Supporting Information

An ^{111}In-Labeled PLA-PEG Nanoparticle for Imaging PSMA-Expressing Tissues

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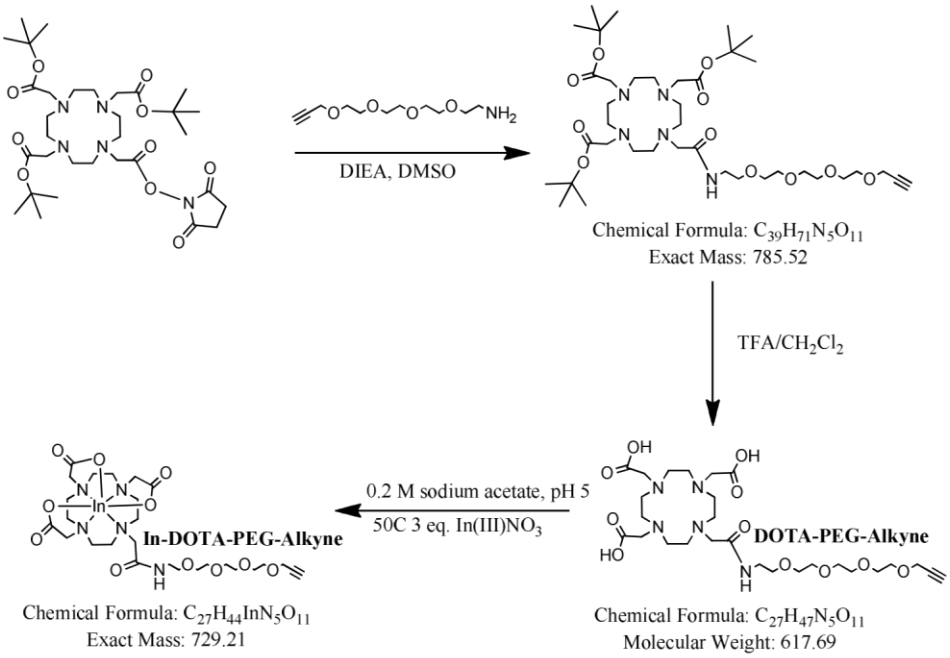
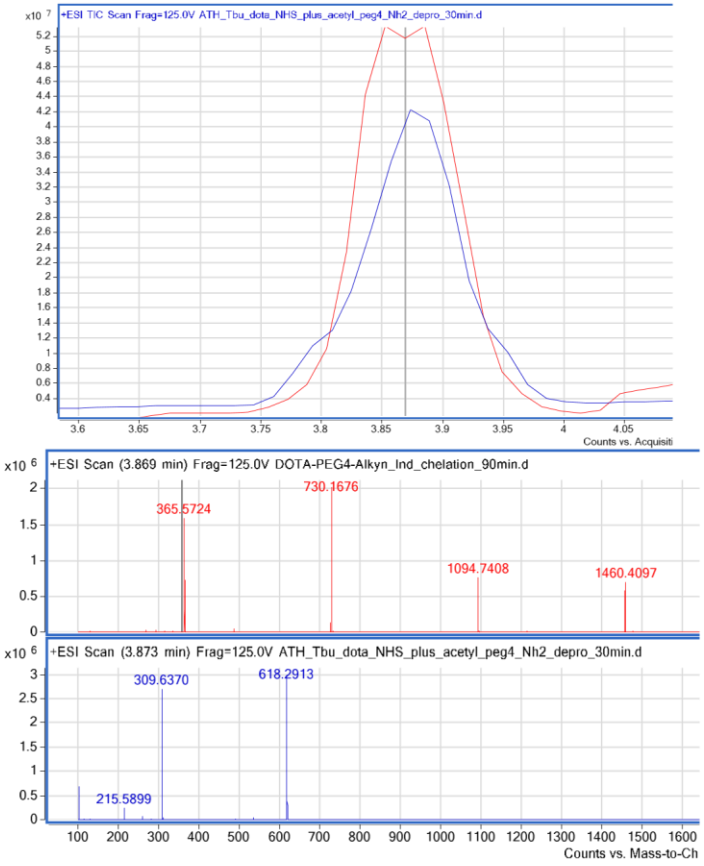
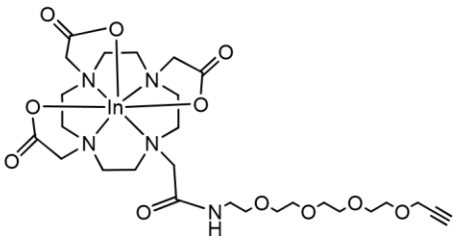
1. Reaction scheme to synthesize **DOTA-PEG-Alkyne** and **In-DOTA-PEG-Alkyne**

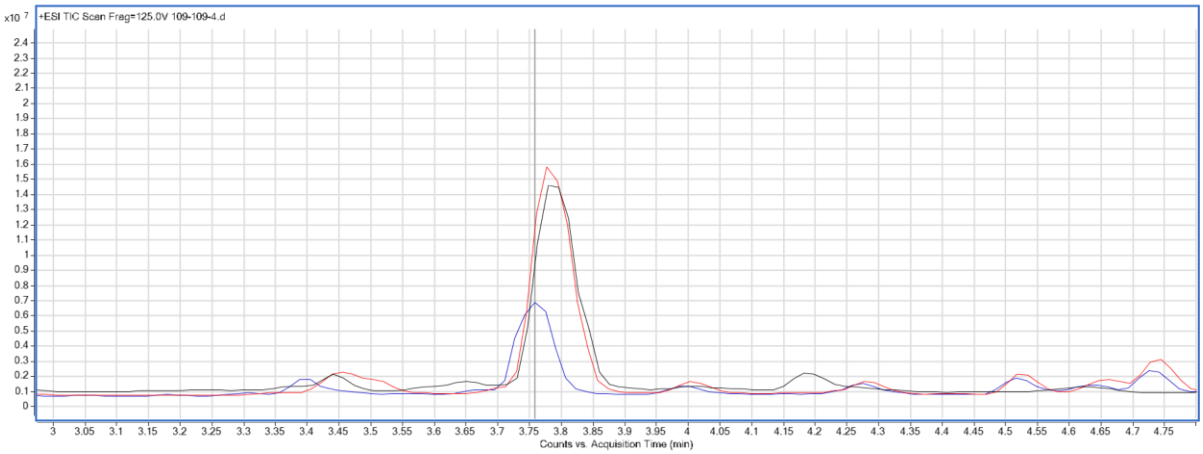


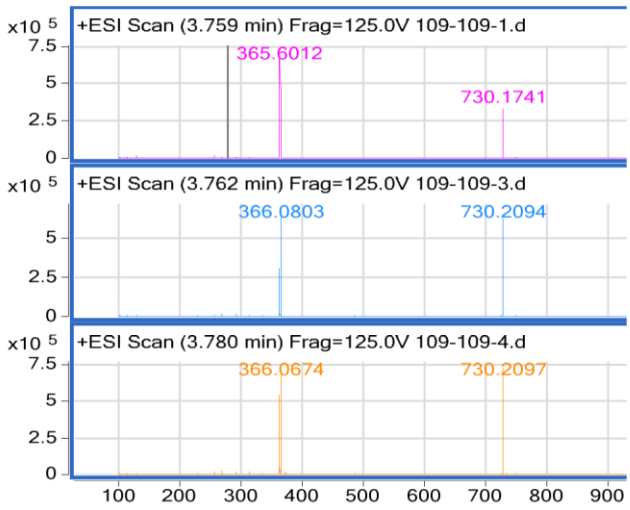


LC-MS spectra for **DOTA-PEG-alkyne** (blue) and **In-DOTA-PEG-alkyne** (red)

2. Stability studies of stable **In-DOTA-PEG-alkyne** (MW: 729.21) by LC-MS) in different experimental condition used for formulation of nanoparticles (click chemistry) formulation







Color	Cu (1mM)	Ascorbic Acid	30% N_3 NP's
Red	-	-	-
Black	+	+	-
Blue	+	+	+

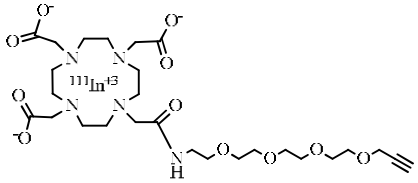
3. Calculation radiolabeling yield and specific activity of ^{111}In-labeled nanoparticles

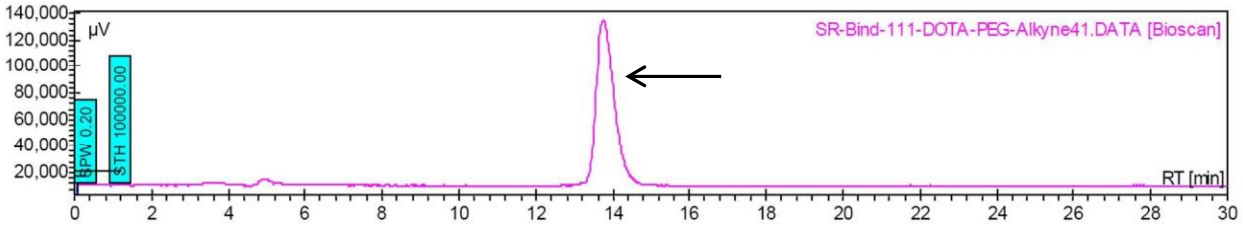
Four separate spin-columns were used to purify the nanoparticles to remove low-molecularweight impurities and free ^{111}In-DOTA-PEG-alkyne from the nanoparticle formulation before performing the biological experiments. A table was prepared to show the calculation of radiolabeling yield and specific activity.

Table S1

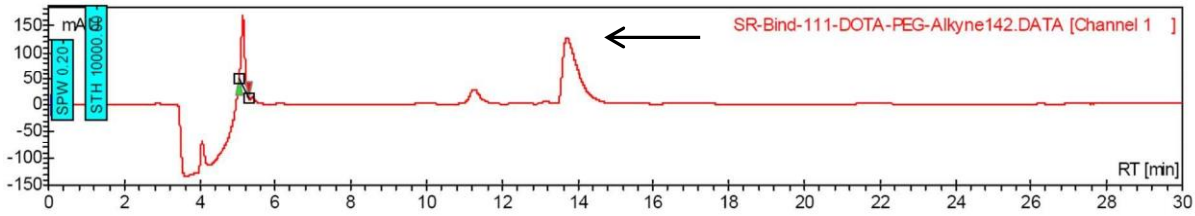
Untargeted Particles **(UNP)**		Column 1 (filtration 1)	Column 2 (filtration 2)	Column 3 (filtration 3)	Column 4 (filtration 4)
Before purification	8.55 mCi ($18.4*10^{-11}$)				
Vial + pipette tips (physical waste)		560 µCi	82.6 µCi	43.7 µCi	114.2 µCi
Activity retained in the column		3.29 mCi	404 µCi	253 µCi	206 µCi
Activity retained in Filtrate		4.73 mCi	4.25 mCi	3.96 mCi	3.65 mCi (Final)
Radiochemical Yield	(3.65/8.55)*100 = 42.2%				
No. of mol. Of In-111 (final)	$7.85*10^{-11}$ mole				
No. of mol. particle taken (lot# 102-192-C) 2.5% GL2	80 µl from 83 mg/ml stock (10% N3) =6.64 mg Or (0.0066/21000) $=3.16*10^{-7}$ mole polymer 4500 polymers/NP $3.16x10^{-7}$ / 4500 = $7.02x10^{-11}$ moles of NP				
Ratio of In-111/particle	$7.85x10^{-11}$ / $7.02x10^{-11}$ = 1.1				
nanoparticle specific activity	**20 MBq/mg (0.55 mCi/mg)**	**52 mCi/nmol**	**1924 MBq/nmol**		

4. HPLC peak for purified **^{111}In-DOTA-PEG-alkyne** (radio/γ) channel

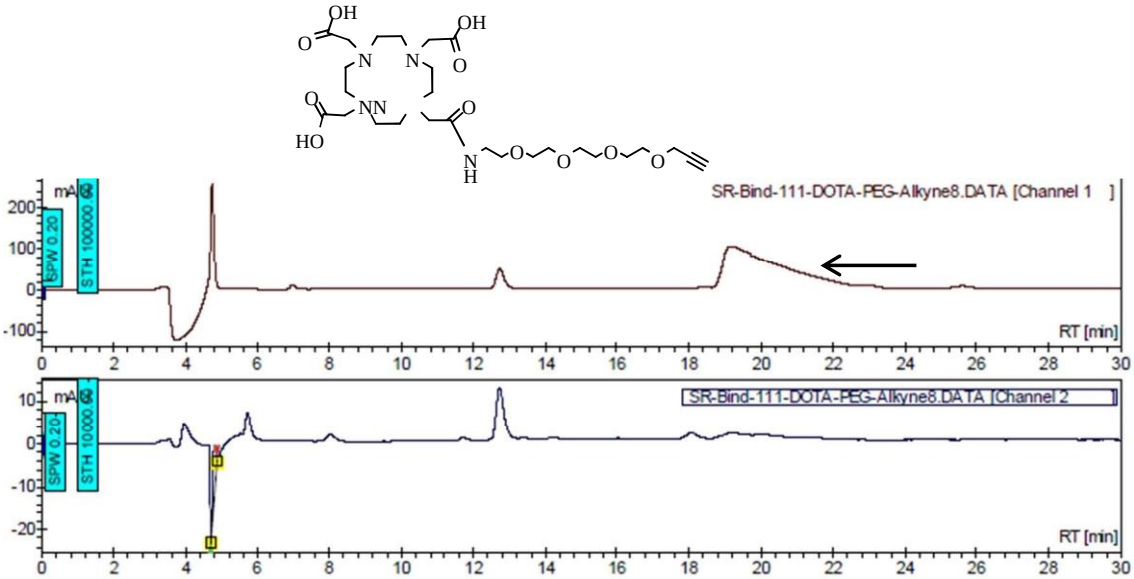




5. HPLC peak for stable (cold) **In-DOTA-PEG-alkyne** (λ =220 nm)



6. HPLC peak for unlabeled DOTA-PEG-alkyne (λ =220 nm). Peak at 12.8 min did not show any mass at ESI-MS. (λ =220 nm, top) and (λ =254 nm, bottom).



7. Supporting Figures for Tissue imaging

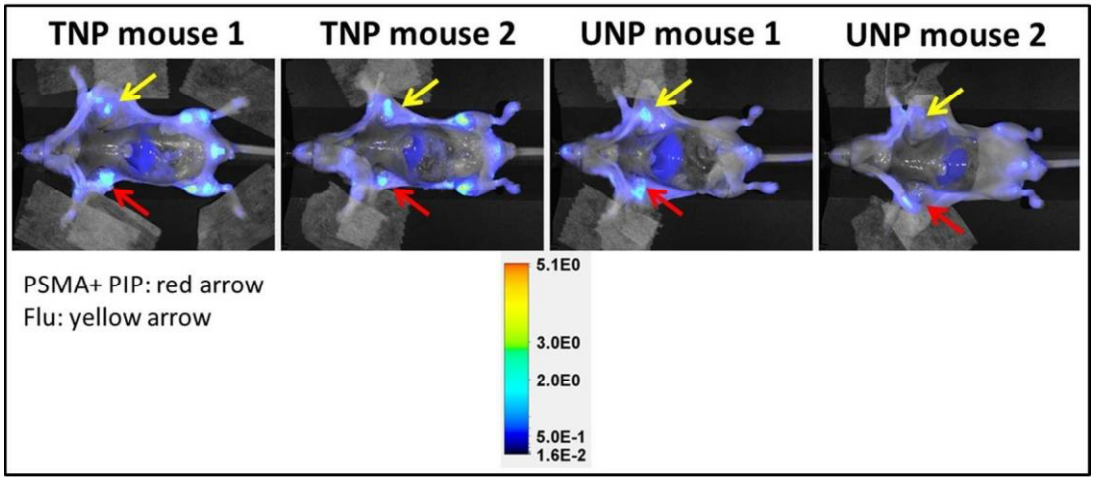


Figure S1. *Ex vivo* NIRF imaging of two mouse pairs each receiving either TNP or UNP as indicated. Mice were sacrificed and imaged 72 h after nanoparticle administration. Tumor types are as indicated. Heterogeneity of tumor uptake is apparent by lack of tumor uptake in either line in UNP mouse 2. All images are scaled to the same acquisition parameters.

Figure S2.

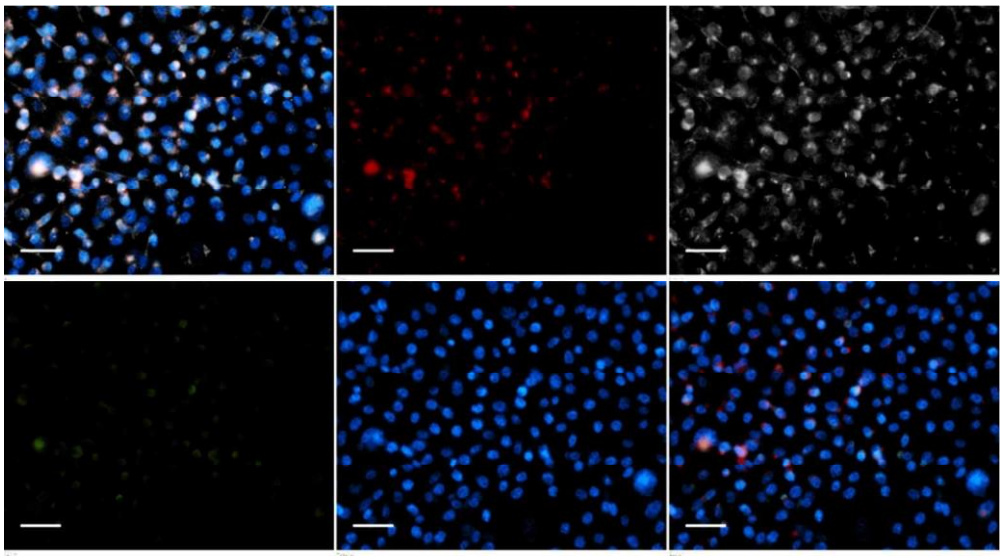


Figure S2. *In vitro* epifluorescence micrograph of cultured PSMA negative PC-3 flu cells containing TNPs. This magnification shows the intracellular distribution of TNPs (red) with PSMA (green, absent), tubulin (white) and nuclei (blue). White arrows indicate some co-localization of TNPs with tubulin (white at the centrosome), possibly due to passive endocytosis. Scale bar = 10 μm.

Figure S3.

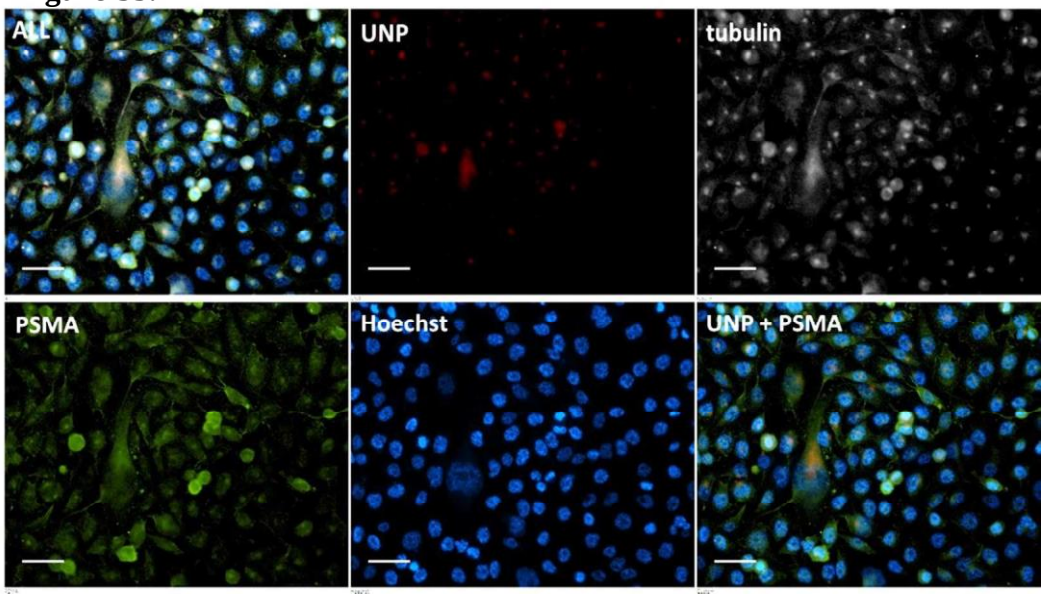


Figure S3. *In vitro* epifluorescence micrograph of cultured PSMA+ PC-3 PIP cells containing UNPs. This magnification shows the intracellular distribution of TNPs (red) with PSMA (green), tubulin (white) and nuclei (blue). White arrows indicate some co-localization of TNPs with tubulin (white at the centrosome), possibly due to passive endocytosis. Scale bar = 10 μm.

Figure S4.

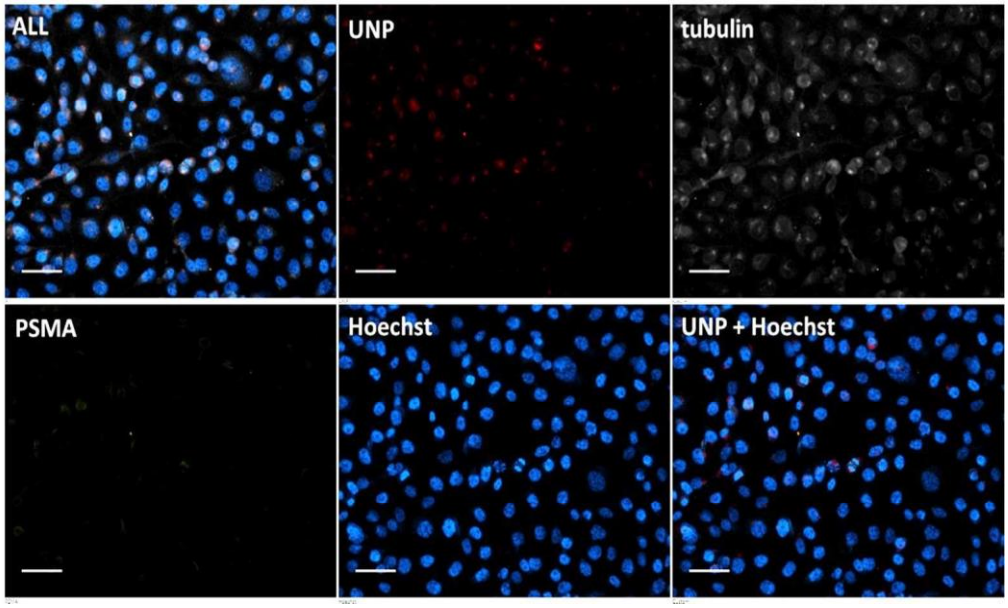


Figure S4. *In vitro* epifluorescence micrograph of cultured PSMA negative PC3 flu cells containing UNPs. This magnification shows the intracellular distribution of UNPs (red) with PSMA (green, absent), tubulin (white) and nuclei (blue). White arrows indicate some colocalization of TNPs with tubulin (white at the centrosome), possibly due to passive endocytosis. Scale bar = 10 μm.

Figure S5.

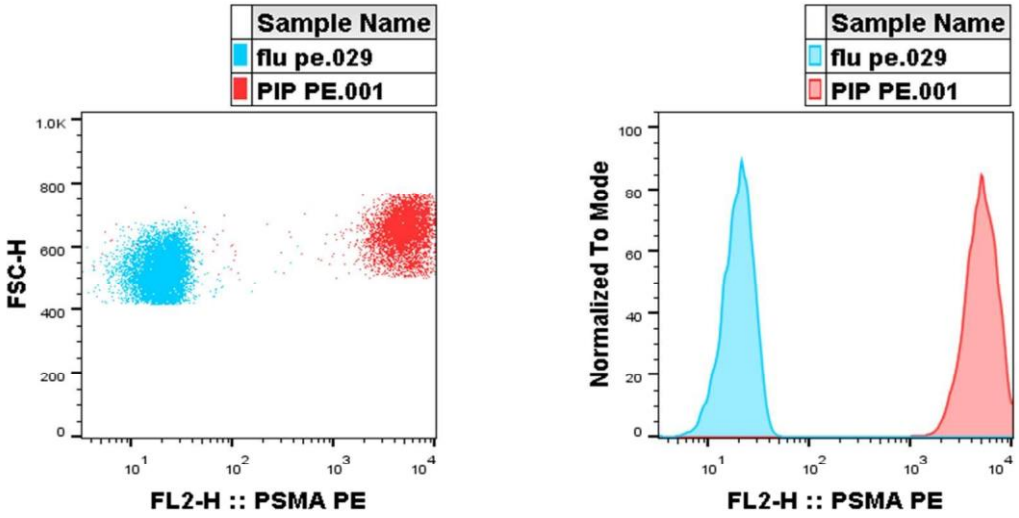
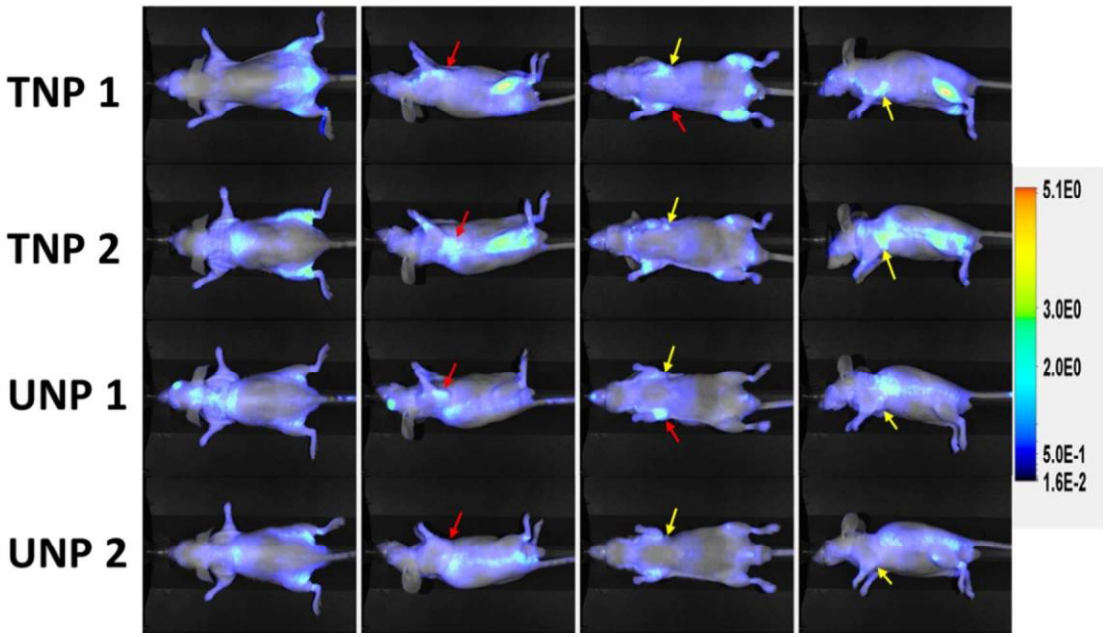


Figure S5. Flow cytometry analysis of PSMA surface expression in PSMA(+) PC-3 PIP & PSMA(-) PC-3flu cell lines. The dot plot (left panel) and the histogram (right panel) show that PC-3 PIP has a uniformly high level of PSMA expression where as PC-3flu is negative.

Figure S6.



Supplementary Figure 6. *In vivo* NIRF imaging of TNP-IRDye and UNP-IRDye distribution in tumor bearing mice at 72 h post-injection. Four athymic nude mice bearing PC-3 PIP (red arrows) and PC-3 flu (yellow arrows) xenografts were injected as indicated with either fluorescent TNP or UNP. Images at 72 h show TNP accumulation around both PIP and flu xenografts as well as around knee joints. UNP accumulation had largely cleared from both tumors except from a $\sim$ 4 mm diameter PIP tumor within the UNP 1 mouse. Color bar indicates relative fluorescence units. PC-3 PIP tumor (red arrow), PC-3 flu tumor (yellow arrow)