METHODS IN MOLECULAR BIOLOGY

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Photoswitching Proteins

Methods and Protocols

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Preface

On August 4, 2004, Edward Boyden and Karl Deisseroth made a groundbreaking discovery. They had just transplanted channelrhodopsin-2, a light-dependent cation channel from flagellate algae into neurons grown in a Petri dish, and exposed the cells to brief blue light pulses in a microscope. And suddenly, the cells responded, firing axon potentials precisely matching the pattern of the light stimulus—optogenetics was born. Since that day, the ability to remotely control neurons with optogenetics rapidly transformed the neurosciences and revolutionized our understanding of how behavior, memories, and feelings emerge from the complex interplay of cells in our brains.

Today, optogenetics is no more exclusive to the neurosciences. It is a flourishing research field and a maturing technology routinely used by many labs around the world and across various research areas in biology. The term optogenetics generally refers to controlling protein function from outside a cell or organism using light. The advantage of using light as trigger as compared to alternative triggers such as chemicals is the high spatiotemporal precision at which it can be applied. This becomes particularly relevant when studying fast and dynamic cellular processes, may it be the firing of neurons, cell migration, receptor signaling, or the transport of macromolecules within a cell, just to name a few.

At the core of optogenetics are photoswitching proteins, i.e., proteins whose activity is dependent on light. Nature presents us with a highly versatile toolbox of photoreceptors differing with respect to their cellular function, the light-switching mechanism they use as well as the wavelength of light they respond to. Generally, photoreceptors can be harnessed for optogenetic applications in two different ways. One is to transfer a photoreceptor into a non-natural context, all the while preserving its natural function. Here, the most famous example is the algae-derived channelrhodopsin-2 used to control ion flux into neurons mentioned before. A second possibility is to couple the photoreceptor's signaling state to a customized function by protein engineering. This allows adapting optogenetics to cellular functions which, in nature, are not dependent on light, but would be interesting to place under light control for basic research or biotechnological purposes.

Optogenetics has truly revolutionized the neurosciences and enabled new, fascinating insights into cell biology. There also have been considerable advances in developing optogenetics for industrial applications, such as the optimization of chemical production in microbes, as well as therapy of neurological diseases or vision restoration.

The purpose of this Methods in Molecular Biology volume is to provide cutting-edge protocols for using optogenetic techniques across a wide spectrum of applications in biology. On the one hand, we wish to offer these as a resource to newcomers, who are just about to perform their first optogenetics experiment. Therefore, we included several basic protocols from both neuronal and non-neuronal optogenetics, as well as three comprehensive overview articles highlighting emerging topics. On the other hand, the large panel of cutting-edge techniques contained in this volume should be of interest to researchers already in the field, who look for the latest updates on the methodology as well as inspiration on how to further improve their established workflows.

Chapter 1 details a method to investigate functional properties of local and long-range connectivity ex vivo by applying channelrhodopsin-2-mediated optogenetics and patch-

clamp to acute brain slices. Chapter 2 introduces and discusses optogenetic and optochemical techniques to investigate G-protein-coupled receptors and their downstream signaling. In Chapter 3, the use of melanopsin, a mammalian G-protein-coupled photopigment, for timely activation of astrocyte-neuron networks in vitro and in vivo is detailed. Chapter 4 describes the use of upconversion nanoparticles for deep brain simulation of neurons by transcranial near-infrared light. Chapter 5 details a methodology for cloning, lab-scale production, and purification of recombinant adeno-associated virus (AAV) vectors encoding optogenetic switches. In Chapter 6, an AAV-mediated method and surgical procedures for cone transduction in the fovea of macaques are presented. Chapter 7 provides an overview and discussion of approaches to control proteins with light in cells and animals, with focus on those based on the light oxygen voltage 2 domain from *Avena sativa*. Chapter 8 details a method for controlling the nucleocytoplasmic transport of selected proteins in mammalian cells with blue light. Chapter 9 describes a methodology for light-inducible CRISPR labeling, which allows following in time the recruitment of CRIPSR-Cas9 to selected genomic loci. In Chapter 10, two complementary approaches facilitating light-dependent activation or inhibition of transgene expression based on cryptochrome-2 (CRY2) are detailed. Chapter 11 describes a method to control programmed cell death in mammalian cells with a dual blue light switch. Chapter 12 details a methodology for tracing reversible blue light-induced chromatin recruitment using the near-infrared fluorescent protein iRFP713. In Chapter 13, the construction and application of a multiwell platform for traceless light control of gene expression by melanopsin is detailed. Chapter 14 describes a methodology to control cAMP production with light or luciferins using an engineered, dual-activated adenylyl cyclase. Chapter 15 details a method for synthesizing an extracellular matrix with mechanical properties adjustable by red and far-red light. Chapter 16 provides fundamental considerations in the design of light-regulated receptor tyrosine kinases (Opto-RTKs) and describes protocols for their expression and characterization in mammalian cells. In Chapter 17, a co-culture assay and corresponding optical high-throughput screening method for identification of voltage-gated Ca^{2+} channel blockers is detailed. Finally, Chapter 18 reviews and discusses emerging tools at the intersection of optogenetics and CRISPR, which facilitate genome perturbations with unmet spatiotemporal precision.

In summary, this volume provides a comprehensive list of methods and tools to investigate highly dynamic pathways and behaviors in cells and animals using optogenetics. It is my hope that the protocols contained in this book will give newcomers in the field a head start and provide established labs with important updates on recent methodological advances. The optogenetics field has been extremely successful and has seen rapid growth over the past 15 years. If this book can make a humble contribution to sustaining this successful path, it has fulfilled its purpose.

I would like to thank all authors, who contributed detailed protocols for this book, thereby making their valuable expertise accessible, and for sharing those little tricks that are so critical to get a technique to work in the lab. Furthermore, I am grateful to John M. Walker, the series editor, who provided guidance and advice and without whom this volume would not exist.

I wish to devote this book to my kids, Valerie and Luisa, who were often sleeping when I worked on this book.

Heidelberg, Germany ***Dominik Niopek***

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