Supporting information.

Gold nanoparticles stabilized with MPEG-grafted poly-l-lysine: in vitro and in vivo evaluation of a potential theranostic agent.

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Experimental

S1. Synthesis of MPEG derivatives. All reagents were from Sigma- Aldrich, the solvents from Thermo-Fisher Scientific (ACS grade).

Initially, MPEG5000 (Sigma-Aldrich) in toluene was refluxed in a Dean-Stark apparatus and dried [1].

Both the N-hydroxysuccinimide and the pentafluorophenyl ester of methoxy MPEG5000 carbonate (MPEG derivative **1** and **2,** respectively, Figure 1S) were synthesized by using a modification of the method described by Miron and Wilcheck [2]. Briefly, 1 mmol of dry MPEG was combined with 10 mmol N,N'-disuccinimidyl carbonate or bis(pentafluorophenyl) carbonate in 30 ml of dry dioxane followed by addition of 10 mmol of 4-dimethylaminopyridine in dioxane. The reaction mixture was kept at RT overnight under argon and the product was purified by three re-precipitations as described above. Yield- 70% (from theoretical).

O-MethylPoly(ethylene glycol)-O'-succinate (MPEG derivative **3**, Fig. 1S) was synthesized by refluxing 1 mmol MPEG5000 with a 5-molar excess of succinic anhydride (Sigma-Aldrich) in the presence of 1.1 mmol 4-dimethylaminopyridine (DMAP, Sigma-Aldrich) in dry dioxane for 5 h at 80^{o}C under argon. The reaction mixture was evaporated in a vacuum, dissolved in chloroform, filtered through a layer of Celite, and purified by repeated precipitations using a mixture of diethyl ether:ethyl acetate (4:1 v/v) on ice. The final precipitate was dissolved in water, the pH was adjusted to 3, passed

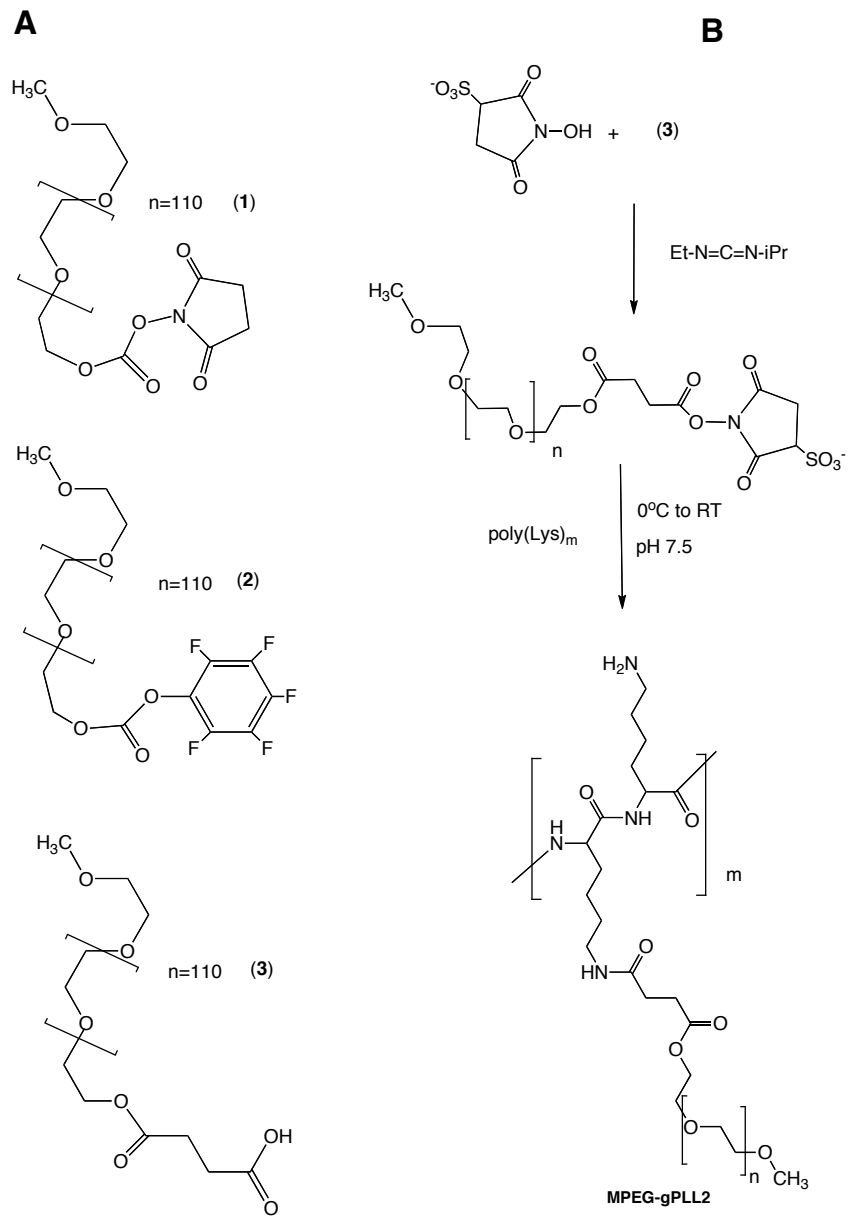


Figure 1S. Synthesis of MPEG-gPLL. A - Methoxy poly(ethylene glycol) derivatives used for covalent grafting to PLL: 1) N-hydroxysuccinimide ester of MPEG5000 carbonate, 2) pentafluorophenyl ester of MPEG5000 carbonate; 3) O-succinyl MPEG5000, **B** - synthesis scheme of MPEG-gPLL2 using water-soluble carbodiimide activation of (3) in the presence of N-hydroxysulfosuccinimide.

through 10 g of ethanol/water washed AG50 W-X8 resin, and lyophilized. Yield-65% (from theoretical).

S2. Synthesis of MPEG-gPLL copolymer. Synthesis of graft copolymer of MPEG5000 and PLL was accomplished using two approaches: 1) using N-hydroxysuccinimide or pentafluorophenyl esters of MPEG carbonate (**1** or **2**, Fig. 1S) for direct N-acylation of poly-l-lysine; 2) by using water-soluble carbodiimide activation of MPEG derivative **3** in the presence of N-hydroxysulfosuccinimide as described in [3,4] with modifications.

Synthesis of MPEG-gPLL1. MPEG-gPLL1 that does not contain esterase-sensitive ester bonds was synthesized by adding 0.84 g of activated MPEG carbonate (i.e. either NHS ester (MPEG derivative **1**) [1] or pentafluorophenyl ester (MPEG derivative **2**) to 100 mg of poly-l-lysine hydrobromide (PLL m.w 34.4 kD, d.p. 164, PDI (GPC)=1.13, Sigma-Aldrich) dissolved in 20 ml of degassed and nitrogen-saturated 0.1 M triethanolamine buffer, pH 7.6 on ice, as ~200 mg portions of dry powders followed by mixing until a complete solubilization of each portion.

Synthesis of MPEG-gPLL2. MPEG-gPLL2 that contains esterase-sensitive ester bonds was synthesized by dissolving 100 mg of poly-l-lysine hydrobromide (PLL m.w 34.4 kD, d.p. 164, PDI (GPC)=1.13, Sigma-Aldrich) in 200 ml of ice-cold, degassed and nitrogen-saturated 0.1 M $NaHCO_3$, pH 8.7 (at approximately 2.5 mM amino groups). 0.9 g of MPEG derivative **1** (0.2 mmol) and 40 mg (0.2 mmol) of N-hydroxysulfosuccinimide (Thermo-Fisher) were dissolved in 2 ml of water and mixed with a solution of 100 mg of 1-(3-dimethyl aminopropyl)-3-ethyl carbodiimide, hydrochloride (EDC, 0.52 mmol) in 0.5 ml water and incubated for 5 min with mixing. The activated solution of N-hydroxysulfosuccinimide ester of

MPEG derivative **1** was added to the solution of PLL and the reaction mixture was incubated overnight at RT under argon.

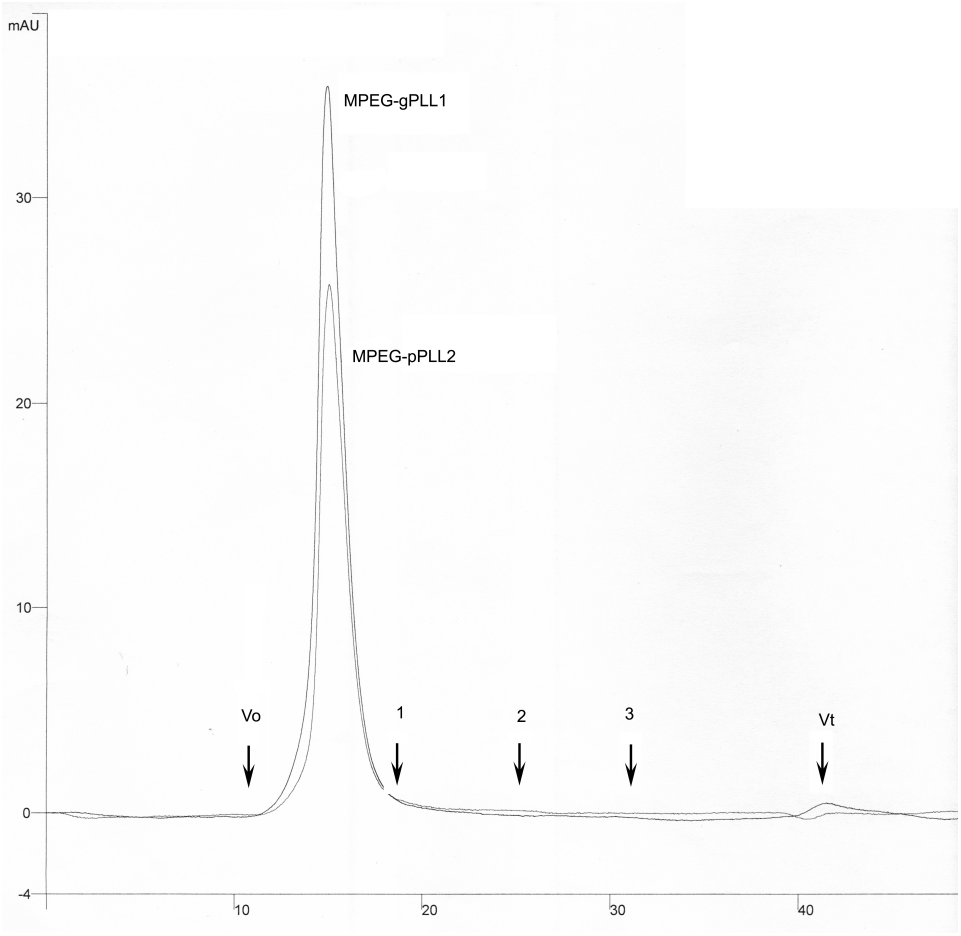
MPEG-gPLL purification and characterization. The obtained graft copolymers were purified by ultrafiltration using UFP-100 cartridges (GE Healthcare) or partially purified using Amicon Ultracel YM-50 (EMD-Millipore) with monitoring of the residual content of MPEG5000 in the product using size-exclusion HPLC (Superdex200, GE Healthcare). The content of free MPEG in the final preparations of MPEG-gPLL did not exceed 2% by weight. The degree of N-ε-amino group acylation was determined using trinitrobenzene sulfonic acid (TNBS). According to TNBS assay the % of PLL amino group modification with MPEG chains was 13-14%. The obtained MPEG-gPLL1 and MPEG-gPLL2 were characterized using Superdex200 size-exclusion HPLC (Fig. 2S A) and NMR spectrometry at 400MHz (Varian). 1H and 13C NMR were recorded in D_2O and $CDCl_3$, respectively, Fig. 2S B,C.

S3. Blocking of amino groups in MPEG-gPLL. Covalent blocking of N-ε-amino groups was performed by using a 10-fold molar excess of dry succinic anhydride over TNBS-reactive amino groups in MPEG-gPLL (20 mg/ml in 0.1 M $NaHCO_3$, pH 7.7) on ice followed by 2h incubation and dialysis. The completeness of N-ε-amino group acylation was determined using TNBS.

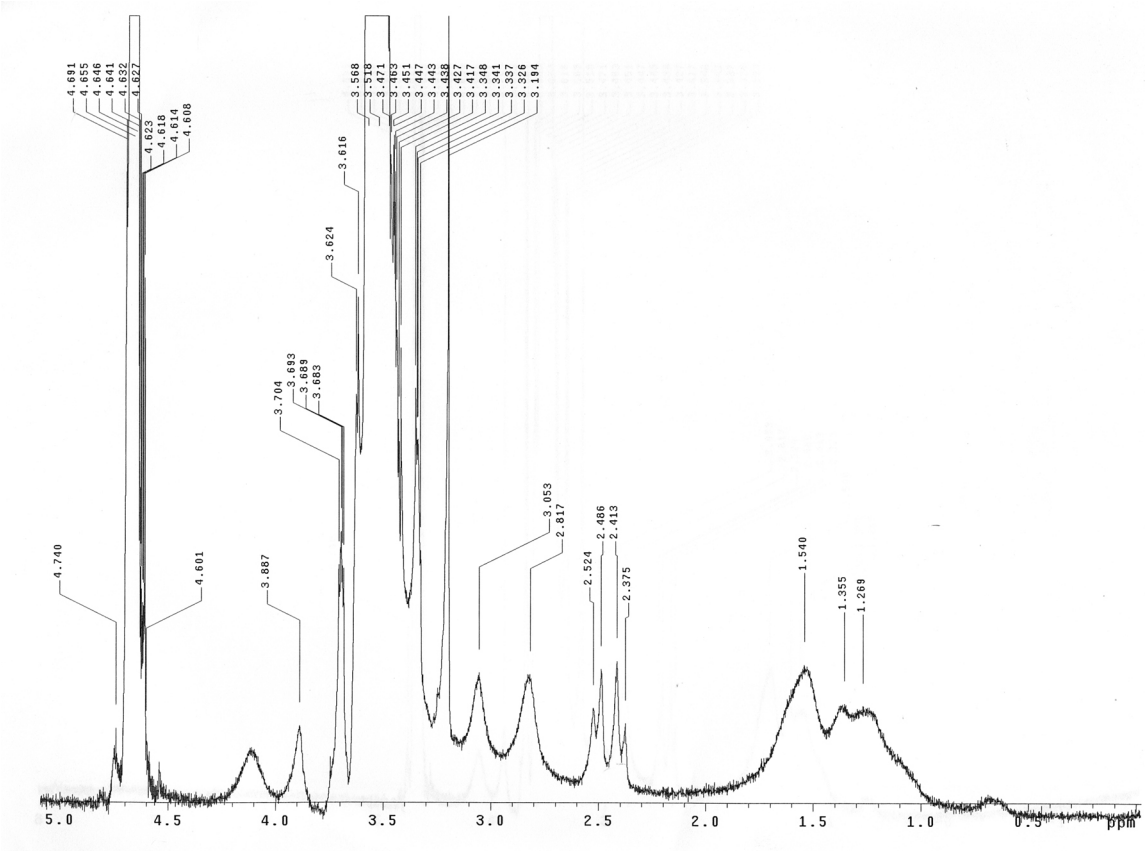
Supporting Results.

Figure 2S

A Size-exclusion HPLC of MPEG-gPLL samples (100 µl, 1 mg/ml) on Superdex200 column (25x 1 cm), eluted with 0.1 M ammonium acetate, pH 7.0 (0.5 ml/min). The column has been calibrated using thyroglobulin (1); IgG (2), BSA (3).



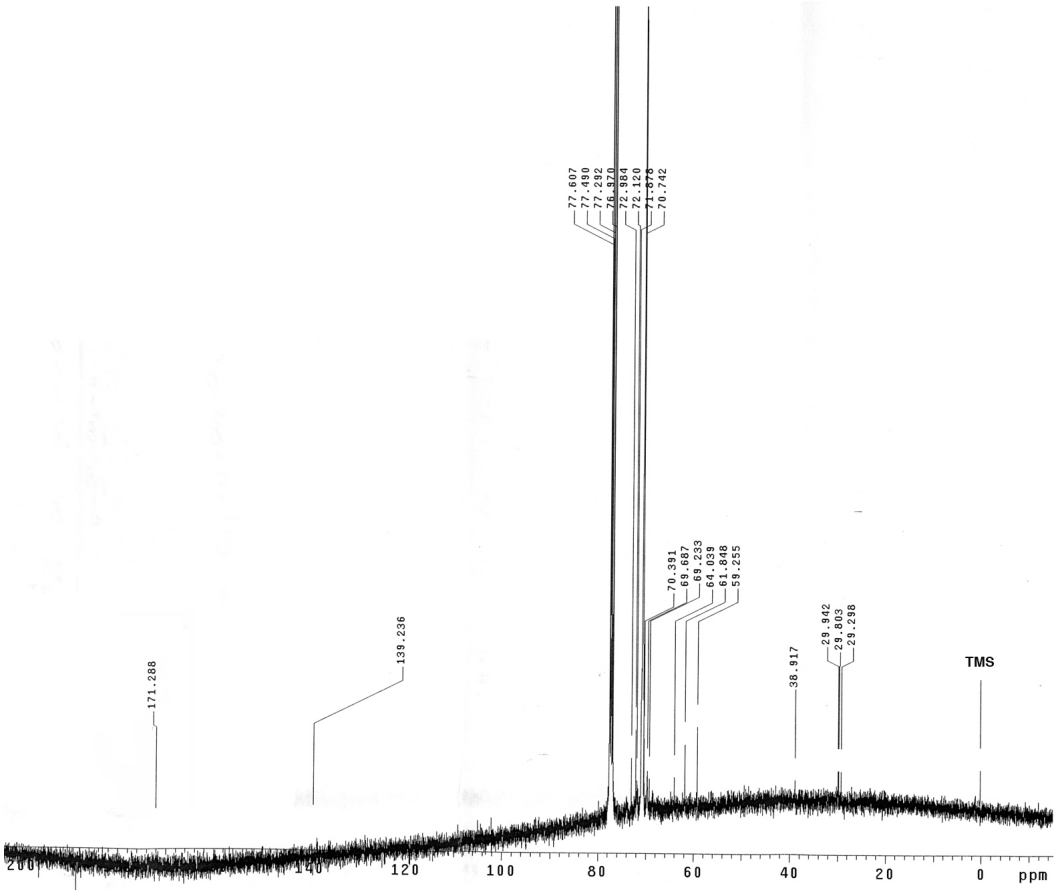
B ^{1}H NMR spectrum (MPEG-gPLL1), 10 mg/ml D_2O



Peak assignment:

Structure element	Protons	
		δ, ppm, D_2O
MPEG	O-CH_3	3.35
	O-CH_2-CH_2-O	3.52
	O-CH_2-C=O	4.1
Lysine, free	α-CH_2	4.55
	N-terminal α-CH_2	3.37
	β-CH_2	1.54
	γ-CH_2	1.269
	δ-CH_2	1.35
	ε-CH_2	2.817
Lysine-PEGylated		
	α-CH_2	4.55
	β-CH_2	1.54
	γ-CH_2	1.269
	δ-CH_2	1.35
	ε-CH_2	3.053
D_2O		4.646

C ^{13}C NMR Spectrum (400 MHz), MPEG-gPLL1 (30 mg/ml, $CDCl_3$)



Structure element	Carbon atoms	
		δ, ppm
MPEG	-O-**C**H_3	61.85
	-O-**C**H_2-**C**H_2-O-	70.74
	-O-**C**H_2-(C=O)-NH	71.88
	-O-CH_2-**C**H_2-O-CH_3	72.12
	-O-CH_2-(**C**=O)-NH-	139.24
Lysine free/PEGylated		
	α-CH_2	59.25
	β-CH_2	29.94
	γ-CH_2	22.30
	δ-CH_2	29.30
	ε-CH_2	38.92
PLL backbone	>CH-(**C**=O)-NH-	171.2-175.08
CDCl_3 (solvent)		76.97-77.61

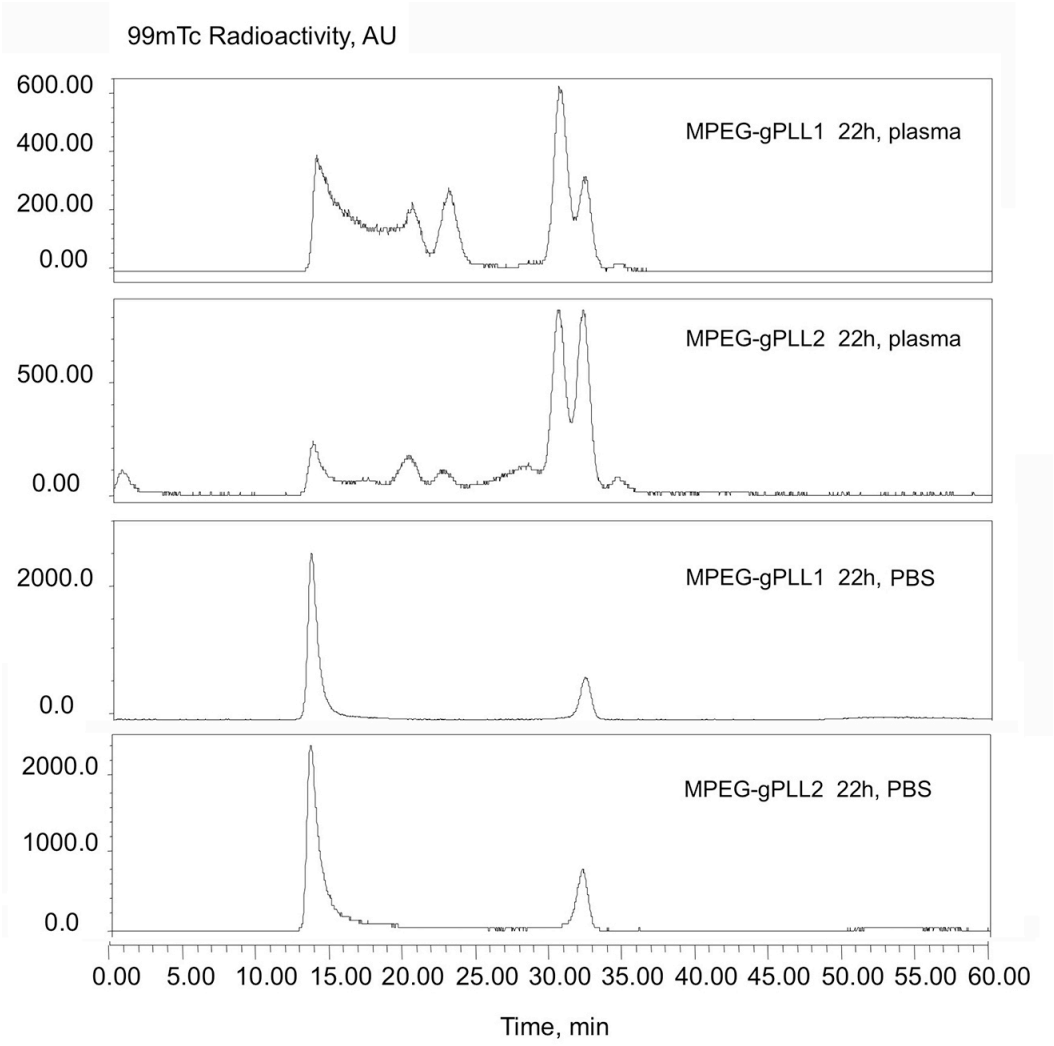


Figure 3S. Size-exclusion HPLC profiles of ^{99m}Tc labeled MPEG-gPLL1 and MPEG-pPLL2 incubated for 22h either in PBS or 80% mouse plasma. Both samples were covalently modified with S-mercaptoacetyldiglycylglycine NHS ester, (S-AcMAG3-NHS, Kerafast, Boston MA) at approx. 5 mol S-AcMAG3/mol MPEG-gPLL prior to labeling with ^{99m}Tc. HPLC was performed on Superose6 GL (1x30 cm) HPLC size-exclusion column eluted with 20% acetonitrile in 0.1M TrisHCl, pH 8.0 (0.6 ml/min). Samples incubated in PBS for 22 h were subsequently succinylated with a 5-molar excess of succinic anhydride/available free amino groups prior to HPLC to prevent MPEG-gPLL binding to Superdex.

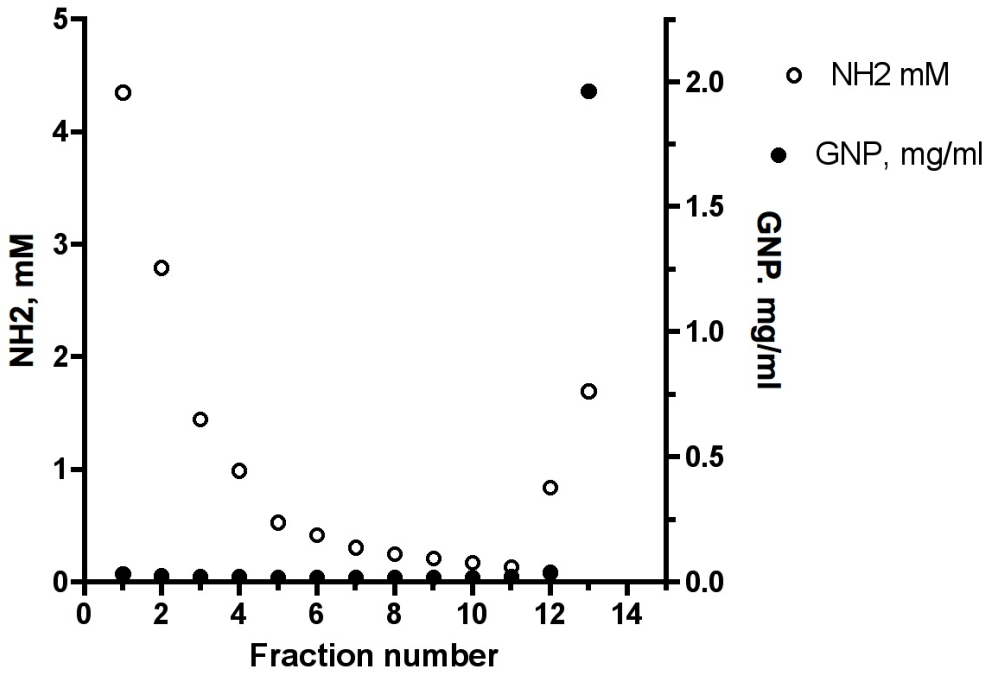


Figure 4S. The results of step-density gradient purification of MPEG-gPLL1 –stabilized GNPs showing amino group and GNP gold content measurements performed in individual fractions. Concentrated GNPs were loaded on top of a step-gradient consisting of 0.2 ml 50% Opti-Prep (60% Iodixanol solution in saline, Sigma-Aldrich, initial density 1.32 g/ml) followed by 4 ml of 8% solution of Opti-Prep (60% Iodixanol solution in saline, Sigma-Aldrich, initial density 1.32 g/ml) and centrifuged in a SW55.1 Ti rotor (Beckman) at 40,000xrpm (RCF 152,000xg) for 40 min.

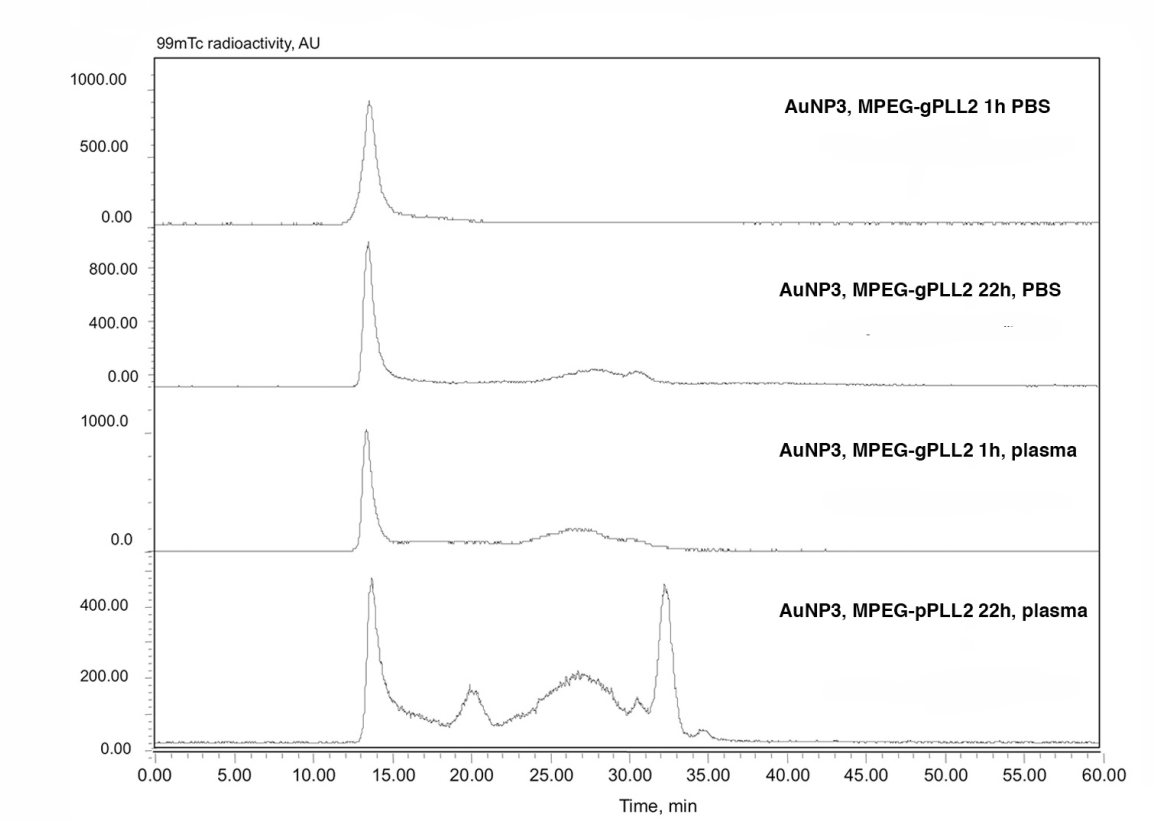


Figure 5S. Fragmentation of MPEG-gPLL2 protective layer in blood plasma. Size-exclusion HPLC profiles of ^{99m}Tc-AuNP3 stabilized with lMPEG-gPLL2 incubated for 1 and 22h either in PBS or 75% mouse plasma. HPLC was performed on Superose6 GL (1x30 cm) HPLC size-exclusion column eluted with 20% acetonitrile in 0.1M TrisHCl, pH 8.0 (0.6 ml/min).

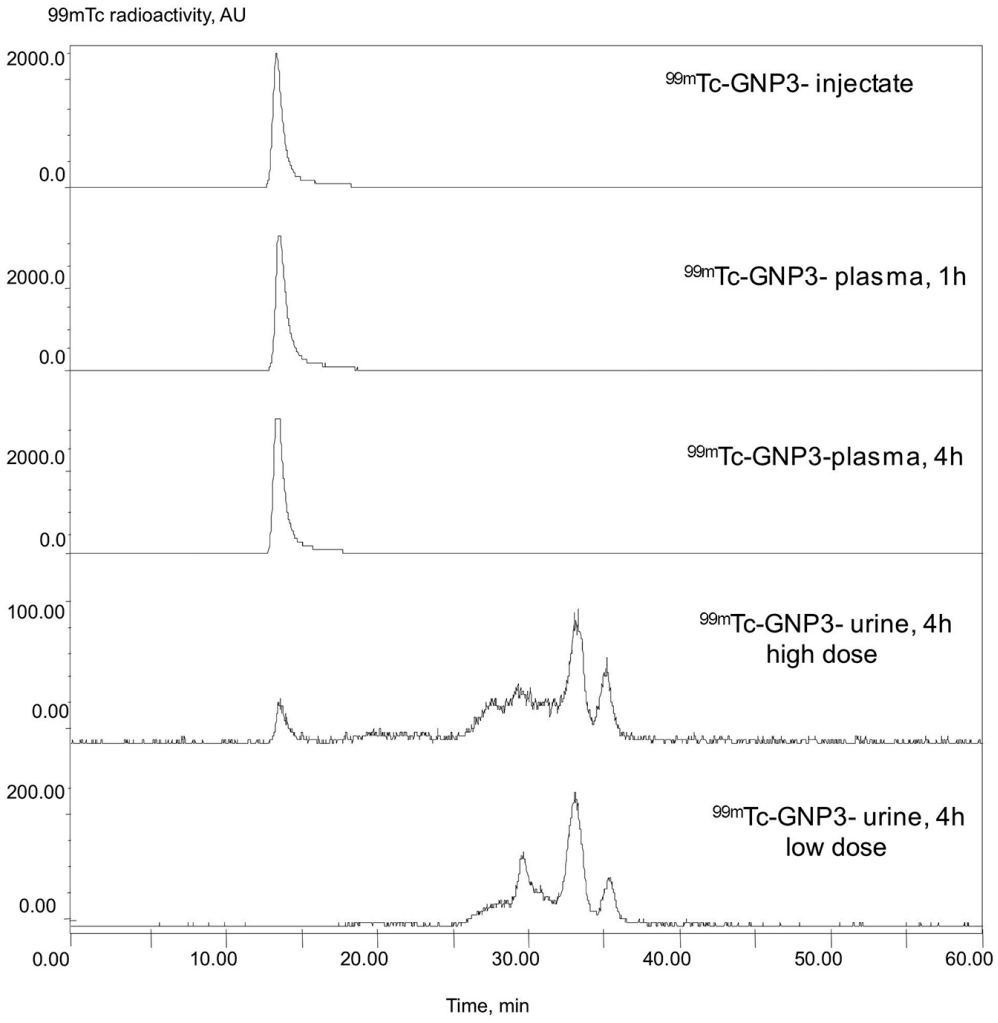


Figure 6S. Stability of ^{99m}Tc-AuNP3 in vivo. AuNP3 were conjugated with S-AcMAG3-NHS prior to labeling with ^{99m}Tc. Size-exclusion HPLC profiles of 1) the initial ^{99m}Tc-AuNP3 injectate that was used for IV administration, 2) mouse plasma obtained from animals at 1h and 4h post injection, and 3) mouse urine samples obtained from the animals injected IV with 10 mg Au/kg and 0.25 mg Au/kg doses of AuNP3 at 4h post administration. HPLC was performed on Superose6 GL (1x30 cm) HPLC size-exclusion column eluted with 20% acetonitrile in 0.1M TrisHCl, pH 8.0 (0.6 ml/min).

Table 1S. Biodistribution of ^{99m}TcMAG3-AuNP3 in mouse models of ectopic pancreatic cancer at 26h post injection*.

Organ	Ectopic PANC-1 tumor xenograft model		Average target/background ratio, (range)
	%ID/g	%ID/organ	
blood	11.50±1.20	10.58±1.04	
liver	5.35±0.83	8.31±0.39	
heart	2.10±0.01	0.29±0.03	
kidney	3.53±0.58	1.52±0.11	
lung	2.95±0.20	0.58±0.01	
spleen	4.00±0.56	0.60±0.01	
stomach	0.44±0.12	0.30±0.01	
small Intestine	0.82±0.22	1.49±0.13	
large Intestine	0.78±0.14	1.07±0.07	
urine and feces		51.30±0.77	
carcass		23.66±0.81	
PANC-1 tumor xenograft	1.15±0.33	0.21±0.22	2.6 (2.0-4.0) (tumor-to-muscle)

* The biodistribution results are presented as mean±SD (n=8) in % injected dose of ^{99m}Tc/ organ weight (g) and in % injected dose per organ.

Reference:

(1). Zalipsky, S., Seltzer, R., and Menon-Rudolph, S. (1992) Evaluation of a new reagent for covalent attachment of polyethylene glycol to proteins, *Biotechnol Appl Biochem 15*, 100-114.