microplate reader (Tecan, Durham, USA). Untreated cells in medium were used as control. All experiments were carried out with four replicates.

17. *In vivo* assays

Female BALB/c nude mice of about 18 g were purchased from Vital River Laboratory Animal Center (Beijing, China), and kept under specific pathogen-free conditions with free access to standard food and water. All the animal experiments were conducted in compliance with the guide for the care and use of laboratory animals of Beijing University Animal Study Committee's requirements. The xenograft tumors were established by subcutaneously (S.C.) injection of 1×10^7/mL SKBR3 cells to the right hind leg of the approximate 6 weeks-old female BALB/c nude mice. 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbonyaineiodide (Dir) with emission maxima centered at 748 nm were purchased from Invitrogen. Tumor growth was measured periodically and

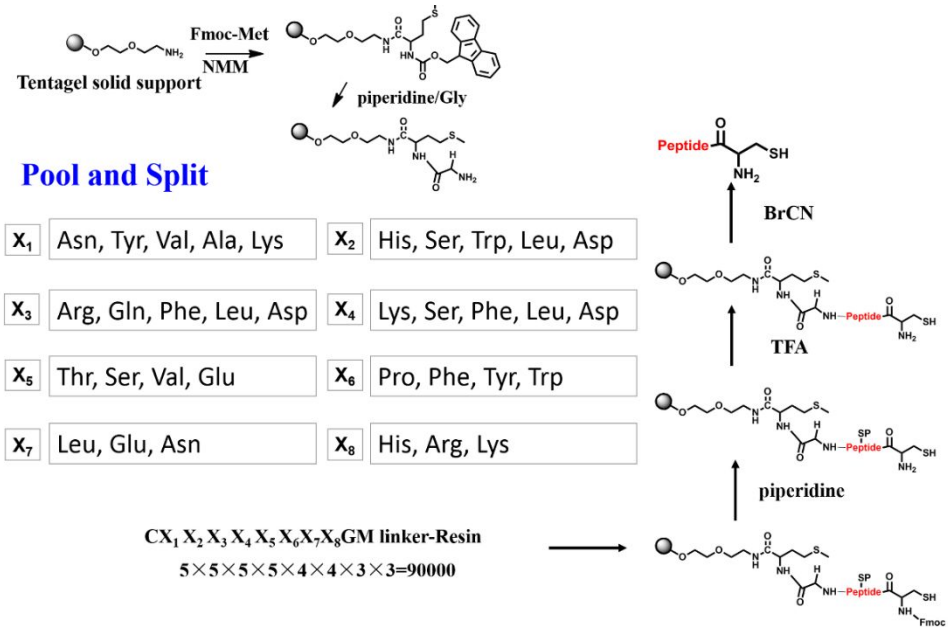
until the tumors reached to grow to about 8 mm in diameter (14 weeks old on arrival). Mice were injected via the caudal vein with PBS, LS^{DiR}, T-LS^{DiR}, H-LS^{DiR} and HT-LS^{DiR} (200μL) at a dose corresponding to 1 μg/mL of DiR. Near-infrared imaging was carried out after 4 and 8 hrs, using a Maestro *in vivo* spectrum imaging system (Cambridge Research & Instrumentation, Woburn, MA). After 8 hrs, organs were excised for *ex vivo* fluorescence imaging. All images were taken with a ten-second exposure time to ensure consistency in the data.

In vivo tumor suppression studies were carried out to examine the toxicity and tumor inhibition efficiency of LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX}. Mice were injected intravenously with PBS (control group), LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} (200μL) at a dose corresponding to 6mg/kg of DOX. Administration was carried out on the first, second, fourth, fifth, seventh, and eighth days. Successive observation was performed in the next 6 days. After the whole assay, tumors were excised. The weights and tumor sizes were recorded daily at the same time. Tumor sizes were measured by a vernier caliper. Tumor volume was calculated by the formula $(L \times W^2)/2$. L is for the longest and W is the shortest in tumor diameters (mm).

18. The RNA-sequencing analysis

The whole transcriptome sequencing reproduced about 0.39 billion reads in all the 18 samples (three independent replicates for each group and twice of the experiments). Each sample have averagely reads of 45 million. The bioinformatic analysis was employed to calculate the gene expressions across in each sample. There are differential expression genes between each sample pair.

Supplementary figures



Scheme S1. Synthesis process of the OBOC peptide library towards HER2

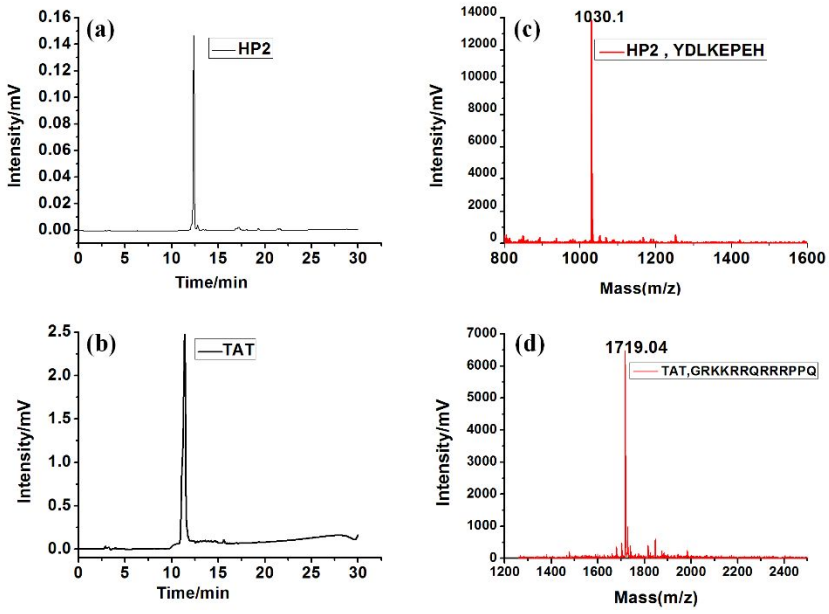


Figure S1. (a, b) HPLC for HP2, TAT peptide (c, d) MALDI-TOF-MS for HP2, TAT

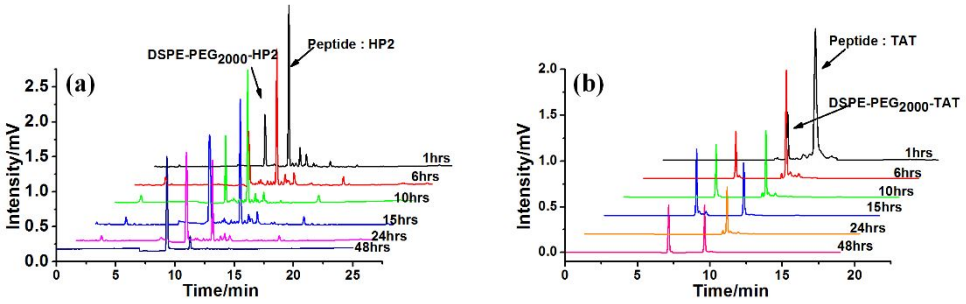


Figure S2. (a, b) Monitoring of the conjugation between TAT, HP2 and NHS-PEG$_{2000}$-DSPE

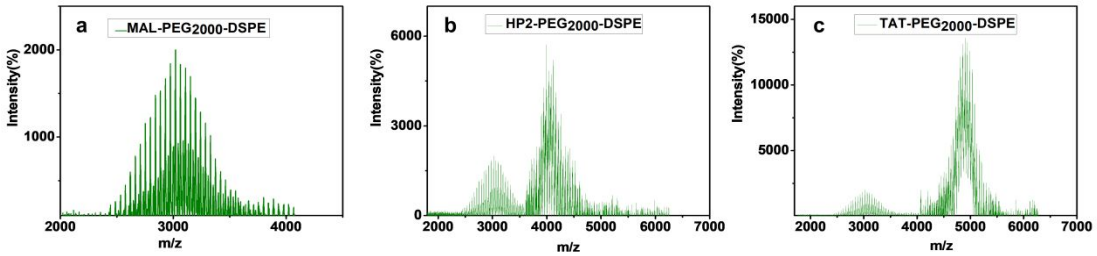


Figure S3. Monitoring of the conjugation between peptides and NHS-PEG$_{2000}$-DSPE by MALDI-TOF-MS. (a) MALDI-TOF-MS spectra of NHS-PEG$_{2000}$-DSPE. (b, c) MALDI-TOF MS spectra of HP2-PEG$_{2000}$-DSPE and TAT-PEG$_{2000}$-DSPE.

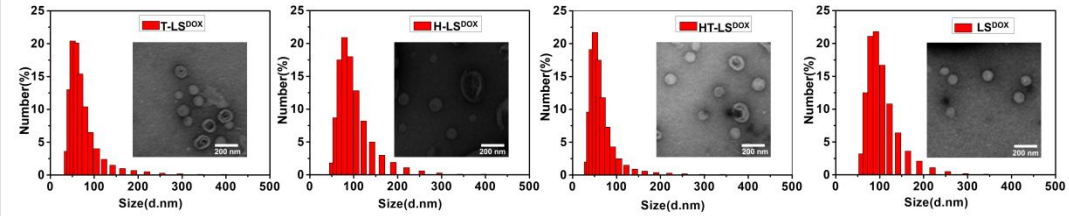


Figure S4. The size distribution and TEM graph of T-LS^{DOX}, H-LS^{DOX}, HT-LS^{DOX} and LS^{DOX}.

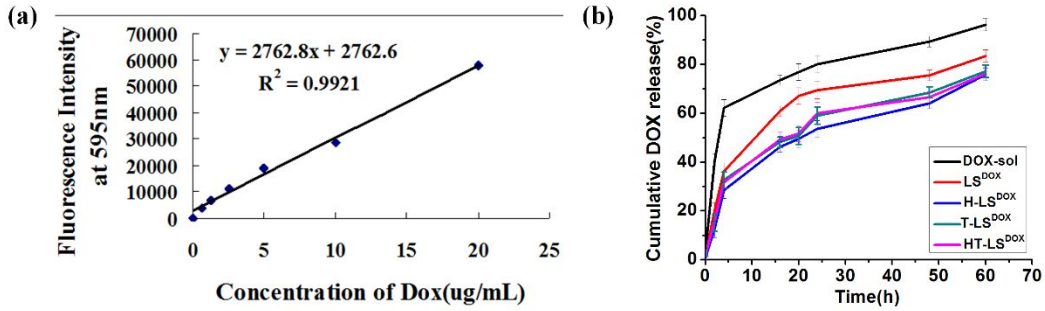


Figure S5. The DOX encapsulation efficiency and the release rates characterization of the probes. (a) A standard curve of DOX in PBS obtained by serial dilution: 20, 10, 5, 2.5, 1.25, 0.625 μg/mL. (b) *In vitro* release of DOX from DOX solution, LS^{DOX}, T-LS^{DOX}, H-LS^{DOX} and HT-LS^{DOX} solution in phosphate buffer solution with 0.5% Tween-80 (mean ± SD, n=3).

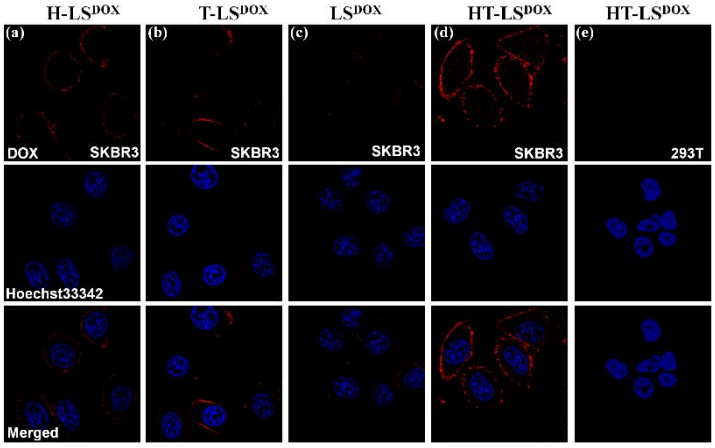


Figure S6. Confocal microscopy images of nanoprobes towards cells. (a-d) SKBR3 cells were incubated with H-LS^{DOX}, T-LS^{DOX}, LS^{DOX} and HT-LS^{DOX}. (e) 293T cells were incubated with HT-LS^{DOX}.

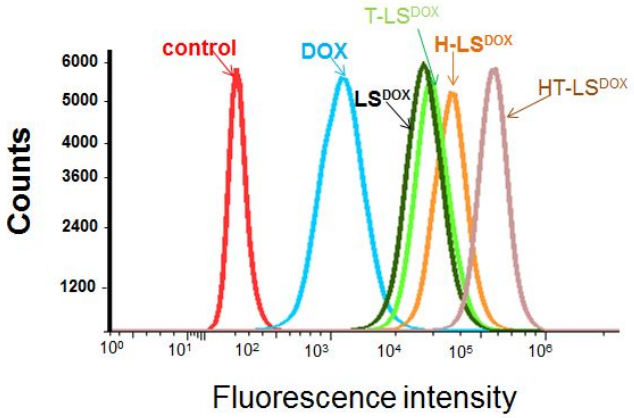


Figure S7. Flow cytometric measurement of DOX uptake by SKBR3 after incubated with DOX solution (DOX-sol), H-LS^{DOX}, T-LS^{DOX}, LS^{DOX} and HT-LS^{DOX}.

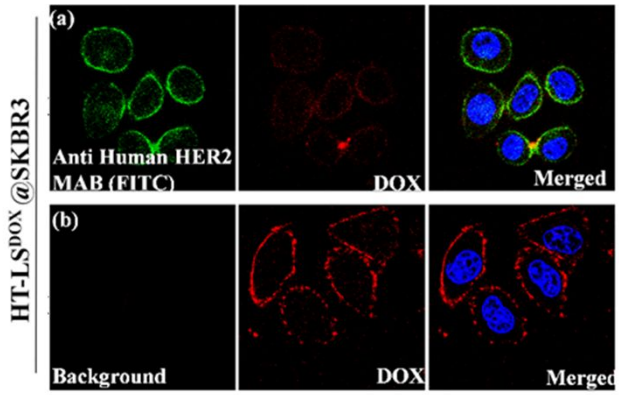


Figure S8. SKBR3 cells were treated with HT-LS^{DOX} and anti-HER2 antibody (a) SKBR3 cells were treated with anti-HER2 antibody and HT-LS^{DOX} together. (b) SKBR3 cells were treated with HT-LS^{DOX} only.

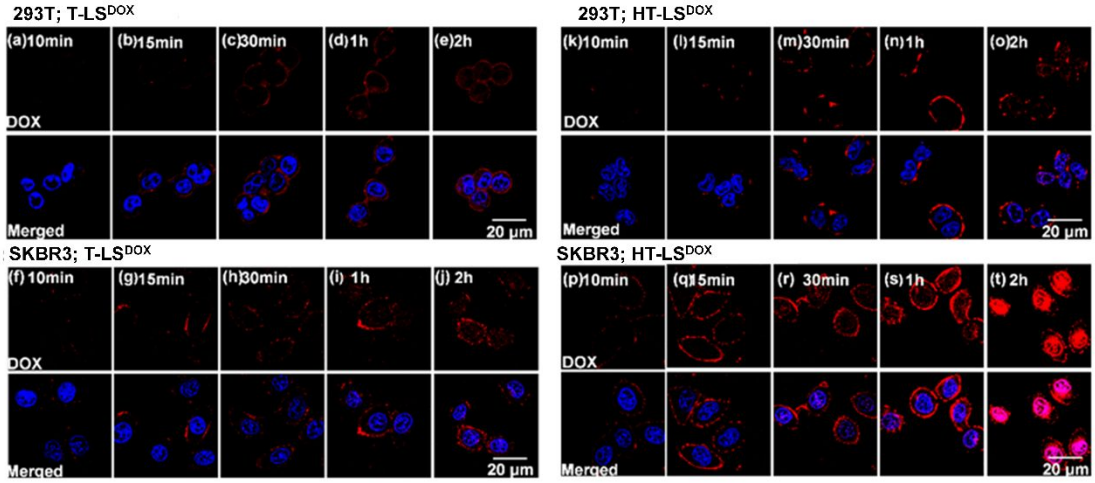


Figure S9 Time-dependent image of the 293T and SKBR3 cells incubated with T-LS^{DOX}, HT-LS^{DOX}. (a-e) T-LS^{DOX} treated 293T cells at different time points. (f-j) T-LS^{DOX} treated SKBR3 cells at different time points. (k-o) HT-LS^{DOX} treated 293T cells at different time points. (p-t) HT-LS^{DOX} treated SKBR3 cells at different time points.

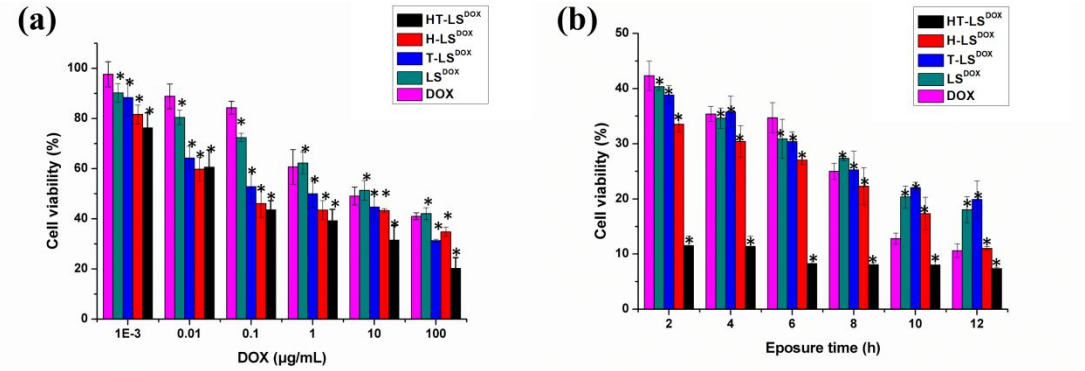


Figure S10. (a) *In vitro* cytotoxicity of H-LS^{DOX}, T-LS^{DOX}, LS^{DOX} and HT-LS^{DOX} solution to SKBR3 cells at various DOX concentration. (b) *In vitro* cytotoxicity of DOX–sol, H-LS^{DOX}, T-LS^{DOX}, LS^{DOX} and HT-LS^{DOX} solution to SKBR3 cells with different exposure time (mean±SD, n=4). (*$p < 0.05$ versus DOX-sol).