Electronic Supporting Information

$^{201}Tl^{+}$-labelled Prussian Blue Nanoparticles as Contrast Agents for SPECT scintigraphy.

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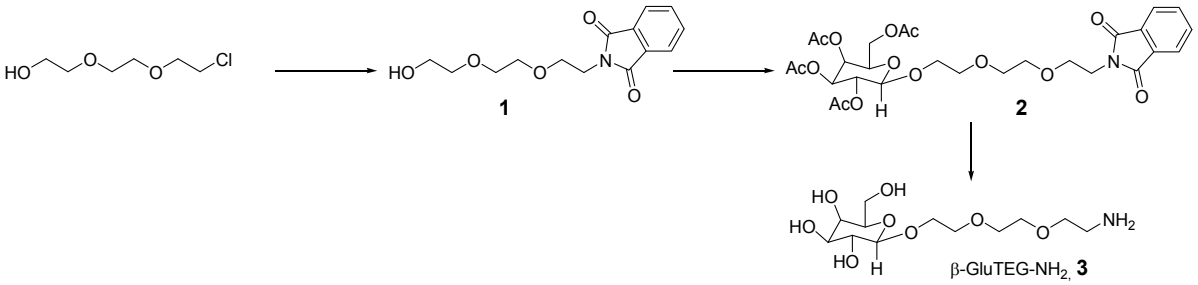
Experimental

Synthesis of glucose functionalized aminotriethyleneglycol (β-GluTEG-NH_2).



Scheme 1. Synthesis of β-GluTEG-NH_2.

2-[2-(2-(2-Hydroxyethoxy)ethoxy)ethyl]isoindole-1-3-dione 1

This step was performed following Haruya Sato, Eiko Hayashi, Naoyuki Yamada, Masanobu Yatagai, and Yoshiyuki Takahara, *Bioconjugate Chem*., **2001**, 12, 5, 701-710.

Briefly, 2-[2-(2-chloroethoxy)ethoxy]ethanol (35.6 mmol, 6 g, 5.2 mL) was added to a solution of potassium phthalimide (39 mmol, 7.3 g) in 43 mL of dry dimethylformamide. The resulting solution was stirred and heated with in oil bath at 100°C overnight. The suspension was cooled to room temperature and the precipitate was filtered-off. The filtrate was evaporated to dryness and dissolved in 100 mL of water; the resulting aqueous layer was extracted by dichloromethane (3x100 mL). The organic layer was dried over anhydrous $MgSO_4$ and then concentrated to afford compound **1** (8.94 g, 89%) as a yellow oil.

NMR spectra were in agreement with the literature data.

RMN ^{1}H (300 MHz, $CDCl_3$): 3.45-3.65 (2m, 8H, OC$\underline{H}_2$), 3.74 (t, 2H, HOC$\underline{H}_2$), 3.89 (t, 2H, NCH_2), 7.67-7.70 (m, 2H, H_{arom}), 7.81-7.85 (m, 2H, H_{arom}).

1-*O*-{2-[2-(2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)ethoxy)ethoxy]ethyl}2,3,4,6-tetracetyl-β-D-glucopyranose, 2

To an ice-cooled solution of compound **1** (32.0 mmol, 8.94 g) and penta-acetyl-D-glucose (16 mmol, 6.24 g) in dry dichloromethane (110 mL) was added dropwise BF_3-Et_2O complex (4 mL of a 1.1M solution). The resulting mixture was stirred at room temperature overnight and then 2 h at 40°C. The reaction mixture was washed with a satured aqueous solution of $NaHCO_3$, the resulting organic layer was dried over $MgSO_4$ and the volatiles were evaporated. The crude was purified by silica gel column chromatography, eluted with petroleum ether and ethyl acetate (40 to 80%, v:v), leading to the desired derivative (7.22 g, 74%) as an oil.

R*f* (petroleum ether/ethyl acetate, 4/6, v/v) = 0.39

RMN ^{1}H (300 MHz, $CDCl_3$): 1.95-2.05 (4s, 12H, Ac), 3.45-3.75 (2m, 10H, OC$\underline{H}_2$), 3.80-3.95 (m, 2H, H-6), 4.0-4.10 (m, 2H, NCH_2), 4.15-4.25 (m, 1H, H-5), 4.50-4.60 (m, 1H, H-4), 4.85-5.20 (m, 3H, H-1, H-2, H-3), 7.65-7.75 (m, 2H, H_{arom}), 7.80-7.95 (m, 2H, H_{arom}).

1-*O*-{2-[2-((2-Amino)ethoxy)ethoxy]ethyl}-β-D-glucopyranose, 3

To a solution of derivative **2** (11.8 mmol, 7.22 g) in ethanol (50 mL) was added hydrazine hydrate (13 mL of an aqueous solution of NH_2-NH_2 80%). The reaction mixture was heated at 80°C during 5 h. After cooling to room temperature, the reaction was quenched by adding 15 mL of acetone, stirred for 15 min, and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with IprOH/H_2O/NH_4OH (80/19/1 to 65/25/10, v:v:v), affording the expected compound **3** as a yellow oil (3.58 g, 97%).

R*f* (IprOH/H_2O/NH_4OH 70/29/1, v/v/v) = 0.11

RMN ^{1}H (300 MHz, D_2O): 2.70-2.85 (m, 2H, NCH_2), 3.20-4.10 (m, 16H, OC$\underline{H}_2$, H-6, H-5, H-4, H-2, H-3), 4.42-4.47 (m, 1H, H-1).

RMN ^{13}C (300 MHz, D_2O): 39.9 (NCH_2), 60.8 (C-6), 68.7, 69.3 (C-5, C-4), 69.7 (OC$\underline{H}_2$), 71.9 (C-2), 73.2, 75.7 (OC$\underline{H}_2$), 76.0 (C-3), 102.3 (C-1).

Synthesis of the nanoparticles.

PB@GluTEG-NH_2. An aqueous solution (6 mL) of $Fe(BF_4)_2.6H_2O$ (43 mg, 1.28×10^{-4} mol) was mixed with 2-[2-(2-aminoethoxy)ethoxy]ethyl-β-D-glucopyranoside stabiliser (30 mg, 9.63×10^{-5} mol) and is added dropwise to a pure aqueous solution (6 mL) of $Na_3[Fe(CN)_6]$ (55.9 mg, 1.70×10^{-4} mol) mixed with 2-[2-(2-aminoethoxy)ethoxy]ethyl-β-D-glucopyranoside stabilizer (30 mg, 9.63×10^{-5} mol). The resulting mixture was stirred 2h at room temperature and then centrifugated (20 000 rpm, 10 min). The solid was filtered off and the resulting solution was filtered with a 0.45 μm syringe filter, then dialyzed in 800 mL of pure water for 24 h using a SnakeSkin Dialysis Tubing, 3.5000 MWCO (3 times). The nanoparticles were precipitated with acetone and dried under vacuum.

Yield: 87 %. El. anal. Found, wt %: C, 32.49; Fe, 23.8; H, 2.89; N, 16.66; Na, 2.18. Determinated formula: $Na_{0.41}Fe[Fe(CN)_6]_{0.85}/(\beta\text{-GluTEG-}NH_2)_{0.6}$. Selected IR (KBr, cm^{-1}): $\nu_{-OH} = 3435\ cm^{-1}$, $\nu_{C-H} = 2917\ cm^{-1}$, $2849\ cm^{-1}$, $\nu_{C\equiv N} = 2071\ cm^{-1}$, $\delta_{H-O-H} = 1618\ cm^{-1}$, $\delta_{Fe-C-N} = 603\ cm^{-1}$.

PB@PEG-NH_2. An aqueous solution (3.5 mL) of $Fe(BF_4)_2\cdot6H_2O$ (50 mg, 1.50×10^{-4} mol) mixed with the commercially available poly(ethylene glycol) bis(3-aminopropyl) stabilizer (55.5 mg, 3.7×10^{-5} mol) was added dropwise to an aqueous solution (3.5 mL) of $Na_3[Fe(CN)_6]$ (64.8 mg, 2.0×10^{-4} mol) mixed with the poly(ethylene glycol) bis(3-aminopropyl) stabilizer (55.5 mg, 3.7×10^{-5} mol). The mixture was stirred for 2 h at room temperature before being centrifugated (20000 rpm, 10 min) to remove a small amount of bulk material. The supernatant was filtered using a 0.45 μm filter and then 3 dialysis (24 h repeated 3 times) were carried out in 800 mL of pure water using a SnakeSkin Dialysis Tubing, 3.5000 MWCO. The nanoparticles were precipitated from acetone and dried under vacuum.

Yield: 83 %. El. anal. Found, wt %: C, 40.68; Fe, 18.02; H, 4.89; N, 13.59; Na, 1.79. Determinated formula: $Na_{0.45}Fe[Fe(CN)_6]_{0.86}/(PEG\text{-}NH_2)_{0.22}$. Selected IR (KBr, cm^{-1}): $\nu_{-OH} = 3389\ cm^{-1}$, $\nu_{C-H} = 2921\ cm^{-1}$, $2869\ cm^{-1}$, $\nu_{C\equiv N} = 2069\ cm^{-1}$, $\delta_{H-O-H} = 1609\ cm^{-1}$, $\delta_{Fe-C-N} = 598\ cm^{-1}$.

Thallium capture experiments.

Tl^+ sorption experiments on nanoparticles were performed by using non-radioactive Tl^+ containing aqueous solutions in order to investigate the sorption kinetic. All Tl^+ extraction experiments were performed in batch solution under shaking at room temperature.

Equilibrium studies in pure water were performed before isotherm sorption experiments also in pure water. For the equilibrium studies, 40 mg of powdered nanoparticles were dispersed in 15 ml of 9.2×10^{-5} M TlCl aqueous solution and shaken for different period of time, from 20 min to 48h. The final Tl^+ containing nanoparticles were purified by dialysis to eliminate non absorbed Tl^+, then precipitated from acetone, centrifugated, and dried *in vacuo*. The Tl^+ contents were determined by elemental analysis. Sorption isotherms' measurements were plotted from data obtained at reaction equilibrium (6h) with different concentrations of TlCl solution.

Nanoparticles Tl^+/PB@GluTEG-NH_2 and Tl^+/PB@PEG-NH_2.

The Tl^+ containing nanoparticles were obtained by simple mixing of PB nanoparticles (40 mg) with 9.2×10^{-5} M aqueous solution of TlCl for 6h. The final Tl^+ containing nanoparticles were purified by several dialyses before being precipitated with acetone.

Tl^+/PB@GluTEG-NH_2: Yield: 87 %. El. anal. Found, wt %: C, 25.81; Fe, 19.03; H, 2.04; N, 13.66; Tl, 22.34. Determinated formula: $Tl_{0.61}Fe[Fe(CN)_6]_{0.90}/(\beta\text{-GluTEG-}NH_2)_{0.6}$. Selected IR (KBr, cm^{-1}): ν_{-OH} = 3430 cm^{-1}, ν_{C-H} = 2920 cm^{-1}, 2851 cm^{-1}, $\nu_{C\equiv N}$ = 2072 cm^{-1}, δ_{H-O-H} =1608 cm^{-1}, δ_{Fe-C-N} = 602 cm^{-1}.

Tl^+/PB@PEG-NH_2: Yield: 91 %. El. anal. Found, wt %: C, 33.57; Fe, 17.80; N, 12.03; Tl, 17.87. Determinated formula: $Tl_{0.58}Fe[Fe(CN)_6]_{0.89}/(PEG\text{-}NH_2)_{0.20}$. Selected IR (KBr, cm^{-1}): ν_{-OH} = 3430 cm^{-1}, ν_{C-H} = 2920 cm^{-1}, 2851 cm^{-1}, $\nu_{C\equiv N}$ = 2072 cm^{-1}, δ_{H-O-H} =1608 cm^{-1}, δ_{Fe-C-N} = 602 cm^{-1}.

Thallium lixiviation experiments.

20 mg of nanoparticles $Tl_{0.58}Fe[Fe(CN)_6]_{0.89}/(PEG\text{-}NH_2)_{0.20}$ are dispersed in 6 mL of ultrapure H_2O and stirred for 48h. Then, the solution is dialysed for 24 h using 40 mL of ultrapure H_2O. The amount of Tl in the dialysis water is measured by ICP-MS to be 0.672 $mg.L^{-1}$. This corresponds to 0.75% of the quantity of Tl present in the nanoparticles. We can therefore consider that in experiments conducted *in vivo*, if there is Tl leaching the leached amount will be less than 1%, of the weight of thallium present in nanoparticles.

Culture cells.

The BALB/c 3T3 cells (murine Swiss albino fibroblasts from Eucellbank- Universitat de Barcelona, Spain) cell lines were grown in Dulbecco's modified Eagle's medium, (DMEM; 4.5 g/L glucose) supplemented by 10% (v/v) Foetal Bovine Serum (FBS), 2 mM L-glutamine,

50 U/mL penicillin and 50 mg/mL streptomycin at 37ºC and 5% CO_2. These cells were routinely cultured in 75 cm^2 culture flasks and were trypsinised using trypsin-EDTA when the cells reached ~80% confluence.

Undifferentiated embryonic stem cells, line D3 (ATCC-CRL 1934), were cultured in high glucose DMEM (4.5 g glucose/L) supplemented by 15% FBS, 2mM glutamine, antibiotics, 50 U/mL penicillin and 50_g/mL streptomycin, 1% non-essential amino acids, 0.1mM mercaptoethanol and 1000 U/mL murine leukaemia inhibitory factor in gelatine-coated 35-mm2 cell culture dishes under 5% CO_2 and 95% humidity at 37 ºC and were routinely passaged every 2–3 days.

Up & Down acute toxicity test.

Up & Down acute toxicity test according OECD 425 Acute Oral Toxicity Up-and-Down Procedure using 29.27 ± 1.21 g female CD1 mice purchased from Harlan Laboratories. Animals were kept in individual cages at room temperature and 12h light/dark cycle. Experiments were approved by the Animal Welfare Committee (CEEA13/0010.V2).

Nanoparticles $^{201}Tl^{+}$/PB@GluTEG-NH_2 and $^{201}Tl^{+}$/PB@PEG-NH_2 for SPECT/CT measurements.

^{201}TlCl was obtained from CisBio, and nanoparticles were radio-labelled at the activity of 10 MBq per mg of NP. In a typical experiment, a solution of 1 mg of the studied nanoparticles, either PB@GluTEG-NH_2 or PB@PEG-NH_2 in 500µL of 0.9 % NaCl was treated with 10 $MBq.mL^{-1}$ ^{201}TlCl aqueous solution in order to obtain $^{201}Tl^{+}$/PB@β-GluTEG-NH_2 and $^{201}Tl^{+}$/PB@PEG-NH_2 nanoprobes.

SPECT-CT imaging.

All animal experiments were performed in compliance with the guidelines of the French government and the standards of Institut National de la Santé et de la Recherche Médicale for experimental animal studies (agreement C34-172-27).

Mice (Nude athymic FoxN1) were obtained from Harlan Laboratories and were acclimated for one week before experimental use. They were housed at 22°C and 55% humidity with a light–dark cycle of 12 h. Food and water were available ad libitum.

Whole-body SPECT/CT images were acquired at various times (20 min, 1h20, 3h20, 7h20, 26h, 48h) after tail vein injection of 8 MBq of radio-labelled $^{201}Tl^{+}$/PB@β-GluTEG-NH_2 and $^{201}Tl^{+}$/PB@PEG-NH_2 nanoprobes. Mice were anesthetized with 2% isoflurane and positioned

on the bed of 4-head multiplexing multipinhole NanoSPECT camera (Bioscan Inc., Washington, USA). Energy window was centered at 73 keV with ±20% width, acquisition times were defined to obtain 30 000 counts for each projection with 24 projections. Images and maximum intensity projections (MIPs) were reconstructed using the dedicated software Invivoscope® (Bioscan, Inc., Washington,USA) and Mediso InterViewXP® (Mediso, Budapest Hungary). Concurrent microCT whole-body images were performed for anatomic co-registration with SPECT data. Reconstructed data from SPECT and CT were visualized and co-registered using Invivoscope®. *In vivo* experiments were repeated three times, and quantification on whole body images provided semi-quantitative data.

	Mean	±	SD
WBC	5,5	±	0,35
LYM	4,85	±	0,4
MID	0,08	±	0,06
GRA	0,56	±	0,12
LY%	88,27	±	2,1
MI%	1,47	±	0,9
GR%	10,27	±	2,65
RBC	5,01	±	0,81
HGB	7,57	±	1,25
MCV	49,67	±	2,52
MCH	15,1	±	0,26
MCH	30,6	±	1,97
RD	16,3	±	0,44
PLT	372,67	±	44,02
PCT	0,24	±	0,05
MPV	6,37	±	0,81
PD	31,4	±	2,08

Table 1S. Hematology data for every animal and mean and standard deviation for all of them. WBC, White Blood Cells (10^9 cells/L), LYM, Lymphocites (10^9 cells /L), MID, Mid cells (10^9 cells /L), GRA, Granulocytes (10^9 cells /L), LYM (%), MID (%), GRA (%), RBC, Red Blood Cells (10^12/L), HGB, Hemoglobin (g/dL), MCV, Mean Cell Volume (fL), MCH, Mean Cell Hemoglobin (pg), MCHC, Mean Cell Hemoglobin Concentration (g/dL), RDWcv, Red Blood Cell Distribution Width (%), PLT, Platelet (10^9 cells /L), PCT, Plateletcrit (%), MPV, Mean Platelet Volume (fL), PDWcv, Platelet Distribution Width(CV) (%).

	Mean	±	SD
Neutrophile	3,33	±	2,08
Band	0,33	±	0,58
Lymphocyt	90,67	±	5,69
Monocyte	5	±	2,65
Eosinophile	0	±	0
Basophile	0	±	0

Table 2S. Results of the leukocyte profile expressed as mean and standard deviation.

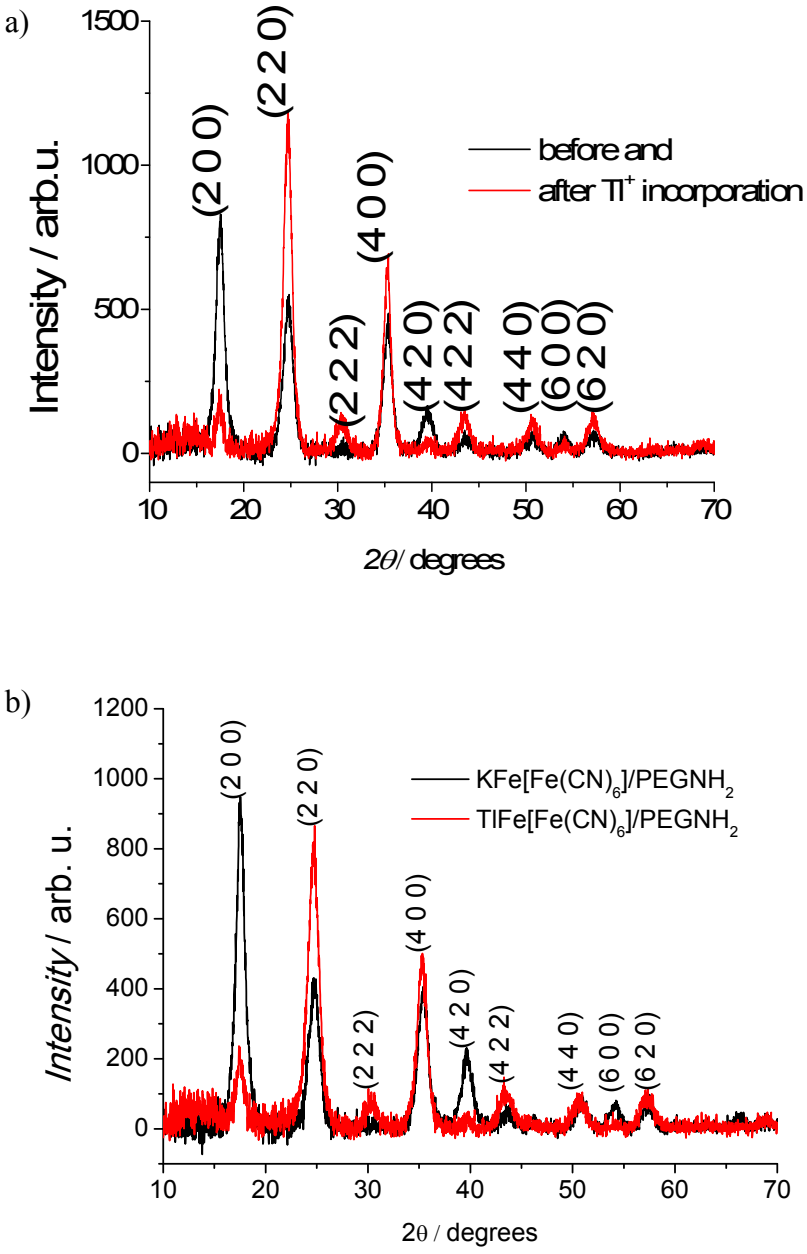


Figure 1S. Room temperature PXRD patterns for a) PB@β-GluTEG-NH_2 and b) PB@PEG-NH_2 before (black) and after Tl^+ incorporation (red), indexed using the space group *Fm-3m*.

The found lattice parameter constant for PB@β-GluTEG-NH_2 is equal to *a* = 10.16 and 10.17 Å, respectively before and after Tl^+ incorporation and for PB@PEGNH_2 is equal to *a* = 10.14 and 10.15 Å, respectively before and after Tl^+ incorporation.

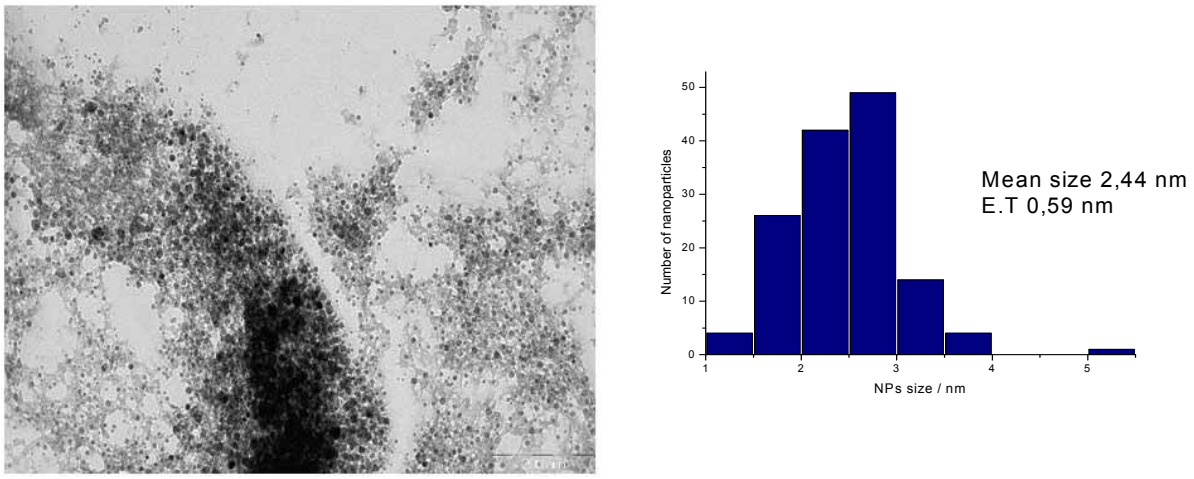
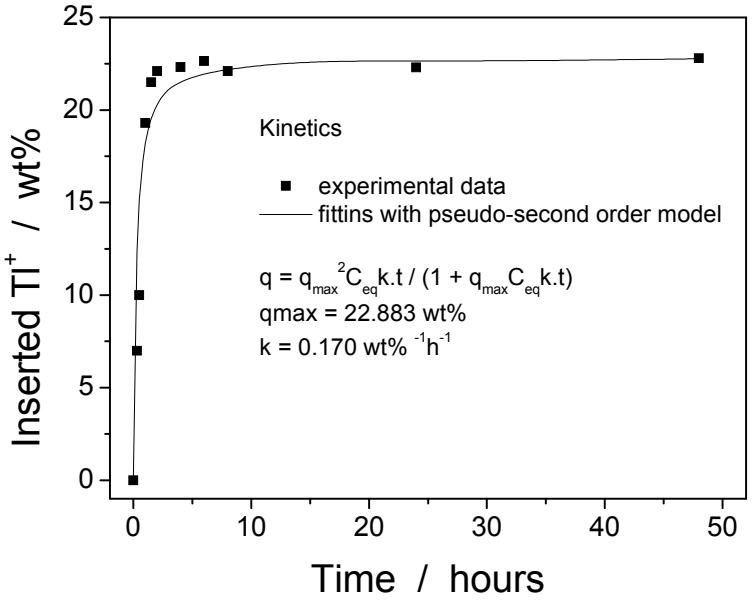


Figure 2S. TEM image of the nanoparticles and the corresponding histograms of the nanoparticle's size distribution (inset) for $Na_{0.41}Fe[Fe(CN)_6]_{0.85}$@)β-GluTEG-$NH_2)_{0.6}$



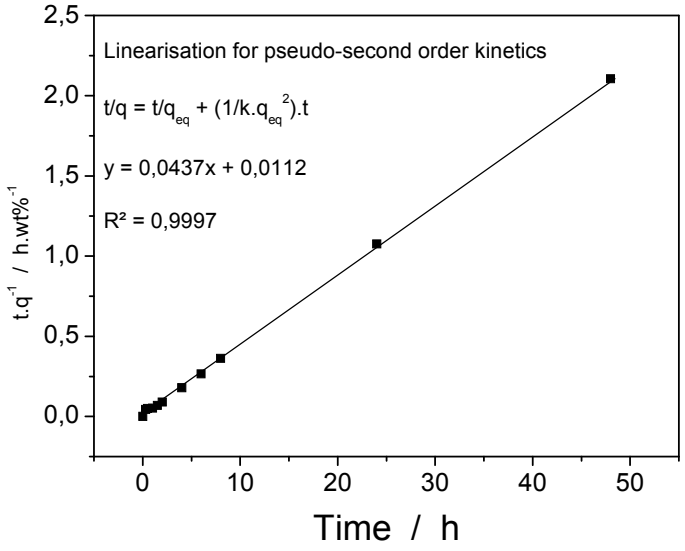


Figure 3S. Kinetics of Tl^+ sorption by $Na_{0.41}Fe[Fe(CN)_6]_{0.85}$@$(PEG\text{-}NH_2)_{0.2}$ nanoparticles at different times. As for most processes involving chemisorption on a solid, a pseudo-second order kinetic is observed.

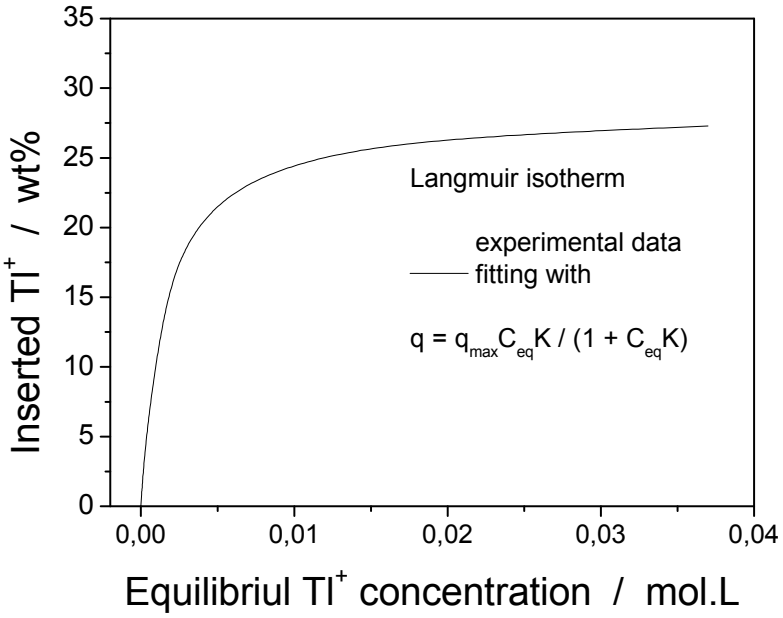
The model for a pseudo-second order has the following form:

$$Q = \frac{kQ_e^2 t}{1 + kQ_e t}$$

where t is the time (min), Q is the entrapped Tl^+ (wt %) at time t, Q_e is the entrapped thallium at equilibrium time and k is the reaction constant ($g \cdot mmol^{-1} min^{-1}$). Qe = 22.883 wt % for a starting concentration, k = 0,17 $wt\%^{-1} min^{-1}$
Obtained from the resolution of pseudo-second order differential equation

$$\frac{dQ}{dt} = k(Q_e - Q)^2$$

Integrations on range 0-t for t andon the range 0-Q for Q.



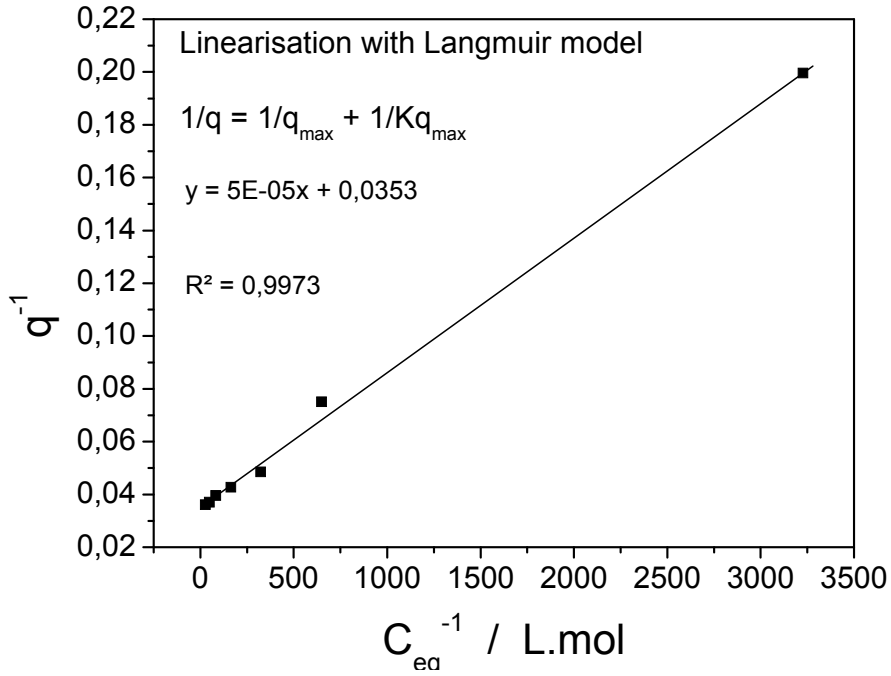


Figure 4S. Tl^+ adsorption isotherms for $Na_{0.41}Fe[Fe(CN)_6]_{0.85}$@$(PEG\text{-}NH_2)_{0.2}$.

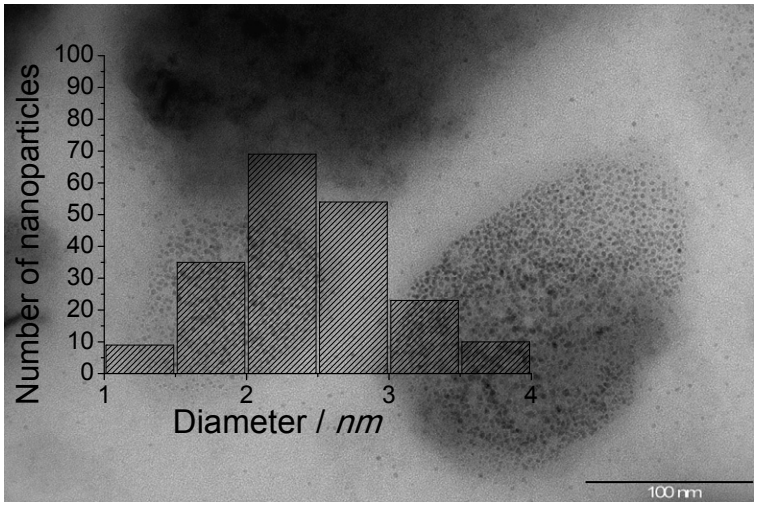
Straight line: fitting of the experimental isotherms with Langmuir model. The experimental isotherms were fitted with the Langmuir model. The linear form for the model is:

$$\frac{1}{Q} = \frac{1}{Q_{max}} + \frac{1}{KQ_{max}}\frac{1}{C}$$

Where C is the equilibrium concentration ($mol.l^{-1}$), Q is the amount entrapped at the corresponding equilibrium concentration (wt %), Q_{max} is the maximal amount of

entrapped thallium (wt %) and K is the sorption energy (l.mol^{-1}). A good fitting was obtained (with R = 0,997, Figure a above). The model gave values of Q_{max} = 26.633 wt % of Tl^{+} and K = 706 L.mol^{-1}. The values for Q in the Langmuir isotherms in Figure b were then plotted with the following formula

$$Q = Q_{max} \frac{KC}{1 + KC}$$



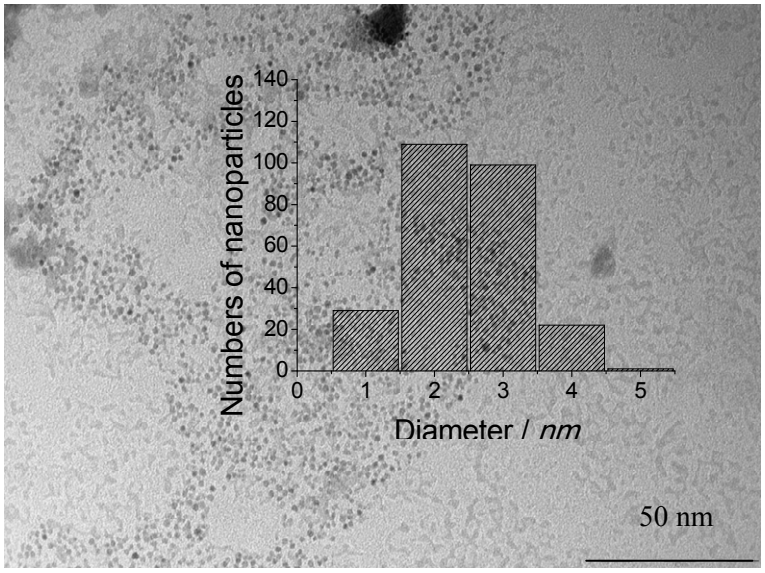


Figure 5S. TEM images of a) $Tl_{0.61}Fe[Fe(CN)_6]_{0.90}/(\beta\text{-GluTEG-}NH_2)_{0.6}$ of 2.33±0.44 nm and b) $Tl_{0.61}Fe[Fe(CN)_6]_{0.90}/(PEGNH_2)_{0.2}$ of 2.05±0.62 nm nanoparticles. Inset: corresponding nanoparticles size distribution histograms.

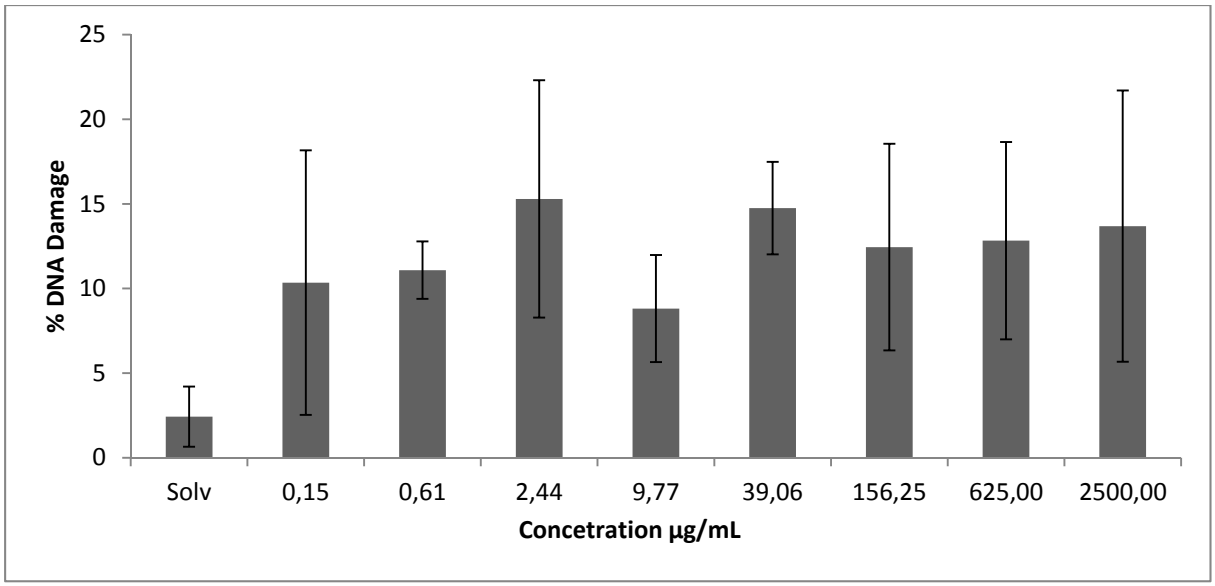


Figure 6S. Genotoxic effect of the $Na_{0.45}Fe[Fe(CN)_6]_{0.86}@(PEG\text{-}NH_2)_{0.22}$ nanoparticles on Balb/c 3T3 cells, by the Comet assay.

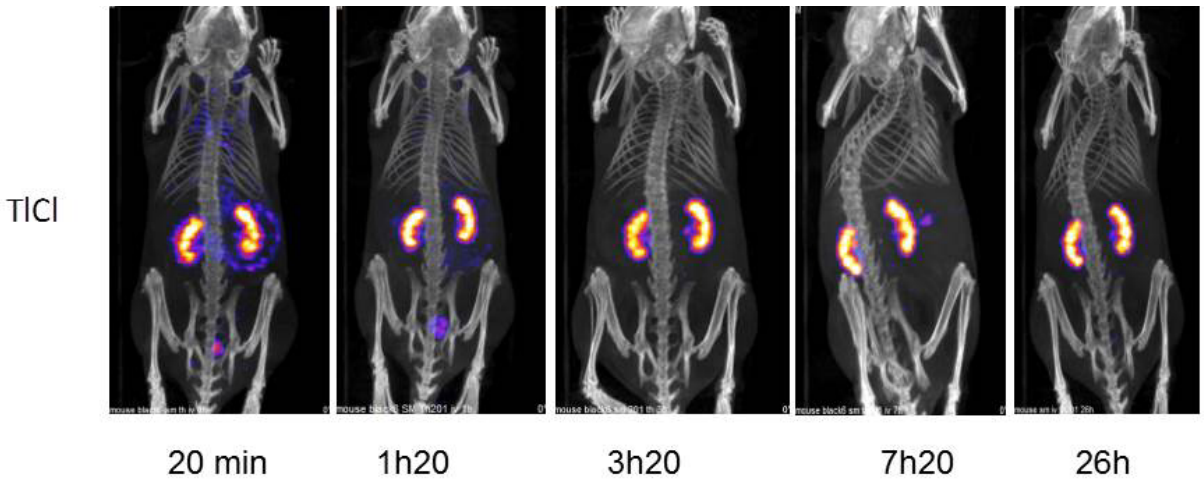


Figure 7S. Longitudinal SPECT/CT images of the mice after intra-venous injection of the ^{201}TlCl for different period of time: 20 min, 1h20, 3h20, 7h20, 26h.

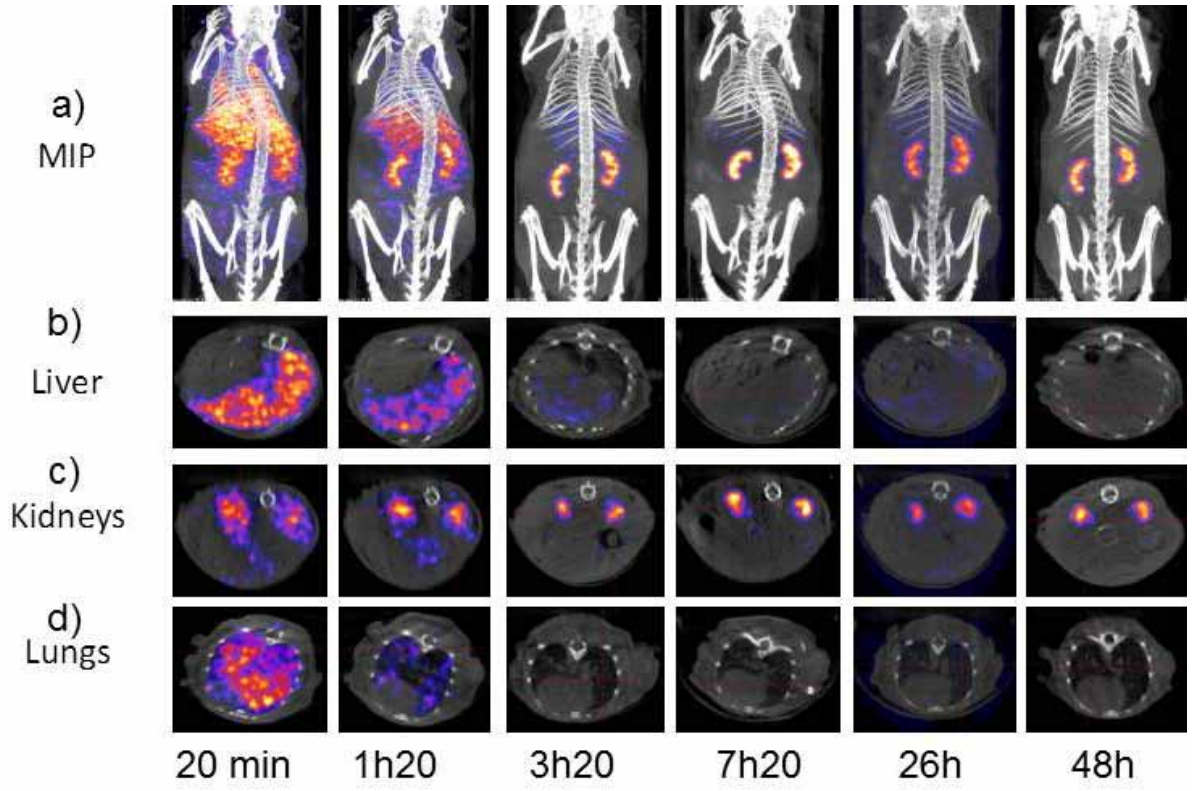


Figure 8S. SPECT/CT images of the mice after intra-venous injection of the nanoparticles $^{201}Tl^{+}$/PB@β-GluTEG-NH_2 for different period of time: 20 min, 1h20, 3h20, 7h20, 26 and 48h with longitudinal (top) and transversal planes shown different organs of interest (bottom: b) liver, c) kidney and d) lungs).

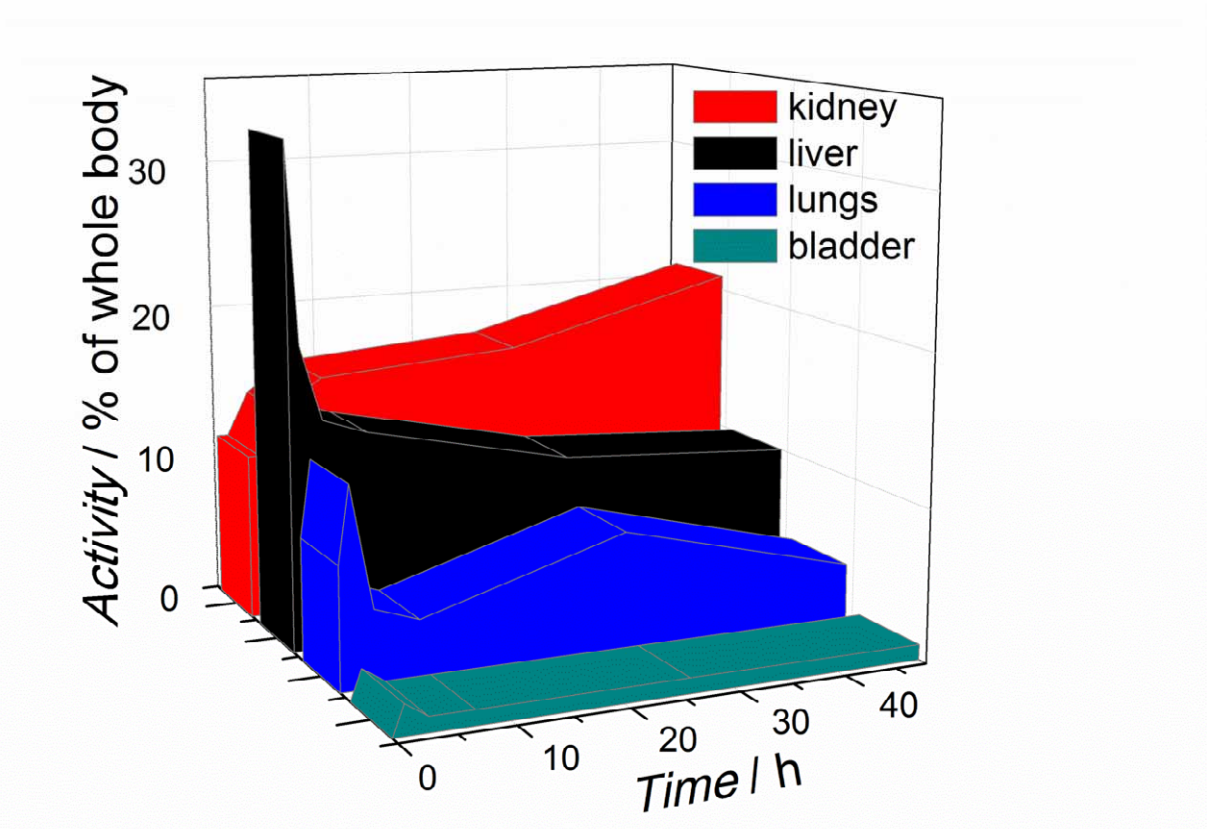


Figure 9S. Captation of ^{201}Tl-labeled nanoparticles $^{201}Tl^{+}$/PB@β-GluTEG-NH_2 by organs of interest in times after *iv* injection.