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Colloidal Gold Conjugates of Cholera Toxin B-Subunit of Alexa Fluor® Fluorescent Dyes for Use in Correlative Studies

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Conjugations of colloidal gold (18.0 nm) to the cholera toxin B-subunit (CTB) of green fluorescent Alexa Fluor® 488 (ex 495 nm/em 519 nm) and to the CTB of the red-fluorescent Alexa Fluor® 594 (ex 590 nm/em 617 nm) show promise for correlative light (*i.e.* differential interference contrast [DIC]), fluorescence, and electron microscopy. Commercially available (Molecular Probes, Inc., Eugene, OR) Alexa Fluor 488 conjugated to CTB and Alexa Fluor 594 conjugated to CTB were separately dialyzed in distilled water for 1hr and separately added to 18.0 nm colloidal gold (pH 8.18) to a concentration of 200 μgm per ml of gold, spun down, and re-suspended in 0.1M phosphate buffer at pH 7.4. The final concentrations of Alexa Fluor 488 and Alexa Fluor 594 conjugates of colloidal gold CTB were approximately 5 x 10 twelfth particles/ml.

Filter paper saturated with Alexa Fluor 488 or Alexa Fluor 594 colloidal gold conjugates of CTB was immediately applied to both ends of peripheral nerve stumps of the anal fin appendicular support of newborn, immature and adult Western Mosquitofish, *Gambusia affinis affinis*. The cut peripheral nerves were exposed for 1 minute, the time found to be sufficient to retrogradely label even the most finely axonal fibers and dendritic branches (Figs. 2-3). *G. a. affinis* were revived, allowed to survive for 6-24 hours, then euthanized, perfusion fixed with 4% paraformaldehyde, washed, bleached, cleared, and visualized using DIC and fluorescence microscopy.

Alexa Fluor 488 and Alexa Fluor 594 colloidal gold conjugates of CTB labeled Mauthner cells and their axons within the hindbrain (Figs. 1-2) and secondary spinal motor neuron cell bodies and their extensive dendritic arbors (Figs. 2-3). The tyramide signal amplification procedure for Alexa Fluor 594 (Molecular Probes, Inc., Eugene OR) combined with a previously described whole-mount clear and stain procedure [1] labeled peripheral nerve fibers of the ano-urogenital plexus at all stages and made them easy to visualize (Fig. 4).

Our results demonstrate that the conjugations of colloidal gold to the CTB of Alexa Fluor 488 and to the CTB of Alexa Fluor 594 have properties that permit their identification in light and fluorescence microscopy and show promise for electron microscopy and correlative studies.

References

[1] Brain Res. Brain Res. Protoc. 1999 (4): 115-123

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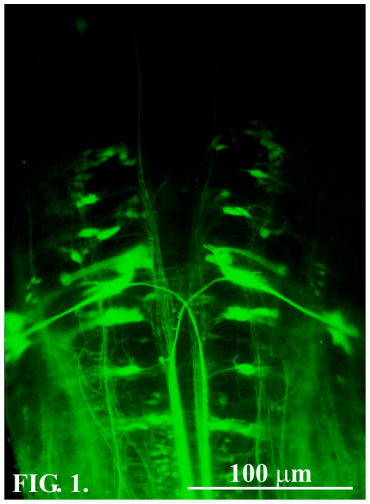

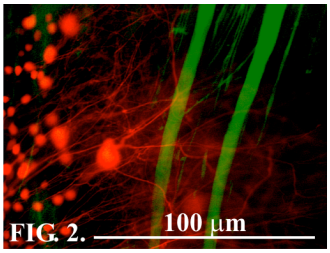

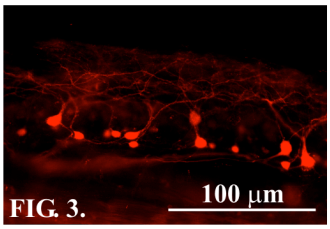

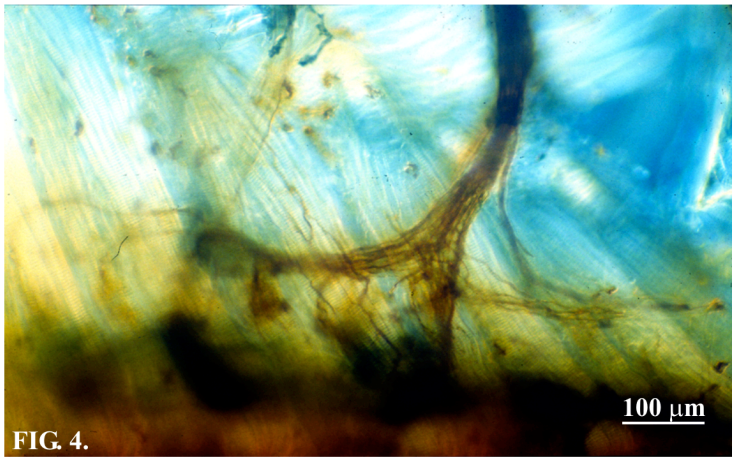

FIG. 1. Mauthner cells and axons in a whole-mount preparation of paraformaldehyde-fixed male Western Mosquitofish, *Gambusia affinis affinis* hindbrain visualized using green-fluorescent Alexa Fluor 488 colloidal gold conjugate of Cholera Toxin B-subunit.

FIG. 2. Mauthner axons (green) and secondary motor neurons (red) in a whole-mount preparation of paraformaldehyde-fixed male Western Mosquitofish, *Gambusia affinis affinis* spinal cord visualized using green-fluorescent Alexa Fluor 488 and red-fluorescent Alexa Fluor 594 colloidal gold conjugates of Cholera Toxin B-subunit.

FIG. 3. Secondary motor neurons in a whole-mount preparation of paraformaldehyde-fixed Western Mosquitofish, *Gambusia affinis affinis* spinal cord visualized using red-fluorescent Alexa Fluor 594 colloidal gold conjugate of Cholera Toxin B-subunit. Note the extensive dendritic arborization.

FIG. 4. Peripheral nerve fibers of the ano-urogenital plexus in a whole-mount preparation of paraformaldehyde-fixed male Western Mosquitofish, *Gambusia affinis affinis* anal fin appendicular support visualized using red-fluorescent Alexa Fluor 594 colloidal gold

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