

# Supplementary Materials: Site-Specific $^{111}\text{In}$ -Radiolabeling of Dual-PEGylated Porous Silicon Nanoparticles and Their In Vivo Evaluation in Murine 4T1 Breast Cancer Model

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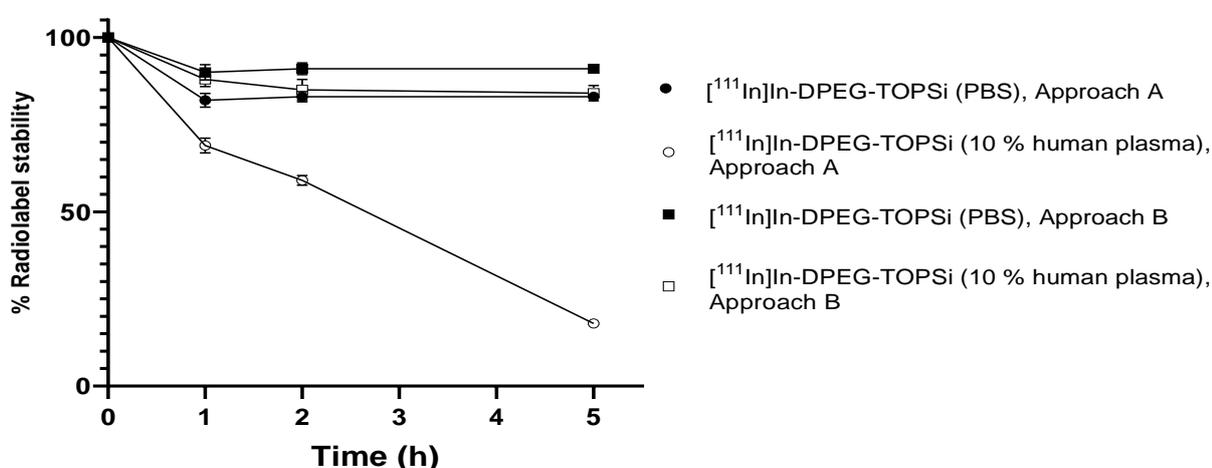
## Experimental procedures

### Materials and chemicals

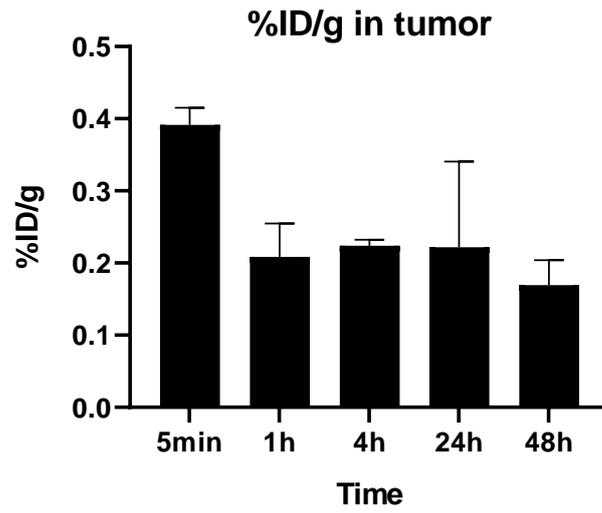
All chemicals and solvents were obtained from commercial providers and they were used without further purification. Ultrapure water (18.2 M $\Omega$ ) was prepared on a Milli-Q Integral 10 water purification system. The cell viability assay, culturing media and other procedures for cells used in experiments are described in the *supporting information* (SI).  $^{111}\text{In}$  was received from Mallinckrodt Medical B.V. (Petten, The Netherlands).

**Cell Culturing.** An animal stage IV human breast cancer cell 4T1 was cultured in RPMI-1640 supplemented with 10% FBS, 1% penstrep, 1 x sodium pyruvate, 1 x glutamex in 75 cm<sup>2</sup> flasks incubated at 37 °C in a humidified atmosphere (95%) and 5% CO<sub>2</sub>.

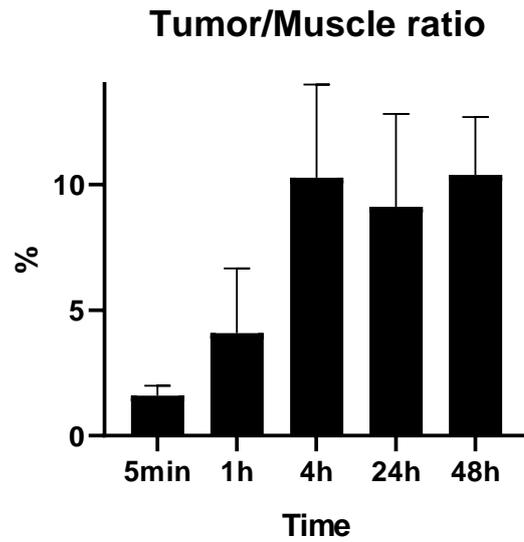
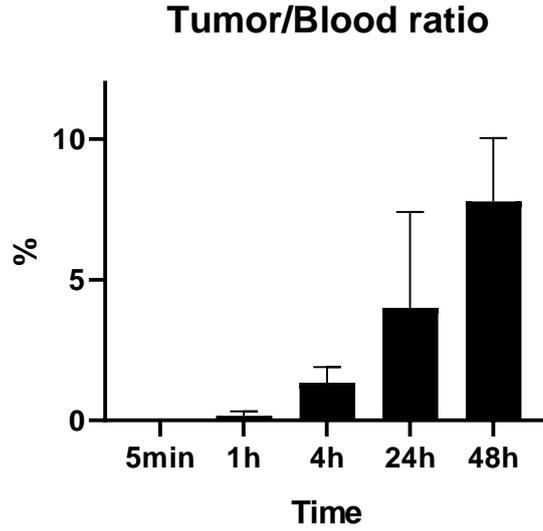
**Radiochemical stability of the  $^{111}\text{In}$ -labeled particles.** The stability of the  $^{111}\text{In}$ -DPEG-TOPSi particles, prepared by both approaches, were investigated *in vitro* in 1 x PBS (pH = 7.4) and human plasma 10%. For the stability tests freshly prepared  $^{111}\text{In}$ -DPEG-TOPSi particles (0.5 mg) were added to 1 ml of PBS or plasma solution in LowBind Eppendorf tubes and incubated at 37°C under constant shaking. At predetermined time points (1 h, 2 h and 5 h) samples were centrifuged and the radioactivity of pellet and supernatant were measured by dose calibrator (VDC-405, Veenstra Instruments). All assays were carried out in triplicate.



**Figure S1.** In vitro stability of  $^{111}\text{In}$ -DPEG-TOPSi particles in 10% human plasma and 1 x PBS (n = 3). The particles were radiolabeled either by using the one step Approach A or the two-step Approach B, in which the particles were radiolabeled by using a presynthesized  $^{111}\text{In}$ -DOTA-PEG<sub>4</sub>-Tz ( $^{111}\text{In}$ 1).



**Figure S2.** %ID/g in tumor for [<sup>111</sup>In]In-DPEG-TOPSi particles at 4T1 tumor model at 5 min, 1 h, 4 h, 24 h and 48 h time points.



**Figure S3.** Tumor/blood ratio and tumor/muscle ratio for [<sup>111</sup>In]In-DPEG-TOPSi particles at 4T1 tumor model at 5 min, 1 h, 4 h, 24 h and 48 h time points.

**Table S1.** Ex vivo biodistribution of [<sup>111</sup>In]In-DPEG-TOPSi, [<sup>111</sup>In]In-TOPSi and [<sup>111</sup>In]In-DOTA-PEG<sub>4</sub>-Tz ([<sup>111</sup>In]1) at 1 h time point.

	[ <sup>111</sup> In]In-DPEG-TOPSi	[ <sup>111</sup> In]In-TOPSi	[ <sup>111</sup> In]In-DOTA-PEG <sub>4</sub> -Tz
Blood	2.32 ± 1.4	0.64 ± 0.08	1.18 ± 0.37
Urine	15.58 ± 11.3	86.23 ± 80.5	810.35 ± 475.2
Spleen	43.72 ± 17.4	122.75 ± 10.4	0.23 ± 0.04
Pancreas	0.44 ± 0.7	0.20 ± 0.04	0.19 ± 0.14
Kidney	0.57 ± 0.3	4.03 ± 0.9	2.03 ± 1.21
Liver	51.38 ± 4.5	69.26 ± 4.6	0.95 ± 0.14
Lung	2.35 ± 1.7	18.04 ± 3.9	1.27 ± 0.34
Heart	0.48 ± 0.2	1.00 ± 0.2	0.47 ± 0.12
Skeletal muscle	0.08 ± 0.06	0.20 ± 0.05	0.14 ± 0.03
Bone (tibia)	0.15 ± 0.05	1.98 ± 0.6	0.26 ± 0.10
Stomach	0.10 ± 0.04	0.21 ± 0.13	0.19 ± 0.06
Small intestine	0.15 ± 0.08	0.15 ± 0.06	0.92 ± 0.17
Large intestine + cecum	0.07 ± 0.03	0.12 ± 0.02	0.16 ± 0.04

# <sup>1</sup>H NMR spectra

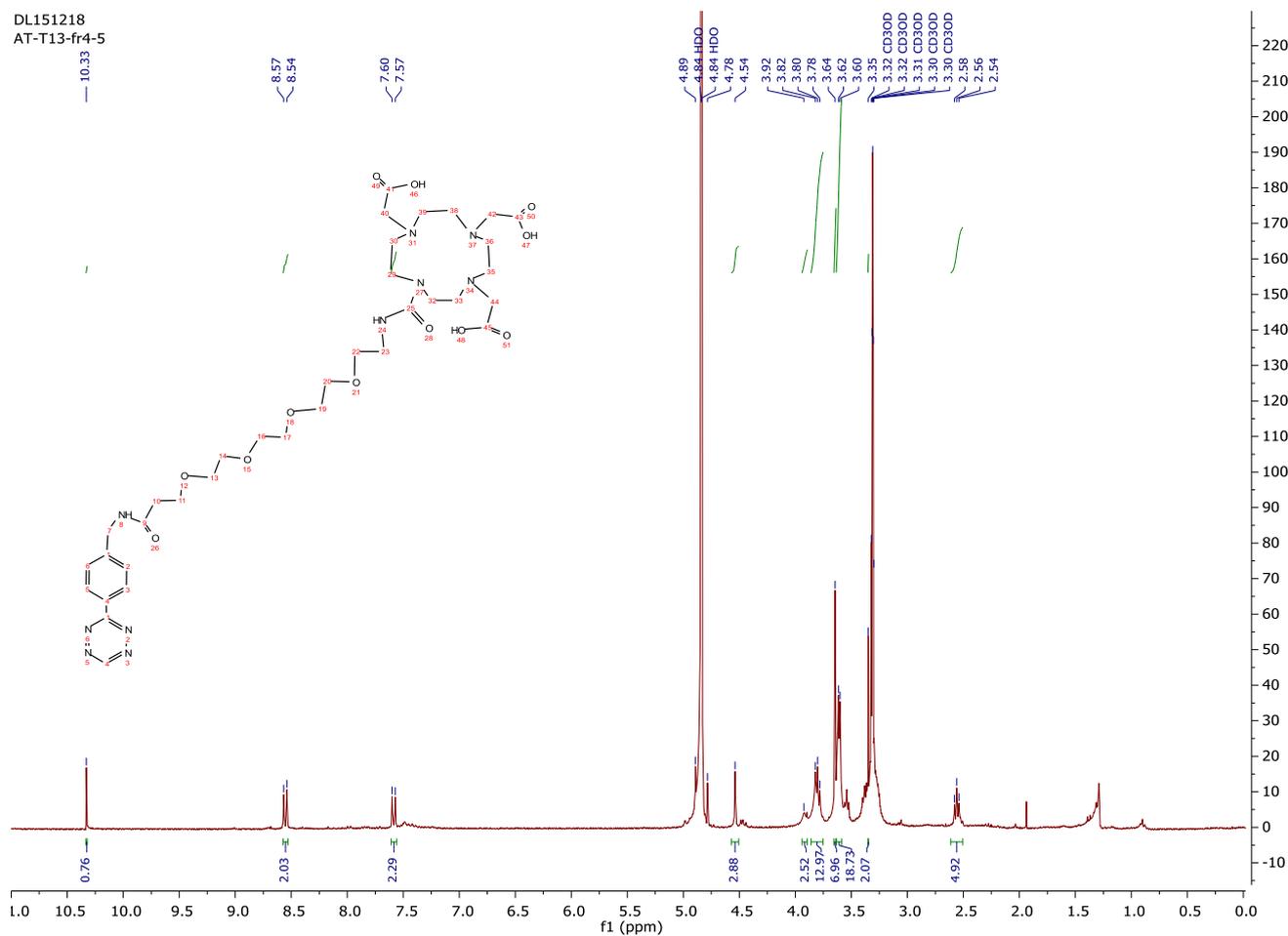


Figure S4. <sup>1</sup>H-NMR spectra from DOTA-PEG<sub>4</sub>-Tz (1).

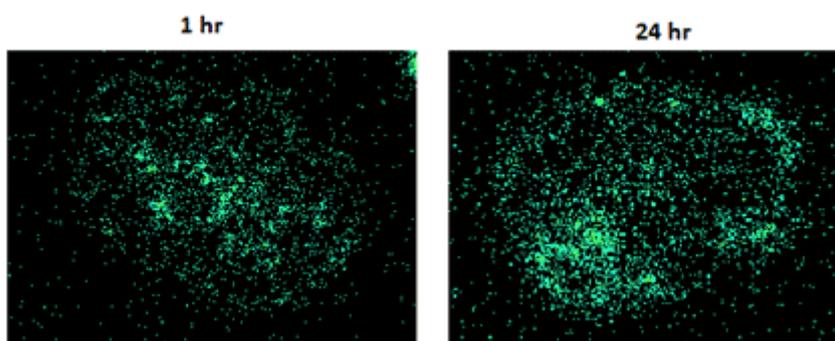


Figure S5. Autoradiography image from 4T1 tumor slices collected at 1h and 24h after [<sup>111</sup>In]In-DPEG-TOPSi administration.