Combined Texas Red and 1.8 nm FluoroNanogold™ for Multimodal Imaging.

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Antifungal drugs generally target the cell wall [1]. Unlike mammalian cell walls, those of microorganisms such as yeast are composed of networks of different interconnected polysaccharide chains. The yeast cell wall is composed of O-mannosylated proteins or "sensors" thought to be involved in signal-transduction. Our goal is to develop nanoparticle (NP) probes to study the function and mechanical properties of macromolecules involved in signal-transduction pathways [2]. However, because the extracellular domains of these plasma membrane "sensors" are embedded within the cell walls, they are not readily accessible. Therefore, we developed a new strategy to bind NP probes to the mannosylated macromolecules in the cell wall of *whi5* mutant *Saccharomyces cerevisiae* (*S. cerevisiae*; **Figure 1**). Biotin was tethered to concanavalin A (Con A; from Jack bean) via a ~ 1.3 nm long linker Sulfo-NHS-LC Biotin (Pierce) and purified by Superose-12 size exclusion chromatography. The biotin-Con A conjugate was used to label surface mannose of *S. cerevisiae* [3]. Unbound biotin-Con A was separated by centrifugation and the cells were washed by centrifugation and resuspension.

The biotinylated cells were labeled and imaged by light and electron microscopy using a novel secondary probe: streptavidin conjugate labeled with both Texas Red and 1.8 nm gold NPs (**Figure 1 A**). The 1.8 nm gold NPs were prepared by direct reduction of Au(III) salt with sodium borohydride in the presence of proprietary thiols [4] under acidic conditions [5] and conjugated to fluorescently-labeled streptavidin following established protocols at Nanoprobes, Inc. The biotinylated *S. cerevisiae* cells were labeled with the combined 1.8 nm gold and Texas Red streptavidin FluoroNanogold™ conjugate, excess conjugate was removed by centrifugation and resuspension, and labeling specificity was monitored by fluorescence microscopy (data not shown). Gold labeling was visualized in the bright field light microscope by catalytic deposition of silver using LI Silver (Nanoprobes, Inc., data not shown), and in the scanning electron microscope (SEM) using HQ silver (Nanoprobes, Inc., **Figure 1 B**).

The key salient features of the protocol presented for successful labeling of mannosylated extracellular domains of the plasma membrane are: (1) ***small probe size*** - larger colloidal gold probes (10-40 nm) give very low and non-uniform labeling due to steric hindrance (**Figure 1 C**); (2) ***use of a reporter with a longer tether between the targeting agent and reporter molecule*** – the

longer (~1.3 nm) tether between Con A and biotin makes specific labeling of plasma membrane "sensors" easy; (3) *covalent linkage between gold NP probes and reporter molecule* – colloidal gold probes are prepared by adsorption of biological macromolecules that tend to dissociate and aggregate over a period of time resulting in lower specific binding and higher nonspecific background while the gold NP probes described in this labeling protocol are cross-linked to the targeting molecule via a stable covalent bond, and therefore produce high specific signal and low background; (4) *use of dual-labeled secondary probes* - although this adds a step to the labeling protocols they enable fast screening of labeled cells by using an inexpensive bench top microscope, thereby minimizing the lengthy sample preparation required for high-resolution electron microscopic studies. The gold and Texas Red dual-labeled FluoroNanogold™ conjugate combined with silver enhancement enables multimodal (fluorescence, bright field and electron microscopy) imaging [6]. The dual labeled *S. cerevisiae* cells enhanced with HQ Silver were also used to demonstrate correlative SEM and high-resolution (10-13 nm) X-ray diffraction microscopy [7].

References

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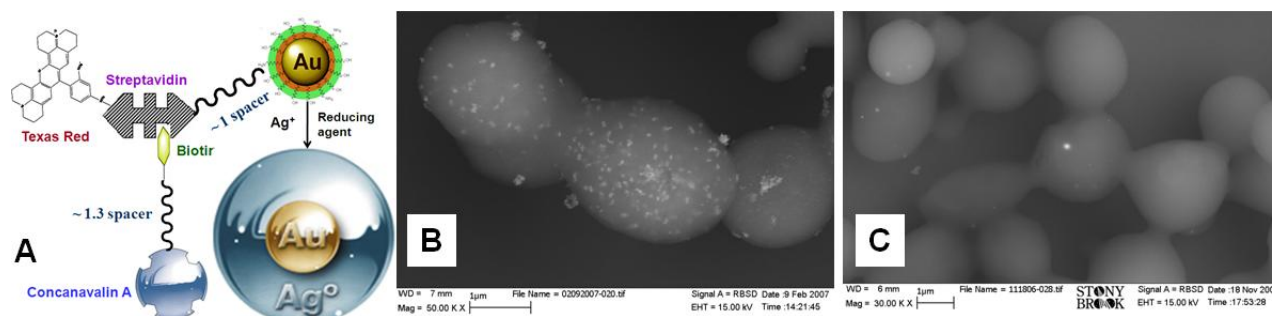[8]   The development of gold-streptavidn conjugate was supported by NIH SBIR grant number 1R43 EB008621.

Figure 1. **A**: Biotin tethered to Con A with ~1.3 nm long linker targeted with a combined 1.8 nm gold and Texas Red streptavidin FluoroNanogold™; **B**: SEM of S. cerevisiae cells labeled with Con A-LC-Biotin targeted by combined 1.8 nm gold and Texas Red streptavidin FluoroNanogold™, then enlarged with HQ Silver (magnification 50,000); **C**: SEM of S. cerevisiae cells labeled with Con A-biotin targeted with 10 nm gold-streptavidin conjugate (magnification 30,000).