# Abstract

Neuronal circuits are complex networks composed of diverse types of neurons that provide the functional flexibility of living organisms. It is generally thought that each type of neuron plays a distinct role, and together they enable the integrated operation of the entire circuit. Therefore, techniques that selectively record the activity of specific neuronal types are indispensable for studies of neural circuits. Microelectrode technique has long been one of the major methods for recording neural activity with millisecond precision. However, because it lacks cell-type specificity, identifying neuronal types requires the use of cumbersome and indirect auxiliary methods. This limitation becomes even more critical in recently developed multi-electrode array technologies, where distinguishing neuronal types has become increasingly difficult.

To confer cellular selectivity on electrodes, this study set out to engineer an orthogonal library of synapse organizers functioning as molecular “lock-and-key” switches. These tools were designed so that a receptor-functionalized microelectrode could induce the formation of presynapse-like structures on axons expressing the corresponding ligand-tagged organizer, in a cell-type-specific manner. The study also sought to determine the ligand–receptor affinities required for such tools.

Three polycistronic constructs—Spot-, V5-, and Alfa-cNrxn1βΔECD—were generated, each fused via a P2A peptide to EGFP-Rab3 as a fluorescent presynaptic marker. Truncation of the LNS domain eliminated binding to endogenous neuroligins, ensuring that synapse induction was strictly dependent on ligand–nanobody interactions. Reported affinities of the peptide–nanobody pairs span from 26 pM (Alfa) to 29 nM (V5). HEK293T surface-display assays confirmed strict one-to-one recognition: only the cognate nanobody produced robust membrane fluorescence, even under saturating ligand concentrations (up to 8 μM). Primary chick forebrain neurons were transfected with the constructs and cultured overnight with nanobody-decorated microbeads. Synapse formation was quantified by EGFP-Rab3 accumulation at axon–bead contacts, and statistical analysis was performed using Kruskal–Wallis followed by Dunn’s post-hoc tests. Targeted contacts (e.g., Spot construct + anti-

SpotNb beads) showed significantly higher Rab3 indices than any off-target combination. No significant differences were detected among the three on-target pairs, indicating comparable synaptogenic potency despite an ~1000-fold range in dissociation constant (Kd). These results establish Spot, V5, and Alfa tags as a bona fide orthogonal trio well suited for multiplexed interfacing. The molecular tools described here are expected to provide a platform for precise, genetically targeted, and multiplexed electrophysiological recording in future. This dissertation is organized into five main chapters. Chapter 1 serves as a general introduction, outlining the basic concepts of key components discussed in this work, including neurons, synapses, and microelectrode technology. Chapter 2 provides a detailed discussion of the background of synapse organizers and their applications in experiments, representing the central focus of this study. Chapter 3 describes the experimental principles and procedures employed in this research, as well as the methods for data acquisition and analysis. Chapter 4 presents and analyzes the experimental results in detail, drawing the corresponding conclusions. Finally, Chapter 5 summarizes the results and conclusions as a whole and offers perspectives for future studies.

**Keywords**

**Neurons, Neuronal Circuits, Synapses, Synapse Organizers, Microelectrode Technique, Dissociation constant, Orthogonality, Engineered synapse organizers**