Supporting Information

Tuning Pharmacokinetics to Improve Tumor Accumulation of a PSMA-Targeted Phototheranostic Agent

Kara M. Harmatys[1,4,†], Marta Overchuk[1, 2,†], Juan Chen[1], Lili Ding[1], Ying Chen[3], Martin G. Pomper[3], Gang Zheng[*,1-2,4]

[1]Princess Margaret Cancer Centre, University Health Network, 101 College Street, Toronto, Ontario M5G 1L7, Canada.

[2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario M5S 3G9, Canada

[3]Johns Hopkins Medical School, 1550 Orleans Street, 492 CRB II, Baltimore, MD 21287, United States

[4]Department of Medical Biophysics, University of Toronto, 101 College Street, Toronto, Ontario M5G 1L7, Canada

[†] First two authors contributed equally to this work.

*Corresponding Author:

Dr. Gang Zheng, University of Toronto, TMDT 5-354, 101 College Street, Toronto, ON, M5G 1L7, Canada, E-mail: gang.zheng@uhnres.utoronto.ca

Supporting Information Contents:

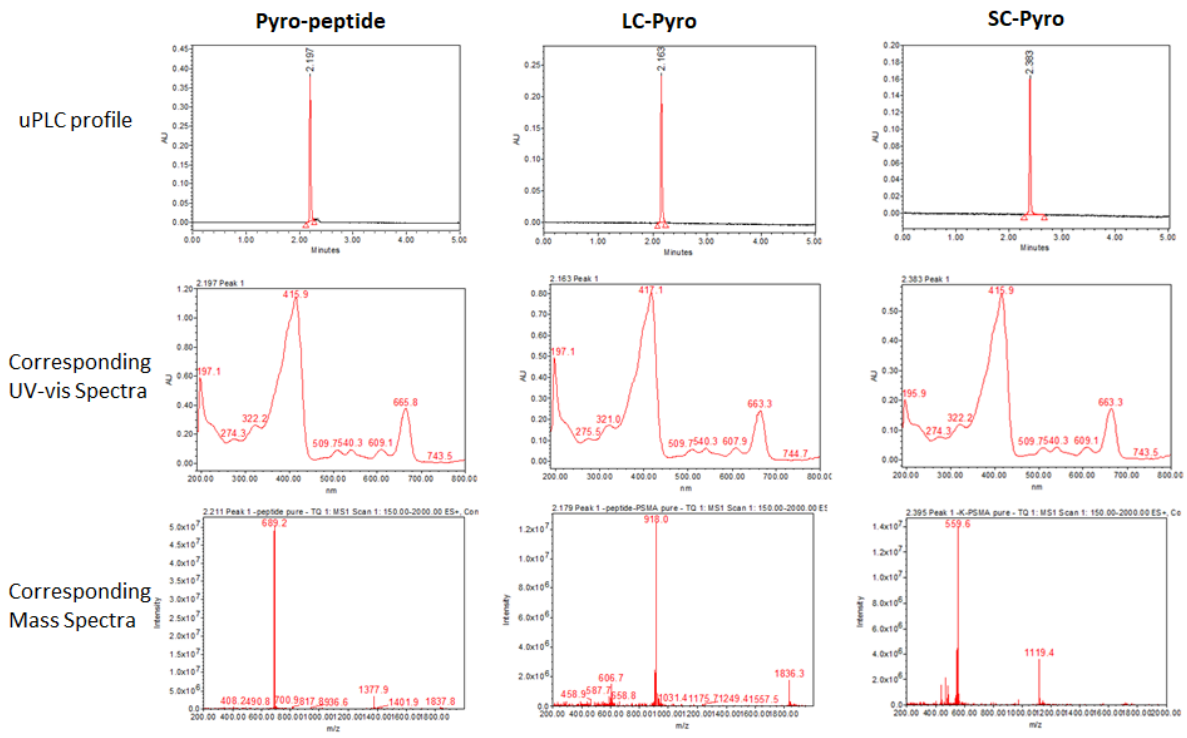

Figure S1. Chemical characterization of Pyro-peptide, LC-Pyro and SC-Pyro; uPLC retention profile (Top); Corresponding UV-vis absorbance spectra (Middle) and mass spectra (Bottom).

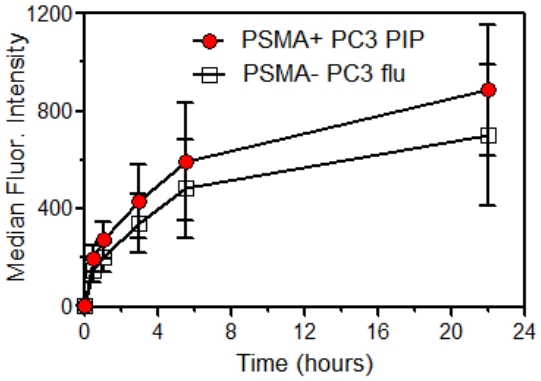

Figure S2. Flow cytometry time-dependent LC-Pyro uptake in PSMA+ PC3 PIP and PSMA- PC3 flu cell lines. Each point represents the median fluorescence intensity ± the SD of three independent measurements.

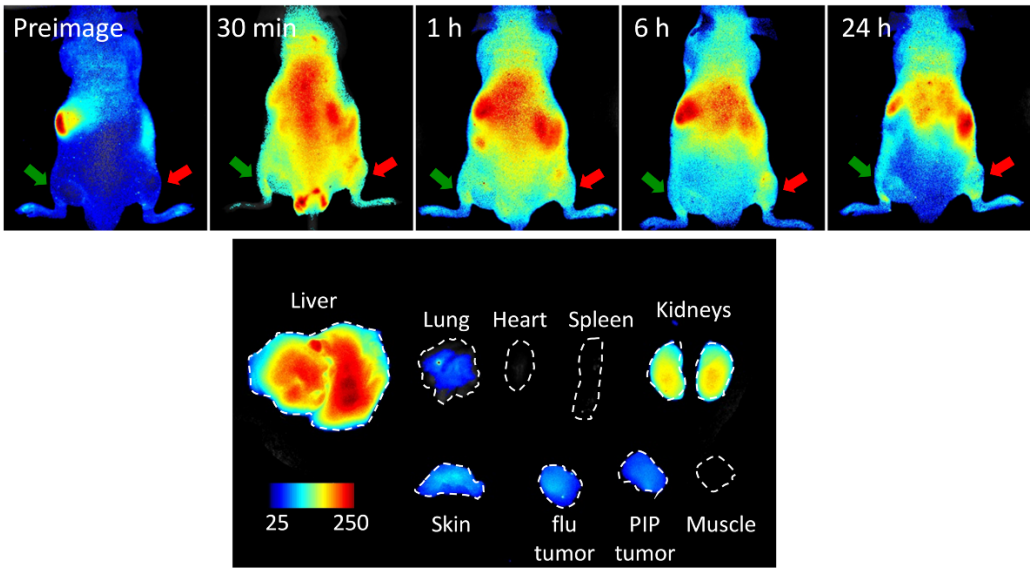

Figure S3. Representative fluorescence images of a mouse bearing dual PSMA+ PC3 PIP (red arrow) and PSMA- PC3 flu (green arrow) tumors that were intravenously injected with an LC-Pyro derivative unconjugated to the PSMA small molecule affinity ligand (20 nmol). Mice were imaged at 0, 30 min, 1 h, 6 h and 24 h post-intravenous injection and major organs were excised and imaged *ex vivo*; n = 3.

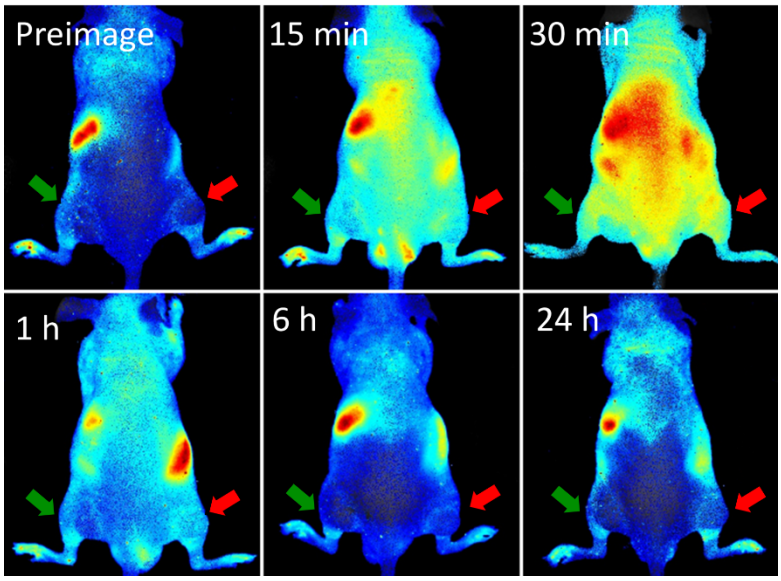

Figure S4. Representative fluorescence images of a mouse bearing dual PSMA+ PC3 PIP (red arrow) and PSMA- PC3 flu (green arrow) tumors that were intravenously injected with SC-Pyro (20 nmol) at 0, 15 min, 30 min, 1 h, 6 h and 24 h post-injection; n = 3.

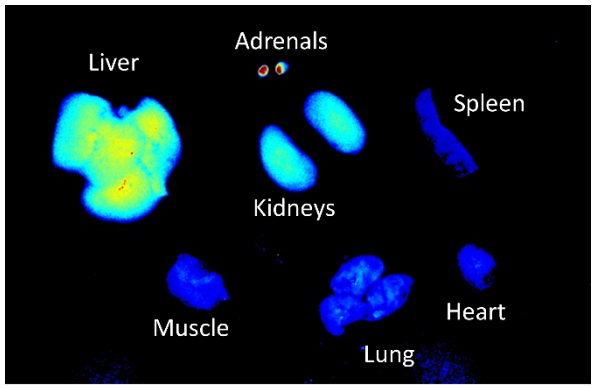

Figure S5. Fluorescence *ex vivo* biodistribution of major clearance organs in a mouse injected with LC-FITC 24 h post-injection.

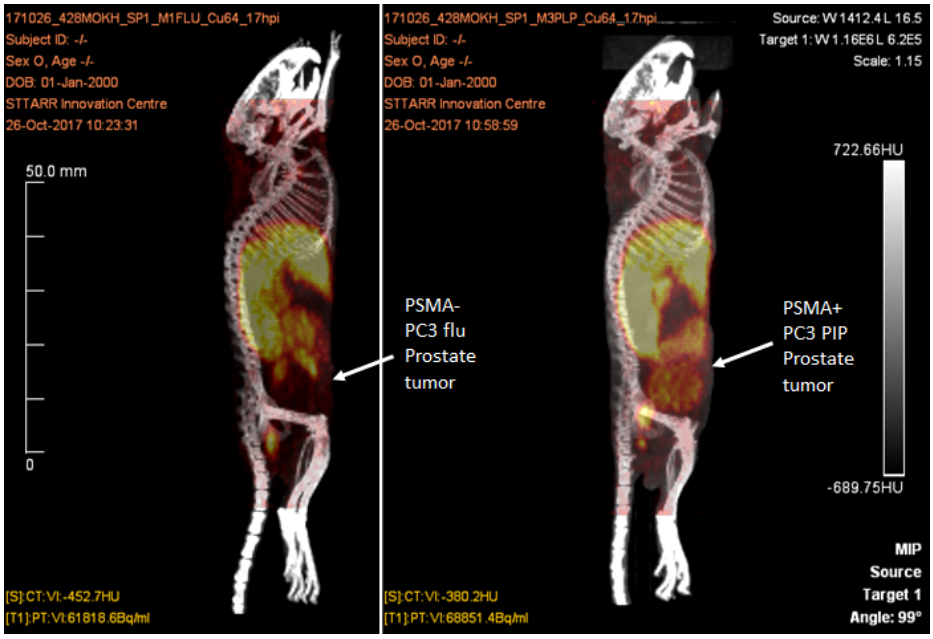

Figure S6. Lateral view PET/CT images of mice bearing either a PSMA- PC3 flu (left) or PSMA+ PC3 PIP (right) orthotopic prostate tumor 17 h post-injection of ^{64}Cu-LC-Pyro.

Organ	PSMA- PC3 flu	PSMA+ PC3 PIP
Liver	18.11 ± 4.43	16.61 ± 3.39
Kidney	21.52 ± 1.27	22.68 ± 3.38
Lung	3.81 ± 1.98	3.47 ± 0.76
Heart	2.00 ± 0.83	1.99 ± 0.45
Spleen	6.85 ± 2.93	8.68 ± 2.10
Adrenals	9.55 ± 0.65	7.36 ± 2.38
Blood	1.74 ± 0.03	2.35 ± 0.92
Bladder	2.60 ± 1.00	5.45 ± 2.51
Urine	1.92 ± 0.71	2.00 ± 0.92
Brain	0.16 ± 0.02	0.18 ± 0.03
Muscle	1.13 ± 0.98	0.98 ± 0.77
Stomach	3.25 ± 0.63	2.85 ± 0.22
Skin	3.81 ± 2.18	2.38 ± 0.69
Fat	2.32 ± 1.88	1.49 ± 1.12
S. Intestine	4.33 ± 2.08	4.70 ± 1.39
L. Intestine	4.44 ± 1.69	4.84 ± 1.96
Tumor	2.30 ± 0.09	9.74 ± 2.26
Prostate	3.38 ± 1.22	4.14 ± 0.53
Sem. Ves.	1.09 ± 0.53	1.16 ± 0.47
Testes	1.59 ± 0.57	1.66 ± 0.26
Feces	14.35 ± 5.79	21.74 ± 8.96

Table S1. Corresponding ^{64}Cu-LC-Pyro biodistribution from **Figure 5** of main organs and PSMA- PC3 flu and PSMA+ PC3 PIP tumors quantified via gamma counting (n = 4 for PSMA+ PC3 PIP; n = 3 for PSMA- PC3 flu, $^{**}P < 0.01$; $^{**}P < 0.001$). Data is represented as mean ± SD.

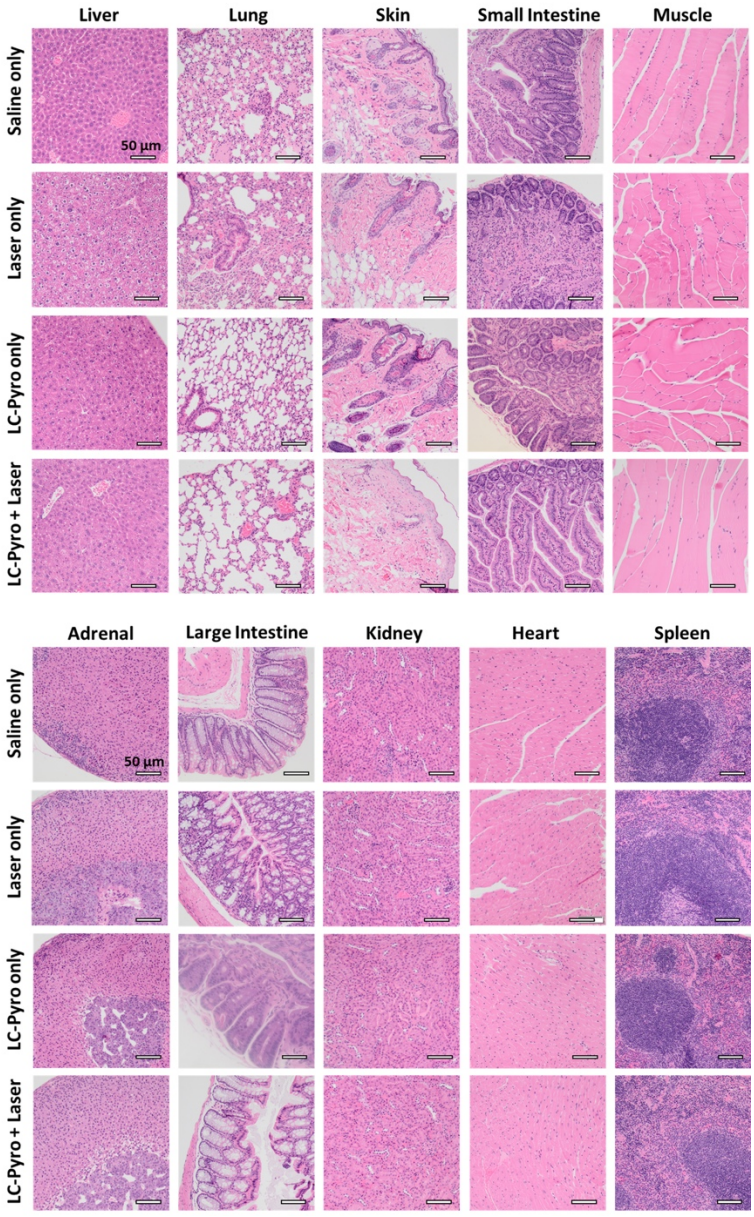

Figure S7. H&E evaluation of organ toxicity 24 h post-treatment from each cohort; 1) Saline only; 2) Laser only; 3) LC-Pyro only and 4) LC-Pyro + Laser. Organs include the liver, lung, skin, small intestine, muscle, adrenal, large intestine, kidney, heart and spleen. Histological slices reveal no adverse side effects on healthy tissues after treatment. Scale = 50 µm.

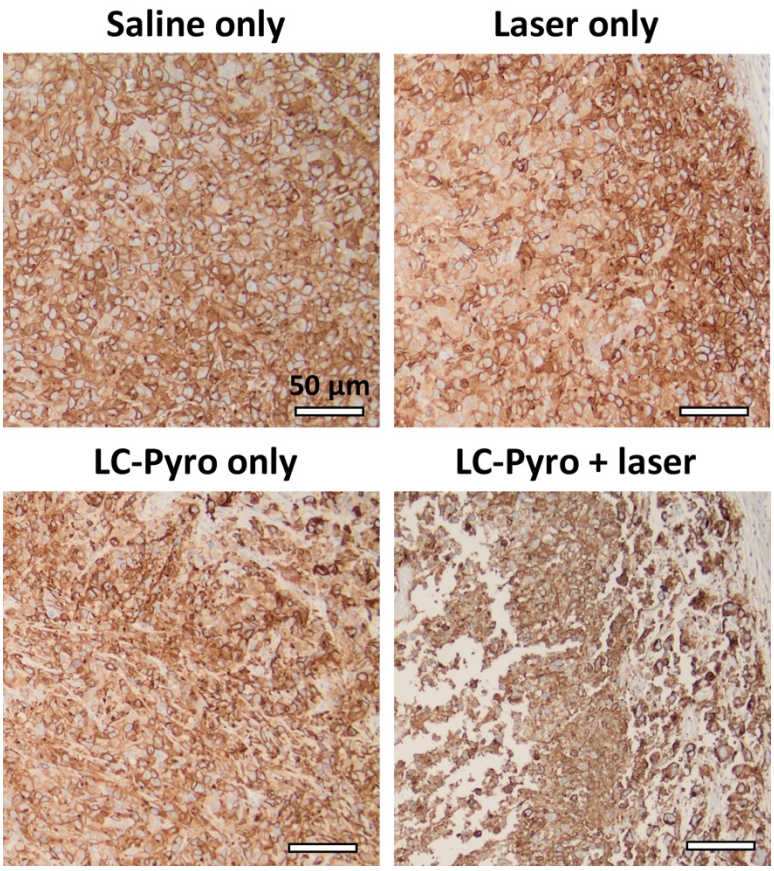

Figure S8. Representative PSMA staining sections of PSMA+ PC3 PIP tumors post-PDT treatment from each cohort; 1) Saline only; 2) Laser only; 3) LC-Pyro only and 4) LC-Pyro + Laser. Scale = 50 µm. PDT did not significantly alter expression of PSMA.

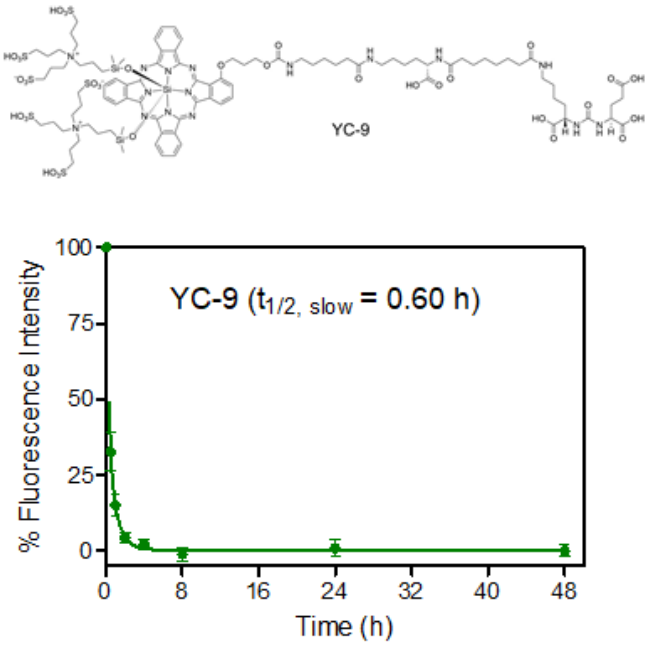

Figure S9. Chemical structure of reported compound YC-9[1] and its plasma blood clearance profile in BALB/c mice (n = 5). The profile fits into a two-compartment model with a slow half-life of 0.60 h.

References:

1. Chen, Y.; Chatterjee, S.; Lisok, A.; Minn, I.; Pullambhatla, M.; Wharram, B.; Wang, Y.; Jin, J.; Bhujwalla, Z. M.; Nimmagadda, S.; Mease, R. C.; Pomper, M. G., A PSMA-targeted theranostic agent for photodynamic therapy. *J Photochem Photobiol B* **2017,** *167*, 111-116.