

PhotonIMAGER™

Real-Time monitoring of luminescent nanoprobe biodistribution

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Summary:

- In vivo distribution of luminescent nanoparticles can be followed in real-time.
- Low background is achieved by recording without external illumination following injection of the photoactivated nanoparticles.
- Homing of the nanoprobe into specific organs can be characterized over more than 1 hour.

Introduction:

A new generation of luminescent nanoparticles emitting in the red to near-infrared range can be optically excited before local, or systemic injection *in vivo*. The afterglow can last for several hours and without external illumination. The high background signal generated during in-situ fluorescent excitation is therefore avoided. Significant signal-to-noise ratio improvements, combined with the superior detection technology of the intensified CCD (iCCD) camera used in the PhotonIMAGER, allows for their detection in deep tissue and Real-Time biodistribution monitoring of active elements hours after injection¹

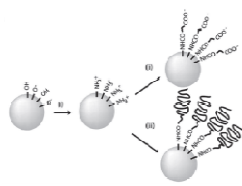


Fig 1. NP surface modification and in vivo biodistribution. (i) Amino-NPs synthesized by reaction with 3-aminopropyltriethoxysilane. (ii) Carboxyl-NPs from a reaction of amino-NPs with diglycolic anhydride. (iii) PEG-NPs achieved by a peptidic coupling of amino-NPs with PEG₅₀₀₀-COOH.

Materials and Methods:

Swiss mice were anesthetized by i.p. injections of a mixture of ketamine (85.8 mg/kg) and Xylazine (3.1 mg/kg). Suspensions containing various concentrations of NPs were excited

with a 6-W UV lamp and directly injected into the anesthetized mice.

In a second set of experiments the luminescent nanoprobe was used to visualize tumor vascularization. A body-shaved C57BL/6 mouse bearing a s.c. implanted Lewis lung carcinoma (3LL) tumor in the inguinal region was pre-injected with liposomes (6 mmol, 100 ml) for 5min followed with an injection of PEG-NPs. For both sets of experiments, the signal was recorded with a PhotonIMAGER and the software Photo-Acquisition. Image analysis was carried out in M³Vision™.

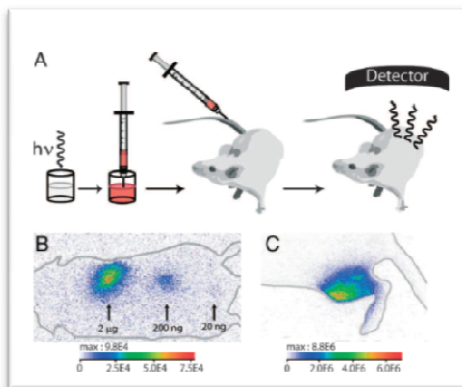


Fig. 2: Principles of in vivo experiments. (A) A suspension containing NPs was excited using a 6-W UV lamp and injected into anesthetized mice. The signal was acquired using an iCCD camera. (B) Image of three s.c. injections of NPs (2mg, 200ng, 20ng). A 2 min acquisition performed immediately after injection. (C) A 90-s acquisition following intramuscular injection (200mg). Luminous intensity is expressed in photons /s/cm²/sr.

Results:

Charged nanoparticles were synthesized with various surface modifications (fig 1), excited with UV light and injected into anesthetized animals (fig 2). Their biodistribution was analyzed after Bioluminescence detection on the PhotonIMAGER. Following injection of positively charged Amino-NPs the bioluminescent signal was retained in the lung within 1 hour, with little change over time (fig 3A).

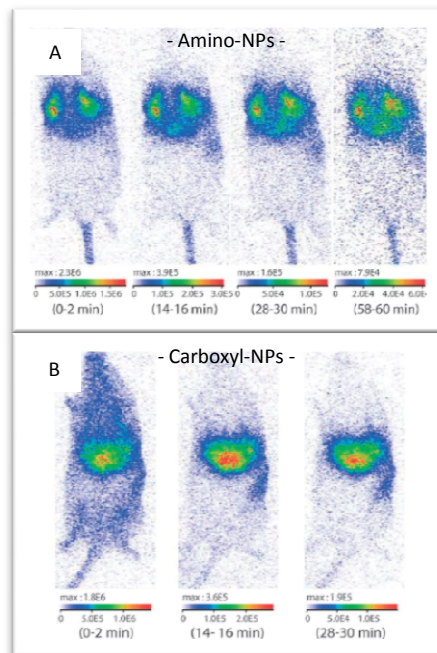


Fig. 3. in vivo biodistribution of various types of nanoparticles. Optical imaging of a mouse following 1-mg tail vein injections of differently charged NPs. (A) Amino-NPs. (B) Carboxyl-NPs. Luminous intensity expressed in photons per s.cm².steradians (sr).

Negatively charged carboxyl-NPs were rapidly cleared from the blood flow by liver uptake and did not present pulmonary sequestration (fig 3B).

Neutral PEG-NPs did not localize to specific organs and remained in the circulation for much longer periods before liver uptake (fig 4A). Note the diffuse signal throughout the mouse body for all three acquisition times.

Increased circulation times were enhanced further with pre-injections of anionic liposomes containing equimolar quantities of phosphatidylcholine, cholesterol, and phosphatidylserine.(fig 4b).

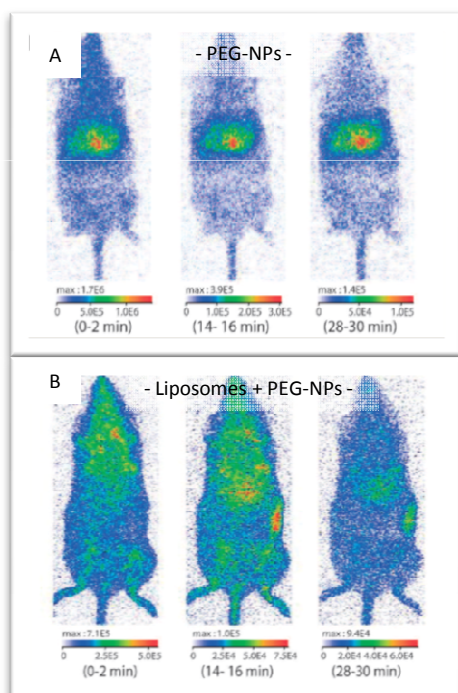


Fig. 4. Effect of liposome pre-injections - (A) PEG-NPs injected as stated in material and methods. **(B)** Pre-injection of anionic liposomes (6 mmol, 100 ml) 5 min before PEG-NP injection. 2-min signal acquisitions were performed between the times indicated above each image. Luminous intensity expressed in photons /s/cm²/sr.

Low luminescence signals in melanin-rich C57BL/6 mice can be detected with the PhotonIMAGER.

The presence of melanin in C57BL/6 mice is a particular disadvantage for *in vivo* optical imaging; because the signal attenuation factor of melanin is very high and covers all of the visible spectrum. As shown in figure 5A, the overall detected intensity was 5 to 7 times lower in C57BL/6 mice compared to Swiss mice. However the luminescent signal from the nanoparticles was still easily detected by the PhotonIMAGER, and biodistribution could be followed over time.

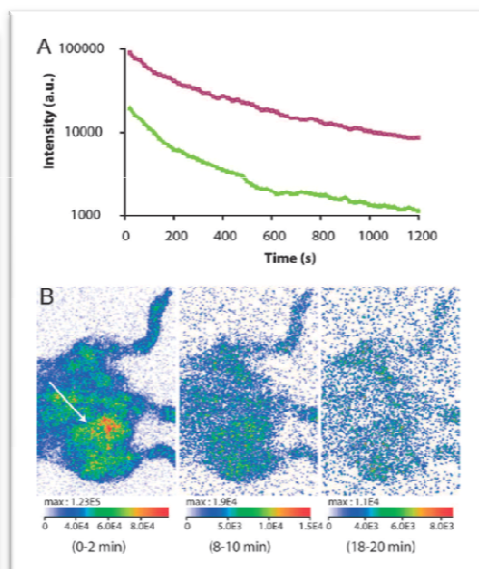


Fig. 5. Imaging of a tumor-bearing mouse. (A) Overall luminous intensity detected in a Swiss mouse (purple curve) and a body-shaved C57BL/6 mouse bearing a 3LL tumor (green curve). Tail vein i.v preinjection of anionic liposomes (6 mmol, 100 ml injected 5 min before NP injection) was followed by tail vein injection of 1 mg of PEG-NPs. A region of interest covering the whole mouse was manually selected and analyzed over a period of 20 s. **(B)** Visualization of the hypervascularization of a 3LL tumor (white arrow) with rapid clearance.

The tumour region was clearly revealed by an increase in local luminous intensity due to high vascularization of 3LL tumours (Fig. 5B).

Conclusion:

The biodistribution of luminescent nanoparticles can be controlled by altering their chemical surface and electrical charge. They can then be monitored in Real-Time using the PhotonIMAGER. This is of particular interest for pharmacological applications.

In addition, the demonstration that tumors in mice can be visualized with long-lasting luminescent NPs provides important perspectives for tumor imaging and cancer therapy.

References:

- [1] Chabottaux V. and Al. (2009) – Membrane-type 4 matrix metalloproteinase (MT4-MMP) induces lung metastasis by alteration of primary breast tumour vascular architecture. *J. Cell. Mol. Med. Vol 13, No 9B, 2009 pp. 4002-4013*