

PERSPECTIVES

## New Frontiers in Gold Labeling: Symposium Overview

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**SUMMARY** The Symposium *New Frontiers in Gold Labeling* was held at the Fifth Joint Meeting of the Japan Society of Histochemistry and Cytochemistry and the United States Histochemical Society. Speakers described technological developments in this area that improved localization of cellular components. Each presentation is summarized in this overview, and complete articles follow that describe these results in more detail.

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In situ localization of molecules in cells and tissues continues to be important in basic and applied biological research. Immunocytochemistry and in situ hybridization of nucleic acids are key methodologies in this context. Because of the relevance of these techniques, advances in technology are highly desirable. The Symposium *New Frontiers in Gold Labeling* was held at the Fifth Joint Meeting of the Japan Society of Histochemistry and Cytochemistry and the United States Histochemical Society on the campus of the University of California–San Diego. Five articles from the Symposium are presented here and advance the theme of this significant meeting.

Hainfeld and Powell describe the development of small gold cluster immunoprobes using the recent technology of organometallic chemistry. Large gold compounds are adapted to link covalently to Fab' antibody fragments to form reagents with exceptional penetration and labeling performance. Their use in electron and light microscopy is described and is compared with previous colloidal gold technology. The 1.4-nm metal cluster Nanogold has been developed as a general reagent for coupling proteins, peptides, carbohydrates, and even DNA and lipids. Some of these novel gold conjugates and their application as new

probes are presented. Newer clusters using other metals, such as platinum and iridium, are also discussed.

Robinson and colleagues present work from their laboratories in which they combine light and electron microscopic immunocytochemistry. These studies were facilitated by the use of FluoroNanogold (FNG), a bifunctional labeling reagent that contains a gold cluster compound, a fluorochrome, and an antibody (e.g., Fab fragment). The authors compare the labeling efficiency of gold cluster probes to colloidal gold probes in cryosectioned neutrophils. They report that the 1.4-nm Nanogold cluster immunoprobes penetrate into these samples to a greater extent than do colloidal gold probes. In addition, they compared the ability of gold cluster immunoprobes and colloidal gold probes to penetrate into glutaraldehyde-fixed and detergent-extracted tissue culture cells. Whereas colloidal gold ( $\geq 5$ -nm) particles do not penetrate under these conditions, the gold cluster probes penetrate throughout the cells. Colloidal gold probes do penetrate into these cells if detergent extraction precedes fixation. A particularly stringent test for the penetration of gold cluster immunoprobes is the immunolabeling of tubulin within centrioles. These structures, which are composed of a characteristic array of microtubules, have been refractory to immunolabeling with colloidal gold as the reporter system. Use of Nanogold immunoprobes as the reporter system leads to heavy labeling of these structures. The authors also demonstrate, at the ultrastructural level, that heavy immunolabeling of centrosomes can be achieved with the mitosis-specific antibody MPM-2 and that the level of labeling varies during the cell cycle. Further evidence for increased labeling efficiency of small gold cluster immunoprobes

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compared to colloidal gold comes from examination of freeze-fracture immunocytochemical preparations, thus establishing the improved labeling efficiency achieved with small gold cluster probes. These results are consistent with work from a number of laboratories showing the inverse relationship between labeling efficiency and colloidal gold size. In addition, the authors review their work using FNG for immunolabeling of microtubules. They show that this reporter system, when coupled to a silver-enhancement procedure, leads to a reaction product that can be imaged by multimodal light microscopy. Moreover, their results suggest the potential of FNG as a reporter system for correlative light and electron microscopic immunocytochemical studies.

Takizawa and colleagues have realized the possibility of rigorous correlative microscopy using FNG as the reporter system, using ultrathin cryosections of human neutrophils as the immunocytochemical substrate. They use primary antibodies directed against marker proteins of the cytoplasmic granules of these cells followed by FNG. Initially, EM grids containing immunolabeled ultrathin cryosections are examined by fluorescence microscopy. The grids are then subjected to a silver-enhancement reaction to enlarge the size of the FNG particles before examination with the electron microscope. The same cell profiles examined by fluorescence microscopy are located in the EM. There is a precise one-to-one correlation between the fluorescence signal and the silver-enhanced gold signal. These results document the utility of FNG for correlative light and electron microscopic immunocytochemistry. The authors also review their work on freeze-fracture immunocytochemistry. Although this is a somewhat specialized application, it has certain unique attributes for assessment of immunolabeling at the ultrastructural level. The thin sections, ultrathin cryosections, or resin sections that are usually used in immunoelectron microscopy offer very limited surface area or volume for analysis. Freeze-fracture preparations, on the other hand, provide relatively large ex-

panses of membranes for immunocytochemical analysis. This feature has been used to advantage in comparing the labeling efficiency of gold cluster probes to that of colloidal gold probes.

Mayer, Bendayan, and colleagues have made significant progress in advancing immunocytochemistry by using a novel gold particle that serves as a substrate for horseradish peroxidase, thus depositing gold clusters, resulting in improved signal detection by electron microscopy. They used the well-characterized systems of localizing amylase in pancreatic acinar cells and insulin antigenic sites in pancreatic islet cells. Results were visible at the EM and LM level using silver enhancement. Such methods may have broad application to the popular CARD (CAlyzed Reporter Deposition) amplification system, permitting an EM-compatible label to be deposited.

Sawada and Esaki have addressed an important detail about the use of small gold probes. For many applications, the 1.4-nm gold clusters require silver enhancement to expand them to a size that is convenient for visibility in the electron microscope. Standard treatment with  $\text{OsO}_4$ , however, oxidizes the silver back into solution, thus diminishing or dissolving the silver enhancement. These researchers found a useful and simple gold toning procedure that coats the silver deposit with a thin layer of gold, making it impervious to attack from the  $\text{OsO}_4$ .

In summary, the articles presented in this Symposium illustrate that there are still frontiers in gold labeling that merit exploration. An important aspect is the development of new and improved labeling reagents. New metal clusters, clusters with novel binding functionalities, new metal cluster conjugates with other molecules, and fluorescent metal cluster probes fit into this category. Of parallel importance is the use of these new probes in a variety of biological systems. The applications presented in this Symposium demonstrate to other investigators the potential of these probes and suggest methods for their use in the resolution of many biological questions.