Supplemental Materials

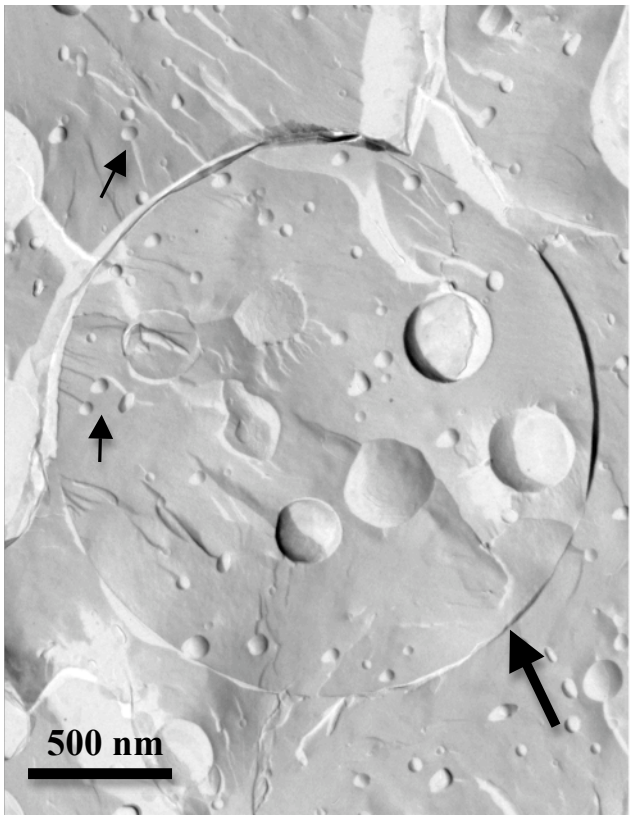


Figure S1: Freeze-fracture TEM image of as-prepared vesosomes. The short arrows point to small, 50 nm diameter liposomes of 55:45 DPPC:Chol that were encapsulated within a 94:4 DPPC:DPPE-PEG750 bilayer (large arrow) using the L_{α} - $L_{\beta'}$ - L_{bI} - $L_{\beta'}$ - L_{α} - $L_{\beta'}$ phase progression described in the text. The interior and exterior density of small liposomes (small arrows) is similar suggesting a passive encapsulation mechanism caused by the softening of the bilayer on heating. The untrapped liposomes can be removed by size exclusion chromatography to leave only the larger vesosomes. There are three distinct environments for ciprofloxacin – one exterior to the vesosome in which fast ciprofloxacin diffusion occurs, and two slow diffusing compartments, one inside the interior liposomes that have a low pH due to encapsulated ammonium sulfate and the space between the interior liposomes and the external bilayer that is at the same pH as the surrounding solution.

Figure S2. ^{1}H NMR spectra showing the chemical shift of histidine at pH 5.19 and 7.64. The calibration curves below for two histidine protons show the change in chemical shift with pH used to determine the vesosome and liposome internal and external pH in situ.

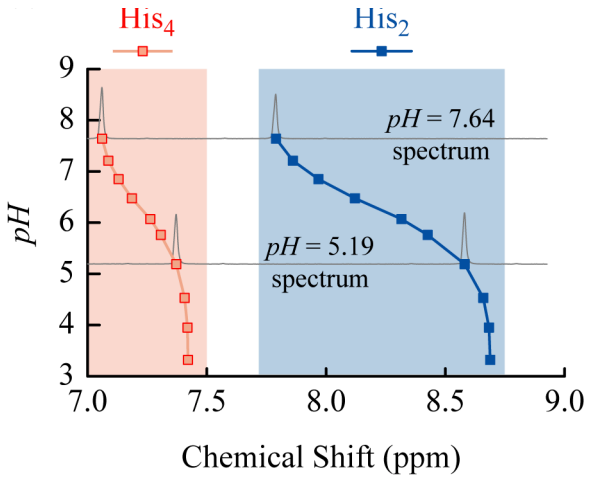
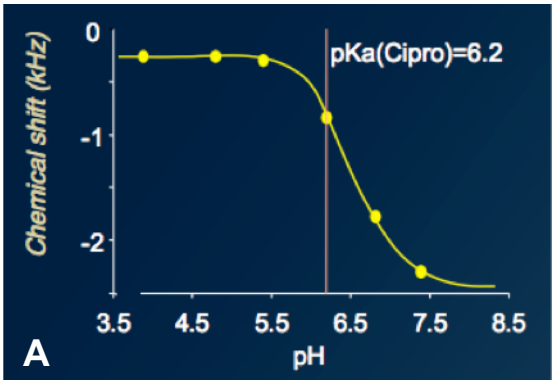


Figure S3. (A) The chemical shift of the ciprofloxacin fluorine in ^{19}F NMR also depends on the pH, similar to the proton spectra of histidine. (B) NMR spectra taken one minute apart of unilamellar liposomes containing cipro loaded with ammonium sulfate. The bottom spectra shows that all the encapsulated ciprofloxacin is in an environment with a pH of ~ 4.5. With time, the encapsulated ciprofloxacin peak decreases in amplitude, but the chemical shift does not change, suggesting that the internal liposome pH does not increase above ~ 5. The free ciprofloxacin in solution has a chemical shift corresponding to ~ pH 7, which also does not change. After ~ 30 minutes exposure to serum, the encapsulated ciprofloxacin peak has disappeared and the free ciprofloxacin peak has increased. From these spectra, the interior liposome pH is not gradually degraded over time by permeation of the ammonium sulfate pH gradient. Rather, it appears that the liposomes equilibrate rapidly, that is, within the time necessary for a single NMR spectra to be taken (~ 1 minute) with the surrounding fluid once the bilayer is compromised by serum components.



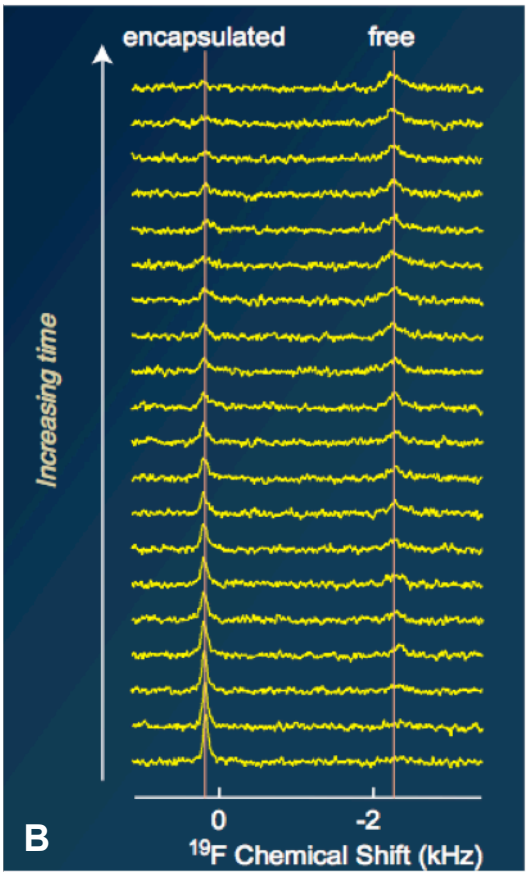
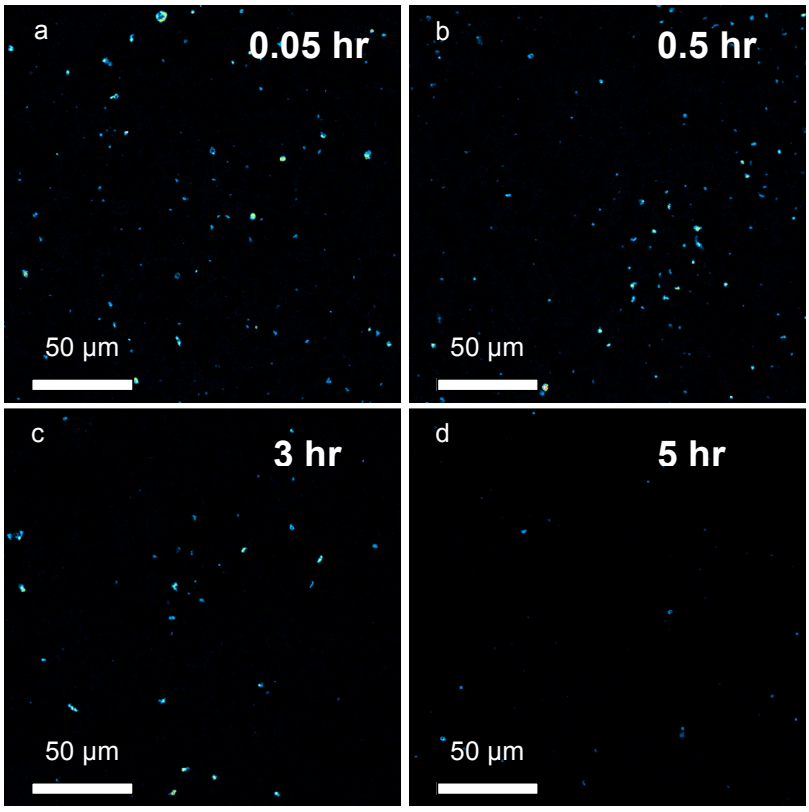


Figure S4. Fluorescence images of Texas red DHPE-labeled vesosomes taken from the optic vein of a mouse at various times after vesosome injection in the tail vein. From the images, the absolute fluorescence intensity and the size distribution and number of vesosomes could be quantified as shown in Fig. 5.



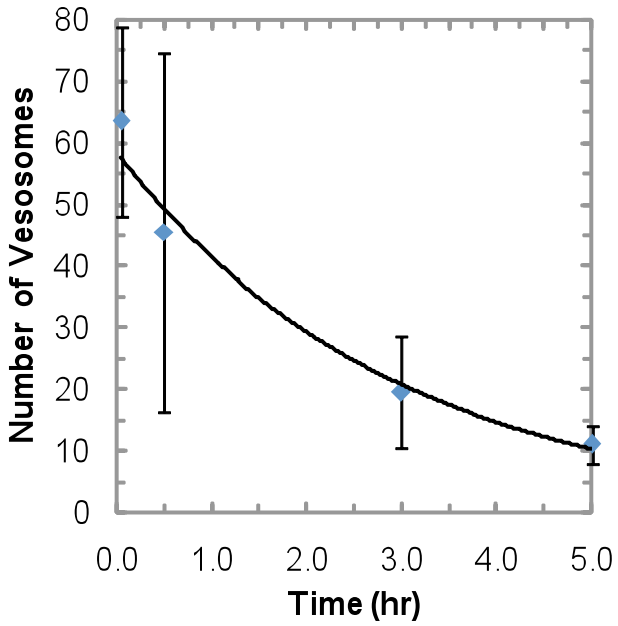


Figure S5. Vesosome half-life measured from average number of vesosomes counted per image as shown in Figure S4. The number decays exponentially with a half life of ~ 2 hours.

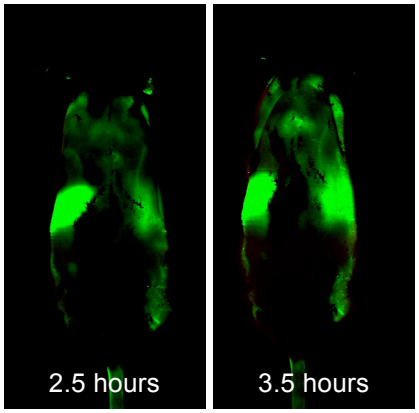


Figure S6. Dorsal view of mouse after injection with Alexa Fluor 750 labeled vesosomes. Bright green areas correspond to kidneys where the vesosomes are likely being filtered out of the circulation, similar to the biodistribution of liposomes in mice.