Supporting Information

Individually-Coated Near-Infrared Fluorescent Protein as a Safe and Robust Nanoprobe for in Vivo Imaging

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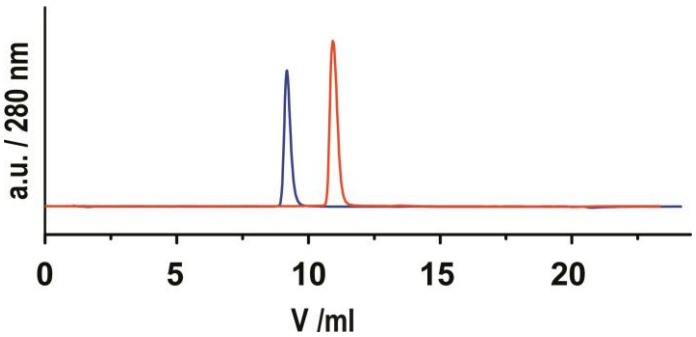

Figure S1. Elution profiles of NIRFP (blue) and APTS-NIRPF (red) on HiTrap Q HP at pH 8.0.

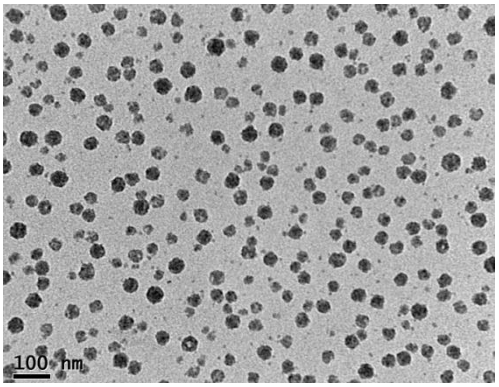

Figure S2. TEM image of NIRFP@silica NPs with an average size of 40 nm.

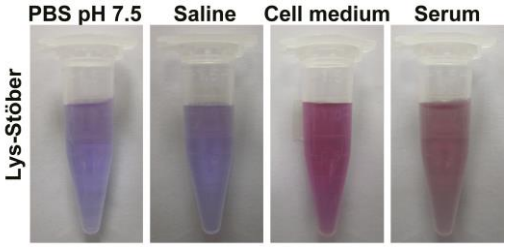

Figure S3. NIRFP@silica NPs stably dispersed in different media. They did not precipitate when centrifugated at 20000 g.

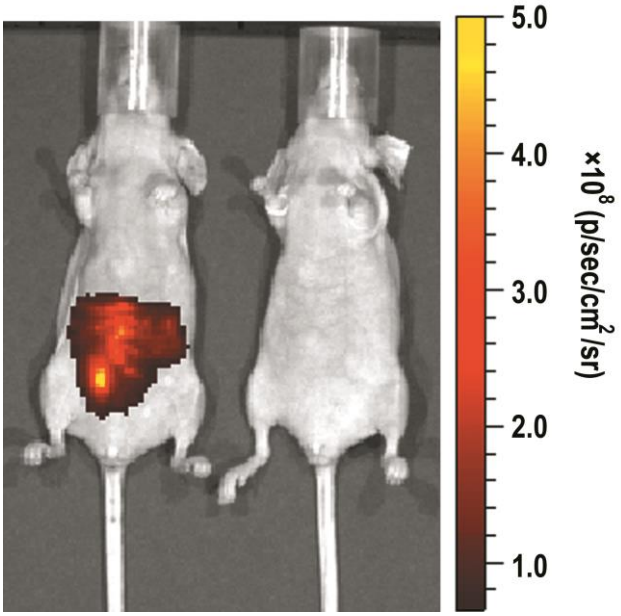

Figure S4. Fluorescence image of nude mice at 5 min after NIRFP@silica NPs exposure. Left: mouse was intraperitoneally injected with 250 μg kg^{-1} body weight of NIRFP@silica NPs; Right: saline control.

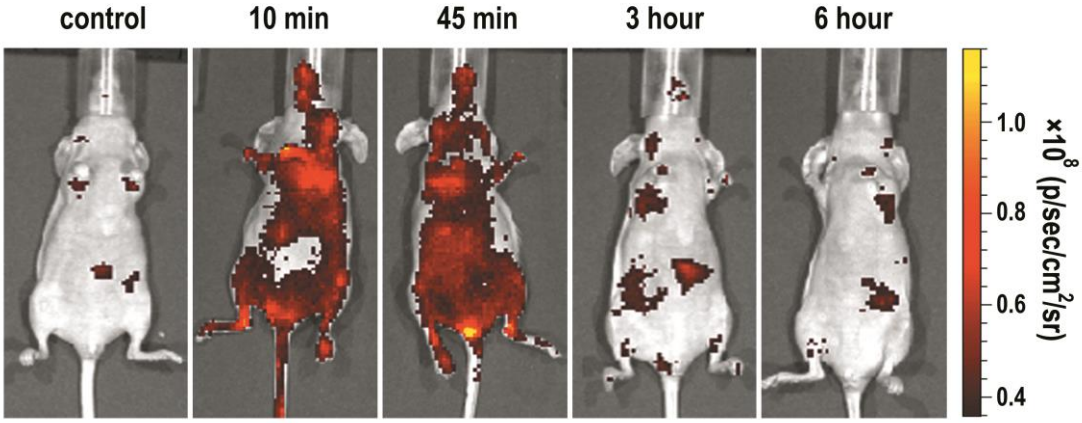

Figure S5. Whole body fluorescence imaging of mice i.v. injected with 3 mg kg^{-1} of NIRFP@silica nanoprobe.

Serum biochemical parameter measurements and histological observation

Male BALB/c mice (8 week) were obtained from SLAC Laboratory Animal, China. They were housed in plastic cages and kept on a 12 h light/dark cycle. Food and water were provided *ad libitum*. Following acclimation, mice were randomly divided into four groups (4-6 mice/group). Mice of two groups were single i.v. (tail vein) injected with NIRFP@silica in normal saline at a

single dose of 30 mg kg^{-1}. One group of mice i.v. exposed to saline solution was taken as the control groups.

All mice showed no symptoms of abnormality, such as lethargy, anorexia, vomiting, or diarrhea, during the experimental period.

Serum samples were obtained at 24 or 72 h post exposure from blood by centrifugation (3000 rpm × 10 min). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total bilirubin levels (TBIL), urea nitrogen (BUN), creatinine (CREA), and uric acid (UA) were assayed using the commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

The results are summarized in Table S1. ALT, AST, LDH and TBIL, the important indicators of the hepatic injury, LDH is also used as an indicator of alveolar macrophage injury in the pulmonary toxicity study. All these parameters keep normal during the experiment period, demonstrating that NIRFP@silica does not induce hepatic and pulmonary damage. BUN and CREA reflect the function of kidneys. Although CREA decreases a little bit at 24 h postexposure, it recovers to the normal level at 72 h postexposure. And the value of CREA is compared to that of the control. All the parameters show that NIRFP@silica at high dosage of 30 mg kg^{-1} is pretty safe to mice after i.v. exposure.

Table S1. Serum biochemical parameters of the control mice and the NIRFP@silica exposed mice at 24 or 72 h post i.v. exposure. Data represent mean ± SD (n=4-6). * $p<0.05$ compared with the control group.

Biochemical parameters	Control	NIRFP@silica (24 h postexposure)	NIRFP@silica (72 h postexposure)
ALT (U/L)	5.45 ± 1.95	5.27 ± 1.28	6.24 ± 0.62
AST(U/L)	8.88 ± 0.95	10.24 ± 1.24	9.64 ± 1.08
LDH(U/L)	278.97 ± 17.92	311.79 ± 39.08	321.88 ± 33.21
TBIL (umol/L)	0.30 ± 0.05	0.36 ± 0.09	0.29 ± 0.05
CREA(umol/L)	66.98 ± 3.61	61.51 ± 1.69*	63.20 ± 3.53
BUN (mmol/L)	6.91 ± 0.98	7.27 ± 0.33	7.64 ± 0.26

At 24 h post exposure, groups of mice were sacrificed and livers, kidneys and spleens were collected. A piece of each organ sample was cut off and fixed in 4% formaldehyde solution. The fixed tissue samples were embedded in paraffin, thin-sectioned, and then mounted on glass microscope slides using the standard histopathological techniques. The mounted sections were

stained with hematoxylin-eosin (HE) and examined by light microscopy. The results are shown in Fig. S6. There is no histopathological change in these organs.

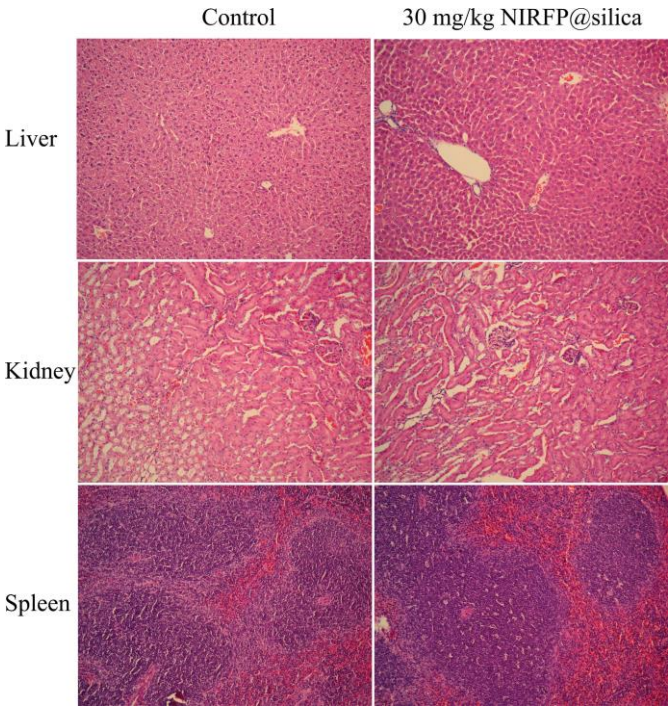

Fig. S6. Opitcal images of HE stained liver, kidney and spleen of mice at 24 h post i.v. injection with 30 mg kg^{-1} NIRFP@silica.