Supplementary Information

Gold-conjugating nanoprobes for targeted molecular imaging using high-resolution secondary ion mass spectrometry

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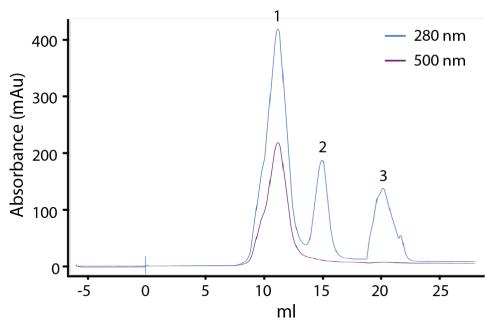


Figure S1. Size-Exclusion Chromatography (SEC) of the nanobody-gold conjugation. Superdex-75 increase column was used to separate 3 nm gold NPs conjugated to anti-mouse nanobody. Peak 1 is the conjugated nanobody and gold NPs, which show their absorbances at the wavelengths of 280 nm and 500 nm, respectively. Peak 2 corresponds to the unconjugated nanobody absorbing at 280 nm. And peak 3 corresponds to a small protein fraction after cleaving the His-Tag from the nanobody.

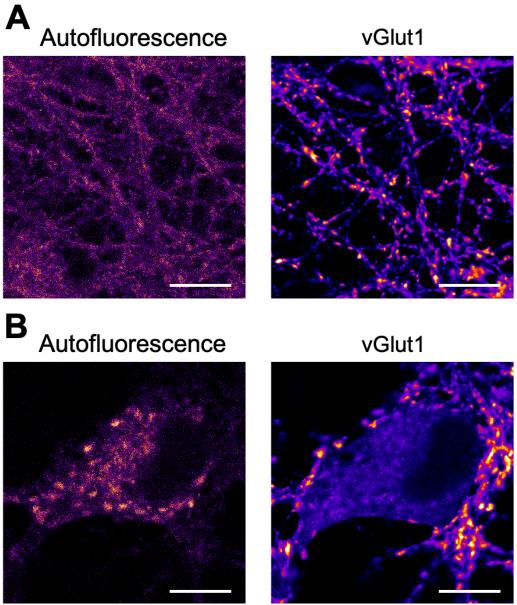


Figure S2. Confocal fluorescence images of vesicular glutamate transporter (vGlut1) primary mouse antibody in hippocampal neurons, which is then revealed by Abberior STAR 580-antimouse secondary antibody. **A.** vGlut localizes as "hot spots" along the neurites. **B.** vGlut localizes dominantly in the neurites compared to the cell body. Scale bars are 10 μ m.

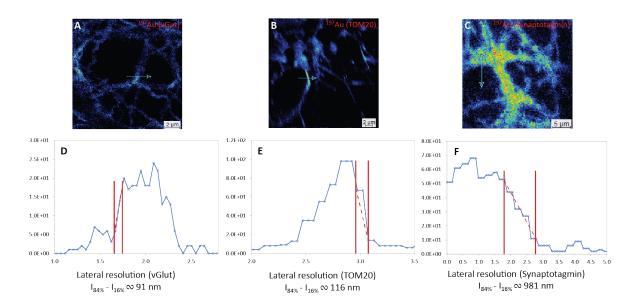


Figure S3. Line-scans on the ¹⁹⁷Au images of different structures in the hippocampal neurons labeled with the gold nanoprobes for determination of spatial resolution. **(A, B, C)** ¹⁹⁷Au images showing the structure of vGlut, TOM20, and Synaptotagmin. The line scan was performed along the arrow in each ion images. **(D, E, F)** Corresponding line scans for the structure of vGlut, TOM20, and Synaptotagmin.

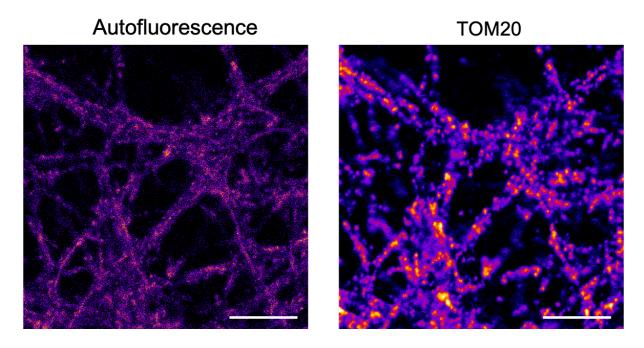


Figure S4. Confocal images of mitochondrial marker TOM20 primary mouse antibody in hippocampal neurons, which is then revealed by Cy5-anti-mouse secondary antibody. Left to right: Autofluorescence and TOMM20. Scale bars are 10 μ m.

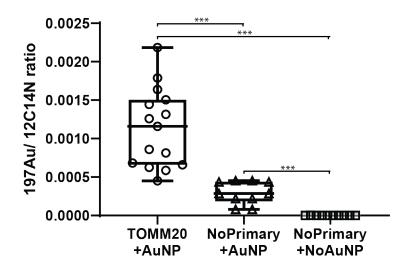


Figure S5. The chart shows a significantly higher signal of ¹⁹⁷Au signal in the neuronal cells labeled with TOM20 plus Au anti-mouse nanoprobe compared to the negative control cells labeled with Au anti-mouse antibody in the absence of primary antibody, and negative control cells labeled with neither primary antibody nor Au anti-mouse nanoprobe. The statistical analysis was performed by the Kolmogorov-Smirnov tests (p<0.0001) (n=15 for labelled cells, n=10 for control cells). Error bars represent SEM.