

*Supplementary Material*

# **Development of PET radioisotope copper-64-labeled theranostic immunoliposomes for EGFR overexpressing cancer-targeted therapy and imaging**

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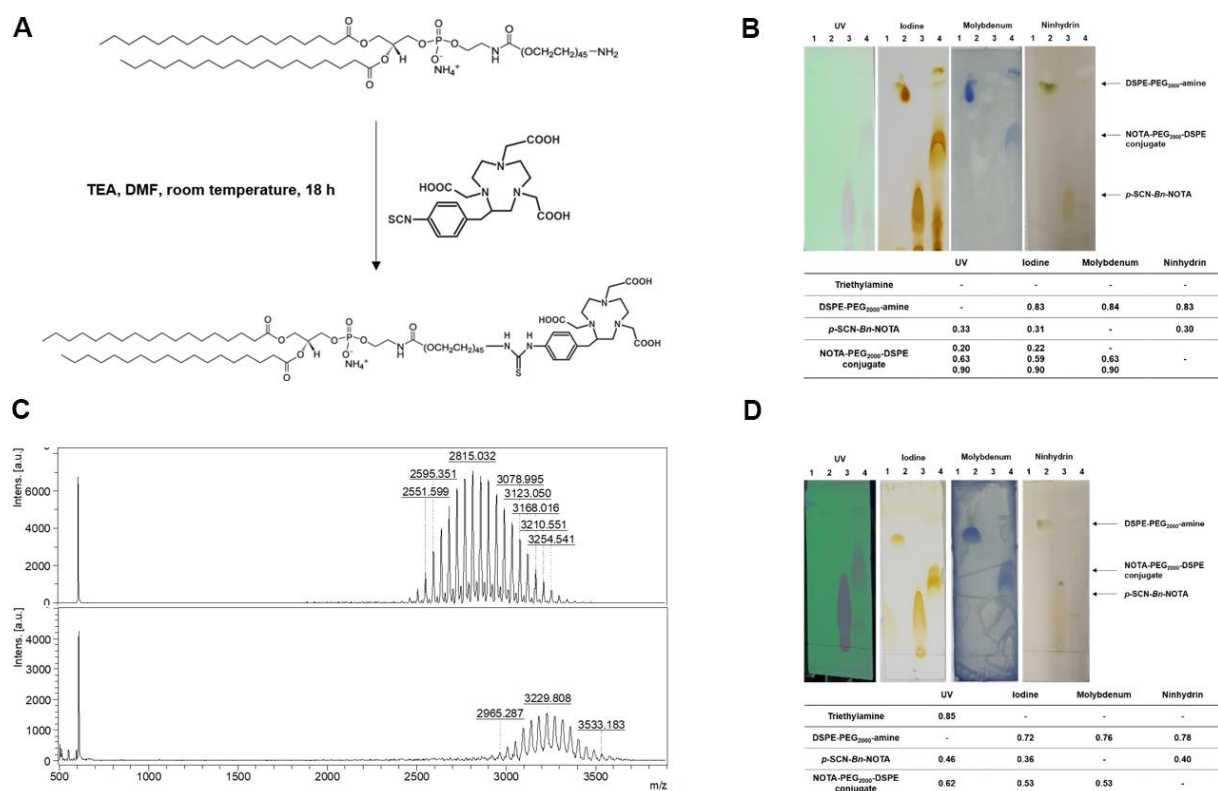
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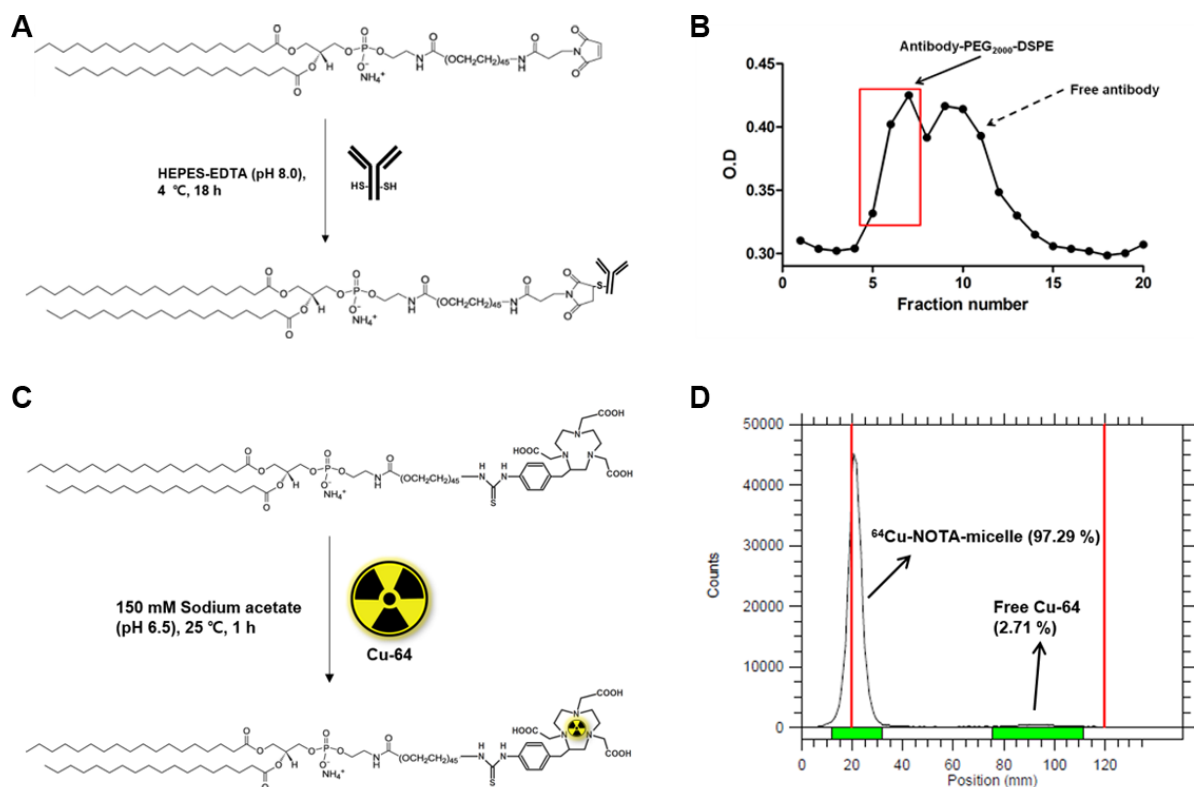
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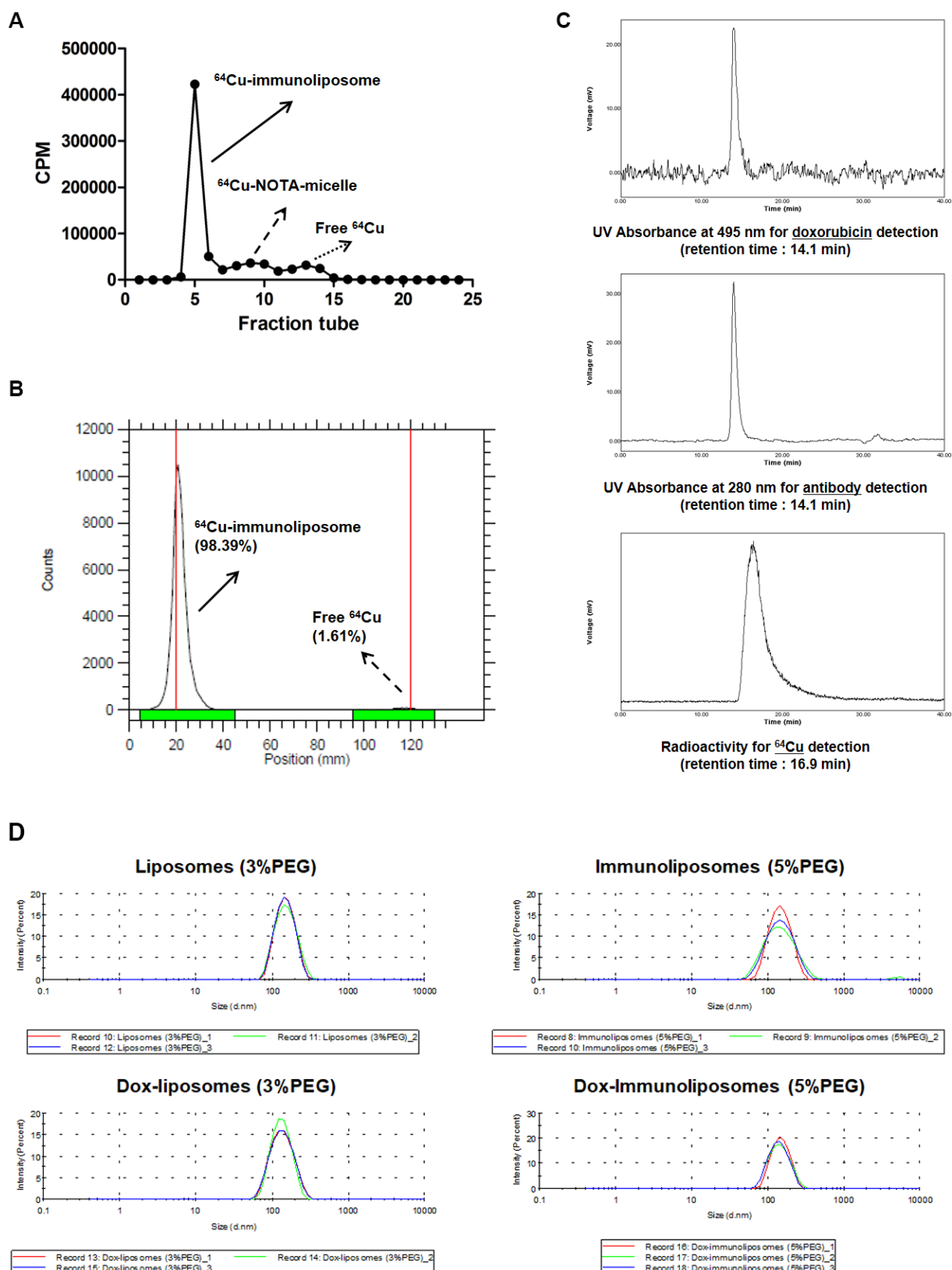
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**Supplementary Figure S1.** Analysis of DSPE-PEG2000-NOTA conjugate. **(A)** DSPE-PEG2000-amine was conjugated with *p*-SCN-*Bn*-NOTA and **(B)** the conjugate was analyzed by a TLC plate. **(C)** MALDI-TOF mass spectrometric analysis of the purified DSPE-PEG2000-NOTA conjugate and DSPE-PEG2000-amine demonstrated an increase in mass from 2859.8 to 3249.1 after conjugation with the *p*-SCN-*Bn*-NOTA. This corresponds to 0.86 NOTA molecules conjugated to one DSPE-PEG2000-amine. The x-axis shows the mass/charge (*m/z*) values, and the y-axis the signal intensity. And **(D)** the purified DSPE-PEG2000-NOTA was confirmed with a TLC plate.

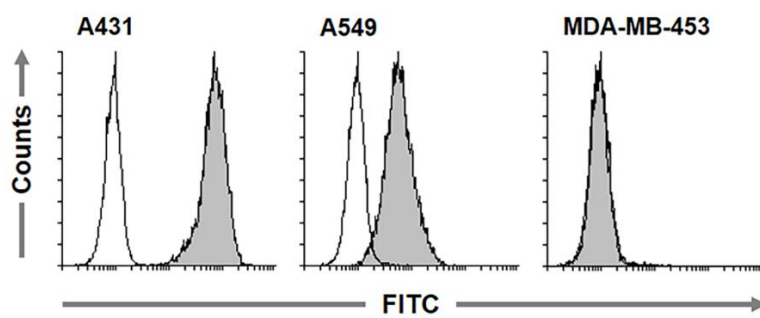


**Supplementary Figure S2.** Preparation of antibody-conjugated or copper-64-labeled micelles. **(A-B)** Anti-EGFR antibody (cetuximab) was conjugated to DSPE-PEG2000-maleimide in micelles, and the antibody conjugation was analyzed with a sepharose CL-4B column. **(C-D)** Copper-64 was labeled to DSPE-PEG2000-NOTA conjugate in micelles, and the copper-64-labeled micelles were analyzed with iTLC-SG.

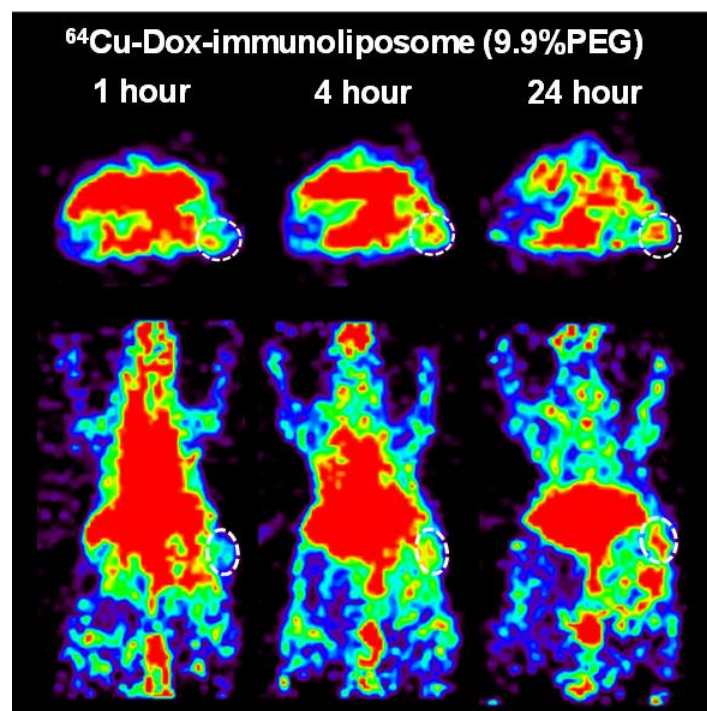


**Supplementary Figure S3.** Purification and evaluation of  $^{64}\text{Cu}$ -Dox-immunoliposomes.  $^{64}\text{Cu}$ -micelles were inserted into doxorubicin-encapsulating liposomes at  $37^\circ\text{C}$  for 4 h. **(A)** The  $^{64}\text{Cu}$ -labeled Dox-immunoliposomes were purified with sepharose CL-4B column and then analyzed using gamma-counter. The  $^{64}\text{Cu}$ -Dox-immunoliposomes in the 4<sup>th</sup> and 5<sup>th</sup> fractions from the column were analyzed with iTLC and HPLC. **(B)** iTLC analysis showed 98.0% or higher purity of the  $^{64}\text{Cu}$ -Dox-immunoliposomes. **(C)** HPLC chromatograms of  $^{64}\text{Cu}$ -Dox-immunoliposomes exhibited a retention time of 14.1 min, detected

by both doxorubicin detection at 495 nm and antibody detection at 280 nm. Meanwhile, the retention time measured with copper-64 was 16.9 min. (D) Size distribution by intensity of various liposomes in this study.



**Supplementary Figure S4.** Evaluation of EGF receptor expression on the cell surface. EGF receptor expression on the surface of A431, A549, and MDA-MB-453 cells was compared by flow cytometry.



**Supplementary Figure S5.** Biodistribution of  $^{64}\text{Cu}$ -Dox-immunoliposomes (10%PEG) in xenografted A549 tumor models. PET imaging of the A549 tumor-bearing mice at 1 h, 4 h, and 24 h after  $^{64}\text{Cu}$ -Dox-immunoliposomes (10%PEG) administration.

**Supplementary Table S1.** Tissue uptakes of  $^{64}\text{Cu}$ -PCTA-Cetuximab

$^{64}\text{Cu}$ -PCTA-Cetuximab	
48 h	
Blood	$9.7 \pm 0.4$
Heart	$3.4 \pm 0.2$
Liver	$10.4 \pm 0.6$
Lung	$6.8 \pm 0.7$
Spleen	$6.8 \pm 0.5$
Kidney	$5.0 \pm 0.3$
Muscle	$1.9 \pm 0.3$
Tumor	$9.2 \pm 1.1$
T/B	$1.0 \pm 0.1$
T/M	$4.9 \pm 0.8$
T/L	$0.9 \pm 0.1$

**Notes:** Tissue uptake of  $^{64}\text{Cu}$ -PCTA-Cetuximab in A549 tumor xenograft-bearing BALB/c-nude mice (n = 3) at 48 h post-i.v. injection expressed as %ID/g; Data (%ID/g) were presented as mean  $\pm$  S.D. (n = 3).



**Supplementary Table S2.** Physicochemical properties of liposomes

Nanoparticles	Size (nm)*	Zeta-potential (mV)*	Polydispersity Index	Doxorubicin Encapsulation Efficiency (%)
Dox-immunoliposomes (5%PEG)	147.5 ± 4.0**	-2.4 ± 0.6**	0.06	96.1
Dox-liposomes (8%PEG)	134.5 ± 1.3	-3.2 ± 0.5	0.08	82.8
Dox-immunoliposomes (10%PEG)	132.7 ± 4.8	-6.2 ± 0.2	0.16	79.8

**Notes:** \*The particle size and zeta-potentials were measured 3 times using a zeta sizer. \*\*The particle size (nm); average particle size ± S.D. Zeta potential (mV); average zeta-potential ± S.D.

**Abbreviations:** PEG, polyethylene glycol.

**Supplementary Table S3.** Biodistribution of  $^{64}\text{Cu}$ -Dox-immunoliposomes containing varied amounts of PEG

	$^{64}\text{Cu}$ -Dox-immunoliposome (5%PEG)			$^{64}\text{Cu}$ -Dox-immunoliposome (10%PEG)		
	1 h	4 h	24 h	1 h	4 h	24 h
<b>Blood</b>	26.4 ± 3.2	24.1 ± 6.4	3.1 ± 0.4	32.0 ± 5.7	28.5 ± 3.1	5.3 ± 1.9
<b>Heart</b>	3.3 ± 0.1	2.8 ± 0.7	0.9 ± 0.0	3.8 ± 0.4	3.5 ± 0.2	1.3 ± 0.3
<b>Liver</b>	11.4 ± 3.2	19.7 ± 0.9	17.8 ± 4.3	11.0 ± 3.8	16.0 ± 1.8	16.6 ± 1.4
<b>Lung</b>	7.4 ± 1.5	7.7 ± 1.8	2.0 ± 0.2	9.7 ± 4.1	8.6 ± 1.0	2.5 ± 0.5
<b>Spleen</b>	21.6 ± 2.9	44.3 ± 8.8	80.9 ± 34.7	28.1 ± 7.2	45.5 ± 12.1	87.5 ± 22.6
<b>Kidney</b>	6.6 ± 0.8	6.0 ± 1.1	3.3 ± 0.7	6.1 ± 1.0	7.8 ± 1.1	4.6 ± 0.4
<b>Muscle</b>	0.7 ± 0.1	0.8 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.6 ± 0.1
<b>Tumor</b>	1.4 ± 0.4	2.9 ± 0.5	2.8 ± 0.0	1.3 ± 0.6	2.7 ± 0.4	4.0 ± 0.4
<b>T/B</b>	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	0.0	0.1 ± 0.0	0.8 ± 0.3
<b>T/M</b>	2.1 ± 0.7	3.7 ± 0.5	6.9 ± 1.7	1.8 ± 0.6	2.7 ± 0.7	7.0 ± 0.2
<b>T/L</b>	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0

**Notes:** Tissue uptakes of  $^{64}\text{Cu}$ -Dox-immunoliposomes (5%PEG or 10%PEG) in A549 tumor xenograft-bearing BALB/c-nude mice (n = 3) at 1 h, 4 h, and 24 h post-i.v. injection expressed as %ID/g; Data (%ID/g) were presented as mean ± S.D. (n = 3).