MATERIALS AND METHODS

Chemistry

Evaluation of aptamer by LCMS. Mass spectral analysis of aptamers was done on Waters (Milford, MA) Acquity UPLC coupling with a Q-Tof Premier high resolution mass spectrometer. An UPLC BEH C18 column (150 x 2.1 mm) eluted with a gradient solvent system consisting of solution A (15 mM ammonium acetate, adding triethylamine to pH 8.5) and solution B (A and 20% CH_3CN) at 0.35 mL/min flow rate. The elution profile was: 100% (v:v) A and 0% B initially; gradient 0 - 40% B over 10 min. Negative ESI mode was used with a source capillary voltage of 2.0 kV, source temperature of 80 ˚C, desolvation temperature of 280 ˚C, cone gas flow of 50 L/Hr (N2), and desolvation gas flow of 500 L/Hr (N_2). The spectra, composed of multiply charged fragments, were transformed into a mass scale using MaxEnt 1 software (Waters).

Synthesis of FB-Aptamer. 13 nmol of aptamer (290 µg) were dissolved in 230 µL of phosphate buffer (pH 8.5). Then 2.4 eq. of N-succinimidyl 4-fluorobenzoate (SFB, 7.6 µg) dissolved in 20 µL of CH_3CN were added. The mixture was incubated at 37°C for 30 min and then purified by HPLC with a retention time of 14.77 min for the non-conjugated aptamer and 17.17 min for FB-aptamer. The two collected HPLC fractions were lyophilized. Deconvolution analysis of LC-MS (supplemental material) confirmed mass of 21620 for the aptamer and 21742 for FB-aptamer.

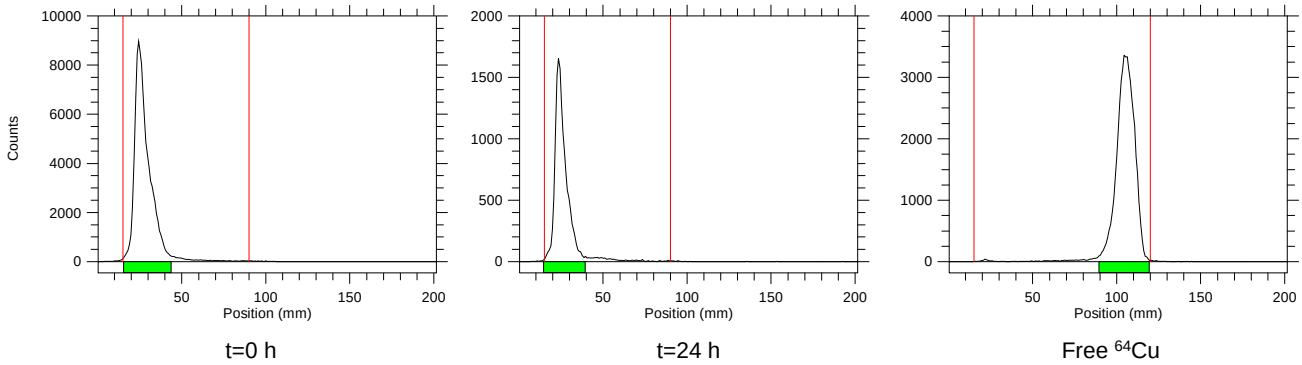
Synthesis of NOTA-Aptamer. 1 mg of aptamer (46 nmol) was dissolved in 0.2 mL of 0.1 M borate buffer (pH 9.3). Then 50 eq. of *p*-SCN-Bn-NOTA in 50 µL of dimethylformamide were added. The reaction mixture was kept at 4 °C overnight. The conjugated aptamer was purified on a 5 kDa Centricon (Millipore). The NOTA conjugated aptamer was washed 4-5 times with water

to remove excess amount of unreacted chelator. LC-MS confirmed the molecular mass of the NOTA conjugated aptamer: 22062 and aptamer: 21612. The difference in 8 mass units for the non-conjugated aptamer is due to the acceptable deconvolution error.

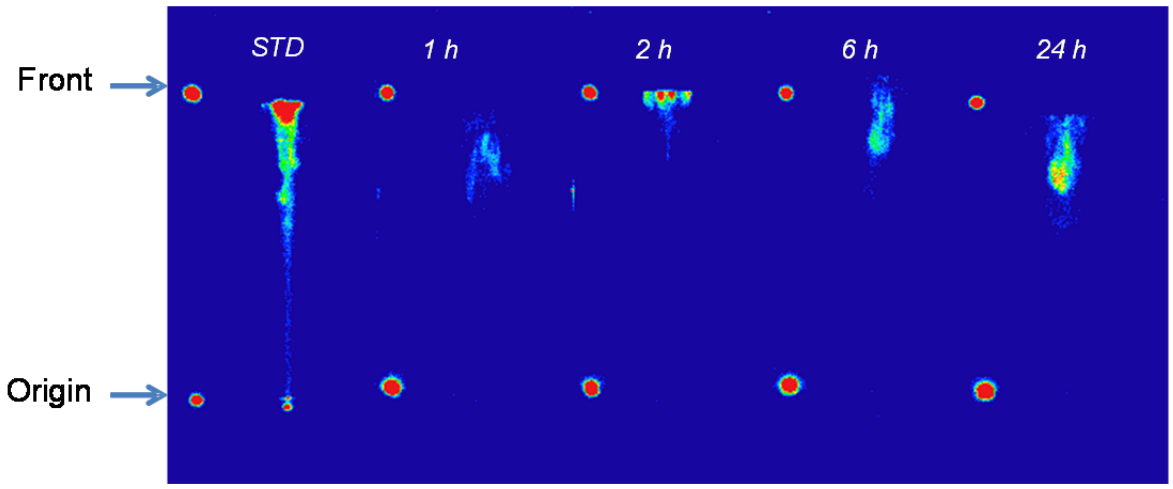
Biology

Biodistribution. 2.48-2.96 MBq (67-80 μCi) of ^{18}F-FB Tenascin C aptamer in a volume of 100 μL PBS/H_2O were injected into a tail vein of tumor-bearing mice (n = 4). At 2 h post-injection blood was drawn from the heart and the mice were then sacrificed. The organs were removed and assayed for radioactivity using a gamma counter.

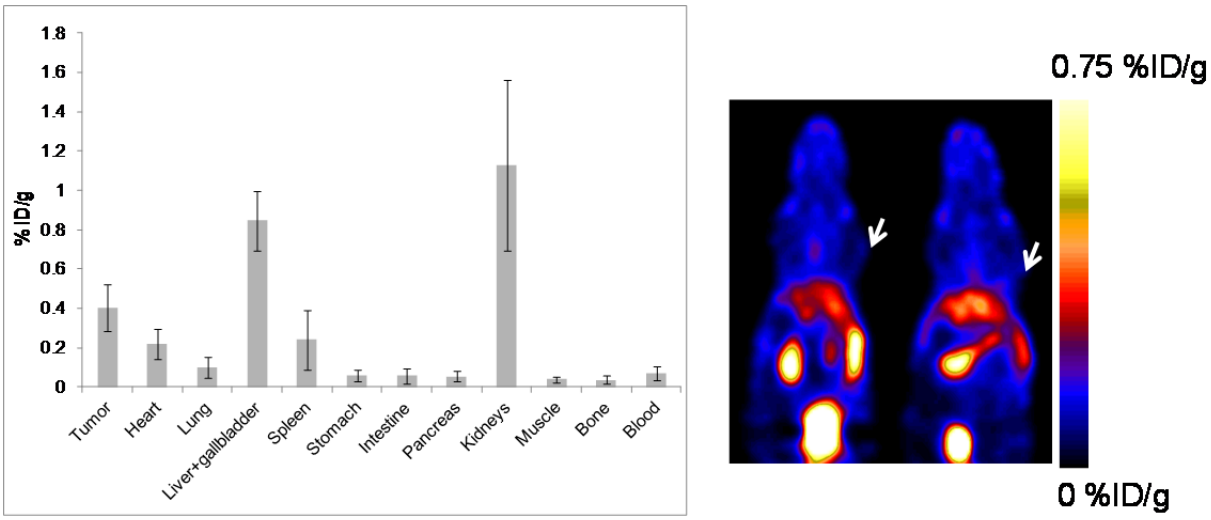
Blocking Studies. Tumor-bearing mice (n = 2) were co-injected with 1.85 MBq (50 μCi) of ^{18}F-FB Tenascin C aptamer and 1 mg of unlabeled Tenascin C aptamer. The mice were scanned using an Inveon DPET and scanned at 30 min, 1 and 2 h post-injection.



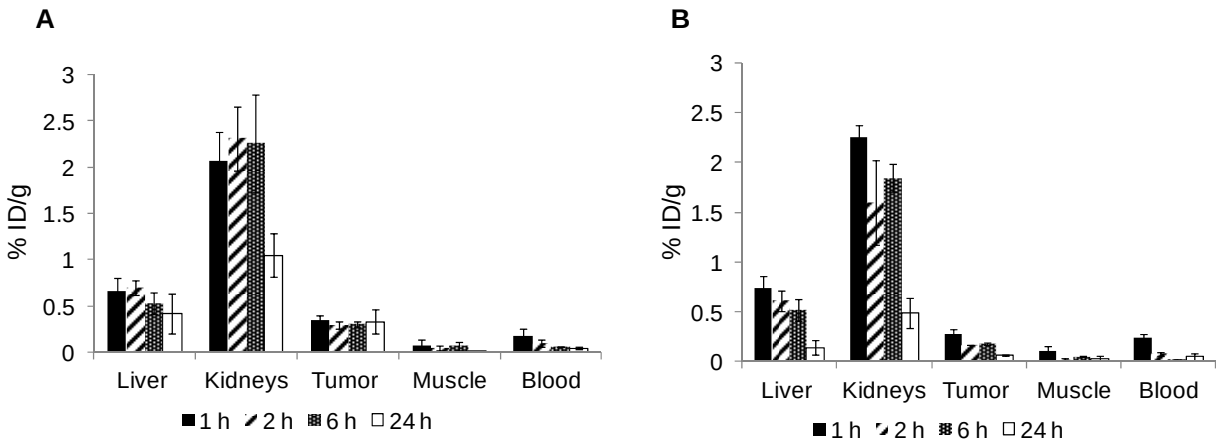
Supplemental Figure 1. Radio-TLC analysis of ^{64}Cu-NOTA-Tenascin C-aptamer in PBS after 24 h using radio-TLC system *1*. R_f[^{64}Cu-NOTA-Tenascin C-aptamer]=0.1, R_f[non-complex ^{64}Cu]=0.9.



Supplemental Figure 2. Radio-TLC analysis of ^{64}Cu-NOTA-Tenascin C-aptamer in mouse serum after incubation at 1, 2, 6 and 24 h using radio-TLC system *2*. R_f[^{64}Cu-NOTA-Tenascin C-aptamer]=0.9, R_f[non-complex ^{64}Cu]=0.1.



Supplemental Figure 3. Left – Biodistribution of ^{18}F-FB-Tenascin C aptamer in female athymic nude mice bearing U87MG tumors at 2 h post-injection. The results are averages of 4 mice ± STD. Right – Blocking studies shown by PET images of U87MG tumor bearing mice (n = 2) co-injected with ^{18}F-FB-Tenascin C and unlabeled Tenascin C aptamer.



Supplemental Figure 4. Biodistribution of ^{64}Cu-NOTA-Tenascin C-aptamer (A) and ^{64}Cu-NOTA-Sc-aptamer (B) at 1, 2, 6 and 24 h post-injection. The results are calculated from PET scans and are shown as averages of 5 mice ± STD.