Supporting Information

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

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1- Synthesis of Au NCs

Thiocetic-zwitterion (Zw, C_{15}H_{30}N_{2}O_{4}S_{3}, M~ 412 g.mol^{-1}) was synthesized following the protocol described elsewhere\textsuperscript{1}. Au NCs with zwitterion (AuZw) were prepared by the addition of gold salt (HAuCl\textsubscript{4}.3H\textsubscript{2}O, 50 mM) to a basic solution (pH 10) containing the ligand in the presence of the strong reducing agent NaBH\textsubscript{4} (50 mM) and stirred for 15 h. Zwitterion stabilized Au NCs were synthesized with the molar ratio Au:Zw:NaBH\textsubscript{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\textsubscript{25}GSH\textsubscript{18} clusters were synthesized in two steps as described by T. Pradeep and co-workers\textsuperscript{2}. Briefly in a first step, the complex formed between HAuCl\textsubscript{4} and glutathione GSH (Au:GSH= 1:4) was reduced in a methanolic solution (0 °C) with NaBH\textsubscript{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 \( \text{Au}_{25}\text{GSH}_{18} \) 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) \( \text{Au}_{25}\text{GSH}_{18} \) with size at 10.5 kDa and (b) \( \text{AuZwMe}_2 \) with size \( \sim \)17 kDa.

Fig. S2. DOSY-NMR of (a) \( \text{Au}_{25}\text{GSH}_{18} \) and (b) \( \text{AuZwMe}_2 \) in \( \text{D}_2\text{O} \) 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 (\( \eta = 1.232\times10^{-3} \) Pa.s at 298K). For \( \text{Au}_{25}\text{GSH}_{18} \), \( D = 1.80\times10^{-10} \text{ m}^2 \cdot \text{s}^{-1} \), which corresponds to 1.90\( \pm \)0.01 nm and for \( \text{AuZwMe}_2 \), \( D = 1.50\times10^{-10} \text{ m}^2 \cdot \text{s}^{-1} \), which corresponds to 2.36\( \pm \)0.01 nm.
Fig. S3. High-resolution TEM images of AuZwMe₂.

Fig. S4. (a) Absorbance spectra of Au₂₅GSH₁₈ (black line) and AuZwMe₂ (red line) in water between 300 and 900 nm. Fluorescence excitation (dashed line) and emission (solid line) spectra of (b) Au₂₅GSH₁₈ and (c) AuZwMe₂ 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; $\lambda_{\text{exc.}}$ 780 nm; $\lambda_{\text{exc.}}>830$ 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).
Fig. S8. *Ex vivo* signal of AuZwMe₂ 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 AuZwMe₂ or Au₂₅GSH₁₈ 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