Detection of immunolabels with multi-isotope imaging mass spectrometry (MIMS)

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ABSTRACT:
Multi-isotope Imaging Mass Spectrometry (MIMS) is a method combining Secondary Ion Mass Spectrometry imaging at high spatial resolution, 30 nm, and quantification at high precision using molecules tagged with stable isotopes. Microscopy, used alone, does not allow for complete recognition of cell-types or for protein identification. We developed a targeted imaging mass spectrometry method, which combine the detection of commercially available antibodies tagged with gold nanoparticles (1.4 nm) in parallel with MIMS analysis of cells or organelles. To study feasibility and tune the protocols we used LR white embedded sections of small intestine of mouse fed with 15N enriched diet. We used well-established antibody immunoassays for anti-actin and anti-synaptophysin. We also synthetized proteins specific for the synaptic vesicles, synaptophysin, or for the synaptic ribbon of photo receptor cells, the ribeye protein on LR-white embedded section of mouse retina. Our results show that we can directly identify antibodies by MIMS analysis of the Au signal of commercially available antibodies tagged with non amplified 1.4 nm gold nanoparticles. They also demonstrate that the gold nanoparticle tagged antibodies do not dilute the 15N/15N signal used for measuring protein turnover. Thus we can simultaneously and directly use MIMS to measure protein turnover and to identify cell type or specific protein. We are working to use antibodies derivatized with Ag nanoparticles and with quantum dots to perform MIMS analysis with multiplexed immuno-assays.

MATERIALS AND METHODS FOR IMMUNOGOLD LABELING

Sulfo-N-Hydroxysuccinimido nanogold (Nanoprobes, NY, USA) were dissolved in 200 μL of deionized water.
Synaptophysin antibody (Abcam, MA, USA) reconstituted in 100 μL of 200 μL of deionized water.

MATERIALS AND METHODS FOR IMMUNOGOLD LABELING

→ Actin antibody (Millipore, CA, USA) 3μg/mL was purified with buffer exchange using spin column (Abcam, MA, USA) to remove tris-glycine.
→ Synaptophysin antibody (Abcam, MA, USA) reconstituted in 100 μL of deionized water.
→ Sulfo-N-Hydroxysuccinimido nanogold (Nanoprobes, NY, USA) were dissolved in 200 mL of deionized water.

3.3 ml of NHS-AuNp were mixed with 0.66 ml of primary antibody, at pH 7.5-8. The reaction mixture was incubated overnight at 4°C. Non-reacted AuNp were removed with MonoBio Spin Chromatography Columns containing tris buffer (Bio-Rad, CA, USA)
→ AuNp-conjugated antibody was diluted 1:10 in blocking solution containing 0.05% Tween (Sigma, MO, USA) and 0.1% BSA (Sigma, MO, USA) in TBS (Sigma, MO, USA). After incubation at room temperature for one hour and overnight at 4°C with AuNp-conjugated primary antibody.
→ Silicon chips were washed five times with TBS (Sigma, MO, USA) and rinsed with distilled water.

IMMUNOGOLD LABELING: MOUSE SMALL INTESTINE

Schematic 1: Antibody labeling with NHS-Nanogold labeled primary amines (N-terminal, Lysine residues) to create a very stable and strong bound between the nanogold and the antibody.

Figure 1: Comparison of anti-actin antibody labeled using 1.4 nm AuNp or Alexa 488-conjugated secondary antibody. (a) MIMS image, 60 μm field, 2 minislices, 200 planes. As expected, the Au signal is observed in the microvilli. (b) Immunofluorescence/CIC image of the adjacent section that was analyzed by MIMS of anti-Actin antibody labeled with Alexa 488-conjugated secondary antibody.

IMMUNOGOLD LABELING: MOUSE RETINA

Figure 2: Combination of immunogold labeling of anti-actin antibody and MIMS analysis from the same section: 100 μm thick, 60 μm field, 2 minislices, 51 planes. The ratio of [15N]/[14N] is obtained from a different analytic run. The geometry of the detectors precludes concurrent acquisition of mass26, mass27 and 197 from a single run.

CONCLUSION
Gold nanoparticles of 1.4 nm size attached to antibodies have been visualized using the MIMS method on the brush border (microvilli) of the intestinal villi and on the synaptic ribbon of retina without Au amplification. We have also compared the signal of anti-actin antibody on successive tissue sections using either traditional immunofluorescence or immunogold combined with MIMS. These results correlate well with traditional immunofluorescence and demonstrate the feasibility of using MIMS to quantify gold nanoparticle-conjugated antibodies.

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