Preface

Biological processes are regulated with high spatial and temporal resolution at the molecular, cellular, and systems level. To control and study processes with the same level of precision, researchers have employed light-sensitive motifs to optically activate and deactivate biological function. Optical control is a noninvasive technique in which the amplitude, wavelength, location, and timing of the light irradiation can be easily controlled. This volume of *Methods in Enzymology* focuses on optical tools that are based on chemical approaches (such as synthetic light-activated small molecules), genetic approaches (such as naturally occurring light-responsive protein domains), and hybrid approaches (such as combining synthetic, light-activated amino acids with their genetic encoding).

The use of light-cleavable protecting groups, so-called caging groups, enables control of biological function with a single, small, covalently linked chromophore that can be removed through exposure to nondamaging UV light, thereby restoring the native molecule. Light activation of small molecules dates back to the first reports on photocaged ATP in the 1970s. Since then, hundreds of compounds with diverse biological function have been placed under optical control, including biological macromolecules such as nucleic acids, peptides, and proteins. In addition to caging group optimization over the years, reversibly switchable approaches have also been developed.

In order to optically activate the function of proteins fused to a modified estrogen receptor, Bensimon synthesized a caged cyclofen molecule, following a new synthetic route, and applied it in zebrafish embryos to the light activation of Cre recombinase (Chapter 1). Excellent off to on switching was demonstrated with spatial and temporal resolution using one- and two-photon excitation. Protein–protein interactions are underlying many cellular processes, and Chenoweth developed optical control of protein association and dissociation through engineering of caged and photocleavable small-molecule inducers of dimerization (Chapter 2). They demonstrated their approach in the recruitment and release of proteins from kinetochores thereby activating and silencing, respectively, the spindle assembly checkpoint in mitosis. Several distinct approaches to optochemical control of nucleic acid function exist. Stafforst photochemically triggered ribonucleotide protein assembly through a caged

benzylguanine SNAP-tag ligand, which enabled recruitment of the enzyme ADAR1 to a guideRNA and thus allowed for precise photocontrol of RNA base editing (Chapter 3). Chen developed optical control of morpholino antisense function through conformational gating by circularizing the oligomer with different light-cleavable linkers (Chapter 4). The introduction of spectrally distinct chromophores enabled combinatorial regulation of multiple genes in zebrafish embryos through irradiation with light of different wavelengths. This strategy of circularization followed by conditional linearization was further adapted by Heckel who installed light-cleavable alkynes into DNA/RNA phosphodiester backbones and then intramolecularly tethered them through cycloaddition reactions with bis-azide reagents (Chapter 5). Multicircular tethering provided oligonucleotides that displayed no background hybridization to their complementary sequence. Peptides and proteins have also been chemically modified with light-responsive motifs. The installation of different photocleavable groups onto insulin was achieved by Friedman through reaction of diazo compounds with carboxylic acid side chains on the protein (Chapter 6). In pioneering work, Woolley developed light-switchable, intramolecular, azobenzene-based cross-linkers that are introduced into peptides and proteins through selective reaction with cysteine residues (Chapter 7). Photochemical switching of the azobenzene configuration switches the protein's structure and thereby regulates its activity, as showcased for Fyn SH3 domains. All of these optochemical approaches benefit from the development of new caging groups and new light-cleavable chromophores, in order to tune activation wavelengths and/or improve quantum yields. Dmochowski developed an interesting ruthenium polypyridyl complex-based light-responsive hydrogel and applied it to the encapsulation and subsequent optically triggered release of the enzyme β-lactamase (Chapter 8). By adjusting the ligands, the absorption wavelength of the metal complex was tuned across the entire visible spectrum. In the context of caging groups in a traditional sense, Ellis-Davies has a long-standing history in improving their properties, in particular toward orthogonal absorption wavelengths for two-color decaging of neurotransmitters with one- or two-photon activation, as demonstrated very efficiently for glutamate and GABA (Chapter 9).

In contrast to chemically synthesized chromophores, "optogenetics" is an alternative approach that makes use of natural, genetically encoded, light-responsive proteins. Optogenetic strategies have been applied to control biological processes such as transcription, protein localization, protein phosphorylation, and ion channel activity. Commonly employed, naturally

occurring photoreceptors include the rhodopsin family of ion channels, light-oxygen-voltage-sensitive domains, as well as phytochrome and cryptochrome proteins.

In order to facilitate optimization and application of optogenetic systems, Tabor developed an open-source hardware platform that is capable of delivering two independent light signals to wells of a 24-well culture plate (Chapter 10). Detailed instructions on how to assemble and program the device were provided. The Möglich lab also constructed a programmable illumination device from readily available parts for the rapid and parallelized testing of biological responses to diverse irradiation programs in a 96-well format (Chapter 11). They went on and applied it to the investigation of pulsed light exposure of defined frequency to maintain two optogenetic systems in their light-adapted state.

In an integration of chemical and genetic light-regulation strategies, photoresponsive proteins have been generated through site-specific incorporation of unnatural amino acids in cells and animals with an expanded genetic code, through the addition of orthogonal protein biosynthetic machinery.

This hybrid approach has been utilized by Wang to insert amino acids that contain an azobenzene chromophore and a reactive handle that performs an intramolecular stapling reaction with a strategically placed cysteine residue (Chapter 12). The resulting light-controlled bridge enabled reversible modulation of the secondary structure of the corresponding protein region. We used genetic code expansion in zebrafish embryos to site-specifically insert a photocaged lysine into the active site of Cre recombinase and were able to trigger DNA recombination events with high spatial and temporal resolution (Chapter 13). This enabled cell-lineage tracing in developing embryos through genomic labeling with a fluorescent protein.

Overall, I would like to thank all authors for their wonderful contributions and for sharing their insightful knowledge with the scientific community. I hope that this volume will be useful to both specialist who have already been working on the development and application of optochemical control of biological systems as well as nonspecialists who are just getting into the field. A wide range of light-activation methodologies exist, and the protocols presented here will facilitate their application to new scientific questions.

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