REVIEW

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Illuminating the Chemistry of Life: Design, Synthesis, and Applications of "Caged" and Related Photoresponsive Compounds

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ABSTRACT Biological systems are characterized by a level of spatial and temporal organization that often lies beyond the grasp of present day methods. Light-modulated bioreagents, including analogs of low molecular weight compounds, peptides, proteins, and nucleic acids, represent a compelling strategy to probe, perturb, or sample biological phenomena with the requisite control to address many of these organizational complexities. Although this technology has created considerable excitement in the chemical community, its application to biological questions has been relatively limited. We describe the challenges associated with the design, synthesis, and use of light-responsive bioreagents; the scope and limitations associated with the instrumentation required for their application; and recent chemical and biological advances in this field.

The extraordinary complexity of the biological world is an irresistible lure that challenges the imagination of biologists and chemists alike. This complexity is evident in multicellular organisms, where individual cells are assigned unique attributes depending upon their location, and within individual cells, where the location, timing, and overall activity of any given biochemical transformation are variables that can have profoundly distinct biological consequences. For example, life and death decisions are determined by intracellular spatial distances of less than 1 μm. Cytochrome *c* is an integral component of the electron transport chain when it resides within the intermembrane space of the mitochondrion. However, upon its release from the mitochondrion, a biochemical cascade is initiated that ultimately results in cell death. Life is organized, be it at the multicellular or cellular level, in a spatially and temporally adaptable fashion. However, in the absence of tools (*e.g.*, antagonists, agonists, *etc.*) that can be modulated in terms of where, when, and how much, it is fiendishly difficult to probe these organizational features.

Chemists have made countless contributions that have impacted the biological realm, ranging from molecules to technology. However, it is clear that the chemical tool kit assembled to date is woefully inadequate for understanding or controlling the biochemistry of life. Arthur Kornberg, back in 1987 noted, "Molecular biology appears to have broken into the bank of cellular chemistry, but for the lack of chemical tools and training, it is still fumbling to unlock the major vaults." (*1*) Certainly, chemistry has played an important role in the isolation and characterization of individual biomole-

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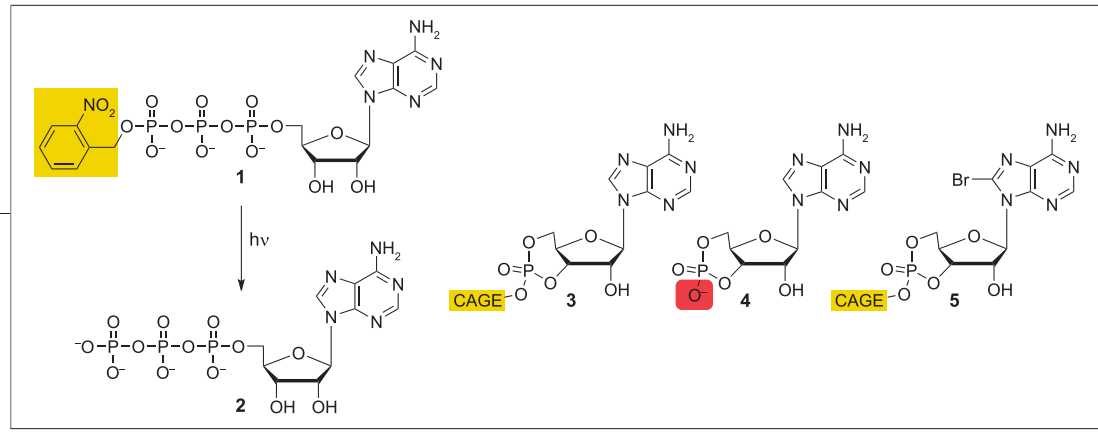

Figure 1. Caged ATP (1) and cAMP analogs (3 and 5). These derivatives illustrate the classical caging strategy *via* covalent modification of an essential functional group required for biological activity. Photolysis converts the caged form of ATP (1) to its active counterpart (2) and the caged form of cAMP (3) to its active counterpart (4). However, species such as cAMP can be rapidly "deactivated" *via* enzyme-catalyzed hydrolysis. By contrast, the brominated cAMP analog 5 is bioorthogonal to the endogenous biochemistry of the cell. Upon photolysis, photoreleased bromo-cAMP analog displays long-lasting cAMP activity since it is resistant to phosphodiesterase-catalyzed hydrolysis.

cules, be they low molecular weight compounds, carbohydrates, nucleic acids, or proteins. However, it is well worth remembering that these biomolecules evolved in conjunction with the cellular environment as a whole. In short, there are many aspects of the biological world that simply cannot be understood outside of the cellular context. The spatiotemporal dynamics of biochemical activity, particularly as it relates to cellular behavior, is but one of many examples.

Biologists have long used chemical and biochemical tools to control intracellular pathways and thereby influence the action of cells and organisms. However, once the agent (inhibitor, activator, *etc.*) enters the cell, all control over that agent is lost. For example, the intracellular spatial distribution of the reagent as well as the precise timing and endurance of its activity are difficult-to-manipulate properties. Light-activatable versions of these compounds offer a means to return control of the reagent to the biologist, even after it has entered the cell. The basic strategy has the appearance of simplicity and elegance. First, identify a key residue or functional group on the agent of interest that is essential for biological activity. Second, covalently modify that key site with a light-cleavable moiety, thereby rendering the agent biologically silent. Third, introduce the inactive agent into the cell or organism. Fourth, use light to activate the agent with pinpoint spatial and temporal accuracy. This strategy is often traced back to the seminal 1978 paper of Kaplan, Forbush, and Hoffman (*2*), who described a light-activatable ATP **1** (Figure 1). The latter is not recognized by a Na/K-ATPase. However, upon photolysis, ATP (**2**) is generated, which is subsequently hydrolyzed by the enzyme. Molecules of this type, in which biological activity is unleashed (or altered!) upon exposure to light, are commonly referred to as "caged" compounds (*2*). Both reviewers and investigators alike have bemoaned the use of this terminology since it conjures up the image of a molecule interred within a molecular prison. Activity, many have argued, rather than the molecule itself, is what's actually released. However, even the latter point is conceptually restrictive. What is really important, from a biological point of view, is the ability to control activity (*i.e.*, on, off, or altered) with light.

Many reviews (*3–16*), including two monographs (*17, 18*), have been devoted to this topic. Indeed, a particularly comprehensive review appeared in 2006 (*8*). A number of important contributions have been reported since the latter was published. However, we've made the decision to use this opportunity to do more than just simply update the field. The concept of designing a reagent that can be controlled after it has entered a cell or an organism is not only intellectually seductive but has broad pragmatic implications as well. Unfortunately, the enormous creativity that encompasses this field has had a rather limited impact on biological research. Photomodulated bioreagents remain a niche science. Our goal, along with describing recent advances, is to highlight the challenges associated with the design, creation, delivery, and photoactivation (instrumentation) of caged compounds. However, it is important to recognize that merely overcoming these challenges will not alter the manner by which biological research is conducted. *The major vaults of cellular chemistry still hold many secrets, but only through the guidance of biologists will the keys to these vaults be forged.* Transdisciplinary cooperation is required to broaden both the appeal and the application of this extraordinary technology to the many outstanding biological questions that remain unasked and therefore unanswered.

We will exemplify, in the discussion that follows, the scope and limitations of caging technology using specific examples that reflect our own unique set of biases. Space limitations preclude the discussion or even cita-

tion of all germane contributions. In addition, we have omitted an explicit description of the range of caging groups and their attributes. The interested reader is referred to earlier reviews on the latter topic (*7, 8, 16–18*).

DESIGN

From a chemical point of view, the design of a caged compound is deceptively straightforward. All that is required are (i) identification of a key functional group essential for biological activity on the molecule of interest and (ii) covalent modification of that group with a photocleavable moiety. We refer to this as the "classical strategy". For example, several caged cAMP analogs (**3**; Figure 1) have been reported (where CAGE represents several different photolabile groups) (*19–29*). Indeed, Engels and Schlaeger's (*30*) description of caged cAMP and its proposed use in living cells actually predates the disclosure by Kaplan, Forbush, and Hoffman of caged ATP. cAMP **4** is generated in cells from ATP and serves as an ancient hunger signal that drives a plethora of intracellular processes. The highlighted negatively charged cyclic phosphodiester moiety in **4** is essential for biological activity, and the caged cAMP motif **3** bears this structural requirement in mind. *However, although chemistry is required to prepare a caged compound, biological insight is critical to design one.* If the biological intent is to constitutively activate a cAMP-dependent pathway, then compound **3** is worthless. It is important to realize that intracellular cyclic nucleotide phosphodiesterases will hydrolyze the photounleashed cAMP, thereby reducing the effect to a temporary burst of cAMP instead of a long lasting phenomenon. A transitory surge may be useful or useless, depending upon the biological question under investigation. Although a short-lived stimulation to any signaling pathway may adequately recapitulate the normal behavior of a properly functioning cell, it may be insufficient to produce a readily observed biochemical or cellular phenotype (as exemplified by aberrant signaling behavior, *e.g.*, cancer). If a more enduring effect is required, then the bioreagent in question must at least be partially impervious to down-regulation by the endogenous biochemistry of the cell. In the case of cAMP, this requires an analog that is resistant to phosphodiesterase-catalyzed hydrolysis. Indeed, many of these derivatives have been described (*31–33*), and one of these has been caged (*e.g.*, **5**) (*20–23*). In short, although it is tempting to directly cage the natural biological molecule, it is important to keep in mind that the biochemistry of life is highly regulated with an emphasis on maintaining biochemical homeostasis. An artificial ortholog, whose structural integrity is orthogonal to the intracellular milieu, may be the molecule most appropriate for the intended biological investigation.

The notion of bio-orthogonality in the design of caged compounds is not limited to just small molecules (*e.g.*, **5**). For example, this concept has been applied to the construction of a caged analog of cofilin, a protein that plays a key role in mediating the structural dynamics of actin (Figure 2) (*34, 35*). Actin exists in two forms, monomeric ("G" for globular) and noncovalent polymeric ("F" for filamentous). Active cofilin alters the equilibrium between G- and F-actin (*36*). Furthermore, phosphorylation of Ser-3 converts active cofilin **6** to an inactive species **7** that no longer influences actin dynamics. At first glance it is tempting to create a cofilin in which the side chain hydroxyl of Ser-3 is modified with a photocleavable moiety (*e.g.*, **8**). However, photolysis of this species would simply generate natural cofilin, which can be rapidly silenced by intracellular protein kinases. Consequently, a bio-orthogonal analog of cofilin was constructed, in which Ser-3 was converted to a cysteine moiety (**9**). Although the cysteine mutant retains the native protein's ability to influence actin dynamics, the cysteine side chain itself is resistant to protein kinase-catalyzed phosphorylation. Consequently, once the corresponding caged derivative **10** is photolyzed, the active analog **9** cannot be switched off by phosphorylation.

An alternative approach ("indirect strategy") has been introduced that circumvents the notion that caged compounds must be derived from the direct covalent modification of an essential functional group. The latter axiom is not only unnecessarily restrictive but also often chemically impossible to achieve. Many biologically active agents, from small molecules (*e.g.*, **4**) to large proteins (*e.g.*, **6**), possess a key functional group that is absolutely essential for activity. However, in many other instances the interaction between two or more biomolecules transpires over a broad molecular surface, reducing the contribution of any particular functionality from essential to merely contributory. Nucleic acid–nucleic acid, protein–protein, and nucleic acid–protein interactions often fall into the latter category. The initial strategy employed to cage these types of molecules relied upon random multisite modification (*37, 38*). For ex-

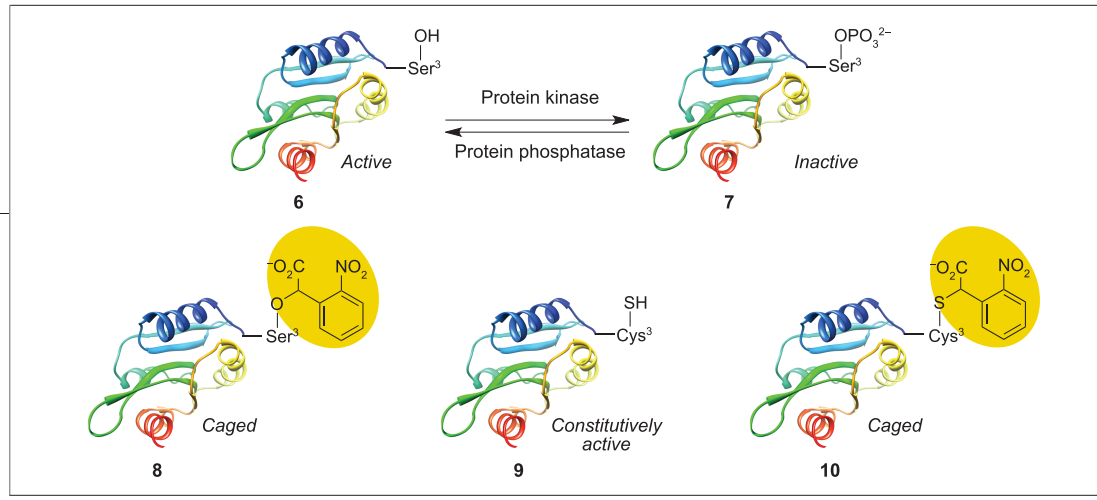

Figure 2. Design of a bioorthogonal caged protein. The actin polymerizing/depolymerizing protein cofilin exists in active dephosphorylated (6) and inactive phosphorylated (7) states. The cage moiety on 8 contains a negatively charged carboxylate, which has been introduced to mimic the phosphate in 7. Although photolysis of 8 should generate active cofilin (6), the latter can be rapidly switched off by the appropriate protein kinases. Consequently, the Cys-3 cofilin mutant 9 was prepared instead, which is impervious to kinase-catalyzed phosphorylation. Photolysis of the corresponding caged derivative 10 generates the constitutively active cofilin 9, which is resistant to intracellular down-regulatory action.

ample, an mRNA coding for green fluorescent protein (GFP) was modified with a caging group at approximately 30 phosphate sites for every 1 kb of RNA sequence (*39*). In the protein arena, one of the first reports of protein caging was described for actin (*40*). The latter was labeled at an average of 3–5 lysine residues per actin molecule (*40*). The GFP mRNA is poorly translated and the polymerization of actin proceeds slowly in the caged forms. In both instances, photolysis leads to significant recovery of activity. However, this strategy not only produces a mixture of caged molecules with potentially variable levels of reduced activity, but subsequent photolysis likely generates an array of partially uncaged species with variable amounts of activity as well. The problem of chemical mixtures has been solved in the nucleic acid field since caged nucleotides can now be simply introduced at specific sites during solid-phase synthesis (*41*).

In addition to single- and multisite modification, a higher order view of biomolecular interactions (secondary and tertiary) has been employed in developing strategies for the design of caged nucleic acids and peptides. For example, a single photocleavable residue (**11**), appropriately positioned in a self-complementary "silenced" oligonucleotide (**12**), provides a clean and efficient method to photochemically unmask biologically inactive forms of nucleic acids so that they can form duplexes (*e.g.*, **13**) with designated targets (Figure 3) (*42*). The biological activity of (**12**) is controlled at a site that is distant from the residues that are directly involved in biorecognition. A number of strategies have been subsequently reported that utilize a single photosensitive moiety, in combination with conformational changes, to control nucleic acid activity and recognition (*12*).

In the peptide/protein field, two approaches have been introduced to skirt the issue of direct caging of a specific key functional group required for biological activity. Although protein–protein interactions are driven by an array of noncovalent interactions between the binding partners, these interactions are typically feasible only if the binding partners can assume the requisite complementary conformation. The light-driven *cis*/*trans* isomerization of the azobenzene moiety (**14**/**15**) has been extensively used to induce both conformationally and biochemically significant changes in nucleic acids (*12*), peptides (*43, 44*), and proteins (*13, 14, 45*) (Figure 4). Unlike coumarin, hydroxyphenacyl (*46, 47*), cinnamate (*48*), and the multitude of *o*-nitrobenzyl caging species, no covalent bond in **14**/**15** is broken upon exposure to light. Consequently, since the azobenzene moiety remains attached to the biomolecule in response to light, caging cannot be achieved in the "traditional" sense, namely, *via* modification and subsequent photolytic release from a critical residue. Instead, an activity-dependent spatial change occurs as a consequence of *cis*/*trans* isomerization. A very recent example of this strategy is the construction of a photosensitive α-helical peptide segment that targets antiapoptotic proteins (*i.e.*, proteins that block cell death) (*49*). Light-mediated disruption of the α-helical conformation compromises

KEYWORDS

Photolysis: Light-induced bond cleavage. In general, the energy required to drive this process is higher (shorter wavelength) than that required for photoisomerization.

Photoisomerization: Light-induced conversion of one isomer (typically configurational) to a second isomer. In general, the energy required to drive this process is lower (longer wavelength) than that required for photolysis.

Caged compound: Any species, but typically a bioactive compound, whose activity is suppressed by a photosensitive functional group. Biological activity is restored by illumination, which results in photolysis or photoisomerization of the photosensitive functionality.

Quantum yield (Θ) of uncaging: A measure of the efficiency with which absorbed light converts a caged compound to its uncaged counterpart. Θ is acquired by dividing the number of uncaged molecules produced by the number of photons absorbed. Consequently, by definition, $\Theta \leq 1$.

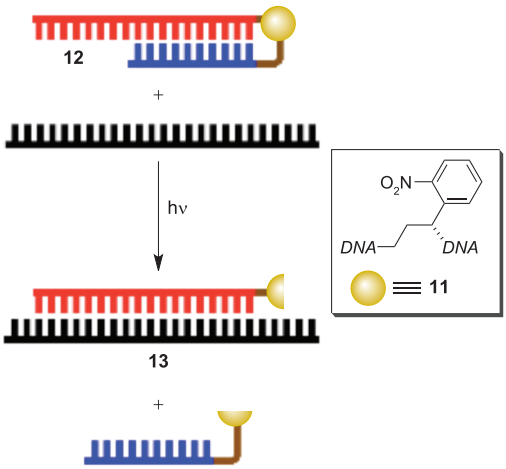

Figure 3. Single site regulation of nucleic acid activity. Self-complementary nucleic acids form *intra*molecular duplexes that prevent *inter*molecular interactions with complementary sequences. An appropriately positioned photocleavable site (11) in a self-silenced species (12) forms, upon photolysis, duplexes with appropriate targets (13).

protein binding affinity (*cf.* **16** and **17**). Photoreversible bioactivity has also been engineered into protein-based ion channels (*45*). For example, illumination at one wavelength occludes the ion channel (**18**) and thus blocks ion flow, whereas exposure to a second wavelength restores activity (**19**) (*50–52*). We note that a variety of photochromic molecules have been described (*53*) with spiropyran-based systems in particular attracting recent interest (*54–57*). A second approach to control peptide conformation is based on the introduction of an *o*-nitrobenzyl moiety on the amide backbone in bioactive peptides (*58–61*). N-Substituted peptides have dramatically altered conformations relative to their unsubstituted counterparts. This strategy has been used to create caged analogs of a protease sensor (*59*), a protein kinase substrate (*59*), a ligand that binds to SH2 domains (a protein recognition motif) (*59*), a sperm activating peptide (*60*), and a cell adhesion peptide (*61*).

Finally, it is not even necessary to directly modify the molecule of interest in order to render it photoactivable ("noncovalent strategy"). Indeed, the first example of a caged biomolecule, namely, cAMP, primarily (but not exclusively) exerts its biological effect by activating the cAMP-dependent protein kinase. Consequently, caged cAMP **3** can be thought of as equivalent to a caged cAMP-dependent protein kinase, although cAMP does act on other biological effectors as well. A very different approach has recently been used to generate a photoresponsive system that behaves analogously to a light-activatable Src tyrosine protein kinase (Figure 5) (*62*). Src kinase has three protein–protein interaction domains, the classic active site region (SH1), where tyrosine on a substrate (peptide or protein) is inserted and undergoes phosphorylation, and two additional protein–protein interaction domains (SH2 and SH3). An inhibitor peptide was designed that simultaneously binds to both the SH1 and SH2 domains (**20**). This bivalent interaction mode is responsible for the high affinity the inhibitor displays for Src. A photocleavable moiety was inserted between the dual binding motif, and thus photolysis slashes the peptide in half. Light-induced cleavage reduces inhibitory potency by >2 orders of magnitude, thereby unleashing Src kinase activity (**21**).

SYNTHESIS AND INTRACELLULAR DELIVERY

The synthesis of caged small molecules typically employs the classical strategy, namely, modification of a key functionality required for bioactivity. Both ATP **1** and cAMP **3** derivatives illustrate the concept. Generally speaking, compounds worthy of caging are commonly associated with a detailed molecular history that highlights the essential nature of specific functional groups. For example, caged versions of estradiol **22** (*63*) and ecdysone **23** (*64*) were prepared by targeting the highlighted alcohol functional groups for modification, since their indispensable contribution to biological activity is well-known (Figure 6). The caged versions of these species have been used to activate gene expression in a light-dependent fashion (*63, 64*). Nevertheless, not all compounds, as exemplified by tamoxifen **24**, have a readily modifiable chemical handle. Analogs of tamoxifen (*e.g.*, **25**) have been described that are biologically active as well. The phenol is known to fit snugly into a critical region of the protein receptor that tamoxifen binds to. Covalent modification of the hydroxyl with a sterically demanding photosensitive *o*-nitroveratryl moiety renders **25** biologically silent (*i.e.*, prevents association with the protein receptor) until activated with light (*65*).

Caged peptides are generally synthesized using standard solid-phase methods. Side chain caged versions of serine (*66–68*) and phosphoserine (*69–71*), tyrosine

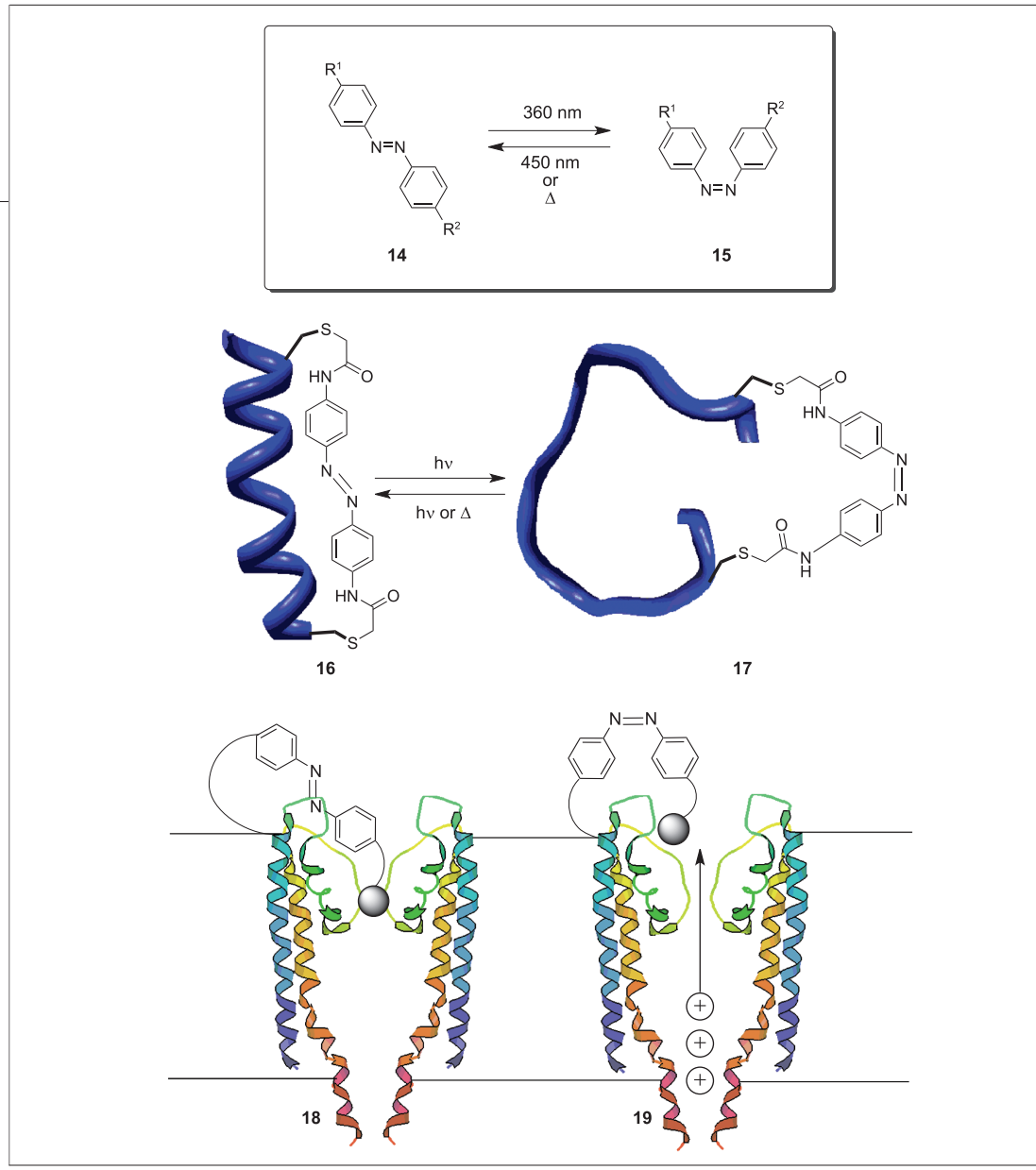

Figure 4. Photoisomerizable azobenzene functionality and its utility as a biochemical photoswitch. The azobenzene moiety is not photolytically cleaved from the biomolecule to which it is appended but rather is reversibly interconverted between *trans* (14) and *cis* (15) isomers. This property has been used to control both peptide/protein conformation (16 ⇆ 17) and activity (18 ⇆ 19).

(*72, 73*) and phosphotyrosine (*69, 70, 74, 75*), threonine and phosphothreonine (*69, 70*), glutamic (*76–78*) and aspartic (*77–79*) acids, lysine (*80, 81*), arginine (*82*), and various unnatural derivatives (*83, 84*) have all been described. The special reactivity of the cysteine side chain allows it to be selectively modified following peptide synthesis, a property that has been used to construct comparatively elaborate photoresponsive derivatives (*e.g.*, **16** and **17** (*49*)). In addition, and as noted above, it is now possible to prepare backbone-caged peptides *via* solid-phase methods as well (*58–60*). Finally, caged nucleic acids are likewise synthesized *via* solid-phase methods thanks to the availability of caged nucleoside phosphoramidites (*41*).

Four different methods have been used to construct caged proteins, each of which has its own unique set of advantages and limitations. Perhaps the most utilized approach is the direct modification of the native protein at a key active site residue (generally the classical strategy; however, the indirect strategy has been employed as well, *e.g.* **18** and **19**). Indeed, chemical modification of enzymatic activity has been an active area of research for more than half a century (*85*). The difficulty commonly encountered in the preparation of these derivatives is the presence of multiple reactive nucleophiles on the target protein, only a few of which actually influence enzymatic behavior. However, with the advent of site-directed mutagenesis, reactive residues (*e.g.*, cys-

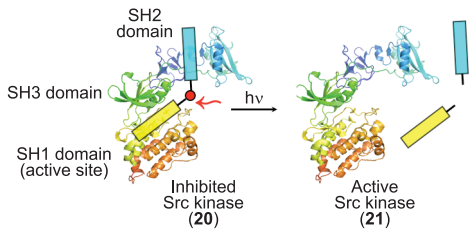

Figure 5. Small molecule caged protein equivalent. A peptide-based bivalent inhibitor binds to two separate domains on the Src protein kinase (20). The high affinity inhibitor contains a photocleavable site (red). Photolysis splits the inhibitor in half, dramatically reducing inhibitory potency and thereby liberating enzymatic activity (21). Figure reproduced from Li, H.; Hah, J. M.; Lawrence, D. S. *J. Am. Chem. Soc.* 2008, *130* (32), 10474–10475.

teine) can be specifically inserted near the active site or at other key regions. For example, Bayley *et al.* (*86*) performed a scanning mutagenesis study on the pore-forming protein hemolysin in order to identify a cysteine mutant that could be caged. Generally, cysteine serves as the most common chemical handle for modification with a photocleavable moiety, although affinity labeling of active site serine residues has been reported (*87, 88*). An extraordinary enzyme-directed caging protocol has been described based on the observation that the cAMP-dependent protein kinase is itself phosphorylated by a second protein kinase at a key near active site threonine residue. However, instead of phosphate, a thiophosphate moiety was introduced onto this threonine *via* the use of a sulfur-containing ATP analog (ATP-γ-S) (*89*). The free thiol of thiophospho-threonine was then selectively caged *via* alkylation. A key advantage associated with the chemical modification strategy is that many native or mutagenized proteins can be bacterially overexpressed in a properly folded state, thereby affording a large quantity of active protein for biochemical studies. Perhaps the primary disadvantage is that even with the presence of a single reactive residue at a highly sensitive site it is often difficult to completely eliminate enzymatic activity (*90, 91*).

Expressed protein ligation (EPL) is an exciting new technology that allows small synthetic peptides to be fused to larger expressed proteins. A detailed discussion of EPL is beyond the scope of this Review, but can be found in refs 92 and 93. There are some limitations associated with EPL, and to the best of our knowledge only a few caged variants of a single protein have been constructed using this approach (*94–96*). Nevertheless, a unique strength of this technology is the ability to ligate a peptide containing multiple modifications (fluorophores, isotopes, post-translational modifications, and cages) to a protein. Indeed, a caged version of Smad2 was constructed by appending a multiply substituted peptide to the C-terminal end of the protein (**26**) (Figure 7). Since a free C-terminus is required for Smad2 activity, the presence of a light cleavable moiety at this position renders the construct light-activatable. In addition, a fluorophore and a fluorescent quencher were positioned on opposite sides of the photocleavable moiety. Consequently, activation of the protein is intimately linked with a dramatic increase in fluorescence (26-fold), thereby providing an immediate visual confirmation of photolysis (**27**). We note that caging groups have been described that likewise display a fluorescent enhancement upon photolytic cleavage from an alcohol-containing bioreagent (*e.g.*, **28** to **29**) (*97, 98*). This property is potentially very useful, particularly in cells, since it can provide a quantitative assessment of the amount of photochemically released compound. An ad-

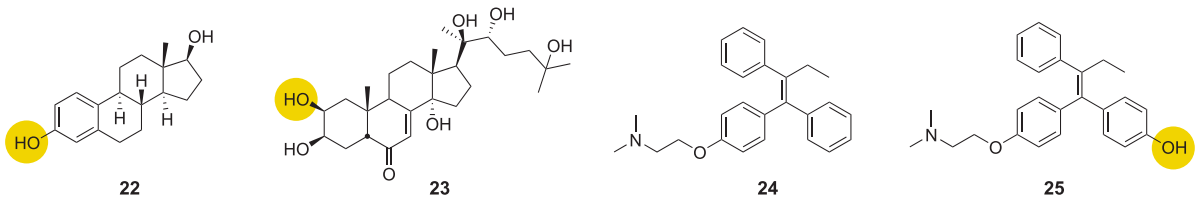

Figure 6. Light-dependent gene activation. Caged versions of estradiol (22) and ecdysone (23) have been used to temporally and spatially control gene expression in living cells. The hydroxyl groups highlighted in yellow are required for bioactivity, which renders these sites ideal for caging (classical strategy). By contrast, some small molecules lack a caging site due to the absence of a functional group handle, such as in the case of tamoxifen (24). However, active derivatives of the latter with readily modifiable functionality (25) have been reported, and covalent substitution of the latter with a photocleavable moiety renders the compound caged.

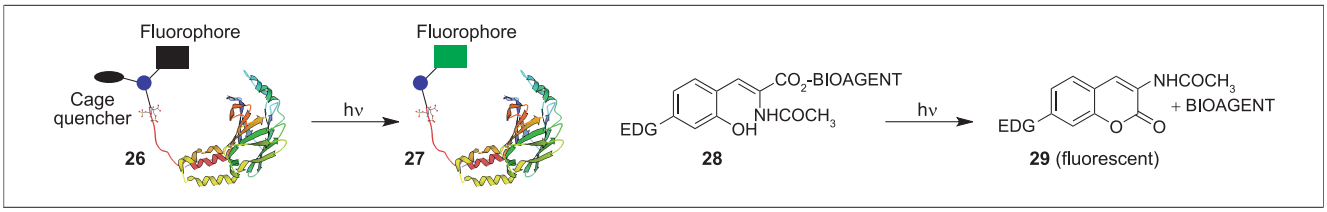

Figure 7. Coupling of photouncaging and fluorescence. Visualization of light-driven release of a caged compound can provide a quantitative assessment of the amount of compound liberated, information that is potentially useful when the experiment is conducted in a living cell. A protein-based system has been described that contains a fluorophore and a fluorescent quencher positioned in close proximity to one another (26). Photolysis simultaneously releases the quencher and activates the protein (27). The *O*-hydroxycinnamic acid caging group 28 is photolytically converted to the fluorescent coumarin derivative 29 simultaneously with active bioreagent formation.

ditional advantage associated with the protein-based system (**26**) is that the fluorophore remains attached to the activated protein, thereby providing the means to observe changes in the spatial localization of the bioreagent following the uncaging event.

Schultz *et al.* (*99*) have pioneered the genetic insertion of unnatural amino acids, including caged derivatives, into proteins. The approach utilizes tRNAs that can introduce unnatural amino acids at certain stop codon sites. Caged proteins generated in this fashion include β-galactosidase (tyrosine (*100*)), superoxide dismutase (cysteine (*101*)) and caspase 3 (cysteine (*101*) at a cleavage site in pro-caspase 3 (*102*)), lysozyme (aspartic acid) (*103*), HIV protease (aspartic acid (*104*)), a restriction endonuclease (lysine (*105*)), dihydrofolate reductase (aspartic acid (*106*)), catabolite activator protein (a diazobenzene moiety (*107*)), and the intracellularly (oocytes) expressed nicotinic receptor (cysteine and tyrosine (*108*)), acetylcholine receptor (tyrosine (*109*)), and inward rectifier channel Kir2 (tyrosine (*110*)). Unnatural amino acid mutagenesis has been used in both prokaryotes and eukaryotes. This technology can potentially introduce a caged amino acid at any position within the open reading frame, whereas EPL has certain restrictions, and the direct chemical modification of an expressed protein must often contend with the presence of multiple nucleophilic residues. Unnatural mutagenesis is more technologically demanding than the other two strategies, requiring non-natural tRNA synthetases for amino acid ligation, expression of multiple unnatural species (proteins and RNA) in the cellular environment, and possible genome wide suppression of (at least some) *bona fide* stop codons. However, expression of the caged protein within a cellular environment bypasses the requirement for cellular delivery (typically by laborious microinjection). As is true for many areas of science, the question being addressed and the comfort level of the scientist with the various technologies will likely determine which strategy is ultimately employed.

Finally, a genetically encoded strategy has recently appeared that takes advantage of the natural photosensory domain of the plant protein phytochrome B (Figure 8) (*111*). The latter contains a covalently affixed tetrapyrrole moiety (a "bilin") that serves as the chromophore. Phytochromes exist in two conformationally (and biologically) distinct forms: far red light (Pfr) and red light (Pf) absorbing states. Red light conversion of Pf to Pfr promotes binding to a transcription factor (Pif3) in plants, which serves as the basis for a light-driven process that promotes (red) or disrupts (far red) protein–protein interactions. Under normal cellular conditions, the protein Cdc42 (with a bound GDP) binds poorly to and therefore fails to activate a second protein, WASP. Two genetic constructs were bacterially expressed: (phytochrome B)-Cdc42(GDP) and Pif3-WASP. In the presence of red light, phytochrome B interacts with Pif3, thereby dramatically enhancing the effective concentration of Cdc42 and WASP, resulting in the latter's activation. Like the unnatural amino acid mutagenesis strategy, this approach requires a significant amount of genetic engineering. For example, bacterial expression of the holo form of phytochrome B (*i.e.*, protein and chromophore) requires the coexpression of heme oxygenase and bilin reductase (required for the biosynthesis of the bilin) (*112, 113*), although a simpler alternative may be possible (*114, 115*). Unlike the first three strategies, this approach does not target a specific residue or site on a single target protein but rather requires the expression of two protein constructs. However, from a biologist's point of view, it offers one potentially distinct advantage: it is chemist-independent.

Although all four strategies have successfully created caged proteins, the ultimate biological utility of any of these species will in large part be determined by the question under study and the tools required to address that question. For example, only adherent cell lines (*e.g.*, most fibroblasts) can be microinjected, which is the primary means by which test tube synthesized proteins are introduced into cells. This not only places a severe restriction on the type of cell that can be studied, but on the nature of the biological readout as well. Only a few hundred cells can be microinjected during the course of an experiment, thereby eliminating readouts that require large cell numbers (*e.g.*, Western blots).

Finally, there are additional concerns that must be considered, whether the caged protein is microinjected or intracellularly expressed: (i) the final concentration of the artificial construct should be as close to that of the

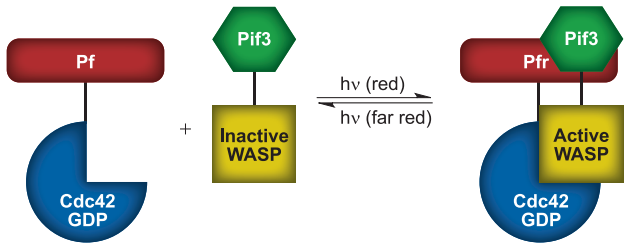

Figure 8. Light-driven formation or disruption of two genetically expressed proteins. Red light-induced conversion of Pf to Pfr promotes the interaction with Pif3. Cdc42 containing bound GDP has a weak affinity for and thus is a poor activator of WASP. However, the Pf-Cdc42-GDP construct does activate Pif3-WASP in a light-dependent fashion by furnishing a dramatic enhancement in the effective concentration of the Cdc42/WASP pair.

native protein as possible, and (ii) native protein background activity may mask or otherwise alter the effect of the caged construct. An obvious way to deal with these issues is to cage the natural protein itself under the endogenous intracellular environment. Although obvious, the task itself is daunting because, as is clear from the previous paragraphs, caging a pure protein under ideal investigator-controlled conditions is nontrivial. However, depending upon the biological question to be addressed, it may not be necessary to actually covalently modify the target protein. In short, the noncovalent strategy that utilizes light-sensitive small molecules to control protein activity may be sufficient. For example, caged small molecules could be used to trap the activated form of an enzyme and then subsequently unleash it at a later point in time. In this sense, an enzyme inhibitor that is destroyed by light is functionally equivalent to a caged enzyme. The Src kinase inhibitor **20** is illustrative of this approach (*62*). Alternatively, caged activators of enzymes (*e.g.*, caged cAMP analogs **3** and **5**) are functionally equivalent to caged enzymes as well. Indeed, such a strategy has recently been applied to phosphatidylinositol 3-kinase (PI3K). PI3K contains an SH2 domain that, upon interaction with the appropriate phosphotyrosine-bearing amino acid sequence from a second protein, results in PI3K activation. The negatively charged monophosphate ester of phosphotyrosine is essential for activation to transpire. The synthesis of a caged SH2 domain-directed peptide was achieved by attaching a photocleavable moiety to this key phosphorylated tyrosine residue (*116*). The caged peptide is ineffective as a PI3K activator since it binds poorly to the SH2 domain. Photolysis generates the active form of the peptide, which in turn activates PI3K, a process demonstrated in NIH3T3 cells.

There are several potential limitations associated with using photocleavable noncovalent inhibitors and activators (noncovalent strategy) as alternatives to caged enzymes. First, there is the issue of selectivity of the inhibitor or activator. A reagent with suspect selectivity precludes a credible correlation between biochemical activity and biological behavior. Second, covalent modification with a photocleavable moiety may only reduce but not completely eliminate the activity of the bioreagent. There must be a significant difference in potency between the free compound and its caged counterpart in order for the caged-reagent-as-a-caged-enzyme strategy to work. For example, a caged inhibitor must be noninhibitory even at the elevated concentrations required for the active inhibitor to dramatically curtail enzymatic activity (*i.e.*, >10-fold above the IC_{50}). Finally, since low molecular weight compounds diffuse rapidly on the experimental time scale, it may be necessary to photodeactivate the majority of inhibitor/activator molecules in the cell (global illumination) in order to observe an enzymatic response. Consequently, subcellular enzyme activation *via* spot illumination (*i.e.*, spatial control) may not be possible using this strategy, although an alternative that may achieve this end has been offered (*62*).

INSTRUMENTATION

The design of new caged compounds not only must take into account the anticipated biological application but also must consider the instrumentation that will be used to uncage the bioreagent. On a laboratory bench, a Hg arc lamp, a Xe flash lamp, or even a simple handheld UV–vis lamp are sufficient to generate a photoresponse. However, it is important to keep in mind the time frame. Under macroscopic conditions (*e.g.*, in a cu-

vette), the photon flux through the sample is relatively modest. Consequently, it is not unusual for complete uncaging to take tens of minutes or even longer, depending on the light source, beam focus, the overlap between wavelength output and absorbance by the cage, and the quantum yield of uncaging. By contrast, photolysis under a microscope commonly focuses light energy through a very narrow spatial window, which can easily be restricted to a single cell or less. Since the photon flux is much greater than what can be achieved in a macroscopic system on the laboratory bench, photouncaging times can drop to under a second from a continuous light source (Hg arc lamp) or in as little as a few pulses from a laser.

In general, uncaging light sources can be divided into two classes: general-purpose light sources that are used both for imaging and uncaging and specific-purpose light sources whose primary role is uncaging. The former includes Hg, Xe, and metal halide lamps, which are commonly employed for widefield imaging. Ultrafast infrared lasers, which have found use in multiphoton imaging, have been utilized for uncaging purposes as well. Specific-purpose light sources consist of a limited number of UV lasers, including N_2 gas lasers and solid-state lasers. The advantage of a general-purpose light source is that a single source coupled to the microscope furnishes a system that can both image and uncage. Switching between these two functions is usually achieved by mechanically switching between excitation filters. By contrast, using a light source whose sole function is uncaging allows the uncaging light to be specifically tailored to give greater temporal or spatial resolution or finer control of the uncaging dose. Any combination of the above sources can be designed for a particular application (for example, two Hg lamps might be present, with one optical pathway optimized for imaging and one pathway optimized for uncaging).

Figure 9 shows the spectra of Hg, Xe, and metal halide lamps. Superimposed on these spectral line shapes is the absorbance cross-section of the 3,4-dimethoxy-2-nitrobenzyl (DMNB) caging group, included as a general reference for uncaging and imaging wavelengths. The Hg lamp has considerable power in the UV region, with prominent spectral lines at 312, 334, and 365 nm. In addition, the excitation intensity in the infrared region (>700 nm) is minimal, which reduces heating at the sample. The Xe lamp has the benefit of flat wavelength dependence throughout the visible and near UV, which enables the user to observe a wide range of fluorophores and efficiently uncage without seeing large differences between channels. However, one needs to include infrared blocking filters to eliminate the heating of the sample that can occur with Xe lamps. Metal halide lamps, on the other hand, which have grown increasingly popular as fluorescence imaging sources, have very little power at wavelengths of <350 nm. Although this property is often beneficial for imaging applications in living cells, these lamps are unsuitable for most uncaging applications. However, when a metal halide lamp is coupled with a dedicated uncaging source such as a UV laser, one can effectively separate the uncaging and imaging wavelengths to eliminate spurious uncaging during imaging.

Ultrafast IR lasers can also be used for both imaging and uncaging, which is one of the advantages of multiphoton microscopy. Furthermore, because multiphoton microscopy is usually implemented in a point scanning mode, there is a precise correspondence between an image pixel and the position of the laser, enabling excellent registration between the image and the uncaging position. The wavelengths for imaging are usually >800 nm, but most of the multiphoton caging groups described to date have peak cross-section absorptions of <750 nm (*78, 117–122*). Consequently, the laser wavelength must be blue-shifted between imaging and uncaging, which can introduce a time delay. In many cases, the benefits of multiphoton uncaging (intrinsic three-dimensional resolution, reduced photodamage out of the plane of focus, and the ability to work in tissue) outweigh this disadvantage. The chemistry and design of 2-photon cages is often different from that of 1-photon cages, a subject that has been discussed (*123, 124*).

The use of a dedicated UV laser for uncaging has several advantages. First, because the laser is not used for imaging, one can design a fixed optical uncaging pathway to produce anything from a diffraction-limited spot (*i.e.*, subcellular) up to full field illumination (tens or hundreds of cells). Second, because the laser is considerably blue-shifted from any wavelength used in imaging, the laser can be relayed to the sample at the same time as the imaging excitation by careful choice of filter and dichroic design. No mechanical shifting of filters or apertures is needed since the dose is controlled by independently modulating the laser power. Thus, imaging and uncaging can occur simultaneously, allowing for ob-

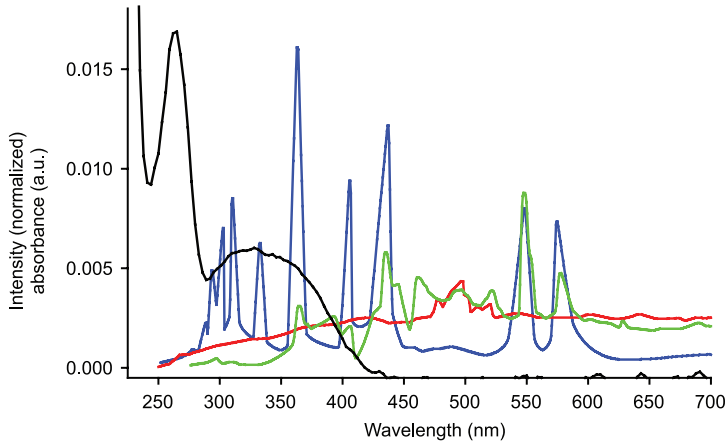

Figure 9. Emission spectra of several common microscope lamps, Hg (blue), Xe (red), and metal halide (green), and the absorbance spectrum of the common caging group 4,5-Dimethoxy-2-nitrobenzyl fluorescein dextran (black). The emission spectra of the lamps are normalized by the integrated area from 250 to 700 nm. The absorbance spectrum of DMNB is in arbitrary units.

servation of fast processes. Finally, it is also possible to use pulsed UV lasers, which allows for precise control of the energy delivered to the sample *via* the number of pulses rather than by a shutter or aperture.

RECENT ADVANCES

The Mayer and Heckel review (*8*) provides an excellent and comprehensive discussion of the literature up through 2005. Consequently, we will focus on recent advances (2006–2008), with a special emphasis on light-modulated peptides and proteins. Several of these studies have been summarized above and will not be recapitulated here. The remainder are segregated into two categories: chemistry, which describes work that emphasizes the synthesis and subsequent physical and/or biological characterization of a caged bioreagent, and biology, which summarizes studies that focus on the elucidation of a biological phenomenon.

Chemistry. Thiophosphate derivatives of alcohol-bearing amino acids have been prepared enzymatically (*89*) or *via* peptide synthesis (*84*). Subsequent *S*-alkylation furnishes the corresponding caged derivatives (*e.g.*, **30**). These thiophosphate analogs of phosphorylated serine, threonine, and tyrosine are useful because they are resistant to phosphatase-catalyzed hydrolysis (Figure 10). Consequently, once uncaged, the freed thiophosphate moiety (and the corresponding biological activity) will not be lost to adventitious phosphatases. The synthesis of preformed caged *O*-phosphorothioyl serine, threonine, and tyrosine (**31**) derivatives has now been reported, along with their direct incorporation into peptides *via* standard Fmoc chemistry (*125*).

The photochromic azobenzene moiety has recently been introduced into the backbone of *N*-glycine-substituted oligomers (peptoids) (*126*). The general attributes displayed by the photoresponsive azobenzene group may ultimately prove useful for controlling both the structure and properties of this peptidomimetic class of compounds.

Caged versions of a few cyclic peptides have been recently described. Urotensin II and endothelin-1 are disulfide-bridged cyclic peptides that display vasoconstrictor activity. Caged urotensin II, modified at either Lys-8 or Tyr-9, is 300-fold less active than its uncaged counterpart (*127*). Caged endothelin-1 analogs (Lys-9 and the C-terminal carboxylate) were likewise acquired against one of the endothelin receptors (ET_A) (*77*). However, in the latter study, unexpected side reactions were observed when attempting to synthesize endothelin-1 analogs caged on the aspartic and glutamic acid side chains. Although caged aspartic and glutamic acids have been previously described (*76, 79*) and enzymes caged at aspartic acid reported (*103, 104*), at the time of this study there had not been any ex-

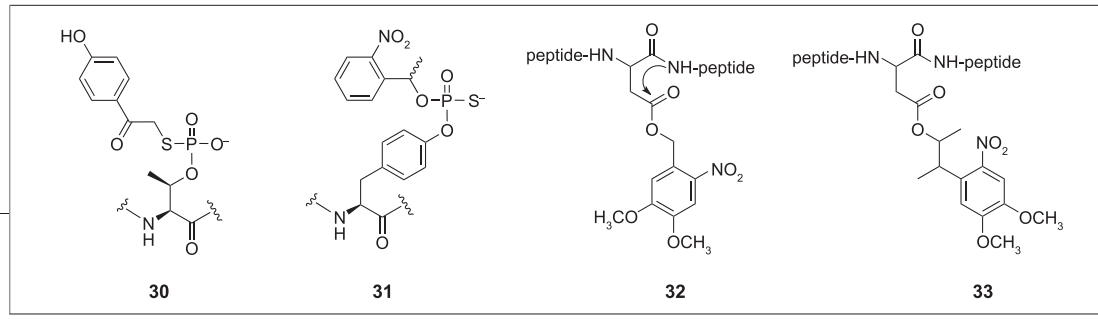

Figure 10. Caged amino acids with acidic side chains. Peptides and proteins containing the *S*- and *O*-modified thiophosphate analogs of threonine (30), serine, and tyrosine (31) have been reported. FmocAsp analogs containing the side chain caging agents depicted in 32 and 33 have been synthesized, but only the latter has been successfully incorporated into peptides *via* solid-phase peptide synthesis. Derivative 32 appears to be susceptible to byproduct formation *via* an intramolecular cyclization reaction.

amples of the use of these caged amino acids in solid-phase peptide synthesis. Unfortunately, the relatively good leaving group ability of the photolabile ester substituent on the aspartic (aspartimide formation, **32** *vide infra*) and glutamic (a truncated peptide) side chains generated unexpected byproduct during solid-phase synthesis.

"RGD" peptides promote integrin-mediated cell adhesion. Cyclic analogues containing the -Arg-Gly-Asp- sequence have been reported, including the selective integrin $\alpha_v\beta_3$ ligand cyclo(-Arg-Gly-Asp-D-Phe-Lys-). The Asp side chain plays an important role in ligand binding and thus was deemed the appropriate site for modification with a photolabile moiety. Goeldner, del Campo, and colleagues (*128*) prepared a linear form of the caged peptide *via* solid-phase peptide synthesis, which was subsequently cyclized in solution. Unlike the endothelin-1 study described in the previous paragraph, the desired RGD peptide containing the modified aspartic acid moiety was obtained in good yield. The difference may lie in the selection of the photocleavable moiety on the aspartic acid side chain. The endothelin study employed the aspartic acid derivative **32** (*77*), whereas the RGD work utilized aspartic acid derivative **33** containing an aryl butyl moiety (*78*). The caged RGD peptide was surface-immobilized through a free lysine residue. An NIH3T3 cell line is unable to adhere to the caged RGB cyclopeptide modified surface. However, upon UV irradiation, cells adopt an adherence pattern that mirrors the irradiated regions of the surface. Ohmuro-Matsuyama and Tatsu (*61*) have also reported a RGD photocontrolled cell adhesion system. However, in this case, a linear peptide containing a photocleavable moiety positioned on the peptide backbone was used to control cell adhesion to the solid substrate. Finally, prior to 2006, several studies employed the azobenzene moiety to create photoswitchable RGD peptides (*129–131*).

Kinesins are motor proteins that employ ATPase activity to move along microtubules. Caged ATP and light has previously been used to initiate kinesin motility (*132, 133*). A recent report described the application of a caged peptide-based inhibitor of ATPase activity to block kinesin movement in a light-dependent fashion (*134*). In short, it is now possible to both initiate and halt kinesin trafficking. However, this work, as well as others using light to initiate and terminate a biochemical process (*82, 91*), highlights *a limitation in current caging technology: it is difficult to distinguish between photocleavable groups on the basis of wavelength.* Unlike the azobenzene series, in which the *cis* and *trans* isomers display a well-resolved response to different wavelengths, the overwhelming majority of photocleavable caging groups respond to light in a relatively narrow range of 320–370 nm. In the absence of two or more photophysically distinct caging agents, it is challenging to place multiple reagents under separate photochemical control.

Photophysically distinct caging groups could be used to create light-activatable biochemical activators and inhibitors that are sensitive to different wavelengths, thereby enabling the investigator to control, for example, both the initiation and termination of a biochemical pathway in a single experiment. Although the wavelength-controlled photolysis of two photosensitive protecting groups has been described in nonbiological systems (*135, 136*), one of these groups, a benzoin derivative, suffers photocleavage at a wavelength (254 nm) much too short to be biologically useful. In addition to this limitation, it may be experimentally desirable to separately control three or more caged compounds in a wavelength-sensitive fashion. This will require both the appropriate instrumentation (a tunable laser or a combination of bandpass filters) and a toolkit of wavelength-distinctive caging agents. *The array of genetically engineered green fluorescent proteins* (*137*), *which stretch from the blue to the red, represents an analogous toolkit that the caging field must seek to emulate.* Initial forays in this direction suggest that considerable effort may be necessary to create a multiwavelength cell-friendly family of caging agents (*124*). For example, although long wavelength (~400 nm) absorbing nitrobenzyl derivatives have been constructed, there is a precipitous drop in quantum yield as a function of in-

creased maximum wavelength of absorption (*124*). In short, it is important to keep in mind that, for any caging group, there will be a minimum amount of energy required to effect efficient photocleavage. Consequently, extending the light absorbance wavelength of the caging group by conjugation could very well produce a species that fails to undergo photolysis. On the other hand, and in marked contrast to the nitrobenzyl family, a long wavelength absorbing (400 nm) coumarin caging group has been described that undergoes efficient photorelease ($\Phi \approx 0.3$) (*22*).

Groves *et al.* (*138*) prepared a caged version of a 16-mer antigenic peptide that, in its uncaged form, is presented by the major histocompatibility complex (MHC) to the T cell receptor (TCR). This elicits a signaling cascade response, the formation of the "immunological synapse", and T cell activation. All of the features were observed in a light-dependent fashion. Anticipated future studies include the dynamic repatterning of the synapse during and following T cell activation.

Biology. Davis *et al.* (*139*) likewise prepared a caged version of the 16-mer antigenic peptide that activates T cells in an MHC-dependent fashion. *One of the clear-cut advantages offered by caging technology is the ability to initiate a biological response with high temporal precision, explicitly providing the biologist with a well-defined start point.* These investigators demonstrated that a TCR substrate known as the Linker for the Activation of T cells is phosphorylated within 4 s of photoinitiation. Other downstream events, such as Ca^{2+} release and diacylglycerol formation, occur in 6–7 s. Cytoskeleton reorganization transpires at 2 min following engagement of the TCR by the active antigenic peptide/MHC complex.

Neurotransmitters are packaged in synaptic vesicles. The latter are transported to the presynaptic terminal and, in response to the appropriate environmental cue, ultimately fuse with the plasma membrane, releasing the neurotransmitter into the synaptic cleft. The protein contents of the synaptic vesicle are recycled for use in future rounds of neurotransmitter release. *N*-Ethylmaleimide sensitive factor (NSF) plays a key role in recycling these protein contents, although it is not clear whether this commences prior to membrane fusion or following neurotransmitter release. A caged peptide-based inhibitor of NSF was microinjected into the presynaptic terminal and subsequently unleashed in a light-dependent fashion (*140*). The inhibitor itself decreases both synaptic transmission and neurotransmitter release. The light-controlled time-dependent studies revealed that this effect is exquisitely rapid, much faster than one would expect if the inhibitor were merely acting in a postneurotransmitter discharge fashion. These results allowed the investigators to conclude that (at least some of) NSF's role(s) precede neurotransmitter release.

Protein kinase C (PKC) activity was recently assessed prior to, during, and after nuclear envelope breakdown (NEB) in dividing cells using a caged peptide-based reporter (*68*). NEB is the mitotic landmark that serves as the transition from prophase to metaphase. Visual snapshots of kinase activity can be obtained at any point during mitosis since the fluorophore-labeled peptide-based PKC reporter substrate is activatable by light. PKC was found to be active prior to NEB, but its activity is dramatically curtailed immediately following NEB. Furthermore, kinase activity is essential for NEB, likely due to lamin phosphorylation, which drives nuclear envelope collapse (*141, 142*). The task of determining which PKC is responsible for this transition is potentially problematic since the PKC family consists of 12 closely related isoforms. However, the Ptk2 cell line employed in this study expresses only five of the isoforms, and only two of these (PKCα and β) phosphorylate the photouncaged peptide reporter. By using inhibitors that selectively target PKCα or PKCβ, in conjunction with the reporter itself, the observed kinase activity responsible for NEB was assigned to PKCβ. This study demonstrates that caging technology makes it possible to follow changes in intracellular kinase activity during rapidly unfolding events and that the observed activity can be correlated with specific cellular signposts. A caged fluorescent reporter for tyrosine kinases, analogous to that for PKC, has recently been described as well (*73*).

These recent studies exemplify the types of questions that are uniquely amenable to photoinitiated biology. First, caging technology offers an extraordinarily precise $t = 0$. Consequently, one can obtain accurate temporal measurements of biochemical and cellular events that transpire downstream of a well-defined start point. Second, caged inhibitors can be used to assess the potential role of biomolecules in distinct phenomena that differ in their biochemical and/or biological lifetimes. Third, caged sensors can be used to acquire visual snapshots of biochemical activity at distinct stages during biological events. There is every reason to be-

lieve that the questions addressed by these recent studies represent but a small sliver of what is likely to prove to be a vast array of potential applications.

What is the nature of these potential biological applications? How does a chemist, with only passing familiarity with biology, identify phenomena that will guide the design of biologically useful caged compounds? The task is daunting, given the abstruse nature of the scientific literature in general and the confounding differences in terminology between even closely related fields of endeavor. Nevertheless, there are biological behaviors, from the subcellular to organismal, that display obvious temporal and spatial components. Mitosis, which has captured the interest of scientists since the 1880s, consists of well-resolved time-dependent stages with clear-cut spatial changes (*e.g.*, chromosome segregation) (*143*). At the biochemical level, a complex interplay of signaling proteins conspire to ensure that division proceeds in the appropriate step-by-step fashion. Although the spatiotemporal role of many of these proteins is presumed, a direct accounting of their action in a temporally sensitive manner often lies just beyond the reach of the biologist. There are many examples of key biochemical participants that drive mitosis. An especially interesting one that has generated significant recent interest is the Ran GTPase (*144*). The latter exemplifies an additional theme that is becoming commonplace throughout biology: *individual proteins often have multiple biological roles, and these roles are determined by where and when the protein is active.* The cAMP-dependent protein kinase may very well be one of the most extreme examples of this theme. Often described as ubiquitously expressed throughout the cell, in reality this enzyme is not fortuitously bumping into an occasional substrate in the cytoplasmic soup. Rather, it is anchored to a plethora of subcellular sites, including the nucleus, the proteasome, mitochondria, the centrosome, various receptors and vesicles, and microtubules, to name but a few (*145*). The protein kinase itself, the mechanism of phosphorylation, and the phosphoryl donor (ATP) are all identical at each of these sites, but the biological ramifications of its site-specific cAMP-driven activation are dramatically different. *Finally, spatial and temporal elements are also present at the organismal level, with an especially acute appearance during embryogenesis.* However, even in the adult, spatially and temporally sensitive activity is responsible for both normal and aberrant behavior. For example, the importance of tissue microenvironment in cancer progression is now appreciated, although still poorly understood at the biochemical level (*146, 147*).

SUMMARY

There is significant chemical interest in the creation of caged compounds, yet the application of these promising reagents to biological questions has been surprisingly limited, with one exception. A large number of biological studies have been performed using caged neurotransmitters (*148, 149*). What sets neuroscience apart from other areas of modern biology? Caged neurotransmitters (glutamate, GABA, acetylcholine, *etc.*) are relatively easy to prepare and straightforward to apply to brain slices, thereby enabling neuronal information processing to be probed in a site- and time-specific fashion. In short, a small collection of compounds has been used to address a wide variety of biological questions using equipment and techniques that are common to most neuroscience laboratories. Why has this technology not played a more substantive role in other areas of biology, especially given the importance of spatiotemporal processes in systems that range from sub- to multicellular? Part of the problem may very well be the sophisticated instrumentation itself. Although many individual laboratories have their own microscopes, these laboratories lack the expertise to modify their existing systems to construct an uncaging apparatus. Many universities and institutes house imaging facilities with personnel capable of assembling an apparatus, but in the absence of a demonstrated need by multiple users, they are under no pressure to do so. *However, in our opinion, the primary barrier for progress in this area is a lack of communication between biologists and chemists*. In general, biologists are unaware of the magic that can be performed by chemists and are thus dependent upon commercially available reagents that, although useful, do not address their specific need. Consequently, in those instances where an exciting spatiotemporal phenomenon is encountered, the biologist is faced with the prospect of ignoring the issue, addressing it in a less than ideal fashion, or convincing a chemist that the problem is an exciting one. In the latter scenario, it is unlikely that the biologist will be capable of recommending anything more than the synthesis of a simple uninspired compound that will do little to whet the appetite of his or her chemically oriented colleague. *Indeed, biologists should not be in the business of designing*

molecules. Rather, the advent of the field of chemical biology has been driven by the recognition that it is the responsibility of chemists to learn biology. Biologists, with their intimate knowledge of the frontiers of their science, must serve as guides for their chemical colleagues. Biological inspiration, when appropriately translated to the molecular level and coupled with chemical innovation, drives the design of biologically useful reagents. Perhaps nowhere is this combination of inspiration, translation, and innovation more needed than in the general arena of photoresponsive compounds. Although it is certainly true that caged reagents have found (and will continue to find) application in the abiotic world, their enormous potential will only be realized in living systems. Let the conversation among biologists and chemists begin!

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REFERENCES

1. Kornberg, A. (1987) The two cultures: chemistry and biology, *Biochemistry 26*, 6888–6891.
2. Kaplan, J. H., Forbush, B., 3rd, and Hoffman, J. F. (1978) Rapid photolytic release of adenosine 5′-triphosphate from a protected analogue: utilization by the Na:K pump of human red blood cell ghosts, *Biochemistry 17*, 1929–1935.
3. Curley, K., and Lawrence, D. S. (1999) Caged regulators of signaling pathways, *Pharmacol. Ther. 82*, 347–354.
4. Curley, K., and Lawrence, D. S. (1999) Light-activated proteins, *Curr. Opin. Chem. Biol. 3*, 84–88.
5. Dorman, G., and Prestwich, G. D. (2000) Using photolabile ligands in drug discovery and development, *Trends Biotechnol. 18*, 64–77.
6. Kramer, R. H., Chambers, J. J., and Trauner, D. (2005) Photochemical tools for remote control of ion channels in excitable cells, *Nat. Chem. Biol. 1*, 360–365.
7. Lawrence, D. S. (2005) The preparation and *in vivo* applications of caged peptides and proteins, *Curr. Opin. Chem. Biol. 9*, 570–575.
8. Mayer, G., and Heckel, A. (2006) Biologically active molecules with a "light switch", *Angew. Chem., Int. Ed. 45*, 4900–4921.
9. Politz, J. C. (1999) Use of caged fluorochromes to track macromolecular movement in living cells, *Trends Cell Biol. 9*, 284–287.
10. Shigeri, Y., Tatsu, Y., and Yumoto, N. (2001) Synthesis and application of caged peptides and proteins, *Pharmacol. Ther. 91*, 85–92.
11. Young, D. D., and Deiters, A. (2007) Photochemical control of biological processes, *Org. Biomol. Chem. 5*, 999–1005.
12. Tang, X., and Dmochowski, I. J. (2007) Regulating gene expression with light-activated oligonucleotides, *Mol. Biosyst. 3*, 100–110.
13. Banghart, M. R., Volgraf, M., and Trauner, D. (2006) Engineering light-gated ion channels, *Biochemistry 45*, 15129–15141.
14. Gorostiza, P., and Isacoff, E. Y. (2008) Optical switches for remote and noninvasive control of cell signaling, *Science (New York, N.Y.) 322*, 395–399.
15. Ellis-Davies, G. C. (2007) Caged compounds: photorelease technology for control of cellular chemistry and physiology, *Nat. Methods 4*, 619–628.
16. Pelliccioli, A. P., and Wirz, J. (2002) Photoremovable protecting groups: reaction mechanisms and applications, *Photochem. Photobiol. Sci. 1*, 441–458.
17. Goeldner, M., and Givens, R., Eds. (2005) *Phototriggers, Photoswitches and Caged Biomolecules: Phototriggers, Photoswitches and Caged Biomolecules*, Wiley-VCH, Weinheim.
18. Marriott, G., Ed. (1998) *Caged Compounds*, Vol. 291, Academic Press, New York.
19. Furuta, T., Momotake, A., Sugimoto, M., Hatayama, M., Torigai, H., and Iwamura, M. (1996) Acyloxycoumarinylmethyl-caged cAMP, the photolabile and membrane-permeable derivative of cAMP that effectively stimulates pigment-dispersion response of melanophores, *Biochem. Biophys. Res. Commun. 228*, 193–198.
20. Hagen, V., Dzeja, C., Bendig, J., Baeger, I., and Kaupp, U. B. (1998) Novel caged compounds of hydrolysis-resistant 8-Br-cAMP and 8-Br-cGMP: photolabile NPE esters, *J. Photochem. Photobiol. B 42*, 71–78.
21. Hagen, V., Dzeja, C., Frings, S., Bendig, J., Krause, E., and Kaupp, U. B. (1996) Caged compounds of hydrolysis-resistant analogues of cAMP and cGMP: synthesis and application to cyclic nucleotide-gated channels, *Biochemistry 35*, 7762–7771.
22. Hagen, V., Frings, S., Wiesner, B., Helm, S., Kaupp, U. B., and Bendig, J. (2003) [7-(Dialkylamino)coumarin-4-yl]methyl-caged compounds as ultrafast and effective long-wavelength phototriggers of 8-bromo-substituted cyclic nucleotides, *ChemBioChem 4*, 434–442.
23. Kaupp, U. B., Dzeja, C., Frings, S., Bendig, J., and Hagen, V. (1998) Applications of caged compounds of hydrolysis-resistant analogs of cAMP and cGMP, *Methods Enzymol. 291*, 415–430.
24. Givens, R. S., Athey, P. S., Kueper, L. W., Matuszewski, B., and Xue, J. Y. (1992) Photochemistry of α-keto phosphate esters: photorelease of a caged cAMP, *J. Am. Chem. Soc. 114*, 8708–8710.
25. Givens, R. S., Athey, P. S., Matuszewski, B., Kueper, L. W., Xue, J., and Fister, T. (1993) Photochemistry of phosphate esters: α-keto phosphates as a photoprotecting group for caged phosphate, *J. Am. Chem. Soc. 115*, 6001–6012.
26. Hagen, V., Bendig, J., Frings, S., Eckardt, T., Helm, S., Reuter, D., and Kaupp, U. B. (2001) Highly efficient and ultrafast phototriggers for cAMP and cGMP by using long-wavelength UV/vis-activation, *Angew. Chem., Int. Ed. 40*, 1045–1048.
27. Hagen, V., Dekowski, B., Nache, V., Schmidt, R., Geissler, D., Lorenz, D., Eichhorst, J., Keller, S., Kaneko, H., Benndorf, K., and Wiesner, B. (2005) Coumarinylmethyl esters for ultrafast release of high concentrations of cyclic nucleotides upon one- and two-photon photolysis, *Angew. Chem., Int. Ed. 44*, 7887–7891.
28. Furuta, T., Takeuchi, H., Isozaki, M., Takahashi, Y., Kanehara, M., Sugimoto, M., Watanabe, T., Noguchi, K., Dore, T. M., Kurahashi, T., Iwamura, M., and Tsien, R. Y. (2004) Bhc-cNMPs as either water-soluble or membrane-permeant photoreleasable cyclic nucleotides for both one- and two-photon excitation, *ChemBioChem 5*, 1119–1128.
29. Wang, L., Corrie, J. E., and Wootton, J. F. (2002) Photolabile precursors of cyclic nucleotides with high aqueous solubility and stability, *J. Org. Chem. 67*, 3474–3478.
30. Engels, J., and Schlaeger, E. J. (1977) Synthesis, structure, and reactivity of adenosine cyclic 3′,5′-phosphate benzyl triesters, *J. Med. Chem. 20*, 907–911.

31. Michal, G., Muhlegger, K., Nelboeck, M., Thiessen, C., and Weimann, G. (1974) Cyclophosphates VI. Cyclophosphates as substrates and effectors of phosphodiesterase, *Pharmacol. Res. Commun. 6*, 203–252.
32. Ogreid, D., Ekanger, R., Suva, R. H., Miller, J. P., Sturm, P., Corbin, J. D., and Doskeland, S. O. (1985) Activation of protein kinase isozymes by cyclic nucleotide analogs used singly or in combination. Principles for optimizing the isozyme specificity of analog combinations, *Eur. J. Biochem. 150*, 219–227.
33. Dills, W. L., Jr., Beavo, J. A., Bechtel, P. J., Myers, K. R., Sakai, L. J., and Krebs, E. G. (1976) Binding of adenosine 3′,5′-monophosphate dependent protein kinase regulatory subunit to immobilized cyclic nucleotide derivatives, *Biochemistry 15*, 3724–3731.
34. Ghosh, M., Ichetovkin, I., Song, X., Condeelis, J. S., and Lawrence, D. S. (2002) A new strategy for caging proteins regulated by kinases, *J. Am. Chem. Soc. 124*, 2440–2441.
35. Ghosh, M., Song, X., Mouneimne, G., Sidani, M., Lawrence, D. S., and Condeelis, J. S. (2004) Cofilin promotes actin polymerization and defines the direction of cell motility, *Science (New York, N.Y.) 304*, 743–746.
36. Van Troys, M., Huyck, L., Leyman, S., Dhaese, S., Vandekerkhove, J., and Ampe, C. (2008) Ins and outs of ADF/cofilin activity and regulation, *Eur. J. Cell Biol. 87*, 649–667.
37. Shah, S., Rangarajan, S., and Friedman, S. H. (2005) Light-activated RNA interference, *Angew. Chem., Int. Ed. 44*, 1328–1332.
38. Monroe, W. T., McQuain, M. M., Chang, M. S., Alexander, J. S., and Haselton, F. R. (1999) Targeting expression with light using caged DNA, *J. Biol. Chem. 274*, 20895–20900.
39. Ando, H., Furuta, T., Tsien, R. Y., and Okamoto, H. (2001) Photo-mediated gene activation using caged RNA/DNA in zebrafish embryos, *Nat. Genet. 28*, 317–325.
40. Marriott, G. (1994) Caged protein conjugates and light-directed generation of protein activity: preparation, photoactivation, and spectroscopic characterization of caged G-actin conjugates, *Biochemistry 33*, 9092–9097.
41. Heckel, A. (2007) Nucleobase-caged phosphoramidites for oligonucleotide synthesis, *Curr. Protoc. Nucleic Acid Chem. Chapter 1*, Unit 1.17.
42. Ordoukhanian, P., and Taylor, J.-S. (1995) Design and synthesis of a versatile photocleavable DNA building block. Application to phototriggered hybridization, *J. Am. Chem. Soc. 117*, 9570–9571.
43. Renner, C., and Moroder, L. (2006) Azobenzene as conformational switch in model peptides, *ChemBioChem 7*, 868–878.
44. Woolley, G. A. (2005) Photocontrolling peptide α helices, *Acc. Chem. Res. 38*, 486–493.
45. Lester, H. A., Krouse, M. E., Nass, M. M., Wassermann, N. H., and Erlanger, B. F. (1980) A covalently bound photoisomerizable agonist: comparison with reversibly bound agonists at electrophorus electroplaques, *J. Gen. Physiol. 75*, 207–232.
46. Givens, R. S., Weber, J. F., Jung, A. H., and Park, C. H. (1998) New photoprotecting groups: desyl and p-hydroxyphenacyl phosphate and carboxylate esters, *Methods Enzymol. 291*, 1–29.
47. Stensrud, K. F., Heger, D., Sebej, P., Wirz, J., and Givens, R. S. (2008) Fluorinated photoremovable protecting groups: the influence of fluoro substituents on the photo-Favorskii rearrangement, *Photochem. Photobiol. Sci. 7*, 614–624.
48. Arroyo, J. G., Jones, P. B., Porter, N. A., and Hatchell, D. L. (1997) In vivo photoactivation of caged-thrombin, *Thromb. Haemostasis 78*, 791–793.
49. Kneissl, S., Loveridge, E. J., Williams, C., Crump, M. P., and Allemann, R. K. (2008) Photocontrollable peptide-based switches target the anti-apoptotic protein Bcl-xL, *ChemBioChem 9*, 3046–3054.
50. Banghart, M., Borges, K., Isacoff, E., Trauner, D., and Kramer, R. H. (2004) Light-activated ion channels for remote control of neuronal firing, *Nat. Neurosci. 7*, 1381–1386.
51. Gorostiza, P., and Isacoff, E. (2007) Optical switches and triggers for the manipulation of ion channels and pores, *Mol. Biosyst. 3*, 686–704.
52. Volgraf, M., Gorostiza, P., Numano, R., Kramer, R. H., Isacoff, E. Y., and Trauner, D. (2006) Allosteric control of an ionotropic glutamate receptor with an optical switch, *Nat. Chem. Biol. 2*, 47–52.
53. Raymo, F. M., and Tomasulo, M. (2005) Electron and energy transfer modulation with photochromic switches, *Chem. Soc. Rev. 34*, 327–336.
54. Young, D. D., and Deiters, A. (2008) Light-regulated RNA-small molecule interactions, *ChemBioChem 9*, 1225–1228.
55. Sakata, T., Jackson, D. K., Mao, S., and Marriott, G. (2008) Optically switchable chelates: optical control and sensing of metal ions, *J. Org. Chem. 73*, 227–233.
56. Fang, J., Sakata, T., Marriott, G., and Iwasa, K. H. (2008) Probing conformational changes of prestin with thiol-reactive optical switches, *Biophys. J. 95*, 3036–3042.
57. Fujimoto, K., Amano, M., Horibe, Y., and Inouye, M. (2006) Reversible photoregulation of helical structures in short peptides under indoor lighting/dark conditions, *Org. Lett. 8*, 285–287.
58. Johnson, E. C., and Kent, S. B. (2006) Synthesis, stability and optimized photolytic cleavage of 4-methoxy-2-nitrobenzyl backbone-protected peptides, *Chem. Commun. (Cambridge, U.K.)* 1557–1559.
59. Nandy, S. K., Agnes, R. S., and Lawrence, D. S. (2007) Photochemically-activated probes of protein–protein interactions, *Org. Lett. 9*, 2249–2252.
60. Tatsu, Y., Nishigaki, T., Darszon, A., and Yumoto, N. (2002) A caged sperm-activating peptide that has a photocleavable protecting group on the backbone amide, *FEBS Lett. 525*, 20–24.
61. Ohmuro-Matsuyama, Y., and Tatsu, Y. (2008) Photocontrolled cell adhesion on a surface functionalized with a caged arginine-glycine-aspartate peptide, *Angew. Chem., Int. Ed. 47*, 7527–7529.
62. Li, H., Hah, J. M., and Lawrence, D. S. (2008) Light-mediated liberation of enzymatic activity: "small molecule" caged protein equivalents, *J. Am. Chem. Soc. 130*, 10474–10475.
63. Cruz, F. G., Koh, J. T., and Link, K. H. (2000) Light-activated gene expression, *J. Am. Chem. Soc. 122*, 8777–8778.
64. Lin, W., Albanese, C., Pestell, R. G., and Lawrence, D. S. (2002) Spatially discrete, light-driven protein expression, *Chem. Biol. 9*, 1347–1353.
65. Shi, Y., and Koh, J. T. (2004) Light-activated transcription and repression by using photocaged SERMs, *ChemBioChem 5*, 788–796.
66. Veldhuyzen, W. F., Nguyen, Q., McMaster, G., and Lawrence, D. S. (2003) A light-activated probe of intracellular protein kinase activity, *J. Am. Chem. Soc. 125*, 13358–13359.
67. Pirrung, M. C., and Nunn, D. S. (1992) Synthesis of photodeprotectable serine derivatives. "Caged serine", *Bioorg. Med. Chem. Lett. 2*, 1489–1492.
68. Dai, Z., Dulyaninova, N. G., Kumar, S., Bresnick, A. R., and Lawrence, D. S. (2007) Visual snapshots of intracellular kinase activity at the onset of mitosis, *Chem. Biol. 14*, 1254–1260.
69. Rothman, D. M., Vazquez, M. E., Vogel, E. M., and Imperiali, B. (2002) General method for the synthesis of caged phosphopeptides: tools for the exploration of signal transduction pathways, *Org. Lett. 4*, 2865–2868.
70. Rothman, D. M., Vazquez, M. E., Vogel, E. M., and Imperiali, B. (2003) Caged phospho-amino acid building blocks for solid-phase peptide synthesis, *J. Org. Chem. 68*, 6795–6798.

71. Nguyen, A., Rothman, D. M., Stehn, J., Imperiali, B., and Yaffe, M. B. (2004) Caged phosphopeptides reveal a temporal role for 14-3-3 in G1 arrest and S-phase checkpoint function, *Nat. Biotechnol. 22*, 993–1000.
72. Sreekumar, R., Ikebe, M., Fay, F. S., and Walker, J. W. (1998) Biologically active peptides caged on tyrosine, *Methods Enzymol. 291*, 78–94.
73. Wang, Q., Dai, Z., Cahill, S. M., Blumenstein, M., and Lawrence, D. S. (2006) Light-regulated sampling of protein tyrosine kinase activity, *J. Am. Chem. Soc. 128*, 14016–14017.
74. Tatsu, Y., Shigeri, Y., Sogabe, S., Yumoto, N., and Yoshikawa, S. (1996) Solid-phase synthesis of caged peptides using tyrosine modified with a photocleavable protecting group: application to the synthesis of caged neuropeptide Y, *Biochem. Biophys. Res. Commun. 227*, 688–693.
75. Humphrey, D., Rajfur, Z., Vazquez, M. E., Scheswohl, D., Schaller, M. D., Jacobson, K., and Imperiali, B. (2005) *In situ* photoactivation of a caged phosphotyrosine peptide derived from focal adhesion kinase temporarily halts lamellar extension of single migrating tumor cells, *J. Biol. Chem. 280*, 22091–22101.
76. Wieboldt, R., Gee, K. R., Niu, L., Ramesh, D., Carpenter, B. K., and Hess, G. P. (1994) Photolabile precursors of glutamate: synthesis, photochemical properties, and activation of glutamate receptors on a microsecond time scale, *Proc. Natl. Acad. Sci. U.S.A. 91*, 8752–8756.
77. Bourgault, S., Letourneau, M., and Fournier, A. (2007) Development of photolabile caged analogs of endothelin-1, *Peptides 28*, 1074–1082.
78. Specht, A., Thomann, J. S., Alarcon, K., Wittayanan, W., Ogden, D., Furuta, T., Kurakawa, Y., and Goeldner, M. (2006) New photoremovable protecting groups for carboxylic acids with high photolytic efficiencies at near-UV irradiation. Application to the photocontrolled release of L-glutamate, *ChemBioChem 7*, 1690–1695.
79. Jasuja, R., Keyoung, J., Reid, G. P., Trentham, D. R., and Khan, S. (1999) Chemotactic responses of *Escherichia coli* to small jumps of photoreleased L-aspartate, *Biophys. J. 76*, 1706–1719.
80. Tatsu, Y., Shigeri, Y., Ishida, A., Kameshita, I., Fujisawa, H., and Yumoto, N. (1999) Synthesis of caged peptides using caged lysine: application to the synthesis of caged AIP, a highly specific inhibitor of calmodulin-dependent protein kinase II, *Bioorg. Med. Chem. Lett. 9*, 1093–1096.
81. Watai, Y., Sase, I., Shiono, H., and Nakano, Y. (2001) Regulation of nuclear import by light-induced activation of caged nuclear localization signal in living cells, *FEBS Lett. 488*, 39–44.
82. Wood, J., Koszelak, M., Liu, J., and Lawrence, D. S. (1998) A caged protein kinase inhibitor, *J. Am. Chem. Soc. 120*, 7145–7146.
83. Pan, P., and Bayley, H. (1997) Caged cysteine and thiophosphoryl peptides, *FEBS Lett. 405*, 81–85.
84. Zou, K., Miller, W. T., Givens, R. S., and Bayley, H. (2001) Caged thiophosphotyrosine peptides, *Angew. Chem., Int. Ed. 40*, 3049–3051.
85. Kaiser, E. T., Lawrence, D. S., and Rokita, S. E. (1985) The chemical modification of enzymatic specificity, *Annu. Rev. Biochem. 54*, 565–595.
86. Chang, C. Y., Niblack, B., Walker, B., and Bayley, H. (1995) A photogenerated pore-forming protein, *Chem. Biol. 2*, 391–400.
87. Turner, A. D., Pizzo, S. V., Rozakis, G. W., and Porter, N. A. (1987) Photochemical activation of acylated α-thrombin, *J. Am. Chem. Soc. 109*, 1274–1275.
88. Loudwig, S., Nicolet, Y., Masson, P., Fontecilla-Camps, J. C., Bon, S., Nachon, F., and Goeldner, M. (2003) Photoreversible inhibition of cholinesterases: catalytic serine-labeled caged butyrylcholinesterase, *ChemBioChem 4*, 762–767.
89. Zou, K., Cheley, S., Givens, R. S., and Bayley, H. (2002) Catalytic subunit of protein kinase A caged at the activating phosphothreonine, *J. Am. Chem. Soc. 124*, 8220–8229.
90. Chang, C., Fernandez, T., Panchal, R., and Bayley, H. (1998) Caged catalytic subunit of cAMP-dependent protein kinase, *J. Am. Chem. Soc. 120*, 7661–7662.
91. Curley, K., and Lawrence, D. S. (1998) Photoactivation of a signal transduction pathway in living cells, *J. Am. Chem. Soc. 120*, 8573–8574.
92. Muir, T. W. (2003) Semisynthesis of proteins by expressed protein ligation, *Annu. Rev. Biochem. 72*, 249–289.
93. Muir, T. W. (2008) Studying protein structure and function using semisynthesis, *Biopolymers 90*, 743–750.
94. Hahn, M. E., and Muir, T. W. (2004) Photocontrol of Smad2, a multiphosphorylated cell-signaling protein, through caging of activating phosphoserines, *Angew. Chem., Int. Ed. 43*, 5800–5803.
95. Hahn, M. E., Pellois, J. P., Vila-Perello, M., and Muir, T. W. (2007) Tunable photoactivation of a post-translationally modified signaling protein and its unmodified counterpart in live cells, *ChemBioChem 8*, 2100–2105.
96. Pellois, J. P., Hahn, M. E., and Muir, T. W. (2004) Simultaneous triggering of protein activity and fluorescence, *J. Am. Chem. Soc. 126*, 7170–7171.
97. Gagey, N., Emond, M., Neveu, P., Benbrahim, C., Goetz, B., Aujard, I., Baudin, J. B., and Jullien, L. (2008) Alcohol uncaging with fluorescence reporting: evaluation of *o*-acetoxyphenyl methyloxazolone precursors, *Org. Lett. 10*, 2341–2344.
98. Gagey, N., Neveu, P., Benbrahim, C., Goetz, B., Aujard, I., Baudin, J. B., and Jullien, L. (2007) Two-photon uncaging with fluorescence reporting: evaluation of the *o*-hydroxycinnamic platform, *J. Am. Chem. Soc. 129*, 9986–9998.
99. Wang, L., Xie, J., and Schultz, P. G. (2006) Expanding the genetic code, *Annu. Rev. Biophys. Biomol. Struct. 35*, 225–249.
100. Deiters, A., Groff, D., Ryu, Y., Xie, J., and Schultz, P. G. (2006) A genetically encoded photocaged tyrosine, *Angew. Chem., Int. Ed. 45*, 2728–2731.
101. Wu, N., Deiters, A., Cropp, T. A., King, D., and Schultz, P. G. (2004) A genetically encoded photocaged amino acid, *J. Am. Chem. Soc. 126*, 14306–14307.
102. Endo, M., Nakayama, K., Kaida, Y., and Majima, T. (2004) Design and synthesis of photochemically controllable caspase-3, *Angew. Chem., Int. Ed. 43*, 5643–5645.
103. Mendel, D., Ellman, J. A., and Schultz, P. G. (1991) Construction of a light-activated protein by unnatural amino acid mutagenesis, *J. Am. Chem. Soc. 113*, 2758–2760.
104. Short, G. F., Lodder, M., Laikhter, A. L., Arslan, T., and Hecht, S. M. (1999) Caged HIV-1 protease: dimerization is independent of the ionization state of the active site aspartates, *J. Am. Chem. Soc. 121*, 478–479.
105. Endo, M., Nakayama, K., and Majima, T. (2004) Design and synthesis of photochemically controllable restriction endonuclease BamHI by manipulating the salt-bridge network in the dimer interface, *J. Org. Chem. 69*, 4292–4298.
106. Lodder, M., Golovine, S., Laikhter, A. L., Karginov, V. A., and Hecht, S. M. (1998) Misacylated transfer RNAs having a chemically removable protecting group, *J. Org. Chem. 63*, 794–803.
107. Bose, M., Groff, D., Xie, J., Brustad, E., and Schultz, P. G. (2006) The incorporation of a photoisomerizable amino acid into proteins in *E. coli*, *J. Am. Chem. Soc. 128*, 388–389.
108. Philipson, K. D., Gallivan, J. P., Brandt, G. S., Dougherty, D. A., and Lester, H. A. (2001) Incorporation of caged cysteine and caged tyrosine into a transmembrane segment of the nicotinic ACh receptor, *Am. J. Physiol. 281*, C195–206.
109. Miller, J. C., Silverman, S. K., England, P. M., Dougherty, D. A., and Lester, H. A. (1998) Flash decaging of tyrosine sidechains in an ion channel, *Neuron 20*, 619–624.

110. Tong, Y., Brandt, G. S., Li, M., Shapovalov, G., Slimko, E., Karschin, A., Dougherty, D. A., and Lester, H. A. (2001) Tyrosine decaging leads to substantial membrane trafficking during modulation of an inward rectifier potassium channel, *J. Gen. Physiol. 117*, 103–118.
111. Leung, D. W., Otomo, C., Chory, J., and Rosen, M. K. (2008) Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway, *Proc. Natl. Acad. Sci. U.S.A. 105*, 12797–12802.
112. Gambetta, G. A., and Lagarias, J. C. (2001) Genetic engineering of phytochrome biosynthesis in bacteria, *Proc. Natl. Acad. Sci. U.S.A. 98*, 10566–10571.
113. Mukougawa, K., Kanamoto, H., Kobayashi, T., Yokota, A., and Kohchi, T. (2006) Metabolic engineering to produce phytochromes with phytochromobilin, phycocyanobilin, or phycoerythrobilin chromophore in *Escherichia coli*, *FEBS Lett. 580*, 1333–1338.
114. Li, L., and Lagarias, J. C. (1994) Phytochrome assembly in living cells of the yeast *Saccharomyces cerevisiae*, *Proc. Natl. Acad. Sci. U.S.A. 91*, 12535–12539.
115. Shimizu-Sato, S., Huq, E., Tepperman, J. M., and Quail, P. H. (2002) A light-switchable gene promoter system, *Nat. Biotechnol. 20*, 1041–1044.
116. Kawakami, T., Cheng, H., Hashiro, S., Nomura, Y., Tsukiji, S., Furuta, T., and Nagamune, T. (2008) A caged phosphopeptide-based approach for photochemical activation of kinases in living cells, *ChemBioChem 9*, 1583–1586.
117. Furuta, T., Wang, S. S., Dantzker, J. L., Dore, T. M., Bybee, W. J., Callaway, E. M., Denk, W., and Tsien, R. Y. (1999) Brominated 7-hydroxycoumarin-4-ylmethyls: photolabile protecting groups with biologically useful cross-sections for two photon photolysis, *Proc. Natl. Acad. Sci. U.S.A. 96*, 1193–1200.
118. Papageorgiou, G., Ogden, D. C., Barth, A., and Corrie, J. E. T. (1999) Photorelease of carboxylic acids from 1-acyl-7-nitroindolines in aqueous solution: a reagent for rapid and efficient photorelease of L-glutamate, *J. Am. Chem. Soc. 121*, 6503–6504.
119. Fedoryak, O. D., Sul, J. Y., Haydon, P. G., and Ellis-Davies, G. C. (2005) Synthesis of a caged glutamate for efficient one- and two-photon photorelease on living cells, *Chem. Commun. (Cambridge, U.K.)* 3664–3666.
120. Kantevari, S., Hoang, C. J., Ogrodnik, J., Egger, M., Niggli, E., and Ellis-Davies, G. C. (2006) Synthesis and two-photon photolysis of 6-(*o*-nitroveratryl)-caged IP3 in living cells, *ChemBioChem 7*, 174–180.
121. Momotake, A., Lindegger, N., Niggli, E., Barsotti, R. J., and Ellis-Davies, G. C. (2006) The nitrodibenzofuran chromophore: a new caging group for ultra-efficient photolysis in living cells, *Nat. Methods 3*, 35–40.
122. Ellis-Davies, G. C., Matsuzaki, M., Paukert, M., Kasai, H., and Bergles, D. E. (2007) 4-Carboxymethoxy-5,7-dinitroindolinyl-Glu: an improved caged glutamate for expeditious ultraviolet and two-photon photolysis in brain slices, *J. Neurosci. 27*, 6601–6604.
123. Gug, S., Charon, S., Specht, A., Alarcon, K., Ogden, D., Zietz, B., Leonard, J., Haacke, S., Bolze, F., Nicoud, J. F., and Goeldner, M. (2008) Photolabile glutamate protecting group with high one- and two-photon uncaging efficiencies, *ChemBioChem 9*, 1303–1307.
124. Aujard, I., Benbrahim, C., Gouget, M., Ruel, O., Baudin, J. B., Neveu, P., and Jullien, L. (2006) *o*-Nitrobenzyl photolabile protecting groups with red-shifted absorption: syntheses and uncaging cross-sections for one- and two-photon excitation, *Chemistry (Weinheim, Ger.) 12*, 6865–6879.
125. Aemissegger, A., Carrigan, C. N., and Imperiali, B. (2007) Caged *O*-phosphorothioyl amino acids as building blocks for Fmoc-based solid phase peptide synthesis, *Tetrahedron 63*, 6185–6190.
126. Shah, N. H., and Kirshenbaum, K. (2008) Photoresponsive peptoid oligomers bearing azobenzene side chains, *Org. Biomol. Chem. 6*, 2516–2521.
127. Bourgault, S., Letourneau, M., and Fournier, A. (2005) Development and pharmacological characterization of "caged" urotensin II analogs, *Peptides 26*, 1475–1480.
128. Petersen, S., Alonso, J. M., Specht, A., Duodu, P., Goeldner, M., and del Campo, A. (2008) Phototriggering of cell adhesion by caged cyclic RGD peptides, *Angew. Chem., Int. Ed. 47*, 3192–3195.
129. Auernheimer, J., Dahmen, C., Hersel, U., Bausch, A., and Kessler, H. (2005) Photoswitched cell adhesion on surfaces with RGD peptides, *J. Am. Chem. Soc. 127*, 16107–16110.
130. Schutt, M., Krupka, S. S., Milbradt, A. G., Deindl, S., Sinner, E. K., Oesterhelt, D., Renner, C., and Moroder, L. (2003) Photocontrol of cell adhesion processes: model studies with cyclic azobenzene-RGD peptides, *Chem. Biol. 10*, 487–490.
131. Milbradt, A. G., Loweneck, M., Krupka, S. S., Reif, M., Sinner, E. K., Moroder, L., and Renner, C. (2005) Photomodulation of conformational states. IV. Integrin-binding RGD-peptides with (4-aminomethyl)phenylazobenzoic acid as backbone constituent, *Biopolymers 77*, 304–313.
132. Higuchi, H., Muto, E., Inoue, Y., and Yanagida, T. (1997) Kinetics of force generation by single kinesin molecules activated by laser photolysis of caged ATP, *Proc. Natl. Acad. Sci. U.S.A. 94*, 4395–4400.
133. Hess, H., Clemmens, J., Qin, D., Howard, J., and Vogel, V. (2001) Light-controlled molecular shuttles made from molecular motors carrying cargo on engineered surfaces, *Nano Lett. 1*, 235–239.
134. Nomura, A., Uyeda, T. Q. P., Yumoto, N., and Tatsu, Y. (2006) Photo-control of kinesin-microtubule motility using caged peptides derived from the kinesin C-terminus domain, *Chem. Commun.* 3588–3590.
135. Blanc, A., and Bochet, C. G. (2002) Wavelength-controlled orthogonal photolysis of protecting groups, *J. Org. Chem. 67*, 5567–5577.
136. del Campo, A., Boos, D., Spiess, H. W., and Jonas, U. (2005) Surface modification with orthogonal photosensitive silanes for sequential chemical lithography and site-selective particle deposition, *Angew. Chem., Int. Ed. 44*, 4707–4712.
137. Shaner, N. C., Steinbach, P. A., and Tsien, R. Y. (2005) A guide to choosing fluorescent proteins, *Nat. Methods 2*, 905–909.
138. DeMond, A. L., Starr, T., Dustin, M. L., and Groves, J. T. (2006) Control of antigen presentation with a photoreleasable agonist peptide, *J. Am. Chem. Soc. 128*, 15354–15355.
139. Huse, M., Klein, L. O., Girvin, A. T., Faraj, J. M., Li, Q. J., Kuhns, M. S., and Davis, M. M. (2007) Spatial and temporal dynamics of T cell receptor signaling with a photoactivatable agonist, *Immunity 27*, 76–88.
140. Kuner, T., Li, Y., Gee, K. R., Bonewald, L. F., and Augustine, G. J. (2008) Photolysis of a caged peptide reveals rapid action of *N*-ethylmaleimide sensitive factor before neurotransmitter release, *Proc. Natl. Acad. Sci. U.S.A. 105*, 347–352.
141. Goss, V. L., Hocevar, B. A., Thompson, L. J., Stratton, C. A., Burns, D. J., and Fields, A. P. (1994) Identification of nuclear β II protein kinase C as a mitotic lamin kinase, *J. Biol. Chem. 269*, 19074–19080.
142. Hocevar, B. A., Burns, D. J., and Fields, A. P. (1993) Identification of protein kinase C (PKC) phosphorylation sites on human lamin B. Potential role of PKC in nuclear lamina structural dynamics, *J. Biol. Chem. 268*, 7545–7552.
143. Mitchison, T. J., and Salmon, E. D. (2001) Mitosis: a history of division, *Nat. Cell Biol. 3*, E17–21.
144. Clarke, P. R., and Zhang, C. (2008) Spatial and temporal coordination of mitosis by Ran GTPase, *Nat. Rev. 9*, 464–477.
145. Edwards, A. S., and Scott, J. D. (2000) A-kinase anchoring proteins: protein kinase A and beyond, *Curr. Opin. Cell Biol. 12*, 217–221.

146. Taylor, R. A., and Risbridger, G. P. (2008) Prostatic tumor stroma: a key player in cancer progression, *Curr. Cancer Drug Targets 8*, 490–497.
147. Lanigan, F., O'Connor, D., Martin, F., and Gallagher, W. M. (2007) Molecular links between mammary gland development and breast cancer, *Cell. Mol. Life Sci. 64*, 3159–3184.
148. Tang, C. M. (2006) Photolysis of caged neurotransmitters: theory and procedures for light delivery, *Curr. Protoc. Neurosci. Chapter 6*, Unit 6.21.
149. Shoham, S., O'Connor, D. H., Sarkisov, D. V., and Wang, S. S. (2005) Rapid neurotransmitter uncaging in spatially defined patterns, *Nat. Methods 2*, 837–843.