Surface Modification

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Surface Functionalization by Strain-Promoted Alkyne–Azide Click Reactions**

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Functionalization of surfaces becomes increasingly important given the ever-decreasing size of active devices and the concomitant increase in surface-to-volume ratios. As a result, efficient routes for such functionalizations through the attachment of functional monolayers or multilayers have become the focus of much research in the last decade, both for hard (typically inorganic) and soft (polymeric, dendritic) surfaces. Specific features of desirable surface modification techniques include the combination of high efficiency with mild, noncorrosive reaction conditions. This approach avoids workup to remove (surface-bound) by-products or excess reactants, which typically is not trivial or is practically impossible. Therefore click reactions, such as the copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) with surface-bound alkynes or azides,[1] have been used to functionalize a wide range of surfaces.

However, the presence of Cu^{I} can be problematic: Cu ions are cytotoxic, disrupt double-stranded DNA, alter the structure of "protein-repelling" ethylene oxide moieties, and can change the intrinsic functional properties of the surfaces, such as the through-monolayer conductivity on semiconductor surfaces and the fluorescence of quantum dots. As a result, alternative approaches have been developed over the last five years, including metal-free ligation chemistry that either requires no further activation (e.g., *N*-hydroxysuccinimide-based amide formations)[2] or employs activated but traceless chemistry (e.g., photoinduced thiol–ene addition reactions).[3] Recently, several examples have been published that aim to combine traceless reactions with room-temperature conditions through the application of strain-promoted alkyne–azide cycloadditions (SPAAC or Huisgen–Bertozzi-type cycloadditions)[4] to surfaces. This Highlight focuses on four such reactions and discusses the current state of affairs and goals for the years to come.

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Boons and co-workers modified the surface of organo-micelles that were constructed from tailor-made block copolymers.[5] When amphiphilic copolymers of poly(ethylene oxide) and poly(ε-caprolactone) were appended with amino groups, the resulting species could be treated with a dibenzocyclooctyne derivative that was functionalized with an activated ester (Figure 1 a). This approach led to the formation of micelles with a cyclooctyne-containing surface (Figure 1 b), which could be readily reacted with a range of azides, including fluorescent dyes, peptides, and azide-linked mannosides. The latter products bound specifically to surfaces onto which concanavalin A had been deposited. Similarly functionalized micelles could potentially also be used for drug delivery, and initial steps were made to demonstrate this potential.

Quantum dots (QDs) are an attractive tool in fluorescence imaging techniques. Functionalization of QD surfaces has been carried out using a metal-free click reaction reported by Texier et al.[6] Cyclooctyne-modified QDs have been functionalized with azido-tagged mannosamine, and the conjugated QDs have been compared with mannosamine conjugates prepared by CuAAC. Interestingly, the QDs—which were used to label cell membrane epitopes—that were prepared in the presence of Cu suffered a 50% reduction in the initial quantum yield, while the QDs prepared by SPAAC even displayed a 30% improvement of the quantum yield.

An application of cyclooctyne-based click reactions on glass surfaces was recently developed by Kuzmin et al.[7] An epoxide-functionalized glass surface was treated with an amine-linked azadibenzocyclooctyne (ADIBO), to yield a highly reactive cyclooctyne-functionalized surface (Figure 2). Its reactivity was studied with azides bearing a fluorescence probe. Since inclusion of the amide functionality speeds up the reaction with respect to that of dibenzocyclooctyne, with just 0.1 mM azide the click reaction reached surface saturation levels after only 100 min at room temperature. This high efficiency at low concentrations is of significance, as the amount of tailor-made azides may be limited either by availability of naturally derived materials or by synthetic complexity. The authors also studied the inverse reaction, in which azide-functionalized surfaces were treated with ADIBO. Use of routinely available azide-functionalized surfaces in combination with ADIBO allowed a versatile entry to surface bioconjugation through biotinylation of the surface,

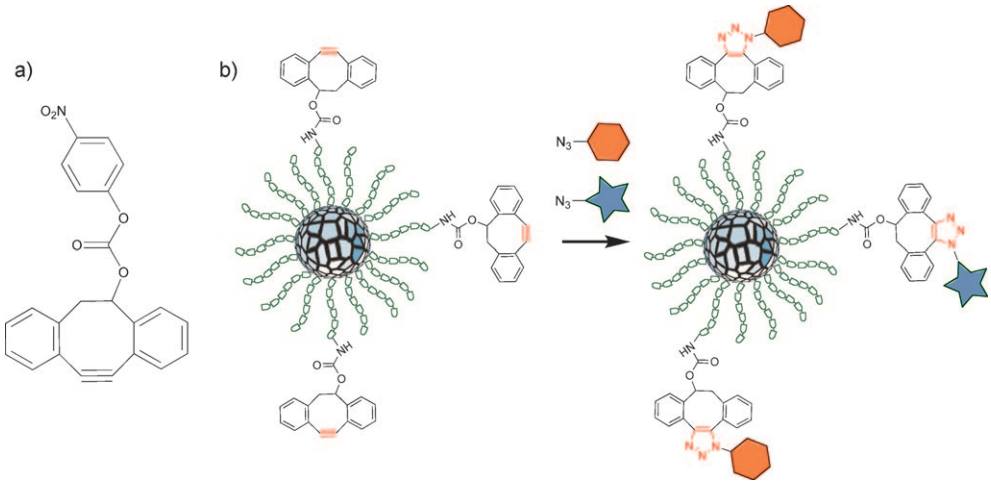

Figure 1. a) Structure of dibenzocyclooctyne with activated ester. b) Micelle with cyclooctyne-functionalized surface. Blue stars and orange hexagons represent different functional groups.

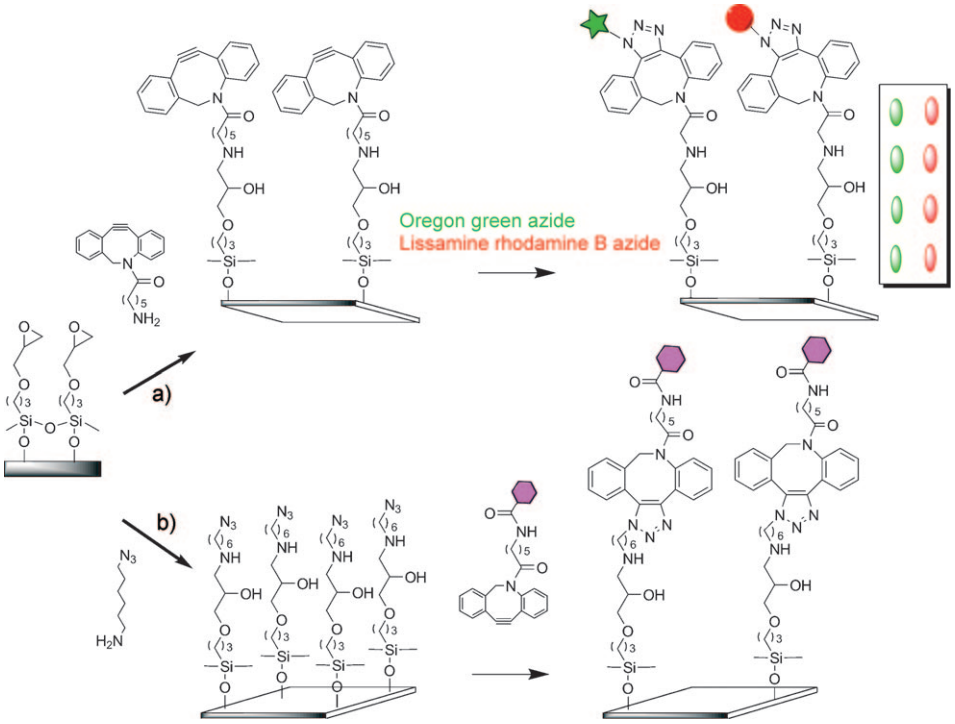

Figure 2. a) Azadibenzocyclooctyne-functionalized glass surface, which was subsequently treated with fluorescent moieties (green star, red circle. b) Inverse copper-free click reaction starting from azide-terminated surfaces and subsequent local immobilization of a fluorescent label (violet hexagon).

immobilization of proteins, and pattering of the surface with fluorescence probes.

While the above reactions proceed in a facile manner, the method of localization is basically determined by locally dropping a reactive solution on a surface. Although spotting is routinely feasible with microaddition systems, a combination with other localization methods would be desirable. Such a technique has recently been developed by Orski et al., who attached a protected dibenzocyclooctyne moiety with an amine linker to a polymer brush on silicon oxide.[8] The dibenzocyclooctyne unit was masked by a photoremovable cyclopropenone moiety, which is on the one hand fully thermally stable but on the other hand can be rapidly activated upon irradiation with UV light (350 nm, 3 min; Figure 3). This photochemical step led to a quantitative loss of CO and yielded reactive C≡C bonds at the irradiated spots. The authors demonstrated this unmasking using a process of local irradiation, reaction with N_3-linked dye 1, subsequent deprotection of all remaining cyclopropenone moieties, and reaction of all newly formed triple bonds with N_3-linked dye 2, which led in a highly smooth way to a patterned surface.

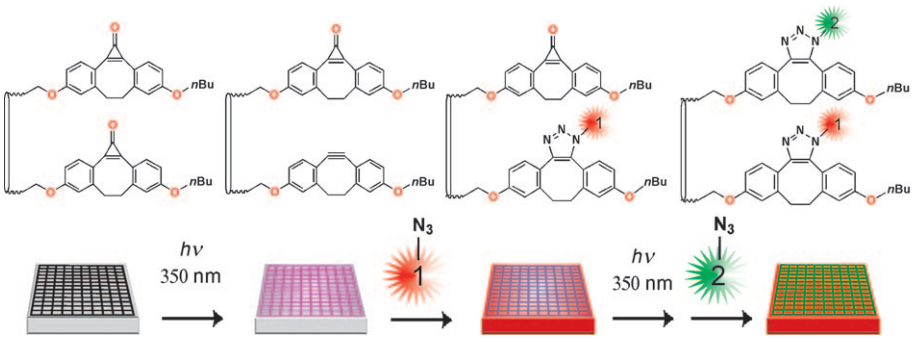

Figure 3. Photopatterning of click surfaces using sequential light-induced local deprotection and cycloaddition reactions.[8]

Like for polymer science,[9] purification on surfaces by nonchromatographic methods is essential and drives the development of true click reactions: after an incomplete reaction, surfaces can behave slightly to significantly different from surfaces with quantitatively modified end groups, yet they typically cannot be converted to a fully substituted surface. At best, a generic passivation method (surface blocking) can be used to minimize the problems. The chemistry displayed above therefore also points to further developments that will be required. First, the reaction efficiency needs to be quantitative. The difficulty in achieving this aim is seen in the elegant work by Boons et al.,[5] who report yields of 58–76% for the cycloaddition reaction. Second, given the size of, for example, the dibenzocyclooctyne moiety, it is unlikely that all azides on an azide-terminated surfaces or in the above-mentioned polymer brush will have reacted. As these surfaces are of specific interest for biodiagnostic purposes, cross-contamination should be as close to zero as possible, thus requiring the development of at least quantitative blocking procedures if the cycloaddition reaction cannot be performed quantitatively. Finally, highly reactive cycloalkyne moieties tend to also react with other groups than just azides, thus reducing the lifetime of cycloalkyne-terminated surfaces. Depending on the application, a tunable reactivity of these moieties is thus in order, requiring further comparative kinetic studies. The work presented above therefore displays both the potential and the challenges of this exciting branch of organic surface chemistry.

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