Supplementary information for “Antibody affinity and valency impact brain uptake of transferrin receptor-targeted gold nanoparticles”

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Figure S1. Optimization of antibody thiolation. The different antibodies used in the study were reacted with 2-iminothiolane (Traut’s reagent) to obtain an average of 1 thiol per antibody. Optimization of the thiolation degree of the different antibodies showed that to obtain 1 thiol per antibody, a 15X molar excess of 2-iminothiolane should be used.
**Figure S2. Biacore sensorgrams.** All antibodies used in the study of TfR-targeted AuNPs were tested for their affinity against the mouse TfR using surface plasmon resonance (Biacore)-based analysis. (A) Isotype IgG control antibodies had a $K_D = 32 \mu M$. (B) High affinity anti-TfR$^A$ antibodies had a $K_D = 21 nM$. (C) Low affinity anti-TfR$^D$ antibodies had a $K_D = 149 nM$. (D) The bispecific anti-TfR$^A$/BACE1 antibodies had a $K_D = 22 nM$ (corrected for drifting baseline). This value correlated with that measured for the high affinity anti-TfR$^A$ antibodies, enabling investigations of the impact of antibody valency on AuNP transport into the brain. (E) The RI7217 antibodies used for immunocytochemistry had a $K_D = 6 nM$. (F) OX26 antibodies (mouse anti-rat, served as negative control for specificity) had a $K_D = 16 \mu M$. 
Figure S3. Expression of the transferrin receptor in relation to tight junction markers. The confocal microscopy images presented in Figure 2 are shown here as individual channel images in addition to the merged variants. Scale bar depicts 10 µm.
Figure S4. Western blot analysis of the TfR expression in primary mouse brain capillary endothelial cells.
To study the purity of the brain fractions after employment of the brain capillary depletion technique, the enzyme activity of γ-glutamyl transferase was measured (n = 12). In the capillary fraction, > 97% of the total enzyme activity was detected, whereas < 3% of the enzyme activity was present in the parenchymal fraction (p < 0.0001).
Figure S6. ELISA determination of β-amyloid protein load after AuNP treatment. To study any potential therapeutic effects of the anti-TfR^A/BACE1 AuNPs, mice were injected with a double dose of AuNPs, and the β-amyloid protein load was determined after 30 hours. Treatment with anti-TfR^A/BACE1 AuNPs had no impact on the β-amyloid protein load in the brain compared to non-treated and isotype IgG AuNP-treated animals.
Figure S7. Representative TEM images of AuNPs in brain capillaries. Scale bars depict 200 nm. ec: endothelial cell. pc: pericyte. cl: capillary lumen. bp: brain parenchyma. bm: basement membrane. vs: vesicular structure.
Figure S8. Representative images showing the difference between larger versus smaller capillaries with respect to uptake of AuNPs. (A) Only few examples of uptake into small diameter capillaries was observed in the TEM analysis, whereas most capillary uptake of AuNPs was observed in ones with larger diameters (B + C). Scale bars depict 1 µm. ec: endothelial cell. cl: capillary lumen. bp: brain parenchyma. tj: tight junction. lc: leukocyte.
Figure S9. Representative TEM images of anti-TfR^4/BACE1 AuNPs in brain parenchyma. Scale bars depict 200 nm. ec: endothelial cell. cl: capillary lumen. bp: brain parenchyma. bm: basement membrane. tj: tight junction. np: neural process. ma: myelinated axon.
Figure S10. Chemical structures of newly synthesized molecules.