

A reliable approach for revealing molecular targets in secondary ion mass spectrometry

Fengxia Li ^{1,2,*}, Eugenio F. Fornasiero ^{1,2}, Tal M. Dankovich ^{1,3}, Verena Kluever ¹, and Silvio O. Rizzoli ^{1,2,*}

¹ Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen, von-Siebold-Straße 3a, 37075 Göttingen, Germany

² Department of Neuro- and Sensory Physiology, University Medical Center Göttingen, Humboldtallee 23, 37073 Göttingen, Germany

³ International Max Planck Research School for Neuroscience, Göttingen, Germany

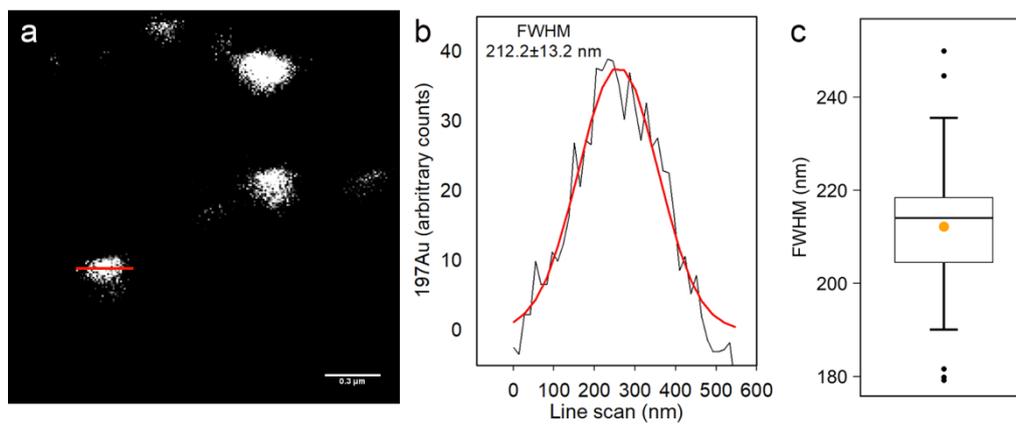


Figure S1. Au standard imaged in nanoSIMS. (a) A representative image of gold spots measured by nanoSIMS; (b) Line scan and fitted Gaussian curve of the gold counts from the red line indicated in (a); (c) Boxplot of FWHM based spatial resolution calculated for multiple detected gold spots ($N = 64$).

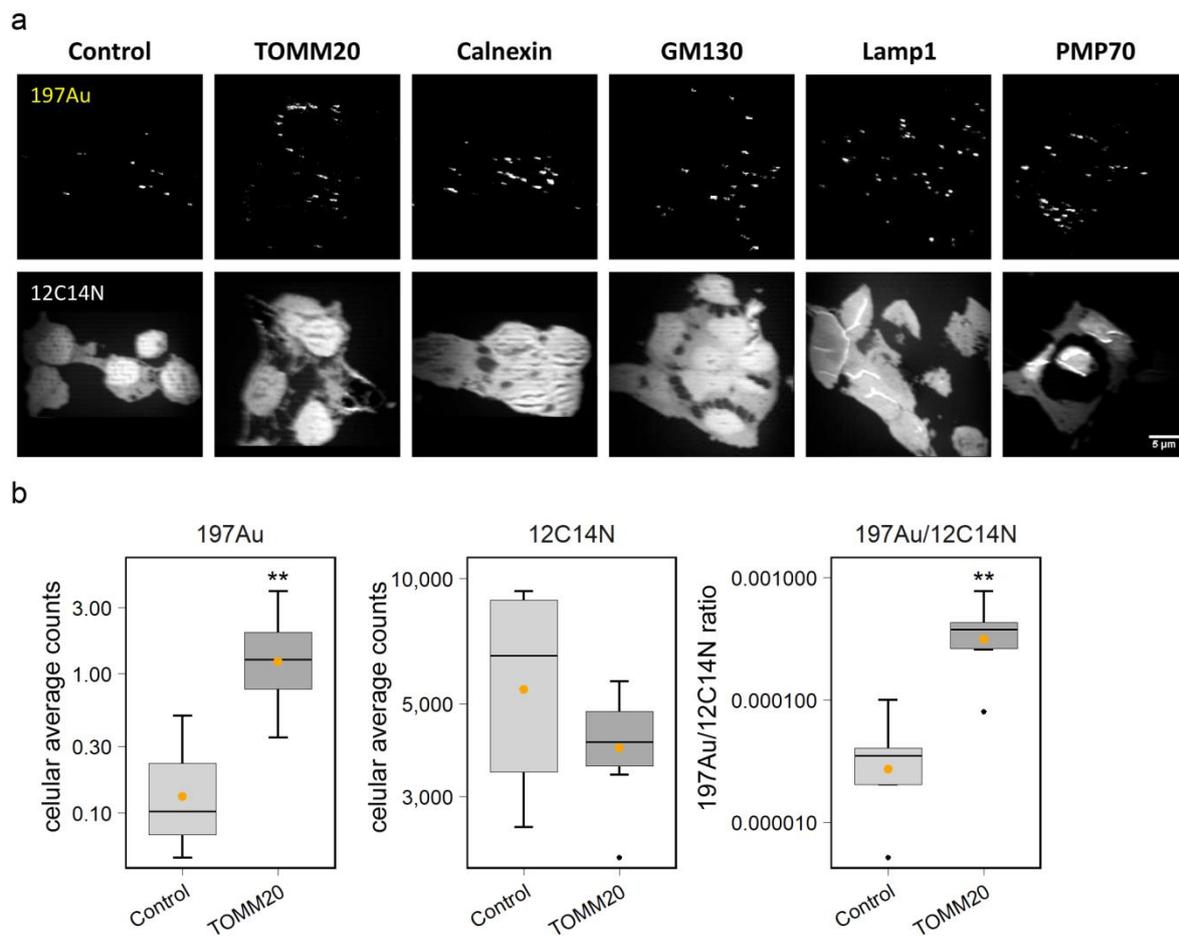


Figure S2. NanoSIMS measurement of 15nm Au_{Np} labelled HEK293 organelle markers. (a) Representative images of each organelle marker labelling and control staining without primary antibody application; (b) Labelling intensity comparison for TOMM20 stained with 15 nm Au_{Np}. Wilcoxon rank sum test; N=5 and 7 for control and TOMM20, respectively; p=0.0051, 0.34 and 0.0051 for 197Au, 12C14N and 197Au/12C14N, respectively.

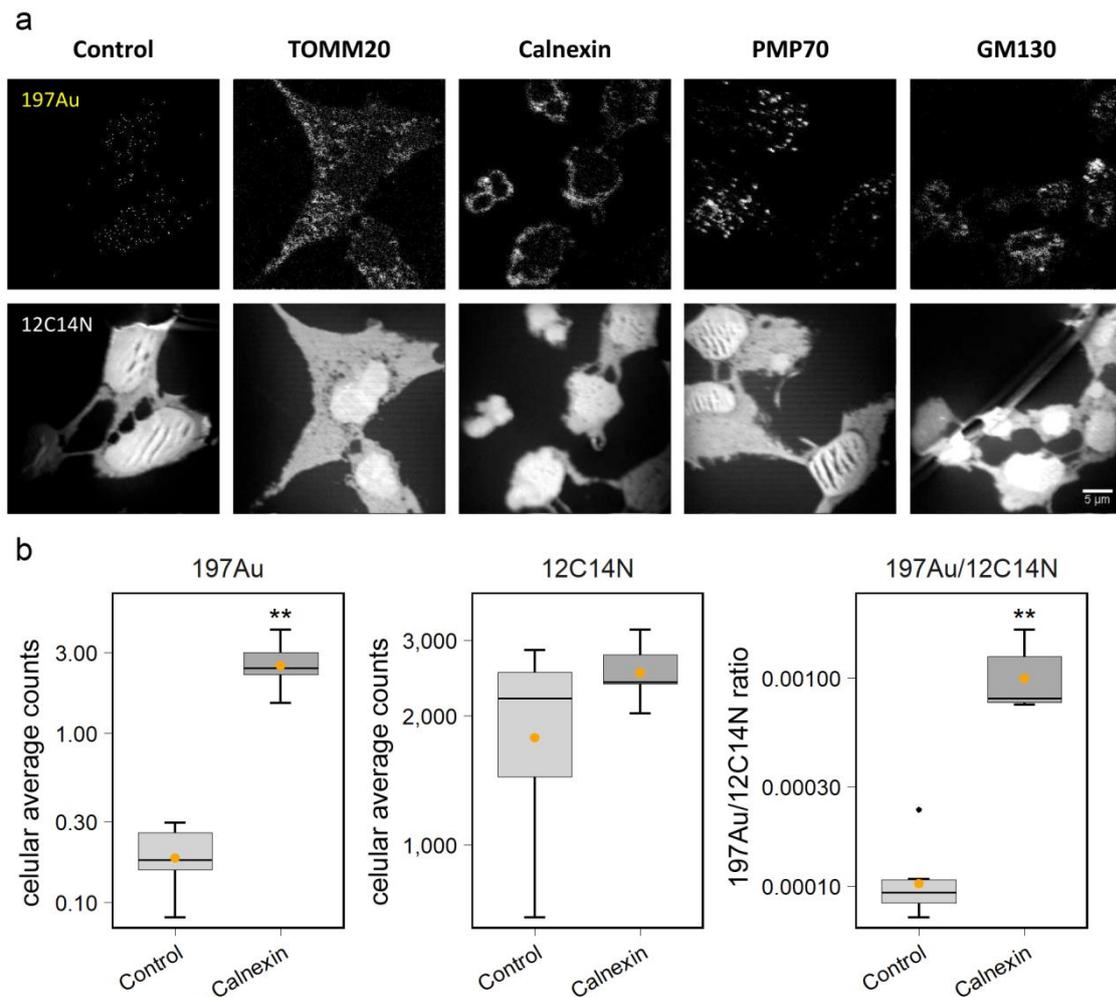


Figure S3. NanoSIMS measurement of 1.4nm Au_{Np} labelled HEK293 organelle markers. (a) Representative images of each organelle marker labelling and control staining without primary antibody application; (b) Labelling intensity comparison for calnexin staining stained with 1.4 nm Au_{Np}. Wilcoxon rank sum test; N=7 and 5 for control and calnexin, respectively; p=0.0025, 0.2 and 0.0025 for 197Au, 12C14N and 197Au/12C14N, respectively.

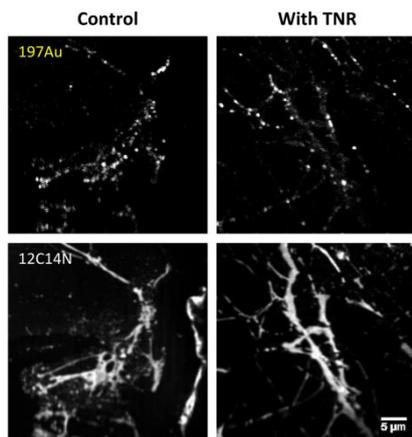


Figure S4. NanoSIMS measurement of 5 nm Ni-NTA-Nanogold lively labelled neuronal cultures detected strong gold signal both in control samples with only gold probe applied and in samples with mixture of His-tagged TNR and gold probe applied.

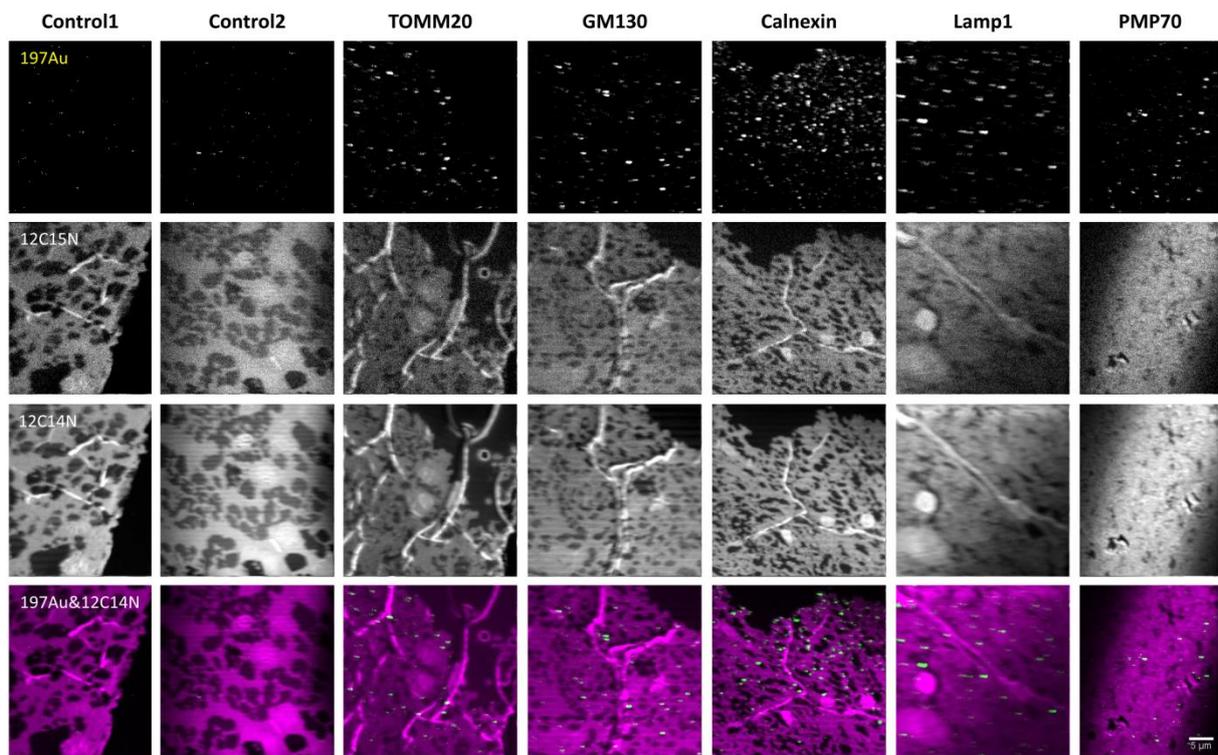


Figure S5. NanoSIMS detection of organelle markers in mouse brain slices tagged by 6 nm Au_{Np}. ¹²C¹⁵N and ¹²C¹⁴N were imaged simultaneously in a single measurement to allow accurate ratio calculation. ¹⁹⁷Au (green) and ¹²C¹⁴N (purple) are merged in the lowest panel.