SUPPORTING INFORMATION

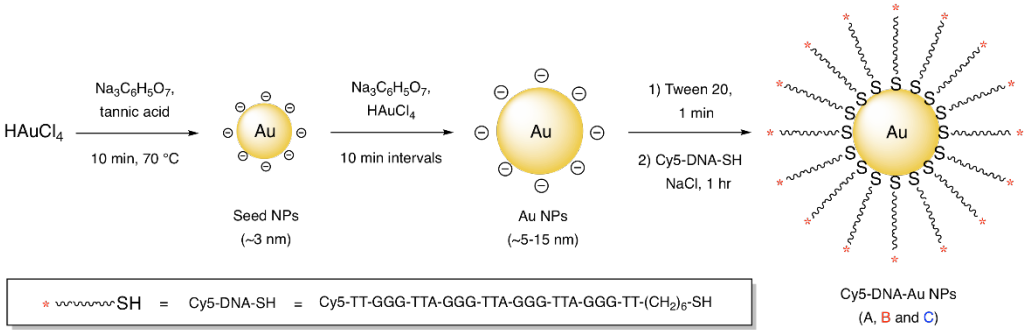
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1. Experimental Procedures

1.1. Nanoparticle Synthesis

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Ultrapure water was used in all reactions and all glassware was washed thoroughly with *aqua regia* (1:3, HNO_3:HCl). UV-Vis spectra were recorded on a Perkin Elmer Lambda 25 UV-Vis Spectrometer. Hydrodynamic diameters and zeta potentials were measured on a Malvern Zetasizer Nano ZS instrument. Fluorescence measurements were made on a Cary Eclipse Fluorescence Spectrophotometer. Synthesised Au NPs were imaged using a JEOL JEM-2100Plus transmission electron microscope. The size distribution of samples was determined using ImageJ by measuring at least 100 NPs. Centrifugation of NP solutions was performed using a Beckman Coulter Optima MAX ultracentrifuge with a Beckman Coulter TLA-55 rotor.



Synthesis of Gold Nanoparticles via a Seeded Growth Method

Au NPs of different sizes were synthesised following seeded growth protocols adapted from Piella *et al*.[1] Briefly, tannic acid (2.5 mM, 17 µL) and K_2CO_3 (150 mM, 170 uL) were added to a solution of sodium citrate (2.2 mM, 25 mL) and heated to 70 °C with stirring. $HAuCl_4$ (10 mM, 0.41 mL) was then added, resulting in a colour change from colourless to pink. Stirring at 70 °C for a further 10 mins resulted in a solution of seed Au NPs. To grow larger NPs, the seed solution was immediately diluted by removing 9.1 mL of the solution and replacing with sodium citrate (2.2 mM, 9.1 mL). Two injections of $HAuCl_4$ (10 mM, 0.2 mL) were added on a time interval of 10 mins before diluting the reaction as before. Further injections and dilutions were carried out until the NPs reached the desired size.

Functionalisation of Gold Nanoparticles with Oligonucleotides

Au NPs were functionalised with fluorescently-tagged oligonucleotides following protocols adapted from Li *et al*.[2] Since oligonucleotides typically used for gene therapies are 20-25 bases in length, a 25 base oligonucleotide sequence was chosen for this study: Cy5-TT-GGG-TTA-GGG-TTA-GGG-TTA-GGG-TT-$(CH_2)_6$-SH (Cy5-DNA-SH).[3] The oligonucleotide has a thiol modifier on the 3' end to allow it to bind to the Au NP surface and a Cy5 modifier on the 5' end to allow for tracking after delivery across the BBB *in vivo*. Before use, tris(2-carboxyethyl)phosphine) (TCEP) was added to Cy5-DNA-SH in a 100x excess and the mixture was incubated for 1 hr at 25 °C. Final concentrations of TCEP and Cy5-DNA-SH were 5 mM and 50 µM respectively.

The concentrations of the synthesised NP solutions were estimated using UV-Vis spectroscopy following reported methods.[4] 1 wt% Tween 20 (10 µL) was added to the Au NPs (50 nM, 1.15 mL) and the mixture was stirred for 1 min. Cy5-DNA-SH (50 µM) was added at a volume that allowed for 100 oligonucleotide strands present per Au NP. NaCl (5 M) was then added until its final concentration in the mixture reached 800 mM. The solution was incubated for 1 hr at 25 °C with gentle agitation and then centrifuged at 30,000 rpm (~55,000 x *g*) for 45 mins at 15 °C. The supernatant was removed and the NPs were redispersed in 500 µL of phosphate-buffered saline with 0.1% Tween 20 (PBST). This centrifugation process was repeated at least 3x to ensure thorough washing of the NPs. Solutions of Cy5-DNA-Au NPs **A**, **B** and **C** in PBS were characterised using DLS. All NP solutions were stored at 4 °C, kept away from light and were used within 2 weeks.

To determine the oligonucleotide loading on the nanoparticles, Cy5-DNA-Au NPs were dispersed in PBST and mercaptoethanol was added to the mixture until it reached a final concentration of 20 mM. After stirring the solution at 25 °C for 48 hrs, the mixture was centrifuged at 30,000 rpm (~55,000 x *g*) for 45 mins and the supernatant (containing the displaced oligonucleotides) was separated. The fluorescence of the displaced Cy5-oligonucleotides was measured and compared to a

standard curve; samples were excited at 656 nm and were scanned from 660 to 760 nm. The concentration of fluorescent oligonucleotides was divided by the initial concentration of NPs to give the number of oligonucleotides attached per NP. The oligonucleotide loading values are summarised in Figure S3b.

1.2. Nanoparticle Characterisation

Table S1. Measured λ_{SPR}, TEM diameters (d_{TEM}), hydrodynamic diameters (d_h) and zeta potentials (ζ) of synthesised Au NPs before functionalisation with Cy5-labelled oligonucleotides.

	Injection No.	λ_{SPR}/nm	d_{TEM}/nm	d_h/nm	ζ /mV
Seed	0	503	-	-	-
A	2	511	6.3 ± 0.7	5.3 ± 0.5	-50 ± 6
	4	515	-	-	-
B	6	517	9.5 ± 1.4	7.9 ± 1.3	-42 ± 4
	8	518	-	-	-
C	10	519	14.2 ± 1.2	11.9 ± 1.3	-50 ± 4

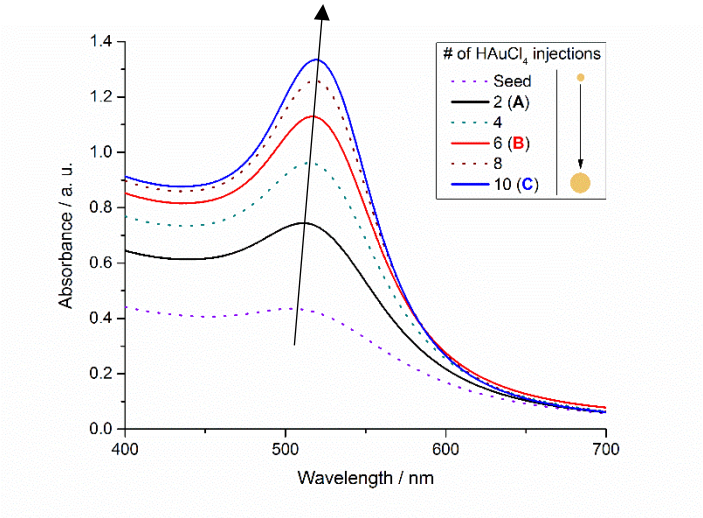
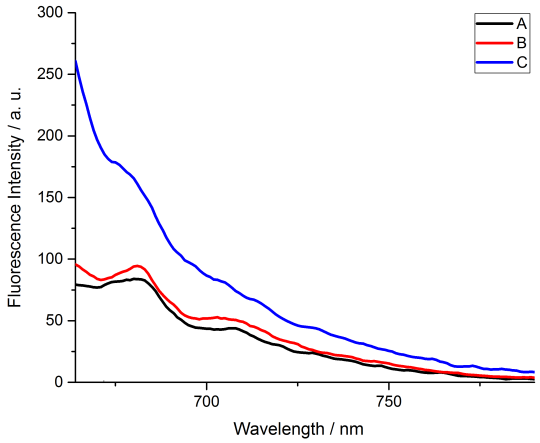


Figure S2. UV-Vis spectra of the synthesised Au NPs. The characteristic surface plasmon resonance band at ~520 nm is observed for all Au NPs, with its absorbance and wavelength increasing with increased NP size.

a)



b)

	Hydrodynamic Diameter / nm	Oligonucleotide Strands / Au NP
A	21.6 ± 3.3	40.7 ± 1.8
B	23.3 ± 2.6	49.2 ± 2.1
C	25.2 ± 1.9	77.0 ± 2.3

c)

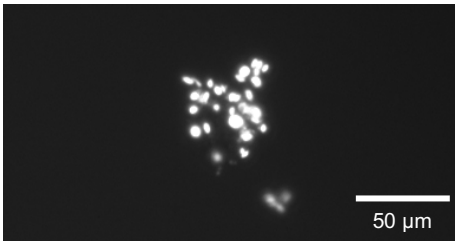


Figure S3. a) Fluorescence emission spectra of Cy5-DNA-Au NPs **A-C**. The oligonucleotide loading was adjusted in each case to ensure that the overall fluorescence intensity was similar to allow for comparison after delivery using focused ultrasound. b) Measured hydrodynamic diameters and oligonucleotide loading of Cy5-DNA-AuNPs **A-C**. c) Synthesised Cy5-DNA-Au NPs (500 nM solution in water) were fluorescent enough to be detected using a conventional widefield microscope. The image shows the remaining material after a drop of NP solution was left to evaporate in a well plate.

1.3. *In Vivo* Focused Ultrasound Experiments

Focused Ultrasound Setup

Ultrasound pulses were emitted from a single-element, spherical-segment focused ultrasound transducer (centre frequency: 1 MHz, active diameter: 90 mm, geometric focus: 77 mm; Sonic Concepts) driven by function generators (33500B Series; Agilent Technologies) through a 50 dB power amplifier (Precision Acoustics). The lateral, elevational and axial full width at half maximum (FWHM) at the focus were 2 mm, 1 mm and 20 mm respectively. Microbubble emissions were captured using a focused passive cavitation detector (centre frequency: 7.5 MHz, focal length: 76.2 mm; Olympus Industrial) aligned co-axially with the FUS transducer, filtered using a 3-30 MHz band-pass filter (Mini Circuits), amplified with a 28 dB pre-amplifier (Stanford Research Systems) and then recorded with an 8-bit oscilloscope sampling at 250 MHz (Picoscope 3205A; Pico Technology). Transducers were mounted onto a 3D positioning system to allow targeting of the desired region.

Before *in vivo* experiments, pressure amplitudes were measured using a needle hydrophone (diameter: 0.2 mm; Precision Acoustics) in a degassed water tank. Peak-positive and peak-negative values were attenuated by 11% to correct for skull attenuation.

General Experimental Procedure

All animal procedures were performed following Home Office regulations and institutional guidelines. Eighteen female C57LB/6 wild type mice (8-10 weeks old, 19.2 ± 0.95 g; Envigo) were used in total. Mice were anesthetised with 98% O_2/2% isoflurane and maintained under anaesthesia for the duration of the experiment. The fur from the head of each mouse was removed using an electric clipper and depilatory cream. The mouse was then positioned and immobilised using a stereotaxic frame. The left thalamus of the brain was targeted using a reported method, in which a metal cross is placed on top of the skull in alignment with the lambdoid and sagittal sutures.[5] A 2D raster scan of the cross was generated using the transducer in pulse-echo mode and used to position the transducer 1 mm laterally from the sagittal suture and 0.5 mm above the lambdoid suture. The right thalamus was used as the control.

Cy5-DNA-Au NPs **A**, **B** or **C** (100 µL, 500 nM) were administered intravenously into the mouse *via* a tail vein catheter. After ten seconds, SonoVue© microbubbles (100 µL, 5 uL/g) were administered over a period of approximately half a minute. The mouse was sonicated using ultrasound pulses (frequency: 1 MHz; peak-negative pressure: 530 kPa; pulse length: 10 ms; pulse repetition frequency: 0.5 Hz; sonication duration: 250 s) as soon as the microbubbles had been injected. Following sonication, mice were sacrificed and transcardially perfused with 20 mL ice-cold phosphate-buffered saline with added heparin (10 units/mL) to reduce blood coagulation. Once the blood was cleared, mice were fixed with 20 mL ice-cold 10% formalin solution. Brains were extracted, submerged in 10 mL 10% formalin solution at 4 °C overnight and then cryoprotected by immersing in 15% sucrose for 6 hrs, followed by 30% sucrose overnight. Cryoprotected brains were sectioned into 30 µm sections and imaged within 24 hrs using a fluorescence microscope (Zeiss Axio Observer Inverted Widefield Microscope). Images of the left (sonicated) and right (control) thalamus were taken using a 10x or 40x objective in both the brightfield and Cy5 (640/30 nm excitation filter, 690/50 nm emission filter) channels to detect the presence of Cy5-DNA-Au NPs.

Fluorescence images of five sections from each brain were used to calculate the normalised optical density (NOD) – the average fluorescence in the targeted region normalised by the control – to give an indication of the amount of Cy5-DNA-Au NPs delivered. Regions of interest were selected using Matlab and any artefacts such as folds were manually segmented and omitted from the calculation. To exclude brain autofluoresence, a threshold of twice the standard deviation of the intensity of the control region was employed. Pixel intensities above this threshold were summed in the targeted and control regions and the NOD was obtained by subtracting the value obtained for the targeted region by that of the control region. A two-sided Student's *t*-test was performed to assess statistical significance, taking $p < 0.05$ to be significant.

For histological analyses, a Texas Red-labelled 3 kDa dextran was injected as a marker of BBB opening. Mice (n = 3) were sonicated as described above. Following sonication, the brains were extracted, paraffin-embedded and cut into 5 µm sections. Eight brain sections covering the entirety of the focal volume were stained with haematoxylin and eosin (H&E) following standard procedures. Briefly, slides were first cleared by submerging in Histoclear II for 5 mins and then again for 10 mins. Sections were then hydrated in water and immersed in Harris Haematoxylin solution for 5 mins. Following rinsing with tap water, slides were dipped 3x in 1% acid-alcohol solution (1% HCl in 70% ethanol) and rinsed. Sections were then dipped in Eosin Y for 50 secs and rinsed with water again. Stained slides were dehydrated by placing in 90% ethanol for 30 secs, followed by 100% ethanol for 30 secs and then left in Histoclear II for mounting. Slides were mounted with DPX, coverslipped and imaged. Unstained sections adjacent to the stained sections were imaged using fluorescence microscopy in the Texas Red channel (562/40 nm excitation filter, 624/40 nm emission filter) to locate the site of BBB opening.

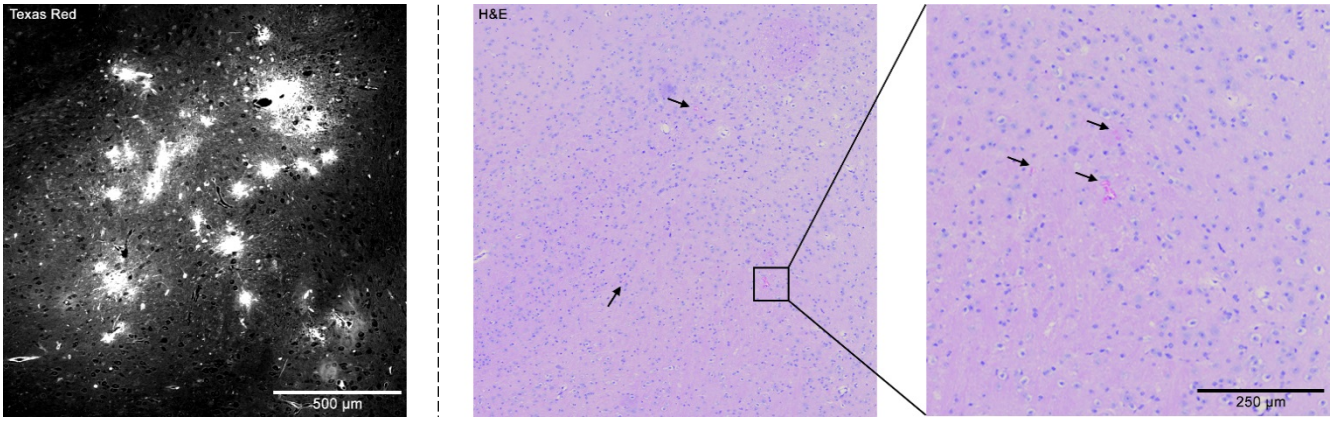


Figure S4. Assessment of safety was conducted by H&E staining. For these experiments, mice were injected with a Texas Red-labelled 3 kDa dextran as marker of BBB opening. Following sonication, mice were immediately sacrificed and brain sections were stained with H&E. A few sites of minor red blood cell extravasation were observed in the targeted region (black arrows).

2. References

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Author Contributions

T.G.C., S.V.M. and M.J.C. carried out experiments and analysed the data; T.G.C., J.C. and R.V. wrote the manuscript and analysed the data; J.C. and R.V. designed the project and secured funding.