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Protocol

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Supplementary information

Non-invasive in vivo microscopy of single neutrophils in the mouse brain via NIR-II fluorescent nanomaterials

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Supplementary discussion on acute inflammation mouse model establishment.

A. Subcutaneous mice ear tissue inflammation

The mice ear tissue acute inflammation was induced by subcutaneous injection of lipopolysaccharide (LPS).

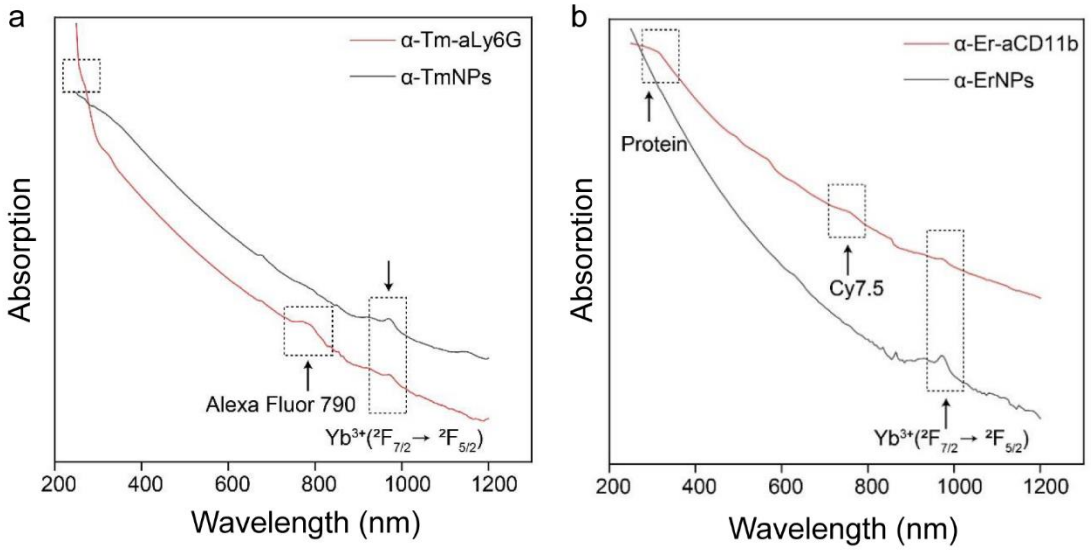
i. Dissolve the LPS power in PBS buffer (1×, pH 7.0) to obtain a working solution of 2.5 mg/ml.
ii. Use an induction chamber to anesthetize the mouse with 4% isoflurane, and deliver 2% isoflurane via nose cone for maintenance during the operation.
iii. Use a sterile insulin syringe to inject 25 μL the above LPS solution subcutaneously into the mouse ear tissue, avoiding transpiercing the mouse ear.
iv. Pull out the needle and press the injection hole for several seconds to prevent LPS exudation.
v. Remove the isoflurane nose cone and place the mouse back into the cage for further use.

B. Ischemic stroke mice.

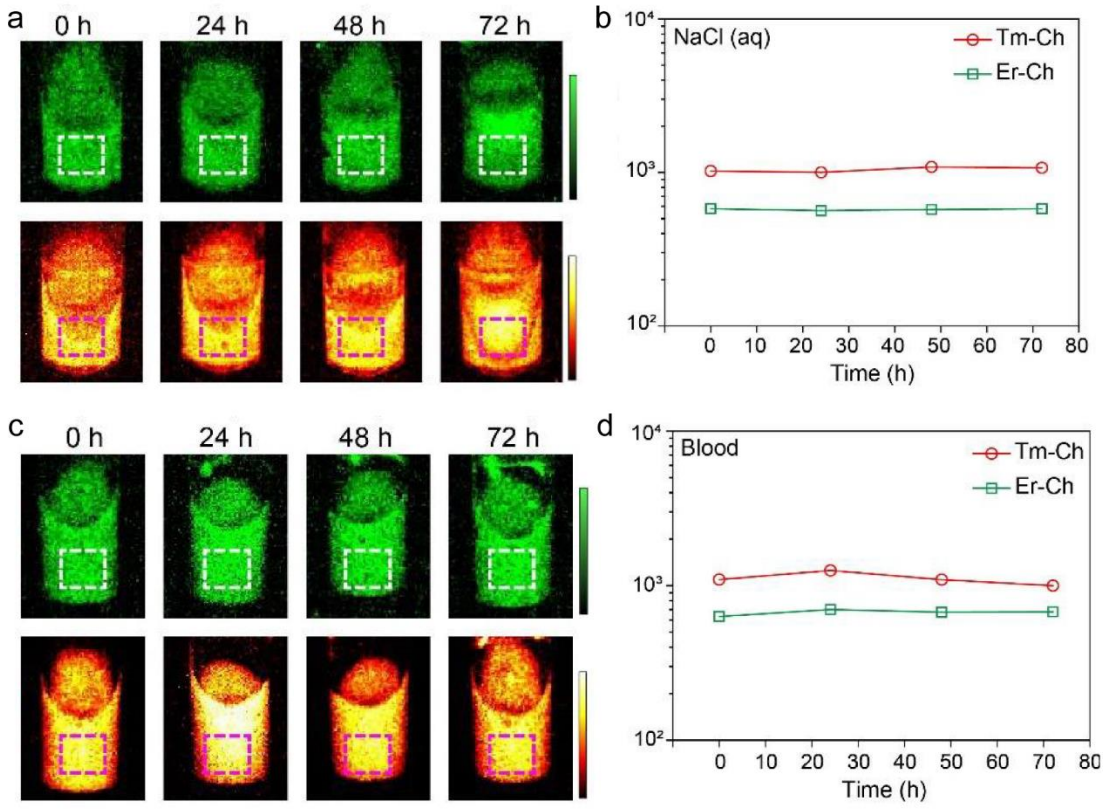
The ischemic stroke mice model was established following a typical middle cerebral artery occlusion (MCAO) method[1]. The MCAO is a well-characterized model in rodents as described otherwhere, thus the procedures are described brief here.

i. Sterilize all instruments prior to surgery by autoclaving.
ii. Weight the mouse (mice of 19-20 g body weight were used) and inject 200 μL avertin to anesthetize the mouse. Deliver 2% isoflurane via nose cone during the surgery.
iii. Shave the hair over the mouse neck and wipe the surgical field with 75% ethanol and povidone.
iv. Place the mouse under the upright objective lens (4× magnification) of a surgical microscope.
v. Make a midline ventral neck incision followed by unilateral MCAO surgery, and use a 1 cm long 6.0 silicone rubber-coated monofilament from the carotid artery bifurcation via an external carotid artery stump.

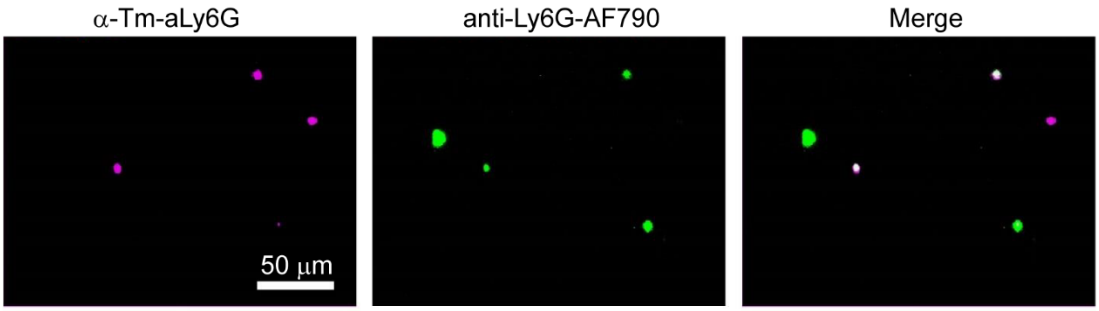
Supplementary Figures



Supplementary Figure 1. **Characterization of the antibody functionalized DSNPs. a**. Absorption spectra of the α-TmNPs and α-Tm-aLy6G nanoprobes. For α-Tm-aLy6G, absorption peaks at around 290 nm corresponds to the characteristic absorption of antibodies containing tyrosine, tryptophan and phenylalanine. The absorption peaks at 786 nm demonstrated successful conjugation of Alexa Fluor 790 labelled anti-Ly6G mAbs with α-TmNPs. **b**. Absorption spectra of the α-ErNPs and α-Er-aCD11b nanoprobes. For α-Er-aCD11b, absorption peaks at around 290 nm corresponds to the characteristic absorption of antibodies containing tyrosine, tryptophan and phenylalanine. The absorption peaks at 760 nm demonstrated successful conjugation of Cyanine 7.5 labelled anti-CD11b mAbs with α-ErNPs.



Supplementary Figure 2. **Stability of α-Tm-aLy6G and α-Er-aCD11b. a&b**. Fluorescent images and statistic data of α-Tm-aLy6G and α-Er-aCD11b in saline solution. **c&d**. Fluorescent images and statistic data of α-Tm-aLy6G and α-Er-aCD11b in blood. The PEGylated nanoparticles showed high aqueous dispersion stability and photostability in both saline solution and mouse blood.



Supplementary Figure 3. **Immunofluorescent staining in a fixed slice of inflamed mouse ear.** Fluorescence images of neutrophils labelled with α-Tm-aLy-6G (Magenta) by in vivo targeting and Alexa Fluor 790 conjugated anti-Ly6G (Green) in vitro staining. Signals of α-Tm-aLy-6G and Alexa Fluor 790 conjugated aLy-6G were aquired in the 1600-1700 nm and 850-900 nm under the excitation of 980 nm and 655 nm laser, respectively. Overlay channel (White) showed partial colocalization of the two signals, demonstrating the feasibility of α-Tm-aLy6G for in vivo neutrophil targeting. Scale bar, 50 μm.

Reference

1. Engel, O., Kolodziej, S., Dirnagl, U. & Prinz, V. Modeling stroke in mice - middle cerebral artery occlusion with the filament model. *J Vis Exp* (2011).