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# Photo-click chemistry strategies for spatiotemporal control of metal-free ligation, labeling, and surface derivatization*

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*Abstract*: Three photo-click ligation strategies described in this account provide scientists with efficient and selective tools for derivatization of various molecules, polymers, and surfaces. Fast photochemical reactions that are utilized in these techniques permit spatiotemporal control of the process. The absence of activating reagents and catalysts, as well as compatibility with aqueous media, makes photo-click ligations suitable for biomedical applications. The first of these approaches relies on the photochemical decarbonylation of cyclopropenones to produce cyclooctynes. The latter undergo rapid catalyst-free strain-promoted azide–alkyne cycloaddition (SPAAC) to azide-tagged substrates. The second method is based on a very fast (>$10^4$ $M^{-1}$ $s^{-1}$) light-triggered hetero-Diels–Alder reaction and permits efficient derivatization of substrates bearing vinyl ether moiety. An even faster reaction between photochemically generated naphthoquinone methides (*o*NQMs) and thiols (~2 × $10^5$ $M^{-1}$ $s^{-1}$) serves as a basis for a third method. This thiol photo-click chemistry allows for the selective derivatization of thiol-functionalized substrates or labeling of free cysteine residues in proteins. The thioether linkage produced by the reaction of *o*NQMs and a thiol is stable under ambient conditions, but can be cleaved by UV irradiation, regenerating free thiol. This feature permits the removal or replacement of immobilized compounds, as well as traceless substrate release.

*Keywords*: alkynes; click chemistry; cycloadditions; Diels–Alder; photochemistry; polymers; quinone methide; strain-promoted azide–alkyne cycloaddition (SPAAC); surface modification; thiols.

## INTRODUCTION

"Click chemistry" is a term describing a set of bimolecular reactions that permits the efficient formation of covalent link between two substrates or between a substrate and a surface [1]. The majority of "click" strategies are based on 1,3-dipolar or Diels–Alder cycloadditions, nucleophilic ring openings,

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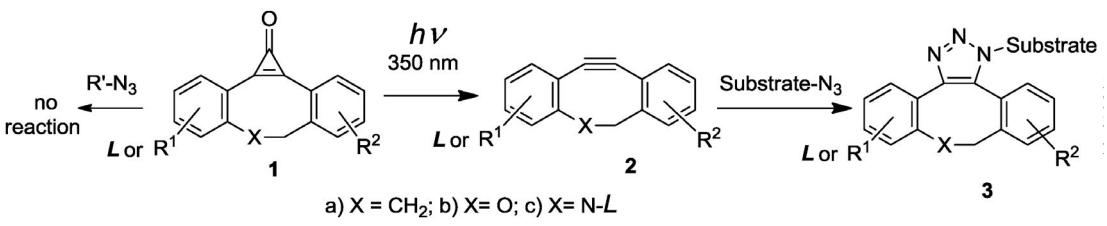
non-aldol carbonyl chemistry, and addition to carbon–carbon multiple bonds [2,3]. Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides to terminal acetylenes (CuAAC) became the gold standard of click chemistry and has found applications in many areas ranging from materials science [3,4] to chemical biology [5,6] and drug development [7]. Use of cytotoxic Cu catalyst, however, somewhat limits the utility of CuAAC [8]. Recently discovered strain-promoted cycloaddition (SPAAC) of azides to cyclooctynes [9], dibenzocyclooctynes [10], aza-dibenzocyclooctynes [11], and thiacycloalkynes [12] offers a bio-compatible catalyst-free version of the azide click reaction [13]. "Click" methods based on a Diels–Alder cycloaddition are also gaining popularity since this reaction does not require catalysts, can proceed in high yield under physiological conditions, and does not produce any by-products [14]. The Diels–Alder click reaction has found applications in materials chemistry [15], derivatization of nanoparticles and surfaces with various bioactive molecules [16], as well as the labeling of oligonucleotides, proteins, and oligosaccharides [17]. However, the generation of a reactive diene–dienophile pair often requires either thermal activation [18] or the use of chemical promoters for the in situ generation of highly reactive dienes [19].

The utility of "click chemistry" can be further extended by employing photochemically triggered click reactions, as this approach permits the spatiotemporal control of the process. Several useful photo-click methods have been reported recently, including cycloaddition of alkenes to photochemically generated nitrile imine [20], photo-initiated thiol-ene [21], and thiol-yne [22] reactions, as well as photochemical uncaging of hydroquinone moieties [16d,e].

## RESULTS AND DISCUSSION

The development of the photo-click chemistry tools in our group employs two very efficient photochemical reactions: decarbonylation of cyclopropenones [23] and photo-dehydration of *o*-hydroxybenzyl alcohol analogs [24,25]. These reactions are characterized by high quantum efficiency, quantitative chemical yield, and can be conducted in an aqueous solution without the loss of efficiency or the formation of by-products. Dibenzocyclooctyne (DIBO) precursors (**1**, Scheme 1), in which triple bond is replaced with the cyclopropenone moiety, are thermally stable compounds that do not react with organic azides. Irradiation of cyclopropenones **1** results in the loss of carbon monoxide and the formation of dibenzocyclooctynes **2** [26]. The latter undergo facile catalyst-free addition to azides (SPAAC). Photochemical dehydration of 3-hydroxy-2-naphthalenemethanol derivatives (NQMP, **6**) produces *o*-naphthoquinone methides (*o*NQMs, **7**). *o*NQMs selectively react with vinyl ethers in aqueous solutions at a very high rate to produce photo-stable Diels–Alder adducts (**8**, Scheme 5) [27]. Thiols are the only endogenous nucleophiles that react with *o*NQMs (**7**) in neutral aqueous solutions. This reaction permits selective derivatization of thiol groups in various substrates (Scheme 10) [28]. Photochemically produced thioethers (**12**) can be cleaved by irradiation under different conditions. Photogeneration of reactive acetylenes has the advantage of being compatible with well-developed azide labeling protocols. The *o*NQM–vinyl ether and *o*NQM–thiol reactions are very fast and allow for high spatial resolution of labeling or patterning. In addition, these two methods represent ligation techniques, which are orthogonal to the popular azide click reaction.




**Scheme 1**

### Light-triggered alkyne–azide cycloaddition (photo-SPAAC)

Cyclopropenones are arguably the most convenient photolabile masking group for alkynes. The excellent thermal stability of these compounds both in aqueous solution and in organic solvents [23a,26c] is combined with high photochemical reactivity [23]. We have employed photochemical decarbonylation of cyclopropenones for the development of a novel phototriggered click strategy for metal-free ligation of azides. Cyclopropenones, such as **1**, do not react with azides under ambient conditions in the dark but efficiently produce reactive dibenzocyclooctynes **2** upon irradiation [26a,c,29]. Photogenerated acetylenes **2**, on the other hand, readily add azides to produce triazoles **3** (Scheme 1). In this and subsequent schemes, ***L*** represents the linker to a surface, label, or another substrate; $\boldsymbol{R^1}$ and $\boldsymbol{R^2}$ represent substituents that are used to modify solubility, photophysical, and other properties of the click reagent.

Introduction of heteroatoms in the DIBO structure results in significant rate enhancement of the cycloaddition reaction. Thus, the rate constant for the reaction of the parent DIBO (**2a**) with benzyl azide in methanol is $k \sim 0.06\ M^{-1}\ s^{-1}$ [26a]; azadibenzocyclooctyne (ADIBO) (**2b**) under the same conditions reacts seven times faster ($k \sim 0.4\ M^{-1}\ s^{-1}$) [11c], while oxadibenzocyclooctyne (ODIBO) (**2c**) is the most reactive cyclooctyne reported ($k \sim 2\ M^{-1}\ s^{-1}$) [27c]. In wholly aqueous addition solutions, the addition of organic azide to cyclooctyne **2c** becomes even faster, exceeding $45\ M^{-1}\ s^{-1}$ [26c].

It is important to note that in the course of the photo-decarbonylation and subsequent azide addition, the $\lambda_{max}$ of the substrate undergoes significant blue shift (Fig. 1). Thus, UV spectra of methanol or PBS solutions of cyclopropenones **1a–c** show a cluster of two or three close-lying intense bands between 310 and 360 nm. The longest-wavelength band is observed at ~350 nm. Irradiation of **1** with 350-nm light resulted in efficient ($\Phi = 0.16$–$0.42$) decarbonylation of the starting material, which could be observed by bleaching of the 310–350-nm bands, and the quantitative formation of cyclooctynes **2**. The acetylenes **2** possess a similar cluster of bands that are 25–30 nm blue-shifted in relation to **1** (Fig. 1). Dibenzocyclooctynes **2** and triazoles **3** have virtually no absorbance at the $\lambda_{max}$ of **1**, thus allowing for selective irradiation of cyclopropenones in their presence and for the convenient monitoring of the reaction progress. In addition, cyclopropenones show no appreciable fluorescence, while triazoles **3** are fluorescent.

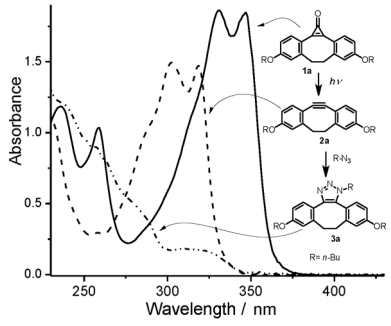
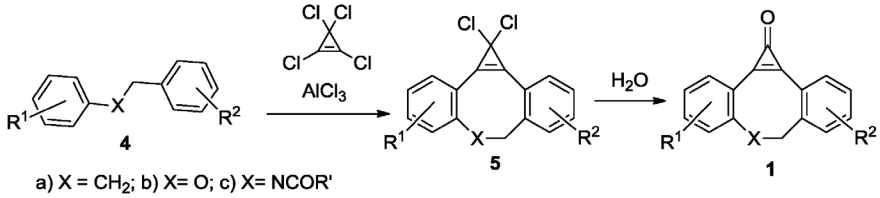



**Fig. 1** Spectra of ~0.5 mM methanol solutions of cyclopropenone **1a** (solid line), acetylene **2a** (dashed line), and triazole **3a** (dash-dotted line).

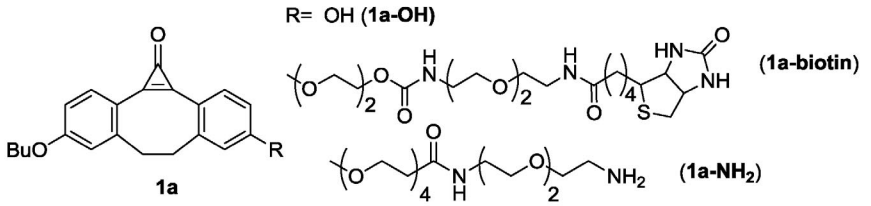
Preparation of cyclooctynes is a simple two-step procedure starting from appropriately substituted diphenylethane (**4a**), *N*-phenyl-*N*-benzyl amide (**4b**), or phenyl benzyl ether (**4c**). The double Friedel–Crafts alkylation of the latter with tetracholorocyclopropene produces corresponding dichlorocyclopropene (**5a–c**). Substituents play a crucial role in this reaction, as they direct the attack of cyclopropenium cation into position *ortho* to the linker that connects two aromatic rings. Controlled hydrolysis of dichlorides (**5a–c**) produces target cyclopropenones (**1a–c**) in moderate yield (20–67 % over two steps, Scheme 2).




**Scheme 2** Preparation of cylopropenones **1a–c**.

The resulting cyclooctyne photoprecursors can be further derivatized by introducing an appropriate linker and/or functional moiety. Thus, cyclopropenone **1a-OH** was conjugated to biotin (**1a-biotin**) for cell labeling experiments [26a] or equipped with amino-terminated linker (**1a-NH$_2$**) [26b] for immobilization on brush polymers (Scheme 3).




**Scheme 3**

The biocompatibility of the photo-SPAAC technique was demonstrated by labeling live cells. Jurkat or CHO cells were grown in the culture medium containing peracetylated *N*-azidoacetylmannosamine, leading to the metabolic incorporation of the corresponding *N*-azidoacetyl sialic acid into their cell surface glycoproteins. These azide-tagged cells were placed in the medium containing 30 μM of **1a-biotin** and exposed to 350-nm light from a fluorescent lamp (4 W) for 1 min. After incubation for 1 h at r.t., the cells were washed and stained with avidin-Fluorescein for 15 min at 4 °C. Fluorescence intensity of the cell lysates indicate efficient labeling of the cells with **1a-biotin** [26a]. As expected, low fluorescence intensities were measured when cells were exposed to cyclopropenone **1a-biotin** in the dark at 25 and 37 °C (Fig. 2). In situ activation of **1a-biotin** was found to have no detectable effect on cell viability and morphology [26a]. For the imaging experiment (Fig. 2), azide-tagged CHO cells were incubated with **1a-biotin** for 1 h in the dark or irradiated with 350-nm lamp for 1 min before incubation. Next, cells were incubated with avidin-Alexa Fluor 488 for 15 min at 4 °C and, after washing, fixing, and staining for the nucleus with the far-red-fluorescent dye TO-PRO-3 iodide, imaged. Images obtained at 488 nm (Alexa Fluor) and 633 nm (TO-PRO-3 iodide) are merged and shown in green and red, respectively (Fig. 2).

We envision that cyclopropenones such as **1a–c** will make it possible to label living organisms with spatiotemporal control.

Cyclopropenone-based photo-click chemistry is also suitable for light-directed surface derivatization. A poly(*N*-hydroxysuccinimide 4-vinyl benzoate) [poly(NHS4VB)] polymer brush coatings (125 nm) were prepared using surface-initiated ATRP [26b]. The electrophilic *N*-hydroxysuccinimide (NHS) ester pendant group allowed for quantitative derivatization of brush matrix with amino-functionalized cyclopropenone **1a-NH$_2$** (Scheme 4). When irradiated with UV light, **1a** undergoes instant decarbonylation to yield the immobilized DIBO (**2a**). DIBO then undergoes catalyst-free cycloadditions with azides to yield the triazole-linked conjugate in quantitative yield under ambient conditions. Unexposed poly(NHS4VB)-**1a** does not react with azides under ambient conditions.

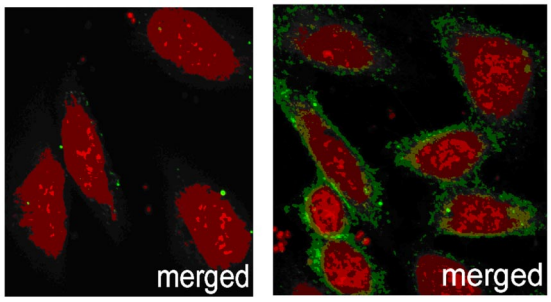
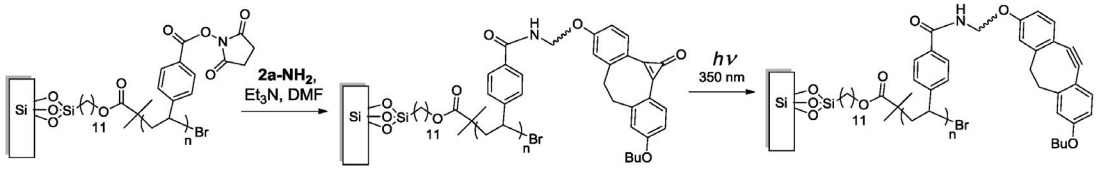



**Fig. 2** Fluorescence images of cells labeled with **1a-biotin** and avidin-Alexa Fluor 488 in the dark (left panel) or after 1 min of 350-nm irradiation. Nuclei were stained with TO-PRO-3 iodide.




**Scheme 4**

To further demonstrate the versatility of the surface photo-SPAAC, poly(NHS4VB)-**1a**-coated quartz slides were irradiated with 350-nm lamp (3.5 mW/cm$^2$) through a shadow mask (12-µm pitch TEM grid) for 150 s. Substrates were then immersed in a solution of Lissamine rhodamine B-azide conjugate. The cycloaddition was complete within 20 min and occurred only in the exposed areas, where cyclopropenone groups underwent decarbonylation to generate DIBO moieties (Fig. 3a).

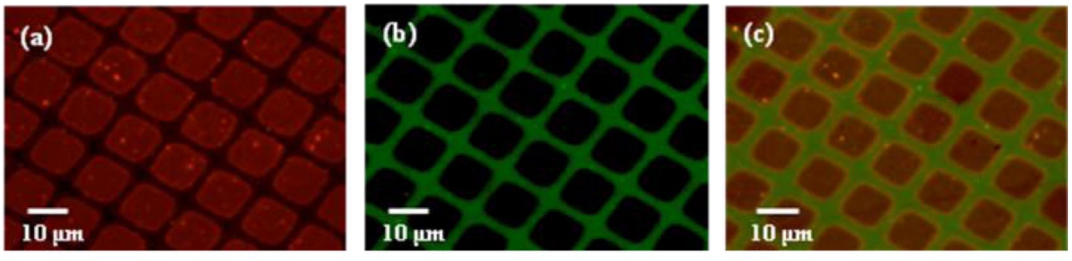



**Fig. 3** Fluorescence microscope images of a photopatterned surface fabricated by sequential photoactivation of poly(NHS4VB)-**1a** coating: (a) photo-click functionalization with azido-rhodamine; $\lambda_{exc}$= 550 nm (b) after flood irradiation and incubation with azido-fluoresceine; $\lambda_{exc}$= 477 nm, and (c) both dyes imaged under $\lambda_{exc}$= 477 nm.

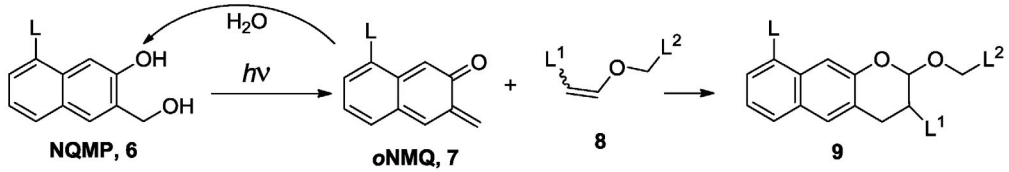
A subsequent flood irradiation of the substrate liberates the remaining DIBO (**2a**) and allows for the functionalization of the protected regions for further click reactions. Azido-fluorescein was then immobilized to generate a multifunctional substrate (Figs. 3b,c). There is negligible cross-contamination between the two dyes, with excellent segregation between the selectively activated regions.

Photo-SPAAC surface functionalization technique allows for the creation of multifunctional surfaces with spatially resolved chemical functionality. This photo activation strategy is versatile, and can be extended to other types of surfaces, as well as to nanoparticle applications.

### Photochemical hetero-Diels–Alder cycloaddition (photo-HDAC)

Irradiation of *o*-hydroxybenzyl alcohols and their analogues results in the dehydration and the formation of *o*-quinone methides. *o*-Quinone methides are very reactive species and readily undergo nucleophilic addition or can be trapped as a Diels–Alder adduct with electron-rich alkenes [24,30]. In aqueous solutions, *o*-quinone methides undergo very rapid re-hydration to starting material. The combination of electrophilic and Diels–Alder reactivity makes 2-napthoquinone-3-methide (*o*NQM, **7**) especially suitable for the development of photo-click methodologies. The lifetime of *o*NQMs in aqueous solution is very short ($\tau_{H2O}$ ~ 7 ms), and only vinyl ethers and enamines are reactive enough to form the Diels–Alder adduct (Scheme 5) [25,27a]. No cycloaddition was observed with other types of alkenes under these conditions, and NQMP does not react with vinyl ethers in the dark. In addition, photo-dehydration of *o*NQM precursor, NQMP (**6**), can be achieved using 350-nm fluorescent lamps or 355-nm output of Nd:YAG laser. It is also important to note that NQMP shows no detectable cytotoxicity in the dark or under irradiation [27a].



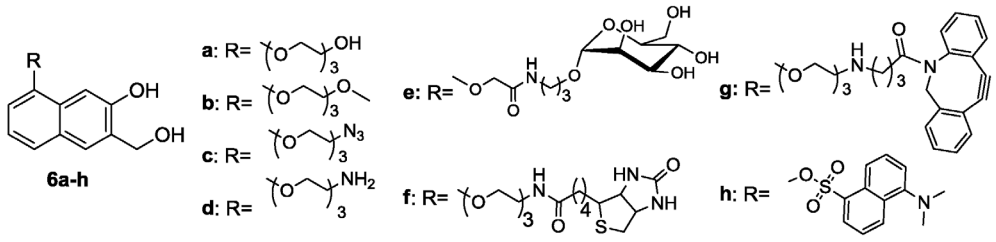

**Scheme 5** Photo Diels–Alder click ligation in aqueous solution.

To achieve photolabeling or photoligation of two substrates, one is derivatized with a vinyl ether moiety (**8**), while 3-(hydroxymethyl)-2-naphthol (NQMP, **6**) is attached to the other. Irradiation of substituted NQMP (**6**) results in efficient ($\Phi$ = 0.20) dehydration of the substrate and the formation of *o*NQM **7** (Scheme 5). In the presence of vinyl ethers (**8**), *o*NQMs undergo very rapid ($k$ ~ 4 × $10^4$ $M^{-1}$ $s^{-1}$) and quantitative Diels–Alder cycloaddition to yield photostable benzo-[g]chromans (**9**, Scheme 5) [25]. The unreacted *o*NQM is rapidly hydrated ($k_{H2O}$ ~ 145 $s^{-1}$) to regenerate NQMP (**6**). The photo-click ligation technique comprising of *o*-napthoquinone methide precursor and vinyl ether can be tailored to produce permanent or a hydrolytically labile linkage. If substrate is connected to the vinyl ether moiety via ether oxygen (***L*²**, Scheme 5), the resulting link is acid-sensitive and undergoes slow hydrolysis at pH < 3 [27a]. Position ***L*¹** in benzochroman linker **9**, produced by conjugation via vinylic carbon, is hydrolytically stable. In aqueous solutions at pH ~ 7, only thiols are reactive enough to outcompete water in Michael addition to *o*NQM. The thioether produced in the reaction, however, is photolabile and regenerates *o*NQM upon excitation, while cycloadduct **9** is photostable.

The label or second component for ligation has to be attached to the naphthalene ring of the *o*NQM precursor **6** via an appropriate linker (***L***, Scheme 5). We have demonstrated that various functional fragments can be introduced into 8-position of NQMP either directly (**6h**) or via the triethyleneglycol (TEG)-linker (**6a–g**, Scheme 6) [27,31]. These substituents do not affect NQMP photochemistry. The azide group in NQMP **6d** remains intact at the Diels–Alder photo-click conditions [27a]. Such bifunctional linker molecules allow for combining light-directed ligation with azide–acetylene click chemistry. To demonstrate the compatibility of Diels–Alder photo-click with SPAAC ligation methods, we have prepared NQMP-ADIBO conjugate **6g** [27b]. Direct labeling with fluorescent dye can achieved using NQMP-dansyl **6h** [32].

The photochemical Diels–Alder cycloaddition described offers a new platform for light-induced ligation, which is orthogonal to the popular acetylene–azide click chemistry. The high photochemical stability of Diels–Alder adducts eliminates secondary photoreactivity. The fast rate of the *o*NQM addition to vinyl ethers coupled with short lifetimes of quinone methides makes this method especially well

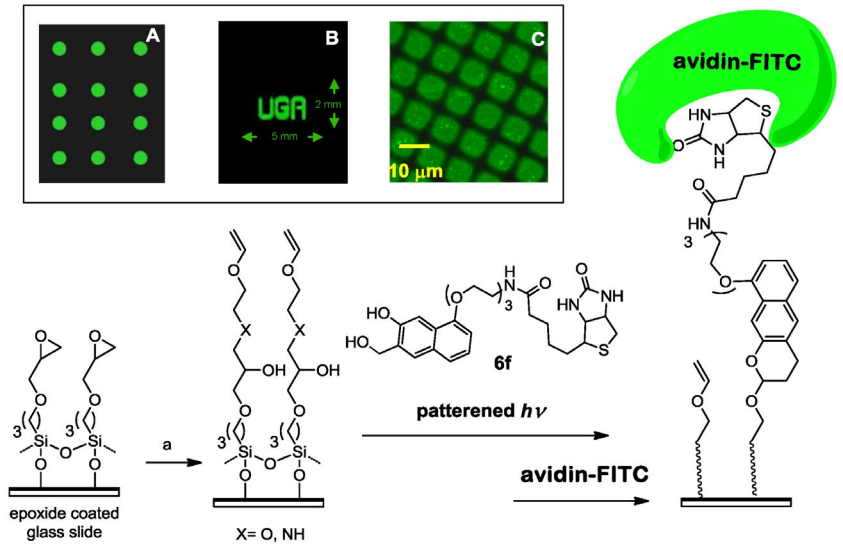
 




**Scheme 6** Functional derivatives of NQMP.

suited for applications requiring spatial and temporal resolution. Photochemically generated *o*NQMs are also very selective: in aqueous solution only electron-rich polarized alkenes produce Diels–Alder adducts.

To demonstrate the utility of photo-HDAC for the light-directed surface derivatization and patterning, epoxy-coated glass slides were functionalized with vinyl ether moieties (Scheme 7). These slides were covered with aqueous solution of NQMP-biotin (**6f**) and irradiated via shadow mask. In the exposed areas, NQMP underwent an efficient conversion into reactive *o*NQM **7f**, which in turn reacted with immobilized vinyl ether moieties producing a photochemically stable covalent link between a substrate and a surface (Scheme 7) [27b]. This photo-click strategy represents an unusual paradigm in photo-patterning: the surface itself is photochemically inert, while the photoreactive component is present in a low viscosity solution. The short lifetime ($\tau \sim 7$ ms) of the active form of the photo-click reagent in aqueous solution prevents its migration from the site of irradiation, thus allowing for the spatial control of surface derivatization. The photo-biotinylated slides were stained with FITC-avidin and imaged.




**Scheme 7** Schematic representation of preparation and light-directed biotinylation of vinyl ether-coated slides followed by immobilization of FITC-avidin. The insert shows fluorescent images of vinyl ether-coated slides spotted with 2 µL drops of $10^{-4}$ M NQMP-biotin (**6f**) aqueous solution and flood-irradiated (A); covered with $10^{-4}$ M NQMP-biotin (**6f**) aqueous solution and irradiated via mask where "UGA" letters were micro-machined in a steel plate (B); or irradiated via 12-µm pitch TEM Cu grid (C).

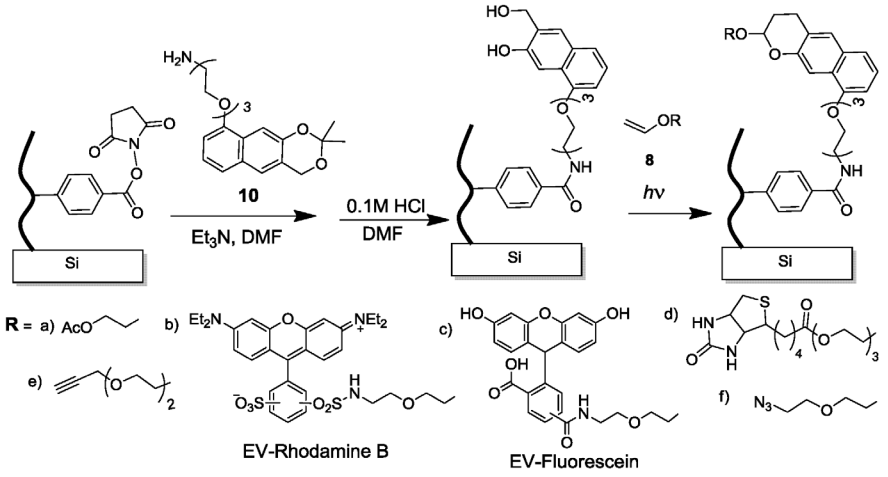
 

Since unreacted *o*NQM groups are rapidly hydrated to regenerate NQMP and consumption of the reagent for the derivatization of self-assembled monolayer (SAM) is minimal, the NQMP solutions can be re-used many times without loss of efficiency [27b].

Protein patterning procedures often require extensive washing to remove substrate non-specifically absorbed on the surface. In our experiments, the FITC-avidin-stained surfaces (e.g., A and B, Scheme 7) were washed by sonicating the glass slides in PBS solution for 30 min followed by overnight incubation in fresh phosphate buffer. From a practical point of view, a shorter washing procedure could enhance the efficiency of photo-click protein patterning. In order to reduce non-specific protein binding, we have developed a photochemical PEGylation procedure. After the initial patterning of NQMP-biotin (**6f**) on the vinyl ether-derivatized surfaces, slides are subjected to flood irradiation in the aqueous solution of NQMP-TEG (**6a**). This procedure makes previously unexposed areas highly hydrophilic and significantly reduces protein binding. Thus, excellent signal-to-noise ratio was achieved when slides, post-irradiated with **6a** and stained with FITC-avidin, were incubated for just 1 h in fresh PBS solution and then rinsed with water [27b].

The sensitivity of 2-alkoxybenzochroman linker (Scheme 7) to acid hydrolysis permits removal of the previously immobilized molecules from the surface. Overnight incubation of photo-biotinylated slides (Scheme 7) in 0.1 N perchloric acid resulted in >98 % loss of the surface-bound biotin groups. To produce hydrolytically stable surface coating, epoxide glass slides were treated with 6-methoxyhex-5-en-1-ol. The resulting slides were irradiated in an aqueous solution of NQMP **6f** to yield biotinylated surfaces, which withstand overnight incubation in 0.1 M perchloric acid solution without loss of Avidin binding capacity [27b].

The SAMs have low surface concentration of reactive groups and, therefore, produce a relatively weak readout signal. The polymer brush platform allows for much higher surface density of the probe molecules and insures high mechanical and chemical stability of the functionalized surfaces. To explore the compatibility of the NQMP-based surface photo-patterning technologies with reactive polymer brush [32], poly(NHS4VB)-coated (50 nm) silicon wafers were treated with acetal-protected NQMP-amine **6d** (Scheme 8) [27c]. The conversion was quantitative as evidenced by complete disappearance of the imide bands in GATR-FTIR spectra. Removal of acetal protection from immobilized **6d** was achieved by treating the wafers with 0.1 HCl in dimethyl formamide (DMF) (Scheme 8).



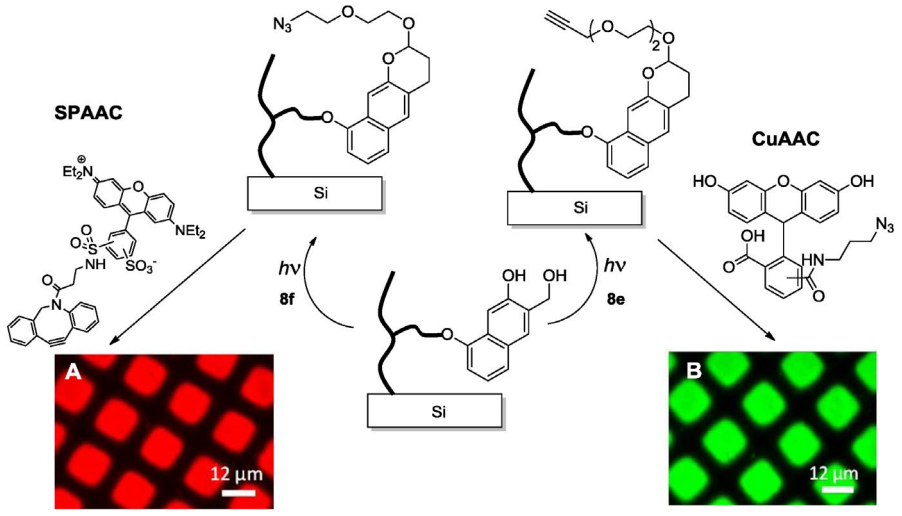

**Scheme 8** NQMP functionalization of poly(*N*-hydroxysuccinimidyl 4-vinyl benzoate) [pol(NHS-4VB)] brush polymer.

To test the efficiency of the new photo-immobilization platform, the wafers coated with the NQMP-functionalized polymer brush [poly(NQMP-4VB)] were immersed in 0.1 mM aqueous solutions of various vinyl ether derivatives (**8a–f**) and irradiated using a 300-nm handheld lamp for 2 min (3.5 mW $cm^{-2}$). Changes in the thickness and hydrophobicity of the polymer layer, as well as the appearance of characteristic features in IR spectra, confirmed the efficient functionalization of poly(NQMP-4VB) brushes.

Overnight incubation of the irradiated wafers in acetic anhydride/pyridine solution did not change the surface characteristics, confirming complete conversion of the NQMP moieties to benzochroman linkers (Scheme 8).

We have directly compared the binding capacity of the 65-nm poly(NQMP-4VB) layer with a NQMP-TEG monolayer [27c] by irradiating both slides in a 0.1-mM aqueous solution of EV-fluorescein **8c** (Scheme 8). The averaged surface emission of the Fluorescein-functionalized poly(NQMP-4VB) layer was 61 ± 5 times more intense than that of the NQMP-derivatized SAM. This experiment demonstrates the increased surface density of solvent-accessible functional groups in the polymer brush layers as compared to SAMs. The NQMP-polymer brush platform is also more efficient than NQMP-SAM for the immobilization of much larger molecules. However, the difference is less pronounced. Thus, photo-biotinylation of both types of slides using **8d**, followed by staining with FITC-avidin, produced only 15 ± 2 times difference in fluorescent intensity. This muted fluorescence intensity enhancement and relatively small changes in layer thickness suggest relatively low loading of the protein in the polymer matrix. This reduced loading is most likely due to hindered diffusion of large protein molecules into the brush and potential cross-linking of the biotinylated polymer brushes with Avidin [27c].

Orthogonality of the photo-HDAC and alkyne–azide click chemistry permits sequential applications of these ligation methods for one-pot derivatization of substrates with multiple moieties, or for the light-directed patterning of photosensitive groups. For example, azide (**8f**) or alkyne-containing vinyl ether (**8e**) is photopatterned onto a poly(NQMP-4VB)-coated surface. The substrate of interest is then immobilized using CuAAC or SPAAC chemistry (Scheme 9).



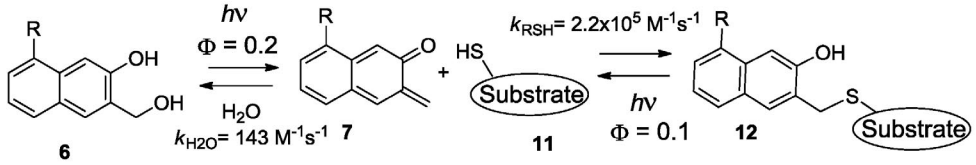

**Scheme 9** Sequential click: photo-HDAC surface patterning followed by azide–alkyne click immobilization of fluorescent dyes: (A) SPAAC of ADIBO-rhodamine B on azide-derivatized surface; (B) CuAAC of azido-fluorescein.

In summary, the click chemistry based on the addition of photochemically generated *o*NQM to vinyl ether works well in aqueous solution, proceeds at a high rate under ambient conditions, and does not require catalyst or additional reagents. Both *o*NQM precursors and vinyl ethers are stable in the dark, and the reaction is orthogonal to other popular derivatization techniques, such as acetylene–azide click reaction.

### Reversible quinone methide–thiol click chemistry (photo-QMTC)

In neutral aqueous solutions only few very reactive nucleophiles, such as thiols, can outcompete in addition to *o*NQMs (**7**) [25]. The resulting thioether (**12**) produced in the reaction of thiols with *o*NQM is hydrolytically stable but can be quantitatively cleaved under 300- or 350-nm irradiation back to **7** with 10 % quantum yield (Scheme 10) [25,28]. In other words, irradiation of NQMP (**6**) in the presence of thiols in aqueous solution results in the establishment of a photochemically driven equilibrium between thiol-bearing substrate **11** and NQMP **6** on one side, and thioether **12** on the other (Scheme 10).




**Scheme 10**

The position of equilibrium is defined by the rate of reaction of the common intermediate **7** with water ($k_{hydr} = k_{H2O}[H_2O]$) and thiol ($k_{RSH}$), as well as by the efficiency of the photoelimination reaction of NQMP (**6**, $\Phi_{OH}$) and NQMP-SR (**12**, $\Phi_{SR}$). Since under the established equilibrium conditions, concentrations of **6** and **12** remain constant, we can write the following equation (eq. 1), where [NQMP], [RSH], and [NQMP-SR] are equilibrium concentrations of corresponding substrates:

$$\frac{\Phi_{OH}[\text{NQMP}]}{k_{hydr}} = \frac{\Phi_{SR}[\text{NQMP-SR}]}{k_{RSH}[\text{RSH}]} \quad (1)$$

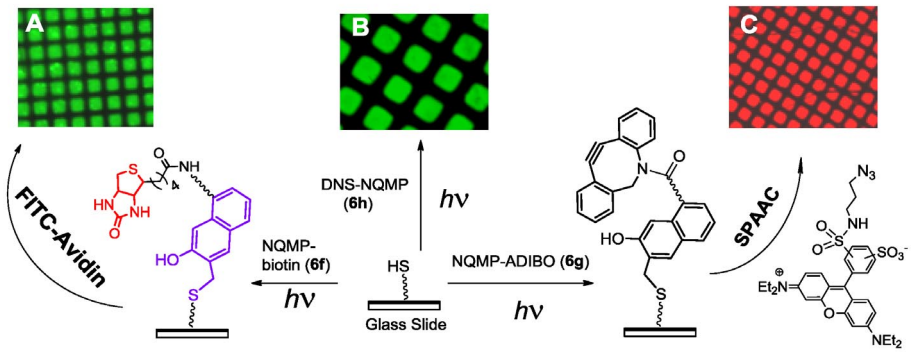
$$\frac{[\text{NQMP-SR}]}{[\text{RSH}]} = \frac{\Phi_{OH} k_{RSH}[\text{NQMP}]}{\Phi_{SR} k_{hydr}} \quad (2)$$

The ratio of caged to free thiol can be further expressed as a function of NQMP concentration (eq. 2). Since $k_{RSH}$ is about five orders of magnitude larger than $k_{H2O}$, and $\Phi_{OH}/\Phi_{SR} \sim 2$ [27a], the equilibrium is shifted in the favor of NQMP-SR (**12**) formation at relatively low concentrations of thiol and NQMP in aqueous solutions. Using experimental rates of *o*NQM hydration and reactions with simple thiols [25], as well as photochemical efficiencies of NQMP and NQMP-SR reactions, we can evaluate that 90+ % conversion of a substrate is achieved at starting NQMP concentration equal [NQMP] = $3 \times 10^{-4}$ $M^{-1}$ + [substrate]. In biochemical labeling experiments, where concentration of a substrate is in μM range or lower, 0.4 mM of NQMP derivative should be sufficient to achieve complete functionalization of all available cysteine residues. On the other hand, the NQMP label can be removed by irradiating the NQMP-tagged substrate in a dilute solution (40 μM or lower) in the absence of free NQMP. Quantitative release of NQMP-caged substrates at higher concentrations can be achieved in the presence of vinyl ethers, as the latter trap intermediate *o*NQM **7** to form photostable benzochroman **9**. We have also employed *o*NQM-thiol photo-click chemistry for the selective and reversible labeling of solvent-accessible cysteine residues in peptides and proteins [28b].

Quinone methide–thiol click chemistry allowed us to develop a method for the reversible photo-derivatization of thiolated surface. This method not only allows for the patterned immobilization of various substrates on the surface but also for the light-directed release or replacement of the immobilized substances. Commercially available thiol-derivatized glass slides are immersed in an aqueous solution of substrates conjugated to NQMP (**6**) and irradiated using 350-nm fluorescent lamps [28]. Photochemically produced *o*NQM moieties **7** react with surface thiols to yield thioether **12** (Scheme 10). The unreacted *o*NQMs are rapidly hydrated to regenerate NQMP (**6**). The very short lifetime of *o*NQM species in aqueous solutions prevents their migration from the site of irradiation, thus allowing for the high spatial resolution of the derivatization (vide infra). Due to much higher nucleophilicity of thiols, their quantitative conversion in the exposed areas is achieved despite large excess of the nucleophilic solvent.

## Direct photo-patterning of a fluorescent dye

We have developed a dansyl-NQMP conjugate (DNS-NQMP, **6h**), which can be employed as a fluorescent photolabile protecting group [31], for surface patterning [28a], or for the direct fluorescent labeling of thiol moieties in proteins [28b]. Upon excitation with 400–450 nm light, **6h** emits intense green emission ($\lambda_{max}$ = 559 nm) with 21 % fluorescence quantum yield. Fluorescent readout at these wavelengths does not cause formation of *o*NQM **7h**. Irradiation of DNS-NQMP (**6h**) with 300- or 350-nm light, on the other hand, results in efficient ($\Phi$ = 0.2) formation of quinone methide **7h** (Scheme 10). We have employed **6h** for the direct fluorescent patterning of thiol-coated glass slides. Slides were immersed in 0.2-mM solution of DNS-NQMP (**6h**) and irradiated via transmission electron microscopy (TEM) grid using hand-held fluorescent UV lamp (350 nm, 4 W) for 4 min (Scheme 11B).



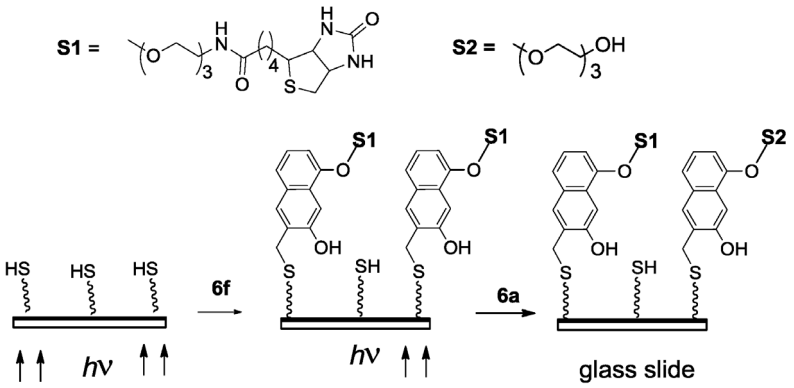

**Scheme 11** Schematic representation of light-directed patterning of thiol-coated slides using 12-µm pitch TEM Cu grid. (A) Biotinylation with **6f** followed by FITC-avidin development; (B) direct patterning of dansyl dye using **6h**; (C) Immobilization of ADIBO (**6g**) followed by click derivatization (SPAAC) with azido-rhodamine.

The quinone methide–thiol click reaction is orthogonal to the majority of other derivatization techniques, including well-developed alkyne–azide click chemistry. Concurrent or sequential applications of photo-click and alkyne–azide click ligations permit one-pot derivatization of substrates with multiple moieties, or for the light-directed patterning of photosensitive substances. To demonstrate the efficiency of such sequential click immobilization, we employed hetero-bifunctional click reagent, NQMP-ADIBO (**6g**). The ADIBO moiety permits efficient conjugation with azide-tagged substances via SPAAC click reaction [11b,c]. NQMP-cyclooctyne conjugate was photo-patterned onto the thiol-coated surface, washed, and immersed in a 0.1-mM DMF solution of Rhodamine B azide for 1 h. The fluorescent microscopic image of the resulting slide is presented in Scheme 11C. The *o*NQM-thiol click

chemistry is suitable for protein immobilization. Thus, FITC-avidin was photopatterned on the thiol-coated glass slide using a two-step procedure. First, NQMP-biotin conjugate (**6f**) was micro-patterned on a slide, which was then developed with FITC-Avidin. The fluorescence microscopic images demonstrate that FITC-Avidin was immobilized only in the exposed areas (Scheme 11A).

The reversibility of the thiol-*o*NQM chemistry permits straightforward generation of a positive or negative pattern (Scheme 12).




**Scheme 12** Reversible derivatization of the thiol-coated surface.

The reversibility of this immobilization technique was demonstrated by exhaustive photo-TEGylation of the thiol-coated glass slide in NQMP-TEG (**6a**) solution. The biotin pattern was then introduced by irradiating of the slide in NQMP-biotin (**6f**) solution through a shadow mask (12 μm TEM grid). FITC-avidin treatment produces a high-resolution protein pattern shown in Fig. 4A. The high contrast of the image indicates the efficient replacement of **6a** with **6f** in the exposed areas. A reversed process, which starts from flood irradiation of the slide in NQMP-biotin (**6f**) solution and followed by NQMP-TEG (**6a**) photo-patterning, produces a negative image (Fig. 4B). To further demonstrate the reversibility of this patterning strategy, the resulting slide (4B) was flood-irradiated in NQMP-biotin (**6f**) solution and stained with FITC-avidin (Fig. 4C). The uniform fluorescence of the resulting slide demonstrates the complete replacement of NQMP-TEG fragments with NQMP-biotin moieties (Fig. 4C).

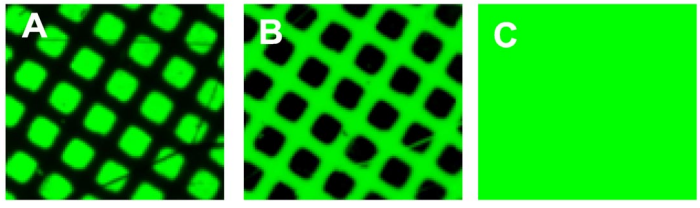



**Fig. 4** Fluorescent images of FITC-avidin stained photo-biotinylated slides. (A) Flood irradiation with **6a** and masked irradiation with **6f**; (B) Flood irradiation with **6f** and masked irradiation with **6a**; (C) Flood irradiation with **6f**, masked irradiation with **6a**, followed by flood irradiation with **6f**.

Since thiol-derivatized surfaces are readily available and a wide variety of substrates can be derivatized with NQM precursor group, 3-(hydroxymethyl)-2-naphthols (NQMPs), this method offers a new platform for light-directed surface functionalization. A photo-QMTC patterning approach is

orthogonal to other popular derivatization techniques and can be used in conjunction with well-developed acetylene–azide click chemistry. A solution of NQMP-conjugated substrate can be re-used numerous times without loss of efficiency because a very minute amount of the reagent is consumed for the derivatization of SAM and all unreacted *o*NQMs are quenched with water to regenerate NQMP. A unique feature of the *o*NQM-thiol photo-click chemistry is the reversibility of the process, which allows for the release of immobilized substrates from a surface or for the replacement of one substrate with another. This feature can be used in the development of light-healable surface coatings, time-resolved photo-release of bioactive molecules, and renewable and repairable microarray technologies. The high stability and robustness of the NQMP group and compatibility of *o*NQM-thiol chemistry with aqueous solutions make photo-click immobilization suitable for biological applications.

## CONCLUDING REMARKS

Photo-click strategies discussed in this account expand the utility of click chemistry by allowing for the spatial and temporal control of the process. The absence of potentially detrimental catalysts and/or activating reagents is an additional beneficial feature of this approach. Photochemical reactions employed for the development of photo-click tools have high quantum efficiency and quantitative chemical yields. As a result, photoligation requires only short irradiation with low-intensity UVA fluorescent lamps, reducing or eliminating light-induced toxicity in cells and photodamage to other substrates. The dark steps of these photo-click reactions are very fast (from 0.1 to above $10^5$ $M^{-1}$ $s^{-1}$) allowing for high spatial and temporal resolution. The photogeneration of cyclooctynes is compatible with well-developed azide labeling protocols; photochemical hetero-Diels–Alder reactions and quinone methide–thiol ligations, on the other hand, are orthogonal to the azide click chemistry. These photoligation methods are fully compatible with aqueous media.

## REFERENCES

1. H. C. Kolb, M. G. Finn, K. B. Sharpless. *Angew. Chem., Int. Ed.* **40**, 2004 (2001).
2. (a) L. Nebhani, C. Barner-Kowollik. *Adv. Mater.* **21**, 3442 (2009); (b) L. S. Wong, F. Khan, J. Micklefield. *Chem. Rev*. **109**, 4025 (2009); (c) B. D. Ratner. In *Biomaterials Science: An Introduction to Materials in Medicine*, A. S. Hoffman, F. J. Schoen, J. E. Lemons (Eds.), Academic Press, San Diego (2004).
3. (a) R. K. Iha, K. L. Wooley, A. M. Nyström, D. J. Burke, M. J. Kade, C. J. Hawker. *Chem. Rev*. **109**, 5620 (2009); (b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless. *Angew. Chem., Int. Ed.* **41**, 2596 (2002); (c) J. E. Hein, V. V. Fokin. *Chem. Soc. Rev.* **39**, 1302 (2010); (d) S. K. Mamidyala, M. G. Finn. *Chem. Soc. Rev.* **39**, 1252 (2010); (e) S. G. Im, K. W. Bong, B.-S. Kim, S. H. Baxamusa, P. T. Hammond, P. S. Doyle, K. K. Gleason. *J. Am. Chem. Soc*. **130**, 14424 (2008).
4. (a) S. Fleischmann, K. Hinrichs, U. Oertel, S. Reichelt, K.-J. Eichhorn, B. Voit. *Macromol. Rapid Commun.* **29**, 1177 (2008); (b) D. Fournier, R. Hoogenboom, U. S. Schubert. *Chem. Rev. Soc.* **36**, 1369 (2007); (c) X.-L. Sun, C. L. Stabler, C. S. Cazalis, E. L. Chaikof. *Bioconjugate Chem.* **17**, 52 (2006); (d) O. Michel, B. J. Ravoo. *Langmuir* **24**, 12116 (2008); (e) S.-Y. Ku, K.-T. Wong, A. Bard. *J. Am. Chem. Soc.* **130**, 2392 (2008); (f) J. E. Moses, A. D. Moorhouse. *Chem. Soc. Rev.* **36**, 1242 (2007); (g) H. Nandivada, X. Jiang, J. Lahann. *Adv. Mater.* **19**, 2197 (2007).
5. (a) P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless, V. V. Fokin. *Angew. Chem., Int. Ed.* **43**, 3928 (2004); (b) S. Punna, E. Kaltgrad, M. G. Finn. *Bioconjugate Chem.* **16**, 1536 (2005); (c) S. Punna, J. Kuzelka, Q. Wang, M. G. Finn. *Angew. Chem., Int. Ed.* **44**, 2215 (2005); (d) J. A. Johnson, D. R. Lewis, D. D. Diaz, M. G. Finn, J. T. Koberstein, N. J. Turro. *J. Am. Chem. Soc.* **128**, 6564 (2006); (e) R. J. Thibault, K. Takizawa, P. Lowenheilm, B. Helms, J. L. Mynar, J. M. J. Frechet, C. J. Hawker. *J. Am. Chem.*

*Soc.* **128**, 12084 (2006); (f) M. J. Joralemon, R. K. O'Reilly, C. J. Hawker, K. L. Wooley. *J. Am. Chem. Soc.* **127**, 16892 (2005); (g) M. Malkoch, R. J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T. P. Russell, C. J. Hawker. *J. Am. Chem. Soc.* **127**, 14942 (2005).

6. (a) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn. *J. Am. Chem. Soc.* **125**, 3192 (2003); (b) E. Strable, D. E. Prasuhn, A. K. Udit, S. Brown, A. J. Link, J. T. Ngo, G. Lander, J. Quispe, C. S. Potter, B. Carragher, D. A. Tirrell, M. G. Finn. *Bioconjugate Chem.* **19**, 866 (2008).
7. (a) G. von Maltzahn, Y. Ren, J.-H. Park, D.-H. Min, V. R. Kotamraju, J. Jayakumar, V. Fogal, M. J. Sailor, E. Ruoslahti, S. N. Bhatia. *Bioconjugate Chem.* **19**, 1570 (2008); (b) H. C. Kolb, K. B. Sharpless. *Drug Discov. Today* **8**, 1128 (2003); (c) K. B. Sharpless, R. Manetsch. *Exp. Opin. Drug Discov.* **1**, 525 (2006).
8. (a) L. M. Gaetke, C. K. Chow. *Toxicology* **189**, 147 (2003); (b) C. J. Burrows, J. G. Muller. *Chem. Rev.* **98**, 1109 (1998); (c) Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn. *J. Am. Chem. Soc.* **125**, 3192 (2003).
9. (a) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi. *Proc. Natl. Acad. Sci. USA* **104**, 16793 (2007); (b) E. M. Sletten, C. R. Bertozzi. *Org. Lett.* **10**, 3097 (2008); (c) J. A. Codelli, J. M. Baskin, N. J. Agard, C. R. Bertozzi. *J. Am. Chem. Soc.* **130**, 11486 (2008); (d) J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl, F. L. van Delft. *Angew. Chem., Int. Ed.* **49**, 9422 (2010).
10. X. Ning, J. Guo, M. A. Wolfert, G.-J. Boons. *Angew. Chem., Int. Ed.* **47**, 2253 (2008).
11. (a) J. C. Jewett, E. M. Sletten, C. R. Bertozzi. *J. Am. Chem. Soc*. **132**, 3688 (2010); (b) M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest, F. L. van Delft. *Chem. Commun.* **46**, 97 (2010); (c) A. Kuzmin, A. Poloukhtine, M. Wolfert, V. V. Popik. *Bioconjugate Chem*. **21**, 2076 (2010).
12. G. de Almeida, E. M. Sletten, H. Nakamura, K. K. Palaniappan, C. R. Bertozzi. *Angew. Chem., Int. Ed.* **51**, 2443 (2012).
13. (a) D. H. Ess, G. O. Jones, K. N. Houk. *Org. Lett.* **10**, 1633 (2008); (b) J. C. Jewett, C. R. Bertozzi. *Chem. Soc. Rev.* **39**, 1272 (2010); (c) M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes, F. L. van Delft. *ChemBioChem* **11**, 1168 (2010); (d) M. F. Debets, S. S. van Berkel, J. Dommerholt, A. J. Dirks, A. F. P. J. T. Rutjes, F. L. van Delft. *Acc. Chem. Res*. **44**, 805 (2011); (e) E. M. Sletten, C. R. Bertozzi. *Acc*. *Chem. Res*. **44**, 666 (2011).
14. D. B. Ramachary, C. F. Barbas. *Chem.—Eur. J.* **10**, 5323 (2004).
15. (a) H. Durmaz, B. Colakoglu, U. Tunca, G. Hizal. *J. Polym. Sci., Part A* **44**, 1667 (2006); (b) A. Dag, H. Durmaz, G. Hizal, U. Tunca. *J. Polym. Sci., Part A* **46**, 302 (2008); (c) B. Gacal, H. Durmaz, M. A. Tasdelen, G. Hizal, U. Tunca, Y. Yagci, A. L. Demirel. *Macromolecules* **39**, 5330 (2006); (d) J. L. Delgado, P. de la Cruz, F. Langa, A. Urbina, J. Casado, J. T. Lopez Navarrete. *Chem. Commun.* **40**, 1734 (2004).
16. (a) H. A. Latham-Timmons, A. Wolter, J. S. Roach, R. Giare, M. Leuck. *Nucleosides, Nucleotides Nucleic Acids* **22**, 1495 (2003); (b) X.-L. Sun, C. L. Stabler, C. S. Cazalis, E. L. Chaikof. *Bioconjugate Chem.* **17**, 52 (2006); (c) M. Proupin-Perez, R. Cosstick, L. M. Liz-Marzan, V. Salgueirino-Maceira, M. Brust. *Nucleosides, Nucleotides Nucleic Acids* **24**, 1075 (2005); (d) M. N. Yousaf, B. T. Houseman, M. Mrksich. *Proc. Natl. Acad. Sci. USA* **98**, 5992 (2001); (e) W. S. Dillmore, M. N. Yousaf, M. Mrksich. *Langmuir* **20**, 7223 (2004); (f) F. Rusmini, Z. Zhong, J. Feijen. *Biomacromolecules* **3**, 1775 (2007).
17. (a) B. Seelig, A. Jaschke. *Tetrahedron Lett.* **38**, 7729 (1997); (b) D. Graham, A. Enright. *Curr. Org. Synth*. **3**, 9 (2006); (c) V. Pozsgay, N. E. Vieira, A. Yergey. *Org. Lett.* **4**, 3191 (2002); (d) R. J. B. H. N. Van den Berg, D. Noort, E. S. Milder-Enacache, G. A. Van der Marel, J. H. Van Boom, H. P. Benschop. *Eur. J. Org. Chem.* 2593 (1999); (e) C. C. A. M. Peeters, P. R. Lagerman, O. de Weers, L. A. Oomen, P. Hoogerhout, M. Beurret, J. T. Poolman. In *Vaccine Protocols*,

A. Robinson, G. Farrar, C. Wiblin (Eds.), p. 111, Humana Press, Totowa, NJ (1996); (f) *Methods in Enzymology*, Vol. 242, Academic Press, San Diego (1994); (g) In *Neoglycoconjugates. Preparation and Applications*, Y. C. Lee, R. T. Lee (Eds.), p. 325, Academic Press, New York (1994); (h) J. B. Robbins, R. Schneerson, P. Anderson, D. H. Smith. *J. Am. Med. Assoc.* **276**, 1181 (1996).

18. (a) B. Seelig, A. Jaschke. *Chem. Biol.* **6**, 167 (1999); (b) G. M. Husar, D. J. Anziano, M. Leuck, D. P. Sebesta. *Nucleosides, Nucleotides Nucleic Acids* **20**, 559 (2001).
19. (a) J. L. Segura, N. Martin. *Chem. Rev.* **11**, 3199 (1999); (b) N. Martin, C. Seoane, M. Hanack. *Org. Prep. Proc. Int.* **23**, 237 (1991); (c) W. Oppolzer. *Synthesis* 793 (1978).
20. (a) Y. Wang, W. Song, W. J. Hu, Q. Lin. *Angew. Chem., Int. Ed.* **48**, 5330 (2009); (b) W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin. *Angew. Chem., Int. Ed.* **47**, 2832 (2008).
21. (a) C. A. De Forest, B. D. Polizzotti, K. S. Anseth. *Nat. Mater.* **8**, 659 (2009); (b) M. Fiore, A. Marra, A. Dondoni. *J. Org. Chem.* **74**, 4422 (2009); (c) M. Fiore, A. Chambery, A. Marra, A. Dondoni. *Org. Biomol. Chem.* **7**, 3910 (2009); (d) K. L. Killops, L. M. Campos, C. J. Hawker. *J. Am. Chem. Soc*. **130**, 5062 (2008); (e) L. M. Campos, K. L. Killops, R. Sakai, J. M. J. Paulusse, D. Damiron, E. Drockenmuller, B. W. Messmore, C. J. Hawker. *Macromolecules* **41**, 7063 (2008); (f) J. W. Chan, B. Yu, C. E. Hoyle, A. B. Lowe. *Chem. Commun*. 4959 (2008).
22. R. M. Hensarling, V. A. Doughty, J. W. Chan, D. L. Patton. *J. Am. Chem. Soc.* **131**, 14673 (2009).
23. (a) A. Poloukhtine, V. V. Popik. *J. Org. Chem.* **68**, 7833 (2003); (b) N. K. Urdabayev, A. Poloukhtine, V. V. Popik. *Chem. Comm.* 454 (2006); (c) A. Poloukhtine, V. V. Popik. *J. Phys. Chem. A* **110**, 1749 (2006).
24. (a) S. E. Rokita (Ed.). *Quinone Methides*, Wiley Series of Reactive Intermediates in Chemistry and Biology, Vol. 1, John Wiley, Hoboken (2009); (b) P. Wan, D. W. Brousmiche, C. Z. Chen, J. Cole, M. Lukeman, M. Xu. *Pure Appl. Chem.* **73**, 529 (2001); (c) E. A. Leo, J. Delgado, L. R. Domingo, A. Espinos, M. A. Miranda, R. Tormos. *J. Org. Chem.* **68**, 9647 (2003); (d) Y. Chiang, A. J. Kresge, Y. Zhu. *J. Am. Chem. Soc.* **122**, 9854 (2000).
25. S. Arumugam, V. V. Popik. *J. Am. Chem. Soc.* **131**, 11892 (2009).
26. (a) A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G.-J. Boons, V. V. Popik. *J. Am. Chem. Soc.* **131**, 15769 (2009); (b) S. V. Orski, A. A. Poloukhtine, S. Arumugam, L. Mao, V. V. Popik, J. Locklin. *J. Am. Chem. Soc.* **132**, 11024 (2010); (c) C. D. McNitt, V. V. Popik. *Org. Biomol. Chem.* **10**, 8200 (2012).
27. (a) S. Arumugam, V. V. Popik. *J. Am. Chem. Soc.* **133**, 5573 (2011); (b) S. Arumugam, V. V. Popik. *J. Am. Chem. Soc.* **133**, 15730 (2011); (c) S. Arumugam, S. Orski, J. Locklin, V. V. Popik. *J. Am. Chem. Soc.* **134**, 179 (2012).
28. (a) S. Arumugam, V. V. Popik. *J. Am. Chem. Soc.* **134**, 8408 (2012); (b) S. Arumugam, J. Guo, N. E. Mbua, E. E. Nekongo, G.-J. Boons, V. V. Popik. *Chem. Sci.* (2013). Submitted for publication.
29. (a) F. R. Fischer, C. Nuckolls. *Angew. Chem., Int. Ed.* **49**, 7257 (2010); (b) F. Starke, M. Walter, H.-J. Pietzsch. ARKIVOC 350 (2010).
30. (a) R. W. Van De Water, T. R. R. Pettus. *Tetrahedron* **58**, 5367 (2002); (b) H. Wang, Y. Wang, K.-L. Han, X.-J. Peng. *J. Org. Chem.* **70**, 4910 (2005); (c) M. Fischer, Y. Shi, B.-P. Zhao, V. Snieckus, P. Wan. *Can. J. Chem.* **77**, 868 (1999).
31. S. Arumugam, V. V. Popik. *Photochem. Photobiol. Sci.* **11**, 518 (2012).
32. S. V. Orski, K. H. Fries, G. R. Sheppard, J. Locklin. *Langmuir* **26**, 2136 (2010).