SUPPLEMENTARY METHODS

Nanoparticle characterization

COOH- and PEG-coated fluorescent NPs of all sizes were measured by laser Doppler anemometry for net surface charge (ξ-potential), PDI, and hydrodynamic diameter using a Zetasizer NanoZS (Malvern Instruments). Size measurements and PDI were performed at 25°C at a scattering angle of 90°. Samples were diluted 500-fold in ACSF, pH 7.0. Nanoparticles in the brain microenvironment are exposed to approximately a 300 mM ion concentration in ACSF *in vivo* and *ex vivo*; however, at this high ion concentration, the accuracy of ξ-potential measurements can be negatively impacted. ACSF was diluted from 100 mM NaCl to 10 mM NaCl to obtain accurate ζ-potential measurements.

PEG chains have a Flory radius defined as $R_f \sim \alpha N^{3/5}$, where N is the degree of polymerization and α is the effective monomer length. An unconstrained 5000-Da PEG chain has a diameter of 5.4 nm and occupies a surface area of 22.7 nm^2 (assuming an unconstrained random walk). PEG surface concentrations (chains/100 nm^2) and Γ/SA was calculated from the ^1H integrals of the bis(trimethylsilyl) benzene (BTSB, Sigma) peak (0.2 ppm, internal standard) and the ethylene oxide peak of PEG (3.6 ppm) using an adapted method published previously (49). The lyophilized PEG-PS NPs were weighed and fully dissolved in a mixture of chloroform ($CDCl_3$, Sigma), trifluoroacetic acid-d (TFAd, Sigma), and a known concentration of BTSB (0.5% w/v). ^1H NMR spectra were obtained at 400 MHz using Bruker REM400. A calibration curve was obtained by plotting the ^1H NMR integrals of various concentrations of PEG5000 polymer (3.6 ppm) in the same $CDCL_3$-TFAd solvent containing 0.5 % (w/v) BTSB. The average PEG surface density (chain/100 nm^2) on the surface of the NPs was calculated by taking into account of the total quantity of PEG detected by NMR and the total NP surface area. The surface area of PS NPs was calculated by assuming that the NPs are made of individual particles of diameter equal to that measured by laser Doppler anemometry, with smooth surfaces, and a density of 1.055g/ml provided by the manufacturer.

PLGA (50:50 lactic:glycolic acid; 58 kDa) and block copolymers of PEG (5 kDa) and PLGA were purchased from Lakeshore Biomaterials and Daigang Biomaterials Co., respectively. PLGA and PEG-PLGA were fluorescently labeled by covalent dye attachment to the free COOH on PLGA, as described previously (50). The fluorescent polymer solution (10 mg/ml in acetonitrile) was added dropwise into 40 ml ultrapure water where NPs spontaneously formed and were stirred for 3 h to remove solvent. Particles were collected, washed with ultrapure water, and resuspended. For paclitaxel (PTX)-loaded PEG-PLGA particles, PTX (NetQem) and PEG-PLGA were dissolved in acetonitrile [10% (w/w) PTX] and added dropwise into 30 ml of ultrapure water and stirred for 3 h to remove solvent. Particles were collected, lyophilized, dissolved in acetonitrile to extract excess PTX, and filtered through a 0.2-μm PTFE filter. Filtrate was injected into a Shimadzu HPLC system equipped with a C18 reverse phase column (5 μm, 4.6×250 mm; Varian Inc). PTX was eluted using an isocratic mobile phase

containing 65% acetonitrile in water at 1 ml/min and detected at 229 nm using a UV detector. The data were analyzed using LC solution software (Shimadzu Scientific Instruments). Drug loading was defined as the weight ratio of drug to total weight (polymer plus drug).

MPT in neocortical slices

Particle transport rates were measured by analyzing the trajectories of PS particles that were recorded using a silicon-intensified target camera (VE-1000, Dage MTI) mounted on an inverted epifluorescence microscope equipped with a 100× oil-immersion objective (NA 1.3). Trajectories were analyzed for at least 100 particles per particle type per sample. Three separate rat brain tissue specimens and five separate human brain tissue specimens were used for each NP type. Movies were captured using Metamorph software (Universal Imaging) at a temporal resolution of 66.7 frames/ms for 20 seconds. The experimental setup resulted in a tracking resolution of 10 nm. The coordinates of NP centroids were transformed into time-averaged MSD, defined as $\Delta r^2(\tau)$ (equation S1):

$$\langle \Delta r^2(\tau) \rangle = [x(t + \tau) - x(t)]^2 + [y(t + \tau) - y(t)]^2 \qquad \text{(S1)}$$

where τ is the time scale (or time lag), x is the distance traveled in the x-coordinate direction, y is the distance traveled in the y-coordinate direction, and t is the initial time of acquisition. Distributions of MSDs and effective diffusivities (D_o) were calculated as described previously (51). The MSD of the NPs as a function of τ can also be fit to equation S2:

$$MSD = 4D_o\tau^{\alpha} \qquad \text{(S2)}$$

Based on this relationship, the slope of the MSD versus time curve on a log-log scale is given by α, a unitless parameter that represents the extent of impediment to particle diffusion. Theoretical diffusion coefficients (D) are calculated based on the Stokes-Einstein equation (equation S3):

$$D = kT/6\pi\mu a \qquad \text{(S3)}$$

where k is Boltzman's constant, T is temperature, μ is the viscosity of the medium, and a is the particle radius.

Particle transport mode classification

The mechanism of particle transport was classified based on the concept of relative change (RC) of effective diffusion (D_{eff}) (51). Briefly, RC values of particles at varying time scales were calculated by dividing the D_{eff} of a particle at a known time scale by the D_{eff} at an earlier (reference) time scale. By calculating RC values for two time regimes (short and long), the transport mode that describes the particle transport properties over different distances and temporal scales can be obtained. The short relative change interval (RC_{short}) was defined at τ_{ref} = 0.2 s, and τ_{probe} = 1 s; the long relative change interval (RC_{long}) was defined at τ_{ref} = 1 s and τ_{probe}

= 2 s. The accuracy of the transport-mode classification was confirmed by the slopes of the MSD versus time plots, where diffusive particles generated α values of ~1, and more hindered particles gave progressively lower values with increasing time scale.

MOVIE CAPTIONS

Movie S1. Multiple particle tracking of 91-nm PLGA NPs in normal rat brain tissue ex vivo. Movie is representative of 12 movies from $n = 6$ total slices.

Movie S2. Multiple particle tracking of 83-nm paclitaxel-loaded PEG-PLGA NPs in normal rat brain tissue ex vivo. PTX loading was 2.5% (w/w), defined as the weight ratio of drug to polymer. Movie is representative of 9 movies from $n = 3$ total slices.