Designing Molecular Printboards: A Photolithographic Platform for Recodable Surfaces

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Abstract: A light induced strategy for the design of β-cyclodextrin (CD) based supramolecular devices is introduced, presenting a novel tool to fabricate multifunctional biointerfaces. Precision photolithography of a modified β-CD was established on a light sensitive tetrazole surface immobilized on a bioinspired polydopamine (PDA) anchor layer via various shadow masks, as well as via direct laser writing (DLW), in order to craft any desired printboard design. Interfacial molecular recognition provided by light generated cavitate domains was demonstrated via spatially resolved encoding, erasing, and recoding of distinct supramolecular guest patterns. Thus, the light directed shaping of receptor monolayers introduces a powerful path to control supramolecular assemblies on various surfaces.

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Supramolecular assemblies on interfaces are of high interest as selective tools to mimic living stimuli responsive recognition systems since complementary host (acceptor)/guest (donor) modules readily allow controlled incorporation and release of specific donor substrates on demand.[1–6] Thus, controlled patterning of host receptors establishes specific features to surfaces in precise locations while surface properties can be easily adjusted by exchanging a guest species in contrast to permanent attachment of a functional component via covalent ligation. So far, the fabrication of molecular printboards as host surfaces "on which multivalent guest molecules can be positioned",[7] is predominantly conducted under thermal stimulation in order to form global self-assembled monolayers (SAMs), as well as layer by layer (LbL) assemblies of receptor precursors.[8,9] Spatially resolved host ligation has been targeted by employing stamping techniques such as nano imprint lithography (NIL) to control the formation of cyclodextrin (CD)-SAMs or microcontact printing (μCP) as a host delivery system in order to covalently bind a CD precursor on a flat surface in predetermined areas.[10,11] However, the positioning of the delivered receptor is restricted by the dimensions of the stamp limiting the flexibility to readily generate any desired host pattern. In addition, stamping technologies are non-adaptable for implementation on topographically challenging interfaces or 3D scaffolds. In order to drive host self-assembly on interfaces to the next level, photodirected engineering of supramolecular materials is a viable option. By pursuing photochemical strategies, individual receptor monolayers can be manufactured at ambient temperature conditions, opening frontiers to involve temperature sensitive substances. Herein, precision photolithography of oligosaccharide cavitate β-CD has been realized by employing micropatterned shadow masks to provide non-irradiated and light exposed domains in order to obtain spatial β-CD grafting. Low μm patterns of covalently attached β-CD have been crafted via direct laser writing (DLW). In addition, interfacial molecular recognition was demonstrated by spatially resolved encoding, erasing, and recoding of distinct guest patterns.

Avoiding the presence of cytotoxic catalysts during host immobilization, light induced ligation techniques such as the nitrile imine mediated tetrazole ene cycloaddition (NITEC) provide bioorthogonality while being simple in implementation.[12–16] The versatility of the NITEC approach on multiple surfaces, such as bioinspired polydopamine (PDA)[19,20] or cellulose sheets has been recently introduced by our team.[15–18] To es-

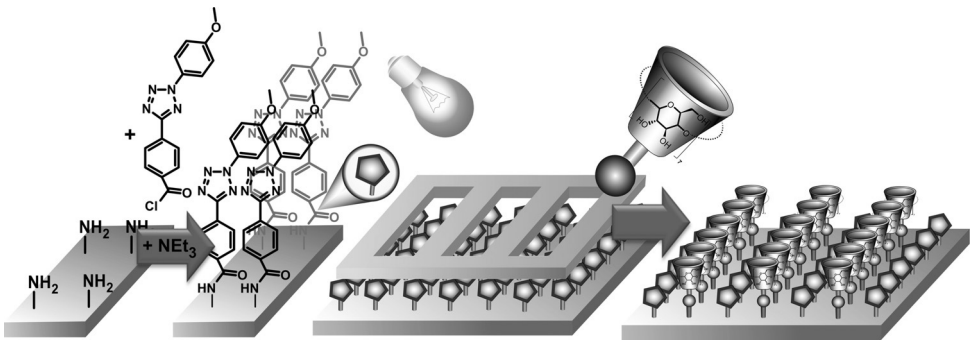
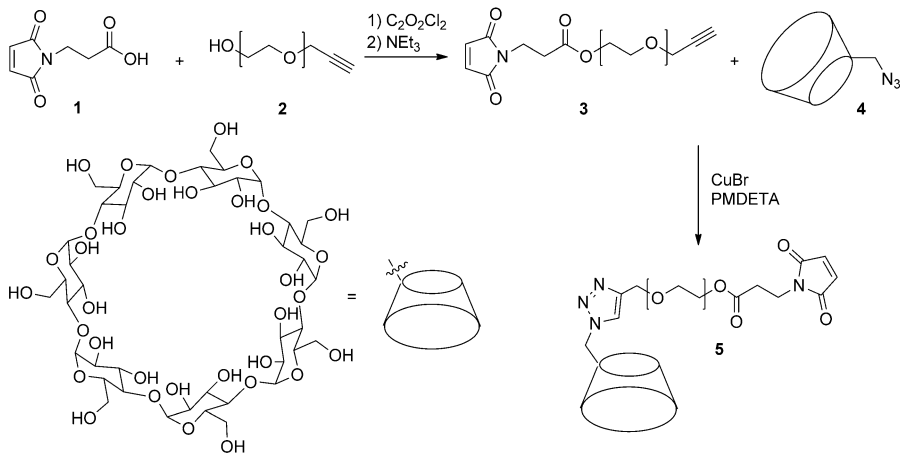


Figure 1. Amidation of an amine exhibiting PDA layer with the acid chloride species of photoactive diaryltetrazole and subsequent light triggered CD ligation employing a micropatterned shadow mask to obtain spatial resolu tion.

tablish a light controlled method for the generation of spatially defined molecular printboards, a photoactive surface was prepared. As illustrated in Figure 1, global coating with a photoactive tetrazole agent was accomplished via amidation of the free amine functionalities provided by the PDA anchor layer polymerized on a silicon substrate. The resulting photosensitive surface enables consecutive host ligation due to light triggered nitrogen cleavage from the tetrazole, resulting in a nitrile imine dipole with affinity towards enes.[21,22] Given that NITEC is envisaged as a tool for facile receptor ligation on surfaces, the corresponding host species needs to feature an electron deficient dipolarophile to enable a 1,3-dipolar cycloaddition with the nitrile imine moiety.[23–25] Thus, oligosaccharide β-CD was modified to entail a maleimide functionality connected to a tetraethylene glycol spacer, as depicted in Scheme 1, to additionally enable the outer rim of the β-CD to be positioned on the surface in an unhindered manner to adjacent grafted hosts. The light induced generation of a nitrile imine dipole enables the spatially controlled cycloaddition with the electron deficient maleimide entity of the manufactured β-CD precursor (**5**) by providing light exposed and non-irradiated areas via implementation of various shadow masks, as illustrated in Figure 1.[18]

The β-CD precursor **5** is immobilized on the photoactive tetrazole surface via irradiation with a lamp emitting at $\lambda_{max} = 320$ nm over a time period of 1 h. The covalent binding resulting from pyrazoline formation of **5** with the tetrazole interface due to light exposure is evidenced by X-ray photoelectron spectroscopy (XPS). Figure S3 (Supporting Information) illustrates the comparison of the C 1s XP spectra of an untreated tetrazole surface as well as from non-irradiated (blank) and irradiated tetrazole surfaces in the presence of **5**. Comparing the XP spectrum recorded from the blank sample with the spectrum of the untreated tetrazole surface reveals no significant differences in the C 1s region, thus excluding physisorption of **5**. Since the oligosaccharide receptor precursor features 34 C-O-C/H units, an increase in intensity of the signal at 286.7 eV assigned to the binding energy of the C-O-C/H species is expected for successful ligation of **5**.[16,26] Indeed, the sample exposed to irradiation shows an increase of the C-O-C/H moiety indicating successful attachment of **5**. Targeted receptor patterns were manufactured by selectively covering photoactive domains with a shadow mask in order to irradiate predetermined areas in the presence of modified β-CD **5**, as displayed in Figure 1. Characterization of the photolithographic patterns was carried out by time-of-flight secondary ion mass spectrometry (ToF-SIMS) to allow fragments resulting from **5** to be assigned to specific areas of the surface, as depicted in Figure 2.

ToF-SIMS imaging revealed multiple fragments ($C_3H_5O_2^+$, CH_3O^+, $C_2H_5O^+$, $C_3H_5O^+$) referring to the β-CD moiety being grafted on light exposed areas, as displayed in Figure 2 (left, middle). In addition, precision photolithography of **5** was accomplished by employing direct laser writing (DLW) as a two-photon laser lithographic technique.[27–30] To the best of our knowledge, DLW is herein combined with tetrazoles for the first time. The light directed β-CD ligation induces covalent attachment of **5** triggered by a focused laser beam, thus enabling us to write any predefined cavitate pattern in a low μm scale (Figure 2 (right), Supporting Information, Figure S8) as evidenced via ToF-SIMS. The versatility of the β-CD printboards is exemplified by encoding and erasing guest patterns resulting from complexation and induced dissociation from the receptor patterned interface.



Scheme 1. Esterification of maleimide propionic acid **1** with the hydroxyl functionality of alkyne monofunctional ized tetraethylene glycol **2** and subsequent copper(I) catalyzed 1,3 dipolar cycloaddition of the resulting spacer precursor **3** with β CD azide **4** to form a maleimide functionalized β CD **5**.

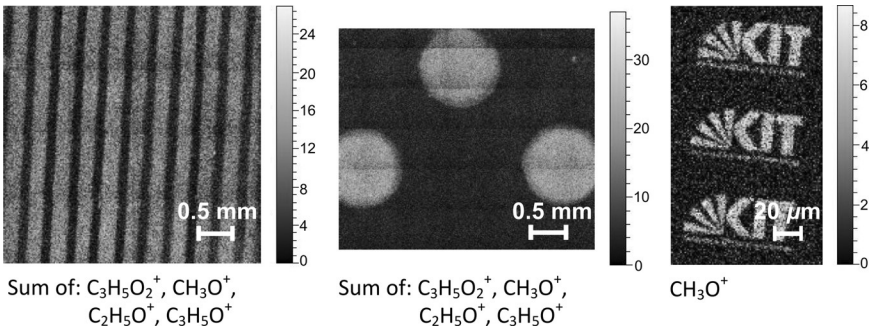


Figure 2. ToF SIMS images of photopatterned receptor printboards derived from the sum of the signals detected at 73.0 u ($C_3H_5O_2^+$), 31.0 u (CH_3O^+), 45.0 u ($C_2H_5O^+$), and 57.0 u ($C_3H_5O^+$) assigned to fragments of the corre sponding β CD. A corrugated (left) and a holey (middle) shadow mask were used for micropatterning. The image on the right depicts the CH_3O^+ fragment in spatial resolution derived from a direct laser written KIT logo.

der structure established by the poly(CBMAA) brushes was scanned with a stylus to obtain the corresponding 3D topography image (Supporting Information, Figure S12). Remaining photoactive domains are able to link an additional maleimide species onto the surface. Thus, the selectively passivated surface is globally irradiated in order to induce attachment of **5** conducted under the same experimental conditions as described before. A two-armed guest (**6**, Figure 4)

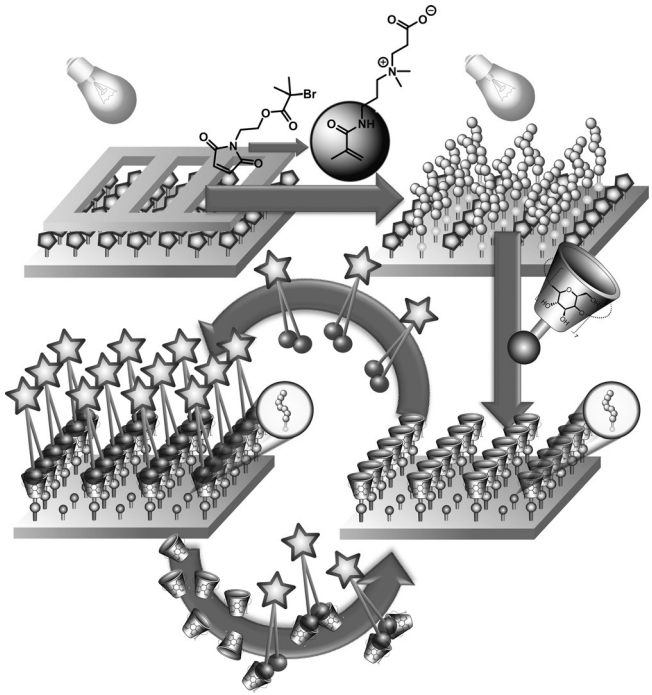


Figure 3. Spatially resolved passivation via photopatterning of an ATRP ini tiator enabling polymerization of poly(CBMAA) brushes and subsequent photoligation of the β CD receptor **5**. Consecutive guest delivery generates a guest pattern to enable reversible erasing and recoding of the guest spe cies.

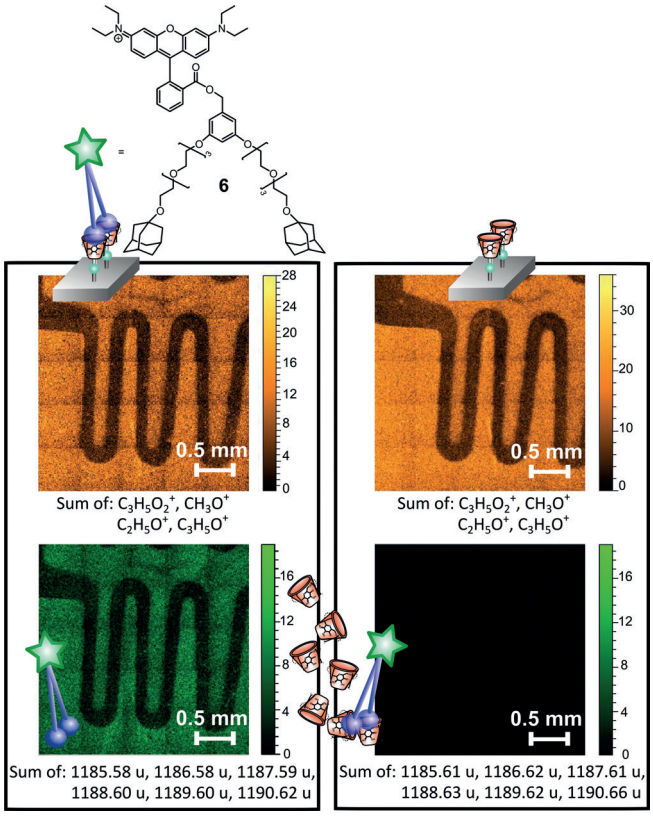


Figure 4. ToF SIMS experiments were conducted employing the same sur face. ToF SIMS images recorded from spatially resolved encoding (left) and erasing (right) of guest **6** from a β CD printboard. The meander line is pas sivated by poly(CBMAA) brushes, whereas **5** is attached elsewhere, exhibit ing signals detected at 73.0 u ($C_3H_5O_2^+$), 31.0 u (CH_3O^+), 45.0 u ($C_2H_5O^+$), and 57.0 u ($C_3H_5O^+$) assigned to fragments of the corresponding β CD **5** (top images). Spatially controlled guest incorporation was imaged by the sum of the signals derived from the isotope distribution of **6** (bottom left). The ToF SIMS image on the bottom right reveals a lack of fragments as signed to **6**, thus indicating successful erasing of the guest pattern.

As depicted in Figure 3, the supramolecularly and spatially resolved printboard applied for guest delivery experiments initially underwent passivation of the PDA anchor layer implemented by spatially grafted polymer brushes.[31] The passivation stage was conducted by deploying a meander shaped shadow mask on the tetrazole-functionalized PDA surface to control light triggered NITEC with a maleimide-functionalized atom-transfer radical-polymerization (ATRP) initiator (**7**).[18] Poly(carboxybetaine methacrylamide) (CBMAA) brushes were subsequently polymerized from the prepatterned initiator-functionalized surface, thus exhibiting unscathed tetrazole entities on non-irradiated sections.[31] Due to crucially changing the topography of the initial photosurface, the profile of the meanentailing β-CD-affine adamantyl anchor groups was synthesized and delivered from a 0.05 mM aqueous solution to the receptor pattern to induce complexation. Guest functionalization was oriented on established guests applied for β-CD complexation.[32–35]

Successful encoding of **6** on predetermined sections of the supramolecular surface was verified by ToF-SIMS imaging (Figure 4) depicting characteristic fragments of guest **6** (bottom left) located on the identical sections where fragments assigned to receptor **5** were detected (top left). The guest encoded surface is immersed into a saturated β-CD solution to offer an excess of competitive host in the solvent phase in order to erase the guest pattern from the interface.[8,36] Figure 4 (right) displays the recorded ToF-SIMS images after erasing the guest pattern from the molecular printboard. Though characteristic fragments attributed to the receptor layer (top right) are detected in a defined location, the pattern resulting from the presence of the guest **6** has vanished (bottom right). In addition, Figure S7 (Supporting Information) illustrates the pertinent section of the mass spectrum (top) according to the isotope distribution of **6** on the encoded surface, matching the simulated spectra of **6** (top), as well as the complementary spectra deduced from the erasing experiment (bottom).

The dynamics of the encoding and erasing procedure was further investigated via surface plasmon resonance (SPR, Supporting Information, Figure S9 (left)). The sensograph resulting from the injection of a 0.05 mM solution of guest **6** to a β-CD surface, immobilized on a gold sensor substrate following similar functionalization steps as described before, revealed successful guest complexation evidenced due to a wavelength shift between the baselines before and after injecting **6** for 15 min. Subsequent injection of an aqueous, saturated solution of β-CD and consecutive purging with water in order to remove the excess of β-CD results in a sharp descent of the sensor response stabilizing with constant water flow, thus indicating guest dissociation from the host interface. In addition, the supramolecular ability of the β-CD printboard to reversibly encode and erase was demonstrated by multiple guest in- and exclusion cycles monitored via ellipsometry and contact angle measurement, revealing an alternating behavior of the dry layer thickness respectively wettability of the surface (Supporting Information, Figure 5, Table S2 and Figure S10). Modeling **5** in bulk attached on a surface with and without complexing a single or both anchor groups of guest **6** by molecular dynamics simulations revealed elevated values for the maximal *z*-distance in a comparable ratio to the difference in layer thickness determined by ellipsometry (increase on printboard by a factor of 1.3, increase in model by a factor of 1.2 or respectively 1.3, Supporting Information, Figure S11).

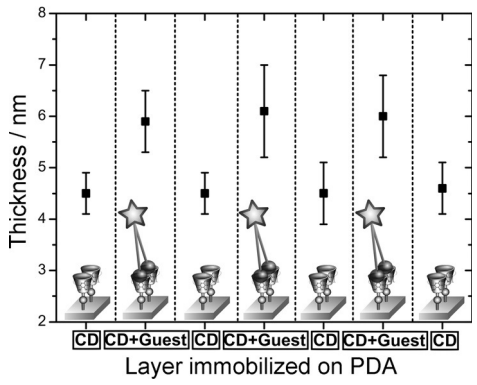


Figure 5. Alternating averaged dry layer thickness of the crude β CD layer on PDA with and without the incorporated guest species **6** determined via ellipsometry.

In summary, designing β-CD printboards via phototriggered ligation was introduced. Various receptor patterns have been written on a light sensitive tetrazole surface immobilized on a PDA anchor layer covered with micropatterned shadow masks exposed to irradiation of a lamp emitting at $\lambda_{max} =$ 320 nm over a time period of only 1 h. Additionally, successful β-CD lithography on tetrazole surfaces was accomplished via DLW, thus demonstrating the versatility of the light triggered approach to write arbitrary low μm-resolved host patterns. The supramolecular applicability of the β-CD printboard was demonstrated by reversible encoding and erasing of an adamantyl-functionalized guest **6**. Indeed, light controlled passivation of distinct areas of the PDA layer with poly(CBMAA) brushes was conducted before the modified β-CD **5** was photopatterned on unscathed tetrazole domains. Subsequent guest delivery from an aqueous solution to the receptor interface and consecutive washing with a saturated solution of free β-CD revealed controlled guest in- and exclusion within well-defined domains of the supramolecular printboard, as imaged via ToF-SIMS. Further, multiple guest encoding, erasing, and recoding cycles have been monitored via ellipsometry and contact angle measurement.

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Keywords: cyclodextrin · molecular printboard · photoconjugation · supramolecular chemistry · surface modification

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