SUPPLEMENTARY MATERIAL

A novel PSMA-targeted probe for NIRF-guided surgery and photodynamic therapy: synthesis and preclinical validation

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Synthesis and characterization of IRDye700DX-PSMA

All chemicals, reagents, and solvents for the synthesis were purchased from Sigma Aldrich, Merck and Iris Biotech. IRDye® 700DX NHS Ester was obtained from LI-COR, Inc. (Lincoln, NE). Analytical and preparative HPLC−MS was carried out on a Waters AutoPurification system (3100 Mass Detector, 2545 Pump Gradient Module, 2767 Sample Manager, and 2998 PDA detector).

The peptidomimetic glutamate-urea-lysine binding motif (Glu-NH-CO-NH-Lys-2-naphthyl-L-Ala-cyclohexane) was synthesized by solid-phase peptide chemistry according to previously published methods on 0.1 mmol scale. Cleavage from the solid support and amino acid side chain deprotections were performed simultaneously by adding a solution of TFA:TIPS:water (95:2.5:2.5) for 2 hours, then filtered to remove the resin. Cold diethyl ether was then added to precipitate the crude compound. The compound pellet was dissolved in 1 mL of water and purified by HPLC-MS, the separation was performed on an Atlantis dC18 OBD Prep Column, 100Å, 5 μm, 19 mm X 100 mm, Eluent: A: 0.1% TFA in water; B : 0.1% TFA in acetonitrile, Gradient: 10%B→30%B over 6 min, 30%B→40%B over 11 min , Flow rate: 20mL/min. After purification, the product was frozen and lyophilized to obtain 30 mg of a solid with white color (yield 46 %). Analytical HPLC-UV-MS was carried out using an Atlantis RP-C18, 3.5 μm, 4.6 mm X 150 mm column, Eluent: A : 0.1% TFA in water; B : 0.1% TFA in acetonitrile, Gradient: 90%B→10%B over 2min, 10%B→30%B over 8 min, 30%B→40%B over 20 min, 40%B→100%B over 6 min, Flow rate: 1mL/min. The chromatogram revealed at 220 nm showed one major peak corresponding to a degree of purity of 92% (retention time 12.8 min). ESI+MS m/z relative to the peak at 12.8 min (calcd. for C33H45N5O9): [M+H]+ 656.3 (obsd.), 656.7 (calcd.) (Figure S1).

PSMA binding motif (1 mg, 1.5 μmol), was conjugated to IRDye700DX-NHS (1.9 mg, 1 μmol) in buffer phosphate 0.1 M, pH = 8 The reaction was stirred for 2 h at room temperature followed by preparative HPLC-MS, the separation was performed on an Atlantis dC18 OBD Prep Column, 100Å, 5 μm, 19 mm X 100 mm, Eluent: A : ammonium acetate 10 mM in water; B : acetonitrile, Gradient: 10%B→50%B over 12 min, Flow rate: 20mL/min. After purification, the product was frozen and lyophilized to obtain 1,2 mg of a solid with dark blue color (yield 49 %). Analytical HPLC was carried out using an Atlantis RP-C18 column, 5 μm, 4.6 mm x 150 mm, ammonium acetate 10mM (solvent A) and acetonitrile (solvent B); Gradient: 20%B→50%B over 20min, 50%B→100%B over 10 min, Flow rate: 1mL/min. The chromatogram (Figure S1) showed one peak corresponding to a degree of purity of 94,4 %, based on the chromatographic peak area revealed at 225 nm and at 689 nm (retention time 6.8 min). ESI(-) MS m/z relative to the peak at 6.8 min calculated for calculated for C103H140N16O33S6Si32- [M-2H]2- 1202.9, found 1202.8, C103H139N16O33S6Si33- [M-3H]3- 801.6, found 801.0, C103H138N16O33S6Si34- [M-4H]4- 601.2, found 600.3.

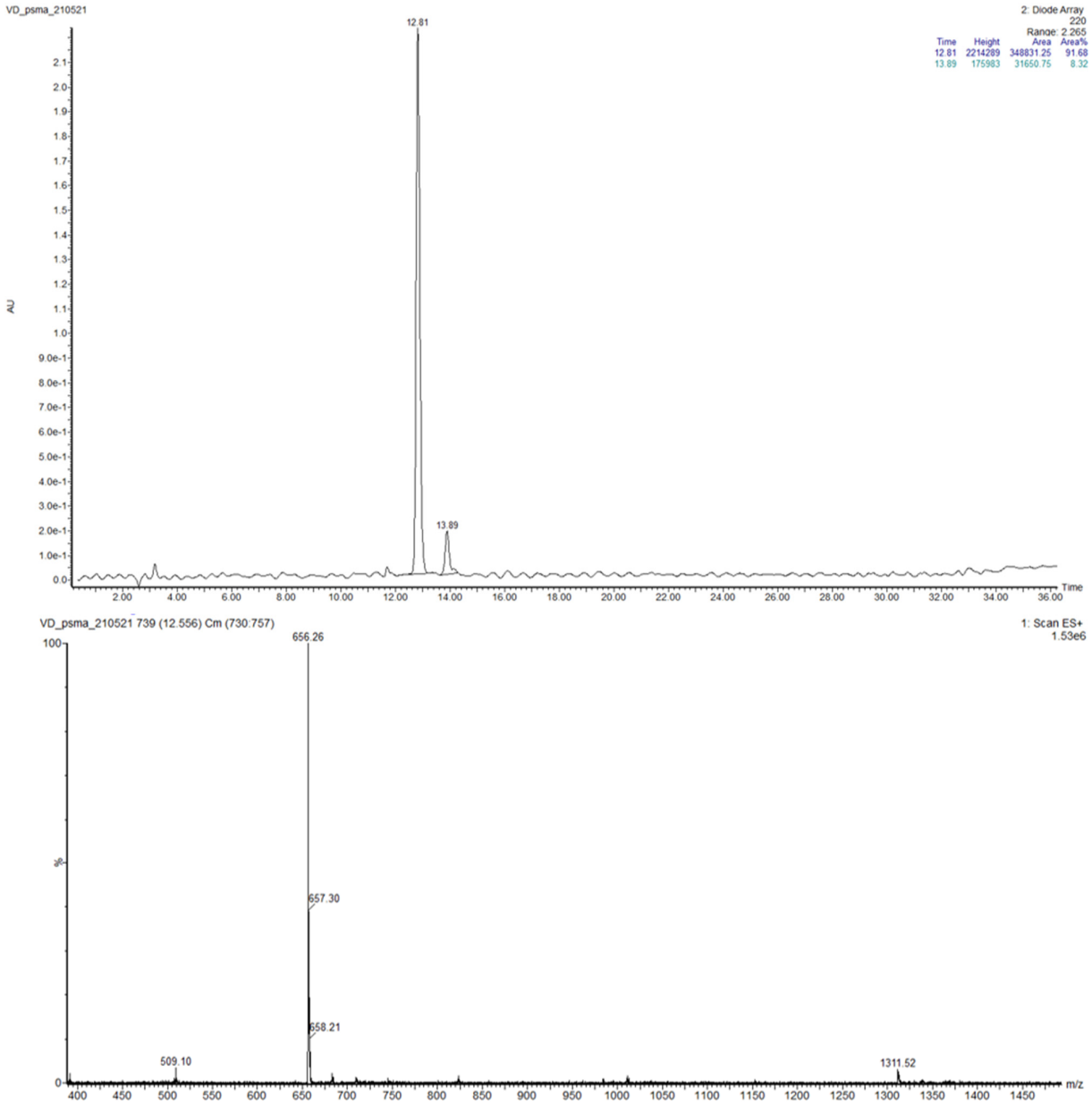

Figure S1. Analytical HPLC of PSMA-617 on Atlantis dC18 column RP, 5 μm, 4.6 mm x 150 mm, H2O-TFA 0,1%, CH3CN-TFA 0,1% on HPLC-UV-Vis-MS system, A: Chromatograms recorded at wavelengths 220 nm. B: mass spectrum ESI(+) MS m/z relative to the peak at 13.48 min.

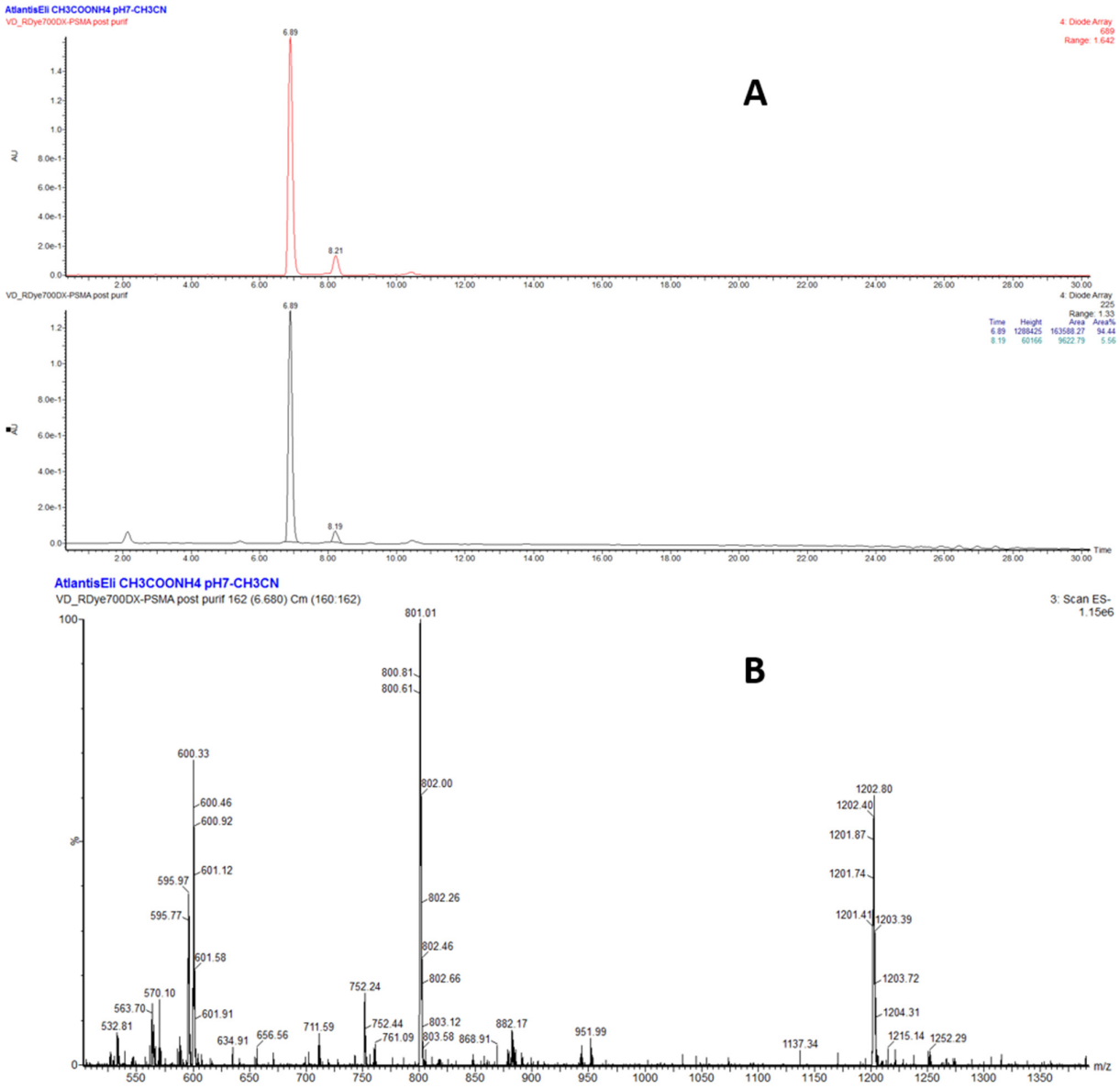

Figure S2. Analytical HPLC of IRDye700DX-PSMA on Atlantis dC18 column RP, 5 µm, 4.6 mm x 150 mm, ammonium acetate 10mM in water and acetonitrile on HPLC-UV-Vis-MS system, A: Chromatograms recorded at different wavelengths: 689 nm and 225 nm. B: mass spectrum ESI(-) MS m/z relative to the peak at 6.8 min.

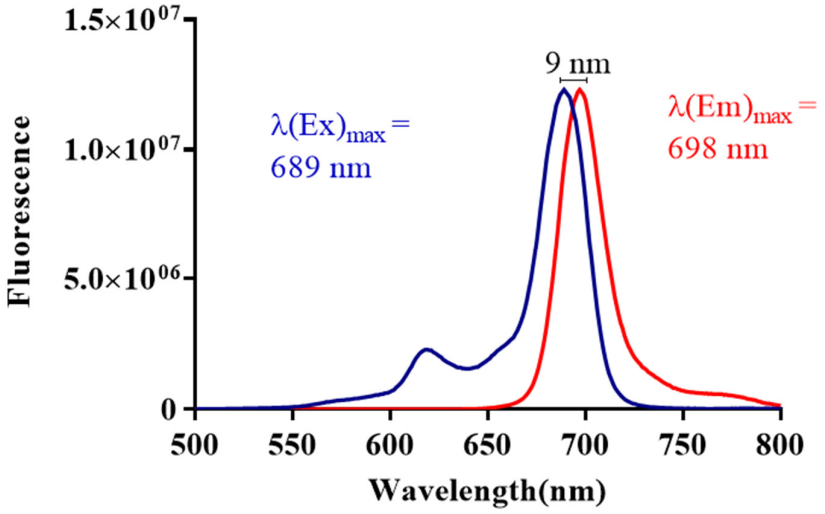

Figure S3. Fluorescence excitation and emission spectra of IRDye700DX-PSMA

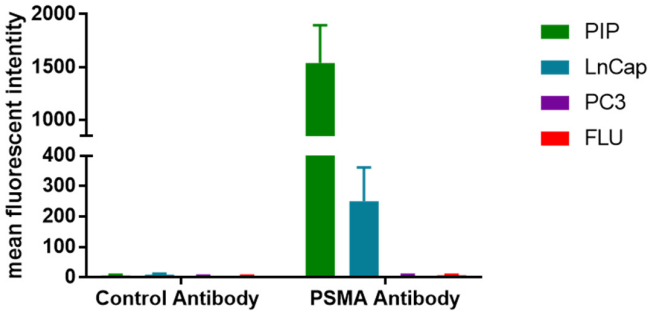

Figure S4. PSMA expression measurement via Flow cytometry in PC3-PIP, LNCaP, PC3-FLU and PC3 cell line

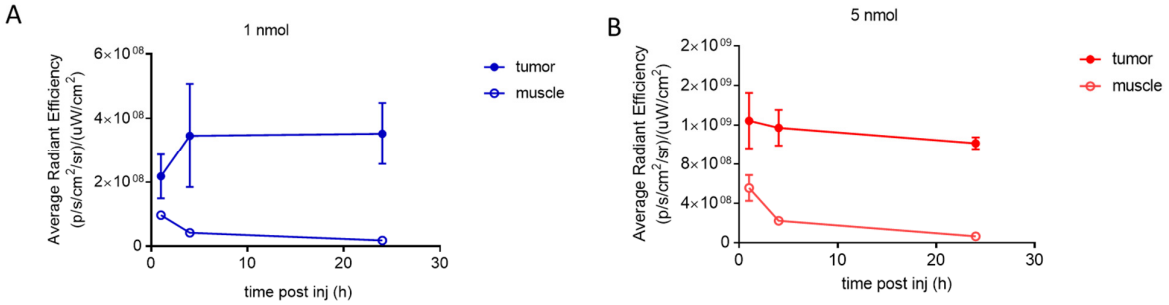

Figure S5. Fluorescent signal calculated from in vivo optical imaging in tumor and muscle region post 1 nmol (A) and 5nmol (B) of IRDye700DX-PSMA.

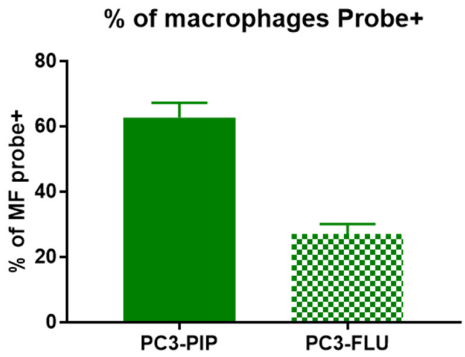

Figure S6. Ex vivo tumour characterization. Flow cytometry data of % of macrophages that taken IRDye700DX-PSMA up to the total macrophages population in the tumor.