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Ratiometric *in vivo* auditioning of targeted silver nanoparticles†

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Abstract

Attaching affinity ligands to nanoparticles (NPs) increases selectivity for targeting cells and tissues, and can result in improved sensitivity and reduced off-target toxicity in diagnostic and therapeutic systems. The decision over key features – NP size, shape, coating strategies and targeting ligands for clinical translation is often hampered by a lack of quantitative *in vivo* NP homing assays. Sensitive, internally controlled assays are needed which allow for quantitative comparisons (auditions) among various formulations of targeted NPs. We recently reported the development of peptide-functionalized, isotopically-barcoded silver NPs (AgNPs) for ultrasensitive studies centered on measuring relative ratios of NP internalization into cultured cells. Here we evaluated the application of this technology for NP homing studies in live mice using peptides with previously described tissue tropism; one peptide that favors vascular beds of the normal lungs (RPARPAR; receptor neuropilin-1, or NRP-1) and another that is selective for central nervous system vessels (CAGALCY). Equimolar mixtures of the peptide-targeted Ag107-NPs and Ag109 control particles were mixed and injected intravenously. Distribution profiles of Ag107 and Ag109 in tissue extracts were determined simultaneously through inductively coupled plasma mass spectrometry (ICP-MS). Compared to non-targeted particles up to ~9-fold increased lung accumulation was seen for RPARPAR-OH AgNPs (but not for AgNPs functionalized with RPARPAR-NH₂, which does not bind to NRP-1). Similarly, AgNPs functionalized with the brain-homing CAGALCY peptide were overrepresented in brain extracts. Spatial distribution (mapping) analysis by laser ablation ICP-MS (LA-ICP-MS) was used to determine the ratio Ag107/Ag109 in

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Conflict of interest

None.

tissue cryosections. The mapping demonstrated preferential accumulation of the RPARPAR-AgNPs in the perivascular areas around pulmonary veins, and CAGALCY AgNPs accumulated in discrete areas of the brain (*e.g.* in the vessels of cerebellar fibrillary tracts). Based on these results, the internally controlled ratiometric AgNP system is suitable for quantitative studies of the effect of targeting ligands on NP biodistribution, at average tissue concentration and distribution at the microscopic level. The platform might be particularly relevant for target sites with high local variability in uptake, such as tumors.

Introduction

Over the past 15 years, a multitude of nanoparticles with different compositions, sizes, shapes, and surface coatings have been designed for therapeutic, diagnostic, and theranostic applications.^{1,2} Whereas it is agreed that nanomedicine holds great promise, especially in improving cancer diagnosis and treatment, expectations have not been fully met and implementation in clinical applications is falling behind.^{3–5} Achieving precision delivery of nanoparticles to target sites *in vivo* remains an important, yet only partially achieved, goal. Quantitative biodistribution studies using multiplexed nanoparticles would facilitate optimization of NP coatings and affinity ligands to deliver nanoparticles to diseased tissues or specific organs of the body. Internal controls in target-homing experiments can include a nanoparticle with a similar coating but lacking the affinity ligand, co-injected with the targeted nanoparticle and thus controlling for various circulation and clearance parameters. Langer and collaborators recently reported the development of DNA-barcoded lipid nanoparticles for multiplexed *in vivo* evaluation.⁶ Whereas this approach can be used for quantitation of the nanoparticles across a wide dynamic range with high sensitivity, the system has potential pitfalls. For example, DNA-barcoded particles are not readily distinguishable on tissue sections for spatial mapping, barcodes must be protected inside the nanostructure to limit degradation, and differences in barcode sequences may affect nuclease sensitivity, efficiency of PCR during amplification, or NP behavior. Lammers and coworkers reported on *in vivo* and *ex vivo* optical imaging using a cocktail of nanoparticles labeled with two fluorophores (DY-676 and DY-750) to assess active *vs.* passive targeting.⁷ However, whereas the optical imaging technique has the advantage of allowing noninvasive longitudinal homing studies, it is not quantitative. Recently, we reported development of an ultrasensitive ratiometric nanosystem for simultaneous profiling of the expression of multiple homing peptide receptors on cultured cells.⁸ The system is based on isotopic barcoding of phenotypically identical AgNPs and it can be used for comparative studies on the NP affinity targeting of cellular receptors.⁸

Many nanoformulations are believed to reach tumor tissue nonspecifically, through the enhanced permeability and retention (EPR) effect.^{3,9} However, the permeability of tumor vasculature is not uniform, potentially compromising the therapeutic efficacy of a NP drug, particularly in slow-growing non-angiogenic tumors with a low level of vascular leakiness. Target selectivity of NPs can be improved by functionalization with targeting ligands (*e.g.* monoclonal antibodies, proteins and homing peptides) that recognize accessible molecular signatures at target sites.^{10,11} Homing peptides identified by *in vivo* phage biopanning screens are particularly well suited for NP targeting, as the phages are themselves biological

nanoparticles, acting as a scaffold to display multiple copies of random peptides during the biopanning. Over the years, many laboratories have reported on homing peptides capable of delivering systemically-administered NPs to healthy organs, such as brain, lung, and pancreas,^{12–15} to damaged and inflamed tissues,^{16,17} and to tumors.^{11,18} Here we show that isotopically barcoded AgNP can be used for quantitative internally controlled *in vivo* homing studies, such as studies aimed at improving the homing of peptide-targeted NPs.

Materials and methods

Materials

CHCl₃, MeOH, isopropanol, dimethylformamide (DMF) were purchased from Sigma-Aldrich. Phosphate-buffered saline (PBS) was purchased from Lonza, Belgium. Biotin-tagged RPARPAR-OH, RPARPAR-NH₂, CAGALCY peptides were synthesized using Fmoc/*t*-Bu chemistry on a microwave assisted automated peptide synthesizer (Liberty, CEM Corporation). Peptides were purified by HPLC using 0.1% TFA in acetonitrile–water mixtures to 90%–95% purity and validated by Q-TOF mass spectral analysis.

Animal procedures

The animal experiments were performed with healthy BALB/c mice according to animal experimentation protocols approved by Estonian Ministry of Agriculture, Committee of Animal Experimentation (permit #48).

Isotopic AgNP synthesis

Isotopic AgNP were synthesized using modified Lee and Meisel citrate method.^{19,20} Isotopically pure (Ag107 or Ag109) AgNO₃ (50.4 mg) was dissolved in the dark in 1 mL of Milli-Q water (MQ, resistivity 18 MΩ cm) and added to a piranha-cleaned (H₂SO₄ and H₂O₂ cleaned; *Caution*: highly oxidizing acid solution) glass flask containing 500 mL of water heated at 65 °C. Tannic acid (1.2 mg) was dissolved in 10 mL of MQ and 200 mg of citrate tribasic dihydrate was added to the solution. This mixture was added to the AgNO₃ solution in the reaction vessel. The mixture was vigorously stirred at ~70 °C and the reaction was allowed to proceed for 3 min, at which point the solution turned yellow. The flask was then transferred to a preheated hot-plate, brought to a boil, and boiled for 20 min. The flask was left to cool to room temperature and any boiled-off volume of water was replaced with fresh MQ by bringing the volume back up to 500 mL.

Fluorescently labeled “wild type” wtAg NP synthesis

Citrate particle synthesis, for the wtAg particles, was done by Lee and Meisel method using naturally occurring silver (mix of Ag107 and Ag109 isotopes). In a piranha-cleaned 4 L Erlenmeyer flask covered with foil, 2 L of Milli-Q water (MQ, resistivity 18 Mohm) was brought to a boil. In the dark, 360 mg of AgNO₃ was measured out, added to flask, and allowed to dissolve. Citrate tribasic dihydrate (400 mg) was dissolved in 40 mL of MQ in plastic tube, previously rinsed with MQ. All of citrate solution was added to the vessel and the solution was boiled for 30 minutes, after which it was allowed to cool to room temperature and the boiled-off volume of MQ was replaced.

AgNP characterization

For size and zeta potential measurements, the samples were diluted in MQ water and measured with a Zetasizer Nano ZSP (Malvern Instruments Ltd, Worcestershire, United Kingdom); refractive index of 2.0 and absorbance value of 0.2 was used for the measurements, each sample was run 3 times. The ultraviolet-visible (UV-Vis) absorbance spectrum of the AgNPs was measured with a Nanodrop 2000c spectrophotometer (Thermo Scientific Inc., Washington, USA).

For transmission electron microscopy (TEM) images, the AgNPs were diluted in MQ water, dropped onto the carbon film covered side of a Cu-300 copper mesh grid (Agar Scientific, Ltd, Essex, UK), air dried at RT, and imaged with a Tecnai 10 (FEI, Oregon, USA) at 80 kV.

AgNP functionalization

The particles were functionalized with NeutrAvidin (NA) for biotinylated-peptide conjugation, and with lipoic acid-polyethylene glycol (1k)-NH₂ (PEG) molecules as previously described.²¹ The terminal amines of PEG were used for coupling of CF555-*N*-hydroxysuccinimide-dye (NHS-dye). Four different types of particles were prepared by attaching the following biotinylated peptides: biotin-X-RPARPAR-OH; biotin-X-RPARPAR-NH₂; biotin-X-CAGALCY (X = aminohexanoic acid), or by blocking the biotin binding pocket with free D-biotin. The products were washed to remove free peptides by centrifuging at 20 000*g* for isotopic and 3500*g* for larger weight particles, decanting, and resuspension in fresh buffer (0.1 M HEPES pH 7.2, 0.1 M NaNO₃, 0.005% Tween 20) with sonication.

In vivo homing studies

For *in vivo* homing studies, 200 µL of AgNPs at 50 O.D. suspended in 200 µL of PBS were injected into tail vein of Balb/c mice. 1 h or 5 h post i.v. administration the mice were perfused *via* the left ventricle of the heart using 20 mL PBS. Organs were snap-frozen in liquid N₂ for cryosectioning and for ICP-MS analysis.

Microscopy

For microscopic imaging, 10 µm cryosections were fixed in 4% paraformaldehyde in PBS. The nuclei of cells were stained using 10 µg mL⁻¹ DAPI, and the sections were coverslipped in aqueous mounting medium for confocal imaging with fluorescence confocal microscopy (Olympus FV1200MPE, Germany).

For silver enhancement, cryosections were fixed in MeOH (−20 °C for 60 s), washed in PBS, and treated with Silver Enhancement kit (Molecular Probes) for 30–40 min with fresh solution added every 13 min. The reaction was stopped in 75 mM Na₂S₂O₃ in water. The nuclei of cells were stained with 10 µg mL⁻¹ DAPI, washed with PBS, followed by dehydration of sections through graded ethanol series, xylene, and mounting in DPX mounting medium.

Fluorescent confocal images and dark-field microphotographs were quantified with Image-J freeware.

ICP-MS analysis on tissue extracts

For radiometric ICP-MS-based biodistribution studies, Balb/c mice were injected i.v. with 200 μ L of a mixture of peptide–AgNPs (prepared from ^{107}Ag) and control biotin-blocked AgNPs (prepared from ^{109}Ag). At indicated time points, the mice were perfused with PBS and the organs were collected and snap-frozen.

Tissue samples were dissolved for ICP-MS using an in-house-developed open-vessel digestion protocol. Frozen tissue samples were thawed at room temperature for 2 h and 0.1–0.2 g of tissue was transferred into perfluoroalkoxy alkane vessels. HNO_3 (5 mL of 69%, Carl Roth, ROTIPURAN® Supra) was added, the samples were transferred to a hotplate, ramped to 90 °C over a period of 1 h and maintained at constant temperature for 2 h. After nitric oxide gas fuming ceased, the mix was cooled to room temperature and 2 mL of 30% H_2O_2 (Fluka, TraceSELECT® Ultra) was added. The samples were returned to the hotplate and, after vigorous effervescence had ceased, the vessels were capped and left to digest overnight (~12 h). The next morning the samples were cooled and an additional 3 mL of 30% H_2O_2 was added. The samples were returned to the hotplate and once effervescence had ceased the vessels were cooled and capped. After digestion, the mixture was diluted with 2% HNO_3 solution to a final dilution factor of 400. ERM® – CE278k (Mussel Tissue) was used as a quality control sample for digestion and NIST SRM 1640a (Trace Elements in Natural Water) for ICP-MS analyses.

Isotope ratio and chemical composition of dissolved tissue samples was analyzed using Agilent 8800 ICP-MS in MS/MS regime using 6 mL min^{-1} of He in the collision/reaction cell to eliminate possible polyatomic overlaps of $^{91}\text{Zr}^{16}\text{O}^+$ and $^{92}\text{Zr}^{16}\text{O}^{1}\text{H}^+$ on ^{107}Ag and ^{109}Ag . Indium in 2% HNO_3 was added on-line and used as an internal standard. MassHunter software (Agilent) was used for data analysis. The detection limit (DL) and background equivalent concentrations (BEC) of the measurement procedure were 2 ng mL^{-1} and 6 ng mL^{-1} respectively.

Each resulting isotope ratio was normalized to the input ratio, *i.e.* the specific Ag107/109 value in the administered AgNP cocktail, in order to showcase in a single graph the preferences in tissue distribution for each of the peptides.

Laser ablation-ICP-MS on tissue sections

For radiometric LA-ICP-MS-based biodistribution studies, mice were injected i.v. with 200 μ L of a mixture of peptide–AgNPs (prepared from ^{107}Ag) and control biotin-blocked AgNPs (prepared from ^{109}Ag). At indicated time points, the mice were perfused with PBS and the organs were collected, snap-frozen, sectioned at 30 μm on Superfrost+ slides, and air-dried.

Bioimaging of $^{107}\text{Ag}/^{109}\text{Ag}$ isotope ratio on tissue sections was performed using a Cetac LSX-213 G2+ laser ablation (LA) system using a HelEx 2-volume ablation cell, coupled to Agilent 8800 ICP-MS. The LA-ICP-MS setup was optimized using NIST 612 glass. The same reference sample was used to monitor the ThO/Th ratio during the analytical run, which remained under 0.3%. The experimental parameters for the LA system are presented in ESI Table 1. † ^{13}C , ^{107}Ag and ^{109}Ag isotopes were monitored with dwell times of 47.5

ms, 95 ms and 95 ms respectively, corresponding to a duty cycle of 0.25 s. ^{13}C was used as internal standard to account for differences in the carbon content of the ablated tissue. Measurements were performed using MS/MS mode to enhance abundance sensitivity for ^{13}C .

Results and discussion

The ratiometric nanoparticle (NP) system we describe herein uses isotope-tags embedded inside the core of AgNPs to track their *in vivo* biodistribution for the purpose of comparing peptides that target specific tissues or cell types. To optimize the system for use in mice we attached to the AgNPs tissue-specific homing peptides previously identified by *in vivo* phage display: RPARPAR – a NRP-1-targeting peptide that, when injected systemically in normal mice, accumulates in the lung microvasculature;¹⁵ and CAGALCY – a CNS-targeting peptide that accumulates in the mouse central nervous system.¹⁴

To confirm that the peptide functionalization confers on AgNPs the expected tissue targeting tropism, we first studied the biodistribution of fluorescent AgNPs using optical imaging. The CF555 dye-labeled AgNP cores are well suited for fluorescence imaging as the AgNP increases the brightness of the surface-tethered dye by about 10-fold *versus* its free state, through a mechanism called plasmonic enhancement.²¹ Fluorescently-labeled AgNP stock was synthesized from silver without any isotope enrichment; naturally occurring wild-type (wtAg) is a mix of Ag107 and Ag109 isotopes. The AgNPs were coated with neutravidin (NA) and PEGylated as described previously, creating a stable colloid ready for loading with biotinylated peptides.²² The size of the wtAg-NA NPs was 106.1 ± 1.2 nm (mean $\pm$ SD) with zeta potential -18.6 ± 0.2 mV (Fig. S4†). For homing studies, the wt-Ag-NA were functionalized with either biotinylated peptides RPARPAR-OH, RPARPAR-NH₂ (a control peptide for RPARPAR-OH that does not interact with receptor NRP-1 and does not home to the lungs), or CAGALCY-OH. Another non-targeted control was prepared by blocking wtAg-NA with free biotin.

The dye labeled CF555-peptide-AgNPs were injected into the tail vein of the Balb/c mice and 5 h post i.v. administration the animals were perfused with PBS to remove NPs circulating in the blood. Biodistribution of AgNPs was characterized using fluorescence confocal imaging with *post hoc* quantitative analysis by ImageJ. In the lungs, CF555-RPARPAR-OH-AgNPs showed robust accumulation in and around the microvessels in the lung parenchyma (Fig. 1A), as expected based on our earlier studies with this peptide,¹⁵ whereas the RPARPAR-NH₂-AgNP signal in the lungs was similar to the negative control, biotin-AgNPs (Fig. 1A). Quantitative analysis revealed a ~7-fold increase of the fluorescence signal in the lungs for RPARPAR-OH AgNPs relative to the untargeted biotin AgNPs (Fig. 1B). To ensure the optical imaging approach was detecting intact AgNPs we subjected the tissue sections from AgNP-injected mice to a silver enhancement procedure,²¹ followed by dark-field microscopy. This treatment allowed us to visualize silver grains in tissue sections by detecting the light that is obliquely scattered into the detector by the reflective metal deposits. This analysis demonstrated robust uptake of CF555-RPARPAR-OH-AgNPs in the lungs, but not in control organs (Fig. S1†).

Intravenous (i.v.) injection of nanoparticles can deposit high numbers of particles to the lungs as a result of a first-pass effect and extensive vascularization. To evaluate the *in vivo* targeting performance of the AgNP system to a tissue with lower baseline uptake than the lungs, we also studied the biodistribution of AgNPs functionalized with a central nervous system-homing peptide, CAGALCY.14 5 h post i.v. administration, confocal analysis demonstrated enhanced accumulation of CF555-CAGALCY-AgNPs in a subpopulation of brain microvessels and in discrete areas in the brain parenchyma. Only low background signals were detected in other organs of these mice (Fig. 2A). In contrast, the CF555-biotin-AgNPs showed no brain homing (Fig. S3†). Quantitative confocal imaging of CF555 fluorescence demonstrated ~9-fold stronger accumulation of CAGALCY AgNPs in the brain regions that were positive for these NPs than of the control biotin NPs (Fig. 2B). These experiments show that functionalization with homing peptides can be used to modulate *in vivo* tissue biodistribution of systemic AgNPs.

For ICP-MS studies, we prepared isotopically pure Ag107NPs and Ag109NPs. An equimolar mixture of peptide-107AgNPs (RPARPAR-OH-AgNPs, RPAPAR-NH₂-AgNPs, or CAGALCY-AgNPs) and biotin-Ag109NPs was i.v. injected in mice, and NP distribution was studied as shown in Fig. 3A. Ag107/109 ratio in the tissue extracts, as determined by ICP-MS, represented specific accumulation of peptide-Ag *versus* biotin-Ag. In the lungs we observed preferential accumulation of RPARPAR-OH-AgNPs, with ~9-fold overrepresentation compared to biotin-AgNPs at the 1 h time point (Fig. 3B). At the 5 h time point, the RPARPAR-OH-Ag107NP/biotin-Ag109NP ratio in the lungs was ~5, which agrees well with the optical imaging-based biodistribution analysis (Fig. 1). In addition, elevated levels of Ag107NPs were seen in the heart – another known target tissue of the RPARPAR peptide.¹⁵ Selective tissue distribution (ratio >1) was also seen for the CAGALCY-AgNPs. Compared to biotin-Ag109NPs, the CAGALCY-Ag107NPs were overrepresented in the brain (Fig. 3C), although the difference was lower than seen in optical imaging studies (Fig. 2B) likely due to the effect of sample averaging during bulk tissue digestion.

ICP-MS analysis on tissue extracts provides information on the average representation of the isotopically-coded nanoparticles. Most tissues, including tumors, are composed of areas of different extracellular composition and functional importance; this heterogeneity translates into differences in the distribution of affinity-targeted payloads.^{23,24} Laser ablation (LA) ICP-MS was originally established for spatially-defined isotopic detection in geological and archeological samples²⁵ and used later to study elemental distribution in sections of biological tissues,^{26–29} including tumors.^{30,31} To study spatial distribution of AgNP in tissues we dosed mice with a cocktail of RPARPAR-Ag107NPs and non-targeted biotin-Ag109NPs followed by perfusion and collection of organs after 5 h, cryosections of the tissues were subjected to LA-ICP-MS analysis. Profiling of the RPARPAR-OH-Ag107NP/biotin-Ag109NP ratio ablation paths showed that RPARPAR-OH-Ag107NPs were generally overrepresented in the lungs and that the Ag107/Ag109 ratio was particularly high (>8) in the regions close to pulmonary vascular structures and bronchioli (Fig. 4A and B). In the same animals the liver Ag107/Ag109 ratio was similar to the input AgNP cocktail (Fig. S5†). Notably, when we averaged the Ag107/109 ratios from multiple ablation paths (16 LA-ICP-MS for the lungs and 13 for the liver) the resulting Ag107/Ag109 values were

similar to those obtained from tissue extracts (lung: ~6.1 for the lung with LA-ICP-MS vs. ~8.9 with ICP-MS; liver: ~1.3 with LA-ICP-MS vs. ~1.1 with ICP-MS; Fig. 4B and Fig. S6†).

Mice injected with a cocktail of CAGALCY-Ag107NPs and non-targeted biotin-Ag109NPs were demonstrated by LA-ICP-MS to have overrepresentation of Ag107 in several areas in the brain, such as cerebellar fibrillary tracts and pontine microvessels (Fig. S7†). In contrast to the other tissues analyzed, the brain had on average very low levels of the silver nanoparticles. Therefore we thresholded the LA-ICP-MS data and evaluated the Ag107/Ag109 ratio only for spots with a total Ag signal above 500 counts per second (Fig. S7†). The thresholded data show that LA-ICP-MS can be used following systemic administration of barcoded NPs for spatially resolved mapping in tissues. Together, these results suggest that tissue microheterogeneity plays an important role in the targeting of peptide-coated AgNPs to tissues. The spatial resolution of LA-ICP-MS clearly provided additional insight in studying targeted therapies, given that spatial differences in biodistribution are likely related to patterns of receptor expression across the vascular system, and colocalization with specific cells would result in better penetration of drug with reduced side effects. Combining ICP-MS with fluorescence or histology appears well suited for *in vivo* optimization of peptide-NP constructs.

Conclusions and perspectives

Our study shows that the isotopically barcoded AgNPs can be used for quantitative non-optical *in vivo* biodistribution studies. Ratiometric internally controlled AgNP system generates tissue distribution profiles and maps for comparing or auditioning of NP targeting ligands and other surface modifications. The isotope platform could be expanded by barcoding metal NPs with additional isotopes, such as palladium, gold, rhenium, or platinum leading to a greater degree of multiplexing with existing LA-ICP-MS instrumentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Systemic CF555-labeled RPARPAR-OH-AgNPs home to lung tissue. Balb/c mice were injected in the tail vein with CF555-labelled RPARPAR-OH and biotin-AgNPs in 200 μl PBS. After 1 h or 5 h post i.v. administration, the animals were perfused with 15 ml PBS to wash out blood and to remove unbound plasma AgNPs, and the organs of interest were snap-frozen. (A) Confocal imaging of lung cryosections from mice injected with CF555-AgNPs, 5 h post i.v. administration. Arrows point at areas of accumulation of CF555-positive RPARPAR-OH AgNPs. *N* = 3; scale bars = 50 μm. (B) Quantitation of the CF555

fluorescence in tissue sections. AgNP signal (red in panel A) expressed as percent of pixels with intensity above threshold. Five randomly chosen fields from 3 animals per group were analyzed. Data represent mean $\pm$ SD, ** $p < 0.01$.

Fig. 2. Biodistribution of systemically administered CF555-CAGALCY-AgNPs. (A) Confocal imaging of tissues from mice injected with CAGALCY-functionalized AgNPs labeled with CF555 fluorescent dye, 5 h post i.v. administration. Arrows point at areas of accumulation of CAGALCY AgNPs (red). Scale bars = 100 μm. (B) Quantitation of CF555 fluorescence in tissue sections. Ag pixels/field were plotted for each type of nanoparticle as fold over control. $N=3$; 5 fields were analyzed per tissue. Data represent mean $\pm$ SD.

Fig. 3.

ICP-MS analysis of tissue distribution of homing peptide-functionalized AgNPs. (A) Outline of the ICP-MS ratiometric homing experiments. Balb/c mice were injected in tail vein with an equimolar mix of peptide-AgNPs (Ag107) and biotin-AgNPs (Ag109) in 200 μ l PBS. 1 h or 5 h post i.v. administration, the animals were perfused with 15 ml PBS to remove free plasma AgNPs, and the organs of interest were snap-frozen. Tissues were subjected to $\text{HNO}_3/\text{H}_2\text{O}_2$ extraction protocol for ICP-MS, or cryosectioned for laser ablation ICP-MS (LA-ICP-MS). (B) ICP-MS analysis of tissue distribution of RPARPAR-OH-AgNPs after 1 or 5 h post i.v. administration. Data represent mean values $\pm$ SD ($n = 3$). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. (C) ICP-MS-based analysis of tissue distribution of CAGALCY AgNPs. Balb/c mice were intravenously injected with an equimolar mixture of targeted CAGALCY AgNPs and biotin-AgNPs. 5 h post i.v. administration the animals were perfused with PBS, and the extracts of organs were subjected to ICP-MS to for relative quantitation of silver isotopes. Data represent mean $\pm$ SD ($n = 3$), *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Fig. 4.

Ratiometric LA-ICP-MS profiling on sections of lung. (A) Balb/c mice were injected i.v. with 200 μ L of a mixture of RPARPAR-OH-Ag107NPs and control biotin-Ag109NPs. At 5 h time point mice were perfused and organs collected. Tissues were snap-frozen, sectioned at 30 μ m, and laser ablation line scans using 40 μ m spot were performed. Hematoxylin–eosin staining of a lung cryosection used for LA-ICP-MS. The laser ablation path is indicated by arrow. Note the microanatomical heterogeneity of the lung section. The region of the graph indicated by the dashed box and pink shading correspond to the inset showing the laser ablation path in panel B. (B) Areas along the ablation path with Ag107/Ag109 > 9 are indicated in red. Elevated Ag107/Ag109 ratios are associated with vascular and bronchial structures. Data are representative of 16 laser ablation paths. Scale bars: (A) 200 μ m; (C) 100 μ m.