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Optimized and Improved Immunogold Labeling on Ultrathin Sections of Nervous Tissue Following High Pressure Freezing, Freeze Substitution and Low Temperature Embedding

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Immuno-electron microscopy is a powerful technique for localizing proteins in cells and tissues. To enable labeling of ultrathin sections of nervous tissue, we developed an immunogold-silver labeling protocol that optimizes antigen detection and preserves neuronal ultrastructure. Specimens from different species including mouse, rat and dragonfly were processed with high pressure freezing, freeze-substitution, and embedded in Lowicryl HM20 resin at -50℃ (HPF/FS/HM20). Indirect immuno-gold labeling was applied on ultrathin sections for detection of target antigens. To visualize primary antibodies, secondary antibodies were conjugated to 5nm or 10 nm gold particles. Gold labeling was followed by strictly controlled silver-enhancement (i.e. duration and temperature). This approach is reliable and sensitive; a positive signal is readily observed at low magnification on the electron microscope (Figure 1). 5 nm and 10 nm gold particles are enlarged proportionally during silver-enhancement while keeping their uniform shape (Figure 2). This makes this approach suitable for double labeling studies, especially when both antigens are located in the same cellular compartment. In our lab, this protocol was used successfully in co-localization analysis, quantification and 3D reconstruction with double labeling on serial ultrathin sections (Figure 3). We provide examples from our studies and compare the sensitivity of immunolabeling with different size gold particles at the light and electron microscopic level.

Reference:

[1] Sarada Viswanathan et al. High-performance probes for light and electron microscopy. Nature Methods (2015), in press.

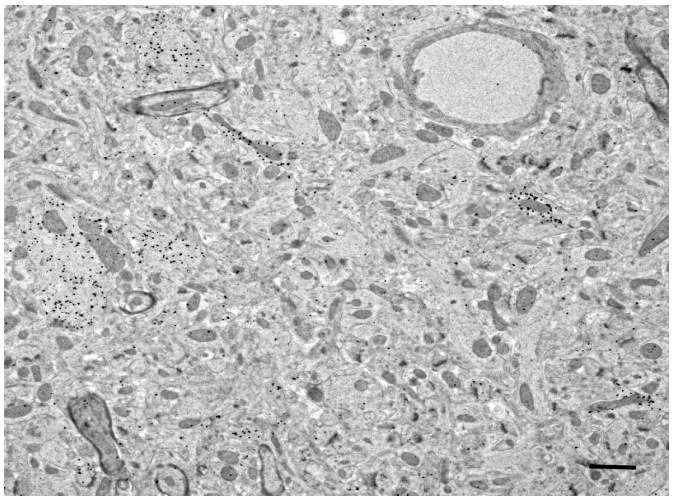

Figure 1: Low magnification image of immunogold-silver labeling on mouse brain following HPS/FS/HM20. Bar = 2 µm.

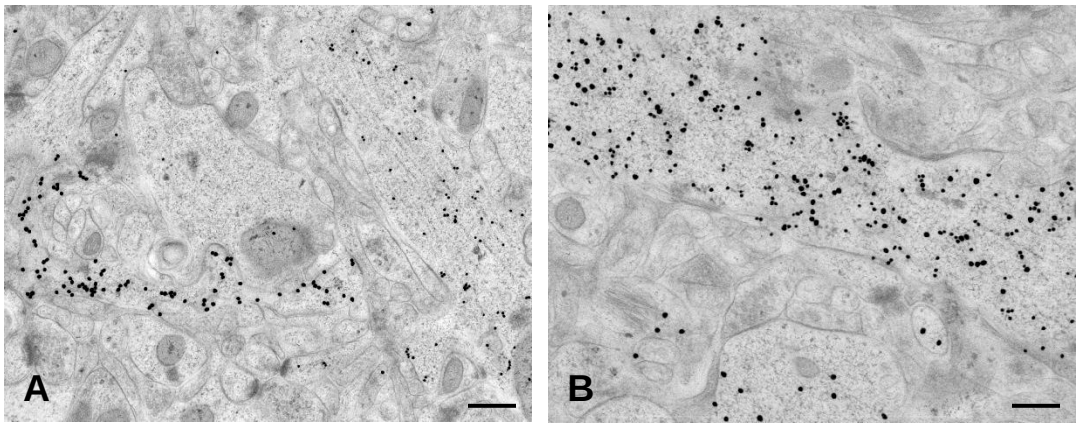

Figure 2: Double immunogold-silver labeling on mouse brain following HPS/FS/HM20. A) Localization of two target antigens in two neuronal dendrites. B) Co-localization of two antigens in one neuronal dendrite (upper). Bar = 200 nm.

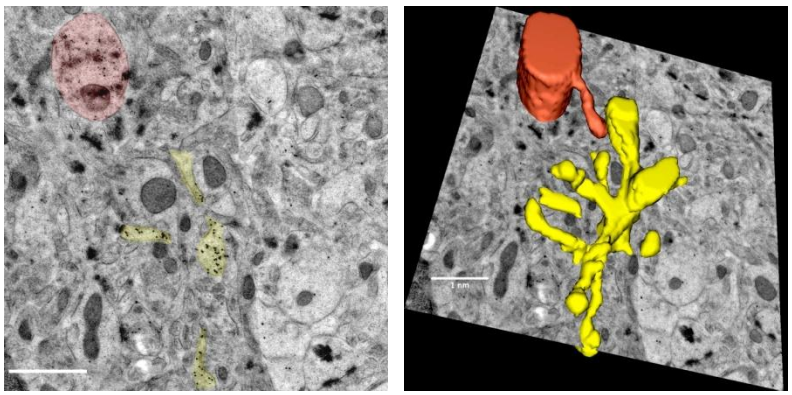

Figure 3. Double immunogold-silver labeling on mouse brain and serial 3D reconstruction of labeled dendrites. Bar = 1 µm.