Methodology Advancements in Electron Microscopy Immunolabeling of Hydrated Brain Tissue Using Subnanometer Colloidal Gold Conjugates Hong Yi, Rahmat Peijper \*\*, and Jan LM Leunissen \*\*
\*Emory Neurology Microscopy Core Facility, Emory University, Atlanta, GA, USA, \*\*Aurion Immunogold Reagents, Accessories, and Custom Labeling, Wageningen, the Netherlands

The ultrastructure of the central nervous system is characterized by the abundance of membrane enclosed cellular and sub-cellular elements, including neuronal and glial cell bodies and processes. Maintaining membrane ultrastructural integrity is essential for identification of these elements, and is a challenge in brain ultrastructural localization experiments. The availability of ultrasmall gold conjugates and silver enhancement solutions allows the penetration of the immunoreagents into hydrated brain tissue without compromising ultrastructure quality, making pre-embedding immunogold labeling a valuable method for high resolution localization of intracellular antigens in brain tissue. Further refinement of the reagent and technique has also made it possible to co-localize multiple antigens using exclusively ultrasmall gold conjugates and sequential silver enhancements.

We present here a few examples of our immunogold localization studies in brain tissue (Figure 1 and 2) through which issues such as localization resolution, reagent penetration, antigen accessibility, and particle size control will be addressed. We will also discuss the development of new conjugates, and demonstrate preliminary localization results using these new conjugates.

## References

1) H. Yi, et al., J. Histochem. Cytochem. 49, 279 (2001)

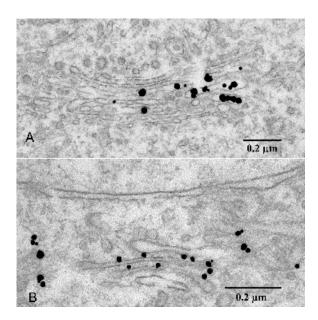


Figure 1. Pre-embedding immunogold labeling of MGP-160 (A) and Huntingtin Interaction Protein Interacter (B) in brain tissue. Note the localization of the gold particles in the inner (A) and outer (B) surface of Golgi membrane

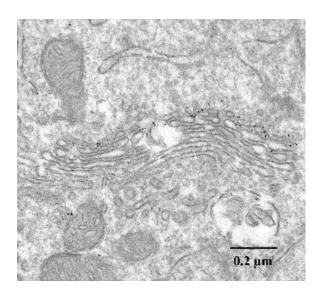


Figure 2. Pre-embedding immunogold labeling of MG-130, a cis-Golgi protein in mouse brain tissue.