Supporting Information

Real-Time Imaging of Vaccine Biodistribution Using Zwitterionic NIR Nanoparticles

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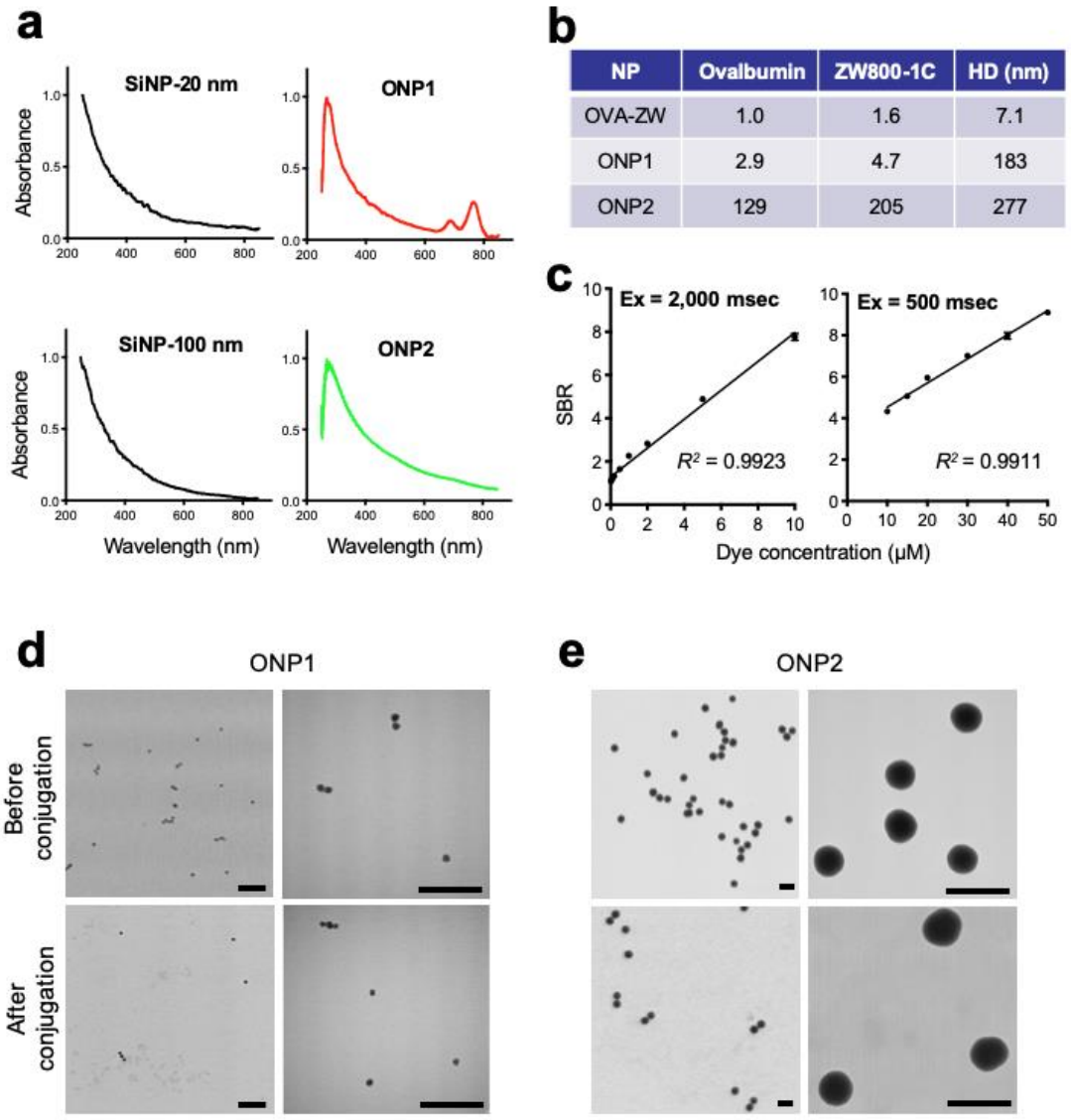
Supporting Figures:



NP	Ovalbumin	ZW800-1C	HD (nm)
OVA-ZW	1.0	1.6	7.1
ONP1	2.9	4.7	183
ONP2	129	205	277

Figure S1. Determination of dye labeling ratio on model vaccines. a) The conjugation ratio of each ONPs was determined based on the Beer-Lambert's law by measuring absorbance spectra of each model vaccine before and after conjugation using a UV-Vis-NIR spectrophotometer. b) Summary of conjugation ratio and HD of OVA-ZW, ONP1 and ONP2. c) Relationship between the concentration of ZW800-1C and its fluorescence signal on the FLARE system among different exposure times (left: 2,000 msec vs. right: 500 msec). Note that there is a linear relationship between the signal and dye concentration (n = 3, mean ± SEM.). d-e) TEM images

of d) ONP1 and e) ONP2 dispersed in pure water at different magnifications. Scale bars = 200 nm.

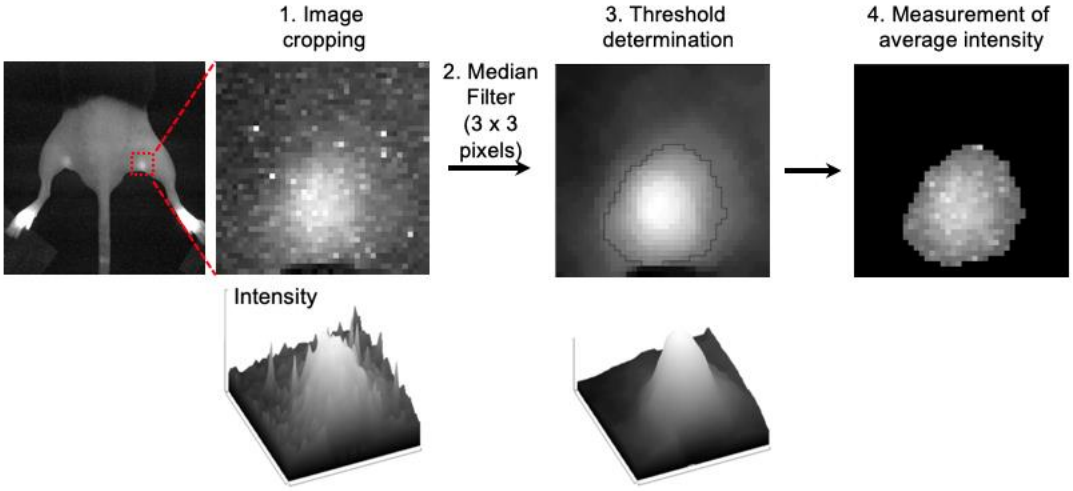


Figure S2. Quantitative ROI determination and intensity measurement scheme. All NIR fluorescence images were normalized identically for all conditions of an experiment to determine the ROI quantitatively. First, approximately 50×50 pixels of an image were cropped around the signal region of LN. Second, a 3×3 median filter was applied to the average noise. Third, a LN signal threshold was identified by an iterative selection method to determine the ROI. Finally, the average intensity of the ROI was measured on the original image.

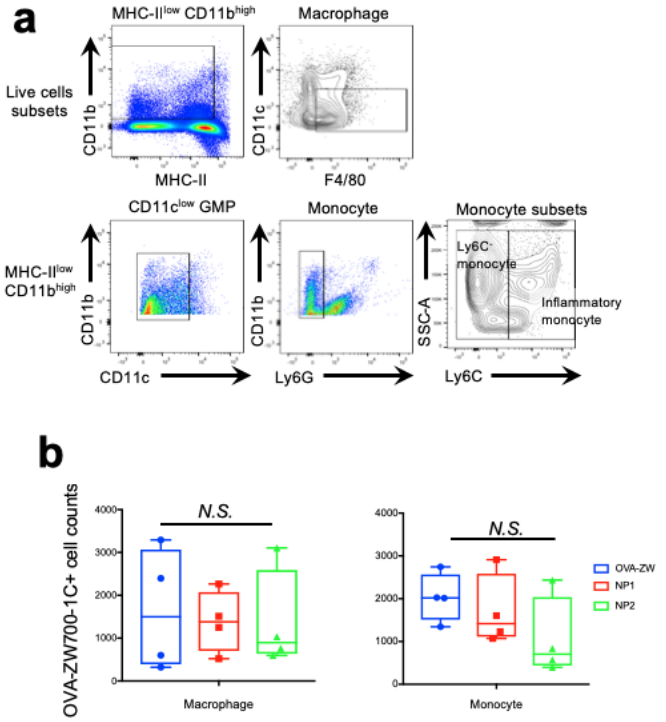


Figure S3. Flow cytometry analysis of vaccine uptake by macrophages and monocytes. Uptake of the conjugated vaccine in macrophage and monocyte populations in the draining LNs was assessed by flow cytometry 72 h post-intradermal injection. a) Gating schematic to identify macrophage and monocyte populations within LNs. b) Cell counts of OVA-ZW positive macrophage and monocyte populations within LNs (n = 4, median ± 95% confidence interval). A P value of less than 0.05 was considered significant: *N.S.*, not significant by one-way ANOVA.

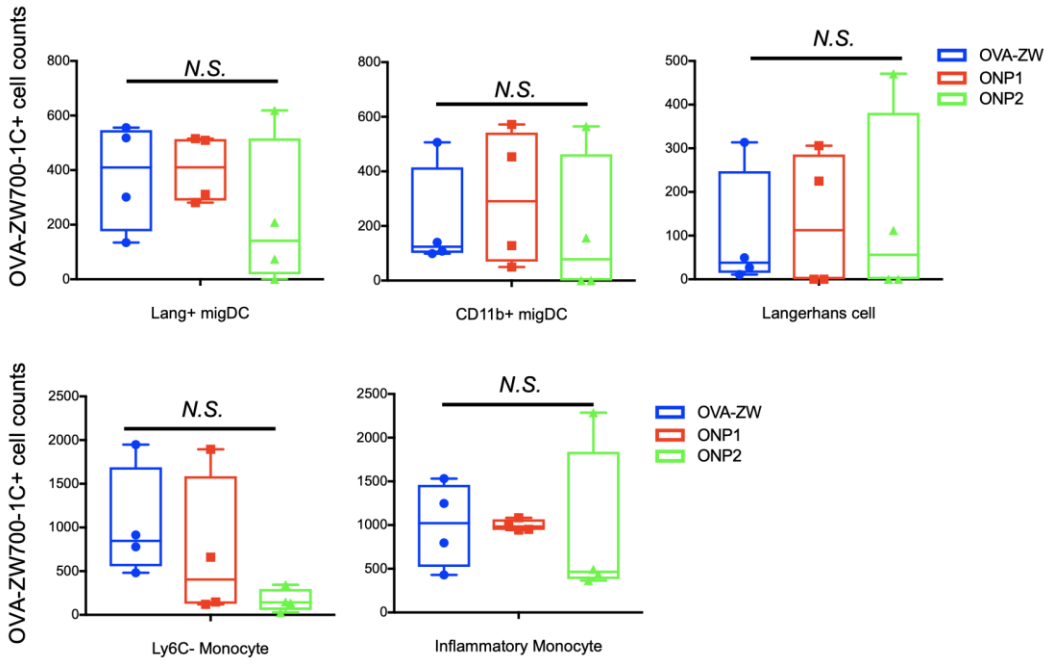


Figure S4. Flow cytometry analysis of vaccine uptake by APCs. Uptake of the model vaccines in DC and monocyte populations in the draining LNs was assessed by flow cytometry 72 h post-intradermal injection. Cell counts of OVA-ZW positive migDC and monocyte subsets (n = 4, median ± 95% confidence interval). A P value of less than 0.05 was considered significant: *N.S.*, not significant by one-way ANOVA.

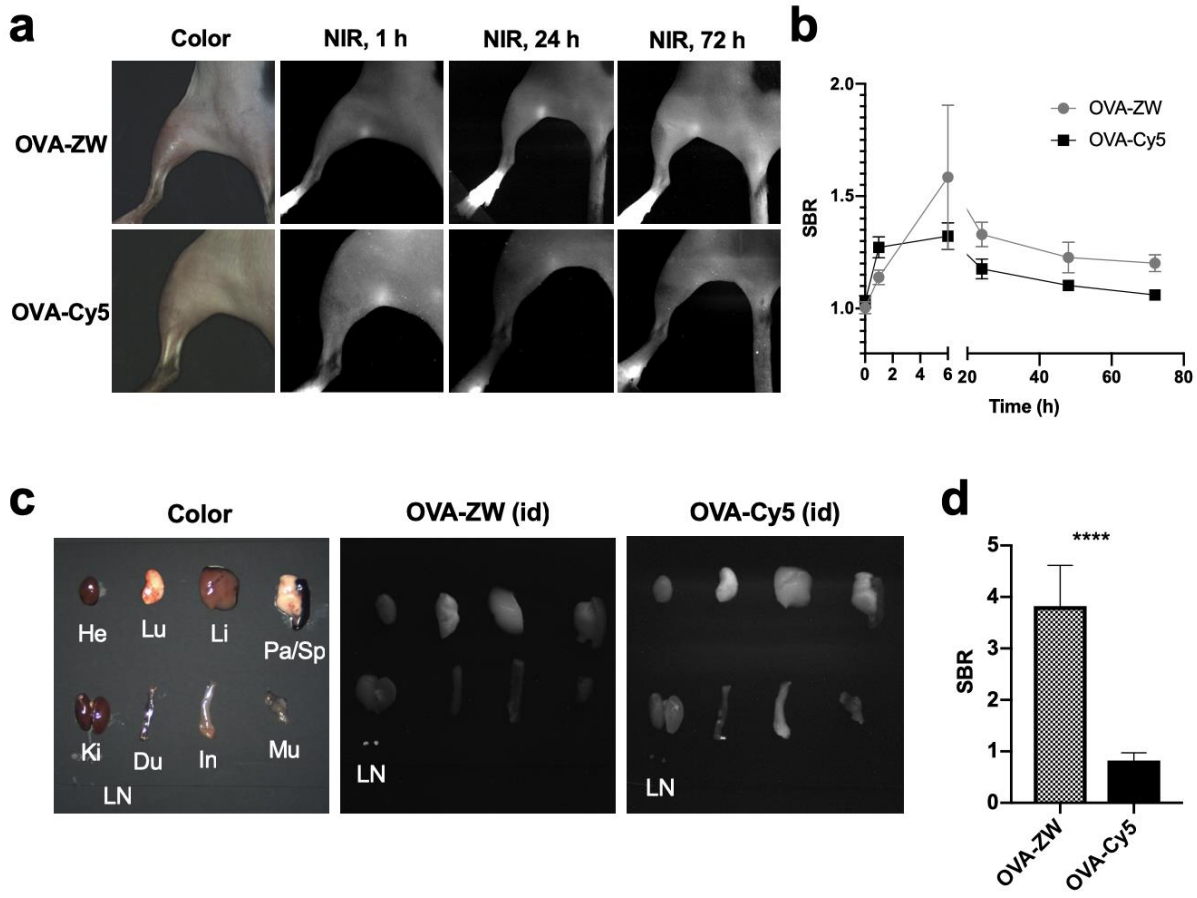


Figure S5. Comparison of fluorescence signals from a model vaccine conjugated to ZW800-1C or Cy5 after intradermal injection. a) Representative images of mice 1, 24 and 72 h post-injection with the conjugated model vaccines. Both 660 nm and 760 nm lasers' irradiance were adjusted to be 1.5 mW/cm^2 on the FLARE system. b) The SBR of popliteal LNs was calculated by comparing with background skin signals (n = 3-4, mean ± SEM.). c) Major organs including heart (He), lungs (Lu), liver (Li), pancreas (Pa), spleen (Sp), kidneys (Ki), duodenum (Du), intestine (In), muscle (Mu), and lymph node (LN) were resected and imaged 72 h post-intradermal injection of the conjugated model vaccine. d) The SBR of LNs are compared with the fluorescence signals of muscle (n = 4-6, mean ± SEM.). d) A P value of less than 0.05 was considered significant: ****P<0.0001 by Student's t-test.