Supplementary Information

A highly selective iron oxide-based imaging nanoparticle for long-term monitoring of drug-induced tumor cell apoptosis

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Supplementary text

Calculation of number of PSA per IONP-Neu-PSA particle:

MW of 10 nm IONP: 1,175,018.742 g/mol = 1,175,018,742 mg/mol

MW of Biotin-PSA: 53,000 g/mol = 53,000,000 mg/mol

1.5 mg of IONP after purification = 1.5 mg / 1,175,018,742 mg/mol = 1.277×10^{-9} mol

1 mg of PSA = 1 mg / 53,000,000 mg/mol = 1.887×10^{-8} mol

27.9% (BCA) of PSA bound by IONP-Neu = $27.9\% \times 1.887 \times 10^{-8}$ mol = 5.265×10^{-9} mol

Number of PSA per IONP-Neu-PSA = 5.265×10^{-9} mol / 1.277×10^{-9} mol = 4.12

20.1 % (SDS-PAGE) of PSA bound by IONP-Neu = $20.1\% \times 1.887 \times 10^{-8}$ mol = 3.793×10^{-9} mol

Number of PSA per IONP-Neu-PSA = 3.793×10^{-9} mol / 1.277×10^{-9} mol = 2.97

Calculation of PSA concentration in mouse blood for in vivo toxicity evaluation

High concentration of PSA can agglutinate erythrocytes and cause hematological toxicity. To ensure minimum in vivo toxicity of IONP-Neu-PSA-Cy5.5, the following calculation of PSA concentration in mice blood was performed. For each mouse, we injected 100 μL of 2.8 [Fe] mg/mL IONP-Neu-PSA-Cy5.5. Since each nanoparticle has 3-4 PSA molecules, the maximum 4 PSA/nanoparticle is equivalent to ~0.95 nmol of PSA per mouse. Given a 25 g mouse with a typical 2.0 mL total blood volume, the PSA blood concentration in a mouse is ~0.475 μM. In comparison, the lowest concentration for WGA (36 kDa) to start agglutinate erythrocytes at 37 Celsius is 0.889 μM (32 μg/mL).[1] Systemic toxicity should be at minimum level at such a low PSA dosage.

Supplementary Figures

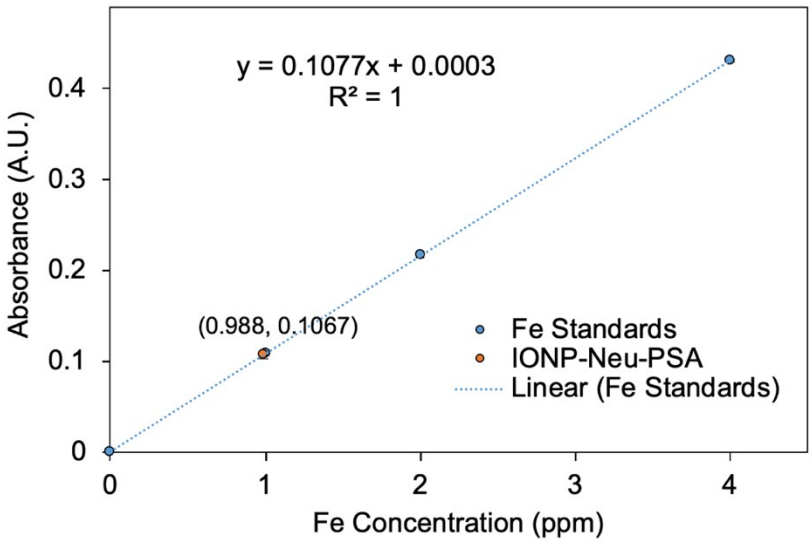

Figure S1. Ferrozine assay for quantifying [Fe] concentration of IONP-Neu-PSA. 0 ppm, 1 ppm, 2 ppm, 4 ppm Fe standards were used to construct the linear standard curve as shown. Absorbance of the 500× diluted IONP-Neu-PSA sample was 0.1067 which corresponds to 0.988 ppm. The Fe concentration of the undiluted IONP-Neu-PSA is 0.494 mg/mL

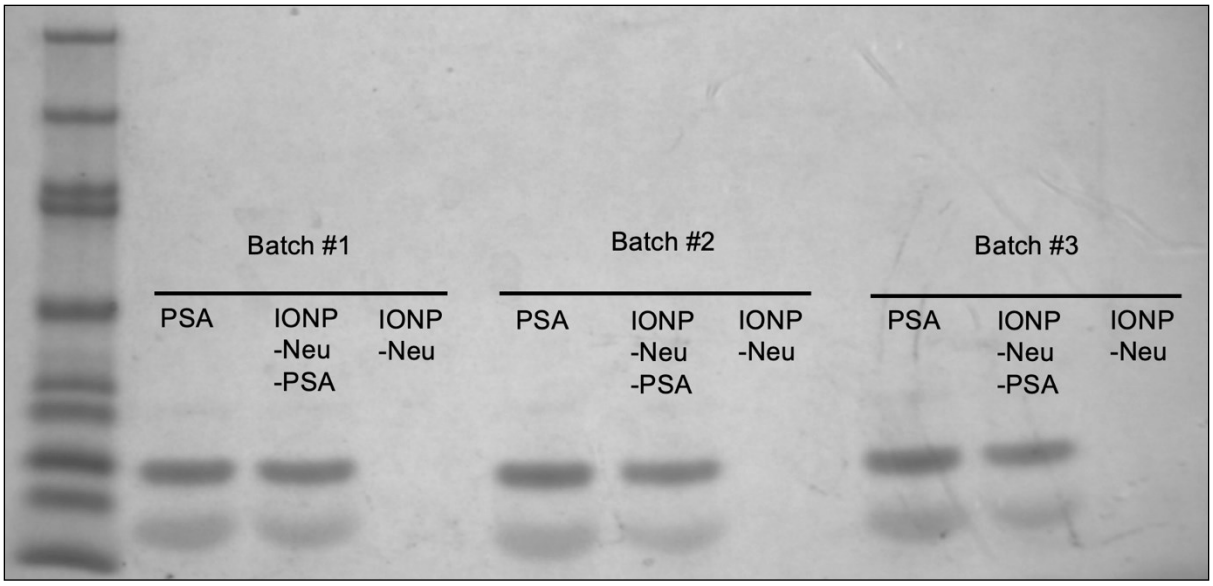

Figure S2. Full SDS-PAGE image for quantification of PSA bound on NPs. Three batches of IONP-Neu NPs were synthesized followed by PSA-biotin attachment independently. PSA controls were diluted to the same concentration as the unpurified IONP-Neu-PSA solution

before spin filtering. The gel was run for 90 mins under a constant voltage of 100 V. The image was taken in a Gel Doc XR imaging system (Bio-Rad).

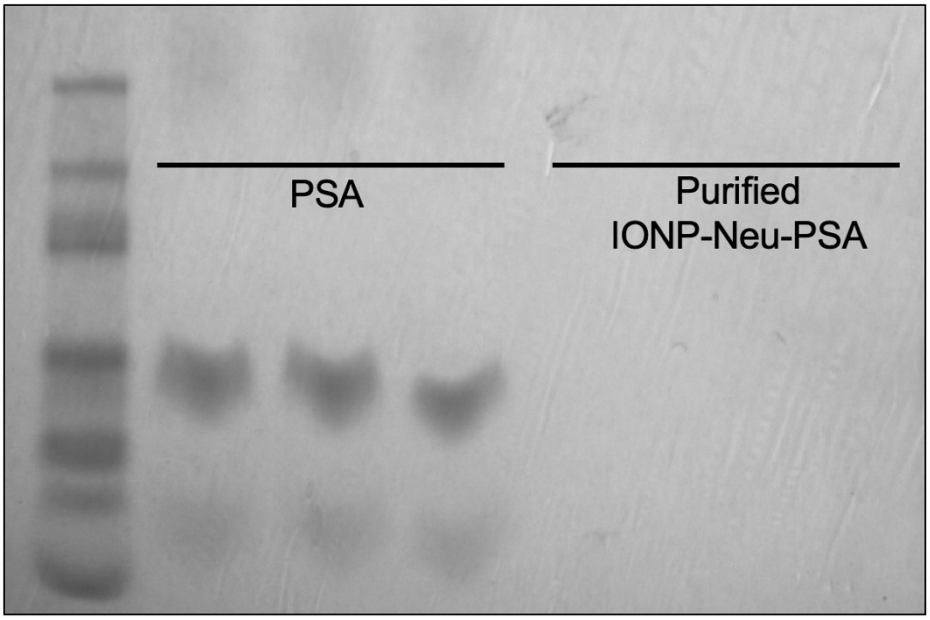

Figure S3. Image of the non-reducing SDS-PAGE for examining the purity of IONP-Neu-PSA. PSA controls were diluted to the same concentration as the purified IONP-Neu-PSA solution. Protein samples were not reduced by 2-mercaptoethanol under heating. The gel was run for 90 mins under a constant voltage of 100 V. The image was taken in a Gel Doc XR imaging system (Bio-Rad).

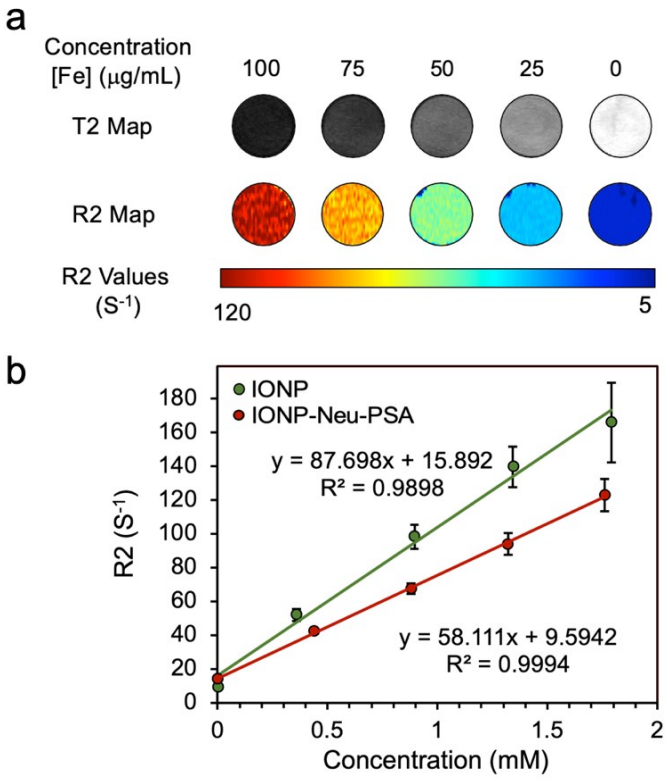

Figure S4. MRI characterization of IONP-Neu-PSA. (a) T2 and R2 maps of IONP-Neu-PSA at various particle concentrations. Water is used as the background. (b) Comparison of transverse relaxivity (slope S^{-1} mM^{-1}) plots between IONP-Neu-PSA and IONP.

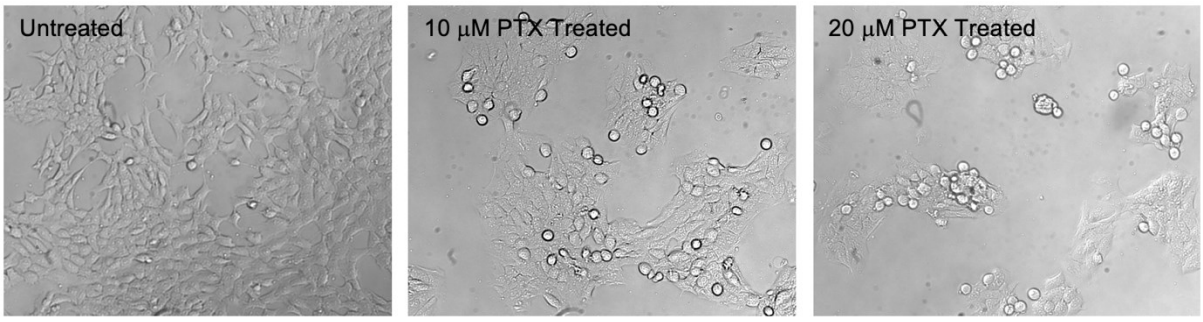

Figure S5. Bright field images of untreated, 10 μM PTX and 20 μM PTX treated 4T1 breast cancer cells at lower magnification.

Reference

1 H. P. Schnebli and T. Bächi, *Experimental Cell Research*, 1975, **91**, 175–183.