

## Supplementary Materials

# Gold-Conjugated Nanobodies for Targeted Imaging Using High-Resolution Secondary Ion Mass Spectrometry

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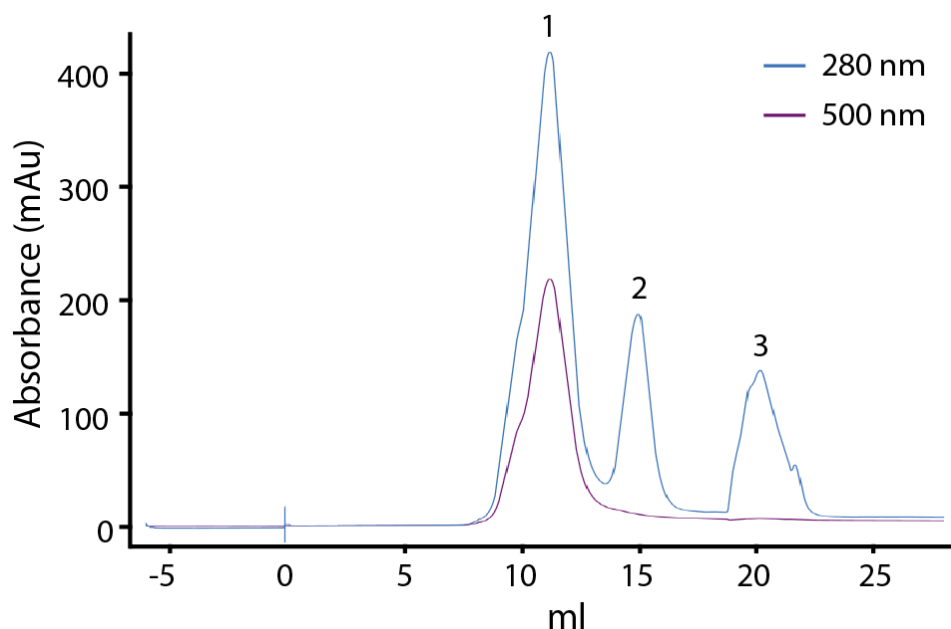
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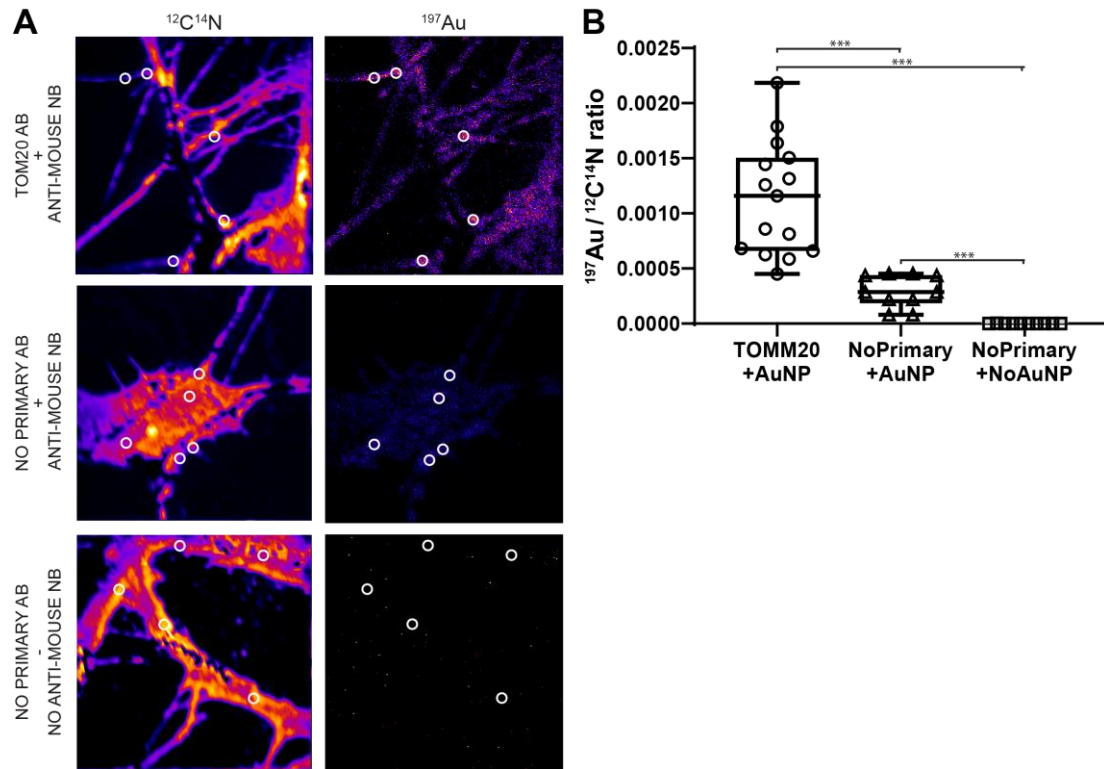
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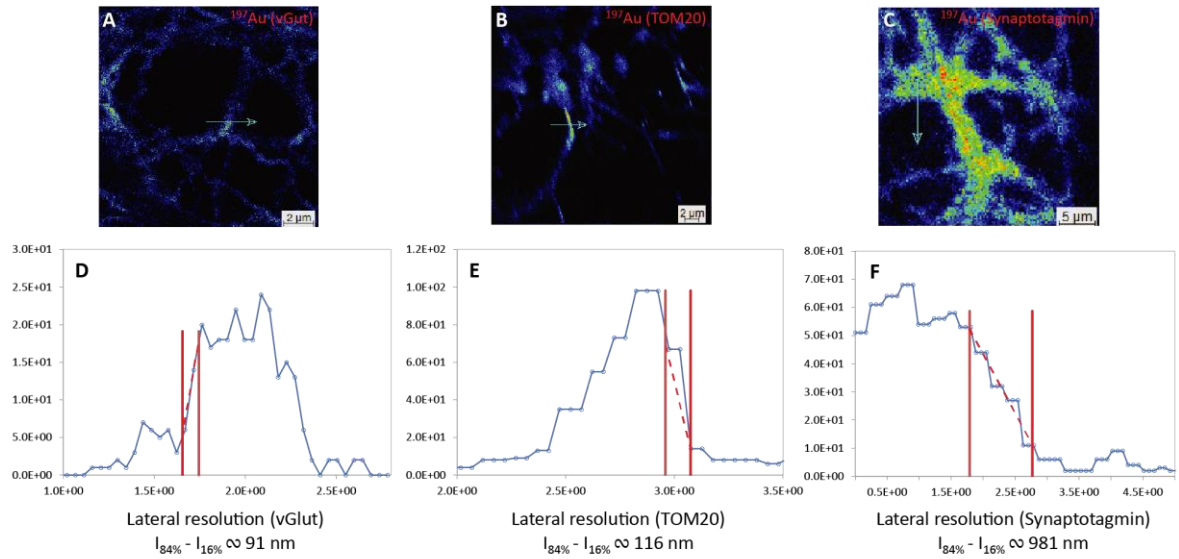
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**Figure S1.** Size-Exclusion Chromatography (SEC) of the nanobody-gold conjugation. Superdex-75 increase column was used to separate 3 nm gold NPs conjugated to anti-mouse nanobody. Peak 1 is the conjugated nanobody and gold NPs, which show their 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. (A)** Selection of ROIs. Representative example of how regions of interest (ROIs) were defined to compare the  $^{197}\text{Au}/^{12}\text{C}^{14}\text{N}$  ratio between different conditions by selecting circular ROIs of 5 pixels diameter. **(B)** The chart shows a significantly higher signal of  $^{197}\text{Au}$  signal in the neuronal cells labeled with anti-TOM20 primary antibody (AB) plus Au anti-mouse secondary nanobody (NB) compared to the negative control cells labeled with Au anti-mouse secondary nanobody in the absence of anti-TOM20 primary antibody, and negative control cells labeled with neither the anti-TOM20 primary antibody nor Au anti-mouse secondary nanobody. Each data point represents the average  $^{197}\text{Au}/^{12}\text{C}^{14}\text{N}$  ratio value from all the pixels of individual ROIs ( $n=15$  for labelled cells,  $n=10$  for control cells). The statistical analysis was performed by the Kolmogorov-Smirnov tests (\*\*\*  $p < 0.0001$ ). Error bars represent SEM.



**Figure S3.** Line-scans on the  $^{197}\text{Au}$  images of different structures in the hippocampal neurons labeled with the gold nanoprobe for determination of spatial resolution of NanoSIMS. **(A, B, C)**  $^{197}\text{Au}$  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.