Supporting Information

Elemental and optical imaging evaluation of zwitterionic gold nanoclusters in glioblastoma mouse models

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1- Synthesis of Au NCs

Thioctic-zwitterion (Zw, $C_{15}H_{30}N_2O_4S_3$, M~ 412 g.mol^{-1}) was synthesized following the protocol described elsewhere[1]. Au NCs with zwitterion (AuZw) were prepared by the addition of gold salt ($HAuCl_4.3H_2O$, 50 mM) to a basic solution (pH 10) containing the ligand in the presence of the strong reducing agent $NaBH_4$ (50 mM) and stirred for 15 h. Zwitterion stabilized Au NCs were synthesized with the molar ratio Au:Zw:$NaBH_4$ = 1:2:2, Afterwards, solutions were filtered twice with Amicon 3 kDa cut-off filters at 13,600 rpm for 20 min to remove excess free ligands, adjusted to pH 7, concentrated to 5 mg gold/mL (~600 μM) in PBS and kept refrigerated until use.

Protected $Au_{25}GSH_{18}$ clusters were synthesized in two steps as described by T. Pradeep and co-workers[2]. Briefly in a first step, the complex formed between $HAuCl_4$ and glutathione GSH (Au:GSH= 1:4) was reduced in a methanolic solution (0 °C) with $NaBH_4$. The resulting precipitate (Au@SG) was washed with methanol and dried. In a second step, Au@SG was

dissolved in water with 1 mM of GSH and heated at 55 °C for 12 h. Solution was centrifuged and $Au_{25}GSH_{18}$ is precipitated from the supernatant by adding methanol. The precipitate was washed several times with methanol before drying in vacuum.

2- Au NC characterization

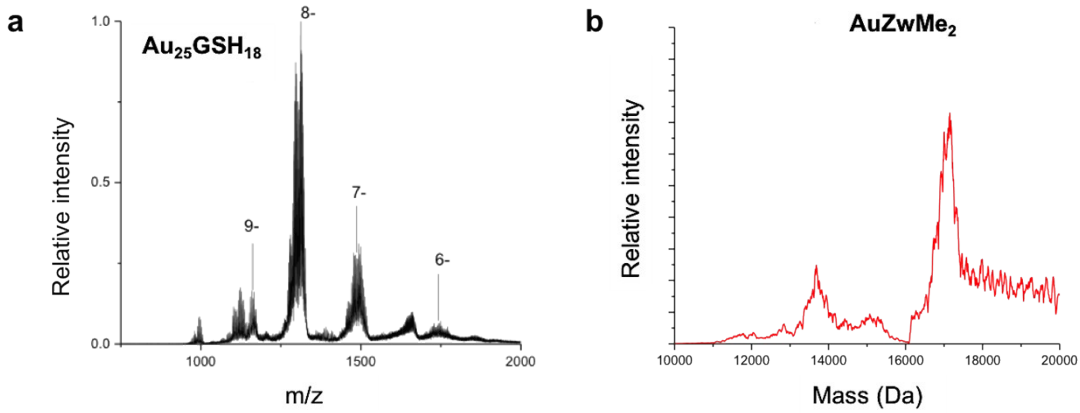

Fig. S1. ESI-MS of (a) $Au_{25}GSH_{18}$ with size at 10.5 kDa and (b) $AuZwMe_2$ with size ~17 kDa[3].

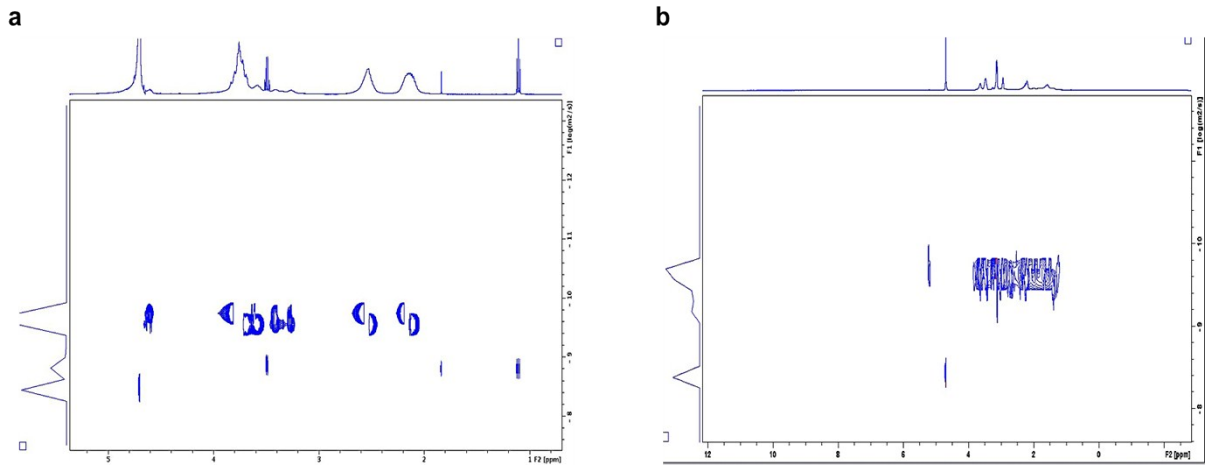

Fig. S2. DOSY-NMR of (a) $Au_{25}GSH_{18}$ and (b) $AuZwMe_2$ in D_2O performed on Bruker Avance 500 MHz spectrometer. Hydrodynamic diameter of the Au NC is calculated from the average diffusion coefficient D using the Stokes-Einstein equation[4] (η = 1.232x10^{-3} Pa.s at 298K). For $Au_{25}GSH_{18}$, D= 1.80x10^{-10} m^2.s^{-1}, which corresponds to 1.90±0.01 nm and for $AuZwMe_2$, D = 1.50x10^{-10} m^2.s^{-1}, which corresponds to 2.36±0.01 nm.

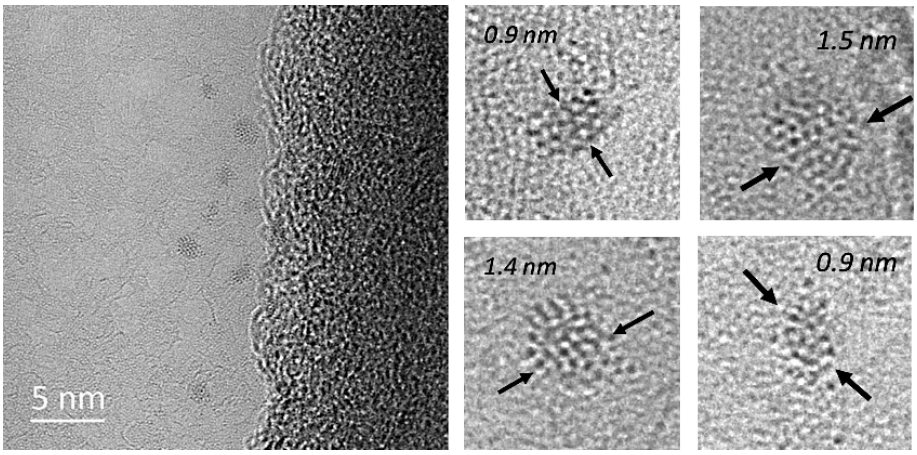

Fig. S3. High-resolution TEM images of $AuZwMe_2$.

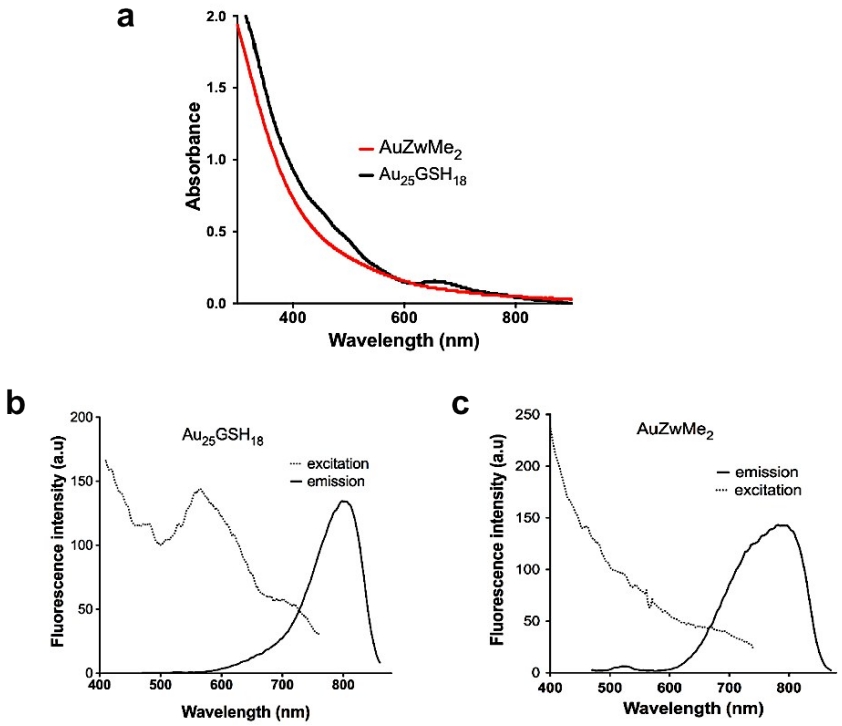

Fig. S4. (a) Absorbance spectra of $Au_{25}GSH_{18}$ (black line) and $AuZwMe_2$ (red line) in water between 300 and 900 nm. Fluorescence excitation (dashed line) and emission (solid line) spectra of (b) $Au_{25}GSH_{18}$ and (c) $AuZwMe_2$ dispersed in aqueous solution.

3- *In vivo* measurements

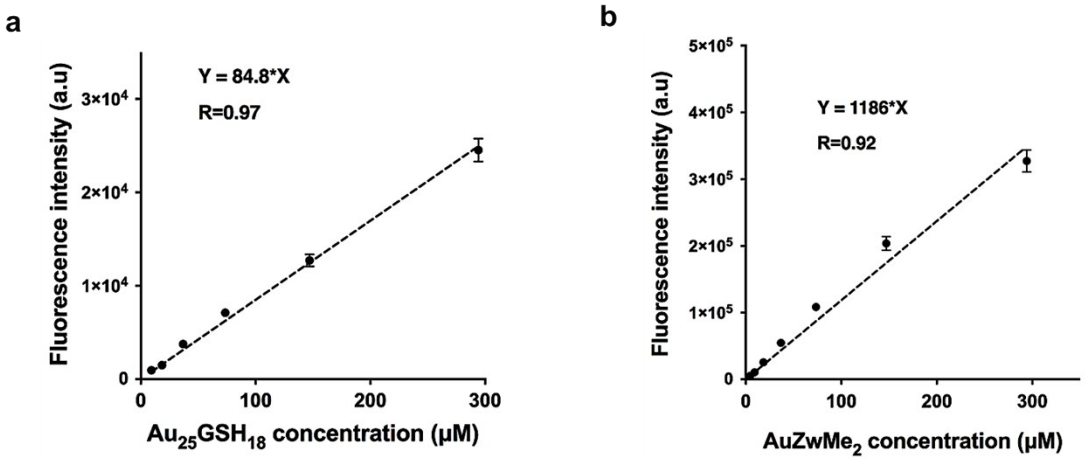

Fig. S5. Fluorescence calibration of (a) $Au_{25}GSH_{18}$ and (b) $AuZwMe_2$ in PBS using a Fluobeam800 system (10 µL; $\lambda_{exc.}$ 780 nm; $\lambda_{exc.}$>830 nm).

Inductively coupled plasma-mass spectrometry (ICP-MS). Au content in organs was determined by means of ICP-MS using a Thermo X serie II, spectrometer (Thermo Electron, Bremen, Germany), which was equipped with an impact bead spray chamber and a standard nebulizer (1 $mL.min^{-1}$). For sample preparation, the organs were weighted before addition of nitric acid (final concentration 1%) and Au content was determined using an external linear calibration curve (between 10 and 100 µg/L of Au(III)). Indium was used as the internal standard. Determinations were carried out in triplicate.

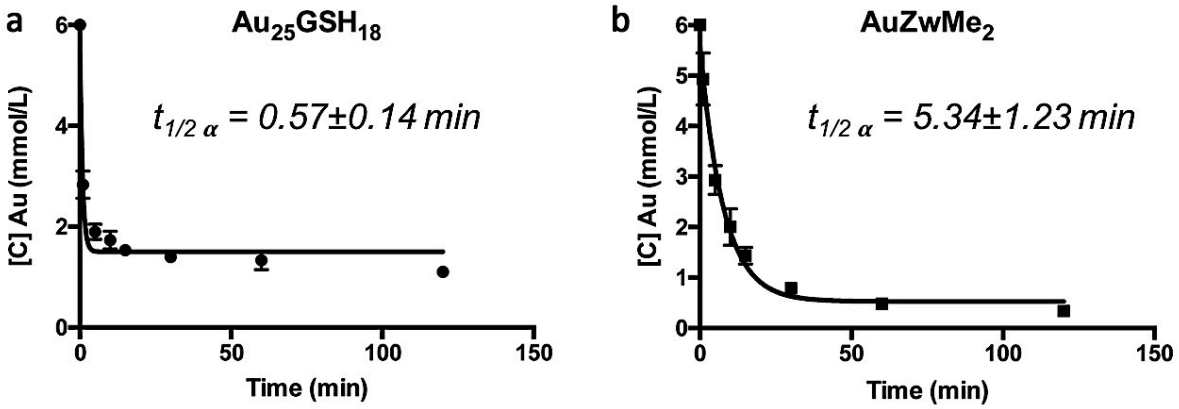

Fig. S6. $Au_{25}GSH_{18}$ and $AuZwMe_2$ pharmacokinetics determined by plasma ICP-MS measurements (n = 3 mice).

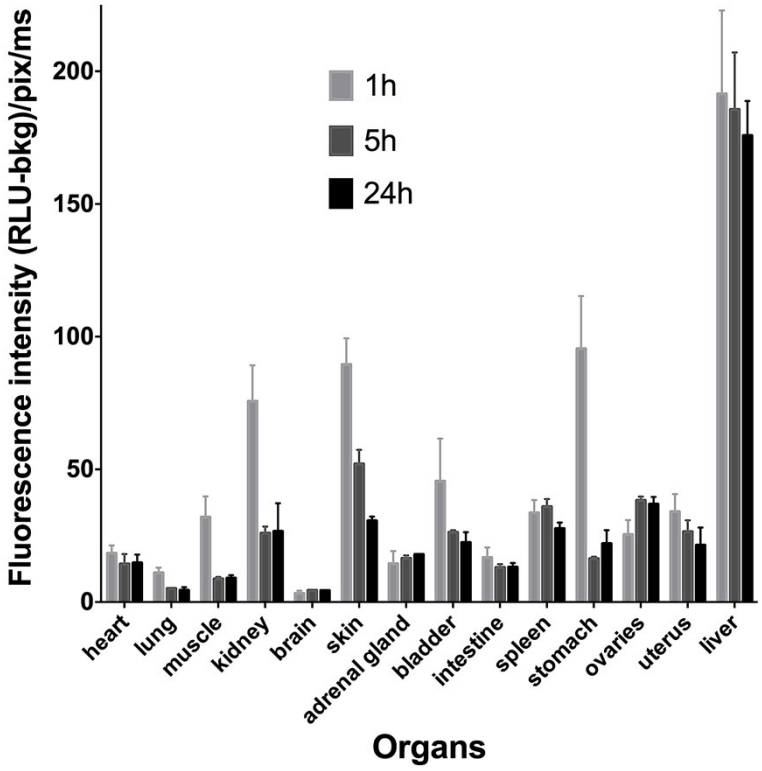

Fig. S7. *Ex-vivo* fluorescence signal of $AuZwMe_2$ in the isolated organs 1 h, 5 h, and 24 h post injection (600 μM; 200 μL) (n = 3 mice/time point)[3].

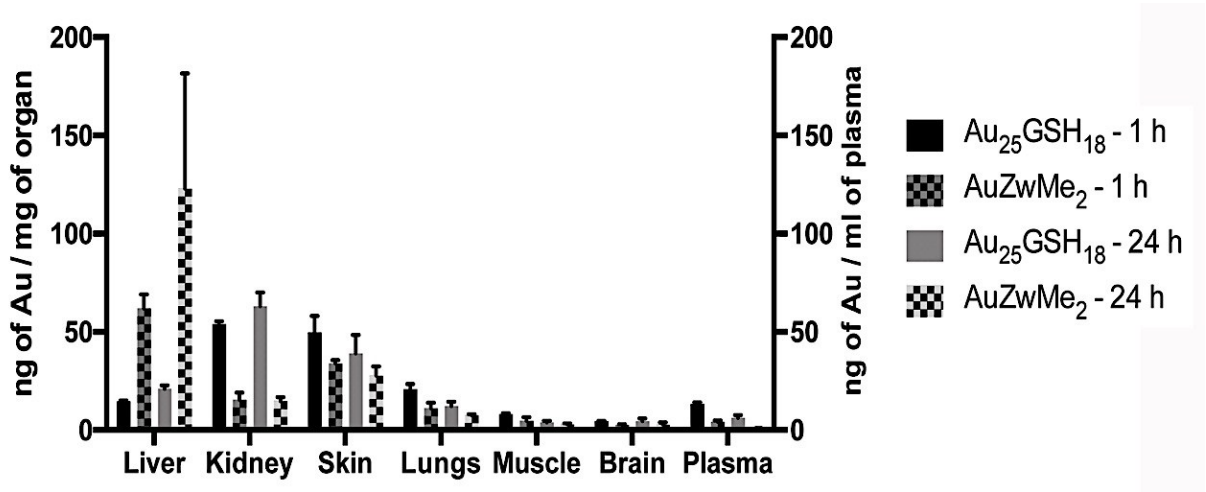

Fig. S8. *Ex vivo* signal of $AuZwMe_2$ from ICP measurement in the isolated organs 1 h and 24 h post injection (300 µM; 200 µL) (n = 3 mice/time point).

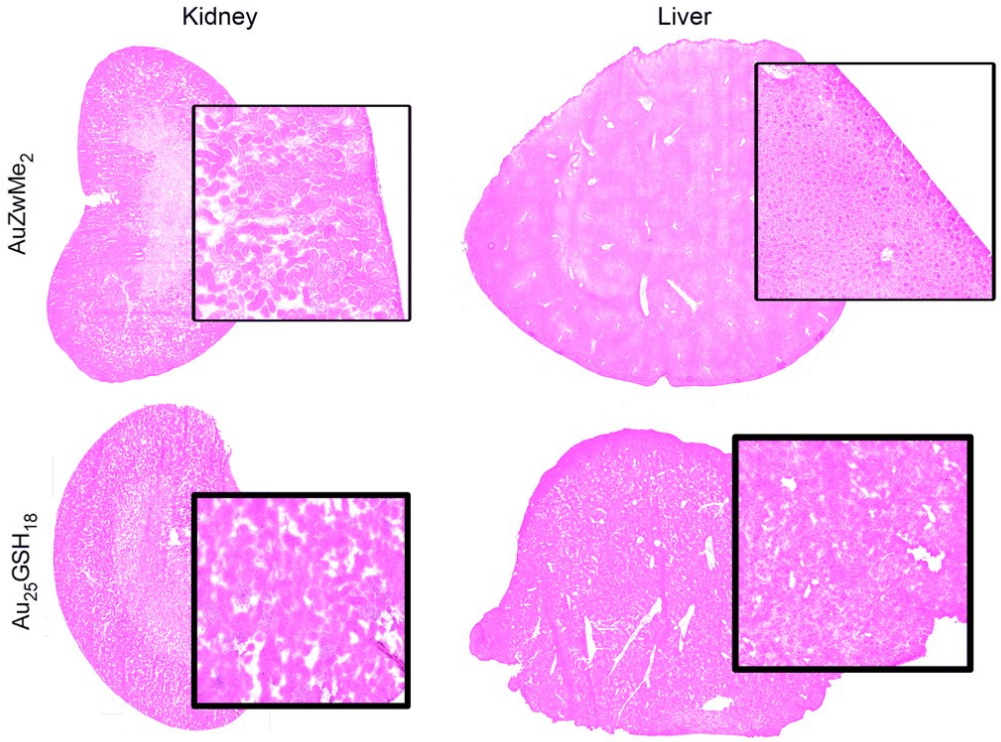

Fig. S9. Histology of kidney and liver samples after $AuZwMe_2$ or $Au_{25}GSH_{18}$ at 24 h post-injection (300 µM; 200 µL).

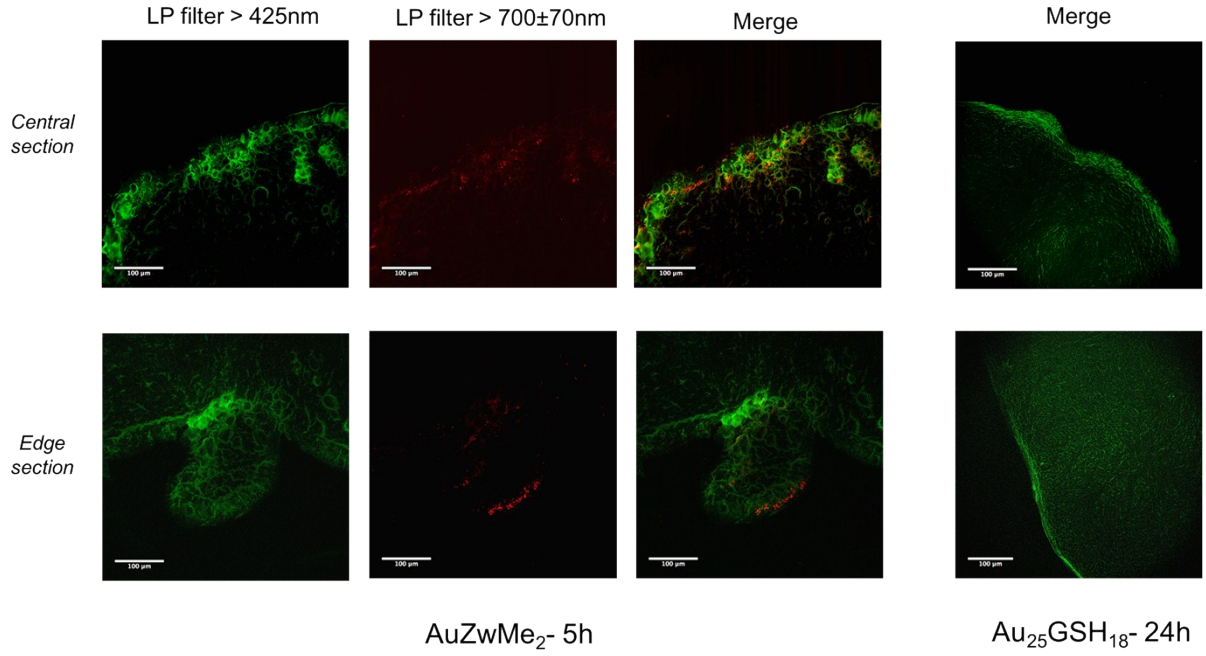

Fig. S10. Fluorescence microscopy of 100 μm tumor section in the edge and in the centre with $AuZwMe_2$ and $Au_{25}GSH_{18}$. Autofluorescence from the tissue (green) is detected using filter LP>425 nm and Au NCs signal (red) using BP filter at 700±70 nm. ($\lambda_{exc.}$ 365 nm).

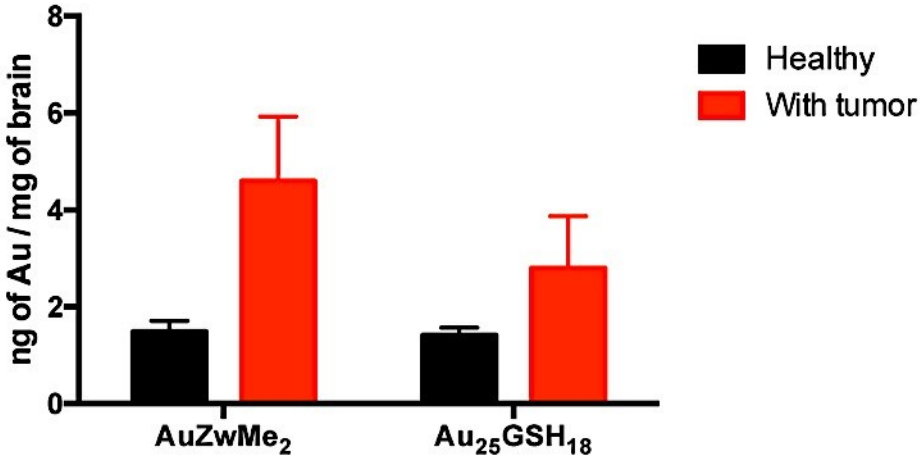

Fig. S11. *Ex vivo* signal of $AuZwMe_2$ and $Au_{25}GSH_{18}$ from ICP measurement in the brain with and without tumor grafted orthotopically in mice 1 h post-injection (300 μM; 200 μL) (n = 3 mice/time point).

References

1. X. Le Guével, O. Tagit, C. E. Rodríguez, V. Trouillet, M. Pernia Leal and N. Hildebrandt, *Nanoscale*, 2014, **6**, 8091-8099.
2. E. S. Shibu, M. A. H. Muhammed, T. Tsukuda and T. Pradeep, *Journal of Physical Chemistry C*, 2008, **112**, 12168-12176.
3. D. Shen, M. Henry, V. Trouillet, C. Comby-Zerbino, F. Bertorelle, L. Sancey, R. Antoine, J. L. Coll, V. Josserand and X. Le Guével, *APL Materials*, 2017, **5**.
4. R. H. Terrill, T. A. Postlethwaite, C. H. Chen, C. D. Poon, A. Terzis, A. Chen, J. E. Hutchison, M. R. Clark, G. Wignall, J. D. Londono, R. Superfine, M. Falvo, C. S. Johnson Jr, E. T. Samulski and R. W. Murray, *Journal of the American Chemical Society*, 1995, **117**, 12537-12548.