

Colloidal Gold Conjugate of Recombinant Cholera Toxin B-Subunit of Alexa Fluor® and Dextran-Texas Red®-Nanogold® Fluorescent Dyes for Use in Correlative Microscopy and Intravital Imaging

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The sexually dimorphic anterior transposition of the median unpaired fin, specifically the anal fin of the Western Mosquitofish, *Gambusia affinis affinis*, provides a versatile experimental model for gaining insight into a number of complex intercellular mechanisms, processes, and interactions during post-embryonic development. Of particular interest is the fate of the *G. a. affinis* nervous system as it undergoes remodeling to accommodate the radical changes in target tissues and organs and the animal changes from a non-internal fertilizing to an internal fertilizing species. However, a major restriction to realizing the full potential of this vertebrate model system, particularly with regard to understanding development and differentiation, is the lack of sufficiently refined and precise mapping techniques and probes. Thus, the cellular decisions underlying motor neuron remodeling during the anterior transposition of the anal fin have received no attention. In earlier work (Fig. 1A-1B), colloidal heavy metals, such as gold (18.0 nm) were directly conjugated to commercially available (Molecular Probes, Inc., Eugene, OR) recombinant cholera toxin B-subunit of green fluorescent Alexa Fluor® 488 [ex 495 nm/em 519 nm; Lot: 73B2] (AF 488 cgrCTB) and to recombinant cholera toxin B-subunit of red-fluorescent Alexa Fluor® 594 [ex 590 nm/em 617 nm; Lot: 73B1] (AF 594 cgrCTB) and shown to be transported retrogradely to the central nervous system, specifically spinal motor neurons, without quenching of the fluorescence (Fig 1). The lack of quenching seen in AF 488 cgrCTB and AF 594 cgrCTB is not well understood and is currently under investigation [1, 2].

Recently (Fig. 1C) we used mono-sulfo-NHS Nanogold® (Nanogold Inc., Yaphank, NY), a 1.4 nm Nanogold compound with a single reactive group, a sulfo-N-hydrixysuccinimide ester (sulfo-NHS) that reacts with primary amines under mild conditions (pH 7.5 to 8.2) to covalently label red-fluorescent dextran Texas Red® [ex 595 nm/em 615 nm; Lot: 65B1], 10,000 MW, lysine fixable (Nanogold TDA). The final stoichiometry of this probe was approximately 0.2 Nanogold's per Texas Red®.

AF 488 cgrCTB, AF 594 cgrCTB, Nanogold TDA, and 3,000 MW fluorescein dextran-amines (FDA) were used to retrogradely label spinal motor neurons innervating a median unpaired fin, the sexually dimorphic anal fin musculature in female and male *G. a. affinis* in order to assess sex differences in spinal motor nuclei organization.

The spinal cord of *G. a. affinis*, as that of other teleost fishes, contains three distinct motor neuronal types: three primary that innervate the axial muscles of a given hemisegment and secondary motor neurons that innervate the appendicular support muscles of the pectoral, pelvic and anal fin spanning several spinal segments (Fig.1D). Retrograde tract tracing using AF 488 cgrCTB, AF 594 cgrCTB, Nanogold TDA, and FDA revealed a unique spinal cord region associated with the 12th through 14th vertebrae, a portion of the unique ano-urogenital region. These segments contain a population of secondary motor neurons that branch extensively and have extensive dendritic arborization; these

segments also contain a plexus innervating the muscles of the median unpaired fins. AF 488 cgrCTB, AF 594 cgrCTB, Nanogold TDA, and FDA revealed the most fine axonal fibers and dendritic branches as well as sex differences (Fig.1E), showing that female *G. a. affinis* had fewer and smaller secondary motor neurons than did males and that the neurons branching and dendritic arborization were more reduced than those in males. Transmission electron microscopy and intravital imaging studies remain to be completed.

References

- [1] E. Rosa-Molinar et.al., *Microsc. Microanal.* 8 (Suppl.2) (2002) 132-133.
 [2] I.K. Kandela, et al., *Microsc. Microanal.* In press.
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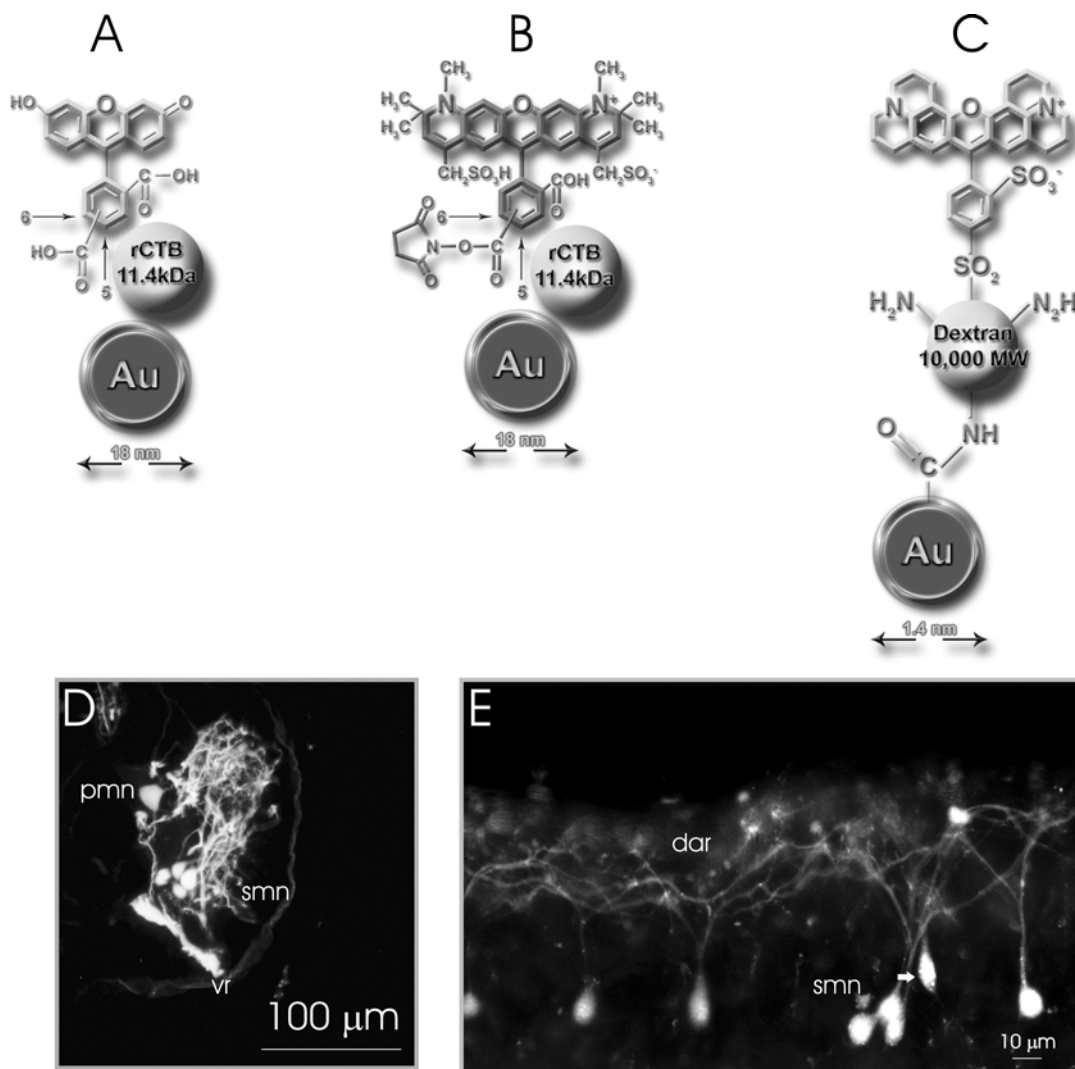


Fig.1. Cartoon of (A) Alexa Fluor® 488 cgrCTB, (B) Alexa Fluor® 594 cgrCTB, and (C) Dextran Texas Red® Nanogold. (D) Retrogradely labeled primary (pmn) and secondary (smn) motor neurons. (E) High magnification of retrogradely labeled secondary motor neurons (smn) showing extensive dendritic arborization (dar). Note the gold clustering (arrow).