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Orthogonal Covalent and Noncovalent Functionalization of Cyclodextrin-Alkyne Patterned Surfaces

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Abstract: The creation of cyclodextrin patterns on a fluorescent reporter surface by microcontact printing provides a versatile orthogonal surface modification method. The alkyne-β-cyclodextrin surface is prepared through a "click" reaction on alkyne-terminated coumarin monolayers. The resulting alkyne-β-cyclodextrin surface can be functionalized through supramolecular microcontact printing on cyclodextrin host patterns and by reactive microcontact printing-induced click chemistry on the alkyne-terminated patterns. The orthogonal covalent and supramolecular "host−guest" functionalization of the surface, and its specificity as well as selectivity, is demonstrated by sequential and one-step printing procedures.

Here we present a versatile orthogonal surface modification strategy creating cyclodextrin patterns on a coumarin-alkyne functionalized surface by reactive microcontact printing (μCP), which can be employed for consecutive covalent and noncovalent immobilization reactions. The concept of orthogonal self-assembly as the selective immobilization of multicomponent systems on heterogeneous substrates was first introduced by Wrighton and Whitesides,[1] and different strategies have been reported since to prepare patterned surfaces with orthogonal functionalities. Photolithography has been used to pattern surfaces for subsequent orthogonal self-assembly of particles, polymers, and carbohydrates,[2] and Gleason et al. made patterned orthogonal reactive nanodomains with amine and acetylene groups using capillary force lithography.[3] Soft lithography techniques like microcontact printing, μCP,[4] can be used to covalently couple molecules onto reactive surfaces, e.g. via amide,[5] imine,[6] urethane, or carbamate[5] bond formation, and also the Huisgen 1,3-dipolar cycloaddition of azide and alkynes, so-called "click chemistry",[7] has been applied for surface modification[8] and for reactive μCP.[9] An illustrative example of a functional surface allowing reversible interactions is the so-called molecular printboard,[10] a monolayer of β-cyclodextrins (β-CD), which has been used to immobilize small molecules, dendrimers, particles, and biomolecules from solution,[11] by supramolecular μCP.[12] We now report a surface with orthogonal functionalities, patterning heptakis-azido-β-cyclodextrin (N_3-βCD) on a surface-immobilized monolayer of alkyne-terminated coumarins by μCP. The resulting orthogonal surface provides selectivity and specificity by allowing covalent bond formation on the alkyne-terminated patterns and supramolecular, host−guest, interactions on the β-CD patterns. Both modalities can be addressed independently, from solution as well as through μCP.

The orthogonal surfaces were prepared by μCP of N_3-βCD (**2**) through the "click" reaction on an alkyne-coumarin monolayer, prepared from a bifunctional profluorogenic coumarin probe **1** (Scheme 1),[13] that shows an increase of the fluorescence quantum yield of 27% upon the triazole ring formation (see Supporting Information (SI) for details on synthesis and characterization of the compounds and surfaces).

The surface-immobilized coumarin **1** acts as a reporter for direct probing of the reactive μCP, as the fluorescence intensity enhancement of the coumarin monolayer upon the triazole formation directly and unambiguously proves the formation of covalently immobilized β-CD patterns (see Figure S6A). To further confirm the presence of the β-CD patterns and orientation of the CD, the obtained substrates were incubated with an aqueous solution of a lissamine rhodamine-labeled divalent adamantyl guest (Rhodamine-Ad_2), which binds the β-CD patterns *via* divalent host−guest interactions. After intense rinsing and sonication with a mixture of EtOH/H_2O, the adamantyl guest molecules were only observed in the β-CD patterns (see Figure S6B), demonstrating moreover the availability and correct orientation of the immobilized β-CD for host−guest interactions, as well as the site specificity of the **alkyne-βCD** surface.[11d]

The **alkyne-βCD** surface described above allows several consecutive and/or simultaneous (printing and/or solution) steps to further orthogonally functionalize the patterned surface, exploiting the opportunity to use covalent and/or noncovalent immobilization on the same surface (Scheme 2). For demonstrating the surface's specificity and selectivity we have used azide-functionalized dyes and dye-labeled diadamantyl guests (Schemes 1 and 2, and see SI for the full molecular structures), which were transferred onto the **alkyne-βCD** surface using PDMS stamps with 10×5 μm^2 line patterns, that were printed perpendicular to the line patterns of the alkyne-CD stripes. The azide and adamantyl dyes can be patterned on the **alkyne-βCD** surface through reactive, respectively, supramolecular microcontact printing. The successful immobilization was directly imaged with fluorescence microscopy using different filter settings depending on the excitation and emission wavelengths of the dyes used as indicated in Scheme 2.

As proof-of-concept and to demonstrate the orthogonal reactivity, three different printing protocols were performed. In the first one, a fluorescein-labeled divalent adamantyl guest (Fluorescein-Ad_2) was printed by supramolecular μCP on the **alkyne-βCD** surface (Scheme 2A). Second, azide-labeled Oregon-green was printed by reactive 'click' μCP on the **alkyne-βCD** surface (Scheme 2B). Third, a one-step functionalization was performed by cross-printing a mixture of an azide- functionalized dye (Oregon Green-N_3) and a dye functionalized with adamantyl groups (Rhodamine-Ad_2) (Scheme 2C).

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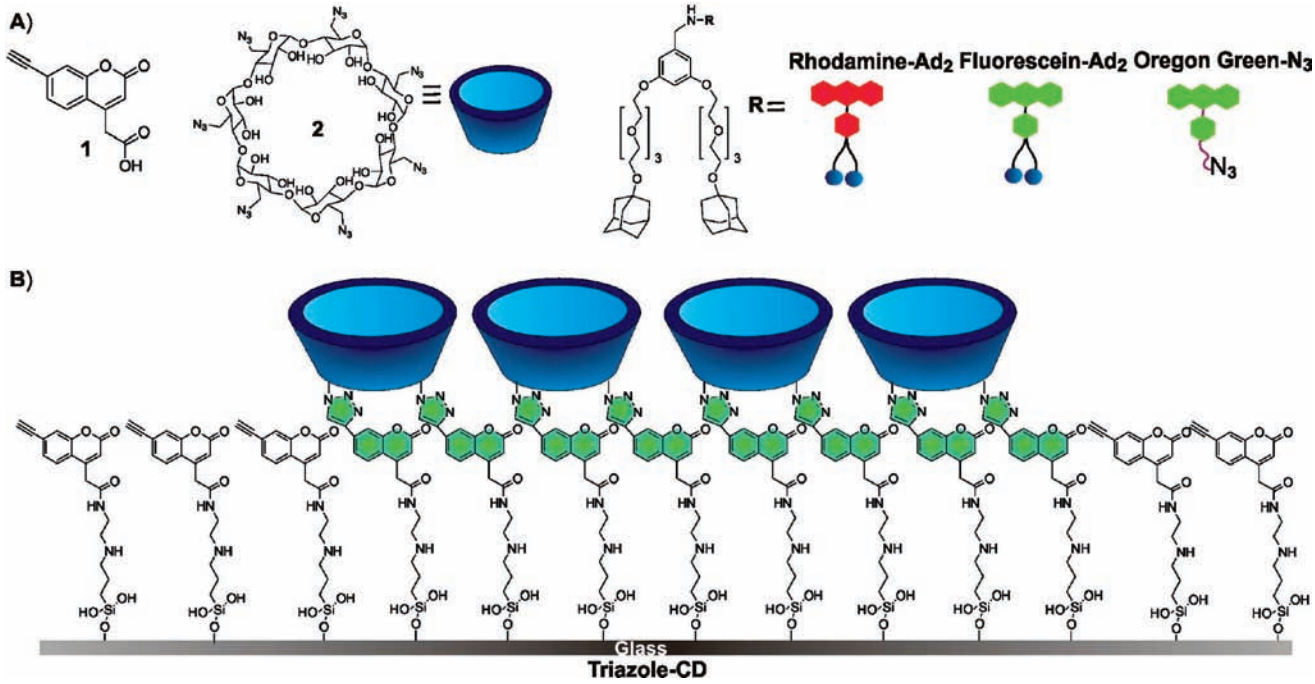
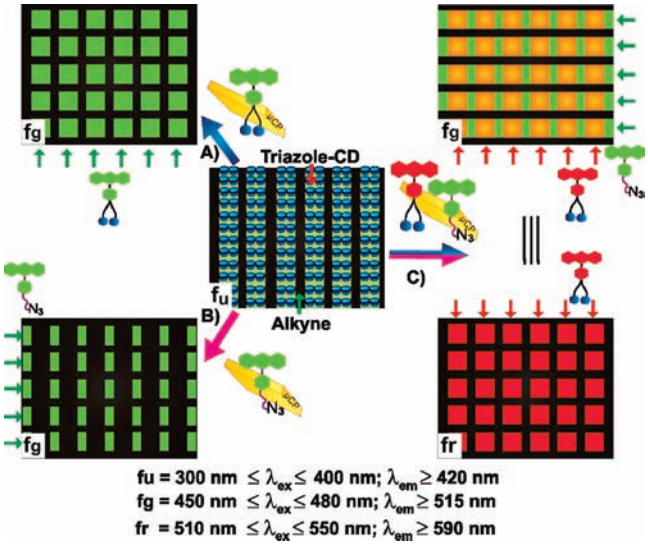
Scheme 1. (A) Chemical Structures of Coumarin (**1**), Heptakis-azido-β-cyclodextrin (N_3-βCD) (**2**) Dye-Labeled Diadamantyl Guests, and Azide-Functionalized Dye (full structures are given in the SI); (B) 1D Side-View Representation of the Molecular Structure of the Orthogonal Alkyne-βCD Patterned Surface Prepared by Printing **2** on Reporting Alkyne-Coumarin Monolayers



Scheme 2. Schematic Procedure for Further Multiple Functionalization of Orthogonal Patterned Alkyne-βCD Surfaces by Supramolecular μCP with Fluorescein-Ad_2 (A) by Reactive μCP with Oregon Green-N_3 (B) and One-Step Fuctionalization by Reactive and Supramolecular μCP with a Mixture of Rhodamine-Ad_2 and Oregon Green-N_3 (C)[a]



[a] Pink and blue arrows indicate covalent bond formation and supramolecular interactions, respectively. Different filters were used to visualize the patterned surfaces: fu indicates UV filter, fg indicates green filter, and fr indicates red filter.

Upon patterning the coumarin monolayer by reactive μCP with N_3-βCD, an **alkyne-βCD** surface is formed with β-CD-lines 10 μm wide and separated by 5 μm of alkynes. Following the procedure depicted in Scheme 2A, a second stamp, previously inked with an aqueous solution of Fluorescein-Ad_2, was brought into contact with the surface, with the line pattern perpendicular to the β-CD lines. The fluorescence image (Figure 1A), taken with blue excitation light ($\lambda_{em} \geq 515$ nm), shows fluorescent 10 × 10 μm^2 squares on the β-CD patterns as a result of the efficient supramolecular μCP.

Complementary to β-CD lines 10 μm wide on the **alkyne-βCD** surface, also the 5 μm stripes of alkynes are still functional, as was proven by cross-printing an azide-functionalized dye, Oregon Green-N_3, on the **alkyne-βCD** surface by reactive μCP (Scheme 2B; see Figure 1B). The fluorescent microscopy image shows green fluorescent 10 × 5 μm^2 areas, only where alkynes were present. Moreover, the dark 10 μm wide lines separating the fluorescent 10 × 5 μm^2 patterns prove the β-CD stripes to be free of reactive alkyne groups.

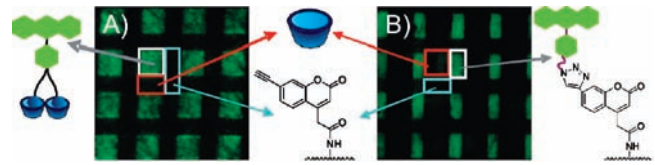


Figure 1. Fluorescence microscopy images of orthogonal functionalized patterned alkyne-βCD surfaces following the process shown in Scheme 2: (A) after cross-printing Fluorescein-Ad_2 onto the patterned alkyne-βCD by supramolecular μCP taken with blue excitation light (green filter, $\lambda_{em} \geq$ 515 nm) and (B) after cross-printing Oregon Green-N_3 by reactive μCP taken with blue excitation light (green filter, $\lambda_{em} \geq 515$ nm).

As final and illustrative evidence of its orthogonality, the selectivity and specificity of the **alkyne-βCD** surface were addressed by a one-step functionalization, according to the procedure described in Scheme 2C. For the two-component site-selective immobilization a stamp was inked with an equimolar mixture of Rhodamine-Ad_2 and Oregon Green-N_3 in EtOH and brought into contact with the **alkyne-βCD** surface, with the pattern perpendicular to the β-CD lines. The consecutively recorded fluorescence microscopy images show that the Oregon Green-N_3 and Rhodamine-Ad_2 are selectively immobilized on the alkyne and β-CD patterns, respectively (Figure 2). When the fluorescence microscopy image was taken with blue excitation light, $\lambda_{em} \geq 515$ nm, green

fluorescent $10 \times 5\ \mu m^2$ squares on the alkyne patterns and brownish $10 \times 10\ \mu m^2$ squares on the β-CD patterns are observed due to the different emission wavelengths of the Oregon Green-N_3 and Rhodamine-Ad_2 dyes, respectively (Figure 2A). On the other hand, when the same area was imaged with green excitation light, $\lambda_{em} \geq 590$ nm, only the $10 \times 10\ \mu m^2$ squares corresponding to the immobilized Rhodamine-Ad_2 on the β-CD patterns are observed (Figure 2B). The different color and size of the fluorescent squares unambiguously and directly demonstrate the high site specificity and selectivity of the **alkyne-*β*CD** surface. The resulting surface shows repeating $15 \times 15\ \mu m^2$ squares in which four differently functionalized areas are present: free β-CD ($5 \times 10\ \mu m^2$) and Rhodamine-Ad_2 filled β-CD ($10 \times 10\ \mu m^2$), alkyne-coumarin ($5 \times 5\ \mu m^2$), and "click"-immobilized Oregon Green ($10 \times 5\ \mu m^2$) areas (Figure 2C).

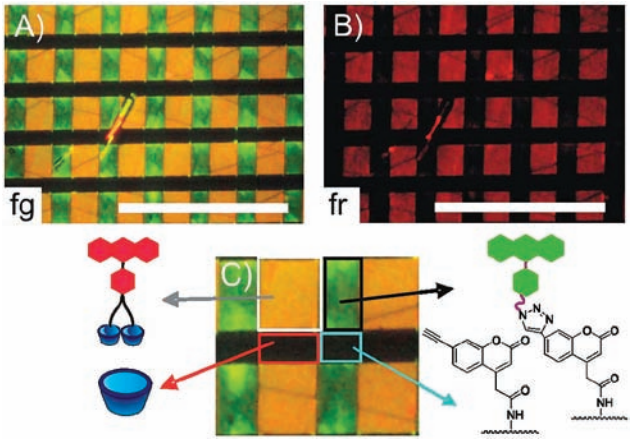


Figure 2. Fluorescence microscopy images of one-step functionalization of orthogonal patterned coumarin-CD surfaces by printing a mixture of Oregon Green-N_3 and Rhodamine-Ad_2 following the process shown in Scheme 2: (A) taken with blue excitation light (green filter, $\lambda_{em} \geq 515$ nm), (B) taken with green excitation light (red filter, $\lambda_{em} \geq 590$ nm), and (C) overview of the four differently functionalized areas. Scale bars represent 55 μm.

In addition to utilizing μCP procedures, the orthogonal surface can also be functionalized addressing the functional groups from solution. In fact, the available cyclodextrin patterns have been visualized, by incubation of the substrate with a solution of Rhodamine-Ad_2, showing good selectivity and directionality over the printed β-CD patterns through the availability of β-CD binding sites before and after the supramolecular μCP (see SI Figures S6B and S7B).

In conclusion, a simple and straightforward approach is presented using μCP to integrate alkyne and cyclodextrin patterns on the same platform, for further orthogonal surface modification through covalent or noncovalent immobilization reactions. The utilization of an alkyne-modified coumarin as a profluorogenic probe allowed the immobilization of N_3-CD and the direct monitoring of the reactive "click" μCP step, with the increased fluorescence of the coumarin unambiguously proving covalent bond formation. The selectivity and specificity of the orthogonal functionalized **alkyne-*β*CD** surface have been demonstrated by sequential and one-step printing procedures with different azide and bis-adamantyl-functionalized dyes. Printing of a mixture of orthogonal reactive dyes allowed patterned tetra-functionalized surfaces in a one-step printing procedure, with full control of the chemical functionality of the patterns. We are currently investigating the applicability of the orthogonal surfaces as a platform for boundary reactions. The concept of orthogonal covalent−noncovalent surfaces is not limited to alkyne and β-cyclodextrin patterns and can readily be extended to other combinations of covalent and noncovalent immobilization reactions, or with different immobilization modalities, e.g. employing functional groups allowing reversible covalent chemistry.

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Supporting Information Available: Full experimental details including synthetic procedures, photophysical properties of **1**, supplementary figures, and tables. This material is available free of charge via Internet at http://pubs.acs.org.

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