SUPPORTING INFORMATION

One-pot synthesis of nanochain particles for targeting brain tumors

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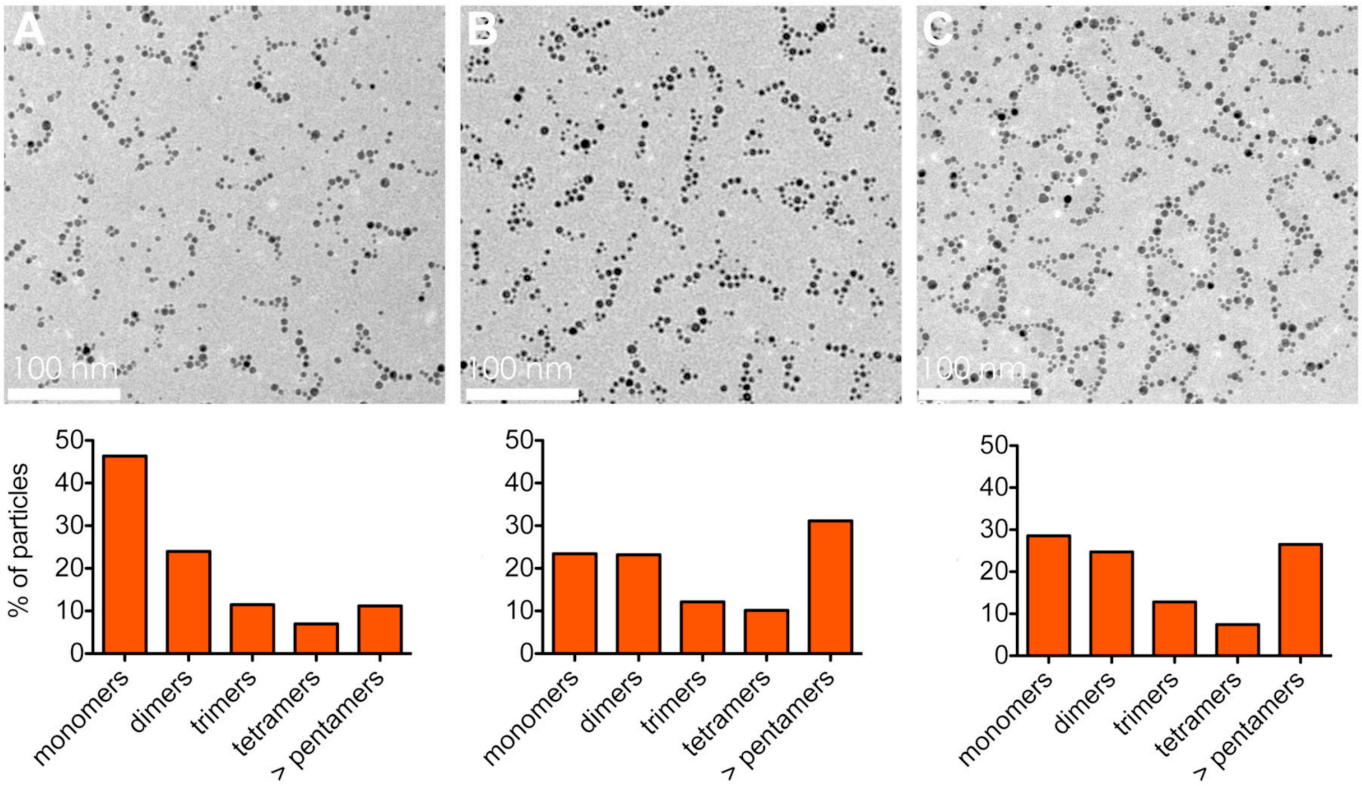
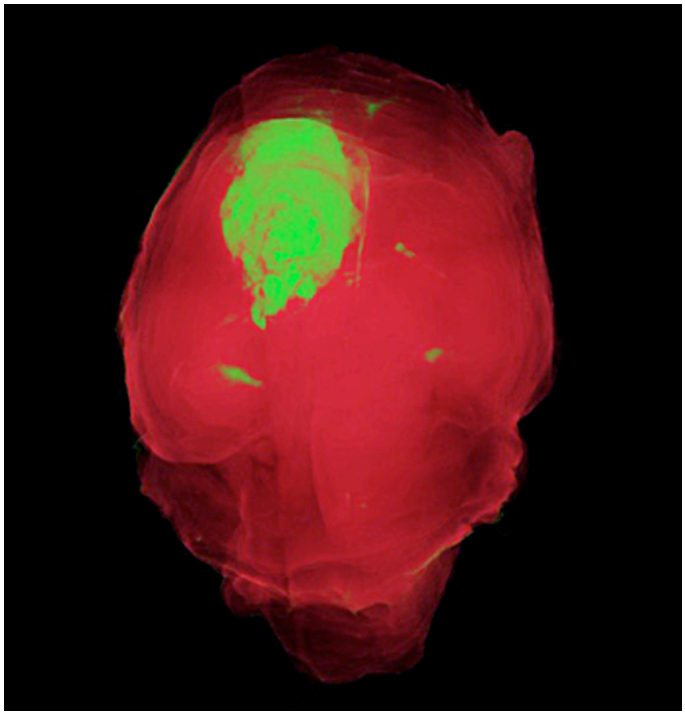


Supporting Figure S1. TEM images of different gold nanochains synthesized by varying the ratio between AuNP-COOH: AuNP-NH_2 and reaction times. By using different stoichiometric ratios between the AuNP-COOH and AuNP-NH_2 particles (*e.g.*, 1.5:1, 2.5:1, 3.5:1) and reaction times (*e.g.*, 10, 30, 60 min), one can control the composition of the resultant chains in terms of their lengths and linearity. The figure illustrates three different conditions resulting in different designs indicating the control of our method over the length of the nanochains. Instead of magnetic separation that we used in the case of iron oxide nanochains, we used centrifugation here to fractionate the nanochains to separate individual AuNPs from smaller chains

(dimers and trimers) and larger chains (tetramers and pentamers). While smaller ratios and shorter reaction times resulted in smaller chains (A), larger ratios and longer reaction times yielded longer chains (B) and, in extreme conditions, networks of chains (C).



Supporting Figure S2. Ultra-high-resolution fluorescence volume of the brain obtained using 3D cryo-imaging. In the end of MR imaging, the brains of the animals were perfused, excised, and imaged *ex vivo* using 3D cryo-imaging. Serial cryo-sections of entire brains were obtained using 3D reconstructions, providing ultra-high-resolution fluorescence images and the location of primary and invasive glioma sites (*i.e.*, CNS-1-GFP).

Supporting methods: Synthesis of gold nanochains

Gold nanoparticles (AuNP) were synthesized using the Brust-Schiffrin method with some modification. Briefly, $HAuCl_4$ solution (367 µL) and tetraoctylammonium bromide (TOAB, 0.1367g) were dissolved in toluene at room temperature. Dodecylamine (0.112 g) was added and stirred at room temperature for 10min. A reducing agent, $NaBH_4$ solution in ice-cold water was added slowly to the reaction mixture. Following the addition of $NaBH_4$, the solution turned from orange to wine red solution and was allowed to stir for 3 hr. The resulting AuNP were precipitated with ethanol, centrifuged at 6,000 rpm for 15 min, and dried under nitrogen for 30 min. Upon dissolving the nanoparticles in chloroform, SH-PEG-NH_2 or SH-PEG-COOH was added in large excess and allowed to react for 2 days at room temperature. Chloroform was removed by air-drying.

Supporting methods: Synthesis of parent iron oxide nanospheres

Iron oxide nanoparticles were synthesized in a three-neck flask by the co-precipitation method.[1] Briefly, 0.6757 g of $FeCl_3.6H_2O$ and 0.2478 g of $FeCl_2.4H_2O$ were dissolved in 5 mL of deoxygenated water. To this solution, 2.5 mL of 0.4 M HCl was added under vigorous stirring. This iron precursor solution was added to a solution of 25 mL of 0.5 M NaOH, which was preheated to 80 °C under a constant flow of argon. The solution became black immediately after the addition of the iron precursor solution, indicating the formation of iron oxide nanoparticles. The reaction mixture was then stirred for another 15 minutes at 80 ^{0}C under argon and the black precipitate was separated by using a powerful magnet. The nanoparticles were then washed several times with Milli-Q water until stable ferrofluid was obtained. To prevent the nanoparticles from agglomeration, citric acid (170 mg of citric acid in 10 mL of water) was introduced and allowed to react at 80 ^{o}C for 1.5 hours. The pH of the reaction mixture was adjusted to 5.2 using concentrated ammonia solution prior to heating. The reaction was protected under argon in

order to avoid any undesired side-reactions. Finally uncoated nanoparticles and aggregates were removed by repeated centrifugation. Excess citric acid was removed by centrifugation with Amicon® Ultra-15 centrifugal filters.

The surface of the iron oxide nanoparticles was modified using silane-PEG-COOH or silane-PEG-NH_2. The pH of 10 mg of citric acid-coated nanoparticles at 1 mg/mL concentration was adjusted to 11 with concentrated ammonia. To this solution, 10 mg of silane-PEG-NH_2 was added and the reaction was allowed to proceed for 24 hours with shaking. The resulting nanoparticle solution was heated at 80°C for 2 hours in order to achieve covalent linkage between polymer chains and the particle surface. Finally, the resulting functionalized nanoparticles (Fe_3O_4@silane-PEG-NH_2) were centrifuged at 4000 rpm for 10 min using Amicon® Ultra-15 centrifugal filters. The concentrated product (termed NS-NH_2 in Fig. 1) was stored at 4 ^{0}C.

The pH of 10 mg of citric acid coated nanoparticles at 1 mg/mL concentration was adjusted to 11 with concentrated ammonia. To this solution, 10 mg of silane-PEG-COOH was added and the reaction was allowed to proceed for 24 hours with shaking. The resulting nanoparticle solution was heated at 80°C for 2 hours in order to achieve covalent linkage between polymer chains and the particle surface. Finally, the resulting mono-functionalized nanoparticles (IONP-COOH or IONP-NH_2) were centrifuged at 4000 rpm for 10 min using Amicon® Ultra-15 centrifugal filters. The concentrated product was stored at 4 ^{0}C. To assess the number of functional groups on the nanoparticles, fluorescence labeling was used to quantify functional groups on nanoparticle surfaces because of its high sensitivity. All labeling reactions were performed in dark at room temperature. To determine the number of amines on the nanoparticle surface the Alexa Fluor® 488 NHS ester (Invitrogen, Carlsbad, CA) was used. IONP-NH_2 nanoparticles were reacted with 10 molar excess of dye for 2 hours. Following the labeling reaction, the sample was dialyzed against PBS using a 100,000 Da MW cut-off membrane to remove unbound fluorescent molecules. All the fluorescence measurements (excitation 480 nm, emission 520 nm) were performed on fluorescence plate reader (Synergy HT; BioTek Instruments, Winooski, VT). The fluorescence intensity of the sample was compared to a standard curve. The iron concentration was measured using ICP-OES after digesting all samples with concentrated HNO_3 acid and nanoparticle concentration was calculated assuming that each particle was made of Fe_3O_4 and a 5.2 g/cm^3 density. In the case of IONP-COOH, Alexa Fluor® 488 Cadaverine dye was used. First, a mixture of EDC: sulfo-NHS was added to the IONP-COOH nanoparticle solution and allowed to react for 15 minutes to activate carboxyl acid groups. The rest of the process was the same as the IONP-NH_2.

Supporting methods: Human GBM Specimens and derivative glioma stem cells

GBM tissues were obtained from excess surgical materials from patients with informed consent. All human tissues were acquired in accordance with a Duke University Institutional Review Board (IRB) protocol, a Cleveland Clinic IRB protocol, or a Case Western Reserve University IRB protocol. Tumor tissue was isolated and enzymatically dissociated with a Papain Dissociation System (Worthington Biochemical). We derived glioma stem cell subpopulations (GSCs) by sorting CD133+ cells from total GBM tumor cells. Functionally validated GSCs and non-GSCs were derived immediately after dissociation of xenograft (transiently) passaged in immunocompromised mice. After removing tumor cells from xenografts, GSCs were enriched based on CD133 expression by flow cytometry. To validate GSCs, they were functionally assayed for self-renewal, multi-lineage differentiation, stem cell marker expression and tumor propagation following established protocols.[2] Briefly, to validate GBM stem cells, we used immunofluorescent staining to confirm that the isolated cells were enriched for GBM stem cells expressing stem cell markers (Nestin, Olig2, Bmi1, Sox2, and Oct4) and that differentiation induced the expression of lineage markers (GFAP and S100b for astrocytes; Map-2 and TUJ1 for neuronal cells; NG2 and GalC for oligodendrocytes). Finally, cells were transduced with a lentivirus to express green fluorescent protein (GFP).

References

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2. S. Bao, Q. Wu, R. E. McLendon, Y. Hao, Q. Shi, A. B. Hjelmeland, M. W. Dewhirst, D. D. Bigner and J. N. Rich, *Nature*, 2006, **444**, 756-760.