Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Recent advances in the optical control of protein function through genetic code expansion

Taylor Courtney and Alexander Deiters

In nature, biological processes are regulated with precise spatial and temporal resolution at the molecular, cellular, and organismal levels. In order to perturb and manipulate these processes, optically controlled chemical tools have been developed and applied in living systems. The use of light as an external trigger provides spatial and temporal control with minimal adverse effects. Incorporation of light-responsive amino acids into proteins in cells and organisms with an expanded genetic code has enabled the precise activation/deactivation of numerous, diverse proteins, such as kinases, nucleases, proteases, and polymerases. Using unnatural amino acids to generate light-triggered proteins enables a rational engineering approach that is based on mechanistic and/or structural information. This review focuses on the most recent developments in the field, including technological advances and biological applications.

Address
Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, United States

Corresponding author: Deiters, Alexander (deiters@pitt.edu)

Current Opinion in Chemical Biology 2018, **46C**:99–107

This review comes from a themed issue on **Synthetic biology**

Edited by **Michael Ledbetter** and **Floyd E Romesberg**

https://doi.org/10.1016/j.cbpa.2018.07.011

1367-5931/© 2018 Elsevier Ltd. All rights reserved.

Introduction

In order to study and manipulate biological processes with the same precision as nature, chemical biologists have developed a number of optical tools [1]. The use of light to control protein activity provides non-invasive, precise, spatiotemporal control and allows for more acute perturbation than other methods (such as RNA interference or gene editing). Optical control of protein function in live systems has primarily been achieved through two approaches: genetic encoding of light-responsive amino acids or optogenetic methods using natural photo-responsive protein domains. Over the last two decades, more than a hundred non-canonical amino acids have been genetically encoded in a range of organisms to provide functionalities not found in the common set of 20 amino acids [2]. The incorporation of light-triggered amino acids into proteins has been used to control a wide range of biological processes in cells and animals [3], and this review highlights select examples from the past 5 years in order to demonstrate the versatility of this approach. Due to space limitations, we are not including other important methodologies, such as protein bioconjugation of photoswitchable ligands [4,5]. Purely optogenetic approaches have been extensively reviewed elsewhere [6–9].

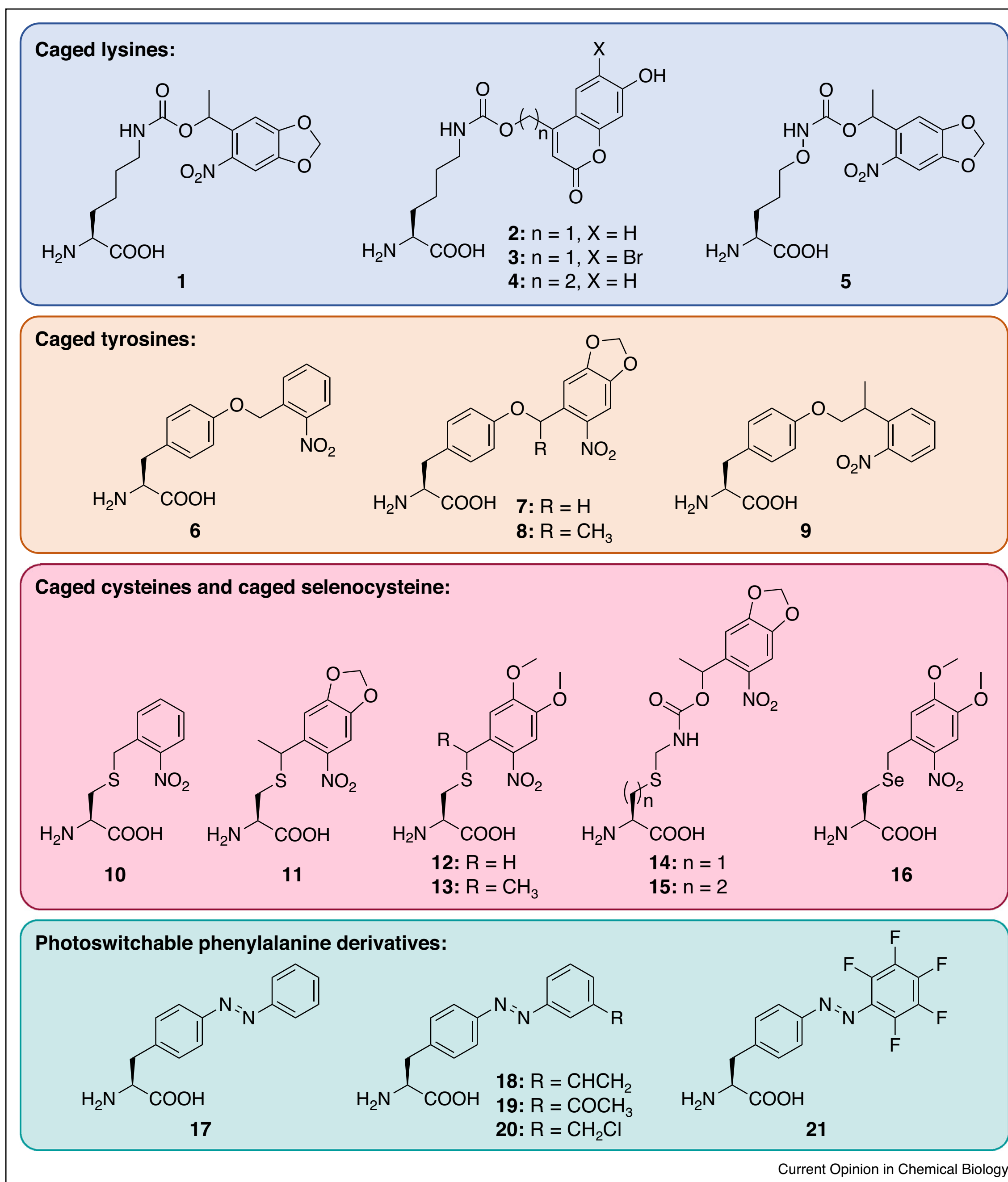
Technical advances in the field

Caged lysine

The photocaged lysine **1** (Figure 1) has been applied toward optical triggering of Cas9 nuclease [10], T7 RNA polymerase [11], Cre recombinase [12], MEK [13,14••] and LCK [15•] kinases, isocitrate dehydrogenase [16], and protein-protein interactions [17,18•]. Optical control of lysine, which plays an essential role in enzymatic catalysis of many biological processes, has been instrumental in gaining a deeper understanding of living systems at the molecular level. This photocaged lysine utilizes 365 nm light for activation and may be incompatible with certain experiments performed in *E. coli* due to the abundance of nitroreductases. In order to develop a system that is compatible with a range of organisms and to provide activation with blue (405 nm) and near-IR (two-photon 760 nm) light, the coumarin-caged lysines **2** and **3** were developed [19••]. Both were applied in mammalian cells for the optical control of luciferase function and of GFP folding and the different decaging wavelengths for **2** (405 nm) and **3** (760 nm) enable sequential, wavelength-selective activation. Additionally, the coumarin chromophore provides fluorescent tracking of the incorporated amino acid prior to decaging, thus **2** and **3** can act as both fluorescent and photo-activatable probes in live cells. Introduction of the additional methylene group in **4**, blocks photolysis and provides a stable and small fluorophore that can be site-specifically placed into proteins. The caged lysine **2** has subsequently been applied to control MEK kinase in zebrafish embryos [14] (see *Optical Control of Cell Signaling* section) and DNA helicase [20] (see *Optical Control of Nucleic Acid Processing* section).

While lysine often plays an essential role in enzymatic catalysis, replacement of the ε-carbon with oxygen generates an amino-oxy functionality which can undergo bio-orthogonal oxime ligation with a ketone or aldehyde. The

Figure 1



Genetically encoded, light-responsive unnatural amino acids. These include caged lysines **1–5**, caged tyrosines **6–9**, caged cysteines **10–15**, caged selenocysteine **16**, and photoswitchable phenylalanine derivatives **17–21**.

Virdee group generated the corresponding lysine analog **5** with a nitrobenzyl caging group to render it unreactive until UV-induced photolysis and encoded it using the same synthetase/tRNA pair engineered for **1** [21]. While incorporation efficiency was low, masking a reactive bio-orthogonal handle with a caging group may minimize off-target reactivity and may enable the encoding of other, more reactive bio-orthogonal handles.

Caged tyrosine

Photocaged tyrosine **6** was genetically encoded a decade ago and has been applied to the optical control of several enzyme classes [22–27]; however, in order to facilitate decaging through red-shifting of the chromophore's absorption maximum, the Deiters group developed three additional photocaged tyrosine derivatives **7–9** [28]. Use of a dual-luciferase reporter allowed for

Download English Version:

https://daneshyari.com/en/article/7693685

Download Persian Version:

https://daneshyari.com/article/7693685

Daneshyari.com