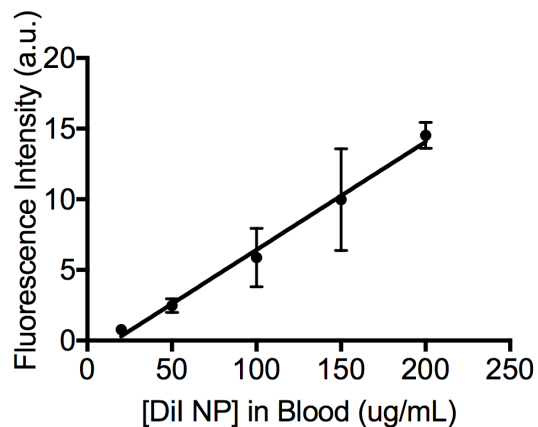


Supplemental Figure 1: Single-color NP controls. Either DiO only or Dil only NPs were diluted in a 50% mouse blood in DPBS solution and separately prepared for quantitative microscopy as described in Methods. Imaging was performed in both GFP and TRITC color channels to assess the presence of any spectral bleed between channels. A) Representative images demonstrate the absence of spectral bleed of the NPs into the second channel for either DiO or Dil NPs. Scale bars represent 150 μm . B) Quantification of multiple images confirms that lack of bleed through between either channel.

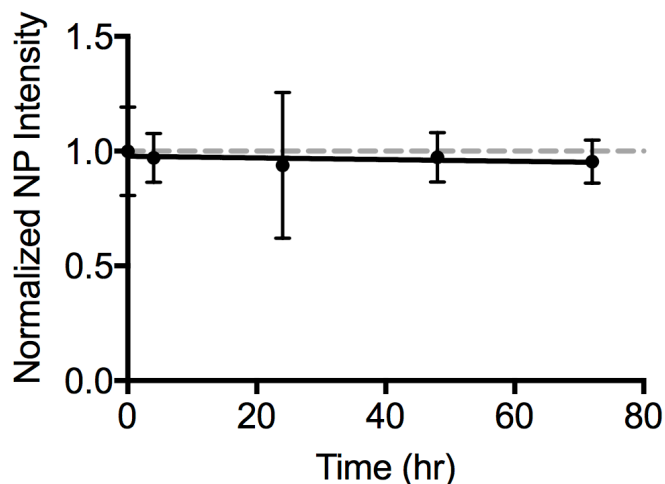


Supplemental Figure 2: Standard curve of Dil-NP using microplate read. Dil-NPs were diluted in 10% mouse blood in DPBS to a range of NP concentrations to assess sensitivity. This used a standardized blood volume of 10 μL diluted with 90 μL of DPBS for each microplate read as would be typical of a larger volume microplate based approach. The x axis reflects the concentration prior to a 10x dilution (i.e. the concentration as it would be in whole blood prior to dilution in DPBS to accommodate the 100 μL volume necessary for reproducible microplate measurements). This approach allows measurements down to $\sim 20 \mu\text{g/mL}$ circulating NP concentration using a SpectraMax M5 instrument.

Supplemental Table 1:

Circulation half-life curve fitting parameters:

Two phase decay fit parameter	Value	95% Confidence interval
Y_0	103.7	99.9 to 108.0
Plateau	6.6	4.3 to 8.9
Percent Slow	72.4	68.1 to 76.6
$K_{fast} (hr^{-1})$	4.4	2.7 to 7.7
$K_{slow} (hr^{-1})$	0.15	0.13 to 0.18
Fast Half Life (hr)	0.16	0.09 to 0.26
Slow Half Life (hr)	4.6	4.0 to 5.5
R^2	0.973	N.A.



Supplemental Figure 3: Stability of Dil-NP Intensity. Dil-NPs were incubated in freshly harvested heparinized blood at 37°C with vigorous shaking. Dashed gray line represents initial mean intensity value; solid black line is linear fit showing minimal loss of individual NP signal over the course of 72 hrs. Data points represent mean intensity value of at least 10 individual NP regions averaged over a 2 pixel x 2 pixel region centered on each individual diffraction-limited NP spot (after image thresholding to isolate individual NP regions). Error bars depict standard deviations.