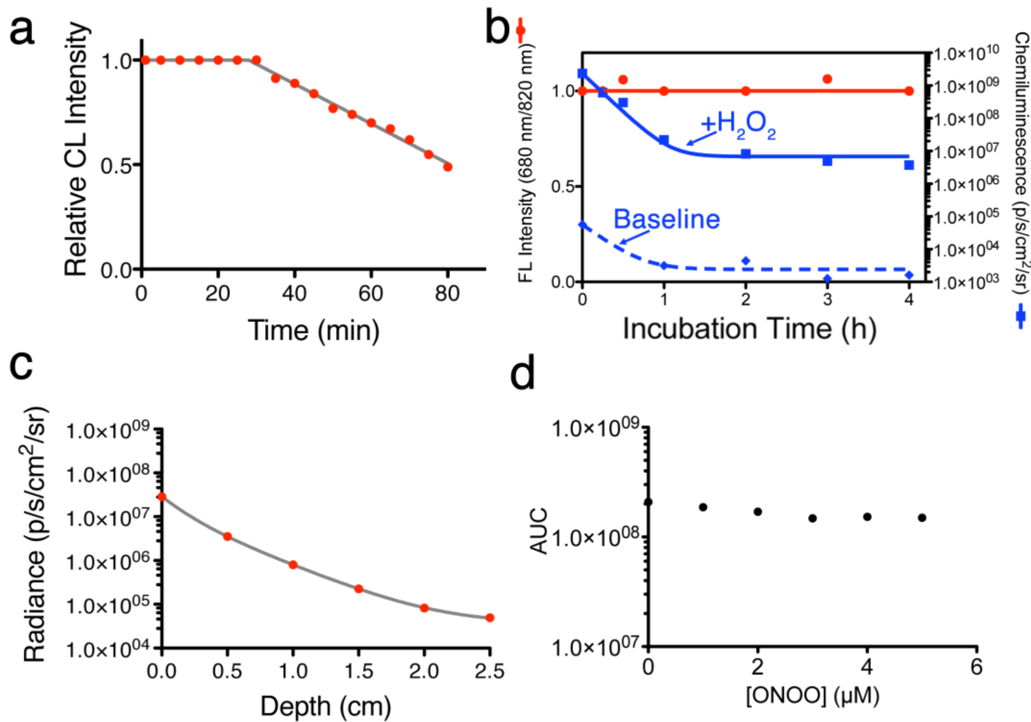
Supplementary Information

Real-time imaging of oxidative and nitrosative stress in the liver of live animals for drug-toxicity testing

Adam J. Shuhendler[1*], Kanyi Pu[1*], Lina Cui[1], Jack P. Uetrecht[2] & Jianghong Rao[1]

[1] *Molecular Imaging Program at Stanford, Department of Radiology, School of Medicine, Stanford University, Stanford, California 94305-5484, USA;*
[2] *Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 3M2, Canada*
**Both authors contributed equally to this work.*



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2 **Supplementary Figure S1.** Signal stability and imaging depth penetration for CF-SPN *in*
3 *vitro*. (a) The lifetime of chemiluminescent signal production from CF-SPN (5 µg/mL) in
4 1x PBS incubated with H_2O_2 (50 mM). (b) Stability of the baseline fluorescence ratio
5 (red) and baseline chemiluminescence emission (dashed blue) upon incubation of CF-
6 SPN (5 µg/mL) in undiluted mouse serum at 37 °C. To demonstrate the prolonged
7 capacity for ROS detection, H_2O_2 (6 µM) as added to CF-SPN incubations at indicated
8 times (solid blue). (c) Chemiluminescence imaging depth of penetration of CF-SPN (5
9 µg/mL) through a gelatin-hemoglobin-intralipid imaging phantom. (d) The total
10 chemiluminescence, as measured by area under the luminescence curve (AUC), from CF-
11 SPN after incubation with different concentrations of $ONOO^-$.

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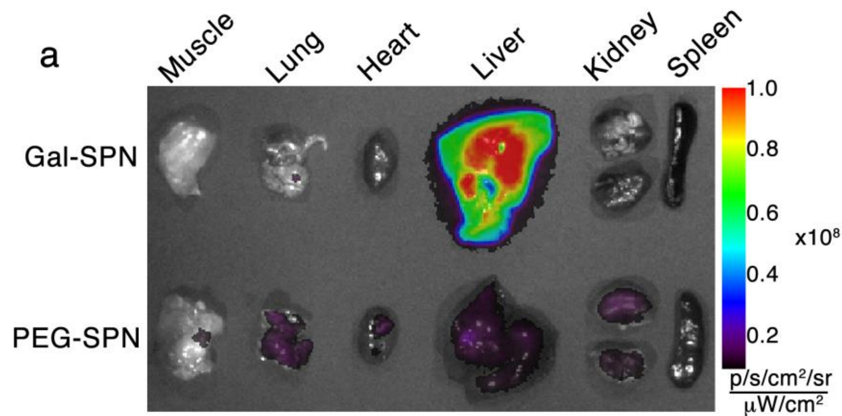

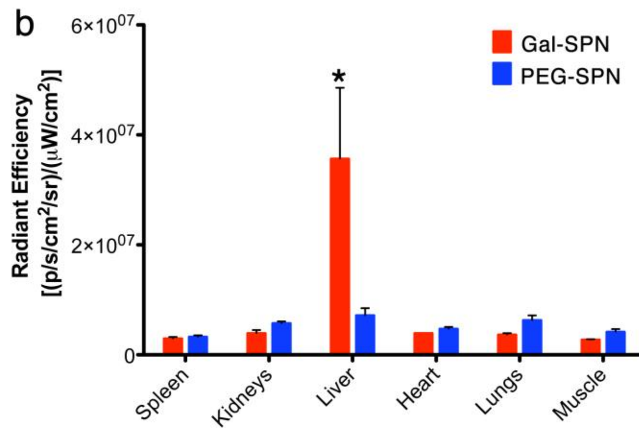

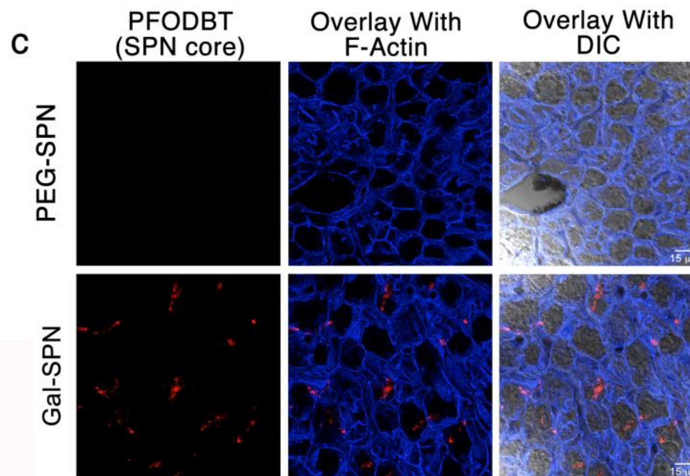

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2 **Supplementary Figure S2.** Effective liver targeting through the conjugation of galactose
3 to the SPN surface. Nanoparticles composed of PFODBT and PS-*g*-PEG-Galactose (Gal-
4 SPN) or PS-*g*-PEG (PEG-SPN) were administered i.v. (0.8 mg each). Tissues were
5 excised and imaged 45 min after nanoparticle administration. (a) Representative image of
6 the biodistribution of Gal-SPN (top) and PEG-SPN (bottom). (b) Organ fluorescence
7 (ex/em=580/680 nm) was quantified and represented as the mean±s.d. (n=3). * $p<0.05$
8 (Mann-Whitney U-test). (c) Uptake of untargeted SPN (top) and asialoglycoprotein

1 receptor-targeted Gal-SPN (bottom) 30 min following intravenous administration. Images
2 are Z-projections averaged over 20 slices and a total z-depth of 7.7 μm. Nanoprobe
3 uptake was marked by fluorescence from the conjugated polymer core composed of
4 PFODBT (red), and cellular boundaries were marked by staining of F-actin (blue).
5 Fluorescence images were overlaid with DIC of the liver sections. Scale bars = 15 μm.
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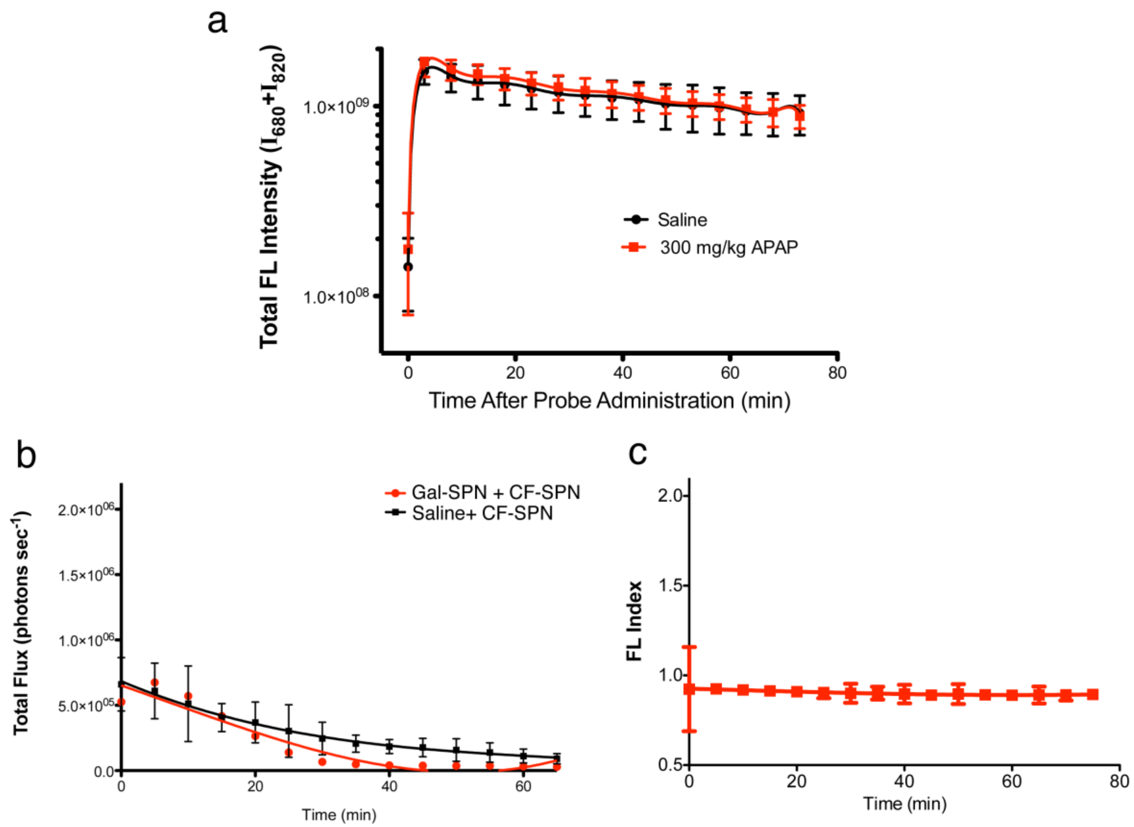

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10 **Supplementary Figure S3.** (a) The uptake and retention of CF-SPN in the liver as
11 measured by tracking total fluorescence intensity over time. Total fluorescence intensity
12 is the sum of emission at 680 nm and at 820 nm ($I_{680}+I_{820}$), which was measured for mice
13 treated with saline (black curve) or with 300 mg/kg APAP (red curve). Data points
14 represent the mean±s.d. of 3 mice. (b & c) *In vivo* assessment of hepatotoxic potential of
15 administered nanoparticles. The galactose-targeted nanoparticles (Gal-SPN, red circles)
16 or saline (black squares) were administered i.v. 15 min prior to CF-SPN, and the
17 chemiluminescence (b) and fluorescence index (c) was recorded. Data represents the
18 mean±s.d. of n=3 mice.

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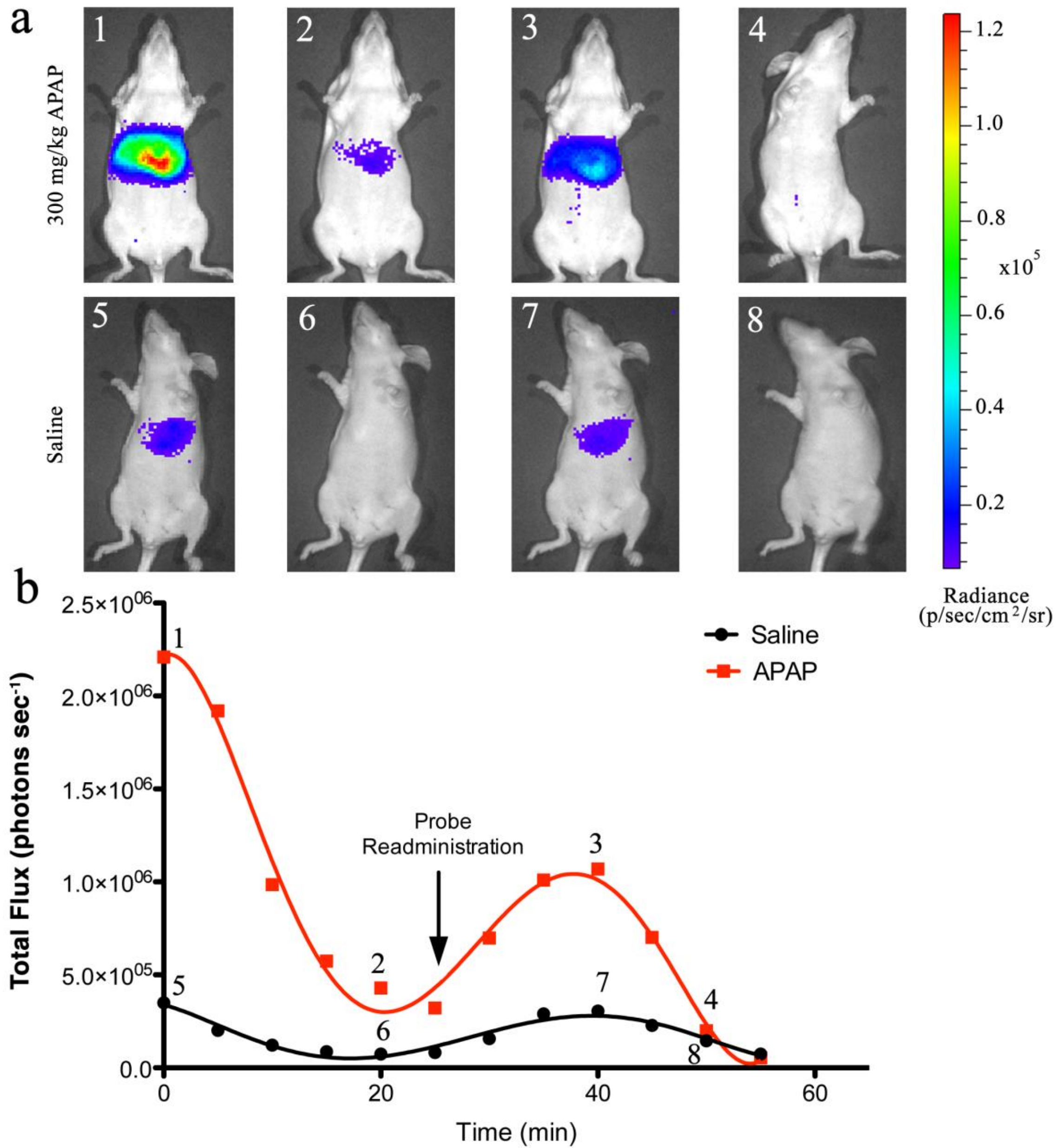

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3 **Supplementary Figure S4.** Extension of the time course of H_2O_2 detection after drug
4 challenge by re-administration of CF-SPN. Mice were administered 300 mg/kg APAP
5 (top row, 1-4) or saline (bottom row, 5-8) i.p., followed by the administration of 0.8 mg
6 CF-SPN i.v. After 25 min, CF-NP was re-administered i.v. (a) Luminescence images and
7 (b) quantitation of liver luminescence are shown. Black arrow in (b) indicates re-
8 administration of CF-SPN. Numbers on images correspond to time points indicated on
9 plot (n=1 mouse per group).

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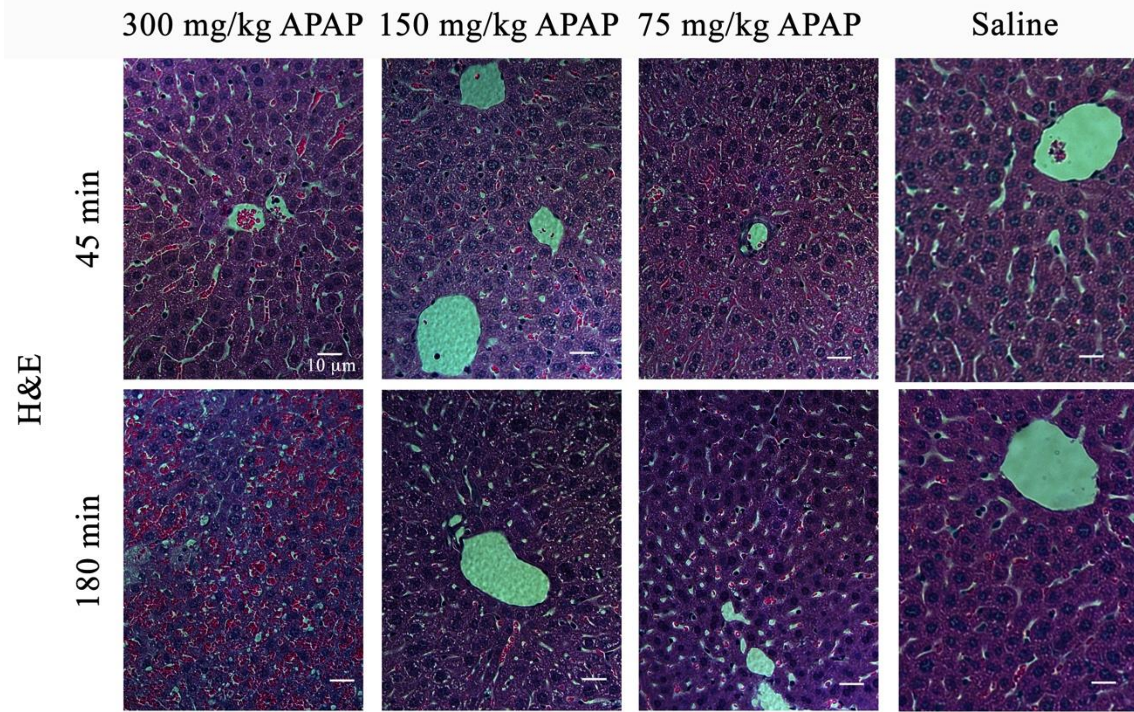

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3 **Supplementary Figure S5.** Histological analysis of liver tissues. Mice were treated,
4 from left to right, with 300, 150, 75 mg/kg APAP, or saline, and euthanized 45 min (top
5 row) or 180 min (bottom row) after drug administration. Sections were stained with
6 hematoxylin and eosin. Scale bar represents 10 µm.

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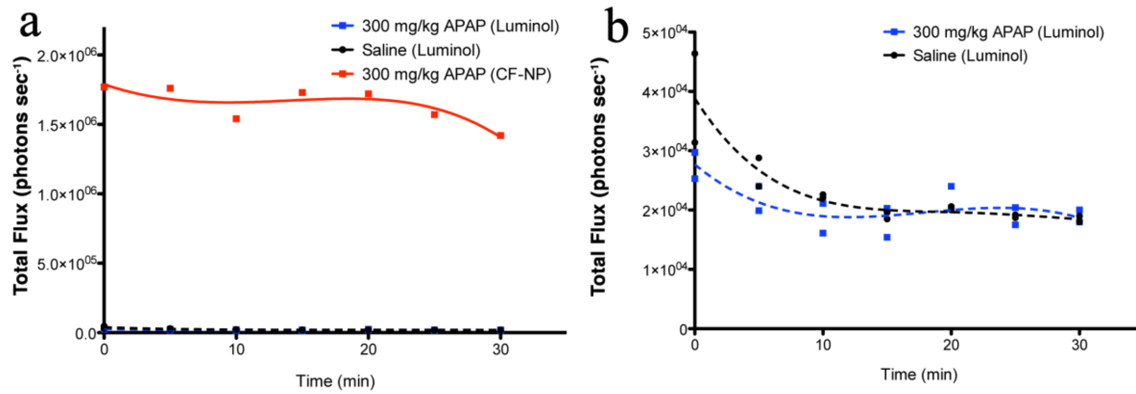

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3 **Supplementary Figure S6.** Comparison between luminol and CF-SPN for their ability to
4 detect drug-induced liver production of H_2O_2. Mice were administered 300 mg/kg APAP
5 i.p., followed either by i.v. injection of 0.8 mg CF-SPN (containing 0.2 mg CPPO), or 0.2
6 mg luminol. (a) Chemiluminescent signals from CF-SPN (red) or luminol with (dashed
7 blue) or without (dashed black) APAP treatment are shown. (b) Rescaled y-axis of (a)
8 showing the lack of any signal generation from luminol. Each group, n=1 mouse.

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