Supporting Information

NIR phosphorescent polymeric nanomicelles: efficient optical probes for tumor imaging and detection

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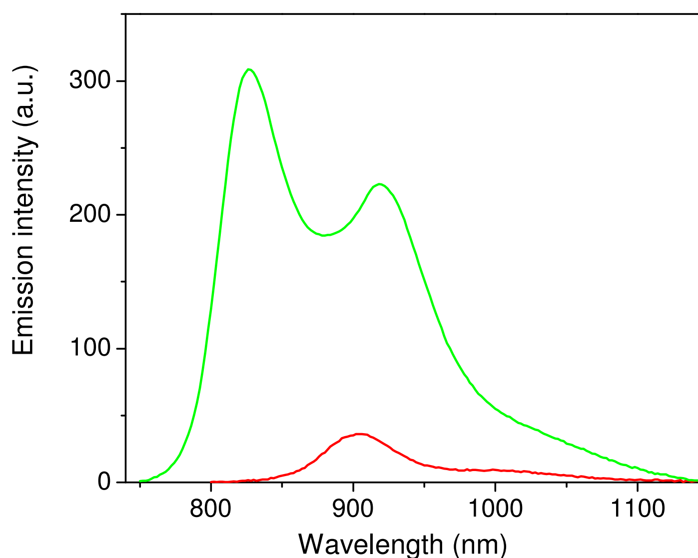
Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) images were obtained using a JEOL model JEM-100CX microscope at an acceleration voltage of 80 kV. The specimens were prepared by drop-coating the sample dispersion onto an amorphous carbon coated 300 mesh copper grid followed by addition of another drop of 1% phosphotungstic acid (for negative staining) which was neutralized with dilute NaOH solution to maintain the pH 7. The grid was placed on a filter paper to absorb excess solvent.

Measurements of the phosphorescence quantum yield.

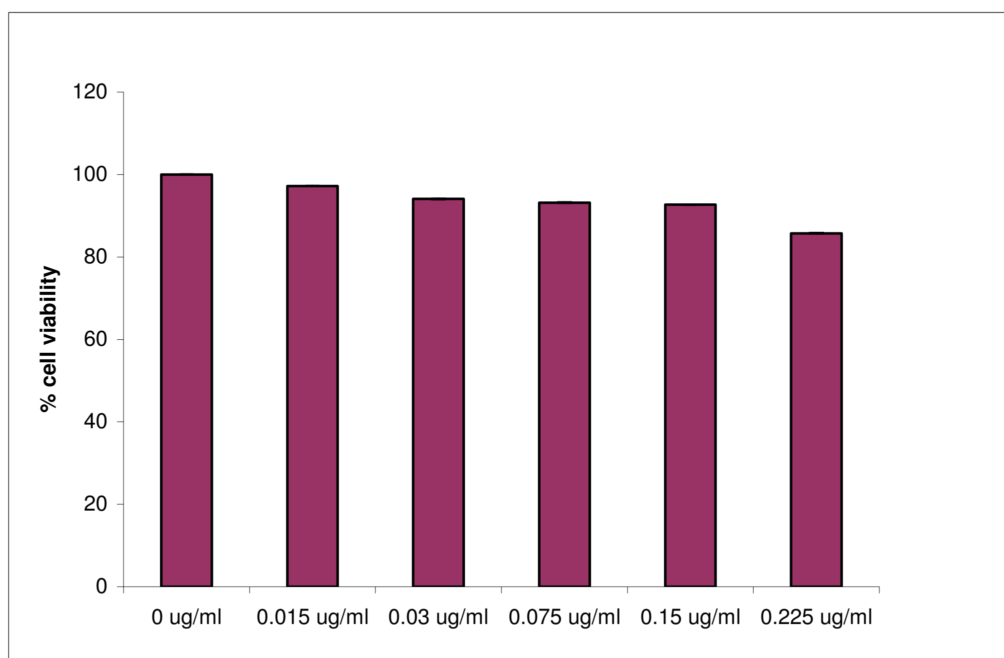
Phosphorescence quantum yield for the Pt(TPNP)/DSPE-PEG/PC nanomicelles was measured using solution of the indocyanine green dye in methanol as a reference. Fluorescence quantum yield for the methanol indocyanine green solution is 0.12.



Supporting Figure 1. Emission spectra of indocyanine green dye in methanol (green) and Pt(TPNP)/DSPE-PEG/PC micellar dispersion. Absorbance was matched at the wavelength of excitation (633 nm).

Cell viability (MTS) assay.

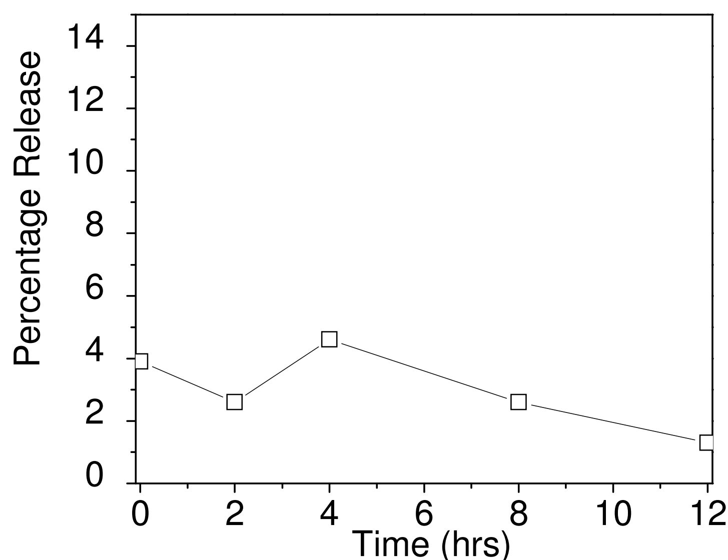
The Panc 1 cells were dispensed into a 96-well flat-bottom microtiter plate (~10,000 cells/well) and allowed to attach overnight using DMEM medium with 10% FBS. The MTS assay has been carried out as per manufacturer's instructions (PROMEGA). It is based on the absorbance of formazan (produced by the cleavage of MTS by dehydrogenases in living cells), amount of which is directly proportional to the number of live cells. In brief, after 24h treatment with Pt(TPNP)/DSPE-PEG/PC nanomicelles, media was changed and 150 µL of MTS reagent was added to each well and well mixed. The absorbance of the mixtures at 490 nm was measured. The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as a percentage. Tests were performed in quadruplicate. Each point represents the mean ±SD (bars) of replicates from one representative experiment.



Supporting Figure 2. MTS assay with Panc 1 cells following treatment with Pt(TPNP)/DSPE-PEG/PC nanomicelles , relative to untreated ‘control’ cells which are being arbitrarily assigned 100 % viability.

Release Kinetics Study.

The release kinetics study was carried out by incubating the Pt(TPNP)/DSPE-PEG/PC nanomicelles at 37°C with 1% Tween-80 solution in water. The samples were spin-filtered after a specified period of time using microfuge membrane-filter (NANOSEP 100K OMEGA, Pall Corporation, USA) at 14,000 rpm for 30 minutes (spin-filtration). The Tween-80 micelles along with any released Pt(TPNP) flowed through this membrane, while the polymeric namomicelle-encapsulated Pt(TPNP) was arrested at the membrane. The filtrate was collected and absorbance of Pt(TPNP) was measured in a spectrophotometer.

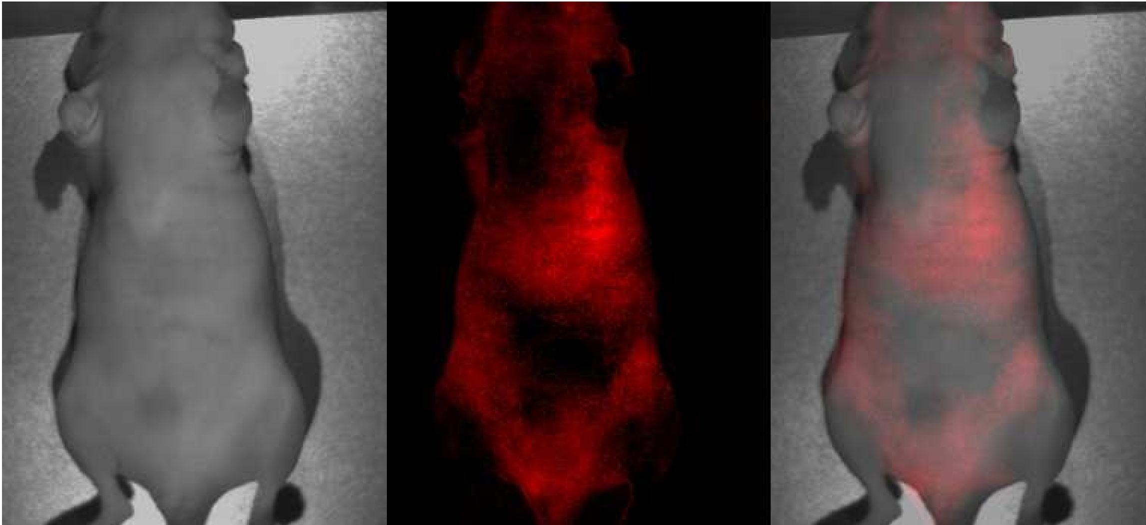


Supporting Figure 3: Release kinetics studies with Pt(TPNP)/DSPE-PEG/PC nanomicelles in 1% Tween-80 suspension at 37°C.

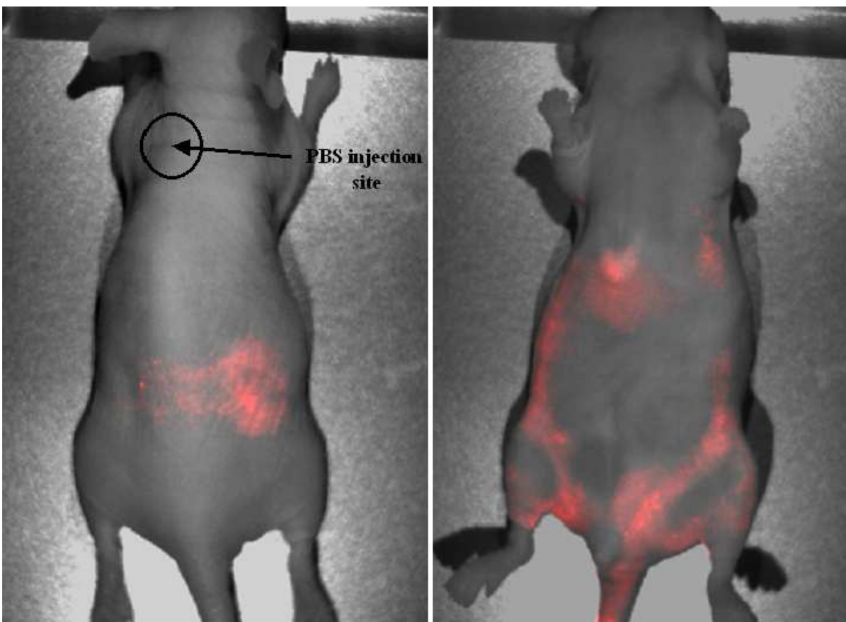
In vivo imaging of NIR Phosphorescent polymeric nanomicelles

In vivo fluorescence imaging was accomplished by using a Maestro GNIR FLEX fluorescence imaging system from (CRi). The FNPs were excited at 975 nm by the defocused emission from the fiber coupled laser diode. Emission filter (850 SP) was used to cut off excitation light.

Wavelength-resolved in vivo spectral imaging was carried out by using a spectral imaging system comprising of an optical head, an optical coupler and a cooled, scientific-grade monochrome CCD camera, along with image acquisition and analysis software. The tunable filter was automatically stepped in 10-nm increments from 750 to 950 nm while the camera captured images at each wavelength with constant exposure. Overall acquisition time was about 10s. The 20 resulting TIFF images were loaded into a single data structure in memory, forming a spectral stack with a spectrum at every pixel. With spectral imaging software, small but meaningful spectral differences could be rapidly detected and analyzed. Autofluorescence spectra and Pt(TPNP) nanomicelles spectra were manually selected from the spectral image using the computer mouse to select appropriate regions. Spectral unmixing algorithms (available from CRi) were applied to create the unmixed images of ‘pure’ autofluorescence and ‘pure’ phosphorescence signal. When appropriately generated, the autofluorescence image should be uniform in intensity regardless of the presence or absence of phosphorescence signal. The identification of valid spectra for unmixing purposes need only be done initially, as the spectra can be saved in spectral libraries and reused on additional spectral stacks.



Supporting Figure 4. Bright field (left), phosphorescence (middle) and merged (right) images of the tumored nude mouse after 2 hrs post-injection with the nanomicelles. The mouse was imaged from the dorsal side showing considerable liver uptake as well.



Supporting Figure 5. Combined bright field/phosphorescence images of the non tumored mouse which was subcutaneously injected with saline (encircled area showing the site of injection), and 30 mins later injected with Pt(TPNP)/DSPE-PEG/PC nanomicelles (tail vein injection). Imaging was carried

out 24 hrs later; no localization of the Pt(TPNP)/DSPE-PEG/PC nanomicelles at the injected site was observed.