Supporting Information

Prostate Specific Membrane Antigen (PSMA) Targeted Gold Nanoparticles for Theranostics of Prostate Cancer

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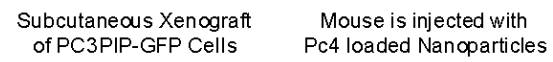
a)

b)



Scheme S1. (a) *In vitro* and (b) *in vivo* studies.

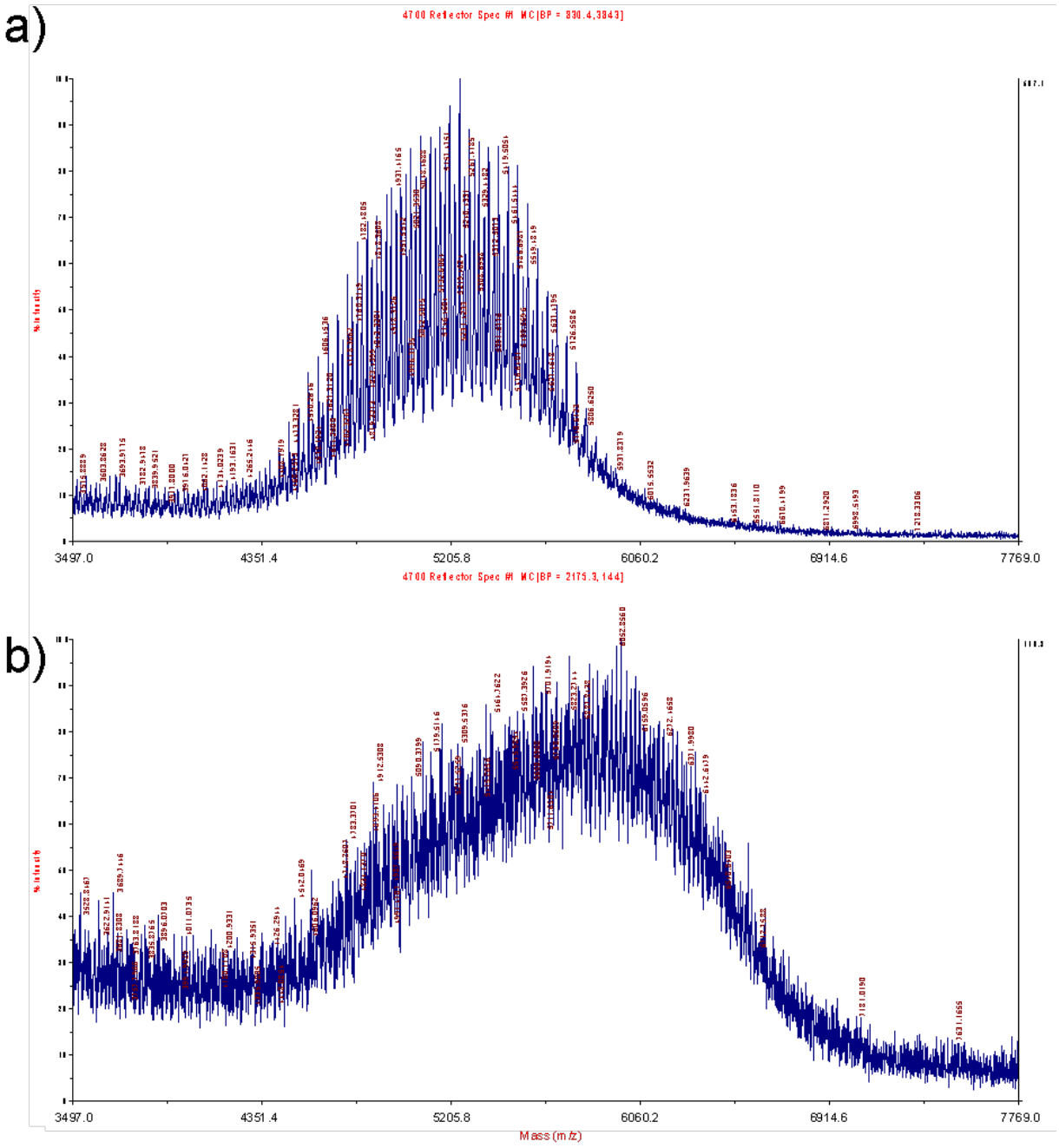


Figure S1. The conjugation of PSMA-1 to (a) OPSS-5kPEG-NHS resulting in (b) SH-5kPEG-PSMA-1 was confirmed by MALDI-TOF MS. The ~1 kDa shift in (b) verifies the successful conjugation.

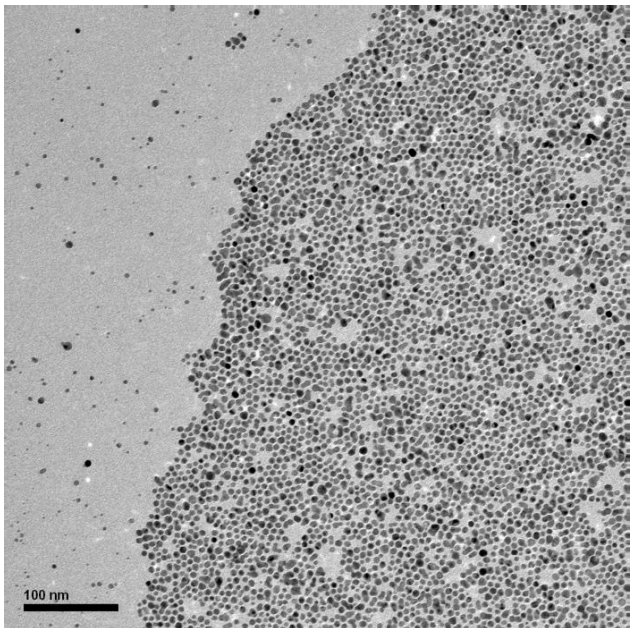


Figure S2. Representative bright field transmission electron microscopy (TEM) image of gold nanoparticles coated with dodecylamine (AuNP-DDA)

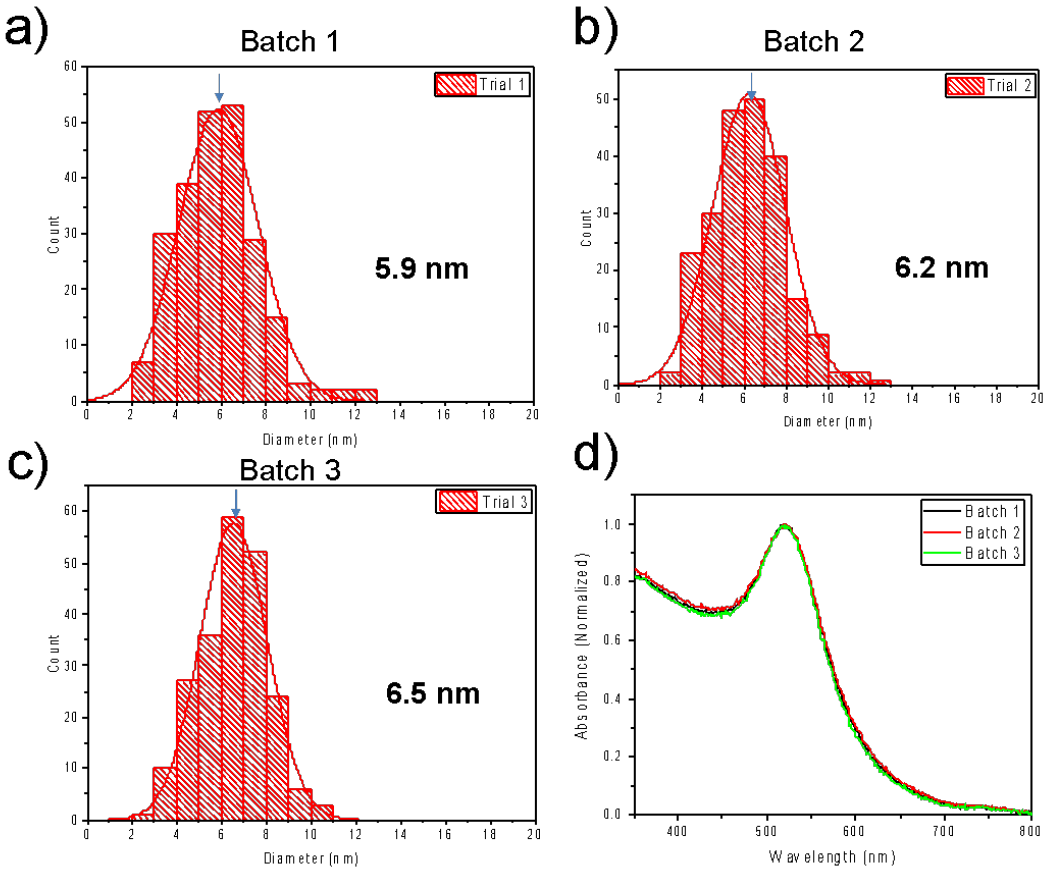
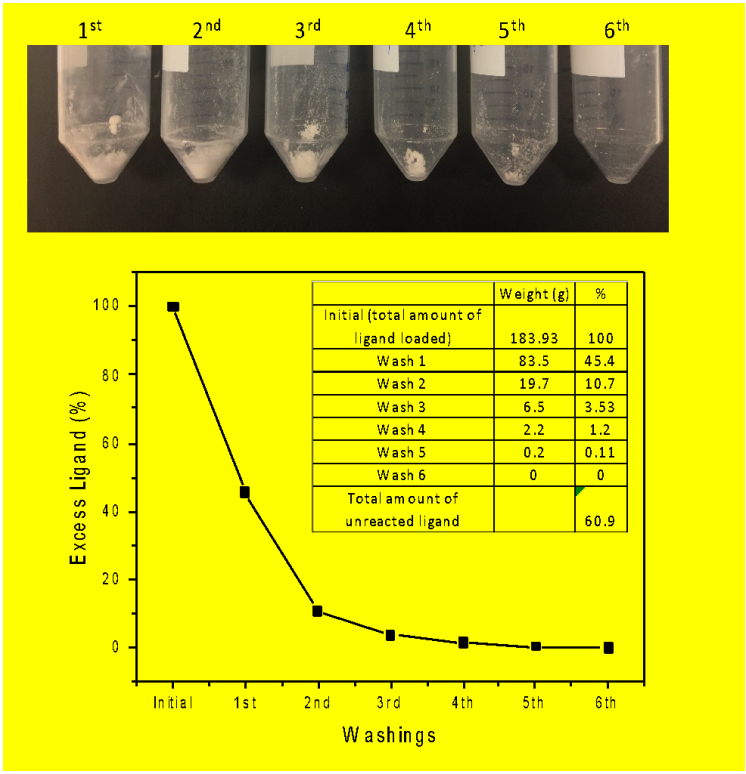


Figure S3. (a-c) Size distribution and (d) UV-Vis spectra of AuNP-DDA synthesized in different batches.



	Weight (g)	%
Initial (total amount of ligand loaded)	183.93	100
Wash 1	83.5	45.4
Wash 2	19.7	10.7
Wash 3	6.5	3.53
Wash 4	2.2	1.2
Wash 5	0.2	0.11
Wash 6	0	0
Total amount of unreacted ligand		60.9

Figure S4. After ligand exchange, the nanoparticle was washed with a total of 300 mL DI water, followed by centrifuge filtration (50,000 MWCO, 4K RPM, 1-1.5h) The washings were kept and lyophilized. Washing was performed until no appreciable remaining solid was observed. The collected solids were then weighed.

a)

b)

c)

Figure S5. UV-Vis absorbance spectroscopy was employed to ascertain the stability of (a) AuNP5kPEG-PSMA-1 and (b-c) AuNP5kPEGPSMA-1-Pc4 in solution by monitoring the surface plasmon resonance(SPR) band of gold (~520 nm) and the signature band of Pc4 (~670 nm). The data suggest that the nanoparticles including the encapsulated Pc4 molecules are stable in a wide-range of solvents/buffers/media for relatively long periods of time.

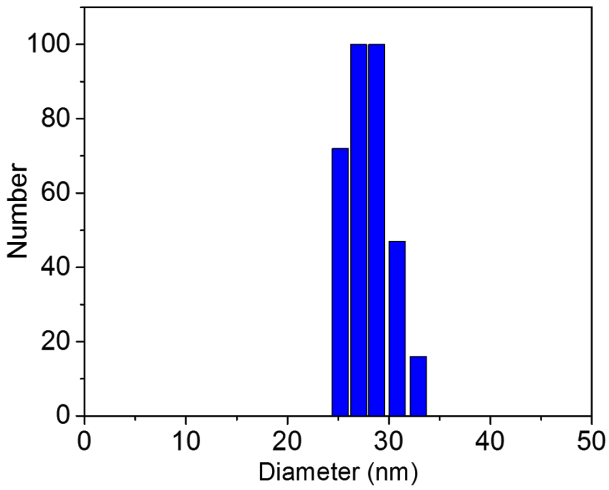


Figure S6. Representative dynamic light scattering (DLS) data of AuNP-5kPEG. Average Size = 26.5 ± 1.1 nm.

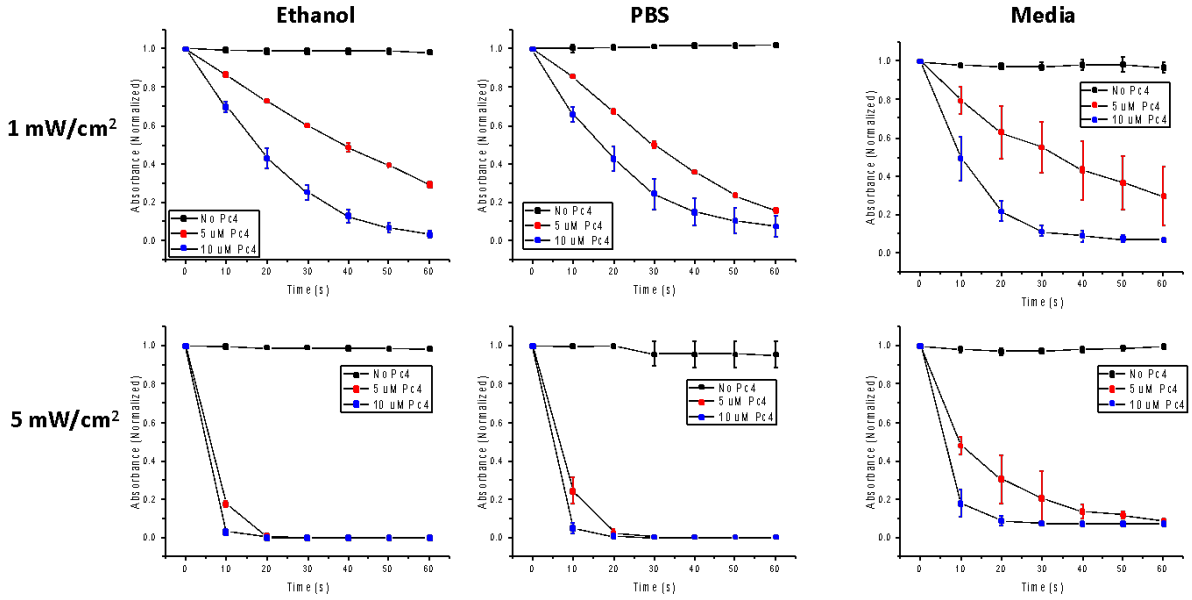


Figure S7. Result of the singlet oxygen generation experiments using DPBF as singlet oxygen trap. The experiment was performed in ethanol, PBS and media.

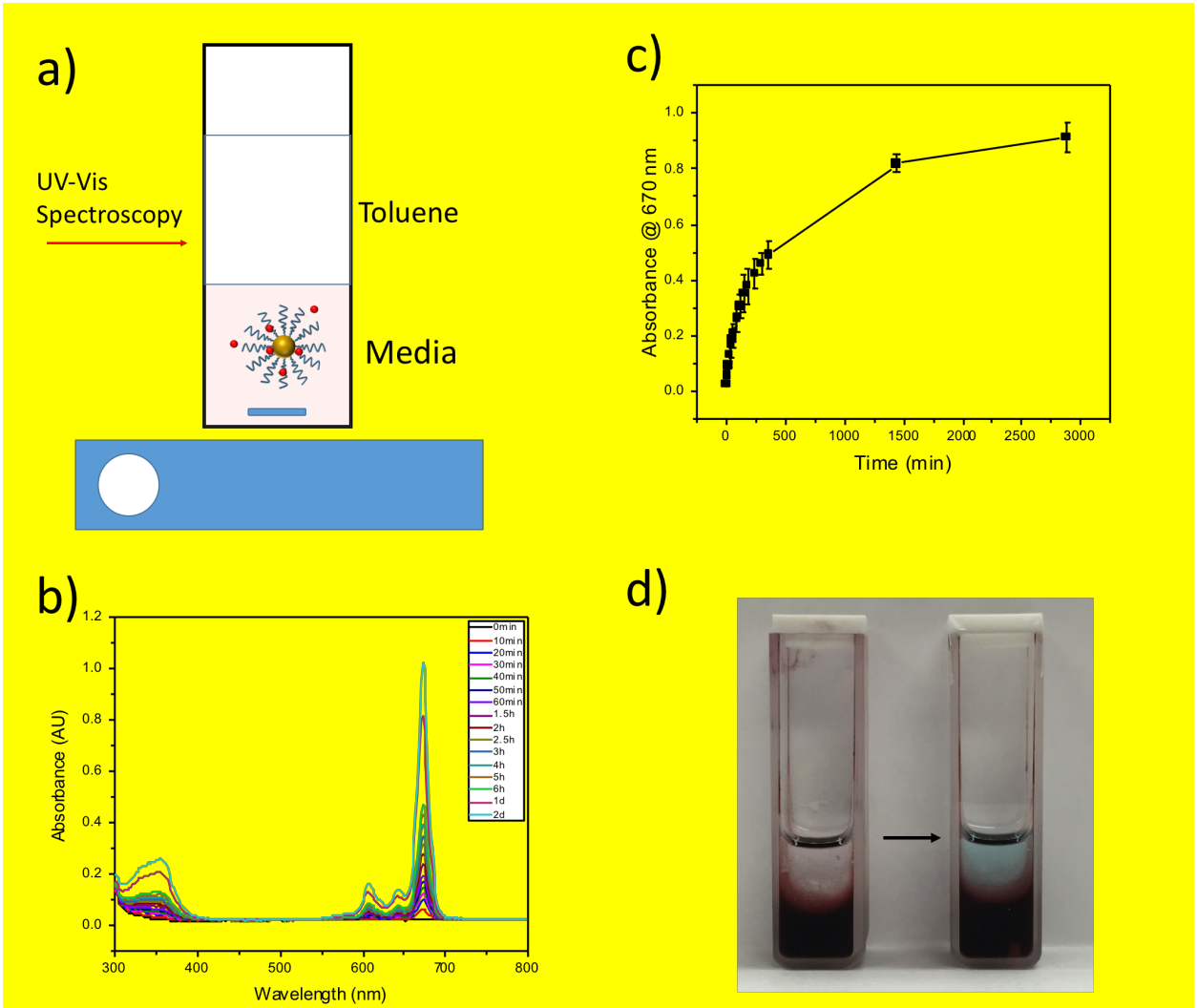


Figure S8. Drug-release studies in two-phase system. (a) The scheme describes the experimental set-up were Pc4 containing gold nanoparticle conjugates were dispersed in media and a toluene layer was added on top. (b) The released Pc4 in the toluene layer was subjected to UV-Vis spectroscopy. (c) A plot of the release of Pc4 (monitored at 670 nm) over time. (c) Image of the two-phase system before and after the experiment. The blue colored toluene indicates the presence of transferred Pc4.

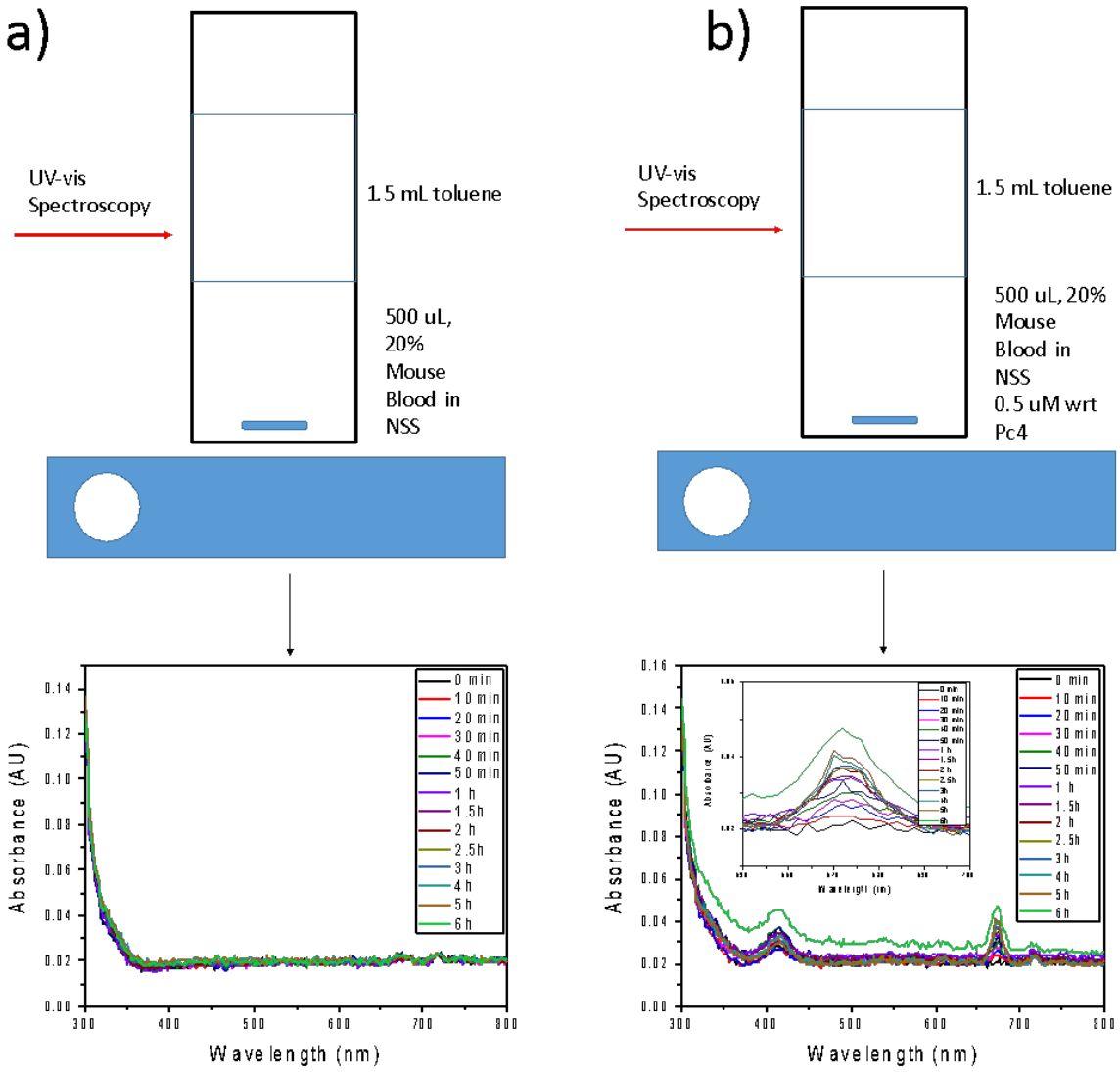


Figure S9. A mouse blood was collected via cardiac puncture and diluted with NSS to which Pc4 containing AuNP conjugates were added. (a) is the control experiment without Pc4 and (b) is the release profile of Pc4 containing AuNP conjugates dispersed in blood.

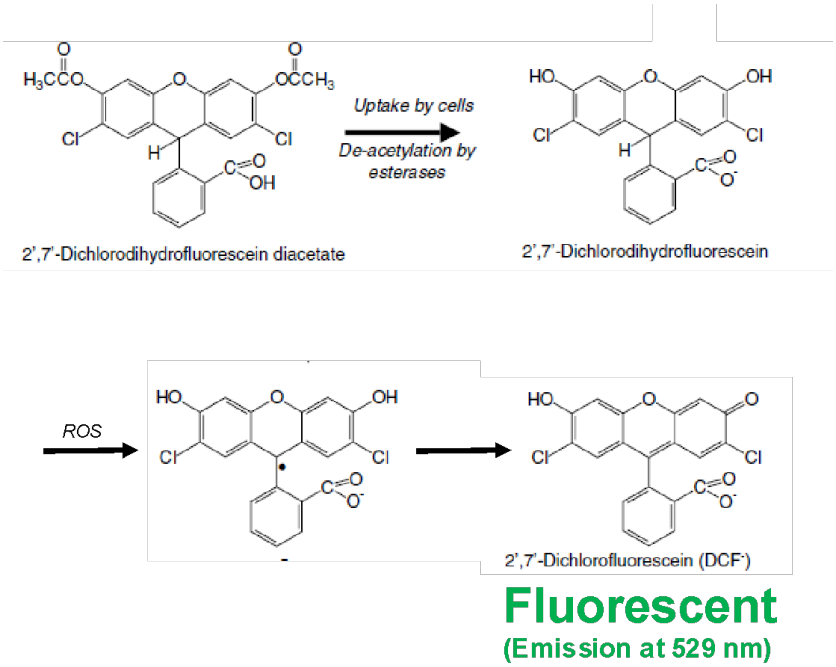


Figure S10. Conversion of non-fluorescent DCFDA to fluorescent DCF.

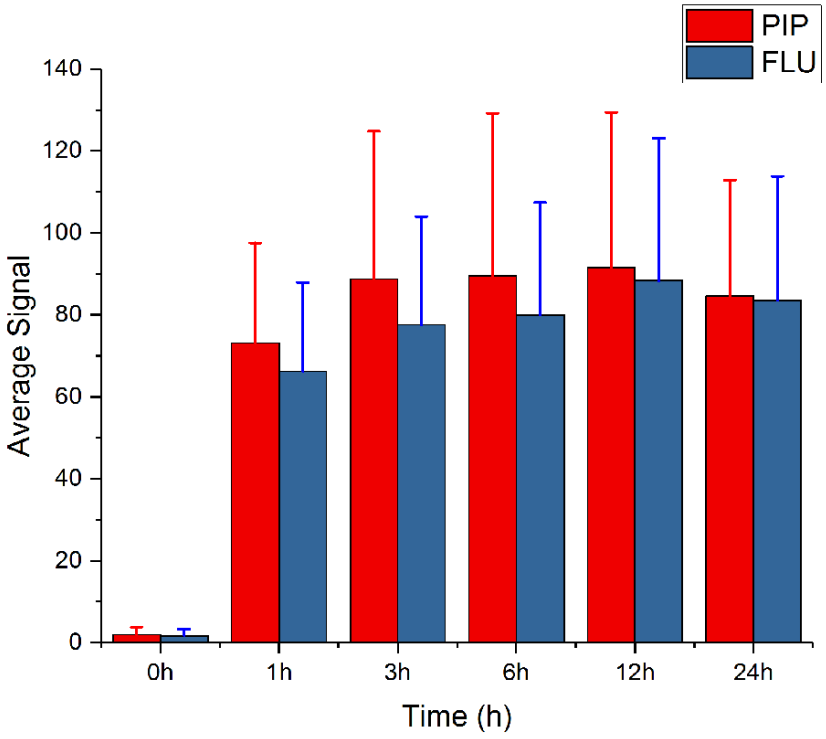


Figure S11. Pc4 signal on PC3pip and PC3flu tumors quantified by Maestro Fluorescence In Vivo Imaging System (0h N=20, 1h N=20, 3h N=17, 6h N=13, 12h N=9, 24h N=5).

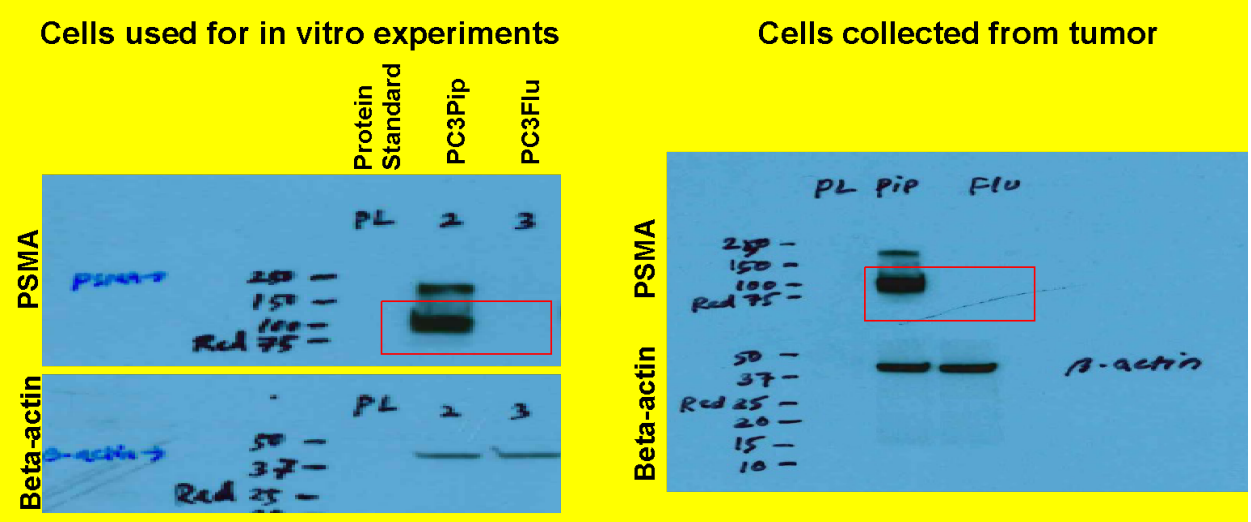


Figure S12. Western Blot to confirm PSMA expression levels in PC3pip and PC3flu tumors. The primary antibody used is J591 (Anti-PSMA antibody) and the secondary antibody is Rabbit anti-mouse HRP.

Sample Calculation of Irradiation Time

For PDT treatment, the tumor was first measured and the laser spot size or treatment area was adjusted to cover the entire tumor. Treatment area was based off the largest diameter and calculated using the formula for area of a circle: A = □ X (diameter/2)^2. Black tape was used to cover the skin surrounding the flank tumor in order to prevent as much of the light as possible from reaching undesired areas. Once the treatment area was determined, the power was adjusted using the formula:

$$\text{Power (W)} = \text{Irradiance (W/cm}^2\text{)} \times \text{Area (cm}^2\text{)}$$

in which irradiance=0.1W/cm^2. The calculated power was then adjusted by the power meter (PM100, Thorlabs). Once the treatment area and power were determined, the treatment time was calculated using the formula:

$$Radiant\ Exposure\ (\frac{J}{cm^2}) = \frac{Power}{Area} \times Time\ (\frac{W}{cm^2} \times s)$$

Therefore,

$$Time\ (s) = \ Radiant\ exposure(\frac{J}{cm^2}) \times \frac{Area}{Power}\ (\frac{W}{cm^2})$$

Typically, the treatment duration is 1500s or 25min for radiant exposure at150J/cm^2 at 672nm.