Supporting Information

DNA-AgNC Loaded Liposomes for Measuring Cerebral Blood Flow Using Two-Photon Fluorescence Correlation Spectroscopy

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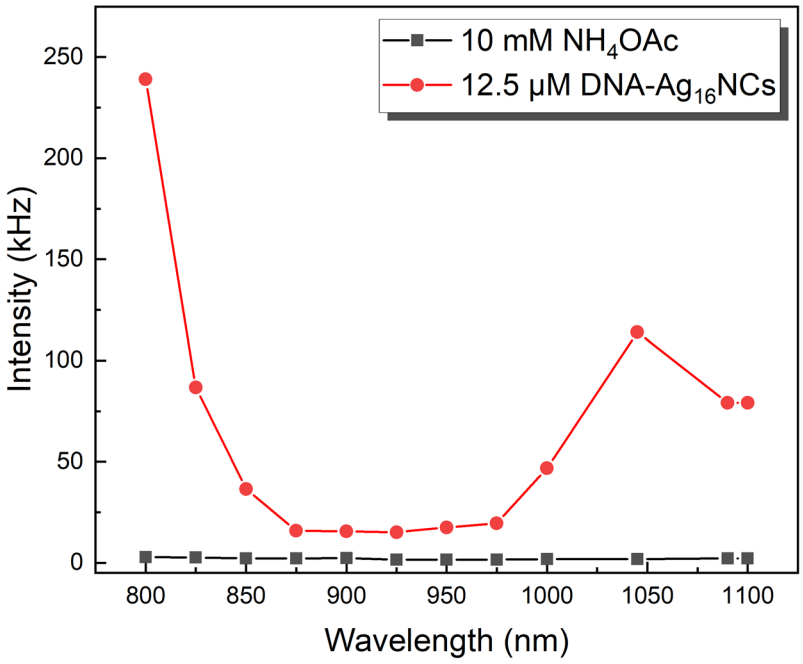


Figure S1. Two-photon excitation spectra of 12.5 μM DNA-Ag_{16}NCs and 10 mM ammonium acetate by measuring the intensity in the red/NIR I channel (590-740 nm).

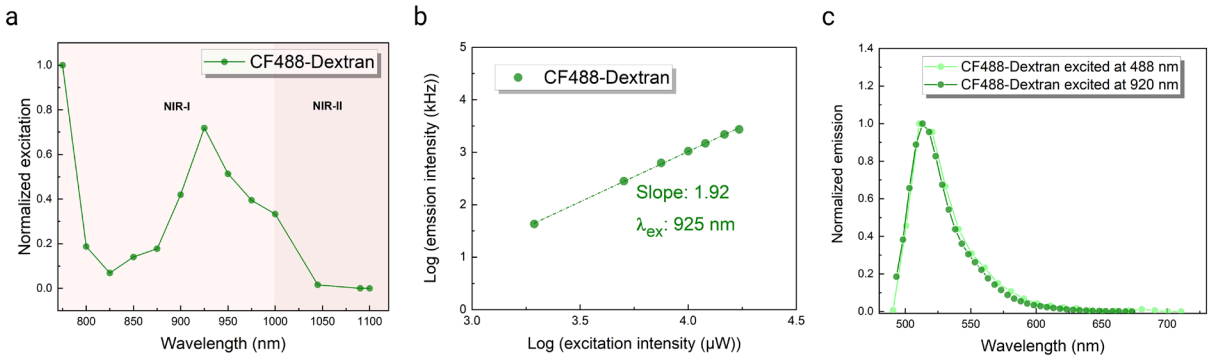


Figure S2. Two-photon optical properties of CF488-Dextran. (a) The normalized two-photon excitation spectrum of CF488-Dextran. (b) Excitation intensity dependence of CF488-Dextran. (c) Normalized emission spectra of CF488-Dextran under one- and two-photon excitation.

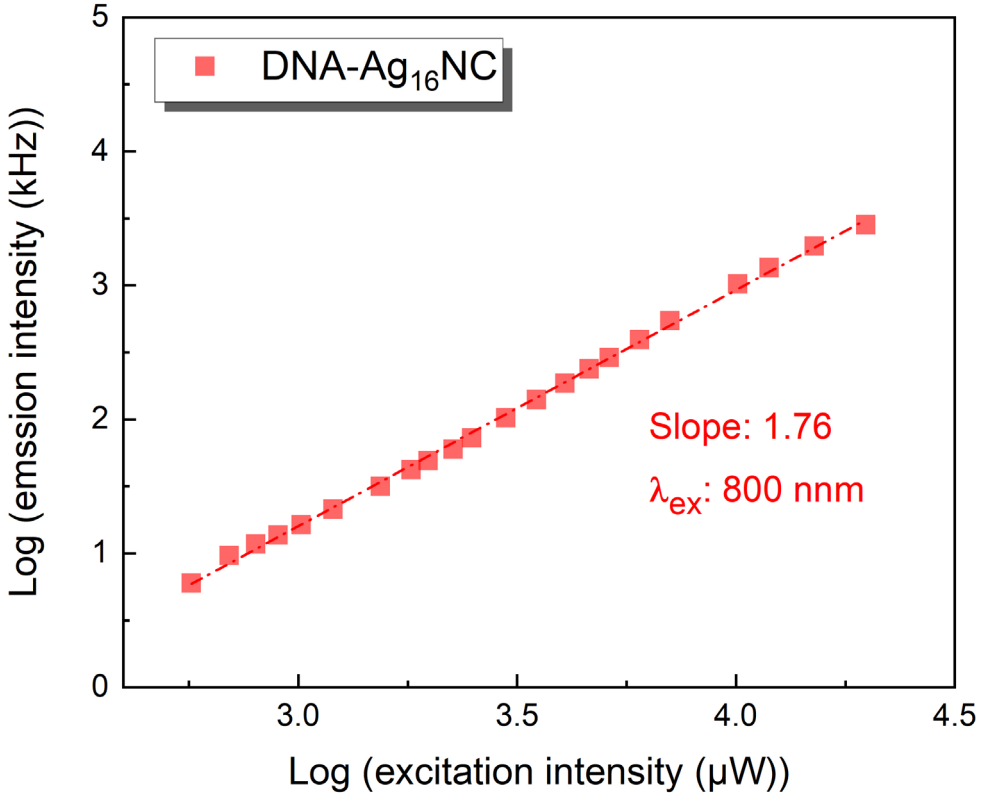


Figure S3. Power dependence of DNA-Ag_{16}NCs under 800 nm excitation with emission collected in the red/NIR I channel (590-740 nm).

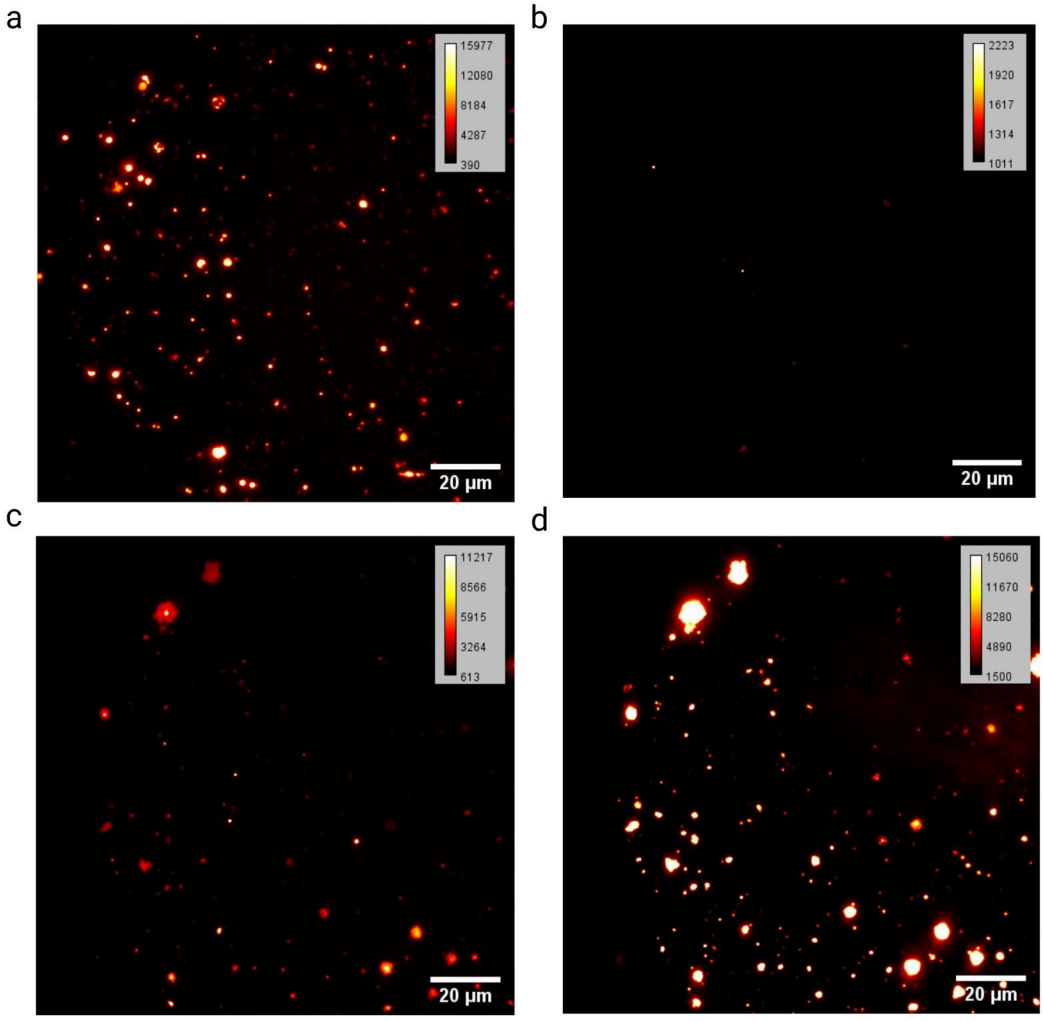


Figure S4. TIRF images of 100 nm liposome loaded with DNA-Ag_{16}NCs under (a) 561 nm and (b) 640 nm excitation. TIRF images of DNA-Ag_{16}NCs-loaded and DiD-stained 100 nm liposomes under (c) 561 nm and (d) 640 nm excitation. Emission was collected from 651 nm to 695 nm.

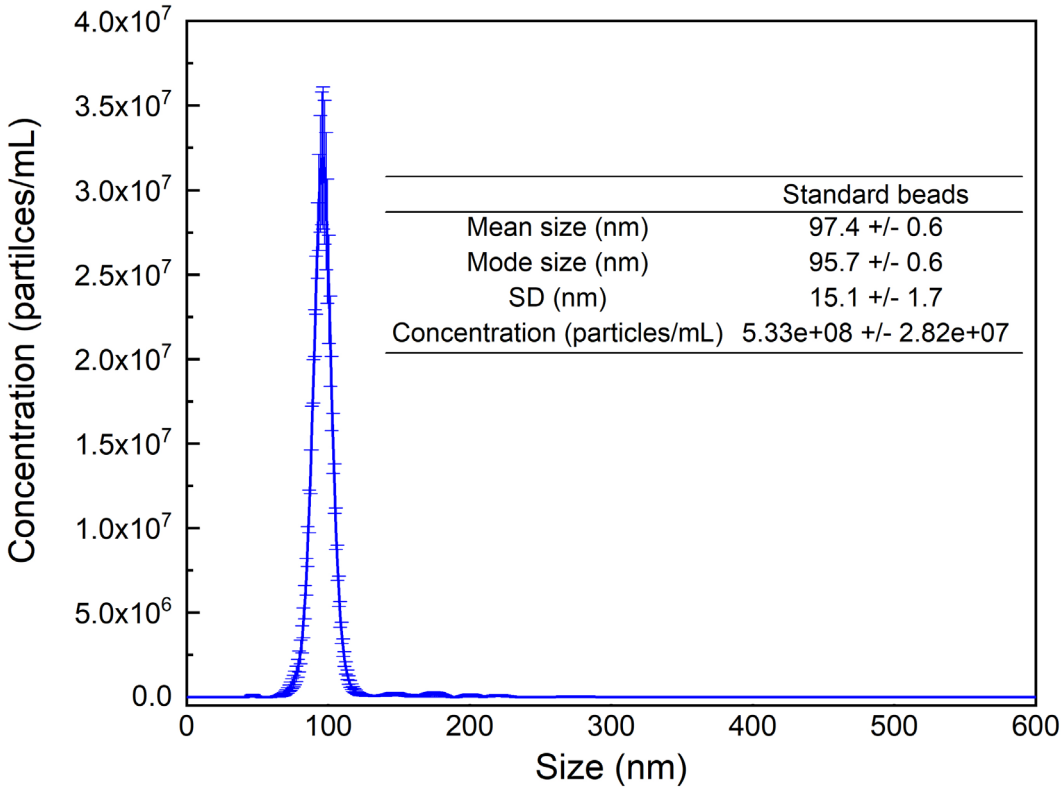


Figure S5. Size distribution of 100 nm beads measured with the NTA system.

Table S1. Size and concentration evaluation of synthesized liposomes.

	100 nm liposome	**50 nm liposome**	**Non-extruded**
Mean size (nm)	110.0 ± 3.0	86.4 ± 1.5	194.2 ± 2.6
Mode size (nm)	91.1 ± 5.0	79.5 ± 1.3	151.3 ± 15.1
SD (nm)	35.2 ± 4.7	29.1 ± 4.3	81.7 ± 4.9
Concentration (particles/mL)	$(2.57 \pm 0.25) \cdot 10^{13}$	$(4.00 \pm 0.42) \cdot 10^{13}$	$(8.39 \pm 2.75) \cdot 10^{12}$

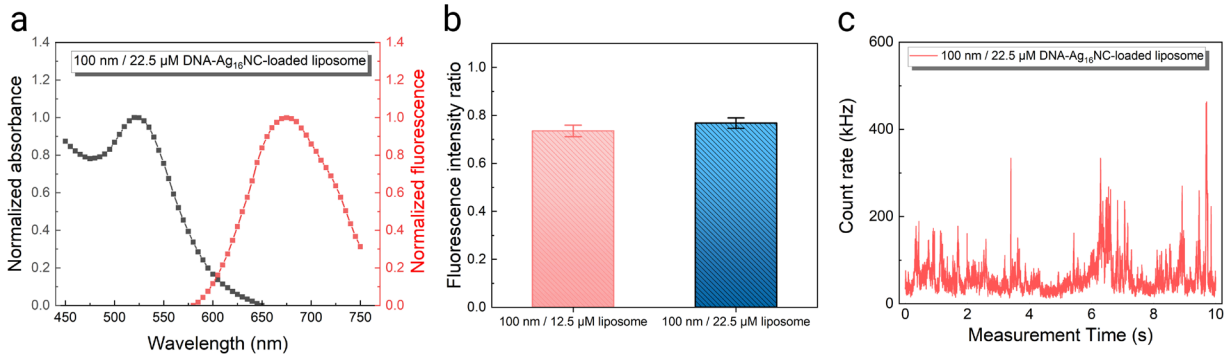


Figure S6. Characterization of 100 nm liposomes loaded with 22.5 μM DNA-Ag_{16}NCs. (a) Normalized absorption and fluorescence spectra of 22.5 μM DNA-Ag_{16}NC-loaded liposomes. (b) Fluorescence intensity ratio before and after the size exclusion column purification of 100 nm loaded liposomes from free DNA-AgNCs. The fluorescence intensity ratios were calculated using emission at 680 nm, before and after purification. The error bar represents the standard deviation of measured ratios at 680±10 nm. (c) Emission intensity fluctuations of 22.5 μM DNA-Ag_{16}NCs loaded liposomes under 1045 nm two-photon excitation. Emission was collected in the red/NIR I channel (590-740 nm).

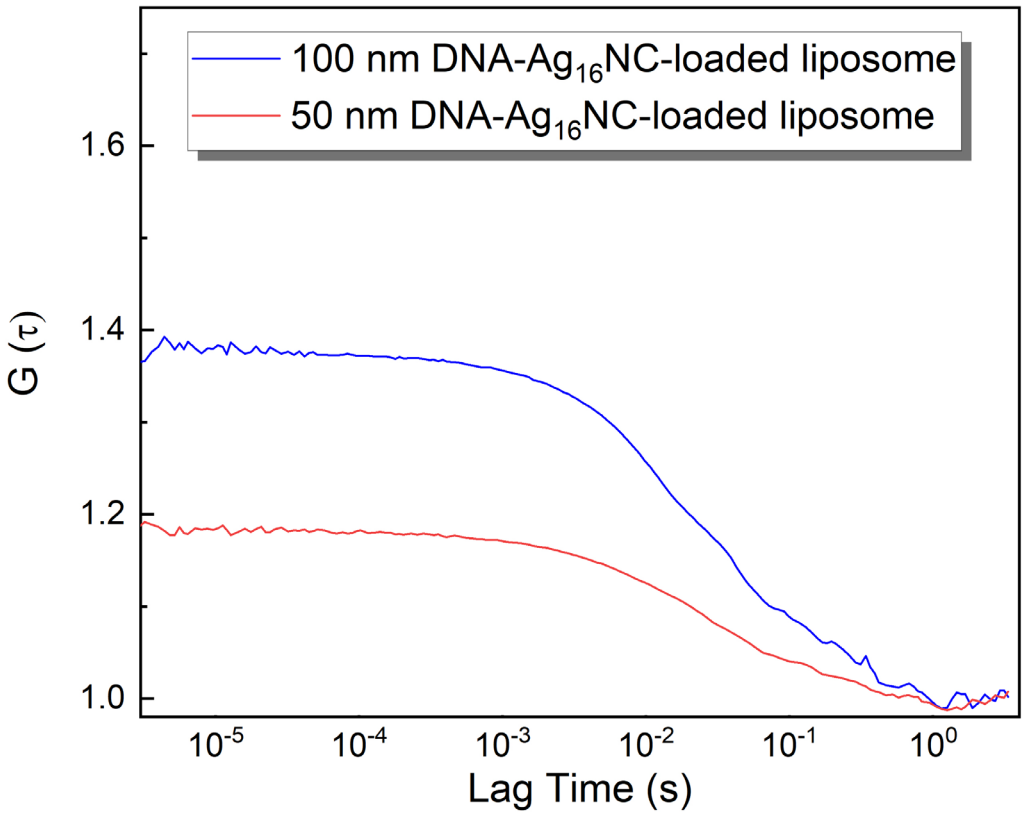


Figure S7. Autocorrelation functions of freely diffusing liposomes loaded with DNA-Ag_{16}NCs.

Table S2. FCS results for freely diffusing DNA-Ag_{16}NC-loaded liposomes with different sizes.

	100 nm liposome	**50 nm liposome**
Diffusion time (μs)	102083.10	53725.18
Particle number	1.48	2.33
Counts per particle (kHz)	7.09	6.37

Table S3. FCS results for liposomes with different sizes and DNA-Ag_{16}NC concentrations at a flow rate of 0.1 mL/min.

	50 nm / 12.5 μM	**100 nm / 12.5 μM**	**100 nm / 37.5 μM**
Residence time (μs)	467.67	485.04	472.56
Particle number	1.54	1.03	1.34
Counts per particle (kHz)	44.73	71.81	171.93

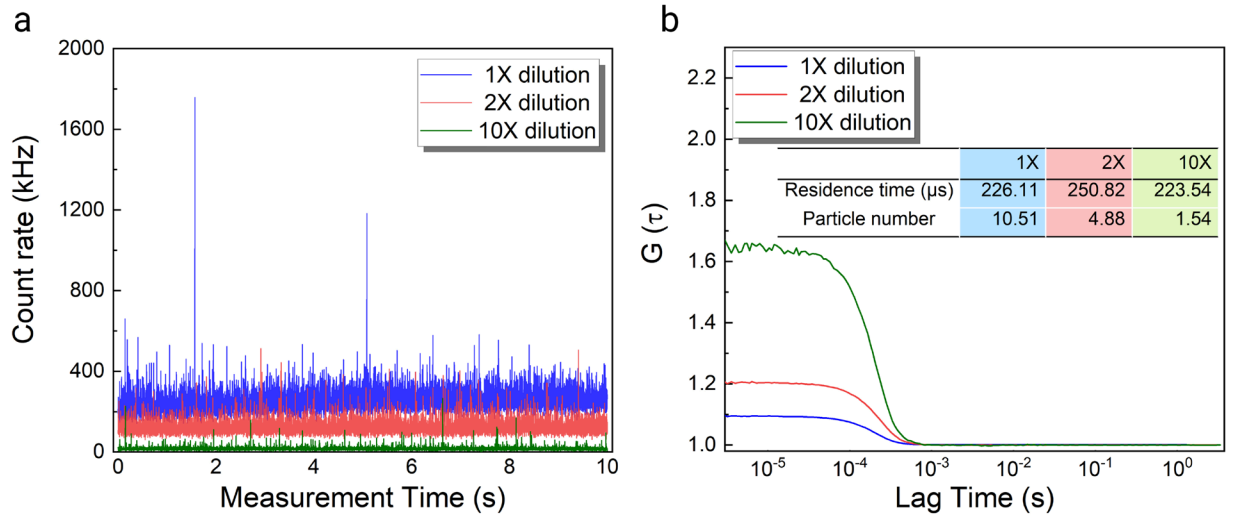


Figure S8. (a) Emission intensity fluctuations and (b) autocorrelation functions of 100 nm /12.5 μM DNA-Ag_{16}NCs-loaded liposomes at different concentrations (two- and ten-times dilutions) in the flow chamber under 1045 nm excitation and 0.2 mL/min flow rate. The inserted table was the summary of the residence time and particle number from the FCS measurements.

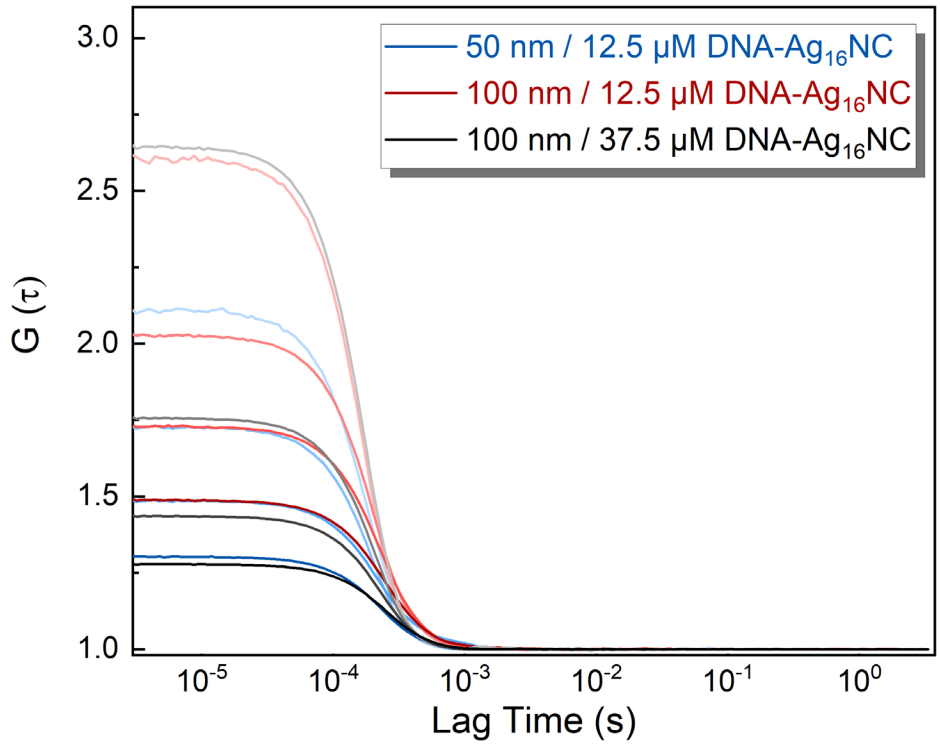


Figure S9. Autocorrelation functions of DNA-Ag_{16}NC loaded liposomes under 1045 nm excitation at different laser powers (5-25 mW) and a 0.2 mL/min flow rate.

Table S4. FCS results of DNA-Ag_{16}NC loaded liposomes excited at 1045 nm with different laser powers and a flow rate of 0.2 mL/min.

	Excitation intensity (mW)	Residence time (µs)	Particle number
50 nm / 12.5 µM	5 .0	202.46	0.89
	11.7	217.66	1.26
	18.3	245.33	2.01
	25.0	271.29	3.26
100 nm / 12.5 µM	5 .0	185.24	0.62
	11.7	233.36	0.96
	18.3	263.90	1.34
	25.0	293.06	2.04
100 nm / 37.5 µM	5 .0	190.30	0.60
	11.7	227.55	1.31
	18.3	261.06	2.27
	25.0	302.83	3.55

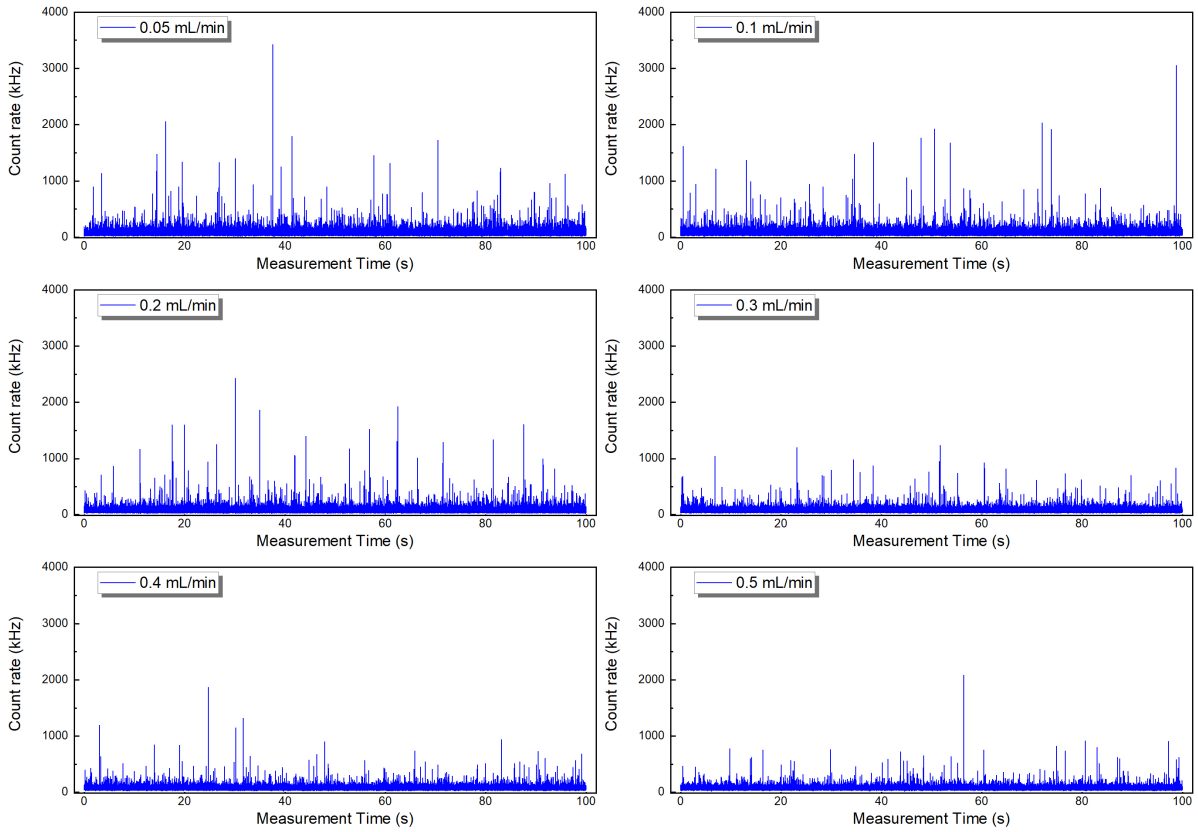


Figure S10. Emission intensity fluctuations of 50 nm liposome loaded with 12.5 μM DNA-Ag_{16}NCs under 1045 nm excitation and different flow rates.

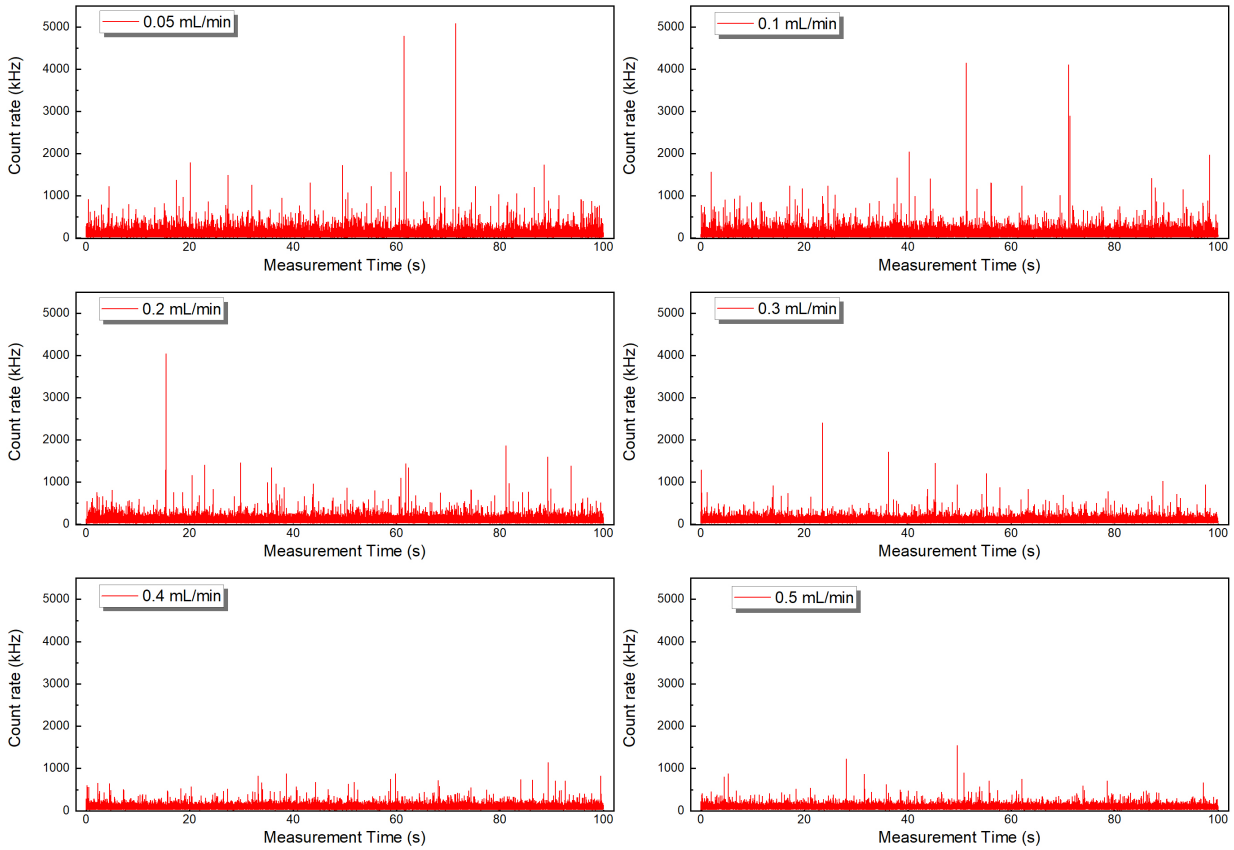


Figure S11. Emission intensity fluctuations of 100 nm liposome loaded with 12.5 μM DNA-Ag_{16}NCs under 1045 nm excitation and different flow rates.

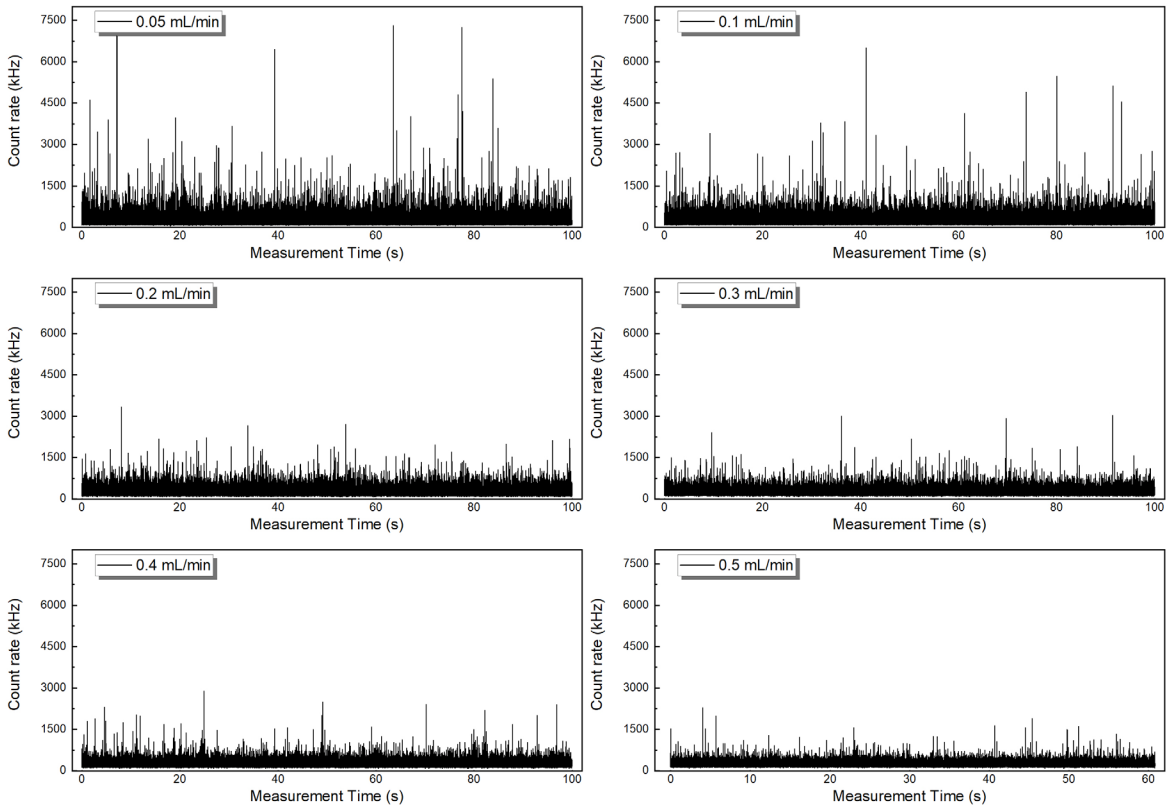


Figure S12. Emission intensity fluctuations of 100 nm liposome loaded with 37.5 μM DNA-Ag_{16}NCs under 1045 nm excitation and different flow rates.

Table S5. FCS results of DNA-Ag_{16}NC loaded liposomes excited at 1045 nm at different flow rates used for calibrating the lateral diameter.

	Residence time (µs)		
Flow rate (mL/min)	**50 nm / 12.5 µM**	**100 nm / 12.5 µM**	**100 nm / 37.5 µM**
0.05	993.71	983.67	977.62
0.10	467.67	485.04	472.56
0.20	217.66	233.36	227.55
0.30	139.59	152.60	150.00
0.40	109.03	105.07	112.28
0.50	86.68	85.70	88.44

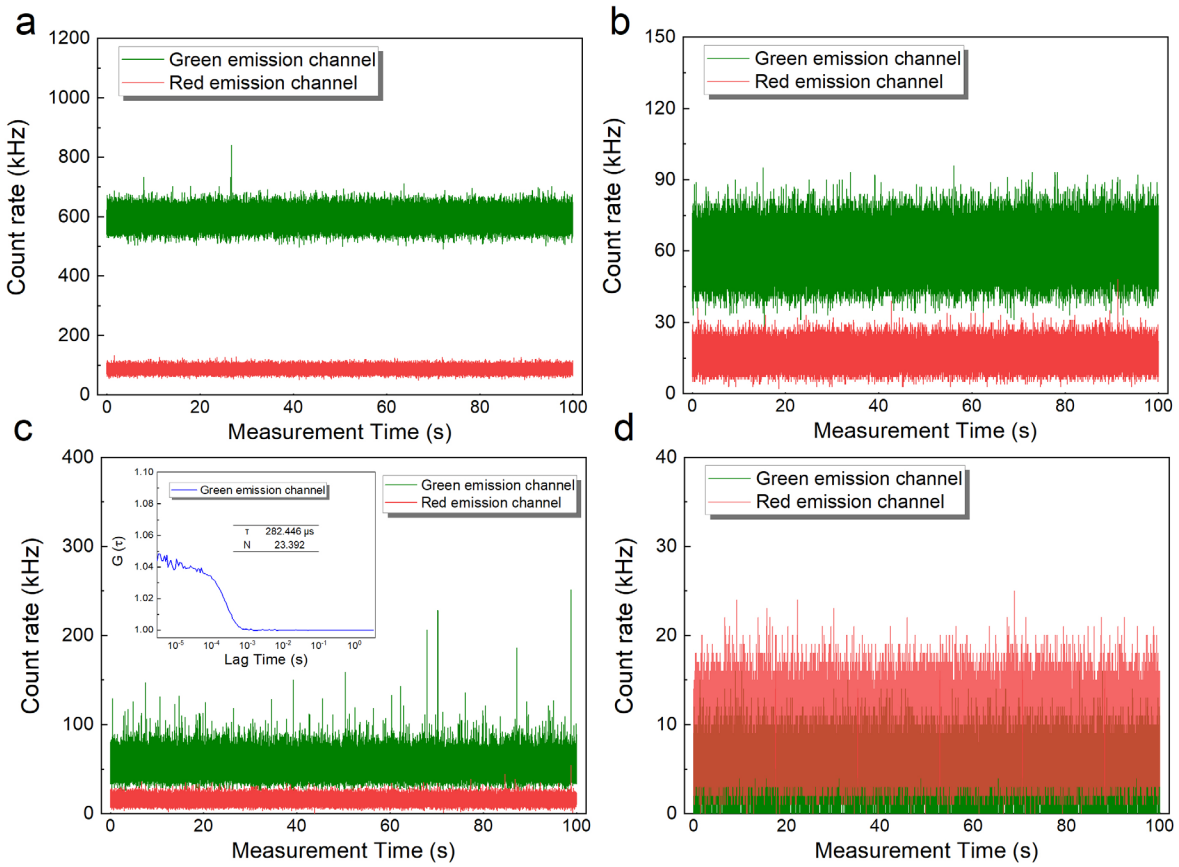


Figure S13. Emission intensity fluctuations of 1 µM FITC-Dextran 70 kDa in the flow channel at a flow rate of 0.1 mL/min under (a) 920 nm (5.5 mW) and (b) 1045 nm (11.7 mW) excitation. Emission intensity fluctuations of 5 nM FITC-Dextran 70 kDa in the flow channel at a flow rate of 0.1 mL/min under (c) 920 nm (25 mW), and (d) 1045 nm (42 mW) excitation. The green and red emission channels collected signals from 525-560 nm and 590-740 nm, respectively. The inserted figure is the autocorrelation function of 5 nM FITC-Dextran 70 kDa under 920 nm excitation and a flow rate of 0.1 mL/min.

Table S6. FCS results of DNA-Ag_{16}NC loaded liposomes excited at 1090 nm and a mixture of these liposomes and FITC-Dextran excited at 1045 nm and 1090 nm.

	Residence time (μs)	**Particle number**
Liposome excited at 1090 nm	453.02	1.07
Mixture excited at 1090 nm	446.35	1.01
Mixture excited at 1045 nm	522.82	1.99

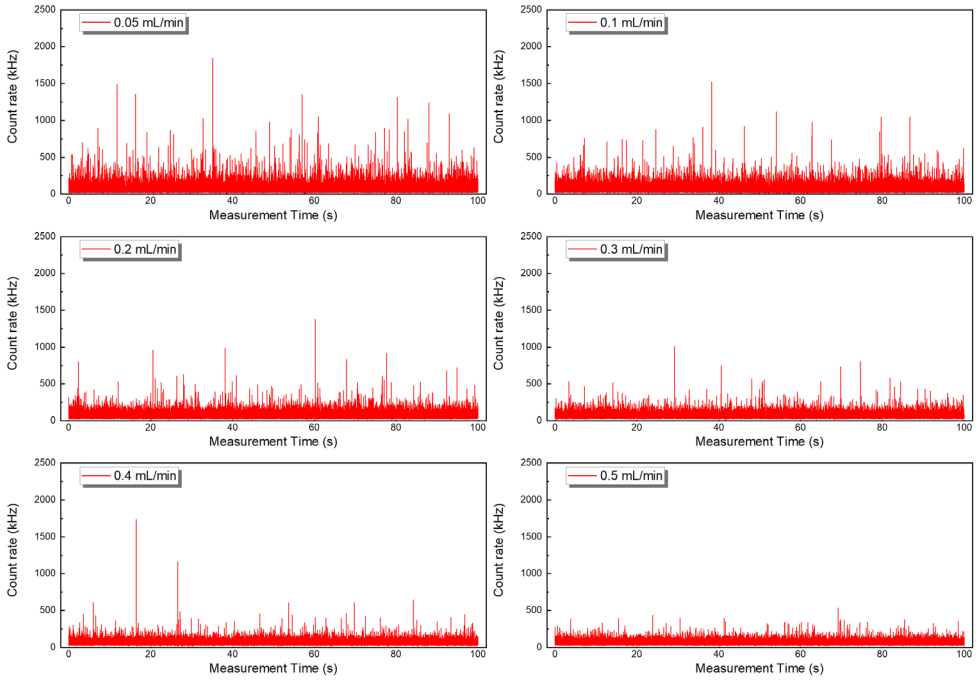


Figure S14. Emission intensity fluctuations of 100 nm liposomes loaded with 12.5 μM DNA-Ag_{16}NCs under 1090 nm excitation and different flow rates.

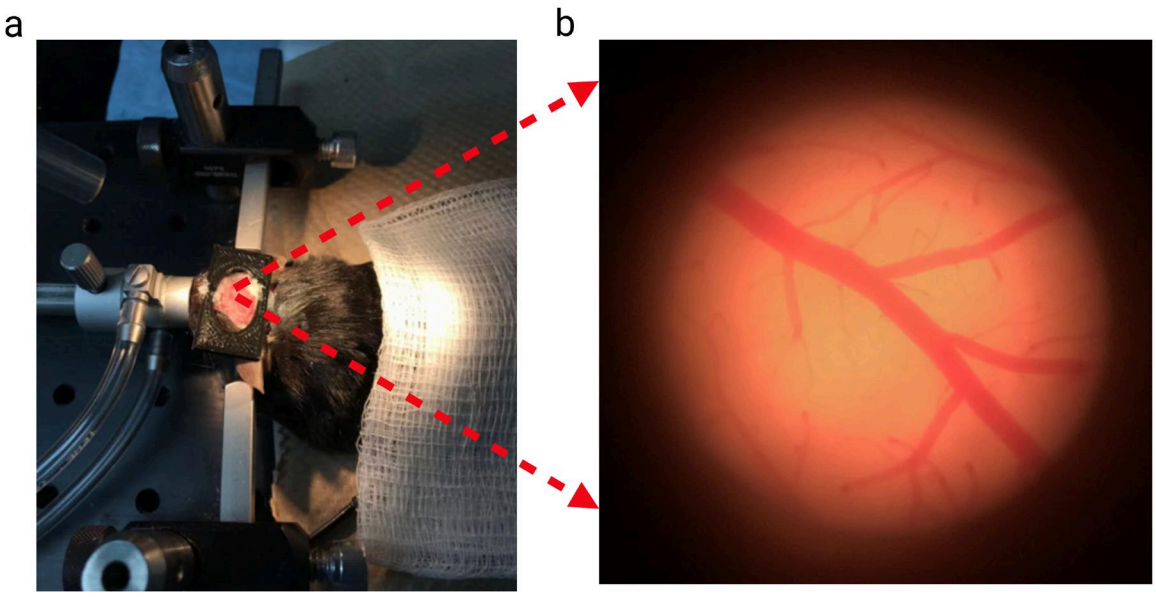
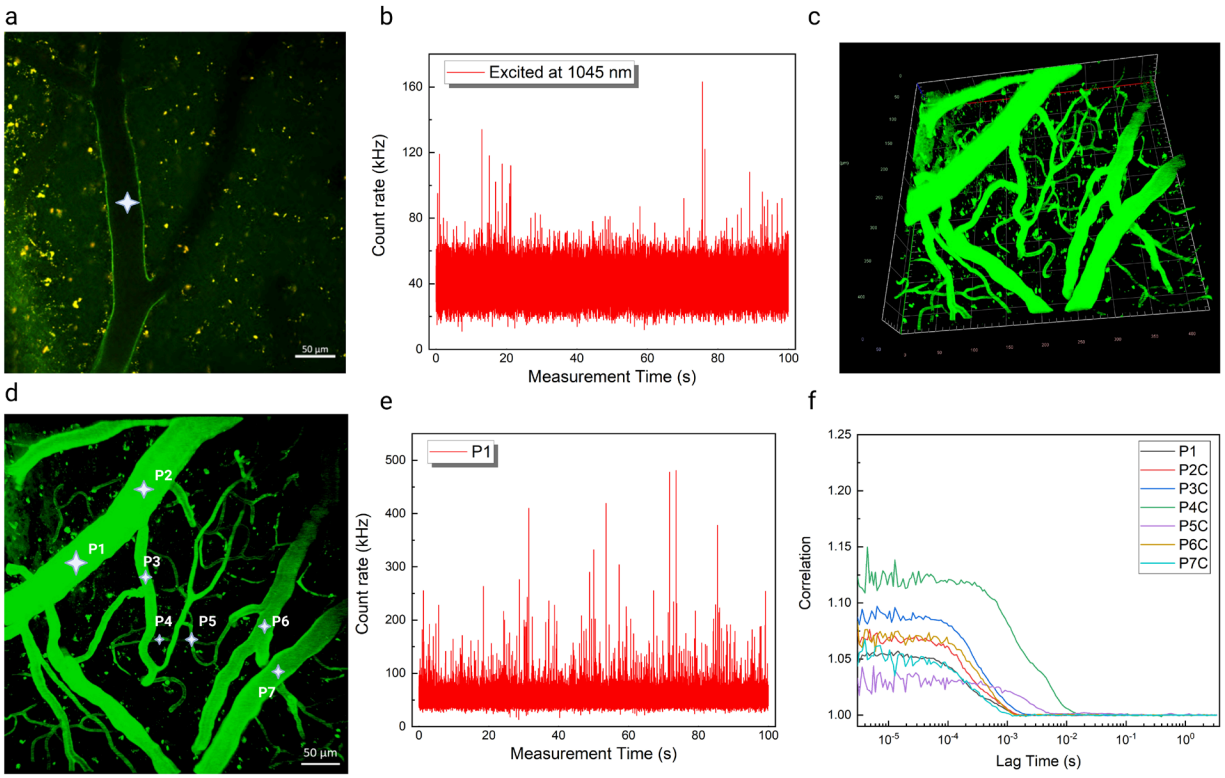


Figure S15. (a) Mouse with a skull-opened cranial window. (b) Microscopic image of blood vessels under the cranial window.



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	Residence time (μs)		Residence time (μs)
P1	416.14	P1	-
P2C	419.24	P2W	989.01
P3C	523.74	P3W	600.98
P4C	4091.58	P4W	4854.17
P5C	2537.93	P5W	2108.86
P6C	492.33	P6W	874.42
P7C	369.31	P7W	509.373

Figure S16. *In vivo* monitoring of the liposome loaded with DNA-Ag_{16}NCs under 1045 nm (11.7 mW) excitation. (a) The image was obtained in the green channel under 920 nm (25 mW) excitation without dye or liposomes present. (b) Emission intensity fluctuations in the red channel under 1045 nm excitation without dye present. (c) *In vivo* 3D image of cerebral vasculature under 920 nm excitation after administrating FITC-Dextran and DNA-Ag_{16}NC-loaded liposomes. (d) Maximum intensity projection from the 3D vascular image. (e) Emission intensity fluctuations at

the center of position 1 under 1045 nm excitation after dye and liposome delivery. (f) The corresponding autocorrelation functions at the indicated positions under 1045 nm excitation. (g) Measured residence time at the center (e.g., P2C) and wall (e.g., P2W) of the cerebral vasculature.

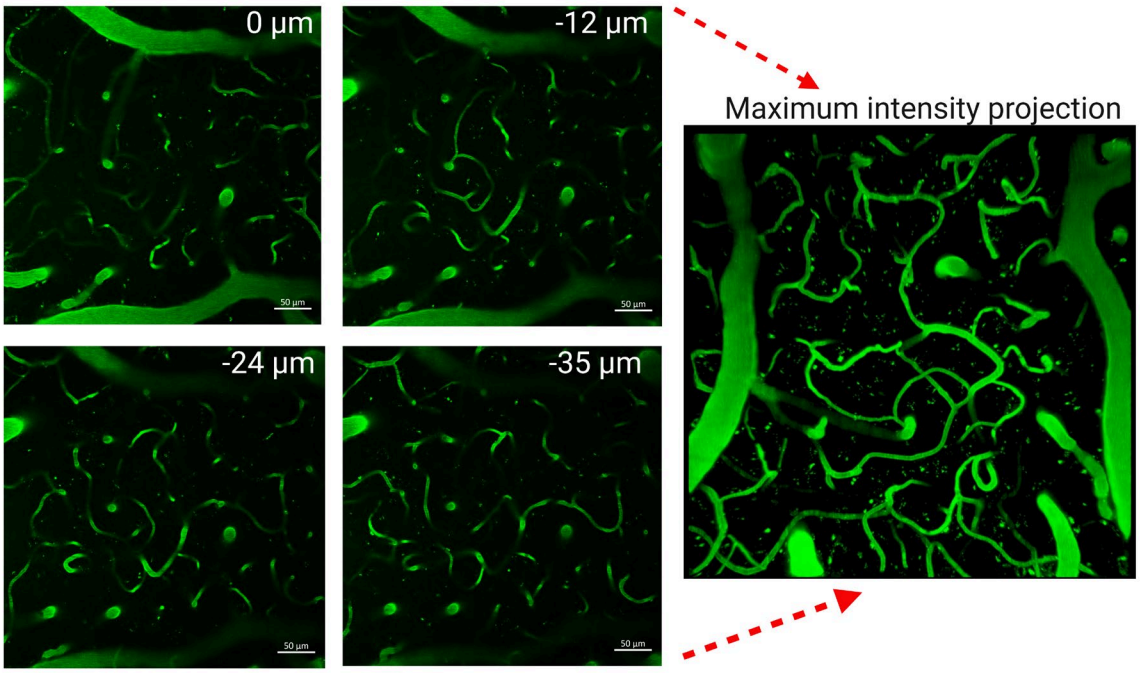

Figure S17. *In vivo* imaging of the cerebral vasculatures after delivery of FITC-Dextran and DNA-Ag_{16}NC-loaded liposomes. Left: images of vasculatures at different depths of the selected area. Right: maximum intensity projection of the selected area. The image was rotated 90 degrees counterclockwise.

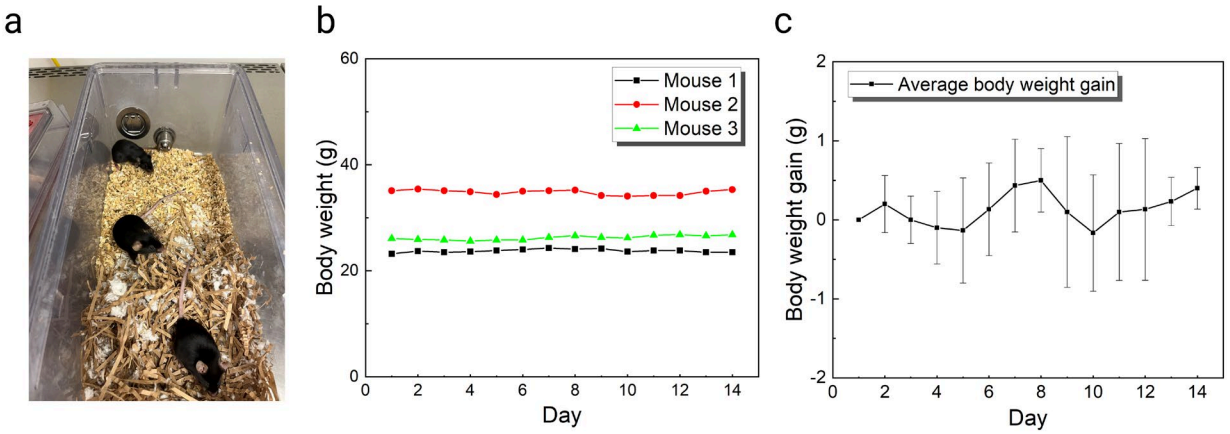

Figure S18. (a) Normal conditions for mice in these studies. (b) Body weights and (c) average body weight gain of three mice after delivery of a mixture of 100 nm/ 12.5 μM DNA-Ag_{16}NCs-loaded liposomes and 70 kDa FITC-Dextran. The liposome and FITC-Dextran concentrations were the same as used in the *in vivo* two-photon FCS experiments.

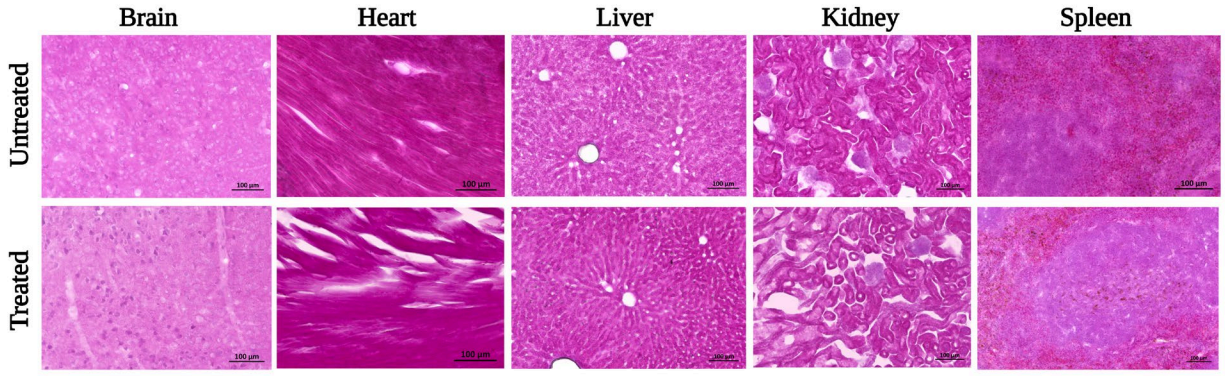

Figure S19. H&E staining images of tissue from the primary organs (brain, heart, liver, kidney and spleen). The treated group were injected with a mixture of 100 nm/ 12.5 μM DNA-Ag_{16}NCs-loaded liposome and 70 kDa FITC-Dextran. The liposome and FITC-Dextran concentrations were the same as used in the *in vivo* two-photon FCS experiments.