Synthesis of ligands conjugated with stearyl chains

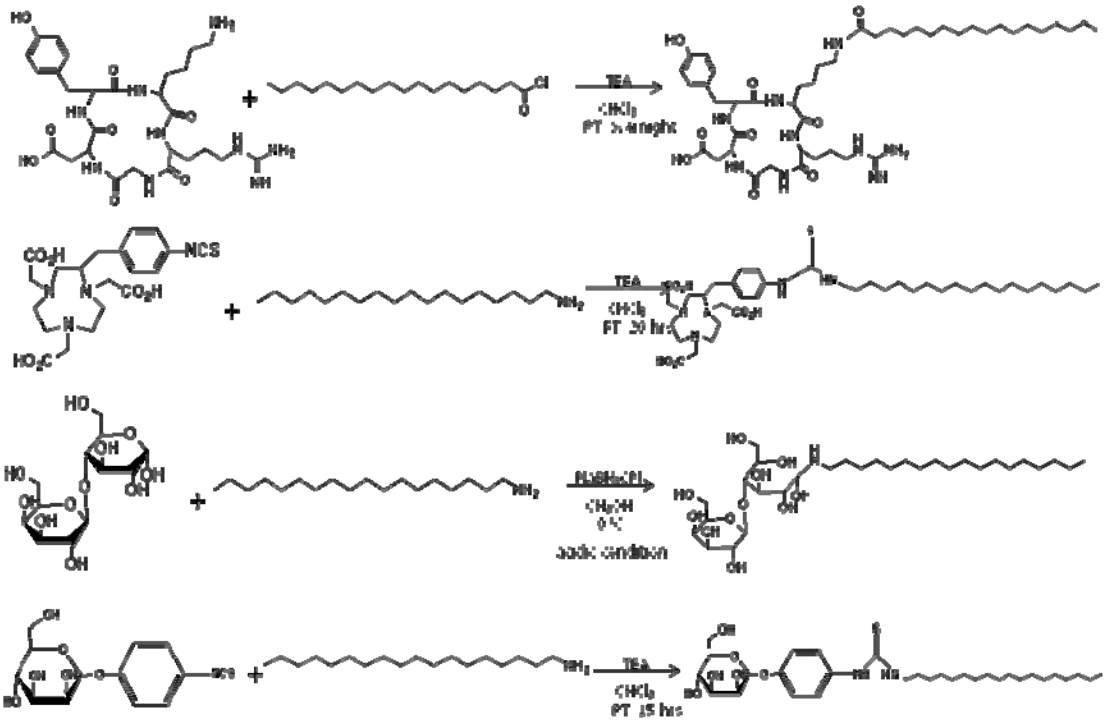
RGD-C_{18}. cRGDyK (10 mg, 0.02 mmol) was dissolved in $CHCl_3$ (0.5 mL), and triethylamine (TEA) (0.007 mL, 0.05 mmol) was then added and stirred at room temperature overnight. Stearoyl chloride (15 mg, 0.05 mmol) was then added and also stirred at room temperature overnight. Reaction completion was checked by mass spectroscopy, and when completed the reaction mixture was washed with water, and the organic layer was concentrated. The solid obtained was washed with MeCN (1 mL). Yield: 8 mg (57%). Mass spectrum (ESI^+), ($M+H^+$): 886.6. HRMS ($M+H^+$): observed 886.5769, calculated 886.5766.

NOTA-C_{18}. 2-(p-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (SCN-Bz-NOTA) (20 mg, 0.04 mmol) was dissolved in $CHCl_3$ (1 mL), and TEA (0.012 mL, 0.09 mmol) was added and stirred at room temperature. Stearylamine (14 mg, 0.05 mmol) was then added to the reaction mixture and stirred for 20 hr. The product was confirmed by mass spectroscopy. Yield: 26 mg (72%). Mass spectrum (ESI^+), ($M+H^+$): 821.8. HRMS ($M+H^+$): observed 720.4731, calculated 720.4734.

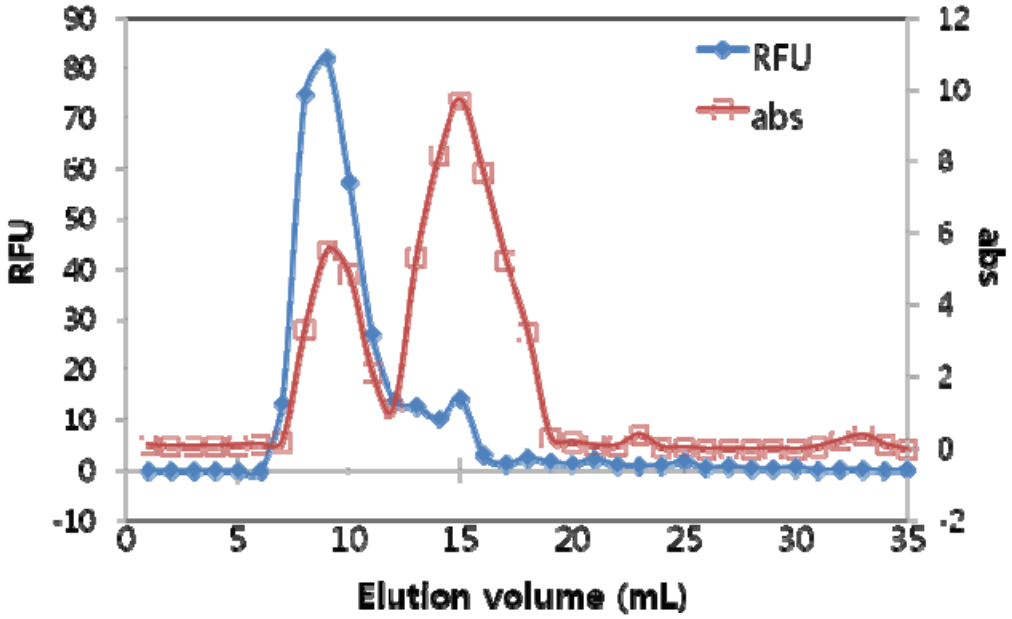
Lac-C_{18}. Stearylamine (20 mg, 0.07 mmol) was dissolved in methanol (2 mL), and α-lactose (267 mg, 0.74 mmol) in water (5 mL) was added and stirred at 0°C. After adding sodium cyanoborohydride (7 mg, 0.11 mmol) in methanol (1 mL), acetic acid was added dropwise and stirred. Reaction completion was checked by mass spectroscopy and the product was purified by silica gel column chromatography. Yield: 30 mg (68%). Mass spectrum (ESI^+), ($M+H^+$): 596.4. HRMS ($M+H^+$): observed 596.4380, calculated 596.4374.

Man-C_{18}. α-D-Mannosepyranosyl-phenylisothiocyanate (14 mg, 0.04 mmol) was dissolved in $CHCl_3$ (2 mL). TEA (0.012 mL, 0.09 mmol) and stearylamine (12 mg, 0.04 mmol) were then added sequentially with stirring. Turbid solution became clear as the reaction proceeded. The reaction mixture was stirred for 15 hr, checked by mass spectroscopy, concentrated, and purified by silica gel column chromatography. Yield: 26 mg

(77%). Mass spectrum (ESI^+), ($M+H^+$): 583.4. HRMS ($M+H^+$): observed 583.3785, calculated 583.3781.

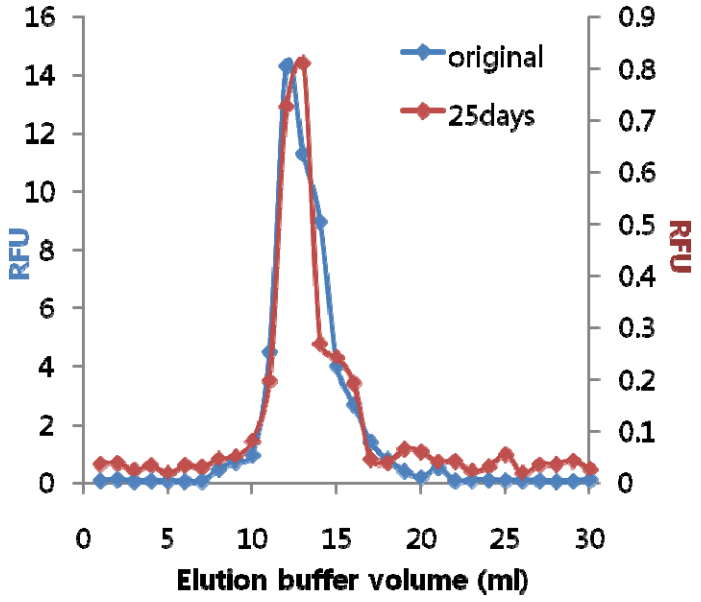


Supplemental Scheme 1. Conjugation of ligands with stearyl chain compounds.



Supplemental Figure 1. Size exclusion chromatography profile of QD655T. Sample was loaded to Sephacryl S-300 (V_o=7.5 mL) and eluted with borate buffer (pH 7.4, 20 mM). The blue line shows the fluorescence of QD in relative fluorescence units (RFU) and the red line shows the absorbance at 273 nm.

A



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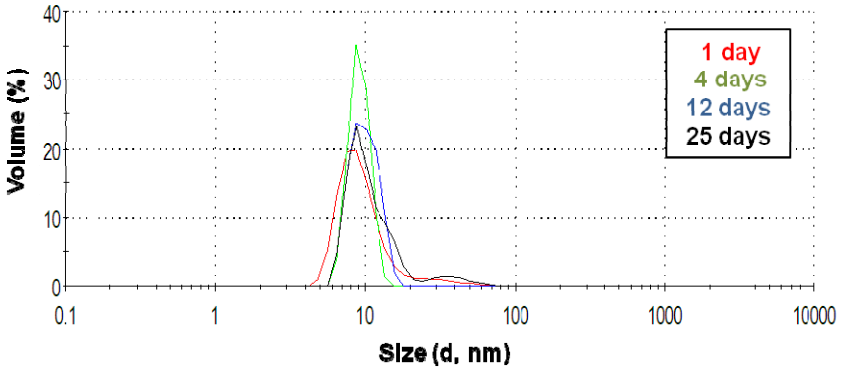
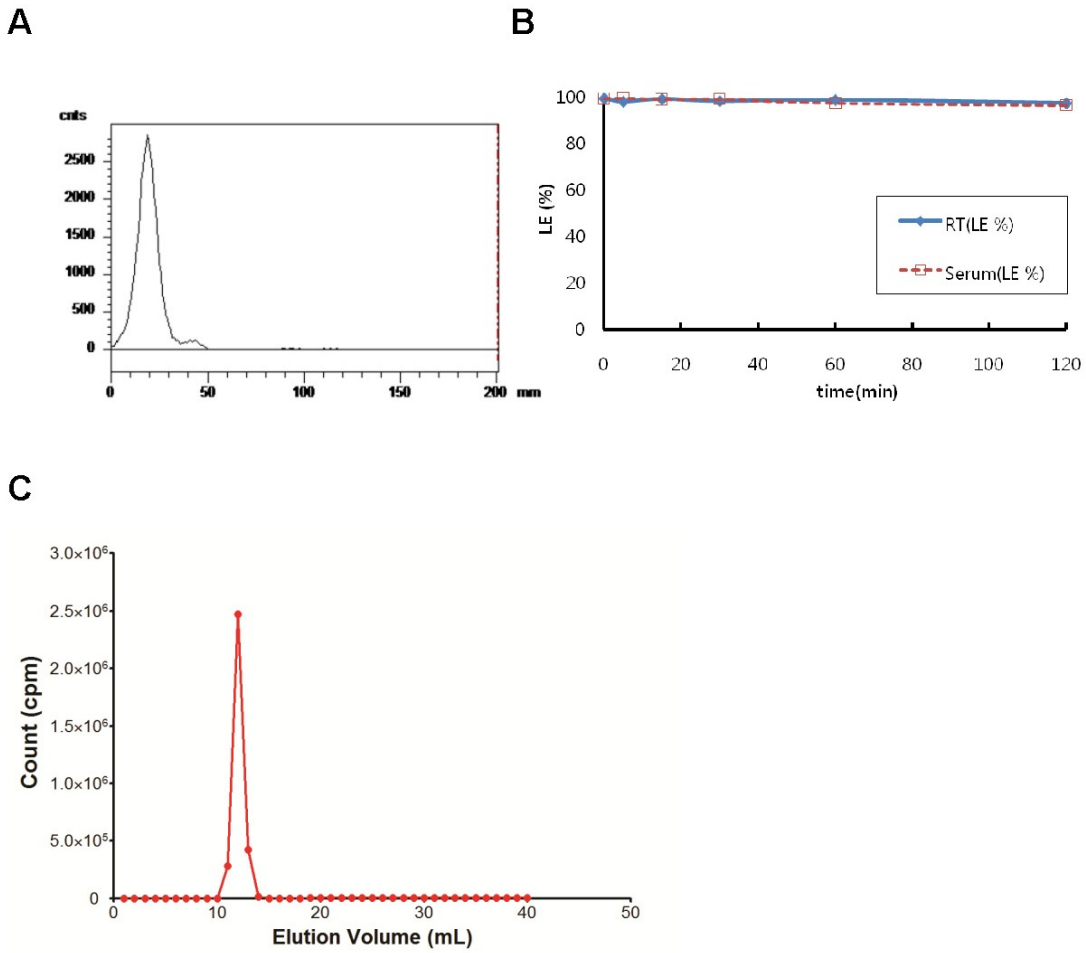
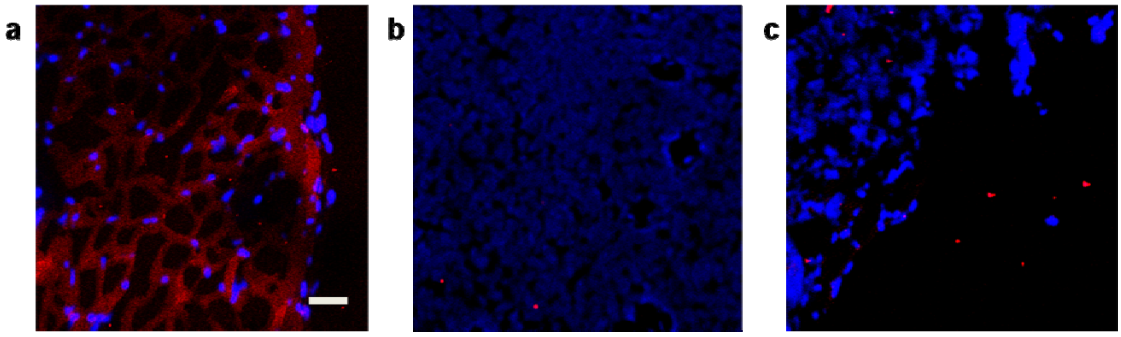


Supplemental Figure 2. Stability testing of encapsulated QDs. Samples were stored for 25 days at 4°C. (A) Size exclusion chromatogram obtained using a Sephacryl S-500 HR column (V_0= 7.5 ml) eluted with borate buffer (pH 7.4, 20 mM). Blue and red lines indicate chromatograms of newly prepared and 25 day old QD545T, respectively. The peak shape change is negligible. (B) Size distributions measured by DLS did not change significantly.

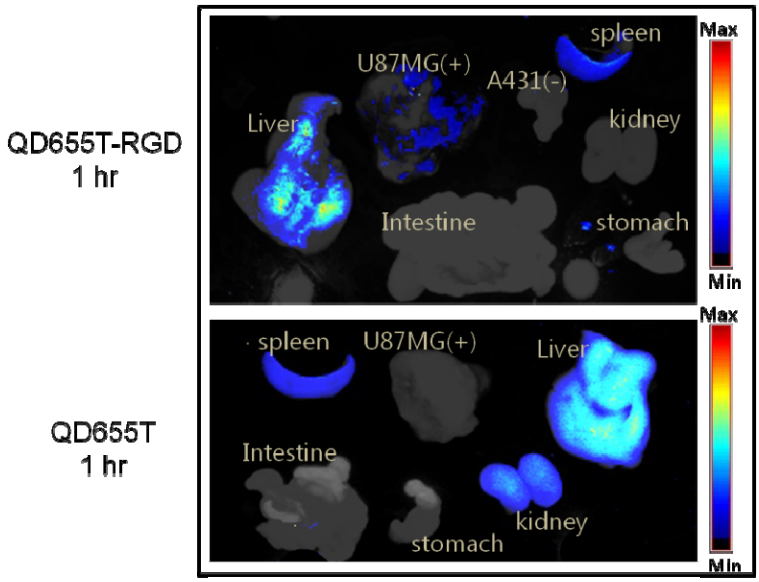


Supplemental Figure 3. Determination of ^{68}Ga-labeling efficiency of NOTA-encapsulated QD and the stability test results obtained for the labeled product. (A) Radio-TLC result of ^{68}Ga-NOTA-QD655T-RGD. No free Ga^{68} was detected by ITLC-SG eluted with 0.1 M citric acid. (B) Stability test of Ga^{68}-NOTA-QD655T in PBS at room temperature and in human serum at 37°C. (C) Gel filtration profile of ^{68}Ga-NOTA-QD655T-RGD using Sephacryl S-500 eluted with 20 mM sodium borate buffer (pH 7.4). No dissociation of ^{68}Ga-NOTA-C_{18} was detected from the encapsulated QD.



Supplemental Figure 4. Confocal fluorescence images of U87MG tumor tissue obtained from a xenografted mouse. Frozen tissue slices (7 μm) were incubated with (A) QD655T-RGD, (B) QD655T, or (C) QD655T-RGD plus cRGDyK (500 nM). All images were obtained under the same experimental conditions and are displayed on the same scale. The scale bar indicates 50 μm. The data shows that QD655T-RGD binds specifically with U87MG which can be blocked by cRGDyK.

A



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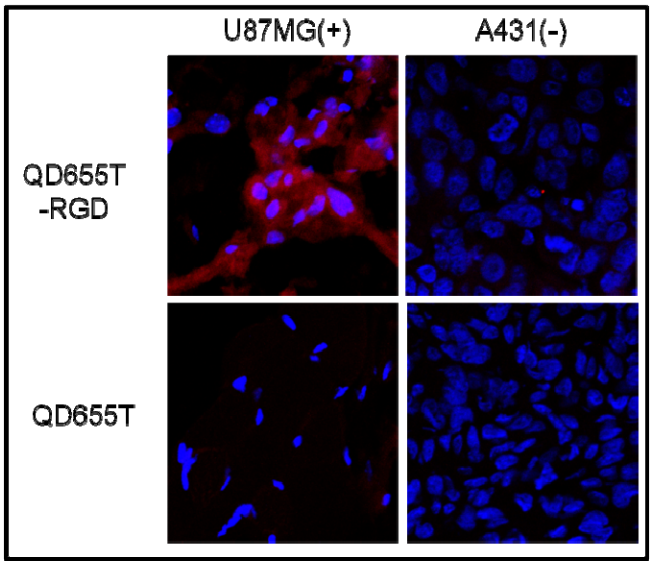

Supplemental Figure 5. *In vivo* targeting and fluorescence imaging of U87MG and A431 tumors xenografted in mice 1 hr after injecting QD655T-RGD intravenously. (A) Fluorescence images of major organs and tumor mass were obtained using Maestro system. (B) DAPI and QD fluorescence images of frozen slices (7 μm) of tumor tissues were obtained using a confocal microscope. All images were acquired under the same experimental condition. The scale bar represents 10 μm. The data clearly shows that QD655T-RGD binds to U87MG but not to A431. QD655T does not bind to the tumors.

Supplemental Videos 1 and 2. 3D rotating video clips of microPET obtained 10 min post-injection of (A) ^{68}Ga-NOTA-QD655T-Man or (B) ^{68}Ga-NOTA-QD545T-Lac. ^{68}Ga-NOTA-QD655T-Man shows both liver and spleen uptakes, but ^{68}Ga-NOTA-QD545T-Lac shows only liver uptake.