Supporting Information

Multistage passive and active delivery of radiolabeled nanogels for superior tumor penetration efficiency

Natascha Drude,[1,2,‡] Smriti Singh,[2,‡] Oliver H. Winz,[1] Martin Möller,[2,] Felix M. Mottaghy,[1,3,*] and Agnieszka Morgenroth[1]*

[1]*Department of Nuclear Medicine, RWTH Aachen University, 52074 Aachen, Germany*

[2]*DWI –Leibniz-Institute for Interactive Materials, RWTH Aachen University, 52074 Aachen, Germany*

[3]*Department of Nuclear Medicine, Maastricht University Medical Centre, 6229 HX Maastricht, Netherlands*

[‡]These authors contributed equally to this work.

*corresponding authors

Correspondence should be addressed to:

moeller@dwi.rwth-aachen.de

fmottaghy@ukaachen.de

Materials

Hydroxy terminated, six arm, star shaped poly (ethylene oxide-*stat*-propylene oxide) sPEG with a backbone consisting of 90 % ethylene oxide and 10 % propylene oxide (M_n= 6100 g/mol, M_w/M_n = 1.12) was obtained from Dow Chemicals (Terneuzen, NL). sPEG was purified by dissolving in tetrahydrofuran followed by precipitation in cold diethylether prior to further use. N,N'-dicyclohexyl-carbodiimide (DCC), (99 %, Acros), 4-(Dimethylamino)pyridine (DMAP) (99 %, Aldrich), 3,3'-dithiodipropionic acid (Aldrich), tris(2-carboxyethyl) phosphine (TCEP) (99 %, Aldrich), dichloromethane (HPLC grade, Aldrich), 2,2'-(7-(1-carboxy-4-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (maleimide NOTA) (94 %, CheMatech), maleimide DOTA-TATE (maleimide(Lys)-chelatorcoupled with (Tyr^3)-octreotate) (95 %, CASLO) 3,6-diamino-9-[2-carboxy-4(or 5)-[[[5-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)pentyl] amino]carbonyl]phenyl]-4,5-disulfo-, inner salt, monosodium salt (Alexa Fluor 488 C_5 maleimide) (Invitrogen), water (ultrapure, Merck), hydrochloric acid (30 %, suprapure, Merck), ammonium acetate (p.a. Pharm Eur, Sigma), were used as received. Dialysis membrane with a molecular weight cut off of 3.5, 25 and 50 kDa were purchased from Spectrum Laboratories, Inc. The ^{68}Ge/^{68}Ga-generator was obtained from ITG Garching, Munich, where the ^{68}Ge is fixed on a modified tin dioxide column. The human monocyte cell line THP-1 and the rat pancreas tumor cell line AR42J were obtained from ATCC.

The cell medium RPMI-1640 and supplementary reagents including fetal calf serum (FCS), L-glutamine, sodium pyruvate and non-essential amino acids (NEA) were obtained from Biochrom. Chemical reagents like phorbol 12-myristate 13-acetate (PMA), dimethyl sulfoxide (DMSO), 4′,6-Diamidin-2-phenylindol (DAPI), N-succinimidyl-3-(2-pyridyldithio)propionate, n-hexane, Span 80, Tween 80, H_2O_2, were procured from Sigma Aldrich. Phosphate buffer (PBS) was purchased from Pan Biotech. Glutaraldehyde was used

as purchased from Agar scientific, (Wetzlar, Germany)**.** The any kD TGX gels, dual color protein marker and native sample buffer were purchased from Biorad.

Experimental procedures

Synthesis of Thiol functionalized sPEG

Thiol functionalized sPEG (HS-sPEG) has been prepared in two steps. In the first step, sPEG was cross-linked with a disulphide cross-linker 3,3'-dithiodipropionic acid via Steglich esterification. In the second step, disulphide bonds were reduced to thiol groups by using TCEP followed by dialysis (MWCO 3500; reg. cell) against water purged with Ar. Typically, four alcohol groups were converted into thiol groups leaving two unreacted OH groups in the polymers. The obtained polymer was lyophilized and stored at -20 °C under argon for further use. In detail for thiol functionalization of sPEG, a solution of DTPA (1.1 mmol, 0.237 g) in dried THF (4 mL) was added dropwise to a solution of purified sPEG (0.25 mmol, 3 g), DCC (1.1 mmol, 0.510 g) and DMAP (1.1 mmol, 0.302 g) in CH2Cl2 (6 mL) in an ice bath at 0 °C over 10 min under stirring. The resulting mixture was stirred at RT for 5 h to allow for complete reaction and cross-linking. For reduction, the hydrogels were immersed in an aqueous solution of TCEP (1.5 eq. with respect to the disulfide units) at room temperature for 4 h. The pH of the solution was adjusted with triethylamine to pH = 6.5. After reduction, the solution was first dialyzed for two days at RT against aqueous HCl solution at pH ~ 3.5, then against pure water under inert gas for one day. Finally, the polymer solutions were lyophilized and stored at +4 °C under argon for further use. The free thiol content of the polymer was determined using NMR. SEC (THF): Mn = 6400 g/mol, Mw/Mn = 1.12.

^{1}H-NMR (400 MHz, D_2O): δ = 1,16 (*d*; -CH_3 (27H)); 2,78 (*m*; -CH_2 (16H)); 3,38-3,87 (*m*; -*backbone*); 4,32 (*m*; -CH_2O-C=O (8H)) *ppm*.

Functionalization of the SH-sPEG with malNOTA or malDOTA-TATE(1)

Synthesis of HS-sPEG labeled with malNOTA (HS-sPEG-NOTA).

HS-sPEG (0.500 g, 7.8×10^{-2} mmol, 1eq. equals 1 SH-group) and NOTA (0.03 g, 3.9×10^{-2} mmol, 0.5 eq.) were reacted in 5mL degased phosphate buffered saline (pH= 7.2) overnight at room temperature under argon. The product was obtained after dialysis (MWCO 3500 reg. cellulose) against water purged with Ar and freeze-drying. It was stored at -20°C under Ar until further use. M_n = 7500 g/mol.

^{1}H-NMR (400 MHz, D_2O): δ = 1,16 + 1,25 (2x*d*; -CH_3 (27H)); 1,93-2,14 (*m*; -CH_2; (0.9H)); 2,37-2,44 (*m*;-CH_2; (0.9H)); 2,78 (*m*; -CH_2 (16H)); 2,98-3,29 (*chelator*); 3,49-3,81 (*backbone+chelator*); 4,31 (*m*; H_2CO-C=O (8)) *ppm*.

HS-sPEG was functionalized with malDOTA-TATE (0.01 mg, 7.8×10^{-3} mmol, 0.1 eq.) according to this procedure.

Labeling of HS-sPEG-NOTA with Alexa Fluor 488 (2)

HS-sPEG-NOTA (0.100 g, 1.333×10^{-2} mmol) and Alexa Fluor 488 maleimide (0.0048 g, 6.666×10^{-3} mmol) were reacted in 5 mL degased phosphate buffered saline (0.01 M, pH 7.2) in dark for 12 h under argon. The product was obtained after dialysis (MWCO 3500 reg. cellulose) against water purged with Ar and freeze-drying.

Size Exclusion Chromatography (SEC)

SEC for estimation of molecular weight and polydispersity of the functionalized was performed in THF with addition of 250 mg/L 2, 6-di-tert-butyl-4-methylphenol. A high pressure liquid chromatography pump (PL-LC 1120 HPLC) and a refractive index detector (ERC 7515A) were used at 35 °C with a flow rate of 1.0 mL/min. Five columns with MZ gel were applied. The length of the first column was 50 mm, and 300 mm for the other four columns. The diameter of each column was 8 mm, the diameter of the gel particles 5 mm, and

the nominal pore widths were 50, 50, 100, 1000, and 10 000 Å, respectively. Calibration was achieved using poly(methyl methacrylate) standards.

Synthesis of nanogels

Nanogels were prepared via inverse miniemulsion technique.[1] For the preparation of the miniemulsion, 37.5 mg of surfactant in a 3:1 weight ratio of Span 80 and Tween 80 was dissolved in 1.25 mL of n-hexane. The aqueous phase consisted of 50 mg (2.66×10^{-2} mmol) of NOTA functionalized HS-sPEG dissolved in 125 µL of PBS buffer (pH 7.2). The organic and the aqueous phases were pre-emulsified by magnetic stirring for 10 min. After stirring the system was ultrasonicated using a Branson sonifier, at a duty cycle of 20 % and output control of 80 % under ice cooling for 1 min. Cross-linking was initiated by subsequent addition of 30 µL of 0.1 M H_2O_2 catalyst in PBS followed by the further sonication for 1 min. The reaction was allowed to proceed for 20 min at room temperature with constant stirring followed by quenching of the unreacted thiol groups by 2-hydroxy acrylate at pH = 7.4. Any further oxidation was stopped by addition of 1.5 mL of acidic water (pH = 3). Separation of the nanogels was achieved by centrifugation at 10,000 rpm for 30 min followed by decantation of the supernatant. Nanogels in the aqueous layer were carefully washed with *n*-hexane (2 × 1.5 mL) and THF (4 × 2.5 mL) in order to remove the surfactants and unreacted polymer. The remaining organic solvents and acid were removed by dialysis (MWCO 50,000; reg. cell.). Purified nanogels were stored in ultrapure water at -20 °C prior further use. malDOTA-TATE-nanogels were synthesized according to this procedure.

In this study two different types of nanogels have been used: with (malDOTA-TATE-nanogels) and without (NOTA-nanogels) the somatostatin targeting peptide. The reason for using two different chelators was solely based on the availability of the chelators with free maleimide functional groups to link with HS-sPEG. Even though NOTA-nanogels can easily

be radiolabeled at room temperature we decided for better comparison to label malDOTA-TATE- as well as NOTA-nanogels at 95 °C.

Radiolabeling of NOTA-nanogel and DOTA-TATE-nanogel with ^{68}Gallium

For the radiolabeling, no carrier added (n.c.a.) $^{68}GaCl_3$ was eluted with 0.05 M HCl in metal free water from a $^{68}Ge/^{68}Ga$-generator (*Isotope technologies Garching GmbH* (*itG*)). The purified NOTA-nanogels and DOTA-TATE-nanogels were labeled with ^{68}Ga at T = 95 °C at a pH of 5.0 by combining 0.3-0.4 mg NOTA-nanogel (which equals 20-25 nmol NOTA) or 0.5-0.6 mg DOTA-TATE-nanogels (40-50 nmol DOTA) in water with ammonium acetate (3.0 M, 1/10th the volume of ^{68}Ga-Cl_3). Finally, 100-200 MBq of ^{68}Ga-Cl_3 (in 0.05 M HCl) were added to the reaction mixture. After 10 min at T = 95°C the radiochemical yield (rcy > 95 %) was estimated by Radio-TLC and radiochemical purity by Radio-SEC (rcp > 90 %).

Competition experiments were performed with $Ga(NO_3)_3$ (14 mM) mixed with $^{68}GaCl_3$. The experiments with non-radioactive Ga^{3+} showed specific labeling at the chelator NOTA/malDOTA-TATE and gave the amount of chelator molecules per star polymer. The quantification was in good agreement with the value estimated by ^{1}H-NMR spectra (of NOTA-nanogels (Fig. S1)). The rcy with both types of nanogels was comparable and reproducible. The specific activity for a typical labeling experiment with maximum 200 MBq n.c.a. $^{68}GaCl_3$ resulted estimated via competition-experiments in 5.1 GBq/µmol malDOTA-TATE (in 500 µg nanogel) and 13.8 GBq/µmol NODAG-GA (in 500 µg nanogel).

Radio-High Performance Liquid Chromatography (Radio - HPLC)

The radiochemical purity were determined by Radio-HPLC equipped with an acrylate based Size Exclusion Column (*PSS SUPREMA*, 3000 Å, 5 µ 8,0 x 300 mm). The HPLC-system assembles a quaternaric HPLC-pump (*Knauer*), UV-detector (*Knauer*) and NaI-detector

(*raytest*) with a recording and analyses software (*raytest*). 0.1 % TFA in HPLC grade water was used as a mobile phase with a flow rate of 0.5 mL/min.

Radio-Thin Layer Chromatography (Radio - TLC)

The radiochemical yield after radiolabeling was determined by Radio-TLC with ITLC-silica strips and citrate buffer as mobile phase. The TLC was developed with a Radio-TLC-Scanner (*mini-Gita*) equipped with a NaI-detector *(Raytest)*. Non complexed ^{68}Ga-Cl_3 was detected as ^{68}Ga-citrate at the front, whereas ^{68}Ga-NOTA/malDOTA-TATE-nanogel remained at the origin. Free ^{68}Ga-NOTA shows an R_f value between ^{68}Ga-citrate and the ^{68}Ga-NOTA/malDOTA-TATE-nanogel.

Native SDS PAGE/phosphor imager analysis

The nanogels were evaluated for purity and stability by a gel electrophoresis (native running conditions). Here for, the nanogel containing samples were mixed (1:1 ratio) with a native sample buffer and loaded into an any kD TGX gel (450 µg/lane of Alexa Fluor 488-labeled nanogel, 20 kBq of ^{68}Ga-labeled nanogel). For visualization, the fluorescence dye labeled nanogels were imaged by an ImageQuant LAS 4010 (GE Healthcare), the radioactive labeled samples were exposed for 10 h to phosphor screens and analyzed by Typhoon FLA 7000 phosphor imager (GE Healthcare).

Scanning electron microscopy (SEM)

SEM was performed with a HITACHI S- 4800 Gatan Cryosystem Alto 2500 in a cryo mode with secondary electron image resolution of 1.0 nm at 15 kV, 2.0 nm at 1 kV and 1.4 nm at 1 kV with beam. The NOTA-nanogel sample dispersed in water was fixed on a holder and was rapidly frozen with boiling liquid nitrogen. It was then transferred to the high vacuum cryo-unit, the Balzers BF type freeze etching chamber. The cryo-chamber is equipped with a knife,

which could be handled from outside by means of a lever. The knife is used to fracture the sample, for imaging of the inner surface structure. In order to remove humidity the samples were cryo-etched by subliming from 5 to 15 min at -95 °C, after which the sample was further inserted into the observation chamber.

Ultra-high Resolution Scanning Electron Microscope SU9000

Hitachi ultrahigh-resolution field emission type scanning electron microscope SU9000 with Energy-dispersive X-ray Spectroscopy (EDX) was used for imaging and mapping of Ga in the nanogels. For the measurement NOTA-nanogels labeled with 'cold' Ga were dialyzed for 7 days. The purified nanogels (5 mg/mL) were spin coated on silicon wafer and dried. Measurements were performed at a resolution of 1 nm and 20 kV.

Dynamic Light Scattering

The particle sizes were measured by photon correlation spectroscopy performed at an angular range between 30 ° and 120 ° with a step of 10 °; **with a setup consisting of an ALV-SP8 goniometer, an ALV-SIPC photomultiplier, a multiple τ digital real-time ALV-7004 correlator, and a solid state laser (Koheras) with a red laser (λ = 633 nm) as light source.** The time resolved signal of two Single Photon Counting Modules was cross-correlated. To prevent multiple scattering highly diluted nanogel solutions of 0.1 - 1.0 mg/mL in bi-distilled water were prepared and passed through a 1.2 µm poly(tetrafluoroethylene) membrane filters to remove dust. Sample cuvettes were immersed in a toluene bath and tempered within an error of ± 0.1 °C. Autocorrelation functions of intensity fluctuations g_2 (q, t) are converted by the Siegert relation and give the field autocorrelation function *f(q,t)*:

$$f(q,t) = \int_0^\infty G(D_0) exp\{-D_0 q^2 \tau\} dD_0 \qquad (1)$$

Where τ is the decay time, $G(D_0)$ is the distribution function of D_0, the diffusion coefficient and q as the scattering vector defined as

$$q = \frac{4\pi n}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \quad (2)$$

with θ being the scattering angle and λ_0 being the wavelength of the laser light in vacuum.

The intensity-weighted decay-time τ distributions obtained from the field autocorrelation function by cumulant analysis were analysed in respect to multimodality. For each diffusive mode the decay rate $\Gamma = 1/\tau$ was plotted against the squared length of the scattering vector q^2. The slope gave the Z-average translational diffusion coefficient D_0 and results in the hydrodynamic radius R_h after use of the Stokes Einstein equation:

$$D_0 = \frac{k_B T}{6\pi\eta R_h} \quad (3)$$

with q, k_B, T and η being the scattering vector, the Boltzmann constant, absolute temperature, and solvent viscosity, respectively. A hydrodynamic radius distribution was calculated from the regularized Laplace inversion of correlation functions with CONTIN algorithm.

For combined Dynamic and static Light Scattering (DLS/SLS) analysis the CONTIN analysis was performed in an angular dependent manner.[2] The respective intensity for each diffusive mode was extracted by subdividing the overall count rate. The Radius of gyration (R_g) was extracted by Guinier plot by plotting the logarithm of the intensity against q^2 and fitting of the linear area where $R_g q < 1$. The slope after linear regression gives the radius of gyration $R_g^2/(-3)$.

$$\ln I_q = \ln I_0 - \frac{1}{3}\langle R_g \rangle^2 q^2 \quad (4)$$

The usefulness of this plot derives from the fact that the extracted Rg is independent of the absolute intensity I_0. The Guinier plot $ln\ I_q$ against q^2 measures the radius of gyration from any shape objects.

The ratio of the radius of gyration and hydrodynamic radius gives indication about the geometry of the particle.[3]

Hard sphere $\langle R_g^2 \rangle = 3/5\ R_h^2$

Vesicle	$\langle R_g^2 \rangle = R_h^2$
Polymer coil	$\langle R_g^2 \rangle = 1/6\ R_h^2$

In vitro cytotoxicity evaluation of the nanogels

The cytotoxicity of nanogels was evaluated in AR42J (rat pancreatic tumor cells), and in stimulated (with PMA 8 nmol) and non-stimulated THP-1 cells. For cytotoxicity assay the cells were seeded on 12-well plates in a culture media and incubated for 24–76 h until 90 % confluence. The cells were incubated with fresh media containing nanogels (0.0, 0.2, 0.4, 0.8 and 1.0 mg/mL) for 4 and 24 h at 37 °C. The cells were harvested by trypsinization and washed with PBS for 5 min followed by centrifugation at 1000 rpm at 20 °C. The supernatant was discarded and the cell pellet was fixed in 70 % ethanol for 30 min at 4 °C. After two washing steps with PBS the cell pellet was dissolved in 50 µL of a 0.1 mg/mL stock solution of RNase and 200 µL Propidium iodide (50 µg/mL stock solution) mixed with additional 300 µL of PBS. The cells were stored at 4 °C before analysed for apoptosis rate the same day by flow cytometry (Cytomics FC500, Beckman Coulter). Samples were analysed in triplicate for each experiment (CXP analysis software, Beckman Coulter).

Cellular uptake of Alexa Fluor 488-labeled nanogels in THP and AR42J cells

The cellular uptake of nanogels was investigated in THP-1 (unstimulated and PMA stimulated, 8 nM for 24 h) and AR42J cells by means of flow cytometry. Alexa Fluor 488-labeled nanogels (0.5 mg/mL) were added to the cell culture (12-well plate with 1.0×10^5/well) for 1 h and 4 h. After two wash steps with PBS the cells were harvested and analysed in terms of nanogel internalization by flow cytometry.

Cellular uptake of ^{68}Ga-labeled unfunctionalized and TATE-functionalized nanogels in AR42J cells

To investigate the cellular uptake and retention the SstR2 expressing AR42J cells (12-well plate with 1.0×10^5 /well) were incubated with of ^{68}Ga-labeled unfunctionalized and TATE-functionalized nanogel (0.5 MBq/well) for 1 h and 4 h. After two wash steps with PBS the cells were harvested and analysed in terms of nanogel internalization by gamma counter. The decay corrected cell accumulated activity was calculated relative to the implemented activity.

In vivo micro-PET imaging of nanogel biodistribution

All animal studies were conducted in accordance with the Federal German Law regarding the protection of animals. The Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) have been followed.

The molecular imaging analyses were performed using a rodent μPET scanner (Invion; Siemens). The general biokinetics of nanogels were evaluated in healthy Wistar rats (male, 250-300 g). The data acquisition started with intravenous injection of 20-25 MBq of ^{68}Ga-NOTA-nanogels and was finished at 2 h p.i.

After data acquisition, PET images were reconstructed by a 3-dimensional ordered-subsets expectation maximum (OSEM) algorithm. All data were corrected for attenuation, scatter, dead time and decay. For calculation of organ accumulated radioactivity during the analysed time period (time activity curve, TAC), regions of interest were drawn over the major organs and tumor on the whole-body images. The rats were euthanized 2-6 h post injection and blood and other main organs were harvested. To evaluate the radioactivity concentration the organs were weighted and the accumulated radioactivity was measured in gamma counter (PerkinElmer Inc.). The decay-corrected radioactivity was expressed as percentage of injected dose per gram (% ID, mean ± SD) which was obtained by dividing tissue radioactivity by injected dose assuming the tissue density as 1 g/cm^3.

The comparative biokinetic studies of ^{68}Ga-DOTA-TATE, ^{68}Ga-malDOTA-TATE-nanogel and ^{68}Ga-NOTA-nanogel were carried out in pancreatic tumor mice model (male

Balb/c nude mice, 30-35 g, subcutaneously xenografted with 5 $\times 10^6$ AR42J cells). The data were acquired directly after intravenous injection of 10-15 MBq of ^{68}Ga-labeled tracer and 4 h p.i. under isoflurane anesthesia (acquisition duration of 60 min). The calculation of TAC was performed as described above. Tumor was defined using co-registered PET and CT image data and subsequently extracted from all PET images. Afterwards the data were averaged in axial direction and visualized using MATLAB mesh functionality (MATLAB R2013a, The Mathworks, USA). To evaluate the radioactivity concentration the organs were weighted and the accumulated radioactivity was measured in a gamma counter. The decay-corrected radioactivity was expressed as percentage of injected dose per gram (% ID, mean ± SD) which was obtained by dividing tissue radioactivity by injected dose assuming the tissue density as 1 g/cm^3.

activity in tissue A/weight of tissue A in g / injected activity = %ID/g;

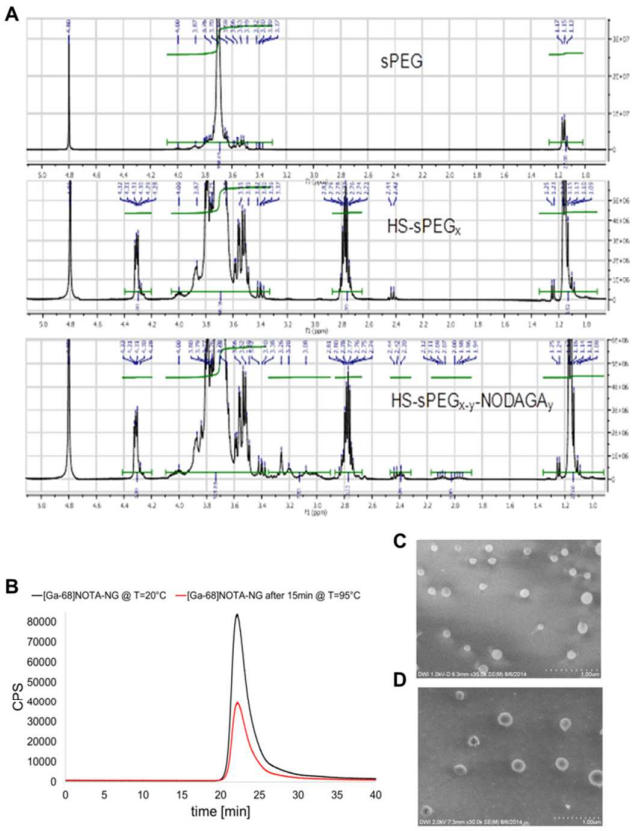
A correction for the overall body weight of the animals to yield % ID was not performed.

Immunohistochemical evaluation of vitality and expression of SstR2 in tumor tissue

Consecutive formalin-fixed, paraffin-embedded tissue sections (2 µm thick) were dewaxed in xylene and rehydrated through graded concentrations of isopropanol to distilled water. Sections were then incubated in 1% BSA- avidin solution (Vector) for 30 min at RT for blocking of unspecific binding. After blocking, the tissue sections were incubated over night at 4 °C with anti-SstR2 antibody and rabbit IgG as an isotype control (1:100 in blocking solution containing biotin, DAKO). Subsequently, the sections were exposed for 30 min to biotin-labeled anti-rabbit immunoglobulin antibody (dilution 1:100; Vector). For visualization of antibody binding, the section were incubated with streptavidin-FITC solution (dilution 1:33 in PBS, Vector) for 30 min at RT. The nuclei were stained by DAPI (1 µg/ml for 5 min on ice).

Fluorometric analysis of GSH concentration in tissue

Samples from tumor, kidney, liver, spleen and serum were homogenized in assay buffer. The homogenates were then mixed with ice cold perchloric acid (6 N) and centrifuged at 13000 × g for 2 min. The clear supernatants were used for measurement of GSH according to the manufacturer's instructions (BioVision).



E

Substance	Zetapotential [mV] @ T = 25°C; pH = 6.5
sPEG-OH	-7.3 ± 1.3
sPEG-malDOTA-TATE	-3.23 ± 0.9
NOTA-Nanogel	-10.16 ± 1.9
malDOTA-TATE-Nanogel	-13.7 ± 3.1

Figure S1. Characterization of nanogels. (A) ^{1}H-NMRs of modified pre-polymer: ^{1}H-NMR in D_2O showing the degree of functionalization of the sPEG. In average we achieved four thiol groups per star with 0.3-0.5 eq. of NOTA. (B) Radio-HPLC/SEC analysis of nanogels at room temperature (black) or after labelling at a temperature of T = 95°C; flow rate: 0.3 mL/min (0.1 % TFA) illustrate that the nanogels remain stable upon short time heating. (C) Representative cryo-FESEM images of NOTA-nanogel. (D) Representative cryo-FESEM images of DOTA-TATE-nanogel. (E) Zetapotentials of NOTA- as well as malDOTA-TATE-nanogels in comparison to the sPEG and malDOTA-TATE functionalized PEG.

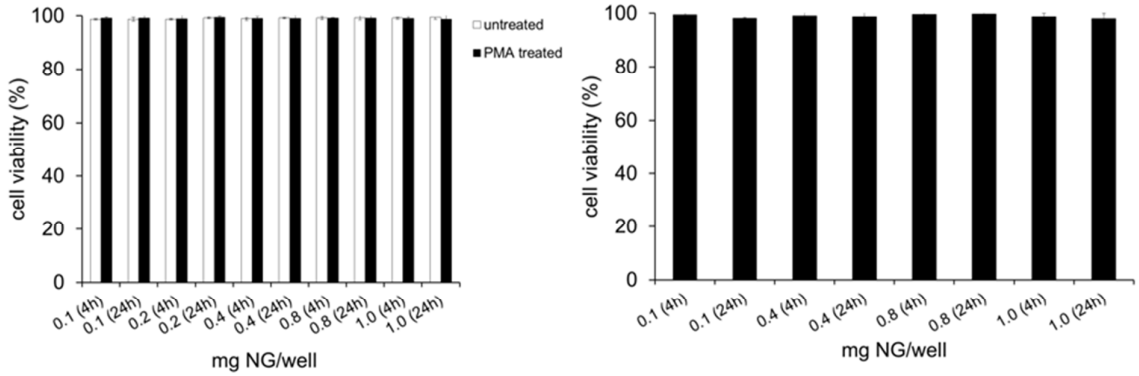


Figure S2. Flow cytometric analysis of nanogel cytoxicity. (A) THP-1 monocytes and macrophages after 4 h and 24 h incubation with nanogel. (B) AR42J tumor cells after 4 h and 24 h incubation with nanogel. Error bars represent standard errors (n = 3).

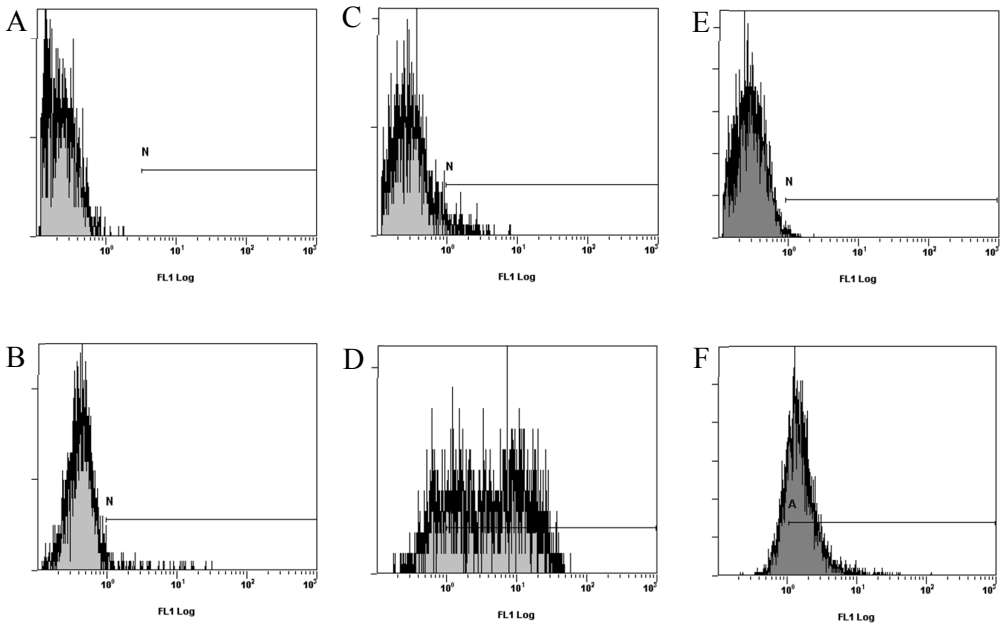


Figure S3. Representative flow cytometric analysis of Alexa Fluor 488-labeled nanogel uptake in monocytes/macrophages (THP-1) and AR42J tumor cells. The unstimulated monocytes, PMA-stimulated macrophages (PMA 8 nmol for 24 h) and tumor cells were incubated with Alexa Fluor 488-labeled nanogel (1 mg/ml) for 4 h at 37 °C. (A) Unstimulated THP-1 cells without nanogel. (B) Unstimulated THP-1 cells incubated with Alexa Fluor 488-labeled nanogel. (C) PMA-stimulated THP-1 cells without nanogel. (D) PMA-stimulated THP-1 cells incubated with Alexa Fluor 488-labeled nanogel. (E) AR42J cells without nanogel. (F) AR42J cells incubated with Alexa Fluor 488-labeled nanogel.

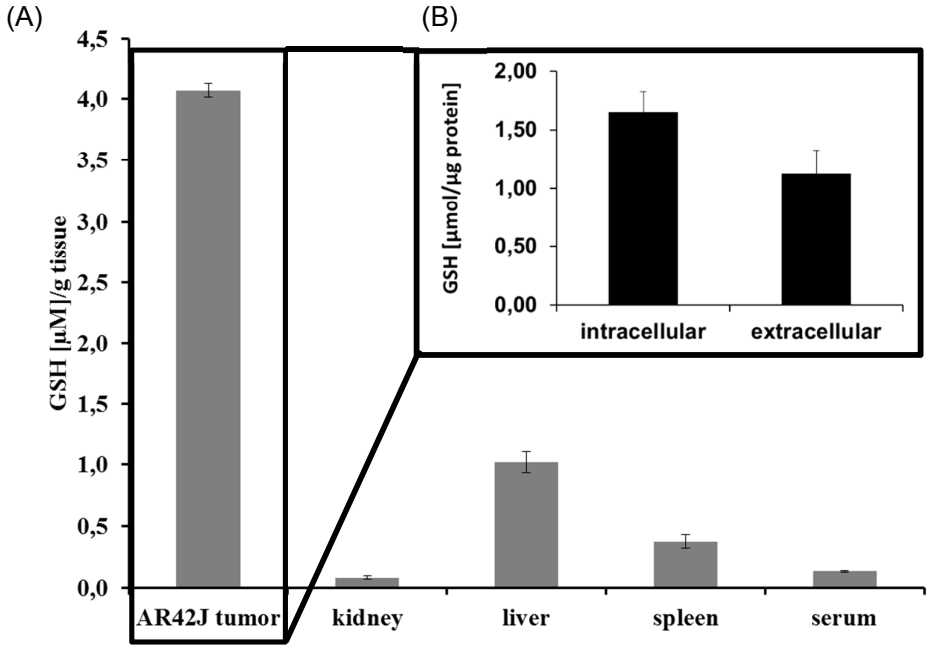


Figure S4. (A) **ELISA analysis of GSH concentration in tissue (calculated in µmol/L per gram tissue).** (B) **ELISA Analysis of extracellular and intracellular (from cell lysates) GSH concentration in AR42J cells.**

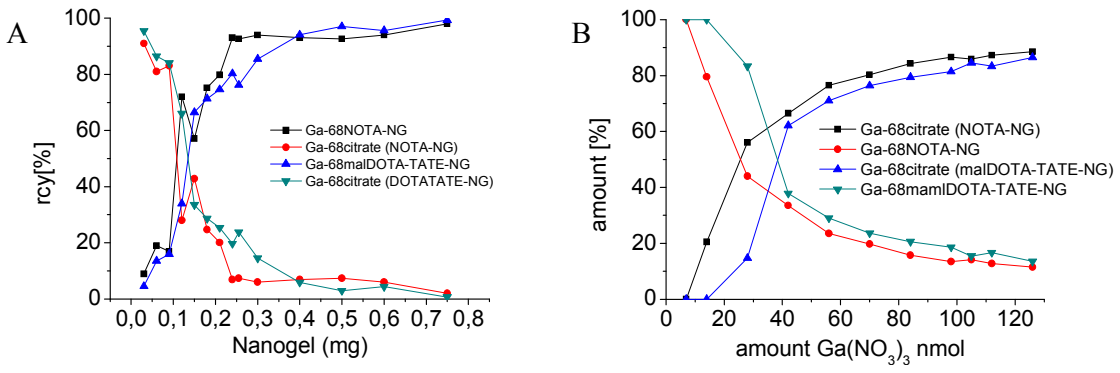


Figure S5. Optimization of labeling conditions for different nanogel derivatives. (A) Evaluation of optimal labeling ratios. Optimal amount of nanogels were determined to be 300 µg nanogel per labeling reaction with NOTA-nanogels and 500µg nanogel per labeling reaction with malDOTA-TATE-nanogels. (B) Competition experiment with cold Gallium(III) for calculation of an optimal amount of NOTA chelator molecules (24 nmol) per polymer (300 µg) and malDOTA-TATE (39 nmol) per polymer (500 µg).

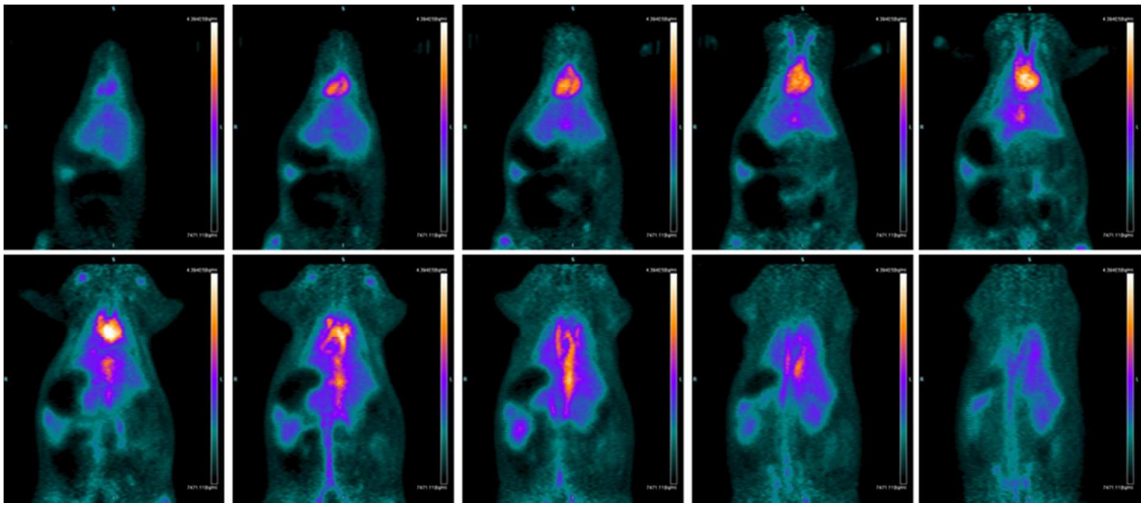


Figure S5. Coronal plane view of a healthy wistar rat after i.v. injection of [^{68}Ga]NOTA-nanogel and 60 min PET acquisition time reconstructed with 3D-OSEM. Plane by plane display of the images shows highest activity in the blood pool (heart, aorta).

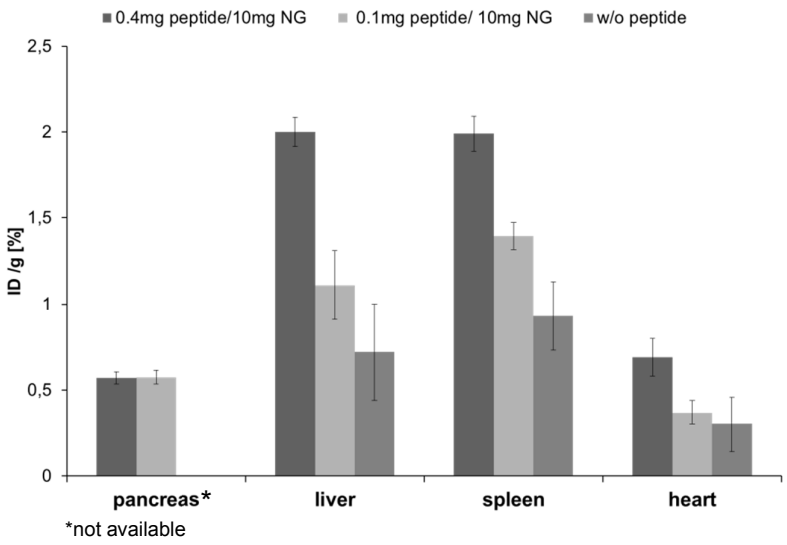


Figure S7. γ-counter analysis of accumulation of [^{68}Ga]NOTA-nanogel, [^{68}Ga]malDOTA-TATE-nanogel (with 4wt% peptide) and [^{68}Ga]malDOTATATE-nanogel (with 1wt% peptide) in % of injected dose per gram tissue (6h p.i.). Error bars represent standard deviation (n=3). The higher the amount of peptide in the nanogels the higher the liver uptake. However, no statistical significant difference was detected for [^{68}Ga]malDOTA-TATE-nanogel (with 1wt% peptide) and [^{68}Ga]NOTA-nanogel regarding blood pool activity.

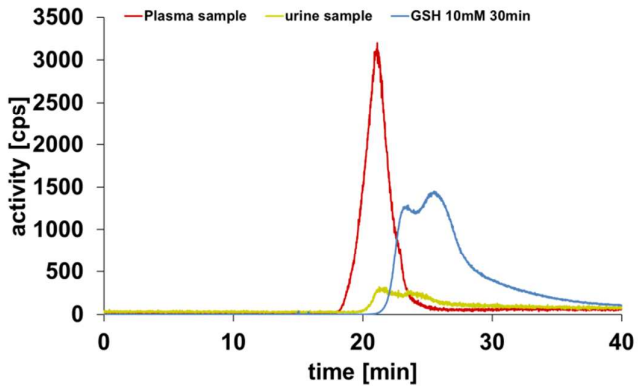


Figure S8. HPLC analysis of nanogel samples taken from blood (red line) and urine (yellow line) and as a control after 30 min of incubation in 10mM GSH (blue line). (Size exclusion column (suprema, PSS) flow rate: 0.3 mL/min (0.1 % TFA)). Supplementary References

1. Groll, J.; Singh, S.; Albrecht, K.; Moeller, M., Biocompatible and degradable nanogels via oxidation reactions of synthetic thiomers in inverse miniemulsion. *Journal of Polymer Science Part A: Polymer Chemistry* **2009,** *47* (20), 5543-5549.

2. Klucker, R.; Munch, J. P.; Schosseler, F., Combined Static and Dynamic Light Scattering Study of Associating Random Block Copolymers in Solution. *Macromolecules* **1997,** *30* (13), 3839-3848.

3. J.Higgins, H. C. B., *Polymers and Neutron Scattering*. Clarendon Press Oxford 1994; p 436.